Preassessment Data Report #10

Chronic exposure of seaducks to oil released by the *Selendang Ayu* at Unalaska Island

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Executive Summary

On 8 December 2004, the M/V *Selendang Ayu* ran aground and broke in half in rough seas off Unalaska Island, Alaska (53°38'N, 167° 07'W). An estimated 354,218 gallons of oil (339,538 gallons of bunker oil [IFO 380] and 14,680 gallons of marine diesel and miscellaneous oils) were released. In the weeks following the spill numerous bird carcasses were recovered. In addition, aerial surveys assessed the abundance of live birds using the oiled area. Our goal was to determine the acute and chronic exposure of these live birds to hydrocarbons associated with this spill. In 2005 and 2006, harlequin ducks (*Histrionicus histrionicus*) were captured in three oiled (Skan, Humpback and Portage) bays and one minimally oiled reference (Chernofski) bay. Liver biopsies were surgically obtained and birds were released at the capture sites. The biopsy samples were analyzed for induction of the cytochrome P450 IA gene (P450), an indicator of hydrocarbon exposure. In 2005 and 2006 the P450 values of ducks in oiled locations were significantly higher than those in Chernofski. The results suggest that harlequin ducks were exposed to hydrocarbons more than a year after the spill. Although it is not known how long these effects will persist, studies following the *Exxon Valdez* suggest that they may persist for years. Elevated P450 in harlequin ducks has previously been associated with reductions in survival of female ducks following exposure to *Exxon Valdez* oil.

Introduction

On 8 December 2004, the *Selendang Ayu* ran aground and broke in half in rough seas off Unalaska Island, Alaska (53°38'N, 167° 07'W). An estimated 354,218 gallons of oil (339,538 gallons of bunker oil [IFO 380] and 14,680 gallons of marine diesel and miscellaneous oils) were released. In the weeks following the spill numerous bird carcasses were recovered despite logistical constraints and the large spill zone. In addition, aerial surveys assessed the abundance of live birds using the oiled area.

During oil spill response and natural resource damage preassessment activities, recovery of oiled carcasses and observations of oiled individuals established that certain bird species were acutely exposed to *Selendang* oil. Because carcass collection and bird observations were limited by logistical constraints, poor observation conditions, and other factors, the trustees initiated an examination of exposure levels to determine if additional species, such as the threatened Steller's eider, had been injured. In addition, re-wash of beached oil, subsequent oil releases from the wreck, and the chance that oil mixed with sediments was entrained in the nearshore environment necessitated an examination to determine if bird injuries likely extended beyond those caused by the initial oil release.

Extensive studies of harlequin ducks in Prince William Sound following the *Exxon Valdez* oil spill revealed that these ducks have high site fidelity and feed on intertidal resources making them susceptible to continuing oil exposure. The research has shown that chronic exposure to oil can have long-term deleterious effects on survival of harlequin ducks (*Histrionicus histrionicus*) (Esler et al. 2002). Accordingly, chronic exposure to sub-lethal levels of oil may have negative effects on population recovery following an oil spill.

We used Cytochrome P450 induction levels in harlequin duck liver as a biomarker to determine if seaducks were being exposed to PAH in the *Selendang Ayu* spill area in the 2 years following the spill. The results are interpreted in regard to geographic and temporal variation in exposure.

Methods

Field Capture and Surgery: Harlequin ducks were captured using mist nets and decoys following standard capture methods (Kaiser et al. 1995). Birds were captured in four locations: Portage Bay, Skan Bay, Humpback Bay, and Chernofski Harbor (Figures 1 and 2). According to shoreline surveys conducted following the spill, these bays differed in the degree to which they were oiled (Figures 1 and 2). Chernofski Harbor was originally selected as an unoiled reference site, but was later found to have been oiled; although to a lesser degree than the other three bays.

After capture, birds were transported a short distance to a mobile field laboratory where they were banded and examined. Their weight, sex, and age (adult or immature) were recorded. A biopsy of liver (approximately 0.05 g) was then extracted from healthy birds according to established procedure (Mulcahy, DVM, Alaska Science Center, Anchorage, unpublished guidelines). The liver samples were immediately frozen and stored in liquid nitrogen and then retained at –80°C until processing. After suitable recovery, as determined by the veterinarian, birds were released near the capture site.

