FIELD AND LABORATORY EVIDENCE FOR REDUCED FITNESS IN PINK SALMON THAT INCUBATE IN OILED GRAVEL

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Introduction

After the *Exxon Valdez* oilspill in March 1989, Alaska Department of Fish and Game (ADFG) biologists observed an apparent increase in pink salmon mortality among embryos incubating in oiled streams. While this was not surprising the Fall after the oilspill because contamination was still obvious, concern arose in the years following the spill when elevated mortalities persisted and streambed contamination was less obvious. Since evaluations of embryo survival had not been made prior to the spill, it was impossible to know if the differences in embryo survival resulted from stream geography, oil contamination, or stock biology. So, ADFG and the National Marine Fisheries Service (NMFS) began two laboratory studies to test the plausibility of these explanations.

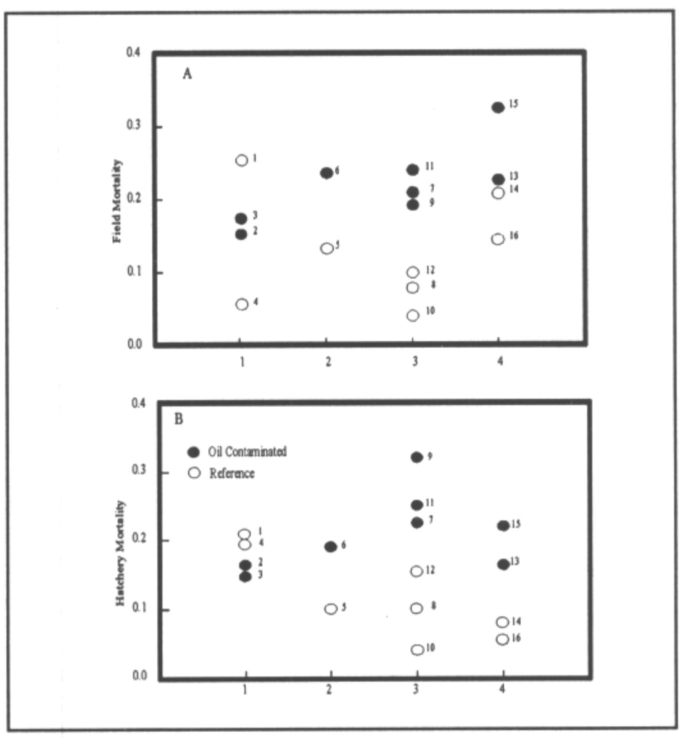

Figure 1. Mortality of pink salmon embryos from different streams in Prince William Sound. **A** depicts differences observed in streams in 1993, 4 years after the oilspill. **B** depicts survival of gametes collected from the same streams as **A**, but incubated in a hatchery. Numbers

The first laboratory study evaluated differences in the environments of oiled and unoiled streams; the oiled streams were mostly found in headlands while unoiled streams were generally found in more protected areas. ADFG biologists removed these environmental influences on embryo survival by spawning pink salmon that returned to oiled and unoiled streams and culturing the fertilized eggs in identical clean hatchery environments.

The second laboratory study examined oil's ability to impart long-term damage to fry that survived incubation in oiled gravel. It was not known if the damage imparted by exposing fish to oil could persist long after the exposure ended. National Marine Fisheries Service (NMFS) biologists incubated pink salmon embryos in gravel contaminated with known quantities of oil. After 16 months in uncontaminated water the surviving fry matured, they were spawned and their offspring were cultured in a clean hatchery environment.

This report broadly describes the field observations and laboratory studies along with their results. The first laboratory study has been done in conjunction with the field observations. The second laboratory study is a multigeneration study aimed specifically at determining the potential for oil to impart heritable genetic damage. The field work and laboratory studies are in progress, and more detailed reports will be published elsewhere.

Field Evidence

The two-year life cycle of pink salmon plays an integral part in the identification of the differences in embryo survival between oiled and unoiled streams. Pink salmon in Prince William Sound spawn in the intertidal environment between August and September each year. Eggs develop in the gravel and hatch in midwinter. The resulting larvae (alevins) remain in the gravel until the yolk has been completely absorbed, at which time the fry emerges from the gravel and emigrates to sea. The fry emerge from the gravel in early spring, and mature approximately 16 months later. Consequently, pink salmon streams usually have two distinct populations: one that spawns in even numbered years and one in the odd years.

In the Fall of 1989, the odd year population returned to spawn. Oil was evident in the intertidal environment of oiled streams especially at a height of approximately 3.7m above mean low water (MLW), the area that came to be known as the "bathtub ring." ADFG biologists selected 31 streams, divided each into 4 zones: three intertidal and one above the "bathtub ring", and estimated the number of live and dead eggs in each zone. In 1989, embryo mortality was highest in the oiled streams in each of the intertidal zones, with no difference in the highest zone. A similar analysis in 1990 revealed elevated embryo mortality only in the zone containing the "bathtub ring." In 1991, the 1989 brood returned and the stream surveys revealed that embryo mortality was elevated in all zones of the oiled streams. A similar, but weaker, result was observed when the 1990 brood returned in 1992.

A possible explanation for this pattern (Figure 1A) is that sublethal damage acquired by survivors in the 1989 brood led to impaired reproductive ability. This explains the initial lack of difference in embryo survival above the "bathtub ring". In 1991, the 1989 brood returned to spawn, reproductively impaired individuals mixed with unaffected individuals throughout the entire spawning zone, and reduced embryo survival in all zones. The same story can account for the even year data, if the less contaminated parts of the beaches are presumed to be "clean" in 1990. Alternatively, the observed differences may have resulted from either environmental differences between oiled streams or from genetic differences between the stocks inhabiting the streams. The substrates and orientations differ between the oiled and unoiled streams which could account for either a direct environmental influence or different selective pressures.

