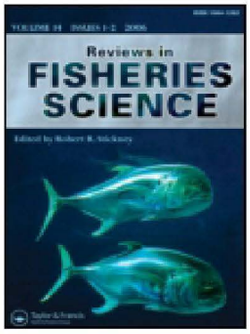

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Reviews in Fisheries Science

ISSN: 1064-1262 (Print) 1547-6553 (Online) Journal homepage: http://www.tandfonline.com/loi/brfs20

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To cite this article: E. L. Brannon , K. Collins , M. A. Cronin , L. L. Moulton , A. L. Maki & K. R. Parker (2012) Review of the Exxon Valdez Oil Spill Effects on Pink Salmon in Prince William Sound, Alaska, Reviews in Fisheries Science, 20:1, 20-60, DOI: 10.1080/10641262.2011.643697

To link to this article: http://dx.doi.org/10.1080/10641262.2011.643697

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Reviews in Fisheries Science, 20(1):20–60, 2012
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ISSN: 1064-1262 print / 1547-6553 online
DOI: 10.1080/10641262.2011.643697

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Review of the *Exxon Valdez* Oil Spill Effects on Pink Salmon in Prince William Sound, Alaska

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The Exxon Valdez oil spill that occurred in Prince William Sound, Alaska, in March of 1989 was the largest crude oil spill in the United States at that time, and it was anticipated to have disastrous effects on the ecology and fisheries of that coastal region. The large pink salmon returns to the sound, a major commercial species in Alaska, were of great concern. The Exxon Valdez Oil Spill Trustee Council, a council of government agencies formed to assess the impact of the spill for recovery purposes, concluded that pink salmon in Prince William Sound were damaged by the oil, based on investigations of the Alaska Department of Fish and Game and the National Marine Fisheries Service. Agency scientists claimed that the oil increased incubation mortality of pink salmon and those exposed to oil experienced less growth, higher long-term mortality, and reduced reproductive success. This contrasted with data and conclusions of the non-agency scientists led by the University of Idaho researchers that showed no impact of oil on incubating eggs or on juvenile pink salmon in marine waters, and no reduction in egg viability of returning adults. In this article the authors re-examine the evidence and resolve the problems that resulted in the different interpretation of research conducted by agency and non-agency scientists. The resolution has far reaching implications on ascertaining the impacts of marine oil spills.

Keywords oil spill, pink salmon, oil toxicity, *Exxon Valdez*

INTRODUCTION

On March 24th of 1989, the *Exxon Valdez* went aground on Bligh Reef spilling ~11 million gallons of crude oil into northeast Prince William Sound (PWS). This was the largest oil spill in the United States at that time and there were widespread concerns that salmon resources in PWS would be seriously affected. The greatest concern involved the pink salmon, *Oncorhynchus gorbuscha*, the most abundant salmon species and the foundation of the commercial fisheries in that part of Alaska.

Pink salmon have a consistent two-year life cycle, and thus are segregated as even- and odd-year runs (Heard, 1991). They spawn in freshwater, as do other salmon species, but the emergent fry go directly to the marine environment for their nursery period before embarking on a 14-month, open-ocean migration that ends with the return to spawn in their stream of origin. A large percentage of the pink salmon populations in PWS spawn in the intertidal reaches of streams entering the sound, and thus the incubation environment of the species was at risk of contamination from oil that washed into those stream reaches. The early marine life history of pink salmon also meant this species could be exposed to oil contaminated marine waters upon emergence as fry entering their nursery areas. However, there was great uncertainty about the actual level of risk that would be experienced by pink salmon. As the *Exxon Valdez* crude (EVC) reached landfall on southwestern shores of the Sound, the 1988 pink salmon brood year alevins were approaching the free-swimming fry stage and were exposed to oil in the incubation environment

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during that short interval of time prior to emergence from their streams in April and May, and subsequently in their marine nursery areas. The pink salmon of the following 1989 brood year experienced a different level of risk. They were exposed to residual oil that remained in the stream substrate during their entire incubation period from the fall of 1989 to the spring of 1990. Upon their emergence oil in marine waters was nearly gone and the potential of risk on their early feeding life stage also diminished.

Because of the uncertainty about the prospects of harm, non-agency scientists sponsored by Exxon Corporation launched extensive studies to determine the effects of oil on incubating pink salmon eggs in PWS streams and on juveniles in the marine environment. Field studies on incubation success that included survival monitoring and in-stream bioassays revealed no elevated mortality of eggs or pre-emergent fry in the ~30 streams that were oiled out of ~1,300 streams entering the sound, nor was there any post-emergent fry mortality observed in the receiving marine environment (Brannon et al., 1995; Moulton, 1996).

This was not true in other studies that assessed the impacts of EVC on pink salmon. Concurrently, a major effort was undertaken by Alaska Department of Fish and Game (ADFG) to monitor incubation success of pink salmon in selected oiled and non-oiled streams entering PWS. In the series of stream surveys that were conducted over a nine-year period, ADFG reported higher egg mortality in oiled streams (Sharr et al., 1994; Bue et al., 1996, 1998), including some of the same streams in which non-agency scientists reported no spill impacts (Brannon et al., 1995; Moulton, 1996).

The Auke Bay Laboratory (ABL) of the National Marine Fisheries Service (NMFS), National Oceanic and Atmospheric Administration (NOOA), was also engaged in the assessment of spill effects on PWS pink salmon. NMFS researchers concentrated their effort on laboratory studies from which they developed an oil toxicity narrative and concluded from those studies that very low concentrations of oil were responsible for the egg mortality observed by ADFG in PWS streams (Marty et al., 1997b; Heintz et al., 1999), and that exposure to EVC from the spill during incubation would have long-term effects (Heintz et al., 2000; Rice et al., 2001, 2007).

In addition to the oiled stream studies the agencies undertook investigations on juvenile pink salmon in nearshore marine waters of PWS. Although their investigations did not reveal any mortality, they reported that in some cases juveniles associated with oiled marine areas of the sound were smaller than those in non-oiled areas. They interpreted smaller size to mean lower initial marine growth (Wertheimer and Celewycz, 1996; Willette, 1996). They felt smaller size would increase susceptibility to predation and result in lower marine survival based on other marine survival studies (Hargreave and LeBrasseur, 1985; Heard, 1991; Parker, 1971).

ADFG took the egg mortality data and the growth data and applied them as variables in a model to predict what those effects would have on the number of returning adults (Geiger et al., 1996). The Geiger model predicted total effects of oil exposure on survival by using five stochastic steps:

$$s_t = Fem_t \times Fec_t \times Dep_t \times Seg_t \times Smr_t$$

where s_t is the total survival, Fem_t is the fraction of the run that were females, Fec_t the mean fecundity, Dep_t the fraction of eggs actually deposited, Seg_t the egg survival, and Smr_t the total marine survival. Using the Geiger model, the total mortality was estimated as a loss of about 2.3 million wild fish in the multiyear (1990–1994) return of 144 million adult pink salmon (Geiger et al., 1996). Although that number represented a relatively minor impact on the entire PWS pink salmon run, it constituted a major effect on those fish actually exposed to EVO. The estimated impact amounted to as much as 27% higher egg mortality in oiled streams as late as 1993. In 1989 the reduced size of juveniles exposed to oil in their marine nursery areas, combined with the egg losses, translated into an estimated 28% higher mortality of wild fish than what would have occurred in the absence of the spill. The *Exxon Valdez* Oil Spill Trustee Council (EVOSTC), a council of government agencies formed to assess the impact of the spill for recovery purposes, concluded from these data that the total mortality amounted to a reduction in returning adults of ~2% of the entire PWS pink salmon population and that the population did not recover from oil exposure until 2001, twelve years after the spill (EVOSTC, 2002).

Therefore, the EVOSTC projection of pink salmon losses due to the *Exxon Valdez* oil spill (EVOS) imbedded in the Geiger model (Seg_t & Smr_t) were in contrast to the reports by non-agency scientists of no oil related increases in egg mortality during incubation or losses of juveniles exposed to oil in marine waters. Based primarily on the laboratory research that showed incubation losses at very low oil concentrations, NMFS investigators suggested that the water quality standard for total polycyclic aromatic hydrocarbons (TPAH) in Alaska should be lowered to 10 pptr (Heintz et al., 1999) and that urbanized areas have an insidious pollution potential (Rice et al., 2001). Those reports have had very serious effects on how oil contamination in the marine environment should be viewed, such as the oil associated with the 2010 BP Gulf of Mexico spill (Maki, personal communication), standards limiting permits for marinas and dredging operations, and it has altered concepts on the effects of chronic exposure (Heintz, 2007; Rice et al., 2007).

Authors' Purpose for Review

The purpose of the authors in this review is to resolve conflicting conclusions from the field and laboratory assessments of the *Exxon Valdez* spill effects on pink salmon embryos and fry by the agency and non-agency investigators. Details of evidence are presented that address the problems by separating the alleged effects on pink salmon as follows: Section I egg mortality in oiled streams of PWS, Section II the interpretation of laboratory results on the chronic toxicity of weathered oil, Section III the reduction of fry growth from oil exposure, and

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then in Section IV the differences in published accounts of spill affects are reconciled. Oil contamination and exposure in this review are presented in parts per billion (ppb) to standardize concentrations in sediment, water, and tissue for comparative purposes. ADFG and NMFS affiliated scientists are referred to as agency scientists. Exxon research contract scientists and affiliated investigators are referred to as non-agency scientists.

SECTION I: PINK SALMON EGG MORTALITY

In the fall of 1989, the determination of no measurable impact of EVOS on incubating pink salmon in oiled streams (Brannon et al., 1995; Moulton, 1996) contrasted with ADFG egg mortality data taken at the same time on surveys of some of the same streams (Sharr et al., 1994; Bue et al., 1996). In the present analysis all of the pertinent evidence was re-examined to resolve the differences by review of the reported mortality in stream surveys, present the field data that dispute the allegation, and reanalyze the mortality in light of pink salmon early life history to reconcile those differences by focusing on the problem of how the original data were interpreted.

ADFG Egg Mortality Stream Survey Results

ADFG routinely survey PWS streams in the spring of the year to estimate pre-emergent pink salmon fry (alevins) survival to assist in predicting run size for the following year. In 1989, prior to the spill, they had just conducted the survey on the 1988 brood, which provided the pre-spill conditions for the opportunity to assess the post-spill impact of the oil contamination in some of those same streams. Therefore, ADFG conducted a second survey and found there were no differences in pre-emergent fry mortality from what was observed in the pre-spill surveys (Sharr et al., 1994). However, in the fall of 1989 ADFG repeated the survey on recently spawned eggs of the 1989 brood. Eggs were flushed from their redds using a hydraulic pump and sampling methods described by Bue et al. (1996), and a higher mortality was detected in oiled streams. They continued their monitoring program of the ten oiled streams that were among the worst contaminated and 15 non-oiled reference streams in close proximity over a period of nine years. The sampling times ran from September 21st to October 28th, coinciding with the very early part of post-spawning period. The results indicated that oiled streams had significantly higher mortality than the non-oiled reference streams from 1989–1993, and again in 1997. Although highly variable among streams and years (Cronin and Bickham, 1998), it was concluded by ADFG that the egg mortality in 1989 and 1990 was the result of direct exposure to oil. Higher losses observed in oiled streams over the next three years, well after oil had essentially left the streams (Brannon et al., 1995), were explained as lingering effects in the form of either genetic damage (Bue et al., 1996, 1998) or physiological anomalies (Rice et al., 2001) experienced by the embryos that affected development of their gametes upon return as adults. Rice et al. (2001) and Craig et al. (2002) suggested that the 1997 mortality resulted from a shift in the intertidal stream, exposing buried toxic oil deposits over which the fish spawned.

Field Studies Conflicting with Oil Induced Egg Mortality

One of the problems with the ADFG egg mortality observations is that other field data collected in many of the same streams at the same time conflict with their results. The following summarizes the evidence from several separate investigations.

Oil Concentrations in PWS Streams

The oil that was spilled from the *Exxon Valdez* was carried over 65 km in open water before it reached landfall, during which weathering exhausted much of the volatile compounds of the oil mass, reducing the monoaromatic components, and leaving the dominance of TPAH (Boehm et al., 1995). Because of tidal dilution and freshwater outflow of the affected streams flushing the intertidal substrate during receding tides, the actual deposit of oil in the stream gravel was very low. Mean sediment-TPAH concentrations in oiled streams, when oil was at its highest concentration in 1989, ranged from 0.5 ppb–267 ppb (Brannon et al., 1995), or only small fractions of the lethal sediment-TPAH threshold of 3,800 ppb–8,300 ppb determined by laboratory bioassays on chronic exposure of pink salmon eggs (Heintz et al., 1999; Brannon et al. 2006a). Mean sediment-TPAH levels in non-oiled reference streams was < 65 ppb. In the subsequent years, the1990 oiled streams still showed that low concentrations of residual oil were present in sediments with mean TPAH ranging between 1 ppb–413 ppb, with one exception (2,818 ppb from one sample), and in the following year means dropped to between 1 ppb–236 ppb. These data show that the contamination of the intertidal stream reaches during the time that oil was present in the stream substrate was well below those concentrations imposing risk of pink salmon incubation mortality in laboratory bioassays (Brannon et al., 2006a; Heintz et al. 1999; Marty et al., 1997b).

Tissue-TPAH Analysis of Embryos Sampled from Oiled Streams

Mean tissue-TPAH concentrations in alevins sampled from oiled streams in 1990 and 1991 were 62.8 ppb and 94.5 ppb dry wt, respectively, and slightly higher than the mean tissue-TPAH of alevins in non-oiled streams of ~39 ppb over the same period (Brannon et al., 1995). These concentrations are far below lethal tissue-TPAH threshold concentrations $> 7{,}800$ ppb shown in long-term laboratory bioassays with pink salmon embryos exposed to naturally weathered EVC (Brannon et al., 2006a). The tissue data of alevins sampled from oil steams were equivalent to the laboratory controls (~90 ppb) that experienced no oil exposure in respective bioassays of Heintz et al. (1999) and Brannon et al. (2006a).

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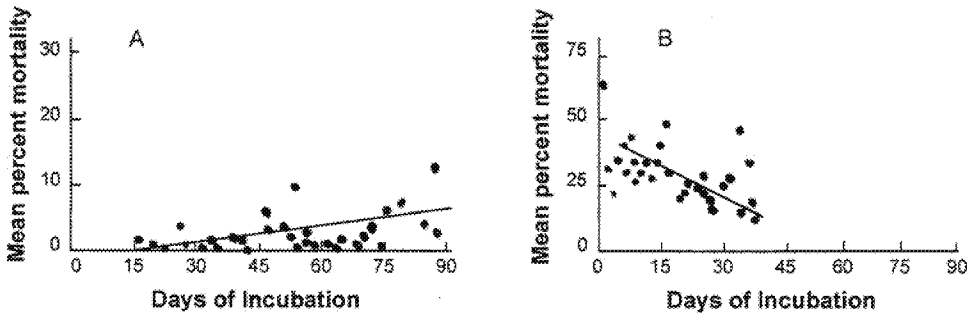

Figure 1 Differences in the pattern of mean mortality versus days of incubation reported in: Figure 1A laboratory bioassay of eggs exposed to an oil-on-gravel concentration of 2,250,000 ppb (Brannon et al., 2006a), and Figure 1B for eggs in ADFG stream surveys from 1989–1997 excluding Port Nellie Juan streams (Craig et al., 2002) (Note different vertical axis).

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Timing of the ADFG Reported Egg Mortality Unrelated to Toxicity Response

Rice et al. (2001, p. 201) stated, "Any tests involving embryos require a long holding period for damage to become detectable." This was the case in all embryo bioassays conducted under laboratory conditions. Bioassay mortality followed a chronic model, spread over the entire incubation period (Figure 1A), and was rarely detected before the eyed stage, but becoming obvious as the live embryos advanced in development (Brannon et al., 2006a; Carls et al., 2005; Heintz et al., 1999; Marty et al., 1997b; Stubblefield et al., 2010). However, the pattern of egg mortality observed in the laboratory was just the opposite of what was reported in the field (Bue et al., 1996, 1998; Craig et al., 2002; Sharr et al., 1994), occurring shortly after spawning completion (Figure 1B). Given that reports on oil toxicity seen in laboratory exposures (Carls et al., 2005; Heintz et al., 1999; Marty et al., 1997b) were inconsistent with the pattern observed in the field surveys, it suggested that other factors had to account for the increased egg mortality in spill-path streams (Brannon and Maki, 1996).

Egg Mortality above the Influence of Marine Oil

ADFG reported higher egg mortality in redds above the high tide level in the freshwater environment, and in streams after oil no longer remained in the intertidal reaches of pink salmon streams. It was suggested that such a result could be inter-generational genetic or physiological damage that occurred in the brood adults during their embryonic development passed on as lethal anomalies to their offspring (Bue et al., 1996, 1998; Rice et al., 2001).

However, empirical and theoretical population genetic assessments indicated that the pink salmon egg mortality observed in oiled streams was not consistent with inter-generational impacts (Cronin and Bickham, 1998; Cronin et al., 2002). Furthermore, no chromosomal damage was observed in incubating pink salmon exposed to 5,700,000 ppb oil-on-gravel mixture in a five year study by Seeb and Habicht (1999) to screen for genetic damage caused by such oil exposure. K-*ras* mutations were induced in the laboratory from exposure to oil in that particular study (Roy et al., 1999), but such mutations were not observed in pink salmon from the oiled streams (Cronin et al., 2002), and thus the field evidence was inconsistent with the hypothesis of genotoxicity.

Also, inter-generational effects could not explain the mortality above tidal influence (13.5% oiled vs. 9.8% non-oiled) in the fall of 1989 (Craig et al., 2002), because those eggs originated from 1987 brood that did not experienced oil exposure. Similarly the 1990 fall egg mortality above tidal influence (24.5% oiled vs. 16.6% non-oiled) were eggs of 1988 brood that only experienced oiling at the very end of their alevin stage, well past developmental differentiation. Consequently, egg mortality above high tide elevation in the fall of 1989 and 1990 cannot be attributed to effects from oil exposure of the parents as incubating embryos.

Inexplicable Persistence of Reported Egg Mortality

A problematic feature of the ADFG data was the long-term nature of the elevated egg mortality in oiled streams from 1989–1993 and reoccurring again in 1997, nine years after the spill. Inter-generational genetic damage or physiological anomalies passed on to the progeny were the explanations offered by ADFG and NMFS scientists (Bue et al., 1996, 1998; Craig et al., 2002; Rice et al., 2001), but that wasn't likely because as many as four generations were involved after oil exposure which was inconsistent with genetic damage (Cronin and Bickham, 1998).

Rice et al. (2001) suggested physiological impairment was consistent with the gonadal histopathology in laboratory exposure of eggs observed by Marty et al. (1997b), but that was reported only as a slight increase in gonadal cell apoptosis in the laboratory exposed alevins in 1989. The apoptosis was not dose correlated, and it was dropped from subsequent assessment in following years. The fact that oil was essentially gone from the streams and intertidal areas of PWS by 1991 (Boehm et al., 1995; Brannon et al., 1995; O'Clair et al., 1996; Short and Babcock, 1996; Wolfe et al., 1996), except in a few on-shore oil deposits, left no mechanism to induce persistent phenotypic damage after 1990, and indicates that something other than oil was responsible for the continuing mortality.

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No Pre-Emergent Fry Mortality Observed in Field Studies

Another compelling piece of evidence against oil as a threat in the incubation environment was the lack of any difference in survival of the pre-emergent fry stage between oiled and non-oiled streams in the spring following elevated egg mortality in the fall (Bue et al., 1996). That was also shown by other investigators monitoring survival in oiled and reference streams, including some of the same streams reported to have high egg mortality, with no statistically significant differences at the emerging fry stage (Brannon et al. 1995; Sharr et al., 1994). This was counter-intuitive since alevins should be especially sensitive to acute oil toxicity (Moles and Rice, 1983). If oil was responsible for egg mortality, not only should the effects of toxicity have continued, but the mortality should have translated into much lower numbers of fry. This suggested that the increased mortality in the fall surveys was confined only to those eggs extracted from the redds, and not among those that continued incubation to the spring fry stage.

Absence of Anomalies among Naturally Incubating Alevins

Alevin samples removed from the incubation substrate in the 1990 by Brannon et al. (1995) were examined for developmental anomalies. No difference ($p > 0.05$) in the proportion of normal alevins in oiled (0.989) versus reference (0.992) streams was seen. The absence of anomalies from field samples contrasted with what was observed in laboratory studies (Brannon et al. 2006a; Marty et al., 1997a), where deformities and blue sac disease (ascites) were observed in alevins exposed to laboratory TPAH doses. The presence of induced deformities and blue sac disease in the laboratory and their absence in the field over the same length of time indicate that laboratory test doses were not representative of that which existed in the oiled streams of PWS.

Retarded Growth Not Shown in PWS Emerging Fry

In laboratory studies at ABL, growth of embryos was affected by exposure to high oil/gravel mixtures (Heintz et al., 1999; Marty et al., 1997b) with a slightly slower rate of development and greater amounts of yolk remaining in the abdominal cavity at emergence compared to controls examined at the same time. This resulted in a slightly greater weight per unit of length, and thus a higher developmental index (kD = 10 × [wt in g]^3/l in mm, Bams 1970). Marty et al. (1997b) suggested this may be a practical assessment of oil effects. Therefore, in 1989 and 1990 when emerging fry from oiled streams were statistically no different in length or weight than in non-oiled streams, and thus no different in their developmental index (Figure 2), this was another demonstration that an oil effect in the field was unlikely. In 1990 fry from reference streams showed a mean kD of 1.76 and fry from oiled streams were at a mean of 1.79, indicating unaltered emergence timing (Brannon et al., 1995).

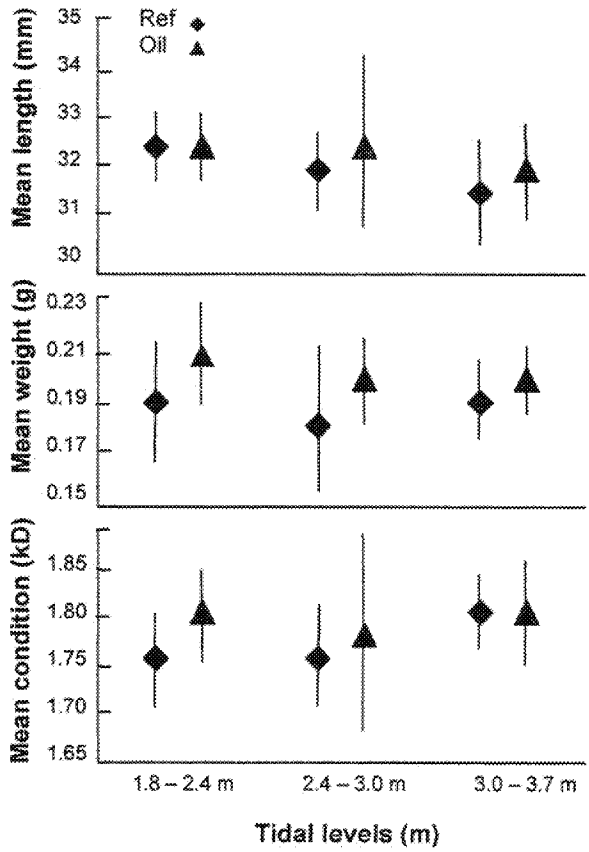

Figure 2 Mean length, weight, and condition index of emerging fry by tide level in oiled and non-oiled streams of PWS in the spring of 1990.

Premature emergence would have been evident by a higher kD index (> 1.9) and that wasn't the case.

