EVOS ANNUAL PROJECT REPORT

All recipients of funds from the *Exxon Valdez* Oil Spill Trustee Council must submit an annual project report in the following format by September 1 of each fiscal year for which project funding is received, with the exception of the final funding year in which a final report must be submitted. Satisfactory review of the annual report is necessary for continuation of multi-year projects. Failure to submit an annual report by September 1 of each year, or unsatisfactory review of an annual report, will result in withholding of additional project funds and may result in cancellation of the project or denial of funding for future projects.

PLEASE NOTE: Significant changes in a project's objectives, methods, schedule, or budget require submittal of a new proposal that will be subject to the standard process of proposal submittal, technical review, and Trustee Council approval.

Project Number: 02476

Project Title: Effects of Oiled Incubation Substrate on Pink Salmon Reproduction

PI Name: Ron Heintz

Time Period Covered by Report: Dec. 1, 2001 to Nov. 30, 2002

Date of Report: May 21, 2003

1. **Work Performed:** Summarize work performed during the reporting period, including any results available to date and their relationship to the original project objectives. Describe and explain any deviation from the original project objectives, procedural or statistical methods, study area, or schedule. Also describe any known problems or unusual developments, and whether and how they have been or can be overcome. Include any other significant information pertinent to the project.

This project involves the culture of multiple generations of pink salmon to examine the potential for oil to cause heritable damage to their reproductive capacity. It requires exposing a generation to oil, incubating their offspring in clean water, and evaluating the ability of the offspring to reproduce. The project began in 1998 when gametes were collected from wild salmon returning to Lovers Cove Creek in southeastern Alaska. The gametes were incubated at the nearby Little Port Walter Hatchery operated by the National Marine Fisheries Service. Eggs were exposed to one of three doses of oil, control, low or high. The high dose was consistent with the Alaska State water quality criteria for total polynuclear aromatic hydrocarbons. Fish surviving the exposure were released in the spring of 1999. In September 2000 we recovered adult salmon that had survived embryonic exposure to oil and approximately 16 months of migrating in the Gulf of Alaska. Gametes were collected from these fish and incubated in clean water. The following May (2001) three groups of fish were marked and released, each representing the original control, low and high dose lines. These were represented by 21,454, 19,816 and 7,387 marked fish from the control, low and high exposure groups, respectively.

Mature adults representing these releases returned to spawn in August through September of 2002. These fish were the offspring of the exposed fish, they had all been treated the same and were never exposed to oil. Thus, any effects observed in their offspring would be due to genetic differences between the gorups.

Returning fish were collected at a weir located at tidewater, so fish ascending the weir were presumed to have been made the transition from saltwater to fresh. Each fish was inspected to identify their exposure group and those with unambiguous marks were spawned. A total of 348 fish returned to the hatchery with identifiable marks. Fish released from the highest exposure group had significantly higher survival ($❹^2 = 18.85$; $P < 0.001$) with a value of 1.1% compared to 0.7% and 0.6% for the control and low exposure groups, respectively. The proportion of females returning was equivalent among all the groups averaging 44.8% and they were all similar in size ($P = 0.809$). In contrast, ANOVA revealed significant differences among male lengths ($P = 0.028$). Males from the highest exposure group averaged more than 15 mm longer than those from the control and low exposure groups.

Upon recovery at the weir, females were palpated to determine if they ovulated, those that had not ovulated were tagged with unique numbers. This permitted control of ovulation timing relative to freshwater entry across all treatments when the fish were spawned. Those that had ovulated prior to their recovery at the weir were discarded. Every four days during the run the tagged females were inventoried and checked for ovulation. Eggs were stripped from females that had ovulated within the last four days, and these were crossed with males from the same exposure group. The time between fresh water entry and ovulation did not differ among the exposure groups ($P = 0.310$), averaging 6.1, 5.9 and 6.1 days for the control, low and high exposure groups, respectively. Nor did the recovery dates differ ($P= 0.214$), the median recovery date was September 10, 2002.

