EVOSTC ANNUAL PROJECT REPORT

Recipients of funds from the *Exxon Valdez* Oil Spill Trustee Council must submit an annual project report in the following format by **Sept. 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). **Please help ensure that continued support for your project will not be delayed by submitting your report by Sept. 1. Timely receipt of your report allows more time for court notice and transfer, report review and timely release of the following year's funds.**

Satisfactory review of the annual report is necessary for continuation of multi-year projects. Failure to submit an annual report by **Sept. 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: 080839

Project Title: Evaluating injury to harlequin ducks (Histrionicus histrionicus) caused by sublethal hydrocarbon exposure in Prince William Sound using species-specific cell lines

PI Name: Hollmen, T. and Springman, K.

Time period covered: Apr 11, 2008 – Aug 31, 2008

Date of Report: September 1, 2008

Report prepared by: Hollmen, T., Springman, K., and Riddle, A.

Work Performed:

Objective: *Develop harlequin duck and surrogate (mallard) cell lines to evaluate injury from site-specific hydrocarbons in harlequin ducks*

FY08 Tasks: Year 1 cell isolation, cell line characterization

Progress:

Year 1 cell isolation

Development of cell lines for bioassays was initiated. Cells have been harvested from two harlequin duck eggs and three mallard (surrogate species) eggs. Fibroblast cells were extracted as described by Docherty and Slota (1988). Connective tissues were digested in media containing trypsin (0.25%) and cells were separated from the suspension by centrifugation. Cell were resuspended and counted. A subsample of all prepared stocks was cultured to determine viability, and the remaining suspensions were cryopreserved with DMSO in liquid nitrogen. For hepatocytes, a modification of a cell extraction process described by Brendler-Schwaab et al (1994) was used. Liver extracts were digested in media containing collagenase (100U/ml) and trypsin (0.125%), and suspensions were centrifuged to separate the cells. A subsample of cells was cultured to determine viability and assess culture conditions. Three different media options (including high and low glucose options) and three different culture well treatments (thin layer collagen, gel collagen, and non-treated) were tested. Remaining cells were cryopreserved with DMSO in liquid nitrogen.

A total of nine cell suspension vials of liver extracts each containing $0.2x10^6$ cells and 25 vials each containing $1x10^6$ fibroblast cells have been cryopreserved from harlequin duck tissue extracts. A total of three cell suspension vials of liver extracts each containing $0.2x10^6$-$0.3x10^6$ cells and 11 vials each containing $1x10^6$ fibroblast cells have been cryopreserved from mallard tissue extracts.

Cell line characterization

Primary cultures have been subcultured to determine stock and passage viability. To date, harlequin duck and mallard hepatocytes have been passaged up to P-3, mallard fibroblasts have been passaged up to P-2, and harlequin duck fibroblast cultures have been passaged up to P-7 or P-10 (ongoing). Culture media and reagents previously used for duck and sea duck fibroblast cell cultures in our laboratory (M199 with 20% fetal bovine serum) are performing well for the maintenance of harlequin duck and mallard fibroblast cultures. Preliminary results of harlequin duck fibroblast yields from year 1 cell isolation are presented in Figure 1. The preliminary results indicate good cell yields through multiple passages.

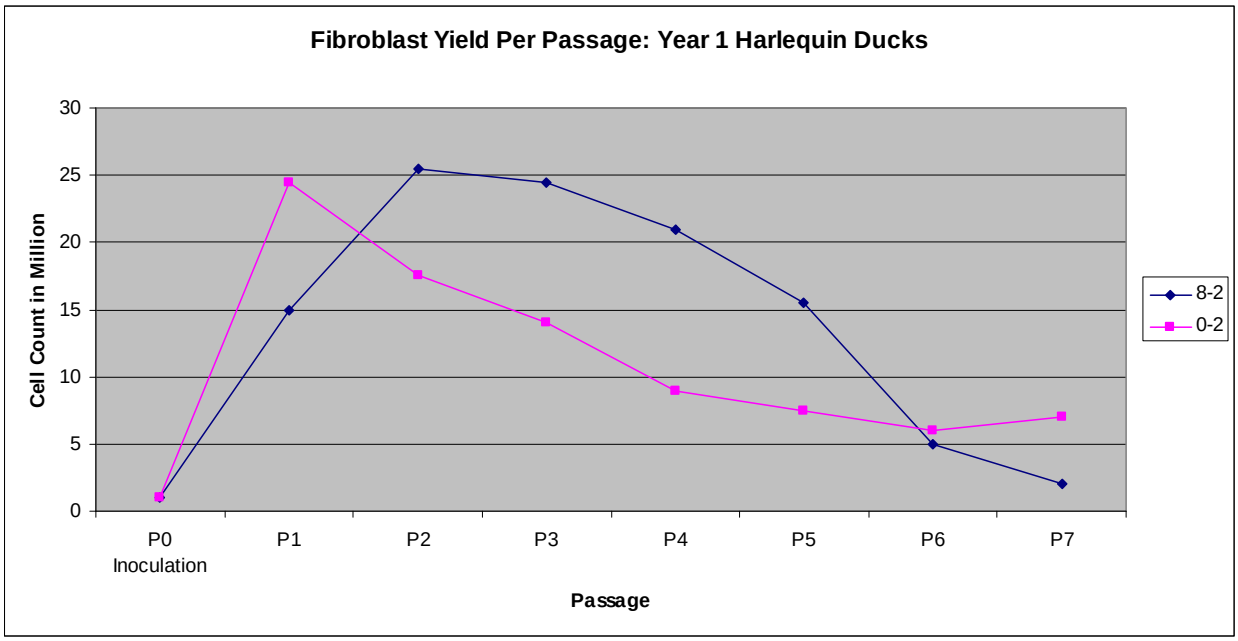

Figure 1. Yields of harlequin duck fibroblasts of two primary cell stocks through passages 1-7. One million cells were inoculated from the primary stock (P-0).