Concurrent Incubation Studies in 1989

In the fall of 1989 Moulton (1996) conducted a field study on egg survival at the same time in some of the same oil contaminated streams reported by Bue et al. (1996, 1998) to have higher egg mortality. Moulton artificially spawned adults from each of nine streams, places the fertilized eggs in 24 perforated incubation boxes per stream, and buried the boxes in the intertidal stream substrate. The incubation boxes isolated effects that would be associated with oil, such as leachate emanating from oil deposits entering redds (Murphy et al.,1999; Rice et al., 2001), from the lethal effects of over-spawning (Collins et al., 2000) and any deleterious impacts of sampling that were unrelated to oil (Brannon et al., 2001). Upon approaching the hatching stage the eggs were removed from the incubation boxes and embryo survival was assessed. Moulton (1996) found that survival was high, averaging 96.5% in oiled streams and 95.1% in non-oiled reference streams ($p = 0.870$). This was evidence that demonstrated embryo survival was unaffected by the oil concentrations that existed in the intertidal reaches of those streams at the height of contamination of beaches and streams along the spill-path.

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Table 1 Mean percent egg mortality in the fall and following spring of the brood years reported by ADFG in the five oiled and five non-oiled streams studied in common by ADFG and Exxon from 1989 to 1993 (Data sources Craig et al. 1995, 2002)

Season	1989 % mort	1990 % mort	1991 % mort	1992 % mort	1993 % mort	Mean
Fall						
Non-oiled	14.9	23.8	23.5	23.5	13.4	19.8
Oiled	15.9	28.4	47.0	42.2	15.0	29.7
Spring						
Non-oiled	5.0	29.0	31.4	14.0	24.4	20.8
Oiled	7.6	31.0	12.2	16.0	9.8	15.3

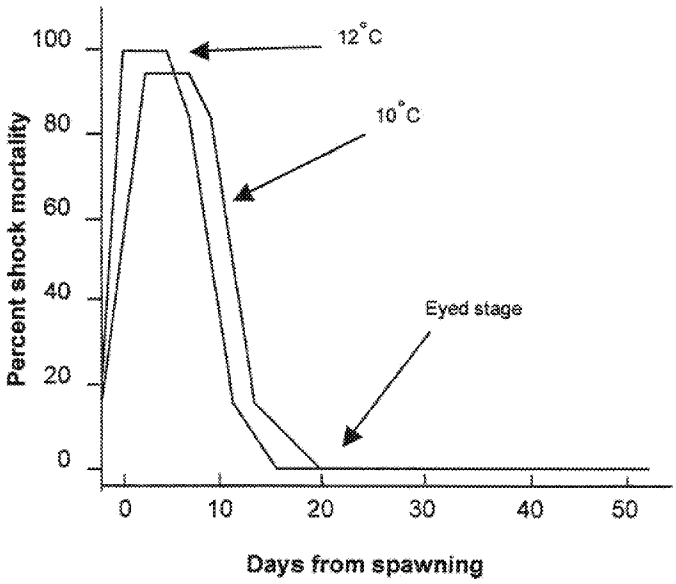

Figure 3 Sensitivity of eggs expressed as% mortality when exposed to mechanical shock by (a) vertical drops into water from a 100 cm height at 10°C (Jensen and Alderdice, 1989), and (b) pouring the eggs submerged in water from one container to another from a 20 cm height at 12°C (Jensen and Collins, 2003).

No Egg Mortality Differences in Spring Samples

The ADFG survey data also provided strong evidence against oil induced egg mortality (Craig et al., 2002). In the same ten streams studied in common by ADFG and non-agency scientists where ADFG reported higher mortality in the fall samples removed from oiled streams, those differences were no longer apparent by the following spring (Table 1). Fall samples showed a mean mortality in oiled streams of 29.7% versus 19.8% in non-oiled streams, while those taken in the following spring from the same streams, using the same techniques, showed mean egg mortality in oiled streams at 15.3% versus 20.8% in non-oiled streams. The same is apparent in the entire 25-stream ADFG data set. Spring egg mortality in oiled streams was at 25.5% and non-oiled streams at 23.4%. If the high fall egg mortality had occurred from oil exposure, those differences would have remained over winter, but clearly that was not the case. The increased mortality was confined to those eggs involved in the fall samples, which leads to the hypothesis that the increased mortality reported in the fall was associated with the sampling process itself rather than oil (Brannon et al., 2001).

Re-analysis of ADFG Stream Survey Egg Mortality

The key to resolving the discontinuity between the reported higher egg mortality in oiled streams and the other field data showing no differences from that in non-oiled streams pointed to a problem in the fall sampling protocol. Re-examination of the ADFG survey data resolved that issue (Brannon et al., 2001).

The Problem with ADFG Sampling Protocol

A general rule in salmon culture is not to disturb eggs before the eyed stage is reached, and that is to prevent mortality from the physical shock of being handled. Beginning within hours of fertilization salmon eggs are highly sensitive to any physical abuse during the process of blastopore closure spanning the first 100 temperature units of development as the embryonic tissue replaces the delicate vitelline membrane surrounding the large yolk at the start of gastrulation (Figure 3). Sensitivity continues to a lesser extent through cellular differentiation. Transition from extreme sensitivity to total tolerance of mechanical disturbance occurs rapidly as the embryos reach the eyed stage 20–28 d post-fertilization, depending on temperatures (Jensen and Alderdice, 1989; Jensen and Collins, 2003).

Comparisons of the end of spawner stream-life with the timing of the ADFG egg mortality surveys revealed a major flaw in the sampling protocol. Annual spawner enumerations in ten streams were conducted for 16 years following the spill (Brannon et al., 2006b; Maki et al., 1995). During the period from 1989–1997 when ADFG made their egg mortality surveys, termination of spawner stream life (the death of the spawning population) in the study streams was as late as the end of September, varying between streams by as much as 13 d, and 5 d or more were common. During those nine years ADFG stream sample timing ranged from September 21st through October 28th (Craig et al., 1995, 2002), which meant that a large percentage of the egg samples were taken within 20 days of post-spawner stream-life. Given that shock sensitivity continues for a period > 20 d (Figure 3) at PWS stream temperatures, it was apparent that a significant proportion of eggs were still in various degrees of susceptibility to mechanical shock at the time they were hydraulically extracted from the gravel by ADFG (Brannon et al., 2001). Where high mortality occurred, the samples had been taken earlier than 20 d after spawning was completed in the streams, and some < 10 d, which placed them in the early stages of embryonic development, highly susceptible to shock (Table 2). Therefore, the timing between the end of spawner stream-life and the initiation of ADFG stream surveys was the clue to the egg mortality problem. Higher mortalities occurred

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Table 2 Mean number of days between the end of adult steam-life and date of sampling, and percent egg mortality in five oiled (O) and five non-oiled (N) intertidal streams studied in common by ADFG and Exxon from 1989 to 1998 (Data sources from ADFG and Exxon stream surveys)

	Brood Year										
Stream class	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998[a]	1998[b]
	Days difference between end of spawning and sampling date										
O	17.4	15.6	4.2	8.6	*	17.6	24.5	26.6	9.4	1.8	24.2
N	18.6	18.8	15.4	12.0	21.8	16.2	25.4	30.2	11.0	2.4	24.2
	Percent Egg Mortality										
O	15.9	28.4	47.0	42.2	15.0	46.9	29.0	19.9	37.2	66.3	26.2
N	14.9	23.8	23.5	23.5	13.4	52.8	32.5	14.9	28.1	59.6	20.0

*Data unavailable.
[a]September field samples 1998.
[b]October field samples 1998.

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with fewer days between the end of stream-life and the time the egg samples were extracted.

The pattern is readily demonstrated by plotting the end of adult stream-life against sampling dates in the ADFG stream survey schedule (Figure 4). For those years from 1989–1997, percent mortality in oiled streams is higher when they were sampled earlier than non-oiled streams because the earlier samples were more vulnerable to shock abuse. When shock abuse occurs the vitelline membrane is ruptured and the yolk material starts coagulating almost immediately, resulting in the death of the embryo. The shock abuse of the ADFG sampling procedure resulted in higher mortality associated with the earlier sampling dates, as would be expected under such conditions, with shock fatalities dropping off in later samples as the embryos acquired greater tolerance to abuse with advancement in development. The four data points for 1998 show when all ten oiled and non-oiled streams were sampled early and then again later in the season, and the effects of hydraulically extracting the eggs before and after achieving tolerance to mechanical shock is very obvious.

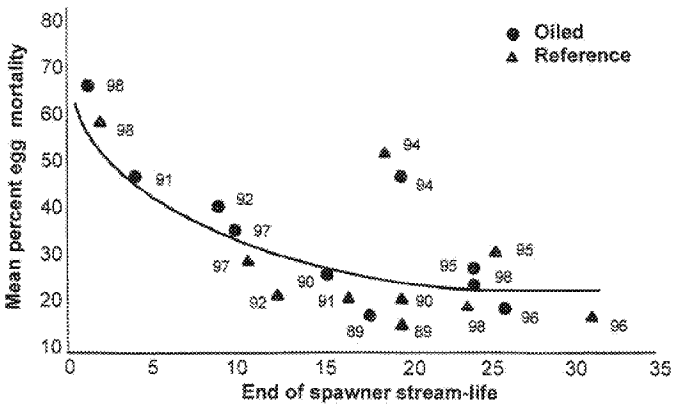

Figure 4 Mean percent egg mortality in oiled and non-oiled streams versus days past spawner stream-life at the time of sampling for the 10 study streams surveyed in PWS from 1989–1998 (line fit by eye). (Mortality data from Craig et al., 2002 and ADF&G unpublished annual data reports 1996 and 1997 and Collins et al., 2000).

Difference in Sampling Effects on Embryos in Oiled and Non-Oiled Streams

The critical question that is pertinent in the sampling shock issue is how did higher shock mortality occur in oiled streams? Oiled streams had higher mortality because spawning in those streams was slightly later and they were sampled earlier more frequently than non-oiled streams. As demonstrated in Table 2 and Figure 4, oiled streams were sampled earlier every year from 1989–1993, and again in 1997 resulting in higher mortality, while the embryos in non-oiled streams had additional days of development before sampling took place in those years. This allowed the eggs in non-oiled streams to acquire greater tolerance to mechanical shock before sampling and thus more frequently escape the higher mortality from the sampling abuse (Brannon et al. 1999, 2001).

This was most apparent in the oiled streams entering Shelter Bay (#663), Sleepy Bay (#678), and Snug Harbor (#682) in 1991. Spawner stream life, based on live counts, ended on September 25th in Shelter Bay, September 24th in Sleepy Bay, and September 25th in Snug Harbor, and they were sampled the 27th, 25th, and 26th of September, respectively, or within 2 days from the end of spawner stream life (Table 3). Since the eggs are sensitive to shock for > 20 d following spawning, any eggs in those samples that had been spawned within that period prior to September 27th were vulnerable to shock mortality at the time of sampling, and thus those three streams showed 65.6%, 57.0%, and 57.1% mortality, respectively. In contrast non-oiled streams were sampled 11–22 days after the end of spawning in 1991, which meant a larger percentage of the populations in oiled streams were sampled after they had acquired resistance to shock.

Evidence that sample timing was the problem is also apparent in the agency data. ADFG records on run-timing and egg sampling dates (Craig et al., 2002) in the 25 streams showed that overall the eggs from oiled streams were sampled at an earlier date and thus at an earlier stage of development than eggs from non-oiled streams, placing the former at greater risk of shock mortality (Figure 5). Run-timing is shown later in oiled streams each year of the survey Figure 5A, with differences in 1989

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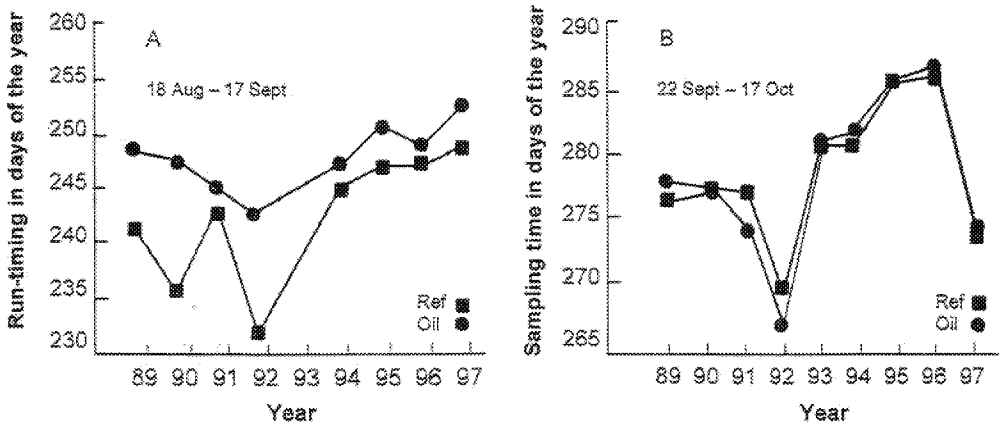

Figure 5 ADFG 1989–1997 PWS oiled and reference stream survey: Figure 5A run-timings based on calendar day when 75% of the spawning population entered the stream, and Figure 5B mean sample dates of eggs removed from redds (Data from Craig et al., 2002).

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(8 d), 1990 (13 d), 1991 (2 d), and 1992 (10 d), indicating that the eggs in oiled streams were developmentally less advanced on any given date. Moreover in 1991 and 1992, superimposed upon the later spawn timing, ADFG sampled oiled streams 3 d and 2 d earlier, respectively, as shown in Figure 5B, which added to the less advanced problem from the later spawning times in those oiled streams. This was confirmed in the ten streams studied in common with ADFG (Table 2) where the difference amounted to over 10 d less advancement in oiled streams for 1991. In contrast in 1994–1996 all sampling was 10 d–15 d later in the season, which allowed more of those respective egg populations to reach the point of resistance to mechanical shock, resulting in no mortality differences between oiled and non-oiled streams.

In 1997 higher egg mortality again occurred in oiled streams. It was suggested the elevated mortality may have been caused by a shift in the stream channel location in the delta, bringing the streams near or through a zone of oil contaminated delta deposits (Craig et al, 2002; Rice et al., 2001). However, records for the 1997 survey summary (Craig et al,. 2002) and adult spawn timing (Brannon et al., 2001) show only two oiled streams of the ten studied in common were responsible for the difference (Table 4). Streams #618 and #628 were sampled only 6 d and 2 d after the end of spawning, resulting in 45% and 60% mortality respectively of extracted eggs. The other oiled streams were sampled on average 13 d after spawning, with a mean of 26.5% egg mortality, similar to the non-oiled streams sampled on average 11 d post-spawning with a mean of 28.1% mortality. The high mortality also occurred in the reach of stream above high tide, above where the "shift in stream location" could take place. While all of the streams were sampled too early in 1997, streams #618 and #628 were exceptionally early. The higher mortality was not the result of channel shifts exposing eggs to oil deposits, but rather insufficient time had passed before sampling, and shock abuse occurred in the eggs being extracted.

Table 3 Days difference between end of spawning and sample timing effect on percent mortality of pink salmon eggs removed from intertidal areas in ten PWS study streams in common by Exxon contract studies and ADFG stream surveys during the September and October sampling periods in 1991 (Craig et al. 2002)

			Inter-tidal	
Stream No.	End of spawn	Sample date	Days diff	% mort
Oiled				
618	9/23	10/1	8	34.4
628	9/22	10/1	9	21.2
663	9/25	9/27	2	65.6
678	9/24	9/25	1	57.0
682	9/25	9/26	1	57.1
Non-oiled				
604	9/20	10/10	20	14.7
621	9/18	10/10	22	37.3
656	9/19	9/30	11	25.0
673	9/16	9/28	12	24.8
695	9/21	10/2	11	15.9

Table 4 Number of days between end of spawning and sample timing effect on percent mortality of pink salmon eggs removed from intertidal areas and above high tide in ten PWS streams studied in common under Exxon contract spawn timing assessment and ADFG stream surveys in 1997

Stream No.	End of spawn	Sample date diff	Days	Inter- tidal % mort	Above tide % mort
Oiled					
618	9/26	10/2	6	45.2	52.2
628	9/28	9/30	2	60.3	37.6
663	9/21	10/8	17	16.7	31.2
678	9/26	10/6	10	28.0	30.0
682	9/27	10/9	12	34.8	26.5
Non-oiled					
604	9/18	10/2	14	22.6	15.0
621	9/22	10/2	10	32.2	35.8
656	9/25	10/5	10	36.6	16.9
673	9/23	10/8	15	23.0	24.7
695	9/21	10/1	10	26.0	31.5

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Table 5 Total eggs, total mortality, percent mortality, percent dense opaque mortality (deaths prior to sampling) percent shock mortality and eyed eggs present at the time of sampling in the ten PWS study streams during the September and October sampling periods selected in 1998 (mean percent based on sum of all streams)

Sample date	Total eggs	Total mort	% total mort	% dense opaque	% shock mort	% eyed eggs
Sept 21 to 27	27,755	17,654	63.6	21.3	42.3	26.8
Oct 16 to 19	34,202	7,807	23.1	22.8	2.2	77.2

Experimental Validation of Early Sampling Effects on Embryo Mortality

To verify the premise that the ADFG reported egg mortality was the result of sampling when the embryos were still sensitive to physical shock, a separate study on sample timing was undertaken in 1998 on the subset of ten streams used in the ADFG stream surveys (Collins et al., 2000). Hydraulic sampling was scheduled to represent the timeframe and protocol used by ADFG stream survey crews reported by Bue et al. (1996, 1998), involving two sampling regimes corresponding with the earlier ADFG survey dates (September 21st–27th), and the later dates (October 16th–19th) from the Craig et al. (2002) data base. Results of the study summarized in Table 5 demonstrate the overwhelming effect of sampling too early within the ADFG sampling timeframe irrespective of oiling, with a 42.3% shock mortality in the September set dropping to 2.2% in the same stream reaches in the later October sample set. It is noteworthy that the mortality referred to as percent dense opaque (21.3%–22.8%) in Table 5, representing those eggs that died from natural causes in the fall prior to the shock mortality induced in the samples taken, represented nearly the same percentage as that in non-oiled stream spring samples in Table 1 (20.8%) taken when there would be no shock mortality occurring.

Hatchery Strays Invalidate Allegations of Oil Induced Egg Mortality

After 1990, when oil had essentially left the streams, the egg mortality was hypothesized to have been caused by inter-generational genetic damage or physiological anomalies passed on to the progeny (Craig et al., 2002; Rice et al., 2001). However, hatchery strays make up a significant proportion of the spawning populations of PWS streams (Collins, 2002; Joyce and Evans, 1999, 2000), and can account for over 80% of the total spawners in streams closest to the hatcheries (Collins et al., 2009a; Cronin and Maki, 2004). Hatchery fish experience only oil-free freshwater incubation conditions and thus would carry no genetic or physiological aberrations from oil toxicity to pass on to their progeny. Consequently, when hatchery strays are discounted from the "oil-exposed" spawners there can be insufficient numbers of wild fish remaining to account for the egg mortality, indicating that the mortality had to include the eggs from hatchery fish and thus oil induced inter-generational effects could not account for the mortality.

For example, in 1991 Shelter Bay (#663) and Sleepy Bay (#678) were reported to have egg mortality of 65.6% and 57% respectively (Craig et al., 2002), or > 2 times that in reference streams (~24%), for a combined mean of ~38% additional mortality attributed to oil in those two streams (Table 3). Considering otolith mark data from hatchery programs in 2001–2003, Collins et al. (2009a) estimated that at the time ADFG samples were taken (September 25th–27th) hatchery fish represented > 90% of the fish in those streams. Assuming equal sex ratio in the total of 1720 intertidal spawners in both stream #663 and #678 (Maki et al., 1995), 860 were females, which meant that only ~86 females were wild and thus potentially exposed to oil as eggs in 1989. Given the estimate of 622,000 dead eggs (Craig et al., 2002), the percentage attributed to oil deaths (236,360 eggs) exceeds the entire fecundity of the native population (~129,000 eggs) in those intertidal streams. The mortality had to include eggs from hatchery spawners that never experienced oil exposure in their embryonic stage, and thus carryover poly aromatic hydrocarbon (PAH) induced genetic or physiological aberrations could not be the source of increased mortality.

The Assessment of Egg Viability

An assessment of potential genotoxic effects on the gametes of returning adults that were exposed to oil as embryos in 1989 was undertaken with the 1991 brood (Brannon et al., 1995). Males and females were removed from the spawning grounds of ten streams, artificially spawned, and the eggs incubated under hatchery conditions. The study found that gamete viability was no different with 96.2% survival of the eggs spawned from adults in non-oiled streams and 96.6% survival of the eggs spawned from adults in oiled streams advancing to the pre-hatching stage (ANOVA; $p = 0.574$) in the hatchery, and no gender differences were detected.

Further, the egg viability study confirmed the effect of sampling shock on the egg mortality in the ADFG stream surveys (Table 3). In the 1991 stream surveys conducted by ADFG, Shelter Bay (#663) and Sleepy Bay (#678) streams showed 65.6% and 57.0% mortality respectively (Craig et al., 2002). But the 1991 viability study just reported the 120 adults from those same two streams removed from intertidal spawning redds and artificially spawned for viability assessment showed egg mortality at < 4%. That comparative 1991 data demonstrated the ADFG high mortality in those two streams could not have been carryover genotoxic or physiological effects from exposure to oil as embryos in 1989, but rather was confined to the eggs extracted from their redds and had to be from the ADFG sampling procedure. The high egg viability in adults artificially spawned from those two steams (> 96%) invalidated any suggestion that high egg mortality in the ADFG samples from those two oiled streams was from reduced gamete quality.

In 1993 Bue et al. (1998) also explored genotoxic effects on egg viability, and included 16 streams and more spawners in a

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study conducted at the Armin F. Koernig (AFK) hatchery. They used an experimental design after Brannon et al. (1995), except the eggs and milt were collected from fish holding off the mouths of streams, transported to AFK, fertilized as separate lots, and then all crosses from a given stream were mixed, providing eight experimental pools of eggs from oiled streams to compare with eight pools of eggs from control or non-oiled reference streams. Survival from oiled streams was 79% and 88% from non-oiled streams. Bue et al. interpreted the lower survival as a genotoxic effect on viability from prior oil exposure as embryos. They felt their results vindicated the ADFG stream survey conclusion, since both the viability study and the field survey showed similar results with ~79% oiled stream and ~89% non-oiled stream egg survival.

However, what appeared to vindicate the stream survey results was unrelated to viability. The eggs that were artificially spawned and brought from Shelter Bay and Sleepy Bay streams and used by Bue et al. (1998) in their study showed mortality of 33% and 22%, respectively, while in contrast the mortality of eggs removed from redds in the ADFG surveys of those same two streams that year was 25% and 8%, respectively (Craig et al., 2002). Under controlled hatchery conditions, survival should be much higher than in the wild where many other factors can cause mortality, but in the Bue et al. study just the opposite was true with the higher mortality of those eggs occurring in the hatchery incubation. These data show that rather than a viability problem the increased mortality had to be associated with the shock of handling the gametes, the delay in fertilization, or problematic incubation conditions at the AFK hatchery. Rice et al. (2001) also attributed the higher mortality of the Bue et al. eggs to greater handling when making the crosses, which underscores the problem. Such sources of additional mortality nullify any ability to assess viability in the absence of unhandled controls.

Another problem in the Bue et al. egg viability study was the hatchery fish. At the time of month when viability samples were taken in 1993, based on subsequent estimates of hatchery fish contributions using otolith marks introduced during incubation, the hatchery fish with no history of oil exposure represented ~75% of the adults in those streams (Collins, 2009a). Those fish would carry no genetic anomalies from previous exposure to oil as embryos because they were incubated under oil-free hatchery conditions, which meant the mortality attributed to oil exposure would fall entirely on the wild fish, and insufficient wild spawners were available to account for the losses. Further, since Bue et al. pooled all egg samples within each stream, they lost any ability to salvage mortality information on individuals among the native fish using the otolith marks to exclude fish of hatchery origin.

The advantage of the Brannon et al. 1991 study was the experimental design. The eggs from each female were kept separate, divided into two lots and each fertilized with a different male, which made it possible to observe each half-sib for potential reduction in viability. Since all of the 400 egg lots showed high survival, regardless of whether or not hatchery fish were well represented in those streams, it was concluded that because there were no differences among the experimental crosses, the wild fish carried no lethal developmental anomalies associated with genotoxicity or physiological infertility. Consequently, we concluded that the mortality in the Bue et al. (1998) egg viability study and the egg mortality in the ADFG stream survey data were caused by something other than oil.