Males arriving at the weir were also tagged and retained for spawning. Average recovery dates did not differ among the exposure groups ($P = 0.541$), and the median recovery date was September 7, 2002 or three days before the females. Males were held for up to 12 days and assigned to one of three groups depending on how long they had been in freshwater: 1 - 4 days, 5- 8 days, and 9-12 days. On a given spawning date, males from the lowest group were transferred into the next highest group and so on, thus most males contributed gametes on a multiple number of spawning dates. Males in the 9-12 day group were killed after spawning.

A total of 519 crosses were made on four different spawning dates. Only females known to have ovulated within the last four days previous to the spawning date were used on a given spawning date. Eggs from each female and male pairing were incubated separately, and males were assigned to females randomly so that each male group was represented by as many crosses as possible up to 20. This approach was designed to account for differences in fertility associated with differences in the amount of time fish had been held in freshwater. Thus, all exposure groups were represented by females that had ovulated within the same time period, and males that had resided in freshwater for equal amounts of time.

Crosses were made after all the gametes had been collected. All gametes were stored in a refrigerator until they were used. Male female pairings were incubated in randomly selected locations in incubator trays. Gametes were collected on September 11, 15, 19 and 23. After 24 hours unfertilized eggs were removed and counted from each cross.

Eggs were shocked and tallied between October 24 and November 15, 2001. After shocking the eggs were returned to the incubators. After 24 hours the eggs were removed from the incubator and the number of live and dead eggs tallied. All eggs were discarded after tallying. Analysis of these data will be presented in the final report, due on October 1, 2003.

2. **Future Work:** Summarize work to be performed during the upcoming year, if changed from the original proposal. Describe any proposed changes in objectives, procedural or statistical methods, study area, or schedule. [**PLEASE NOTE**: Significant changes in a project's objectives, methods, schedule, or budget require submittal of a

new proposal that will be subject to the standard process of proposal submittal, technical review, and Trustee Council approval.]

Future work includes completing the analysis of embryo survivals and producing the final report. The final report is due on October 1, 2003.

3. **Coordination/Collaboration:** Describe efforts undertaken during the reporting period to achieve the coordination and collaboration provisions of the proposal, if applicable.

Not Applicable

4. **Community Involvement/TEK & Resource Management Applications:** Describe efforts undertaken during the reporting period to achieve the community involvement/TEK and resource management application provisions of the proposal, if applicable.

Not applicable

5. **Information Transfer:** List (a) publications produced during the reporting period, (b) conference and workshop presentations and attendance during the reporting period, and (c) data and/or information products developed during the reporting period. [**PLEASE NOTE:** Lack of compliance with the Trustee Council's data policy and/or the project's data management plan will result in withholding of additional project funds, cancellation of the project, or denial of funding for future projects.]

No reports produced

6.**Budget:** Explain any differences and/or problems between actual and budgeted expenditures, including any substantial changes in the allocation of funds among line items on the budget form. Also provide any new information regarding matching funds or funds from non-EVOS sources for the project. [**PLEASE NOTE:** Any request for an increased or supplemental budget must be submitted as a new proposal that will be subject to the standard process of proposal submittal, technical review, and Trustee Council approval.]

No substantial deviations from proposed budget

Report Prepared By: _____

Project Web Site Address: _____

SUBMIT ANNUAL REPORTS ELECTRONICALLY TO katharine_miller@oilspill.state.ak.us. THE REPORTS WILL BE POSTED ON THE TRUSTEE COUNCIL'S WEB SITE AND SHOULD ALSO BE POSTED ON THE PI'S WEB SITE. The subject line of the e-mail transmitting the report must include the project number and the words "annual report" (e.g., "035620 Annual Report"). Electronic reports must be submitted either as an Acrobat Portable Document Format (PDF) file or word processing document (Microsoft Word 2000 for Windows or lower or WordPerfect 9.0 or lower) with any figures and tables imbedded. Acrobat PDF 4.0 or above file format must be used, preferably in 'formatted text with graphics' (called "PDF normal" under Acrobat PDF 4.0) format. Minimally, "PDF searchable image" (called "PDF original image with hidden text" under Acrobat PDF 4.0) may be used if pre-approved by the Trustee

Council Office. In either case, the PDF file must not be secured or locked from future editing, or contain a digital signature from the principal investigator.