ACUTE AND CHRONIC EFFECTS OF CRUDE AND DISPERSED OIL ON CHINOOK SALMON SMOLTS (*Onchorhyncus tshawytscha*)

A Research Preproposal for the Oiled Wildlife Care Network – Year 3, April 2005

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Hypothesis and Specific Aims
This project is designed to compare the toxic actions of the water-accommodated fraction (a naturally dispersed fraction; WAF) and chemically dispersed fraction (a chemically enhanced water-accommodated fraction; CEWAF) of Prudhoe Bay Crude Oil (PBCO) to the smolts of Chinook salmon (*Onchorhyncus tshawytscha*). The null hypothesis to be tested is: *The toxic impacts of crude oil on Chinook salmon smolts are not increased by application of oil dispersants.*

The specific aims of the 3-year project are:
1. To conduct field-modeled laboratory exposures of salmon smolts to declining concentrations of the WAF or CEWAF of PBCO, with the goal of determining the starting concentrations that induce metabolic stress, narcosis, and mortality; metabolic stress will be assessed using an advanced nuclear magnetic resonance (NMR)-based metabolomics approach.
2. To determine the long-term growth and metabolic viability of the acutely exposed fish by culturing them in clean (oil- and dispersant-free) seawater.

Experimental Plan
Due to the large maritime transport of crude oil from Alaska to California, there is serious concern that future coastal oil spills (and response activities) near rivers of spawning importance would cause deleterious impacts to salmon smolts entering the ocean [1]. Therefore, this project will examine and compare the toxic actions of both the WAF and CEWAF of PBCO to Chinook salmon smolts.

We have designed and tested a new flow-through exposure system based on a 20-L polycarbonate exposure chamber. A preliminary WAF exposure was initiated using both fall-run smolts from the Department of Fish and Game's American River Hatchery and winter-run smolts from the UCD Bodega Marine Laboratory to assess sensitivity of salmon to PBCO. Due to different susceptibilities, fish cannot be used interchangeably for this study. Thus, experiments will only use the fall-run smolts. Surviving salmon from these initial exposures were used in preliminary ^{1}H NMR metabolomic analyses to assess usefulness of muscle tissues and to investigate the efficacies of different extraction techniques. Methanol-water (2/1) was determined to be optimal for muscle extraction, as it produces flat baselines, numerous metabolite peaks, and minimal sample-to-sample variation.

Chinook salmon smolts (~6 cm FL; available in early summer 2005) will be exposed for 96 h to a minimum of 5 water concentrations of either the WAF or CEWAF of PBCO. The WAF and CEWAF will be characterized for hydrocarbons by gas chromatography-flame ionization detection (GC-FID).

Declining exposure concentrations will better simulate actual spill conditions, where both dilution and dispersion occur. Additional experiments will involve feed saturated with either the WAF or CEWAF of PBCO. Smolts will be evaluated for metabolic stress (see below), narcosis, and mortality. Experiments will be repeated a minimum of three times for statistical validity.

Surviving smolts will be placed in clean seawater and routinely weighed and measured for another 6 months to determine any sublethal chronic effects. During this period smolts will be sacrificed for assessment of metabolic status using ^{1}H-NMR-based metabolomics. This approach combines the metabolic profiling capabilities of NMR with powerful pattern recognition (chemometric) methods [2, 3]. It will enable comprehensive assessment of the metabolic effects of WAF and CEWAF by profiling the hundreds of low-molecular weight endogenous metabolic compounds. It also is an ideal tool for identifying subtle metabolic changes that could impair growth and reproduction. We have previously conducted NMR-based metabolomic experiments using salmon and medaka embryos, and have established protocols and a demonstrated ability to conduct the proposed studies [4–8].

Significance to Oiled Wildlife Health

The National Marine Fisheries Service and the DFG Office of Spill Prevention and Response (OSPR) have indicated that obtaining an understanding of the impacts of spill remediation on the fisheries of endangered anadromous species is of great importance (J. Dillon, pers. comm., 2002; M. Sowby, pers. comm., 2003) Currently, there is limited information on the effects of oil or chemically dispersed oil on anadromous fish smolts. Thus, this project will provide resource managers basic information on the toxic effects of oil spills on migrating salmon, and the data to support decisions regarding the advisability of applying dispersants under spill conditions where migrating salmon are present.

This project also addresses the desire of the OWCN to investigate the direct and indirect effects of oil on wildlife. It will address applied research questions regarding the risk of oil to endangered species. Experiments on the biochemical responses of smolts to oil will also address basic research questions concerning mechanisms of oil toxicity in early life stages, using a state-of-the-art approach. Due to the large maritime transport of crude oil from Alaska to California, there is significant potential for a catastrophic spill. It would seriously impact salmon populations if one were to occur during key periods of their migration, particularly when salmon smolts are entering the ocean from native streams and rivers. This study will provide data on the toxicity of the bioavailable fractions of oil on salmon smolts, and will provide information on the risk of chemically-dispersed oil to migrating salmon, allowing for more informed decision-making in the event of a coastal spill.

Project Duration

The duration of the project is three years. Years 1 and 2 of the project have already been funded by OWCN, and flow-through exposure systems and the methods for metabolomic analysis of tissues are now developed and ready for use with smolts when they are available from the hatchery in June 2005. Additional equipment necessary for large volume WAF generation is now in place, having been purchased with funds from the OSPR (see below). This current OWCN pre-proposal is for Year 3 the project. During Year 3, the acute tests with WAF and CEWAF will be completed, as well as the metabolomic analysis. The chronic effects on growth will also be completed.

Estimated Budget (note: Year 3 budget request is in bold)

	Year 1	Year 2	**Year 3**	Totals
Personnel (Postdoctoral Researcher; salary and benefits)	27,474.00 (75%)	28,847.00 (75%)	**30,290.00 (75%)**	86,611.00
Equipment	0.00	0.00	**0.00**	0.00
Supplies	5,000.00	4,000.00	**4,000.00**	13,000.00
Travel (Between Davis and Granite Canyon)	500.00	500.00	**500.00**	1,500.00
Other Expenses (NMR recharge)	1,000.00	2,000.00	**2,000.00**	5,000.00
Indirect costs	0.00	0.00	**0.00**	0.00
Totals	33,974.00	35,347.00	**36,790.00**	106,111.00

Budget Justification

The postdoctoral researcher would be primarily responsible for performing the metabolomic analyses. Supplies include glassware and chemicals for the exposures. Travel funds would support travel between UCD (where the metabolomic analyses would be conducted) and the DFG Granite Canyon Marine Pollution Studies Laboratory (near Monterey, CA), where the exposures will be conducted.

Supplemental Funds

A matching award (3 years, $65,000/year) from OSPR was received in July 2004. It provides chemistry and equipment support. Also, a matching award from the NOAA Coastal Research Response Center (formerly the Cooperative Institute for Coastal and Estuarine Environmental Technology; CICEET) was received in January 2004. This award, $75,000/year for 2 years, supports the personnel at the Marine Pollution Studies Laboratory where the smolt exposures will be done.

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