Further, since Bue et al. (1998) seined the fish off the mouth of the streams, there was no way of knowing whether the captured spawners represented the intertidal population, especially in August when the upper non-oiled reach was receiving many of the returning fish. The added disadvantage of using fish holding off stream mouths for the study is there are fish in early stages of ovulation waiting to enter the streams as well as overripe fish that fall back out of the stream and hold off the mouth, either of which will naturally show lower egg viability.

Finally, the egg viability reduction alleged by Bue et al. (1998) to occur in the 1993 brood fish, and thus reputed to carry genetic anomalies, originated from 1991 spawners. Those were fish that as embryos experienced the highest oil concentrations over the entire incubation experience in 1989, and they showed no inter-generational effects from previous exposure. The 1991 eggs had high survival and progeny were not exposed to oil, essentially gone from the intertidal areas by the 1991 fall spawn (see Section 2). The authors consider this as evidence, suggesting that the hypotheses on inter-generational effects (Bue et al., 1998; Rice et al., 2001) are untenable because 1993 spawners should have had no predisposition for reduced egg viability.

Agency Data Validate Sampling Effects as the Cause of Egg Mortality

Although Craig et al. (2002) had concluded that oil caused the increased egg mortality observed in the ADFG stream surveys, they recognized that sampling shock may have influenced their results. Craig et al. reanalyzed their pooled egg mortality data with an adult stream entrance covariate that represented the timeframe when 75% of the population had entered the spawning stream and found that egg mortality was related to sampling date. Craig et al. stated, "using Day 75 as a covariate and pooling data across all years, results were consistent with an effect of sampling induced mechanical shock suggesting that our test for oiling effect may have been affected by sampling date" (p. 27). So ADFG recognized the problem.

Further evidence for shock mortality comes from the NMFS studies. In response to evidence showing mechanical shock was the cause of increased egg mortality in ADFG stream surveys (Brannon et al., 2001; Collins et al., 2000), a laboratory study was undertaken by Carls et al. (2004b) to examine the effects of mechanical shock during incubation. Their results confirmed sampling early in embryo development would be a problem. In a separate study by Thedinga et al. (2005) naturally spawned pink salmon eggs in Lovers Cove Creek, Baronoff Island, in southeast Alaska (i.e., not PWS), were sampled during and after the spawning period to show their resistance pattern to mechanical

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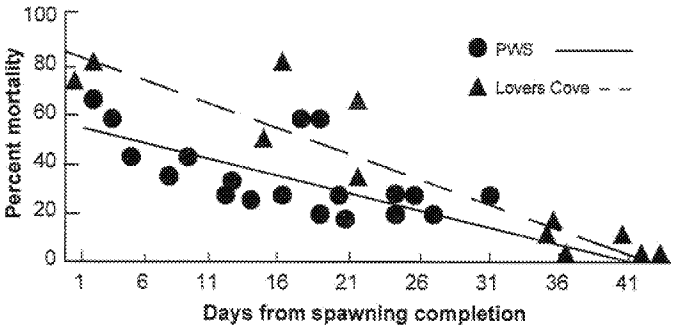

Figure 6 Linear relationship of mortality and sample days after spawning completion when eggs were sampled from Lovers Cove Creek (Thedinga et al., 2005) and from the ten 1989–1998 monitored ADFG survey streams in PWS (Brannon et al., 2001).

shock. The population terminated spawning on the 11th of October, and if the Thedinga et al. data on egg resistance to hydraulic shock is taken and the reciprocal plotted as egg mortality from October 11th–November 10th, it reaffirms the sampling problem. Thedinga et al. data demonstrate that if a population of eggs is sampled shortly after the termination of spawning and continued over the following 4 weeks, high mortality ensues at ~80% and then gradually drops to < 10% by the time the last of the spawn advances past the eyed stage (Figure 6, line ▲). That pattern is the same as shown in ADFG egg mortality (line •) over the early incubation period. Thedinga et al. data independently support the evidence that the Bue et al. (1996, 1998) egg mortality classified as oil induced death was instead caused by mechanical shock.

However, as recent at 2010, NMFS investigators have shown that in addition to increased mortality, protracted shock susceptibility occurs in pink salmon eggs as a consequence of slower development from exposure to high oil concentrations (Carls and Thedinga, 2010), and suggest, therefore, that prolonged susceptibility to mechanical damage will increase mortality when the eggs are sampled during their period of shock susceptibility if observers didn't differentiate between natural morality and shock abuse. Their observations were made in the laboratory simulating intertidal conditions and their results were applied to PWS oiled streams. While lethal shock irrespective of oil exposure was not disputed, Carls and Thedinga implied their data support the view that oil was the cause of the direct increase in egg mortality in the ADFG stream surveys, and indirectly it could have been even higher by the prolonged susceptibility of the embryos to shock from sampling. The authors suggested their results invited review of the events following the *Exxon Valdez* oil spill in PWS.

The oil concentration that induced mortality and the delay in development, and thus prolonged embryo susceptibility, was a 3,000,000 ppb oil/gravel mixture (22.4 ppb aqueous-TPAH). Although not reported, based on similar flows and oil concentrations in the Carls et al. (2005) study, that level of exposure in the Carls and Thedinga (2010) study would result in a tissue-TPAH load > 12,000 ppb, exceeding the measured tissue-TPAH of alevins in oiled streams (<95 ppb) by over 120 times. Therefore, we question the application of their results to eggs in oil contaminated streams following the oil spill in PWS.

Further, the low exposure concentration of 1,000,000 ppb oil/gravel mixture (6.6 ppb aqueous-TPAH) would be expected to result in a tissue-TPAH > 3,000 ppb representing a tissue load at least 30 times higher than what was measured in alevins incubating in PWS oiled streams. It is noteworthy that neither increased mortality nor prolonged susceptibility occurred at that concentration, and it was 30 times higher than what was measured in alevins incubating in PWS oiled streams. Therefore, in retrospect, the Carls and Thedinga (2010) study verifies that neither induced mortality nor the protraction of sensitivity to shock would have occurred at the oil contamination levels experienced in PWS streams.

Conclusion on the Cause of Increased Egg Mortality

The authors have presented data that clearly demonstrate the increased egg morality in oiled stream surveys was induced by mechanical shock from sampling too early while the embryos were susceptible to mortality from such abuse, and not from oil. The field data that is related to egg and alevin quality, the low sediment- and tissue-TPAH levels in oiled streams, and the high survival of embryos in comparative studies in oiled and non-oiled streams, support that conclusion. The latest NMFS research showing that mechanical shock of recently spawned eggs results in high mortality (Thedinga et al., 2005; Carls et al., 2004b) is viewed as independent evidence that is also in support of that conclusion.

SECTION 2: LABORATORY RESEARCH ON OIL SPILL EFFECTS ON PINK SALMON

In this chapter the authors' analysis is on the application of agency laboratory results to explain the increased egg mortality observed in oiled streams. Given the evidence reviewed above that increased egg mortality wasn't associated with oil (Brannon et al., 1995, 2001; Collins 2000), it now becomes a question of how can the NMFS position on oil induced egg mortality in PWS streams be reconciled? In this process the authors will first characterize the level of oil contamination in intertidal areas following the spill, then review the laboratory research and field studies that investigators interpreted in support of the alleged chronic effects, and conclude by presenting the university research that revealed problems in the laboratory reports on EVC toxicity.

Oil Concentrations in Prince William Sound Streams and Deltas

By the time EVC reached landfall in southwestern PWS much of the monoaromatics of crude oil were exhausted (Wolf et al., 1994) and the less toxic TPAH were left dominating the composition of the weathered oil. Further, tidal dilution and

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freshwater flushing of the intertidal streams limited the amount of oil contaminating the stream substrate. As presented earlier, when oil was at its highest level in 1989 the mean sediment-TPAH concentrations in oiled streams ranged from 0.5 ppd–267 ppb, overlapping with the concentrations measured in non-oiled reference streams at < 65 ppb (Brannon et al., 1995). The low contamination level was also evident from the tissue-TPAH of alevins (<95 ppb) sampled from oiled streams (Brannon et al., 1995), which was slightly higher than alevins in non-oiled streams (~39 ppb), and far below lethal tissue-TPAH threshold concentrations of > 7,800 ppb naturally weathered EVC determined in the laboratory (Brannon et al., 2006a).

The intertidal areas around oiled streams also showed limited contamination levels. Although heavy oiling occurred initially on some intertidal areas associated with pink salmon streams in the spring of 1989 (Boehm et al., 1995), most of those areas returned to near background levels by the fall of 1990, and were indistinguishable from non-oiled sites by 1991. The oil content of the upper several centimeters of intertidal substrate showed an average less than the Effects Range-Low toxicity threshold (Long and Morgan, 1990) in intertidal shorelines by 1990, with the mean TPAH concentrations on oiled intertidal beaches < 500 ppb (Boehm et al., 1995). In Sleepy Bay, one of the most heavily oiled beaches, sediment-TPAH levels were < 1000 ppb in 1989, dropped to < 200 ppb by 1990, and only ~33 ppb by 1991 (Short and Babcock, 1996). By 1990 mean TPAH concentrations in sediments at many other oiled sites had declined to 100 ppb–200 ppb, slightly above pre-spill background levels (O'Clair et al., 1996). Except for a few locations where buried oil persisted (Short et al., 2004, 2007), most of the oil was gone by 1991 and toxicity of sediments to larvae oysters and amphipods had diminished to background levels (Wolfe et al., 1996).

Therefore, based on sediment-TPAH concentrations in oiled streams and tissue-TPAH of embryos in those streams, contamination levels were only slightly above that in non-oiled streams in 1989 and 1990. Intertidal contamination was not dictated by TPAH concentrations in oil deposits, but rather by the background sediment-TPAH levels of the intertidal substrate as reported by Boehm et al. (1995), Short and Babcock (1996), O'Clair et al. (1996), and Wolfe et al. (1996). Those levels on the deltas and intertidal areas ranged from means of < 35 ppb to ~500 ppb sediment-TPAH during the first three years following the spill; well below the toxic sediment-TPAH threshold (3,800 ppb to 8,300 ppb) of the laboratory weathered oil (Brannon et al., 2006a; Heintz et al., 1999). There is no evidence suggesting that TPAH concentrations reaching incubating eggs in oiled streams approached lethal toxicity.

Agency Laboratory Studies on EVC Chronic Toxicity

NMFS personnel at the ABL hypothesized a very different scenario. Having assumed the increased pink salmon egg mortality in oiled streams reported by ADFG was caused by exposure to lethal oil concentrations, NMFS conducted a series of laboratory studies to determine the effects of weathered oil on pink salmon. The results of their investigations were used to explain the egg mortality in spill-path streams and to predict long-term damage to pink salmon from oil exposure. The authors will review those studies with analysis.

Laboratory Study on Chronic Effects of Oil

The initial studies on the effects of EVC oil exposure during the incubation phase of pink salmon were conducted by Marty et al. (1997a, 1997b). Laboratory weathered oil/gravel mixtures were prepared, placed in PVC columns, and flushed with water four days prior to testing to remove particulate oil particles. The eggs were then placed on the gravel contained in columns and exposed to the effluent water that upwelled through the columns. Very high mortality occurred at the highest oil-on-gravel concentration of 4,510,000 ppb (aqueous-TPAH dose of 52 ppb), but at the next highest concentration of 622,000 ppb (aqueous-TPAH dose of 32 ppb) mortality was not significantly different from the controls. The lethal threshold concentration fell some place between the two. Maximum tissue-TPAH is unknown, because the highest levels reported (> 10,500 ppb and > 39,000 ppb) were concentrations measured 96 days after initial exposure when much of the oil-on-gravel load had diminished. Sediment-TPAH exposures for those concentrations were19,500 ppb and 140,000 ppb respectively.

Marty et al. (1997b) reported that ascites, commonly referred to as blue sac disease in fish culture, indicated damage to the embryos from oil exposure, and oil-on-gravel concentrations as low as 55,200 ppb resulted in 0.5% incidences of ascites among alevins. Ascites increased as oil concentrations increased. Oil-exposed groups emerged prematurely from the incubators and most of the differences observed disappeared as the fry advanced beyond emergence from the oiled gravel. It was assumed that the test water passing through the columns carried predominantly the water-soluble fraction (WSF), because the ratios of alkanes: PAH were lower in the column effluent than the oil phase, and phytane, a branched chain aliphatic hydrocarbon with very low solubility in water, was thought to be absent in the effluent, and concentrations in the tissue analysis were consistently below the detection limit of the analytical method used. These conditions led Marty et al. to conclude that the TPAH responsible for the embryo deaths and ascites "was in true solution" (p. 1002).

Analysis

There were two problems with the Marty et al. (1997b) study regarding its application to incubation conditions in PWS oiled streams. Oil concentrations tested were far in excess of that measured in the field. The highest sediment-TPAH concentration tested with no lethal affects was 19,500 ppb. That level was 73 times higher than the highest mean sediment-TPAH concentration (267 ppb) measured in oiled streams in 1989 (Brannon et al., 1995), and the highest dose tested was > 500 times the

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highest mean sediment-TPAH concentration. Consequently, test concentrations showing oil effects were not related to contamination levels in spill-path streams.

The second problem was the conclusion that the toxic dose was only the dissolved TPAH. That was based on the assumption that the rinsing of the oil/gravel mixture eliminated particulate oil from being dispersed in the column effluent. But the eggs were incubated in direct contact with the oil-on-gravel, and there was nothing preventing PAH to partition from the oil directly across the egg membrane. This meant the reported aqueous-TPAH levels under-represented the actual TPAH exposures and thus overstated the PAH toxicity. Further, the experimental method didn't assure that particulate oil was absent from the test water. Alkanes:PAH ratios and phytane evidence was thought to show the absence of particulate oil or minute oil droplets that would magnify toxicity. However, mass estimates of PAH losses derived from gravel measurements exceeded losses attributed to the soluble fraction by a factor of nearly 2 times (Marty et al., 1997b), which suggests that oil droplets could account for some of the loss. It also appears that the aqueous-TPAH concentrations reported exceeded the maximum dose that could be dissolved in solution from the oil-on-gravel levels tested and that would suggest droplet contamination.

Laboratory Tests Expand on Oil Toxicity to Pink Salmon Embryos

Heintz et al. (1999) undertook a laboratory study to determine the lethal concentrations of TPAH leached from oil-contaminated gravel, whether direct contact was necessary to accumulate lethal doses, and the persistence of oil toxicity associated with very weathered oil (VWO). Using the Marty et al. (1997a, 1997b) experimental design, seven laboratory weathered oil-on-gravel concentrations were tested, including VWO at a concentration of 2,860,000 ppb oil-on-gravel with water flushing through the columns at 150 mL/min (Table 6). Assuming the dissolved oil in true solution was the source of toxic components, Heintz et al. reported that an 18 ppb aqueous-TPAH concentration of laboratory weathered oil was lethal to embryos. In addition, the exposure of embryos to VWO was reported much more toxic with an aqueous-TPAH lethal concentration of only 1 ppb. It was further concluded that the chronic toxicity had to be related to high molecular weight PAH (HPAH) compounds, because the low molecular weight hydrocarbons (LPAH) were at concentrations thought too low to be toxic. Consequently, Heintz et al. (1999) associated long-term chronic toxicity of VWO with aqueous-HPAH.

Some eggs were also incubated in the upwelling flow on a perforated aluminum plate sitting slightly above the gravel surface at oil-on-gravel concentrations of 74,000 ppb and 717,000 ppb. When those tests on indirect exposure to the oiled gravel in columns emitting aqueous-TPAH concentrations of 18 ppb and 31 ppb showed similar tissue-TPAH levels as eggs in direct contact with the same concentrations, they asserted it was the dissolved fraction responsible for the toxicity in all experimental lots, and not contact with the large oil-on-gravel loads used to produce the dissolved doses (Table 6).

Table 6 Sediment-, aqueous-, and tissue-TPAH levels, in the Heintz et al. (1999) laboratory study on percent mortality of incubating pink salmon exposed to five highest concentrations of laboratory weathered EVC at flows of 150 mL/min

Gravel EVC (ppb)	Gravel-TPAH (ppb dry wt.)	Aqueous-TPAH (ppb d-0)	Tissue-TPAH (ppb dry wt. d-36)	Percent mortality
Control	1	0.8	90	33.9
74,000	1,000	7.8	1,900	44.7[b]
281,000	3,800	18.0	~ 6,000[a]	51.4
717,000	9,600	31.0	15,000	48.8[b]
2,450,000	32,700	48.0	71,000	73.4
2,860,000*	4,400	1.0	470	62.7

[a]Extrapolated
[b]Estimated from Heintz et al. 1999, Figure 2.
*VWO.

Analysis

The Heintz et al. (1999) study also had inconsistencies. The 18 ppb aqueous-TPAH dose emitted from 280,000 ppb oil-on-gravel with a sediment-TPAH of 3,800 ppb was the lowest aqueous concentration showing significantly higher mortality than the controls, but it differed markedly from > 32 ppb aqueous-TPAH reported by Marty et al. (1997b) without mortality from the exposure well above the level in the Heintz et al. study. The authors suggest that because of the laboratory methods followed by Heintz et al. (1999), the reported lethal dissolved dose most likely contained particulate oil, which would have elevated the actual level of TPAH exposure above that which was reported. The lethal sediment-TPAH of 3,800 ppb was also > 14 times higher than the mean EVC measured in oiled streams. Consequently, the laboratory studies had little relevancy to the experience of spill exposed pink salmon eggs, with the exception of demonstrating that mortality would not occur unless sediment-TPAH was much higher than that measured in the oiled streams.

ABL personnel disagreed with the Brannon et al. (2006a) analysis that oil droplets were present in the ABL dosing water reported to carry PAH in true solution. Carls et al. (2008) claimed the absence of phytane was proof that oil micro-droplets were not in the treatment water. However, the pertinent water chemistry data (*Exxon Valdez* Trustees Hydrocarbon Database, 2003) shows that phytane was indeed present in the dosing water and some of the tissue samples. Of the 31 water samples analyzed for the 1993 pink salmon experiments, 29 contained phytane at concentrations above its method detection limit, indicating the presence of un-dissolved oil in the water that could contribute to toxicity. Moreover, the 1993 ABL technician notes recovered in the Freedom of Information Act (FOIA) requests indicated that oil sheen and oil buildup were observed in the incubation containers of the Heintz et al. (1999) study, demonstrating the

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presence of particulate oil in the incubation system, which could elevate tissue contamination.

Although Heintz et al. (2008, p. 1475) stated, "we were keenly aware that introduction of oil microdroplets by the dosing apparatus might seriously confound interpretation of results," they dismissed the effect of eggs resting directly on the oil as a contribution to toxicity. Oil in contact with the chorion allows petroleum hydrocarbons to diffuse directly across the egg membrane (Collins et al., 2009b). Consequently, toxicity determinations based just on dissolved oil are invalidated by the presence of these sources of contamination (Payne and Driskell, 2003) and suggest that the reported dissolved TPAH concentrations alleged to be lethal to incubating embryos under-represented the PAH concentrations that were responsible for the laboratory mortality (Carls et al., 2005; Heintz et al. 1999; Marty et al., 2007b).

The Shift in the Oil Toxicity Paradigm

The interpretations of the laboratory results by NMFS scientists have far-reaching implications. The alleged 1 ppb aqueous lethal dose in the laboratory test with VWO (Table 6) was suggested as the minimum lethal threshold of toxicity of chronically exposed pink salmon embryos to highly weathered oil (Heintz et al., 1999). The LPAH concentrations remaining in weathered oil were too low to be toxic, and thus the more toxic HPAH homologues (3–4 ringed aromatics) in true aqueous-PAH solution were presented as the fraction responsible for the egg mortality in oiled streams (Heintz et al., 1999; Rice et al., 2001), displacing the 1–2 ringed LPAH aromatics previously attributed to toxicity of crude oil. Based on that concept, Rice et al. (2001) proposed a shift in the oil toxicity paradigm that implicated the toxic HPAH analytes as the agents responsible for chronic aqueous toxicity of weathered oil. This shift in the oil toxicity paradigm implies that under chronic exposure of susceptible organisms, weathering increased the toxicity of oil by emitting higher proportions of HPAH per unit of mass, resulting in the alleged highly toxic leachate at concentrations of 1 ppb aqueous-TPAH. They suggested that this is what caused the mortality of eggs in oiled streams, referred to as interstitial toxic water (Heintz et al., 1999; Murphy et al., 1999).

Analysis

Several problems are associated with the Heintz et al. (1999) and Rice et al. (2001) interpretations of results apart from the oil droplets and under-representation of the aqueous-TPAH toxic threshold. The test concentration was an oil-on-gravel mixture of 2,860,000 ppb, but the source of the VWO lethal toxicity in that oil mass is unknown. The disconnect between TPAH levels in the embryos and the alleged lethal dissolved aqueous-TPAH dose of 1 ppb was in the laboratory evidence itself. The reported lethal tissue-TPAH level sequestered by the embryos from the VWO was only 470 ppb (dry wt). That was compared to over 1,900 ppb TPAH (dry wt) in embryo tissue exposed to laboratory weathered oil with no increase in mortality in the same study (Heintz et al., 1999). Furthermore, embryos exposed to naturally weathered oil (NWEVC) taken from the shores of PWS showed a tissue-TPAH concentration of 7,800 ppb that also resulted in no increased mortality (Brannon et al., 2006a), and the concentrations of HPAH analytes in the tissues of those embryos were > 16 times higher than with the VWO (Figure 7). Each of those studies showed an aqueous-TPAH concentration > 7 ppb, compared to the 1 ppb in VWO, and suggests VWO toxicity had little or no relationship to PAH.

Carls et al. (2006) indicated that nominal doses are not appropriate for inter-laboratory comparisons, implying that the composition of the PAH is the critical factor. Although the magnitude of difference in tissue-TPAH concentrations of VWO (470 ppb) with mortality and NWEVC (7,800 ppb) without mortality tends to negate such criticism, comparisons can also be made of specific toxic components, such as the alkyl phenanthrenes, which Heintz et al. (1999) referred to as one of the most toxic PAHs in VWO. The Heintz et al. data show that eggs exposed to VWO had accumulated ~100 ppb alkyl phenanthrenes with 62.7% mortality. In contrast, in the Brannon et al. (2006a) study the tissue alkyl phenanthrenes were 1575 ppb, but

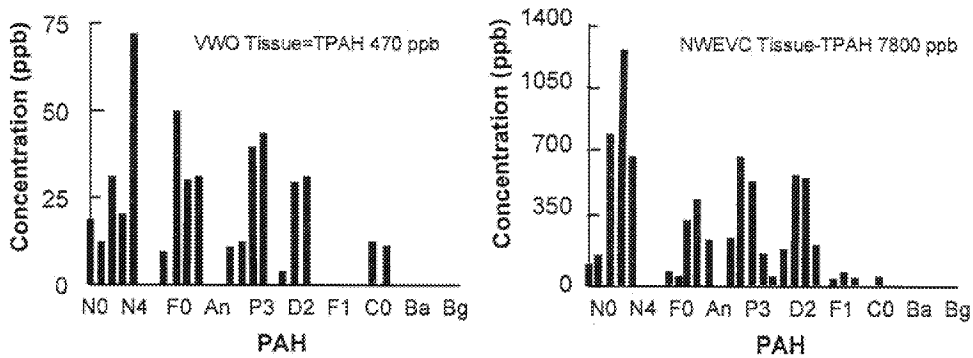

Figure 7 Tissue-TPAH composition in embryos exposed to 2,860,000 ppb VWO with mortality and in embryos exposed to 2,250,000 ppb NWEVC without mortality (Note vertical scale difference. VWO concentrations calculated from Heintz et al., 1999).

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without any increased mortality above that of the controls (Figure 7). Phenanthrenes do not appear to have been the source of VWO toxicity.