Multiple media compositions and tissue plate well treatments were tested to optimize hepatocyte cell performance and viability, and work is ongoing. Best performance to date has been achieved with thin layer collagen well treatment and low glucose growth and maintenance media with 20% fetal bovine serum.

Objective: *Evaluate injury due to site-specific lingering oil in PWS in harlequin ducks at the cellular level*

FY08 Tasks: Deploy SPMDs in PWS, obtain reference compound test mixtures

Progress:

Field work

During June – July 2008, semipermeable membrane devices (SPMD) were deployed in Prince William Sound (PWS) at the following sites:

Site #1: Nellie Juan. This site (60.54263 N, 148.31591 W) served as a true control site. As part of Restoration Project 060740, SPMDs were deployed here. The results from that study demonstrate that total concentration of 44 polycyclic aromatic hydrocarbons (tPAH) was quite low at this site (Short et al., in press). This area has characteristics of good harlequin duck habitat.

Site #2: McClure Bay. This site (60.54814 N, 148.16579 W) was also sampled in 2004. Here, semi-liquid Monterey Formation fuel oil was released from ruptured tanks in the earthquake in 1964 and was uncovered and analyzed as part of Project 060740. At this sampling, some surface asphalt predominated. Upon retrieval, the deployment device was out of the pit entirely, but held in place with an anchor.

Site #3: Eleanor Island, Northwest Bay (60.55075 N, 147.57959 W). At this moderate oil residue (MOR) site, sheen was present at 10 – 25 cm beneath cobble and pebble. Here the Field Blanks (a necessary QA/QC measure) were exposed for a brief period. Sheen was apparent on retrieval of the deployment device.

Site #4: Zaikoff Bay (60.30018 N, 147.06353 W) was selected as a reference site. The beach morphology was of boulder/cobble with veneer armoring.

Site #5: Disk Island (60.49840 N, 147.65935 W). Two replicates were placed at this site as SPMD extracts generated the highest results in the ethoxyresorufin-*o*-deethylase (EROD) assay in 2004. The beach morphology is cobble/pebble over peat, and oil appeared 2 – 3 cm below the beach surface followed by peat. Significant sheen was apparent from both replicates upon retrieval.

Site #6: Herring Bay (60.48481 N, 147.72330 W). This site is one of boulder/cobble with bedrock nearby. Sheen was apparent upon retrieval.

Site #7: Bay of Isles (60.38056 N, 147.71437 W). This MOR site showed oil at 2 – 15 cm below the beach surface, with peat beneath.

Site #8: Mini Mew (60.47746 N, 147.64832 W) (adjacent to Mew Cove). This site has been dug by ExxonMobil in 2003 and 2004. This MOR site has many of the features of good otter and duck habitat, with oil at 5 – 10 cm beneath cobble/pebble beaches. A great deal of eel grass can be found here. Sheen was apparent upon retrieval.

Site #9: Deer Island (60.44900 N, 147.75961 W). This light oil residue (LOR) site shows oil at 15 – 20 cm beneath pebble/cobble beaches with some peat deeper above bedrock. There are numerous otter pits nearby. The deployment area was the saddle between two bays. Upon retrieval, beads of oil as well as sheen were apparent.

At each deployment site, the cages were half-buried in pits excavated on shorelines at the mid-tide level of each site, with the other half exposed to the atmosphere. After placement in a pit, the excavated material was replaced to the level of the beach surface, surrounding, but not covering, the cage. The deployment devices were anchored in place with duckbill anchors. Approximately 100 g of material from each pit was collected at all sites and placed in hydrocarbon-free amber glass jars.

Upon retrieval, SPMDs were sent to Environmental Sampling Technologies Lab in St. Joseph, MO via FedEx. The cooler arrived with gel packs still frozen.

Reference compound test mixtures

Test mixture material has been acquired. Regulations regarding transportation of Hazardous Material (CFR Title 49, Volume 2, Part 172) require special handling, packaging, labeling and insurance. All requirements are being fulfilled, and the material will be shipped before 1 October 2008.

Future Work:

Project plan for FY09 is described in the original proposal.

Coordination/Collaboration:

The advice, collaboration, and assistance of Dan Esler (Simon Fraser University), Dan Rosenberg (ADF&G) and Mandy Lindeberg (NOAA) were requested for several aspects of this study. Dan Esler and Dan Rosenberg have a great deal of familiarity with sites in PWS where harlequin ducks can be found and with research on harlequin duck populations at these sites. In 2004, Mandy Lindeberg deployed the SPMDs in PWS, and her knowledge of MOR and LOR sites is extensive. When selecting sites for SPMD deployment, information was exchanged through conference calls and emails to select relevant sites that could link this study to other EVOSTC-funded research. Mandy Lindeberg worked with us in the field to deploy the SPMDs in the same manner that was used effectively for the 2004 study.

Community Involvement/TEK & Resource Management Applications:

Poster displays are in preparation to communicate about this project to the visitors at the ASLC. Further outreach and community involvement plans will be developed during FY09.

Information Transfer:

Publications, presentations, and information products will be developed when results are available from this research project.

Budget:

No changes from budgeted expenditures.

We can accept your annual report as a digital file (Microsoft Word or WordPerfect), with all figures and tables embedded. Acrobat Portable Document Format (PDF) files (version 4.x or later) are also acceptable; please do not lock PDF files or include digital signatures.

Please submit reports electronically in ProjectView or by email to catherine.boerner@alaska.gov. Also, please be sure to post your annual report on your own website, if you have one.

We appreciate your prompt submission of your annual report and thank you for your participation.