Laboratory Assays: Approximately 4 mg (wet weight) of liver per bird was analyzed for EROD activity (i.e., P 450 induction) at the Department of Animal Sciences, University of California, Davis. For quality assurance and quality control, 5 embryonated mallard (*Anas platyrhynchos*) duck eggs were injected with 2 mg/egg of beta-napthoflavone (BNF), a known P450 inducer, and their livers assayed at 24 hours. Similarly, livers from 5 undosed embryonated mallard eggs were run as baseline controls. Liver samples were prepared by separating the hepatic microsomes using differential centrifugation. Microsome preparations were prepared from livers homogenized in 0.1 M $NaPO_4$ buffer at pH 7.4, centrifuged at 100,000 x g for 1 hour, and then resuspended (ml/g tissue) in 50 mM Tris solution that contained 1 mM EDTA, 1 mM DTT, and 20% v/v glycerol at pH 7.4. EROD activity was measured as described by Trust et al. (2000) according to the method of Bruke and Mayer (1974), adapted to a fluorescence microwell plate scanner (Melancon 1996). Modifications were determined in triplicate in a 96 well plate at 25°C using a Packard FluoroCount microplate fluorometer (Packard Instrument Company, Meriden, CT). Each well contained 1 µl of microsomes, 159 ul of 2.5 uM final concentration 7-ethoxyresorufin in 50 mM Tris-buffer at pH 8.0. The addition of 40 ul of 1.34 mM final concentration of NADPH initiated activity. Fluorescence was measured at excitation wavelength of 530 and emission wavelength of 590 nm at 1-minute intervals for 6 minutes. EROD was expressed as picomoles per minute per milligram of protein (pmol/min/mg protein). Protein was determined using the Bradford reagent (Sigma, St. Louis, MO).

Analyses: The ratio of EROD activity in dosed versus control eggs was calculated for each year's sample. If the ratios differed then we assumed that the absolute values of EROD activity were not directly comparable among years. The P450 data from wild birds were highly skewed with a few very large values, so we log transformed those data prior to analyses to improve normality.

Randomization procedures were used to assess the probability that EROD activity differed in oiled bays compared to Chernofski. We calculated the difference in mean log P450 values between oiled and unoiled locations. We then randomly reassigned individuals to a sampling location, without replacement, such that each location retained its original sample size. We re-calculated the difference in mean log P450 values among locations. We repeated this process 1000 times and we report the *P*-value as the proportion of random trials with greater differences between oiled and unoiled locations than observed in the actual data + 1 divided by the number of trial + 1 (Davidson and Hinkley 1997). Each year was analyzed separately. Conditional on the sample of birds captured, this proportion represents the probability that the variation observed among sample areas occurred by random chance. All analyses were conducted using the Poptools Macro within Microsoft Excel.

Harlequin duck captures were attempted in four locations: Portage Bay, Skan Bay, Humpback Bay, and Chernofski Harbor (Figures 1 and 2). According to shoreline surveys conducted following the spill, these bays differed in the degree to which they were oiled (Figures 1 and 2). Chernofski Harbor was originally selected as an unoiled reference site, but was later found to have been oiled; although to a lesser degree than the other three bays. Age and sex ratios as well as mean body mass of birds captured was compared among birds captured in the oiled bays and Chernofski using randomization procedures as described above.

Results

Capture: One hundred-twenty harlequin ducks were captured in and around the spill zone (Figures 1 and 2). A total of sixty birds were captured in three bays with moderate to heavy oil (Skan, Humpback and Portage) and 60 birds were captured in the minimally oiled reference location Chernofski Harbor. There was no difference in the age and sex ratios, or body weights, between birds captured in oiled and unoiled locations in 2005. There was a lower proportion of adult birds captured in the oiled areas (0.73) compared to Chernofski (0.96) in 2006, but there were no differences in sex ratios or body weights or age ratios in 2005.

Cytochrome P450: The measured enzyme (or P450) response of dosed mallard eggs varied among years. In 2005 the ratio of EROD activity in dosed to control eggs was 62. In 2006, the ratio was only 24.2. Therefore, we concluded that the absolute values of EROD activity were not directly comparable among years.