When all tissue-PAH analytes of VWO were magnitudes lower than what was present in tissues of embryos without mortality, that evidence undermines the shift in the oil toxicity paradigm. Moreover, a critical evaluation of the VWO chemistry was contrary to the conclusion of PAH toxicity at 1 ppb (Page et al., 2011). Therefore, the question remains what made VWO so toxic in the Heintz et al. study? The VWO was excess from research at ABL during the previous year and was salvaged from uncontrolled storage conditions, including exposure to outdoor conditions over a period of nine-months where factors such as ammonia and sulfide by-products of microbial hydrocarbon degradation could occur, and this may have been the case. Extensive analyses of the chemistry data by Page et al. (2011, 2012) have shown that microbial degradation and contamination affected the toxicity of VWO.

The shift in the oil toxicity paradigm implies that HPAH analogues are available. However, the HPAH compounds alleged to be the toxic components responsible for egg mortality actually became increasingly less available to incubating embryos as the oil weathered (Brannon et al., 2006a). While HPAH components, such as chrysenes, may be highly toxic, their limited solubility makes them less bioavailable to incubating pink salmon. This is consistent with weathering effects in other studies where upon continued exposure to decreasing test conditions tissue-TPAH became increasingly naphthalene-dominated and chrysenes least represented (Carls et al., 2005). The HPAH analytes do not appear to dissolve readily enough to be a threat to incubating eggs. While increasing in percentage composition of the oil residue during the weathering process, the actual amount of HPAH remaining in the residue diminishes over time. Furthermore, the flushing of the redds with freshwater and washing by tidal cycles would eliminate any risk of toxicity outside the oil mass.

Cytochrome Enzyme Studies

The enzyme cytochrome P4501A (CYP1A) in vertebrates is generated by exposure to xenobiotics—such as petroleum—in order to metabolize and purge them from their system. The presence of CYP1A in embryo and fry tissues has been used by investigators to indicate whether there was exposure to PAH. They have concluded that the presence of the enzyme indicates the fry had experienced some damage from that exposure (Carls et al., 2005; Rice et al., 2001). Using the Heintz et al. (1999) experimental design, but at flows of 1600 mL/min (10-fold increase) Carls et al. exposed pink salmon embryos to oil/gravel mixtures, with the two highest at ~1,400,000 ppb and 3,800,000 ppb oil-on-gravel concentrations. They reported CYP1A induction levels were related to EVC exposure concentrations, and that it was initially detected in eggs at just over three weeks of embryonic development. Low mortality occurred in all of the egg lots up to the eyed stage, with no statistically significant differences from that of the controls.

Following the eyed stage, however, mortality increased with dose, showing statistically significant differences at emergence in the highest two doses with mortality approximately 21% and 50%, compared to 15% in controls. The higher flows through the system appeared to improve survival compared to results of other studies. Anomalies detected were ascites, bulging eyes, malformed structures around the head, and external hemorrhaging.

Analysis

Several important observations were made by Carls et al (2005). First, differences in mortality between the treatments and the control didn't become apparent in their study until after the eyed stage of the embryos. This was in sharp contrast to the increase in mortality in the oiled streams that was observed in the ADFG stream surveys only shortly after spawning had terminated (Figure 1B). The pattern in the Carls et al. study was the same as that shown in Figure 1A, confirming that oil related incubation mortality continues through the entire incubation period, unlike what was observed in PWS stream surveys.

Second, although all tissue-TPAH decreased with time, the tissue-TPAH became increasingly dominated by naphthalenes, while the heavier molecular weight phenanthrenes concurrently decreased in proportion over the same period. If HPAH were responsible for the toxicity observed, as suggested by Rice et al. (2001), they should have persisted in the tissues, but that wasn't the case. HPAH compounds were in low and decreasing concentrations in the tissue, which is contrary to the idea that they were the source of toxicity to the embryos. HPAH analytes are less soluble and become biologically less available with the advance of the weathering process (Brannon et al., 2006a). In that sense, Carls et al. (2005) results are noteworthy since they demonstrate that while naphthalene concentrations in the water decreased markedly during the weathering process they still dominated tissue-PAH composition over the same time period. By their near absence, HPAH compounds did not appear to be a contributing factor to mortality.

Third, Carls et al. (2005) convincingly demonstrated that pink salmon embryos show CYP1A induction very early in their development, long before the eggs hatch. This is noteworthy because as Carls and Thedinga (2010) stated, "if pink salmon embryos in PWS were exposed to PAHs in PWS—as supported by CYP1A induction (Weidmer et al. (1996) . . . then differential mortality between oiled and reference streams is plausible" (p. 7). But Wiedmer et al. didn't find CYP1A induction in embryos prior to the eggs hatching: only in alevins. They sampled eggs and pre-emergent fry in four oiled and five non-oiled PWS streams in 1989–1991 and found that eggs showed no evidence of CYP1A in any of the streams surveyed. Because CYP1A induction occurred in laboratory eggs exposed to oil at least as early as three weeks after fertilization, the absence CYP1A in eggs from oiled streams indicates that oil exposure was not high

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enough in those streams to induce the enzyme in the incubating population prior to hatching. This was the period coincident with the observed egg mortality in ADFG surveys (Sharr et al., 1994), and thus the absence of CYP1A in eggs sampled from PWS streams adds to the evidence refuting the allegation that oil concentrations were high enough to be responsible for the increased mortality.

While embryos may be damaged when exposed to high concentrations of weathered oil, it can also be concluded that the presence of CYP1A in the embryos isn't an indicator of damage, but rather a bio-indicator of the presence of oil or some other factor that induced the enzyme. Inducible tissues of unexposed groups also showed highly variable CYP1A scores in the Carls et al. (2005) and Marty et al. (1997b) studies. Vertebral cartilage stained higher in the unexposed controls, and CYP1A levels increased in 13 tissues among the controls over the incubation period in the Carls et al. study. In the Marty et al. study among the pre-emergent fry the controls had the highest CYP1A scores in some cases, indicating there is a diversity of conditions that induce CYP1A expression, and not necessarily correlated with damage.

Research on Particulate Oil

The complicating issue with the ABL research (Carls et al., 2005; Heintz et al., 1999; Marty et al., 1997b) was that only the aqueous-dissolved phase was considered the sole cause of toxicity to the eggs; direct contact with oil was not considered to have contributed to the egg mortality. When Brannon et al. (2006a) suggested the direct contact with dissolved oil also contributed to toxicity, Carls et al. (2008) attempted to respond to that criticism by exposing Zebrafish, *Diplodus cervinus,* eggs to dissolved-PAH with and without the presence of un-dissolved oil. Eggs were embedded in an agarose matrix layer on the bottom of a Petri dish to exclude oil droplets, and then the agarose was covered with a solution of mechanically dispersed oil placed in water over the top of the agarose and in which another lot of eggs was incubated. The agarose prevented passage of undissolved oil droplets from reaching the eggs while allowing the diffusion of dissolved PAH. The first lot of eggs incubated in agarose were then compared for induced developmental defects with the second lot exposed to the dispersed oil solution above the agarose layer in the presence of both droplets and dissolved PAH. TPAH composition of water and agarose was determined, alkane and PAH exposure was quantified, and oil particle size distribution in water and agarose was analyzed. Although overall physiological toxic effects averaged 17% lower in agarose embedded eggs, statistically the differences were borderline, and the results were interpreted to mean only the aqueous-dissolved oil was responsible for toxicity with no damage attributed to un-dissolved oil.

Analysis

The difficulty with the interpretation of Carls et al. (2008) results was that while no damage was attributed to un-dissolved oil, there was a significant difference in abnormal heart looping and the extent of toxic injury among embryos was generally lower in the agarose embedded embryos, and thus consistent with greater damage of those exposed directly to mechanically dispersed oil. Apparently there was no analysis of tissue-TPAH that would have helped clarify contributions of the dispersed oil, although agarose would have slowed passage of the more complex hydrocarbons.

However, there is also uncertainty about the experimental design. While oil droplets were prevented from physically contacting the chorion, it is possible that oil in contact with the agarose could partition directly through the matrix to the egg membrane as if the agarose were simply an extension of the chorion. Undissolved oil could have been a source of contamination in this manner without passing through the aqueous-dissolved state.

Conclusions on Application of Laboratory Studies

The authors suggest that the studies on the impacts of weathered oil under laboratory conditions did not represent what PWS pink salmon experienced in EVC contaminated streams. The high oil concentrations used in the laboratory trials (Carls et al., 2005; Marty et al., 1997a, 1997b) were unrelated to conditions that existed in those streams. The highest non-lethal laboratory tissue-TPAH concentrations were > 20 times higher than the tissue-TPAH in samples taken from oiled streams. Attempting to equate results from high oil concentrations employed in laboratory studies on pink salmon eggs with low contamination levels experienced by eggs in the field exaggerates the exposure.

The laboratory experimental design is also an issue. The authors suggest that assuming toxicity to be related only to the aqueous-dissolved TPAH when laboratory test conditions exposed embryos and alevins directly to dispersed oil (Heintz et al., 1999; Marty et al, 1997b), either in contact with the oil/gravel mixture or potentially to oil droplets, underrepresented the actual TPAH levels of exposure. Further, the proposal that HPAH were the toxic components that would put embryos in oiled streams at risk is unsupported by research results.

Do Field Studies Support Oil Toxicity in PWS Streams?

Investigators recognized the evidence that the streambed sediment-TPAH in oiled streams was never high enough to be of any risk to pink salmon embryos (Murphy et al., 1999). Therefore, they reasoned if oil was the cause of the egg mortality reported by ADFG (Bue et al., 1996, 1998; Sharr et al., 1993), the oil source had to come from outside the stream and it had to be made up of highly toxic components to survive the dilution of tidal flow and freshwater flushing. In this section the authors discuss the three primary field studies undertaken by ABL offered in support of shoreline oil deposits as the source of toxicity affecting pink salmon eggs in PWS streams.

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Stream Delta Oil Deposits as a Source of Toxicity to Embryos

Investigators proposed that highly toxic HPAH compounds concentrated in weathered oil deposits outside the stream were the source of egg mortality. The theory was that leachate from the deposits was transported via interstitial flow to the adjacent stream, delivering highly toxic HPAH doses to incubating embryos (Carls et al., 2006; Heintz et al., 1999; Murphy et al., 1999; Rice et al., 2001). To assess the theory Murphy et al. (1999) analyzed sediment samples from oil deposits archived by ADFG in 1989–1991 that included seven ADFG benchmark sites, ranging in sediment-PAH from < 1 to 968,000 ppb, as well as using samples taken by ABL in 1995. Because there were no stream delta sediment-TPAH measurements between those years, Murphy et al. considered the ADFG petroleum hydrocarbon deposit concentration data to be representative of the TPAH level in the delta intertidal substrate in 1989, and then used the 1995 ABL sediment-TPAH data to interpolate concentrations for intervening years. Using those estimates they assumed that sediment-TPAH concentrations equivalent to the Heintz et al. (1999) laboratory determined minimum lethal sediment-TPAH threshold of 3,800 ppb (Table 6), or 18 ppb aqueous-TPAH, characterized some stream deltas until 1995. Referring to that data, Rice et al. (2007) stated that "such concentrations were still present in the interstitial waters of a number of salmon streams in 1995" (p. 477).

Analysis

The critical issue in examining the Murphy et al. (1999) and the Rice et al. (2007) analysis is the recorded data on the actual level of contamination that existed in the delta sediment. The samples archived by ADFG in 1989 did not represent the delta oil-in-sediment concentrations. Those samples were not taken randomly from the delta and were not claimed to be representative of oil concentrations across the delta. They were intentionally taken from visible oil deposits and mostly as single samples per site to simply demonstrate the presence of EVC. As reviewed previously by Boehm et al. (1995), Short and Babcock (1996), O'Clair et al. (1996), and Wolfe et al. (1996) the oil content of substrate on the deltas and intertidal areas ranged from means of < 35 ppb to ~500 ppb during the first three years following the spill, well below the levels hypothesized by Murphy et al. and Rice et al, and returned to normal background levels shortly thereafter. The use of samples from visibly oiled areas, rather than systematic sampling to fully characterize the beaches, is not useful for assessing general shoreline oil conditions. There is no evidence that toxic concentrations of oil leached from the deltas and existed in interstitial waters entering streams. ABL investigators did not analyzed aqueous-TPAH levels in interstitial waters associated with oil deposits, nor did they analyze embryo tissues for PAH contamination in streams adjacent to oil deposits on the deltas in 1989, 1990, or 1991.

The Mechanism Proposed for Toxic Water Transport

To support the feasibility of the interstitial flow hypothesis as a mechanism that transported toxic PAHs from buried intertidal oil deposits on the stream banks to the incubation substrate of PWS streams, Carls et al. (2003) performed dye tests. On two stream deltas they injected dye through well points along the stream bank and then monitored where it resurfaced during the first tidal ebb. They reported that the beaches were very porous and that dye released in the subsurface through tubes inserted in the beach 4 m–7 m from the stream would require as little as 23 min–34 min to reach the stream, and experienced 90% dilution in the process, and in some cases dye appeared on the opposite side of the streams from which it was injected (Carls et al., 2003).

The point of the Carls et al. (2003) study was to substitute dye for PAH-laden water and to show that interstitial flows from oil deposits on the delta could make their way to the incubation environment of the adjacent stream. They were successful in demonstrating how porous the delta substrate was to the infiltration of salt water upon incoming tides and conversely the rapid dewatering of the intertidal area upon receding tides. Based on these data Carls et al. concluded: "The flow of groundwater containing PAH dissolved from oiled intertidal beaches surrounding anadromous streams into the hyporheic zone explains how pink salmon eggs became contaminated after the Exxon Valdez oil spill" (p. 253).

Analysis

The observations of Carls et al. (2003) do not support the supposition that interstitial toxic water contaminated pink salmon eggs. Notwithstanding that PAH transport wasn't observed, their observations showed that water moved from the injection-points to the stream at a mean velocity of ~19 cm/min. At that rate the interstitial flow passing oil deposits would contain petroleum hydrocarbons only at very low concentrations. Further, the oil residues are sequestered in low-porosity sediments that inhibit the flow of water (Li and Bloufadel, 2010; Pope et al., 2011), which means while the HPAH analytes do not readily dissolve in any case, they would leach from the residues even more slowly. As they dissolve and disperse toward the stream they are diluted by ground water and tidal infiltration entering the permeable sediments they are traveling through: only traces of HPAH would actually reach the stream. The process is demonstrated by the dye mixtures that were diluted 90% in the short distance from the injection-point to the stream in the Carls et al. study. Moreover, upon reaching the streams the leachate would be confronted with the high freshwater flushing and diluting flows irrigating the redds, reducing any potential risk to incubating eggs. This is consistent with the low tissue-TPAH measured in incubating alevins (<95 ppb) in the Brannon et al. (1995) analyses at the height of stream contamination. The authors suggest the Carls et al. (2003) data show that the interstitial transport of PAH at concentrations sufficient to kill incubating eggs in adjacent streams is highly improbable.

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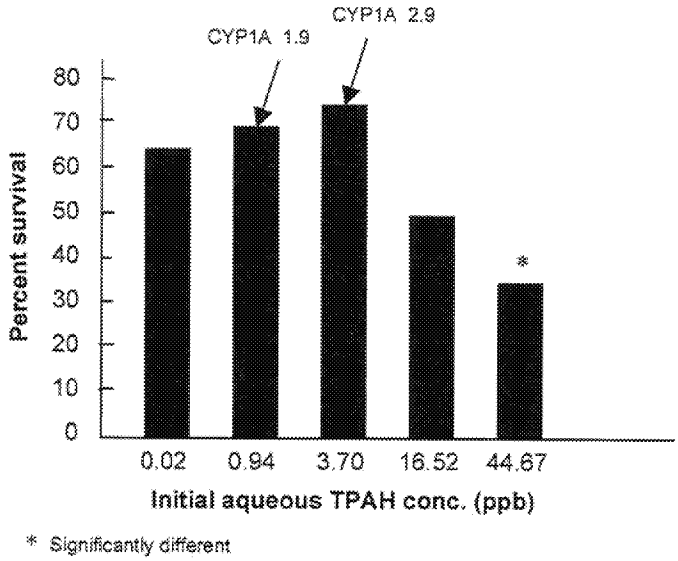

Figure 8 Percent survival of $5\frac{1}{2}$ month old fingerlings in net-pens after exposure to different initial aqueous-TPAH concentrations during incubation as embryos (from Carls et al., 2005).

CYP1A Analysis of Eggs and Fry Applied to the Field

CYP1A as an indication of exposure to petroleum hydrocarbons is consistent with the function of the enzyme. In the discussion of the laboratory studies, NMFS investigators contend that the presence of CYP1A in alevins and fry associated with oiled streams in PWS is indicative of long-term damage reducing survival success of later life stages (Rice et al., 2007; Carls et al., 2005). That position was related to the Carls et al. (1996a) field study where the enzyme was observed in fry in marine waters off from oiled streams. Carl et al. (2005) expanded on that theme in a net-pen study where laboratory exposed embryos were raised to the fingerling stage. The fish were raised in net-pens for $\sim 5\frac{1}{2}$ months after exposure to different oil doses during incubation that elevated tissue-TPAH loads and CYP1A scores. Subsequent survival success was reduced over the ~5 1/2 months in the two highest doses with significant toxic loads represented by aqueous-TPAH concentrations of 16.5 ppb and 44.7 ppb (Figure 8). They concluded their results were consistent with the alleged reduction in PWS adult returns from exposure to oil, and that CYP1A induction is related to a variety of lethal effects and diminished marine survival.

Analysis

It appears there are problems in how CYP1A is interpreted and applied to conditions in PWS. Sub-lethal and lethal concentrations of PAH will trigger CYP1A in pink salmon, but, as indicated in the laboratory studies above, the enzyme does not indicate damage. Laboratory embryos had been exposed to a range in aqueous-PAH concentrations from 0.9 ppb–44.6 ppb in the Carls et al. (2005) study, and only the two highest concentrations showed reduced, post-emergence survival by the end of the study (Figure 8). However, the embryos exposed to 0.9 ppb and 3.7 ppb aqueous-PAH had CYP1A scores of 1.9 and 2.9 respectively and show slightly higher survival rates than the controls after the $5\frac{1}{2}$ months of rearing. It is noteworthy, therefore, that contrary to the investigators' contention those juveniles with at or slightly higher survival than the controls demonstrated that CYP1A scores up to 2.9 had no predictive negative survival value.

The lack of long-term survival effects was also evident in other field studies. Wiedmer et al. (1996) found that CYP1A induction was apparent in pre-emergent fry with scores up to 4.0 in 1989 and 3.0 in 1990, but neither Sharr et al. (1994) nor Brannon et al. (1995) observed differences in pre-emergent fry survival between oiled and non-oiled streams. This again showed that CYP1A was an indicator of exposure, not damage. Longer-term evidence of no damage with CYP1A scores at ~4.0 determined by Wiedmer et al. was also shown by wild adults returning to oiled streams at rates statistically no different from non-oiled streams (Brannon et al., 2006b; Maki et al., 1995).

The Carls et al. (2005) implication that reduced growth and survival of laboratory fry equate with what embryos in oiled PWS stream would experience is problematic. Carls et al. stated, "The combined results from a series of embryo-larvae exposures experiments. . . . demonstrate that CYP1A induction is related to a variety of lethal and sublethal effects, including abnormalities, reduced growth, and diminished marine survival" (p. 264). That is only true when CYP1A induction was coincident with very high oil concentrations in their studies. As shown above, induction scores up to 2.9 had no effect on marine survival in the Carls et al. (2005) study and scores as high as 4.0 in the Wiedmer et al. (1996) assays had no effect on pre-emergent fry survival or adult return success. The Carls et al. high oil exposure concentrations were ~1,400,000 ppb and 3,800,000 ppb oil-on-gravel, with initial tissue-TPAH levels of exposed embryos at ~12,500 ppb and ~30,000 ppb respectively. Those tissue loads exceeded tissue levels in embryos in oiled streams by > 100 and > 300 times respectively (Brannon et al., 1995). The authors suggest there was no relationship between the induction of CYP1A in the laboratory exposures and the growth or survival of pink salmon originating from oiled streams in PWS.

Conclusions from field studies

Although deposits of oil on the beaches were hypothesized to produce leachate resulting in interstitial toxic water entering the redds and causing embryo deaths, actual field data show that was not the case. As confirmed in laboratory studies, HPAH compounds alleged to have been the toxic components of weathered oil are actually increasingly less available to incubating embryos as oil weathers and thus not the source of toxicity contemplated in the new oil toxicity paradigm. CYP1A induction from exposure to oil in PSW streams was not related to any damage that could be identified in pink salmon during or after the incubation experience, and there is no evidence that adults exposed to oil as embryos in oiled streams experienced lower return rates.

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Prospective Long-term Effects of Oil Exposure

Based on the interpretation of the above series of laboratory and field studies and the belief that egg mortality was caused from exposure to the oil, NMFS investigators at ABL concluded that survivorship was reduced in later life stages and that survivors would experience lower reproductive output at maturity (Heintz et al. 2000). The rationale for the long-term effects centered around three field studies summarized below, with analysis involving relevant data.

The Search for Long-term Risk in PWS Streams

Analysis of six oiled streams in 1999, ten years after the EVOS, was undertaken by Carls et al. (2004a) in search of persistent petroleum hydrocarbons in PWS. Low-density polyethylene membrane sampling devices (PEMDs) were deployed in streams in 1998 for periods of 45–56 days to sequester petroleum hydrocarbons from the water. PEMDs were suspended in tubes and buried in each of four tidal elevation zones of the streams at locations corresponding with other studies. Sediment samples were taken from the streams for PAH analysis, pink salmon embryo tissues were sampled to assess for TPAH concentration, and also scored for CYP1A expression.

Two previously oiled streams (Sleepy Bay and Junction creeks) showed elevated concentrations of petroleum hydrocarbons in extracts from PEMDs in the lower intertidal areas with mean PAH estimated between ~70 ppb–110 ppb compared to samples upstream of tidal influence (and thus above the point where oil would have gone) of ~50 ppb (Carls et al., 2004a). The composition of PEMD samples was found to be consistent with moderately weathered EVC. None of the other streams showed EVC contamination.

The sediment samples showed that PAH was elevated to ~33 ppb in the lower tidal area of Sleepy Bay Creek, but that level was consistent with pre-spill background levels (O'Clair et al., 1996). The tissue-PAH (minus naphthalenes and C1 naphthalene concentrations) levels of embryos sampled from those streams were low (< 50 ppb), and EVC wasn't identifiable as the source of PAH. EVC profiles in the water and sediment were also inconclusive (Carls et al., 2004a). CYP1A scores showed that the enzyme was induced in embryos in three of the streams, with two showing higher scores in the lower tidal elevations than above high tide. The relationship of CYP1A scores with exposure to TPAH was uncertain, and the elevated TPAH levels were not identifiable with the EVC profile. However, based on those data Carls et al. concluded that, "Positive identification of *Exxon Valdez* oil in Sleepy Creek water over ten years after the spill, together with evidence of PAH level in sediment remain elevated, and that pink salmon eggs were exposed to PAHs, indicates long-term habitat damage" (p. 841).

Analysis

The authors suggest that the Carls et al. (2004a) conclusion of long-term habitat damage was inconsistent with their data. There is no evidence of toxicity to eggs, or quantification of damage to the habitat, and little evidence of the presence of EVC. Furthermore, sites used from above mean high tide as the base measurements for comparison were not legitimate controls for intertidal stream conditions that are affected by various marine petroleum residues. The data that Carls et al. generated is consistent with what occurs commonly in PWS as background conditions from a variety of non-EVC sources unassociated with the spill (Boehm et al., 1998).

Long-Term Effects of Chronic Exposure on Growth and Adult Returns

Pink salmon eggs exposed to oil concentrations in the laboratory in 1993 and 1995 were used in follow-up investigations to assess the long-term effects of such exposures on growth and survival of juveniles reared in net-pen studies as well as on their subsequent growth and survival to the adult stage after their release in marine waters as fry in 1994 and 1996 (Heintz et al., 2000). After emergence of the 1993 brood fry, which were exposed as embryos to oil-on-gravel concentrations ranging from 15,000 ppb–2,450,000 ppb (aqueous-TPAH 1.3 ppb–48 ppb), one study set remained in marine net pens for 200 days of post-emergence rearing, after which they were weighed and measured, and their survival determined. There were no statistically significant differences in fish lengths among all lots. Fish weight tended to be higher than the controls in those fish originating from the three lowest oil concentrations (aqueous-TPAH 1.3 ppb, 3.6 ppb, and 7.8 ppb), but the three highest exposure concentrations (aqueous-TPAH 18 ppb, 31 ppb, and 48 ppb), resulted in significantly lower weights than the controls.