Laboratory Study 1

In 1993, ADFG biologists began to examine the influence of stream environment on embryo mortality. They collected pink salmon gametes from fish in oiled and unoiled streams, flew the gametes to a hatchery, and incubated the fertilized eggs. They observed survival to eyeing for the progeny from each stream and analyzed the differences in offspring survival between oiled and unoiled streams.

Similar responses were observed in both the laboratory and field observations in 1993, leading ADFG biologists to reject the environmental explanation for differences in mortality between oiled and unoiled streams (Figure 1B). The results suggest that the differences in survival resulted directly from parental influence which could occur in three different ways. First, impaired gametogenesis in the 1991 brood resulted from their exposure to oil when they incubated in oiled streams. Second, oil caused heritable damage to the reproductive ability the 1989 brood which was passed to both the 1991 and 1993 broods. Third, differences in the stream environments between oiled and unoiled streams led to selection for differing rates of offspring survival.

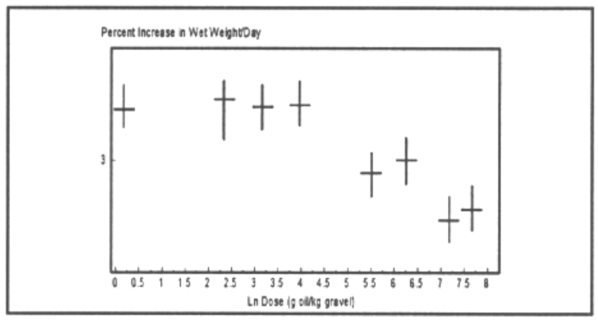

Figure 2. Marine growth of pink salmon exposed to gravel during incubation. Growth was measured between August and October 1994, 4 to 6 months after the oil exposures ended.

Laboratory Study 2

In 1993, NMFS biologists began a multi generational study designed to demonstrate the long-term effects of incubating in oiled gravel. They collected gametes from a pink salmon population that was unaffected by oil in 1989, and incubated them in gravel contaminated with known quantities of oil. Surviving fry were cultured, and once mature, they were spawned and their offspring were incubated in an uncontaminated environment.

The NMFS biologists either captive-reared or coded-wire tagged fry emerging from the oiled gravel to examine long-term effects. Captive reared fish were tagged with PIT tags and held in netpens. Growth rates of the PIT tagged fish were monitored until they died in the winter of 1995. Prior to dying, the PIT tagged fish demonstrated a long-term effect on growth (Figure 2). Growth was significantly lower in fish that were exposed to polynuclear aromatic hydrocarbon (PAH) concentrations as low as 1.0 ppb in water, which is one order of magnitude lower than the Alaska State water quality standard for PAH (15 ppb in water).

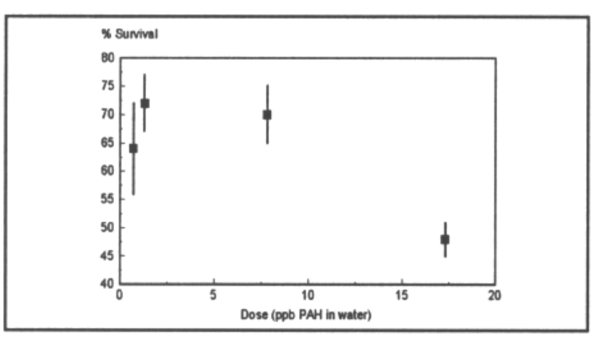

Figure 3. Reproductive success of pink salmon exposed to oiled gravel during incubation. Symbols depict 95% confidence intervals for survival of offspring to eyeing.

The coded-wire tagged groups returned to the hatchery in August 1995 when they were spawned. Only fry from the control and three lowest doses were released, and each dose was represented by four tag codes. When the mature fish returned, they were measured, and their tags were recovered and decoded so that the gametes could be sorted by dose. On each of 4 spawning dates, the gametes were pooled so that all the possible pairwise crosses were made for each dose. Male fish exposed to the highest dose (17 ppb PAH in water) were 10 cm smaller at maturity, and survived, on average, 15% lower than unexposed fish. Furthermore, marine survival was poorest for fish exposed to the highest dose in 3 out of the 4 tag groups. The mean survival of embryos taken from fish exposed to 17 ppb PAH in water was 25% lower than mean survival of the progeny of unexposed parents (Figure 3). Other matings, not reported here, support this result.

Conclusion

The results of these two investigations make it difficult to exclude oil contamination as a culpable agent for the differences in fitness observed between oiled and unoiled streams in Prince William Sound. ADF&G biologists found that the differences in embryo survival were not directly related to the stream environment, and the NMFS investigation has revealed a long-term effect of oil on pink salmon growth and suggested further effects on marine survival and gamete viability. Detailed analyses of the hydrocarbon uptake during incubation in the NMFS investigation demonstrated that residual pockets of oil alongside streambeds could account for the high mortalities observed in 1991. If impaired gametogenesis was among the sublethal effects acquired by the 1991 brood, then embryo increased mortality would persist in the oiled streams in 1993. A genetic effect has not been identified, nor has it been ruled out. The progeny of pink salmon exposed to oiled gravel in the NMFS investigation will return to the hatchery in September 1997, when the genetic explanation will be evaluated.