The experiment was repeated with the spawn of the 1995 return adults with larger numbers of fish than in 1993 and in two experimental lots representing intermediate (83,000 ppb) and highest (726,000 ppb) oil-on-gravel exposures with aqueous-TPAH levels of 5.2 ppb and 19.4 ppb respectively. This net-pen study was run for 300 days, and while the lengths were not reported, only the highest oil-exposed fish were significantly lighter at the end of the study.

The second study set of fry originating from three 1993 test lot exposures of 15, 000 ppb, 74,000 ppb, and 281,000 ppb oil-on-gravel concentrations (aqueous-TPAH 1.3 ppb, 7.8 ppb, and 18 ppb) and released to open marine waters in 1994 to assess adult return survival, was also revealing. Return success in 1995 was 2%, 2.2%, and 1.7% respectively with overlapping standard errors, showing that statistically survival was the same as the controls at 2%. Also, there were no differences in the lengths or weights of the adult returns from the test lots.

The two test lots of 1995 brood fry exposed to oil-on-gravel concentrations of 83,000 ppb and 726,000 ppb (aqueous-TPAH 5.2 ppb and 19.4 ppb) were released as fry in 1996. They returned in 1997 with no size differences, but survival to the returning adult stage was only 1.1% and 0.8%, respectively with overlapping standard errors, compared to the controls at 1.3%.

Heintz et al. (2000) concluded the results in net-pen fingerling growth and adult returns supported the premise that there were

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delayed effects of oil exposure on PWS pink salmon. They claimed there was 15% less survival of the 1997 adult return exposed to 83,000 ppb oil-on-gravel as embryos compared to the controls, and Rice et al. (2007) concluded later that the those adults returning from incubation exposure to the high test dose (726,000 ppb oil-on-gravel) represented nearly a 40% reduction in survival compared to the controls.

Analysis

It is better if the conclusions about adult survival and growth reduction in net pens are put in perspective. The concentrations tested by Heintz et al. (2000) under laboratory conditions were far in excess of what had occurred in PWS oiled streams (Brannon et al., 1995) and thus have little relevance to the effects of the oil spill. Based on tissue-TPAH concentrations, there were no negative effects of exposing embryos to oil on subsequent growth rates of fingerlings in net pens until tissue-TPAH concentrations were > 60 times higher at ~6,000 ppb (Heintz et al., 1999) than what was experienced in PWS streams (<95 ppb, Brannon et al., 1995). Moreover, the lengths and weights of adults returning in 1995 and 1997 that had experienced those high concentrations as embryos under controlled laboratory conditions were statistically the same as the controls after 14–16 months on the high seas. The net-pen studies and marine growth of fry from laboratory exposures appear to verify that embryos that experienced the much lower oil levels in spill-path streams should demonstrate no long-term growth reduction in marine waters.

The authors also suggest that the differences in survival of returning adults in the Heintz et al. (2000) fry marine release studies were not that meaningful in application to fry from oiled streams in PWS. Of the two release years, only one showed differences. The 1995 returns showed that the embryos with > 20 times the tissue-TPAH level of incubating alevins in oil streams survived as well or better than the controls that had no exposure to oil. In 1997, while the adult returns for the medium and high dose exposed embryos were reported respectively at ~15% and 40% lower survival than the controls (Heintz et al., 2000, and Rice et al., 2007), those claims appear to have been based on the comparison of the percentages of fish returning, rather than calculating survival as the percentage of adults returning from the number of fry released for each experimental group. The actual data for the 1995 brood fry releases showed that the medium dose (83,000 ppb oil-on-gravel) returned in 1997 only 0.2% fewer fish than the controls, with overlapping standard errors, and the highest dose (726,000 ppb oil-on-gravel) returned only 0.5% fewer fish than the controls. While these may be considered as different in survival performance, the differences were relatively minor. Given that the controls had experienced 98.7% mortality, the level of difference among the treated groups at 98.9% and 99.2% mortality would be an expected level of variation even among multiple non-oiled control lots. This was demonstrated in adult returns in 2002 from fry released in 2000 that were the progeny of pink salmon exposed to oil during incubation in 1998 (Heintz, 2003). The kin of the experimental brood exposed to the highest oil concentration (aqueous-TPAH 13.3 ppb) had the highest return success at 1.1% compared to the controls at 0.7%. Can we conclude that the long-term effects of oil treatment was a 36% higher survival benefit over the controls? No, the real difference was 98.9% mortality versus 99.3% mortality; virtually the same.

Moreover, exposure to high oil concentrations during incubation tends to delay emergence timing (Carls and Thedinga, 2010; Marty et al., 1997b) and that emerging fry experience differences in survival based on early marine growth (Cross et al., 2008; Mortensen et al., 1991; Moss et al., 2005). However, there were no analyses of the interaction between dose experienced and release time in the Heintz et al. (2000) studies to help adjust survival estimates.

The authors disagree that the Heintz et al. (2000) follow-up studies on long-term growth and survival of pink salmon supported the claims of delayed effects of oil exposure on PWS pink salmon. There were no impacts on growth and little or no effect on return success at oil exposure levels that were many times higher than concentrations experienced by pink salmon embryos in the oiled streams of PWS. Consequently, no negative survival or growth effects would be anticipated from the much lower oil exposure levels that embryos were shown to have experienced in spill-path streams.

Long-Term Effects of Chronic Exposure on Reproductive Success

Heintz et al. (2000) concluded that adults experiencing chronic exposure to oil during their incubation period as embryos would experience "reduced reproductive output at maturity" (p. 214). The egg viability of the adult returns of the 1993 and 1995 brood fry marine releases in that study apparently were not assessed. However, eggs of adults exposed to TPAH concentrations as embryos in 1998 were used to test the viability hypothesis. Eggs exposed to TPAH low and high concentrations in 1998, released as fry in 1999 and returned, and were spawned artificially in 2000 showed that egg viability or survival was no different from the eggs of the returning controls released for comparison. The Heintz et al. (2002) research failed to demonstrate any negative effects of embryo exposure to oil on reproductive success of returning adults, even though high TPAH exposure was experienced. These were the gametes of fish exposed to oil as embryos, and Heintz concluded there was no impact on the reproductive ability of the generation of fish exposed to oil.

Analysis

Although the Heintz (2002) study appeared only in an annual project report and is not published, the data is pivotal evidence. It is contrary to both reduced pink salmon egg viability

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interpreted by Bue et al. (1998) and the hypothesis of reproductive impairment (Heintz et al., 2000) from exposure of embryos to EVC. The egg viability of adults returning from fry releases in 1998 were unaffected from exposure as embryos to two different oil concentrations experienced under laboratory conditions that were higher than what was experienced by embryos in PWS streams in 1989 and 1990. Consequently, the absence of reproductive effects at the Heintz (2002) TPAH levels tested imply there would be little risk to the productivity of pink salmon that were incubated in oiled steams, and is consistent with other data showing there were no inter-generational impacts due to oil-exposure (Brannon et al., 1995; Brannon and Maki, 1996; Cronin and Bickham, 1998; Cronin et al., 2002; Cronin and Maki, 2004).

Conclusions about the Hypothesis on Long-Term Chronic Toxicity

The authors suggest the allegations that pink salmon embryo exposure to the *Exxon Valdez* oil spill would later result in lower fry growth in marine waters, lower adult return survival, and reduced reproductive success are unsupported. The laboratory evidence on much higher oil concentrations compared to the low level of EVC contamination in oiled streams based on tissue-TPAH levels indicates there was little long-term risk to incubating PWS pink salmon. Similarly, embryo exposure to oil concentrations in oiled streams would be expected to have no effect on their gamete viability as adults.

University Studies on Oil Toxicity

Laboratory and field investigations through the University of Idaho and Oregon State University helped resolve the discordance between the field evidence showing no effects on pink salmon from the EVOS and the alternative claims of significant negative effects based primarily on laboratory studies. The authors review the university studies below.

University of Idaho Laboratory Study of Oil Toxicity on Pink Salmon

To assess the conclusions reached by investigators regarding lethal effects of oil on pink salmon embryos, a study was conducted at the aquaculture laboratories of the University of Idaho (UI) in 2001 (Brannon et al., 2006a) with dissolved TPAH doses in generator columns similar to the experimental design described by Marty et al. (1997a, 1997b) and Heintz et al. (1999) that were flushed four days to eliminate particulate oil in the treatment water prior to introducing the eggs. Pink salmon eggs were exposed to EVC removed from the cargo tanks of the *Exxon Valdez* and artificially weathered (AWEVC), and to naturally weathered oil removed from PWS beaches (NWEVC) in 1989 that had been stored under controlled conditions. Four oil-on-gravel concentrations at 200,000 ppb, 750,000 ppb, 1,500,000 ppb, and 2,250,000 ppb were prepared and tested against a control exposed to no oil. Test lots of eggs were placed directly on the oiled gravel and also downstream in the effluent of each oil treatment. Egg mortality was not significantly different at any oil concentration prior to the eyed stage, and became significant among the alevins only at the highest oil-on-gravel concentration with 59.7% mortality by the end of incubation (Table 7) compared to 16.9% mortality in the controls (Brannon et al., 2006a). Mortality of eggs and alevins suspended above the gravel was not significantly different from those directly on the gravel, and mortality of embryos exposed to the NWEVC was no different from the controls, even at 2,250,000 ppb oil-on-gravel contamination. The resulting aqueous- and tissue-TPAH concentrations from a given oiled gravel concentration were much lower than in the laboratory studies of Heintz et al. (1999) (Table 6), and the resulting mortalities were also lower. The lack of difference with the NWEVC was attributed to its high moisture content (64.5% wt) acquired as the weathered oil mixed with water in the form of mousse upon reaching landfall that reduced the TPAH per unit of mass equivalent to AWEVC concentration of 750,000 ppb oil-on-gravel (Table 7).

Table 7 Comparative sediment-, aqueous-, and tissue-TPAH levels, in the Brannon et al. (2006a) laboratory studies on percent mortality of incubating pink salmon exposed to concentrations of (a) laboratory weathered and (b) naturally weathered EVC at flows of 150 mL/min

Gravel EVC (ppb)	Gravel TPAH (ppb)	Water TPAH (ppb)	Tissue TPAH (ppb)	Percent Mortality
Control	< 1	0.01	85	16.9
200,000 [a]	460	1.46	1,500	12.6
750,000	4,200	7.43	5,300	13.3
1,500,000	8,300	7.84	7,100	23.1
2,250,000	12,100	16.39	13,200	59.7
2,250,000 [b]	4,400	8.27	7,800	14.8

[a]Laboratory weathered oil.
[b]Natural weathered oil from Prince William Sound.

It should be noted that the highly weathered VWO used by Heintz et al. (1999) was markedly different in composition from the NWEVC in the UI study (Figure 9). Their VWO contained no C0- through C3-naphthalenes and the most abundant PAH group was the C3-phenanthrenes. NWEVC in the UI study contained substantial amounts of alkyl naphthalenes, with C2-phenanthrenes as the most abundant group. NWEVC was not lethal at an aqueous-TPAH of 8.27 ppb compared to the Heintz et al. VWO inducing mortality at aqueous-TPAH of only 1 ppb (Tables 6 & 7), further supporting the conclusion that VWO toxicity was from something other than just PAH. As shown previously in this section, the most compelling evidence that PAH was not responsible for the toxicity was the tissue-TPAH level of 470 ppb with alkyl phenanthrenes at ~100 ppb in VWO with high embryo mortality compared to the 7,800 ppb tissue-TPAH showing alkyl phenanthrenes at ~1575 ppb in the NWEVC without increased mortality over the controls (Brannon et al., 2006a).

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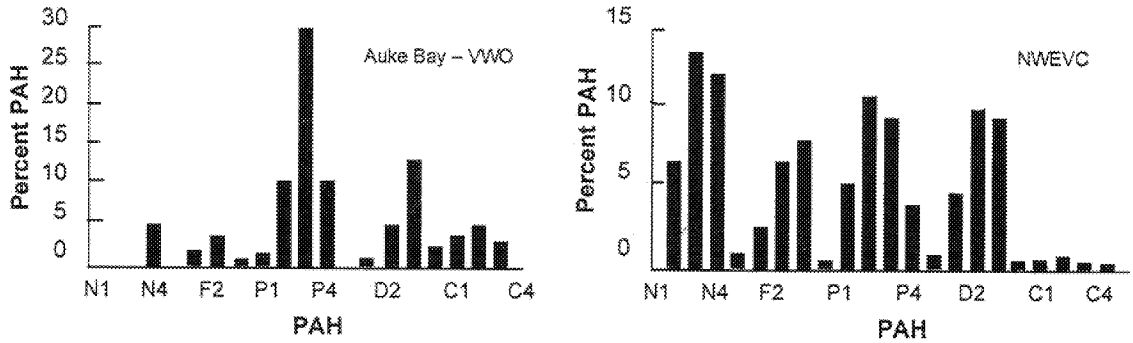

Figure 9 Oil-on-gravel percent TPAH composition of VWO used by Heintz et al. (1999) and NWEVC used by Brannon et al. (2006a) in laboratory bioassays of pink salmon eggs.

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TPAH concentrations in gravel decreased with time during the egg incubation period in the Brannon et al. (2006a) study at all concentrations of oil-on-gravel, with loss of hydrocarbons most rapid early in the exposure period. The low dissolution rate of HPAH was apparent in the tissue-TPAH of embryos during the entire incubation period of the UI study. Tissues were dominated by C2- and C3-naphthalenes, C1-phenanthrene, and C1-dibenzothiophene in contrast to only traces of the higher molecular weight compounds. The low tissue concentrations indicate that HPAH residues were not readily biologically available. Blue sac disease occurred in the controls, but was significantly much higher at 1,500,000 ppb and 2,250,000 ppb ($p < 0.05$) for both on and above oil/gravel mixtures. The presence of the disease in the controls indicate that conditions other than petroleum contamination can induce blue sac disease, a disease observed in some salmon hatcheries.

Brannon et al. (2006a) found that four days of flushing of the oil/gravel mixture prior to starting the Brannon et al (2006a) study did not eliminate particulate oil from the treatments, and thus verified that incubating eggs above the oiled gravel surface were exposed to both dissolved and dispersed oil under that experimental design. Given that eggs were in direct contact with the oil phase and covert oil micro-droplets were present in the treatment solution, TPAH in true solution was not the only source contributing to the tissue-TPAH concentration measured in embryos.

UI Laboratory Study of Oil Toxicity on Steelhead

In 2002 and 2009 a second set of studies was undertaken at the Aquaculture Research Institute, UI, with steelhead trout, *Oncorhynchus mykiss* (Collins et al., 2009b), using the same experimental design and oil concentrations as Brannon et al. (2006a). In this case the objective was to examine the influence of water flow on mortality and the incidence of the blue sac disease in the presence of EVC. At 150 mL/min flow (an apparent velocity of ~1.4 mm/sec) mortality increased at the higher concentrations, but mortality was not significantly higher than the controls at any oil concentration when incubated at 600 mL/min (an apparent velocity of ~5.6 mm/sec). Although exposed to the same oil-on-gravel concentrations, the embryos in the 600 mL/min flow experienced < 50% of the aqueous-TPAH exposure and accumulated only ~50% of the tissue-TPAH load with very low incidence of blue sac disease compared to those incubated at 150 mL/min.

Blue sac disease occurred at 7.5% of the control alevins and 22.2% and 37.7% of those that had experienced a tissue-TPAH loads of 15,974 ppb and 42,460 ppb respectively at 150 mL/min flow downstream from the weathered oil. However, at a flow of 600 mL/min the disease was < 3.8% of the alevins even when they had experienced a tissue-TPAH concentration of 26,445 ppb. Therefore, while oil increased the incidence of the disease, it occurred in the controls at low flow and was nearly eliminated by higher flows in all test lots, including high oil concentrations.

Understandably, the flow under which exposure is experienced affects the dissolution level of petroleum hydrocarbons in the water, and thus the rate at which PAH molecules are absorbed by the eggs. But it is noteworthy that 39.3% mortality occurred in embryos with a tissue-TPAH load of 15,974 ppb when incubated in 150 mL/min flow, while only 9.6% mortality occurred in those with tissue-TPAH of 26,445 ppb incubated in 600 mL/min flow. This indicates that additional factors other than PAH were involved in affecting embryo survival, and Collins et al. (2009b) suggested that it may have been dissolved oxygen. The flow of 150 mL/min may have been insufficient to provide adequate oxygen to the developing embryos, suggesting that low oxygen could have been a synergistic effect on toxicity with oil on the chorion partially compromising the respiratory surfaces of the eggs. The control egg lots incubated at 150 mL/min flow hatched later, were delayed in yolk absorption, and experienced higher mortality (14.8%) compared to controls (6.5%) incubated at 600 mL/min.

Collins et al. (2009b) also addressed the question of whether PAH was absorbed by eggs when in direct contact with oil in the absence of an aqueous medium. After wiping the eggs of moisture, they were placed directly in refrigerated, undiluted non-weathered North Slope crude oil and sampled at 1 d, 6 d, and 56 d over the exposure period. The results showed an immediate and continuing uptake of TPAH over the eight weeks, demonstrating that oil in direct contact with the egg membrane, whether resulting from droplets adhering to the egg surface or in

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contact with oiled gravel substrate, cannot be excluded as a PAH source partitioning directly across the egg membrane from the oil mass. When eggs are in contact with oil, aqueous-dissolved PAH is not the only source of PAH absorbed across the chorion.

Field Studies on the Interstitial Toxic Water Hypothesis

To establish the level of toxicity associated with oil deposits in the stream deltas, a series of bioassay studies were undertaken in the fall of 2003 by Brannon et al. (2007) to test the validity of the interstitial toxic water hypothesis under field conditions and test the allegation that weathered EVC becomes increasingly more toxic as weathering advances (Heintz et al. 1999; Murphy et al. 1999; Rice et al. 2007). Sleepy Bay, Bay of Isles, East Herring Bay, Evans Island and Snug Harbor were the oiled shoreline sites selected where extensive oiling had occurred during the EVOS in 1989, and five non-oiled sites were used as controls. Live spawned pink salmon eggs were placed in mesh bags and buried in beach sediments near the streams. At Sleepy Bay the eggs were buried directly in an EVC oil deposit. Eggs were buried on previously oiled intertidal areas at Bay of Isles, Evans Island and Snug Harbor. All samples were retrieved after 3 weeks. East Herring Bay was included in the study because each year pink salmon spawned naturally over buried oil deposits in the intertidal beach area away from the stream at the site, and egg samples were simply dug from the natural redds over the deposits. TPAH analyses were made of both the eggs and the sediments samples.

Most noteworthy were the results for Sleepy Bay, with a sediment-TPAH of 23,493 ppb in the oil deposit and the low tissue load of 643 ppb dry wt. carried in the eggs after exposure for 3 weeks buried in the deposit (Table 8). Bay of Isles was 170 to 6,608 ppb sediment-TPAH, and the tissue loads were also low. At East Herring Bay, where spawning occurred directly over buried oil with sediment-TPAH at 17,673 ppb, the tissue-TPAH was only 13 ppb dry wt. The results indicated that even when in contact with or sitting directly over weathered oil deposits, the tissue-TPAH levels of the eggs were far from lethal levels shown in laboratory bioassays with AWEVC (> 7,100 ppb, Brannon et al. 2006a), and the eggs absorbed HPAH concentrations only in low amounts. For example, the C1-C4 naphthalenes remaining in the deposits in sample #4 at Sleepy Bay were lower (2,600 ppb dry wt.) than the HPAH analytes (5,670 ppb), but naphthalenes were still much higher in the tissue-TPAH loads (296 ppb) than HPAH analytes (45 ppb). Therefore, even at very low sediment C1 – C4 naphthalene concentrations it is apparent that they were more readily dissolved in the leachate from the deposits than were the elevated concentrations of HPAH that dominated the petroleum hydrocarbon composition of the deposits.

In this regard it is important to note that also in contrast to the interstitial toxic water hypothesis (Heintz et al. 1999, Rice et al. 2001), the weathered oil didn't emit higher HPAH concentrations with time in the actual field areas impacted by the EVOS (Brannonn et al., 2007). In the laboratory bioassays embryos were exposed to a sediment-TPAH of 4,400 ppb in the NWEVC, which resulted in a peak tissue-TPAH level of 7,800 ppb (Brannon et al. 2006a). This contrasts sharply with the field results at Sleepy Bay where sediment-TPAH was at five times higher concentrations in the oil deposit, but 12 fold less in tissue-TPAH of embryos buried in the deposit than in laboratory embryos, with HPAH detected at very low levels. These data indicate that while the percentage of HPAH increases in weathered oil over time, its availability as toxic leachate diminishes because of limited solubility.

Table 8 Sediment-TPAH concentrations from 5 PWS beaches, and tissue-TPAH concentrations of eggs buried on 10 PWS beaches in PWS from mid-August to mid-September in the fall of 2003

	Sediment-TPAH (ppb)				
Sample no.	Sleepy Bay Oiled	Bay of Isles Oiled	Herring Bay Oiled	Bainbridge Control	Culross Control
1	909	6,608	17,673	24	29
2	171	304			
3	1,436	2,445			
4	23,149	170			
	Tissue-TPAH (ppb dry wt.)				
Sample no.	Sleepy Bay Oiled	Bay of Isles Oiled	Herring Bay Oiled	Bainbridge Control	Culross Control
1	11	39	12	14	12
2	16	515	14	6	16
3	18	24		7	6
4	625	287		13	9
Sample no.	Evans Bay Oiled	Snug Harbor Oiled	Horseshoe Control	Ewan Control	Drier Control
1	6	6	6	38	10
2	7	6	7	10	10
3	8	11	7	11	9
4	17	10	9	11	

Research on Dissolved Oil and Oil Droplets at Oregon State University

In the fall of 2005, a study was undertaken at the Parametrix Laboratory, Oregon State University (OSU), to assess the effects of dissolved oil vs. un-dissolved oil droplets on toxicity of oil to pink salmon embryos (Stubblefield et al., 2010). To test exposure in the most severe toxic conditions, fresh North Slope crude oil was used in the bioassays. Thus the toxic components were not just PAH but also BTEX (benzene, toluene, ethylbenzene, and total xylene). Five exposure concentrations with micro-droplets (HMP) and five without micro-droplets (LMP), plus a control, were employed in triplicate as continuous doses from post-fertilization to emergence. This assay represents the truly chronic exposure and thus allows assessment of minimum chronic levels associated with toxic effects. The experimental

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Table 9 Average embryo mortalities for LMP and HMP studies with corresponding aqueous-TPAH (total polynuclear aromatic hydrocarbon) and BTEX (benzene, toluene, ethylbenzene, and total xylene) concentrations and tissue-TPAH for each water soluble fraction (WSF) treatment ($N = 4$ replicates unless otherwise noted)

	Embryo Test Day 51				
Mixing	WSF	TPAH ng/L	BTEX mg/L	Mortality %	Tissue-TPAH ng/g
LMP	0	47	1	36.0	600
	6.3	357	29	36.0	600
	12.5	486	55	37.0	3,776
	25.0	1,383	80	31.5	
	50.0	1,996	150	35.3[a]	10,000
	100.0	6,289	461	36.0[b]	23,149
HMP	0	79	2	26.5	500
	6.3	1,984	85	28.5	4,569
	12.5	2,314	94	26.0	4,454
	25.0	4,569	180	36.1	10,000
	50.0	8,961	533	58.0*	11,000
	100.0	27,746	2095	100.0*	20,000

*Significantly different than 0% WSF.
[a]$N = 3$; one replicate removed due to exposure chamber malfunction.
[b]Complete treatment mortality due to oxygen depletion in exposure chambers at day 48.

design provided a true aqueous solution nearly absent of un-dissolved petroleum hydrocarbons (LMP) to compare with solutions contaminating un-dissolved oil micro-droplets (HMP) covering a range of tissue-TPAH concentrations, but in the presence of BTEX.

The first noteworthy result was the responses of the embryos to test doses of oil. Although the aqueous-TPAH/BTEX concentration represented a near-saturated WSF solution at 10°C (6.3/461 ppb), resulting in a tissue-TPAH of 23,149 ppb, that dose was insufficient to cause mortality significantly higher than the controls (Table 9). Therefore, while the petroleum hydrocarbons in fresh oil are considered the most toxic because of the high concentrations entering solution, Stubblefield et al. (2010) found that when in the dissolved state the high concentrations of TPAH absorbed were not lethal to pink salmon at the near-saturated concentration.

The second noteworthy result was the major effect of un-dissolved oil micro-droplets on toxicity to the pink salmon embryos. With the presence of micro-droplets, mortality started to increase at 25% WSF of TPAH/BTEX (4.6/180 ppb) showing a tissue-TPAH of 10,000 ppb and reached very significant mortality of well over 60% at TPAH/BTEX levels of 50% WSF. This demonstrated that the presence of oil micro-droplets caused a toxicity response resulting in mortality starting at TPAH/BTEX tissue concentrations of 10,000 ppb (about the levels reported in the Heintz et al. 1999 and Brannon et al. 2006a), or ~ 57% lower than what was tolerated with a nearly true solution of dissolved oil (tissue-TPAH load > 23,149 ppb). Even at the eyed stage, only 23 days into the study, the embryos exposed to micro-droplets experienced 58% mortality in the 50% TPAH/BTEX concentrations, compared to no increased embryo mortality in groups with no oil droplets in the nearly 100% true dissolved state of TPAH/BTEX. CYP1A scores increased with dose in some tissues, but not in others, and scores were not correlated with mortality. No significant effects on the growth (measured as weight or length) of pink salmon or anomalies were noted in either the dissolved or oil micro-droplet tests.

Conclusions from University Studies on Oil Toxicity

We realize that the university laboratory studies did not replicate the ABL studies, and the different results of university studies do not invalidate the results of the others. We feel the interpretation of the agency results are the issue. The university laboratory studies suggests that the NMFS reports on toxicity of dissolved aqueous-TPAH need to be re-examined. The ABL experimental type PAH generator columns used in the UI investigations and previously assumed to eliminate particulate oil from laboratory tests was found to contain minute oil droplets that contribute to mortality. It appears, therefore, that reporting only the dissolved petroleum hydrocarbon level as aqueous-TPAH in such experimental procedures would over-state the toxicity of dissolved oil because the contribution of PAH from the presence of covert oil micro-droplets or direct contact with the oil mass wouldn't be included. Further, there was no evidence in the studies that weathered oil increased toxicity, either in the form as TPAH or as HPAH leachate. Field evidence suggests that well-weathered oil deposits on the deltas of the actual areas impacted by the EVOS do not represent a continuing and increasing threat of interstitial toxic water to pink salmon eggs incubating in adjacent streams.

Although preliminary and needing verification, the implications of the Stubblefield et al. (2010) results are far reaching and apply to oil contamination beyond the *Exxon Valdez* incident. The demonstrated absence of mortality from dissolved TPAH in nearly a saturated true solution and the mortality from exposure to the same TPAH concentration of dissolved TPAH in the presence of minute oil droplets, changes the perspective on oil toxicity. The Stubblefield et al. study demonstrated that oil in aqueous solution with un-dissolved oil droplets markedly increased the toxicity to pink salmon embryos compared to just dissolved oil.

These results suggest that given the absence of mortality greater than the controls in the near-saturated WSF solution of TPAH/BTEX, the dissolved oil concentrations reported lethal to pink salmon eggs in other laboratory studies unrelated to the *Exxon Valdez* spill may be incorrect. Unless there was an awareness of the presence of oil micro-droplets and precautions taken to excluded them, we suggest that reports on toxicity will have been over-stated because of the covert contribution of micro-droplets to tissue loads, either by direct contact with the oil mass or by micro-droplets carried in solution that adhere to the chorion. The absence of increased mortality at tissue-TPAH concentrations of > 23,000 ppb exposed to only the

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dissolved TPAH in the Stubblefield et al (2010) study, and > 26,000 ppb in higher flows in the presence of oil droplets in the Collins et al. (2009b) study, demonstrate that toxicity of petroleum hydrocarbons is highly variable beyond the ~6,000 ppb to 10,000 ppb in the presence of micro-droplets reported toxic in the ABL and university investigations, and will depend on the conditions around the exposure experience.

SECTION III: GROWTH OF JUVENILE PINK SALMON IN MARINE WATERS

Pink salmon migrate immediately to nursery areas in nearshore marine waters upon emergence, and while it was anticipated that the oil would have damaging effects on the fry in 1989, much of the surface oil had been carried out of PWS before fry emergence, and no fry mortality or avoidance of the residual surface oil was observed. Notwithstanding the lack of direct evidence, the second variable of the Geiger model (Smr_t) involved the reduction in juvenile pink salmon marine survival based on smaller mean size of fish in some oiled areas. Hypothetically, lower growth can result in reduced survival due to higher predation experienced by smaller fish (Cross et al., 2008; Hargreaves and LeBrasseur, 1985; Heard, 1991; Moss et al., 2005; Parker, 1971). Consequently when agency studies reported that juvenile pink salmon were smaller, it was concluded that oil exposure resulted in lower growth (Wertheimer and Celewycz 1996, Willette 1996), and thus it was assumed that those fry experienced higher predation losses, which was estimated in the Geiger model (1996) to contribute to the 28% higher mortality of the 1989 wild juvenile marine population in the southwestern sound from the 1988 brood year. This review challenges the allegation of reduced growth based on reanalysis of the data. The agency studies are summarized below and the evidence that is contrary to their conclusions is presented.

Allegations of Reduced Fry Growth

Reduced growth as a survival variable in the Geiger model was based on two studies. The first was from size differences of fingerlings captured in PWS by Wertheimer and Celewycz (1996). The second was on marked hatchery fry released and recaptured by Willette (1996).

Size Differences in PWS Pink Salmon

Wertheimer and Celewycz (1996) monitored size of fry captured in bays and migratory corridors in the sound. They contrasted weight and length of the juveniles from oiled regions with those from non-oiled regions over a period from April–July in 1989 and 1990, using beach seines to capture the fish. Juvenile pink and chum salmon were sampled from four oiled and four non-oiled locations, with two sites at each location in bays and two sites between the mainland and island in the migration corridors from PWS to the Gulf of Alaska (Figure 10). Fingerling stomachs were examined for fullness and composition, and temperature and salinity data were taken in triplicate at 1 m and 4 m depths at each location to characterize the environments associated with the sample sites.

Wertheimer and Celewycz found no statistically significant differences in weight or length of pink or chum salmon juveniles in bays or corridors over the two year study except for pink salmon in 1989, and only in corridors, not in bays. Mean length of samples from non-oiled corridors was longer (48 mm) than in oiled corridors (45 mm) in late June. The difference was not attributed to temperature where differences between locations were reported as insignificant. However, in contrast to lengths, weights of fingerlings between oiled and non-oiled corridors were the same and the condition index (weight:length ratio) was higher in oiled areas. Moreover, plankton productivity was higher in oiled areas in 1989, and there were no differences in stomach fullness of fingerlings between oiled and non-oiled areas. Wertheimer and Celewycz concluded from these data that in 1989 growth rate of pink salmon juveniles was less in oiled corridors based on reduced length of fish, notwithstanding that weight was unaffected.

Size Differences from Hatchery Fry Releases

Willette's (1996) assessment compared the sizes of pink salmon fry released and recaptured from the PWS commercial hatcheries in 1989–1991. In this study the growth effects of exposure to oil after release into the marine environment were based on size differences of coded-wire tagged (CWT) early-fed hatchery produced fish recovered in oiled areas compared to tagged fish from the same test lots recovered from non-oiled or lightly oiled areas (Figure 10). Tagged fish were released from AFK and Wally H. Noerenberg (WHN) hatcheries in the spring of those years. Beach and purse seines were used to recover the tagged juveniles. The water temperature and zooplankton abundance data taken twice a week by the Prince William Sound Aquaculture Corporation (PWSAC) in passages adjacent to each hatchery were used in the study to assess their effects on growth.

Willette observed that fry recovered in moderately oiled areas were lighter than those recovered in non-oiled or lightly oiled areas in 1989. The calculated growth rate of hatchery fish recaptured in oiled areas was 4.17%/day from AFK and 4.22%/day from WHN, compared to those from non-oiled areas at 4.93%/day from AFK, and 5.16%/day from WHN. While the weights followed similar trends in 1990 and 1991 as in the 1989 study, the differences were not large enough to be significant. Although temperatures were slightly higher in non-oiled areas, their influence on growth was considered insufficient to account for the weight differences. In contrast to weight, Willette observed no differences in juvenile lengths. Therefore, based on those differences in weight, Willette concluded that exposure of juvenile pink salmon to oil in the study areas resulted in a reduced growth rate in 1989.

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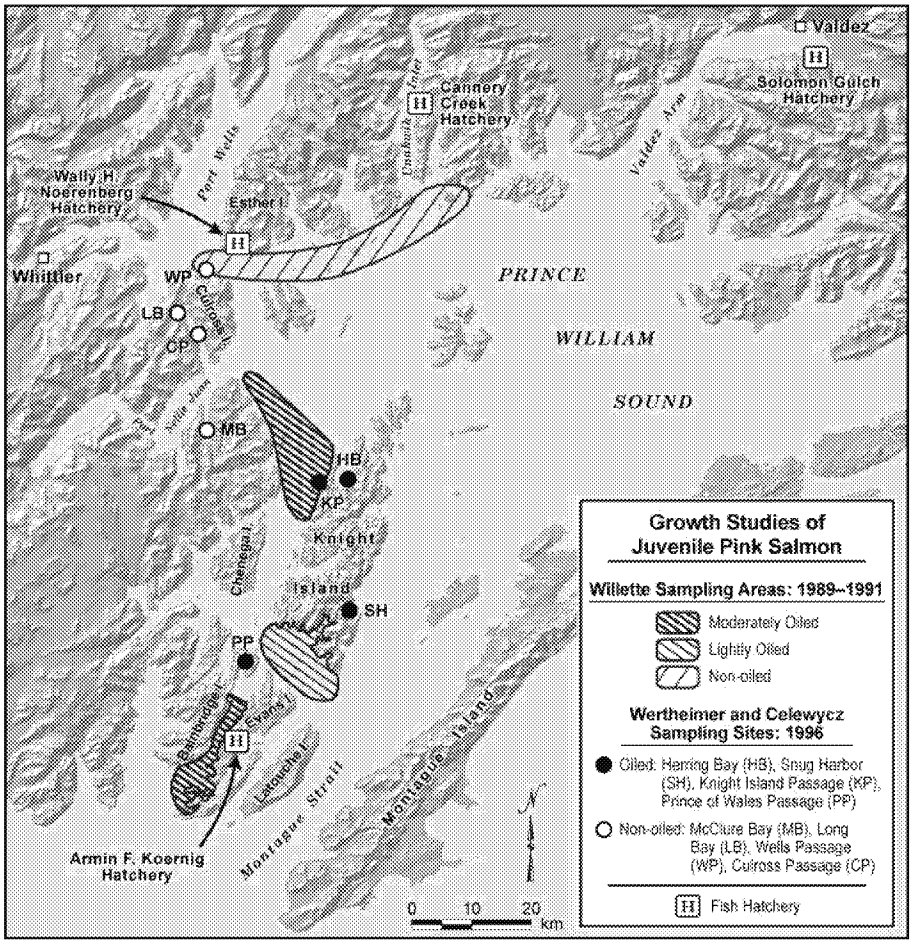

Figure 10 Map of Prince William Sound showing oiled and reference sites used by Wertheimer and Celewycz (1996), and the hatchery release sites and oiled and reference recovery sites used by Willette (1996), in their studies on growth of juvenile pink salmon following the *Exxon Valdez* oil spill.

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Conclusions of the Agency Studies

The conclusions of the investigators in the two agency studies were that the size differences of juvenile pink salmon exposed to oil in marine waters represented a reduction in growth rate. Although growth rates were not directly measured, reduced growth was inferred from differences in size of juveniles captured in oiled and control marine areas in 1989, involving less length in oiled corridors, but no weight differences, in the case of Wertheimer and Celewycz (1996), and lighter weight, but no length differences, in oiled areas in the case of Willette (1996).

Evaluation of the Assumptions Necessary to Analyze for Growth Differences

After re-examination of the Wertheimer and Celewycz (1996) and Willette (1996) studies, the authors conclude that the evidence is insufficient to infer that smaller size of juvenile pink salmon in oiled marine waters of PWS represented a reduction in growth rate. In order to interpret size differences in that manner, at least four assumptions must first be satisfied, those being: (1) the same population was randomly distributed between oiled and unoiled areas, (2) samples represent the same age structure, (3) sample areas were comparable, and (4) sampling gear was unbiased with regard to the sizes of fish caught. The authors will present the argument as to why they believe those assumptions were not satisfied in the Wertheimer and Celewycz and in the Willette studies.

Random Distribution

Distribution of juvenile pink salmon in the Wertheimer and Celewycz study included samples in non-oiled corridor sites that were 50–70 km upstream from the oiled corridor sites (Figure 10). In 1989 the fish in the non-oiled corridor of Wells Passage were dominated by juveniles from the WHN (160 million), Cannery Creek (59 million), and Solomon Gulch (134 million) hatcheries with a total release of 353 million fry, and constituting

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66% of the fish in those corridor samples. As they progressed southward downstream from Wells Passage south of the WHN hatchery to the oiled corridors the percentage of hatchery fish in the samples diminished. By the time they reached the oiled corridors of Herring Bay and further south to Knight Island passage, hatchery fish decreased to 44% and 19% respectively (Celewyzc and Wertheimer, 1994) as wild recruits from the adjacent streams joined the migration. Consequently, the evidence shows a non-random distribution of fish between areas with the dominance of pre-fed hatchery fish in the non-oiled corridors and wild fish in the oiled corridors. The change in composition from the non-oiled corridor downstream in the current to the oiled corridors shows the non-oiled corridor samples were disproportionately representative of hatchery fish compared to the oiled corridors. Consequently, the samples taken in 1989 did not represent the same population of randomly distributed juveniles. Further, since hatchery fish were released in a shorter timeframe and pre-fed before release, there is good justification to assume the length differences observed were due to that disproportionate hatchery composition between oiled and non-oiled corridors. No significant differences were observed in 1990, but hatchery contributions increased by 40% that year, further diluting the wild production that had dropped to half that of 1989, based on the number of wild brood spawners.

In contrast to Wertheimer and Celewycz, the Willette (1996) 1989 studies on hatchery tagged fry found differences in weight, but not length. This is noteworthy since sampling areas for the two studies overlapped. However, differences in Willette's weight data between oiled and non-oiled areas had a major problem in 1989 that was corrected in 1990 and 1991. The problem was that the gain in weight from release to recapture was based on average weights at times of release, not averages of gains by individual fish. In 1989 the early-fed fry were released over a period of 8 d at the WHN hatchery and over a period of 5 d at the AFK hatchery. All fry received the same tag code at their respective hatchery, regardless of the release dates. Therefore, upon recapture the growth rate was determined by the recapture weight subtracted from the mean weight for the 8 d day and 5 d release periods, respectively, from WHN and AFK hatcheries. Since the hatchery fry increased in weight by ~4.6%/d in the wild, the first fish released from WHN or AFK hatcheries would be ~37% and ~20% heavier respectively by the time the last fish in each group emerged, were tagged, and released. With the inability to know which date the individual recaptured fish were released, it was impossible to determine the actual weight-gain/day differences between release and recapture times. Because AFK fish had to pass through an oiled passage off the AFK hatchery release site to reach the lightly oiled beaches of Knight Island 10–15 km further upstream and the uncertainty as to how long the AFK fish remained in the oiled area off from the hatchery before moving upstream to Knight Island, the assumption about random distribution is questionable. For the 5 d release period, early releases should predominate in samples taken further away from the release point, and thus more days of growth in the control area of Knight Island would be expected compared to the late releases that enter the beginning of the oiled site closer to the release point. At WHN releases from the hatchery covered an 8 d period with the same tag code, and thus non-random distribution could not be certain under such a sequence of releases. Given that in the absence of marine oil in 1990 and 1991 when fry were given separate tag codes for each release day and showed the same tendency for size trends as 1989 between sites selected for oiled and non-oiled comparisons, it makes averaging the size for the release periods in 1989 more suspect as having influenced the results. The small number of recaptures of only 250 AFK and 150 WHN fish also added to the uncertainty that size differences represented reliable data between sites.

Age Structure at Study Locations

Unmarked hatchery fish predominant in the northern non-oiled corridors were released in a comparatively narrow timeframe, while wild fry that constituted a growing percentage of samples in the oiled corridors along the migratory pathway represented an extended entry into marine waters (Brannon and Maki, 1996; Wertheimer and Celewycz 1996). This means oiled corridors with lower percentages of hatchery fish would also be expected to have more recent wild recruits and thus slightly younger juveniles, and that invalidates the assumption of the same age structure in the Wertheimer and Celewycz study.

The effect of new recruits is shown with data on tagged fish providing a stabilized composition. Mortensen et al. (2000) measured tagged and untagged juvenile pink salmon in PWS and showed the growth rate of untagged fish in the samples was lower than the growth rate for tagged fish, because new wild recruits entering the untagged population reduced the average weight. Therefore, the 1989 alleged lower growth rate in the oiled corridors downstream in the migratory pathway in the Wertheimer and Celewycz study is explained better by the higher percentage of newly recruited smaller fish entering the population along the migratory passage.

The influence of differential effects on growth is also problematic in the Willette study. Collection sites for the moderately oiled area were mostly downstream from the AFK hatchery, whereas sites for the control (lightly oiled) areas ranged from 10–15 km upstream from the hatchery in the current of the sound. The fact that the collection sites for lightly oiled areas were further away from the release point than in the moderately oiled areas means samples taken would not be expected to represent the same age. Those released first in the 5 d period would have the opportunity to travel further with more days to gain weight than those released later. In the case of WHN the distribution pattern of the hatchery releases over the 8 d period is unknown, making the age structure of the samples also an unknown factor, which leaves the assumption of same ages between oiled and control sites unresolved.

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Comparable Sample Areas

Wertheimer and Celewycz (1996) concluded that sampling sites were matched between oiled and non-oiled corridors. However, selecting sample sites that are the same in terms of their effects on the behavior of juvenile pink salmon is nearly impossible and may be the same only in the broadest criteria. LeBrasseur and Parker (1994) have shown that juvenile populations in littoral areas will differ in the timing (relative age) and period of residence around different sites. Shoreline gradients also differ between locations, which results in different escape patterns to avoid sampling gear, and also influences the escape opportunity of larger fish. Wertheimer and Celewycz (1996) were aware of the problem and reported that they took it into consideration, but it is not evident how that was done. Corridor samples in their study were close to shore and collected by beach seine, which meant they were vulnerable to unequal access to larger or older juveniles.

Temperature is a major influence on growth, and in the present case Brannon and Maki (1996) had concluded that the temperature effects were not adequately accommodated for in the government studies. Spies (2007) disputed the allegation and referred to the temperature data collected in the government studies as evidence to the contrary. However, while Wertheimer and Celewycz (1996) concluded temperatures were no different between oiled and non-oiled corridors, they had confined measurements just to terminal sample sites at approximately two-week intervals. Those measurements were too infrequent to characterize the temperature regime experienced by juveniles during their migration, especially when there was no information on temperatures that existed in a cohort's distribution and migration pathway. Willette (1996) detected temperature differences of 0.9°C in the same regions sampled by Wertheimer and Celewycz, but also depended on infrequent measurements. Other data in overlapping sites from the same year showed oiled corridors were slightly cooler than non-oiled corridors, and in some cases temperatures varied between sites by 1°C at the same time of day, and 2°C over a 24 hr period (Brannon and Maki, 1996). It appears that temperature differences were large enough to be of concern when attempting to estimate growth rates, and were higher in the non-oiled areas giving those juveniles a slight unaccounted for advantage.

Bias in Sampling Gear

The assumption that the sampling gear was unbiased was confounded by the inability of beach seines to sample corridors offshore in deeper water. This is shown by the 1989 fork length distribution in the corridor pink salmon catches ranging from 29 mm–71 mm in beach seine samples of Wertheimer and Celewycz compared to fork lengths ranging from 44 mm–78 mm for the same time period in offshore trawl samples in Knight Island passage reported by Brannon and Maki (1996) (Figure 11).

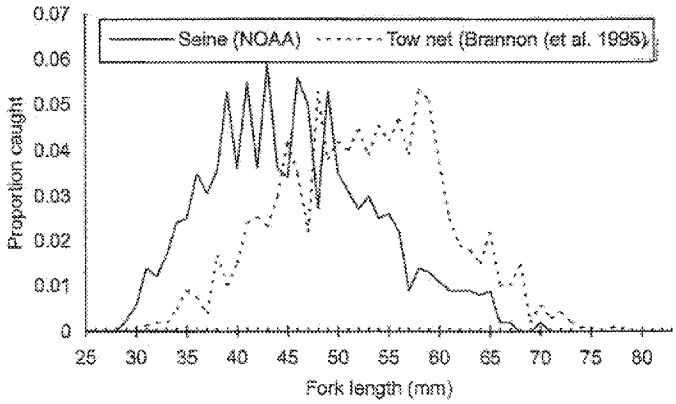

Figure 11 Fork length of seine caught (NOAA) and tow net caught (Brannon, 1995) PWS juvenile pink salmon in early June of 1989.

It is also apparent in Figure 11 that some of the more advanced fish in June had already left the nearshore habitat, and thus nearshore samples taken by beach seine were not representative of the fish available to Wertheimer and Celewycz (1996) and Willette (1996) in the off-shore corridor areas. Mean length of seine-caught juveniles increased from 40.0 mm–45.5 mm from late May to early June with an apparent growth of 5.5 mm. Mean length in trawl samples was 43.5 mm–51.8 mm for the same period were larger with an apparent growth of 8.3 mm, or a 50% increase over beach seine-caught fish. The magnitude of the difference increases with time as larger and thus older fish leave the nearshore habitat for residence offshore along the migratory pathway and would be inaccessible to sampling by beach seines, thus leaving smaller, more recent, recruits in nearshore waters, reducing the average size. Size bias in catches also confounds the assumption of equal age structure of the samples on any given date.

Conclusions on the Validity of Assumptions

The authors conclude that the four assumptions that are necessary before size differences can be related to growth were either not satisfied or there were uncertainties about their validity. Distribution was different between oiled and non-oiled corridors by dominance of the hatchery fish in the control corridors in the Wertheimer and Celewycz study, and age structure was also influenced by the same differences. Using the same tag codes over the 8d and 5d release periods at the WHN and AFK hatcheries confounded accurate determinations of size and time relationships in the Willette study. In both studies there is uncertainty about how similar matched sample sites were, and there was evidence that beach seine sampling gear did not take samples representative of the population that was shown in the comparative trawl data. The authors contend that since there was no assurance that the assumptions were valid, the justification to make conclusions about growth based on size differences cannot be supported.

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Empirical Evidence on Growth Potential from Oil Exposure

Apart from the above assumptions, it is legitimate to look at other empirical evidence that might support the hypothesis that oil exposure reduced early marine growth of pink salmon fry. Under such circumstances reduced growth could occur either by (a) exposure to toxic marine waters negatively affecting growth physiology, (b) toxicity reducing the food base, or (c) a direct toxic effect on fry from consumption of oil in the diet. The authors look at this evidence to determine if it supports the hypothesis.

Environmental Toxicology

Direct toxic effects of oil negatively affecting optimum physiology of the juvenile fish might be an explanation for size differences caused by reduction in growth rate. This would be detectible by differences in the water chemistry or the response of indicator organisms under controlled studies.

Water-column chemistry. Two major research programs were initiated on water-column chemistry within days following the spill in an effort to assess the extent of hydrocarbon contamination. One was undertaken by non-agency scientists, Neff and Stubblefield (1995) referred to as the Battelle study. They undertook 17 field surveys including 110 sampling stations within PWS, and completed the project between March 27, 1989, and March 9, 1990. That was the worst-case period for exposure of pelagic species to spilled oil in PWS. Water samples were taken from just below the surface (top 1 cm) and from depths between 1 m–42 m. The second study was by agency scientists, Short and Rounds (1993), referred to as the NOAA study. In their research water samples were taken one, three, and five weeks following the spill at depths of 1 and 5 meters. All of the samples were analyzed by gas chromatography—mass spectrometry (GC/MS), with individual PAH analytes reported.

The data from the Battelle and NOAA studies show that the highest aqueous-TPAH concentrations, and thus the greatest threat to the pink salmon relative to potential effects on growth, were immediately offshore of the heavily oiled shorelines representative of the bays where oil tended to accumulate. Aqueous-TPAH concentrations at these sites ranged from 2 ppb–7 ppb, with one sample above 10 ppb (Short and Rounds, 1993). In contrast TPAH concentrations in offshore sites most characteristic of the corridor or passage environments were consistently < 1 ppb, with non-oiled background stations measuring between 0.02 ppb–0.08 ppb for the year following the spill (Neff and Stubblefield, 1995). It is most noteworthy, therefore, that the Wertheimer and Celewycz (1996) data demonstrated that in those offshore corridors where the TPAH concentrations were < 1 ppb smaller size juvenile pink salmon were allegedly caused by oil exposure. Conversely, in the confined bays where oil concentrations were the highest, juvenile size was unaffected. Clearly, these data are contrary to the rationale that water column toxicity was responsible for the reduction in juvenile length observed in the corridor environments.

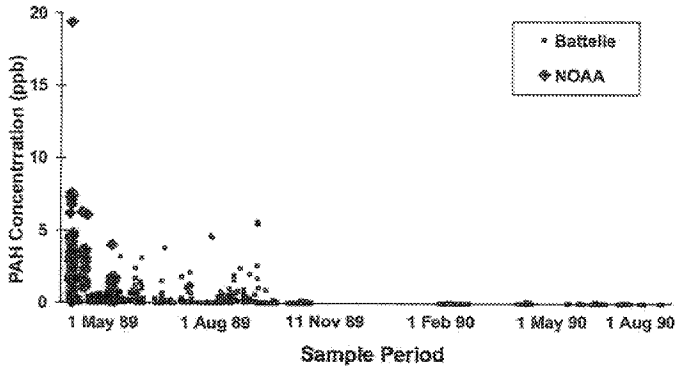

Figure 12 PAH concentrations in the Exxon Valdez oil spill-path of PWS in 1989 and 1990 from water quality samples taken by Battelle and NOAA.

It was suggested that fry residence in the bays was not long enough to show size differences (Wertheimer and Celewycz, 1996). However, histograms of fry length taken from juveniles in the bays in late May and early June included individuals from 40 mm–65 mm in length, or a 40%–100% increase in size over that at emergence (32 mm), constituting > 50% of the fish captures, and suggesting juveniles had considerable residence time in bay areas. Moreover, the water quality monitoring programs were discontinued after the first year, because water column PAH returned to background levels by August of 1989 (Figure 12), just a couple of months after emergence was completed and before pink salmon fingerlings moved into open water of the Gulf. Consequently, there is no evidence from the water chemistry monitoring in PWS that environmental toxicity was present that would have affected fry growth during the spring of 1989.

Toxicity tests with standardized bioassay species. Toxicity tests of water from the corridors of PWS were undertaken in the laboratory on indicator organisms immediately following the spill. Procedures followed the protocols of the U.S. Environmental Protection Agency (USEPA, 1985, 1988) considered as the standard to detect aqueous toxicity. A total of 123 water samples were taken at depths of 1 m and 3 m in PWS from April 3rd–July 6th in 1989 (Neff and Stubblefield, 1995). The depths represented the region of the water column where the largest numbers of pink salmon juveniles reside in the sound and where the highest hydrocarbon levels occurred in the spring of 1989. The toxicity tests represented three trophic levels of the marine ecosystem: the marine diatom *Skeletonema costatum;* the mysid shrimp *Mysidopsis bahia;* and larval sheepshead minnow, *Cyprinodon variegatus* (Suter and Rosen, 1988).

No mortality occurred among the mysid shrimp or minnow exposures to water samples taken at the height of oil contamination of surface waters during April–July. Algal cell growth and larval fish-growth showed no negative effects from exposure concentrations of the PWS water. Hydrocarbon concentrations present in the water column at the time of the most severe oil contamination in the sound were not high enough to result in any toxic responses among the three indicator species. It follows, therefore, that pink salmon juvenile growth should not have been negatively-affected when no effects of oil in the

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water column was demonstrated with the three standard indicator organisms.

Impact of Oil on the Food Supply

Oil effects on the food web might be considered the second most likely candidate for growth reduction of juvenile pink salmon. Reduced growth due to oil affecting the food web could function either by reducing the availability of the food supply for pink salmon fry, or it could have a more direct affect by creating a negative physiological response from having consumed contaminated feed.

Zooplankton biomass. Zooplankton is the main food source of juvenile pink salmon and assessing the effect of the oil spill on zooplankton biomass provides a straight forward approach to ascertaining potential effects of the spill on food. Such data have been collected by the PWSAC since the late 1970s for use in synchronizing hatchery fry releases in the sound with the plankton bloom (Cooney et al., 1981) and provides a relative index for year-to-year comparisons. The sites monitored are Port Ashton and Elrington Passage, both within the oil spill impact area. Large calanoid copepods, dominated by *Pseudocalanus* spp. and *Calanus* spp., are the primary prey of young salmon, and crucial to marine productivity. Data from monitoring sites, representing the top 20 m of the water column, indicate that zooplankton biomass in 1989 was among the highest recorded in western PWS (2 x the previous 8 yr mean), and ostensibly was responsible for exceptional growth among juvenile salmon for that year. These data also correspond with the data on plankton biomass gathered by Celewycz and Wertheimer (1996), showing no differences between oiled and non-oiled areas in any of the eight sample sites except for greater abundance of some species in oiled areas.

The distribution and density of epibenthic harpacticoid copepods was also monitored in heavily and lightly oiled bays by Wertheimer et al. (1996). They found that copepod mean densities on heavily oiled shorelines were higher than on lightly oiled shorelines. Moreover, the percentage of egg-bearing copepods were similar in the oiled and non-oiled sample sites. Wertheimer et al. concluded that copepod abundance appeared to increase from higher nutrients in the oiled areas. Therefore, from the standpoint of food availability, there is no evidence that smaller pink salmon fry in the oiled corridors can be explained by reduced growth from possible effects of oil on the zooplankton biomass. The food supply used by pink salmon in PWS was abundant in 1989.

Plankton consumption by juvenile pink salmon. Wertheimer and Celewycz (1996) also examined feeding performance of chum and pink salmon juveniles in oiled and non-oiled areas by assessing diet composition and stomach fullness. They found no differences in juvenile feeding activity between oiled and non-oiled areas. Moreover, they concluded that pink salmon did not avoid oiled areas in any study site, which suggests that oil concentrations didn't inhibit feeding behavior.

Similarly, Willette (1996) examined feeding performance of the hatchery fry released and also found no difference in stomach fullness of fry in oiled and non-oiled areas. Evaluation of feeding and stomach fullness was also undertaken by Sturdevant et al. (1996) on both pink and chum salmon fry in oiled and non-oiled areas of the sound in the spring of 1989. They determined that stomach fullness and quantity of food items were similar in oiled and non-oiled areas, and thus confirmed that food consumption by juveniles did not decrease in the presence of oil. These observations suggest that feeding performance does not explain the observed size differences in pink salmon between oiled and unoiled corridors.

Oil Contaminated Feed

While it was shown that aqueous-TPAH levels in the water column were below toxic concentrations to zooplankton and appeared to stimulate productivity, it was possible that ingestion of oil could in some way impact fry growth. Consumption of contaminated food items in the water column or direct consumption of oil droplets, for instance, could disrupt metabolic pathways or increase the metabolic cost of depuration. Such circumstances could concentrate petroleum and pass on higher doses to feeding fry than would be absorbed as dissolved TPAH from the water column.

Oil-concentrations absorbed by feeding fry. In 1989 Sturdevant et al. (1996) observed oil droplets in the stomachs of some fry samples taken from the same eight locations as those in Wertheimer and Celewycz (1996) with no apparent effect on their condition. In an analysis of samples from those areas by Carls et al. (1996), the tissue-TPAH concentration of pink salmon fry off oiled bays in 1989 averaged ~200 ppb (ng/g) dry wt. While the tissue-TPAH of juveniles sampled in the corridors was nearly the same, they averaged less at ~175 ppb, or > 12% less than the bays. This was a period when the highest concentrations of oil were present and apparently, based on CYP1A levels in the tissue (Carls et al., 1996), fry ingested more oil contaminated food or droplets than any other time. It is important to recall, therefore, that while Wertheimer and Celewycz found no differences in fry weight or length between the non-oiled and oiled bays, they reported that fry in non-oiled corridors were longer than in oiled corridors. But fish in the oiled corridors showed a lower mean tissue-TPAH concentration than those in oiled bays, which suggests that size differences were not related to oil exposure, although tissue-analysis was limited.

Laboratory feeding study. A laboratory feeding study by Wang et al. (1993) was undertaken to assess the influence of oil in the diet of pink salmon fry. Three levels of oil were selected and proportionally mixed into feed, producing oil-contaminated pellets at oil concentrations of 0.37 mg, 2.8 mg, and 35 mg oil/g of feed. Over a six-week feeding period, the results showed that growth was significantly lower only in those fed the highest oiled feed. Wang et al. demonstrated that pink salmon decreased

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feeding at the highest oil contamination level, but no growth reduction in dry weight was apparent from consuming feed at lower levels of contaminated.

Carls et al. (1996b) apparently reworked the Wang et al. (1993) data to re-examine the level of oil ingestion that would affect growth in wet wt. over the six week period. They concluded that weights among the fry fed 0.37 mg oil/g of feed (the lowest contaminated feed level) were the same as the controls at five weeks, but dropped slightly below the controls by the sixth week. Carls et al. then applied those results in support of the Wertheimer and Celewycz (1996) conclusions that size differences in their study represented reduced growth from oil exposure by stating, "This research strongly supports the hypothesis that ingestion of hydrocarbons was a cause of the reduced growth in pink salmon fry," referring to the smaller size of juveniles in oiled corridors (p. 616).

Unfortunately, Wang et al. and Carls et al. did not report the tissue-PAH levels resulting from consuming the contaminated feed. However, the authors would predict that both the intermediate or lowest feeding levels that showed little or no growth effects would have much higher mean tissue-PAH levels than the ~175 ppb observed in the corridor fingerling samples (Carls et al., 1996a). The authors expect that based on elevated tissue levels in the presence of laboratory aqueous-PAH concentrations, the PAH doses of that magnitude in the feed would result in the accumulation of tissue-PAH loads similar to or greater than the same level in the water. The PAH level in the lowest dose fed was 13,000 ppb (Carls et al., 1996a). Therefore, no growth reduction among fingerlings with tissue-PAH levels as low as ~175 ppb would be anticipated.

Carls et al. (1996b) feeding study also showed that when weight was affected by oil, fish length was also influenced, and to about the same degree. This is an important point about the effect of oil on juvenile growth when confronted with oil in their nursery environment. Those results are contrary to the Wertheimer and Celewycz (1996) field study where only differences in length were observed, and the Willette (1996) study where only differences in weight were observed. The Carls et al. study suggests the size differences observed in the field studies were not oil induced differences in rate of growth.

Marine Growth Studies

The most relevant data on growth in the judgment of the authors are the responses of juvenile pink salmon in marine waters after long-term exposure to known oil concentrations during incubation, in which the embryo and alevins are the most sensitive life stages of pink salmon to oil toxicity. The pink salmon growth data is examined from various studies after long-term incubation exposure to known TPAH concentrations.

Growth response of oil-exposed juveniles in marine net pens. The growth in net-pen studies and marine releases presented in the previous chapter are also relevant here in the marine growth studies (Heintz et al., 1999, 2000). Juveniles reared in net pens for 200 and 300 days after experiencing tissue-TPAH loads of > 70,000 ppb showed no differences in lengths compared to the controls. Although exposed at different life stages, those results are of interest in light of the Wertheimer and Celewycz (1996) conclusion that length was alleged to be affected in corridor marine waters where the mean tissue-TPAH load (~175 ppb) was several hundred times lower (Carls et al., 1996a). Similarly, no weight differences were detected in juveniles reared in the Heintz et al. (2000) study after embryos experienced tissue-TPAH concentrations up to 1,900 ppb, which is contrary to the allegation of Willette (1996) that weight was reduced in marine waters when mean tissue-TPAH levels were ten times lower.

The Carls et al. (2005) net-pen study is also relevant here. Having grown juveniles in net pens for $5\frac{1}{2}$ months after embryos had experienced aqueous-TPAH levels ranging from 0.94 ppb–44.67 ppb, differences were reported. The lowest exposure resulted in a tissue-TPAH load of ~800 ppb, and the highest at ~30,000 ppb, neither of which resulted in less weight and length than the controls, although weight and length of juveniles were reduced when exposed to intermediate concentrations. The mitigating circumstance, however, may have been the mortality that occurred in the highest exposure group that could have removed the smaller individuals, leaving the more healthy fish that had tolerated the contamination. Mortality reached ~70% in the highest dose compared to ~34% in the controls.

It should be noted that the size of the Heintz et al. (2000) pen-reared juvenile controls were only about 23 g after 200 d of rearing and 60 g after 300 d of rearing. Net-pen reared juveniles in the Carls et al. (2005) study were < 10 g after ~162 d of rearing. These sizes are quite small for such long rearing periods compared to growth of open water juvenile pink salmon that would be much larger over the same period. While the relative sizes may reflect the influences of exposure to high oil concentrations under artificial and restrictive rearing conditions, the application of the net-pen study results where the growth of the controls was limited may not be justified in relating to the effects of oil on growth of free-ranging pink salmon.

Growth response of oil-exposed juveniles released in marine waters. The study by Heintz et al. (2000) also examined the effect of oil exposure on the size and survival of fry releases into the marine environment. The three test lots of the 1993 brood year embryos initially exposed to 200 ppb, 1,000 ppb, and 3,800 ppb sediment-TPAH concentrations during incubation, or aqueous-TPAH concentrations of 1.3 ppb, 7.8 ppb, and 18 ppb, were released as fry in 1994. Although effects of exposure to oil are most pronounced on developing embryos and alevins, long-term effects of exposure could also be apparent in returning adults, but that was not the case. The adults returning in 1995 were not statistically different in weight or length after exposure to those oil doses which were much higher than any measured in the marine corridor waters of PWS. The 1995 brood releases of fry in 1996 that had experienced sediment-TPAH concentrations of 860 ppb and 7,500 ppb (aqueous-TPAH 5.2 ppb and 19.4 ppb) also returned in 1997 with no differences in weight or length.

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Size variability of oil-exposed wild juveniles in marine waters. Field surveys in corridors with surface-trawls in 1989 (Brannon and Maki, 1996) also provided relevant information. The mean lengths of pink salmon juveniles sampled off the entrance of Snug Harbor and Knight Island Passage on the 8th and 10th of June were 55.8 mm and 60.1 mm respectively. Lengths off Herring Bay on the 6th of June and in Montague Strait on the 8th of June were smaller at 49.4 mm and 50.4 mm respectively. These sample areas were in oiled corridors, and larger than those measured in oiled corridors (~46 mm) on the corresponding sample dates by Wertheimer and Celewycz (1996), and also larger than those in non-oiled corridors (~48 mm). the authors conclude from these data that in 1989 substantial length differences were apparent in the populations sampled in oiled corridors at the same time of year, and there is no evidence those differences were associated with oil concentrations.

Growth response of oil-exposed juvenile in other studies. A five year study on the growth and marine survival of pink salmon fry exposed to concentrated sub-lethal doses of dissolved oil by Birtwell et al. (1999) has direct application to the conclusions of Wertheimer and Celewycz and the work by Willette. On three different years pink salmon fry were exposed for 10 d to a low water soluble fraction (WSF) of oil ranging from 54 ppb up to 349 ppb (benzene, toluene, xylenes, ethybenzene, naphthalene) as sub-lethal exposures, but markedly higher than the dissolved fraction of oil that fry experienced in PWS following the *Exxon Valdez* oil spill. Growth of the fry was monitored before release from the holding facilities and while they noted there were growth differences among the test groups and controls, the differences were not dose-dependent.

After the 10 d exposure period the fry were released into the waters of Discovery Passage in Johnston Strait, British Columbia, for natural rearing and return in the 1991 to 1993 brood years. The returns showed no statistically significant differences in weight, length, or survival. The results applied to the situation in PWS, and demonstrated no size reduction from oil WSF exposure as juveniles higher than what was experienced in PWS and no impact on the return size or survival to the adult stage.

The authors suggest the Birtwell et al. (1999) field results are contrary to laboratory results interpreted to show growth of juveniles in PWS was reduced from previous exposure to oil (Carls et al, 1996b, 2005; Heintz et al., 2000). The results also do not appear to support the allegation that smaller juvenile sizes observed by Wertheimer and Celewycz (1996) and Willette (1996) in oiled areas were growth related.

Conclusions from Empirical Evidence on Growth Potential

The authors conclude from studies on potential effects of the *Exxon Valdez* spill that the evidence does not support growth reduction of juvenile pink salmon from EVC in marine waters of PWS. The concentration of oil in the water column and the level of tissue contamination appear too low to limit growth. The toxicology assessment, abundance of food items, level of oil contamination of the food supply, and actual growth performance of juveniles exposed to known oil contamination levels also suggest that oil concentrations in spill-path waters were not high enough to limit growth. In contrast, there was strong support available from field research on plankton productivity that showed conditions in PWS were conducive to good growth and perhaps even superior growth in areas along the trajectory of the EVC. The authors suggest, therefore, that the size variations of juvenile pink salmon reported by Wertheimer and Celewycz (1996) and Willette (1996) in the summer of 1989 were more likely caused by samples representing differences in the origins of sampled cohorts, age structure of the juveniles, variation in habitat associated with the study areas such as temperatures, and/or sampling bias which they believe has been demonstrated in the variations of juvenile sizes in the 1989 marine seine and trawl samples taken from oiled corridors.

SECTION IV: RESOLUTION OF EXXON VALDEZ OIL SPILL EFFECTS ON PINK SALMON

When the *Exxon Valdez* grounded and spilled nearly 11 million gallons of oil in PWS it was anticipated that there would be a major impact on the pink salmon that use the intertidal stream reaches for incubation and the marine nursery waters of that large inland sea. While the anticipated major negative effects of the spill never occurred, the preconception that there would be damage nevertheless had a major influence on the interpretation of research results. That is understandable given the magnitude of the spill and visual evidence of shoreline oiling, but superimposing that preconception on the evidence was also the essence of the problem, coupled with litigation priorities. Once attention to the effects of the spill was taken over by the process of litigation, there was the inevitable alignment of those researching the spill effects to be identified with the opposing camps of the plaintiffs and the defendants. The EVOSTC—the council of government agencies formed to assess the impact of the spill—tended to depend on agency research and conclusions in assessment of damage, and in general the agencies didn't give serious consideration to the work of the opposing non-agency scientists. This was exemplified by Spies (2007), who was appointed as chief scientist for EVOSTC at that time, in his statement about dueling scientists in *Long-Term Ecological Change in the Northern Gulf of Alaska*. He said:

> To stave off government litigation or regulation, some industry groups manufacture uncertainty about solid scientific finding. Use of study designs by Exxon scientists that were underpowered to detect significant differences, or where study sites were not picked in impact areas,. . suggest that causing doubt is a strategy that Exxon has employed (p. 428).

The best way to answer such an allegation is to present the full evidence on the extent of the impacts, which the authors have

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endeavored to do on the effects of the *Exxon Valdez* oil spill with considerable detail from the scientific perspective that turned out to show the PWS pink salmon were not damaged at observable levels. The purpose of the authors in this discussion now turns to the reconciliation of the agency position on the immediate and long-term damage to the pink salmon population. Three issues drove the allegation of damage to pink salmon. Egg mortality was higher in oiled streams, laboratory evidence of egg mortality was assumed to represent embryo experiences in oiled streams, and smaller juveniles were associated with oiled corridors in the sound.

Increased Egg Mortality as an Artifact of Sampling

Review of the stream survey data and the large data base on the studies of the PWS oiled streams provided a convincing account as to why the ADFG observed an increase in mortality of eggs in oiled streams. The process of flushing the eggs from their redds during the critical period of embryo sensitivity to physical shock in a matter of days after the last of the eggs were spawned was responsible for the increased mortality in the ADFG stream surveys (Figure 4). The problem was sample timing (Collins et al., 2000; Brannon et al., 2001). In the fall of 1989–1993 and again in 1997 returning adults in oiled streams spawned later and the incubating eggs were sampled earlier than in non-oiled streams. The result was that higher lethal sampling shock occurred in oiled streams that mistakenly appeared as though it was related to oil.

Evidence revealing the situation was forthcoming from a series of studies executed on the condition of the incubating environment, eggs, and alevins exposed to oil. The problem was confirmed in records on egg mortality in the IDFG data base (Craig et al., 2002). The higher mortality in oiled streams sampled in the fall of the year was absent by the following spring. If the higher egg mortality in the fall was from oil exposure, that mortality would have still been evident in the number of dead eggs retained in the redds over winter, and that wasn't the case. Although NMFS denied sample timing was responsible (Rice et al., 2001), most recently they have come to recognize the lethal effects that occur from sampling eggs too early after the spawning event (Carls et al., 2004b; Thedinga et al., 2005; Carls et al., 2010).

The problem was compounded as demonstrated by Collins et al. (2000) showing the latest spawners entering the stream dig over earlier redds. Consequently, nearly 40% of the post-spawning egg population can be attributed to the last 20% or fewer spawners, and thus a disproportionate number of eggs sampled during the post-spawning period were from the latest spawners. Because resistance to shock occurs rapidly after embryos complete blastopore closure, a difference of a few days in sample timing during the period of embryo development can make the difference in embryo shock mortality when sampling is in close proximity to the end of spawner stream-life (Figure 4). The later spawning and slightly earlier removal of egg samples from oiled streams that overlapped embryo sensitivity to mechanical shock made timing the crucial factor in the increased level of mortality observed in oiled streams (Collins et al., 2000; Brannon et al., 2001).

Reconciliation of the Egg Mortality Allegation

When ADFG reported higher egg mortality in oil streams, it was assumed that oil was responsible for those losses. Although Bue et al. (1996) stated that their conclusion was not definitive, nonetheless the results fit with the preconception that oil would cause damage, and with that assumption there was no attempt by the agencies to confirm whether toxic conditions existed in pink salmon redds or embryo tissues in oiled streams. The higher mortality was convincing, but wrongfully assumed as an oil effect. In the 1993 Proceeding of the Exxon Valdez Oil Spill Symposium it was reported that pink salmon eggs and juveniles were directly affected, with egg mortality *"the most compelling evidence of long-term damage,"* (Spies et al., 1996, p. 7).

However, the most compelling evidence was the origin of the problem. The ADFG survey crews were unaware that eggs are highly sensitive to physical shock during early embryo development. The stream surveys were conducted within the four weeks following the end of spawning that overlapped the egg sensitivity period, and because oiled streams had been sampled earlier than non-oiled streams, in some cases extracting eggs from the redds as little as 2 d after the end of adult stream-life, those embryos were less developed and experienced a higher shock mortality, unintentionally biasing the survey against the oiled streams (Brannon and Maki, 1996; Brannon et al., 1999; Collins et al., 2000; Brannon et al., 2001).

After shock sensitivity of embryos was presented as the problem (Brannon and Maki, 1996) and the agencies recognized that sampling too early in embryonic development would result in such mortality, Carls and Thedinga (2010) were uncertain whether or not ADFG sampling crews were aware of embryo sensitivity to shock at the time of sampling and suggested that observers could have been easily trained to detect mechanical shock effects. Therefore, it is important to leave no doubt about that matter. Neither Sharr et al. (1994), Craig et al. (1995), nor Bue et al. (1996) made any statements about egg sensitivity in the sampling routine conducted in their analyses of ADFG stream survey data, nor did they give any recognition of methods to avoid confusing shock mortality with natural mortality or to compensate for mechanical shock in their procedures. It is apparent that the ADFG survey crews were unaware that their sampling protocol compromised their surveys.

Further, Rice et al. (2001) challenged the assertion of shock mortality of pink salmon eggs by characterizing the ADFG stream surveys as a routine followed for many years with no egg mortality resulting from the procedure: that was correct. Historically, surveys were done for management purposes to help predict adult return for the respective brood, and they were conducted from mid-October through November. However, starting

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in 1989, the year of the spill, and proceeding to 1997, ADFG moved their sample timing forward by nearly three weeks, beginning by the third week of September (Sharr et al., 1994; Bue et al., 1996, 1998). That change meant that the first of the sampling schedule overlapped with eggs that had been spawned during the first three weeks of September and those eggs hadn't yet development tolerance to mechanical shock by the time the earlier samples were taken. While the sampling method was routine, the sample timing schedule was not, and proved fatal for the eggs spawned later in the season.

The Problems with the Laboratory Studies

It is important in the review to distinguish between what has been shown in laboratory research and the results of investigations conducted in the field on the *Exxon Valdez* spill. The authors' assessment and conclusions in the review apply to conditions that deal with the effects of the oil in PWS. The laboratory work is not an issue except where we believe it has been wrongfully interpreted or applied to spill conditions. The greatest incongruity between agency and non-agency research has been on what levels of contamination were experienced by incubating pink salmon in oiled streams and what that experience meant in the long-term health of those fish.

NMFS investigators at ABL accepted the conclusion from the ADFG stream surveys that oil was the cause of the higher egg mortality. The laboratory bioassay work that followed was to determine the effects of weathered oil on pink salmon during incubation (Marty et al., 1997a, 1997b; Heintz et al., 1999). Based on their published works, after the laboratory toxicity levels were determined ABL investigators assumed that because the increased egg mortality observed by ADFG was caused by oil, then the concentrations that were shown to be toxic in the laboratory must have been the concentrations the eggs experienced in the oiled streams. This is deduced from their statements that the observed effects of oil corroborate Bue et al. mortality claims (Heintz et al., 1999; Rice et al., 2001), that their results were consistent with field data on egg mortality (Carls et al., 2005), that residual oil persisted at oiled streams and may have exceeded the lethal threshold through 1993, (Murphy et al., 1999), and egg mortality was consistent with drainage of oil-contaminated water from the surrounding sediment (Rice et al., 2007). The laboratory test concentrations of oil resulted in a tissue-TPAH lethal toxicity threshold around 6,000 ppb and the VWO was toxic with a corresponding aqueous-TPAH level as low as 1 ppb (Heintz et al., 1999). Those laboratory results initiated the oil toxicity narrative that was developed by ABL to explain the mortality and thereafter the narrative was expanded to involve long-term damage to the pink salmon that had been exposed to oil.

However, the contamination levels measured in the non-agency assessment of oil in the stream substrate were well below the concentrations tested in the laboratory. Mean sediment-TPAH of the oiled streams ranged from 0.5 ppb–267 ppb in the fall of 1989, remained at that level or higher by the fall of 1990 and returned to near background levels of < 39 ppb in most streams by1991. When that became apparent and the contamination levels of stream sediments were not high enough to cause mortality, ABL investigators looked to the oil deposits on the stream deltas and alleged that it was leachate emanating from the deposits that was the source of toxicity entering intertidal stream reaches killing the eggs (Heintz et al., 1999; Murphy et al., 1999). Leachate passing through the subsurface substrate to the incubation redds was presented as the interstitial toxic water hypothesis.

Further, it meant the leachate would have to be extremely toxic to overcome the dilution from the high flushing rate of freshwater irrigating the incubation environment. High toxicity was not consistent with the LPAH compounds in the weathered oil, so investigators turned to the more toxic HPAH analytes as the primary source of toxicity, and those petroleum compounds were good candidates because of their increased concentration in percentage of the oil mass as it weathered. The low aqueous-TPAH concentrations of VWO (1 ppb) that ABL thought to be responsible for the egg mortality in their laboratory trials supported such a hypothesis, and attention shifted to the HPAH compounds as the responsible agents for the chronic toxicity of weathered oil (Rice et al., 2001) rather than the light more soluble low molecular weight hydrocarbons previously considered the most hazardous. This "shift in the oil toxicity paradigm" to HPAH was the next step in the oil toxicity narrative.

With egg viability of spawners reported to be lower in adults exposed to oil as embryos (Bue et al., 1998), and with earlier reports of lower marine growth of oil exposed juveniles, further laboratory tests were conducted. Growth rates of fingerling pink salmon that experienced high oil concentrations during laboratory incubation were lower compared to controls when reared in marine net-pens, and return of adults from release of fry that had experience oil during incubation showed variability in return success. Consequently, the narrative then went on to implicate oil exposure of embryos to further impacts with the statement that, "fish populations whose natal habitats are contaminated with PAHs at low ppb levels can be expected to experience the compound effects of mortality during exposure, reduced survivorship afterward,s and reduced reproductive output at maturity" (Heintz et al., 2000, p. 214).

Reconciliation of the Laboratory Research

There were several problems to reconcile with the laboratory work at Auke Bay. The first was the assumption that oil was the cause of the ADFG reported higher egg mortality in oiled streams. That established the foundation on which the subsequent laboratory bioassay work was based and set the oil toxicity narrative pathway that, in the opinions of the authors, led away from what the preponderance of the data was showing.

The second problem was to apply the laboratory results to the field situation in PWS streams. The oil concentrations tested

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in the laboratory that showed growth effects and variability in long-term survival were not low. The laboratory trials exposed embryos to oil concentrations many times higher than what contamination levels were experienced by naturally incubating pink salmon (Marty et al., 1997b; Heintz et al., 1999; Carls et al., 2005; Carls and Thedinga, 2010). Those data had no application to conditions in oiled streams. The tissue loads of incubating pink salmon were decisive data on exposure experience during incubation in oiled streams that was also overlooked. Heintz et al. (1999) stated:

> Measurements of PAH concentrations in developing pink salmon embryos collected from redds of streams on the oiled beaches would have been a more sensitive indication of exposure, but unfortunately measurements were not reported by either Bue et al. or Brannon et al. (p. 494)

And Murphy et al. (1999) also remarked that the non-agency studies, "did not measure PAH. . . . in salmon embryos" (p. 917). That was mistaken. ABL scientists failed to notice the data reported in non-agency publications showed that mean tissue-TPAH contamination levels in alevins did not exceed 95 ppb at the height of oil encroachment in the intertidal reaches of oiled streams (Brannon et al., 1995; Brannon and Maki, 1996). As it turned out the tissue-TPAH levels of alevins in the experimental controls of the agency studies (~90 ppb) were more compatible to the tissue concentrations of alevins in oiled streams (<95 ppb) than were the laboratory test concentrations. The sediment- and tissue-TPAH levels exposures in the laboratory tests had no relationship to the contamination levels measured in actual stream sediments or alevins tissues from EVC exposure, and the interpretation of damage encouraged the toxicity narrative further along the path of irreconcilability.

The third problem was the lack of field work at the time when the greatest potential for damage was present. As late as 2007 it was stated:

> Both field and laboratory experiments show that exposure to PAH at low parts-per-billion concentrations impairs development of pink salmon. . . . Reduced growth and survival were noted long after exposure had stopped and long after PAH was eliminated from the tissue, demonstrating that long-lasting or permanent damage had occurred (Rice et al. 2007, pp. 476–477).

If that were true, it applied only with the high oil concentrations tested in the laboratory, not with the low concentrations observed in the field. The agency investigators never analyzed for stream sediment- and tissue-TPAH concentrations to assess the level of oil exposure of incubating eggs or alevins in the field during the first three critical years following the grounding of the *Exxon Valdez*. There is no record that interstitial water adjacent to or in salmon redds was analyzed to support the narrative that toxic aqueous-TPAH concentrations occurred in stream substrate during that period. No long-term studies of marine mortality of juveniles exposed to oil during incubation or reduction of adult returns of fish originating from oiled streams were conducted.

In the field the intertidal sediments acted as a filter limiting particulate oil entering the redds, which wasn't the case in the laboratory. One of the concerns about the laboratory data in that regard was the evidence of oil droplets in the treatment water as a covert source of PAH contributing to the toxicity of what was reported only in terms of the dissolved fraction. Preliminary laboratory work showed that toxicity of dissolved oil was markedly increased by the presence of micro-droplets in the treatment water (Stubblefield et al., 2010). Therefore, when it was demonstrated that micro-droplets were present in the oil toxicity studies at ABL, it suggested the low parts-per-billion reported to be toxic most likely overstated the toxicity associated with the dissolved fraction.

The fourth problem was the analyses of embryos exposed to weathered oil in the laboratory trials were inconsistent with the oil toxicity narrative. Tissue-TPAH concentrations were dominated by low molecular weight PAH, with naphthalenes well represented, and tissue-HPAH at low and decreasing concentrations as the oil weathered (Heintz et al., 1999; Carls et al., 2005; Brannon et al., 2006a), challenging the shift to HPAH in the oil toxicity paradigm proposed by Rice et al. (2001, 2007). The shift in the oil toxicity paradigm was largely conceived by the alleged VWO toxicity at 1 ppb in the laboratory study by Heintz et al. (1999) and that too was later challenged by Page et al. (2011, 2012).

Further, the low rate of HPAH dissolution from oil deposits and low and decreasing tissue-HPAH as oil weathered doesn't support the allegation of long-term chronic threats of toxic leachate infiltrating adjacent pink salmon incubation streams. HPAH increases in percentage of the oil mass as oil weathers, but its total concentration diminishes and is less biologically available because of its limited solubility. Moreover, the background sediment-TPAH levels on deltas and intertidal areas as the alleged source of toxic leachate were too low to be a threat (Boehm et al., 1995; Short and Babcock, 1996; O'Clair et al., 1996; Wolfe et al., 1996). Further, eggs experimentally buried on oil contaminated beaches or buried in weathered EVC deposits on the stream deltas in 2003 (Brannon et al., 2007) did not absorb TPAH at concentrations that imposed any lethal risk to incubating pink salmon embryos. There is no evidence that such toxic interstitial water ever reached incubating redds or that HPAH leachate was ever concentrated enough to overcome the diluting effects of tidal washing and freshwater flushing of stream substrate.

Finally, the concluding problem is that egg mortality, long-term effects, and lower egg viability from exposure of brood year embryos to oil are still cited as an impact of the spill (Spies, 2007; Rice et al., 2007; Heintz, 2007; Carls and Thedinga, 2010). Conflicting evidence has not changed the mindset on damage. The extensive data showing egg mortality was not oil related and that exposure to high oil concentrations of embryos in the laboratory do not apply to field conditions in PWS are simply dismissed. For example, after the annual restoration project

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report by Heintz (2002) showed that the adults returning from exposure to high oil levels as embryos experienced no impairment in reproductive ability, that study has not been referenced by NMFS investigators in subsequent publications. However, his study and other complementary data, such as the very low egg morality (< 4%) in spawners removed from streams in 1991 where very high egg mortality was observed in ADFG surveys (i.e., 65.6% and 57% respectively in streams in Shelter Bay and Sleepy Bay), essentially nullify inter-generational effects alleged about oil exposure of pink salmon embryos in PWS.

Juvenile Growth Reduction from Oil Exposure

Coincident with the egg mortality observed in oiled streams, the size of juvenile pink salmon in oiled corridors of the sound and hatchery marked fry recovered in oiled areas were found to be smaller than in non-oiled reference areas. Wertheimer and Celewycz (1996) observed reduced length of juveniles in oiled corridors, and Willette (1996) observed lower weights of hatchery fish captured from oiled areas. The size parameters that varied were different between studies, but smaller size in each case was interpreted to be the result of reduced growth rate from oil exposure in marine waters.

Although Brannon et al. (1995) pointed out that if the health of the fish was deteriorating it would be apparent by a lower condition factor, which was the case in the feeding trails with high oil concentrations (Carls et al., 1996b), just the opposite was true when oil was fed at the intermediate level, showing a higher condition factor in their study. However, the condition factor of fingerlings fed low concentrations of oil was the same as the controls for the most part. Therefore, when Wertheimer and Celewycz observed that the condition factor of juvenile samples from oiled areas was higher than non-oiled areas, rather than correlating juvenile condition with the higher level of food availability in oiled areas, they related the condition to oil exposure, even though the concentration in the contaminated areas was < 1ppb and much lower than the lowest oil exposure in the Carls et al. feeding trials. If the relationship that Wertheimer and Celewycz was making with the feeding trials was legitimate, it would suggest that the source of contamination was from the consumption of oil, and the level would have to have been in the range of the intermediate levels in the Carls et al. feeding study. That would amount to a PAH of ~130,000 ppb, representing a substantial oil intake for which there is no evidence.

The smaller size was interpreted as a reduced growth rate from oil exposure, and based on the literature (Hargreaves and LeBrasseur, 1985; Mortensen et al., 1991; Parker, 1971), that was estimated to have had a significant reduction in adult pink salmon returning to PWS as a result of increased predation on the smaller fry in 1989. However, Willette (1996) was cautious in the growth conclusion by stating, "Sufficient data were not available to determine if the level of hydrocarbon exposure was great enough to cause the estimated reduction in growth rate attributed to oil contamination" (p. 533).

Reconciliation of the Claim of Reduced Growth in Oiled Marine Waters

In the investigators' conclusions about growth rates from the size differences observed in samples, similar to the conclusion about egg mortality from oil exposure, the alternative possibilities that could explain size differences were not given much attention. In the present re-examination of the evidence about growth reduction of juvenile pink salmon, the authors conclude that the data does not support the claim. Foremost, the assumptions that were necessary before size variation could be attributed to differences in growth rates were not satisfied. The evidence showed that juvenile samples from the study sites were not representative of the same population and that age structure differed based on the origin of samples collected from oiled and non-oiled areas. The trends that occurred in subsequent years (Boldt and Handorson, 2004), suggests it was a typical pattern, but not different enough to be statistically significant. There was also uncertainty whether the paired study areas were similar enough for comparative analysis of size differences. Also the sampling gear used had resulted in juvenile catches that were biased against the larger size fish.

Moreover, the allegation that growth was reduced in oil-exposed pink salmon juveniles in marine waters is not supported by the empirical evidence. The marine waters were not toxic based on water chemistry and bioassays using EPA approved protocols with indicator organisms (Neff and Stubblefield, 1995). PWS was particularly productive in plankton, feeding of juvenile pink salmon was not inhibited by oil (Celewycz and Wertheimer, 1996; Sturdevant et al., 1996; Wertheimer and Celewycz 1996), and petroleum contamination levels in juvenile tissues in the field (Carls et al., 1996b) were well below concentrations shown to negatively affect growth (Heintz et al., 2000; Carls et al., 2005). Laboratory studies and field trials by Heintz et al. (2000) showed that juvenile length and weight were unaffected at tissue-TPAH concentrations many times higher than what was experienced by pink salmon in oiled corridors of the sound (Brannon et al.,1995).

Research results on net-pen studies conducted by Heintz et al. (2000) and Carls et al. (2005) are also problematic for support of the Wertheimer and Celewycz 1996 and Willette 1996 interpretation that smaller size represented reduced growth rather than differences in population structure or age of fish in the samples. Body weight was not affected in those juveniles that had experienced tissue-TPAH concentrations of 1,900 ppb in the Heintz et al. studies (2000), and those fish were exposed to much higher oil concentrations and at a life stage much more sensitive to oil toxicity than post-emergent fry (Moles and Rice, 1983). The Heintz et al. (2000) and Carls et al. (2005) net-pen studies also showed no survival reduction of pink salmon juveniles when as embryos they experienced tissue-TPAH levels ~1,900 ppb–3,000 ppb, > 10 times higher than the mean tissue load of ~175 ppb–200 ppb pink salmon fry showed in oiled marine waters (Carls et al., 1996a). Moreover, adults returning from marine releases of fry that experienced tissue-TPAH levels

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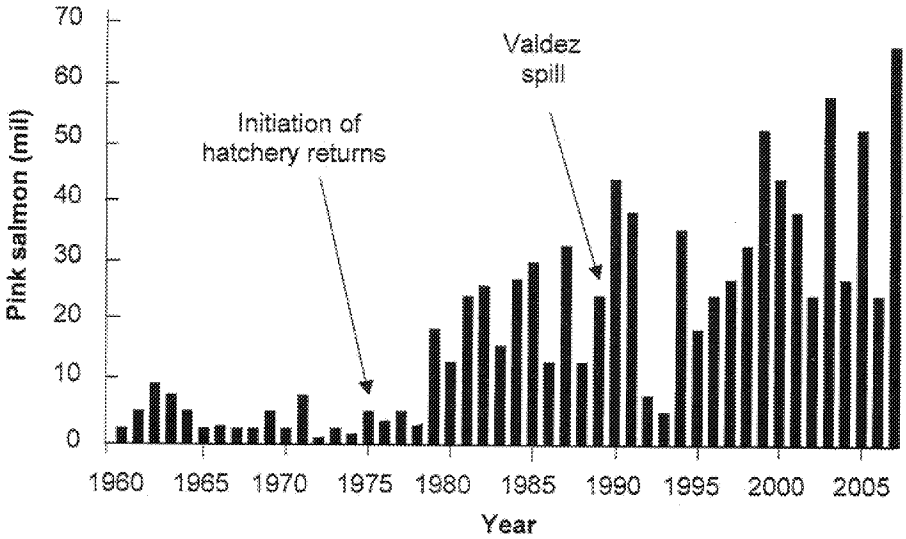

Figure 13 Adult pink salmon returns to PWS from 1960–2007, showing the record returns of adults in 1990 and 1991 that had experienced the highest oil concentrations of EVC during incubation in 1989 and 1990. By 2007 ten major return years have occurred since the EVOS, representing the good health of the PWS pink salmon population.

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of ~1,900 ppb during incubation showed no differences in size at return from that of controls. The evidence that growth of PWS pink salmon was unaffected by oil is also consistent with other studies exposing pink salmon juveniles to oil concentrations (Birtwell et al., 1999) and with the published literature showing open-water spills generally do not cause extensive injury to fish or other water-column organisms (Longhurst et al., 1982; Rice, 1985; Sherman and Busch, 1978; Straughan, 1970; Wells et al., 1985).

CONCLUSIONS

The results of the present review on the impact of oil have major implications on the previous assessment of reduced adult returns under the Geiger model (Geiger et al., 1996). The EVOSTC (2002) made the determination that PWS pink salmon didn't recover from the spill until 2001, six generations or twelve years after the spill. That determination was based on the higher egg mortality in oiled streams from 1989 through 1997 (Bue et al., 1996, 1998; Craig et al., 1995, 2002), and the alleged reduced growth in oiled marine waters (Wertheimer and Celewycz, 1996; Willette, 1996). Those impacts were represented as variables (Seg_t and Smr_t) in the Geiger model reducing survival (s_t) of returning adults by as much as 2.3 million wild fish. As demonstrated in this review the authors challenge the values assigned to the Seg_t and Smr_t variables.

Regarding the issues that drove the allegations of damage to pink salmon, the authors have demonstrated in the present review that the increased egg mortality observed by ADFG was caused by their sampling protocol, unrelated to oil (Brannon et al., 2001; Collins et al., 2000), and thus consider that the value of Seg_t variable in the Geiger model is essentially zero. Furthermore, the bioassays in the laboratory, including those that were below the toxicity threshold of pink salmon, were so far in excess of the oil concentrations in oiled streams that they have no application to what embryos in those streams experienced. The authors conclude that the contamination levels in those streams posed no measurable risks to the subsequent long-term growth and survival of pink salmon juveniles in marine waters.

The authors have also shown in this review that the weight of the evidence does not imply that size differences represent differences in growth of PWS juveniles once they were exposed to oil contaminated marine waters. Consequently the authors believe the review has shown that the Smr_t variable in the Geiger model is unsupported and incomplete.

Contrary to the Geiger et al. estimate of reduced returns, the authors submit the observed increase in plankton productivity and reduction of bird predation following the spill contributed to the record survival success of the 1989 and 1990 pink salmon brood year classes exposed to the oil spill, and those elements were not included in the Geiger marine survival estimates. The authors suggest that with the correction of the variables in the Geiger model it will eliminate the estimate of reduced numbers of returning adult pink salmon. Consequently, EVOSTC needs to revise their official assessment of the effects of the *Exxon Valdez* oil spill on PWS pink salmon. The published work sponsored by EVOSTC is misleading in the understanding of the effects of the spill on pink salmon and its implications on oil contaminated waters.

The long-term effects of the *Exxon Valdez* oil spill on the PWS pink salmon survival can best be demonstrated by the success of adult returns. The general good health of the PWS population immediately following the spill is shown by the record returns of pink salmon to the sound in 1990 and 1991 (Figure 13), which were the returns from embryos exposed to the oil in 1989 and 1990 (Brannon et al., 2006b), and their successful productivity since then by nine additional record years. Nearly

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3.5 times the number of 1988 adults returned in 1990, although 6% fewer fry were released from the PWS hatcheries that produced that large run. A stock-specific run reconstruction model for PWS pink salmon by Templin et al. (1996) also concluded there was no reduction in adult returns from the spill. These data present a compelling case that pink salmon were not measurably damaged by the *Exxon Valdez* oil spill. There is no evidence supporting the projected losses to the PWS pink salmon that were anticipated at the time of the spill event or estimated by the Geiger model.

The authors recognize that the large hatchery programs in the sound are major contributors to the success in cultivating the large runs of PWS. We also realize that natural variability will occur in numbers of adults returning as a reflection of PWS productivity, such as the low returns in 1992 and 1993 caused by low temperatures affecting growth and reduced plankton productivity (Cooney and Willette, 1997; Willette, 1992). But the success of the hatchery runs is also evidence that oil passing through their marine nursery areas and migratory pathways in the spring of 1989 didn't inhibit the record returns of those fish in 1990, and the demonstrated success of the natural returns to oiled streams in the twelve years of study thereafter (Brannon et al., 2006b; Maki et al., 1995). Recovery efforts for pink salmon were not applied nor were they necessary because the evidence shows they were not damaged in any measurable way.

The authors suggest the EVOSTC conclusions about oil effects on pink salmon were influenced by the need for litigation to identify damages and recover monetary compensation for the injuries to natural resources. This was evident in the tenor of the proceedings of the *Exxon Valdez Oil Spill Symposium* in 1996 where the contributors were agency researchers reporting on the extent of oiling and their assessment of damages. Unfortunately this is part of the process that characterizes damage assessment in such major environmental perturbations. Exxon also presented its side on the effects of the spill in the symposium on the *Exxon Valdez Oil Spill: Fate and Effects in Alaskan Waters* in 1995. Both of these were productive contributions that presented the data and the interpretation of the research results, but they left no resolution to the differences in conclusions arrived at on spill effects beyond the readers ability to work through the experimental procedures and resulting data. The present review undertook that task on pink salmon in an attempt to reconcile the interpretations of the research based on the science. The implications of these findings are significant in our understanding of the impact of oil spill on PWS pink salmon. Accurate information is the key in establishing confidence that we understand the ecological impact of the *Exxon Valdez* oil spill and that such understanding is founded on reliable science.

ACKNOWLEDGEMENTS

The work performed on the effects of the *Exxon Valdez* oil spill on pink salmon in Prince William Sound was sponsored by Exxon and Exxon Mobil Corporation through contracts with consultants and funds provided to the *Exxon Valdez* Oil Spill Trustee Council over the last 23 years. The review of the effects of the spill on pink salmon is the compilation of those works and the assessment of that research by the co-authors. The authors thank the in-house and external reviewers for their helpful comments and critique of the article, and appreciate the willingness of *Reviews in Fisheries Science* to publish the review.

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