Samples analyzed for EROD activity to date include: 60 from oiled areas (n = 22 from Skan, n = 18 from Humpback, and n = 20 from Portage) and 58 from Chernofski Harbor. We deleted two extreme samples from Chernofski as outliers: one value was more than double the next nearest value and the other point was a negative value; both points were suspected to be laboratory errors and were having a disproportionate effect on the results. Additionally, two samples from Chernofski were damaged during preparation; thus statistical analyses for Chernofski were based on 56 samples.

There was a significant difference in log EROD activity between oiled and unoiled areas in both years (2005: $P < 0.001$, 2006: $P < 0.003$) (Figure 3).

Discussion

Because the dosed egg standards did not yield equivalent results across years we assumed that absolute values of EROD activity were not directly comparable among years. Thus, all analyses were based on P450 values between oiled and reference sampling locations within years. This accounted for any systematic bias in laboratory procedures between years.

The study was designed to determine sublethal hydrocarbon exposure of sea ducks within the *Selendang Ayu* spill zone. Harlequin ducks were observed in all areas and were captured consistently from all study bays. The study design utilized a control/exposed comparison where birds captured in oiled area were compared with birds captured in a single “reference” area within years. However, after the study was initiated, oil was observed on and around Chernofski Harbor. Thus, birds using this area may have also been exposed to hydrocarbons from the *Selendang Ayu*. The effect of such exposure would decrease the relative difference between the oiled and reference bays in our statistical analyses, thus making true differences more difficult to detect. Given that higher levels of P450 induction were still detected in the more heavily oiled bays compared to the oiled ‘reference’, sampling birds in an unoiled reference area would only increase the magnitude of the oiling relative effect. So while we likely underestimated the true magnitude of the P450 response resulting from exposure to *Selendang Ayu* oil, an effect is nonetheless clear.

The control/exposed study design assumes that all other potential factors influencing the variable of interest (i.e., P450 induction in this case) are equal across sampling areas such that the only difference between the control and exposed sample is the effects of the *Selendang Ayu* spill. Evidence of non-*Selendang Ayu* oil was found in both the oiled bays as well as Chernofski (see source allocation of tarball samples) and we assumed that these background levels of exposure were equivalent among areas. The age and sex ratios, as well as body weights, of birds captured in each area were comparable with the exception that more hatch year birds were captured in the oiled bays compared to Chernofski in 2006. Given the comparable sex ratios and body weights among sampling areas in 2006, we doubt this difference in age ratios would cause us to reach an erroneous conclusion. In the absence of any data regarding age and sex specificity in P450 response levels in birds, we assumed that samples were equivalent across control and exposed areas.

Harlequin ducks in the spill zone were exposed to hydrocarbons at significantly higher levels than harlequin ducks in a minimally oiled bay immediately following the spill (Figure 3). Thus, in addition to birds killed in the initial spill event (i.e., carcasses found on beaches), oil from the *Selendang Ayu* spill appears to have continued to affect other birds using the spill areas. Studies in Prince William Sound following the *Exxon Valdez* spill indicate that exposure resulting in elevated P450 levels correlate with reduced over-winter survival of harlequin duck females (Esler et al. 2002). Thus, mortality estimates based solely on dead birds found immediately after the spill event likely underestimate the magnitude of the overall injury as birds chronically exposed to low levels of hydrocarbons may also suffer increased mortality.

Harlequin ducks can be exposed to oil long after it is no longer visible on the surface of beaches. For example, harlequin ducks were still being exposed to *Exxon Valdez* oil, sixteen years after the spill (Trust et al 2000; Esler et al. 2002). More than a year following the wreck of the *Selendang Ayu*, levels of P450 inductions were still significantly higher in oiled bays compared to Chernofski. In spite of clean-up efforts in the oiled bays, birds utilizing these habitats were apparently still being exposed to hydrocarbons from the *Selendang Ayu* more than a year following the spill. Given the known effects of oil exposure on survival of harlequin ducks (Esler et al. 2002), we hypothesize that harlequin ducks were still dying as a result of exposure to hydrocarbons released by the *Selendang Ayu* more than a year after the spill. Although it is not known how long harlequin ducks in the spill area will be exposed to *Selendang* oil, based on studies conducted in response to other spills, and depending on factors such as the geomorphology of beaches and oil toxicity, the spill effects may persist for years.

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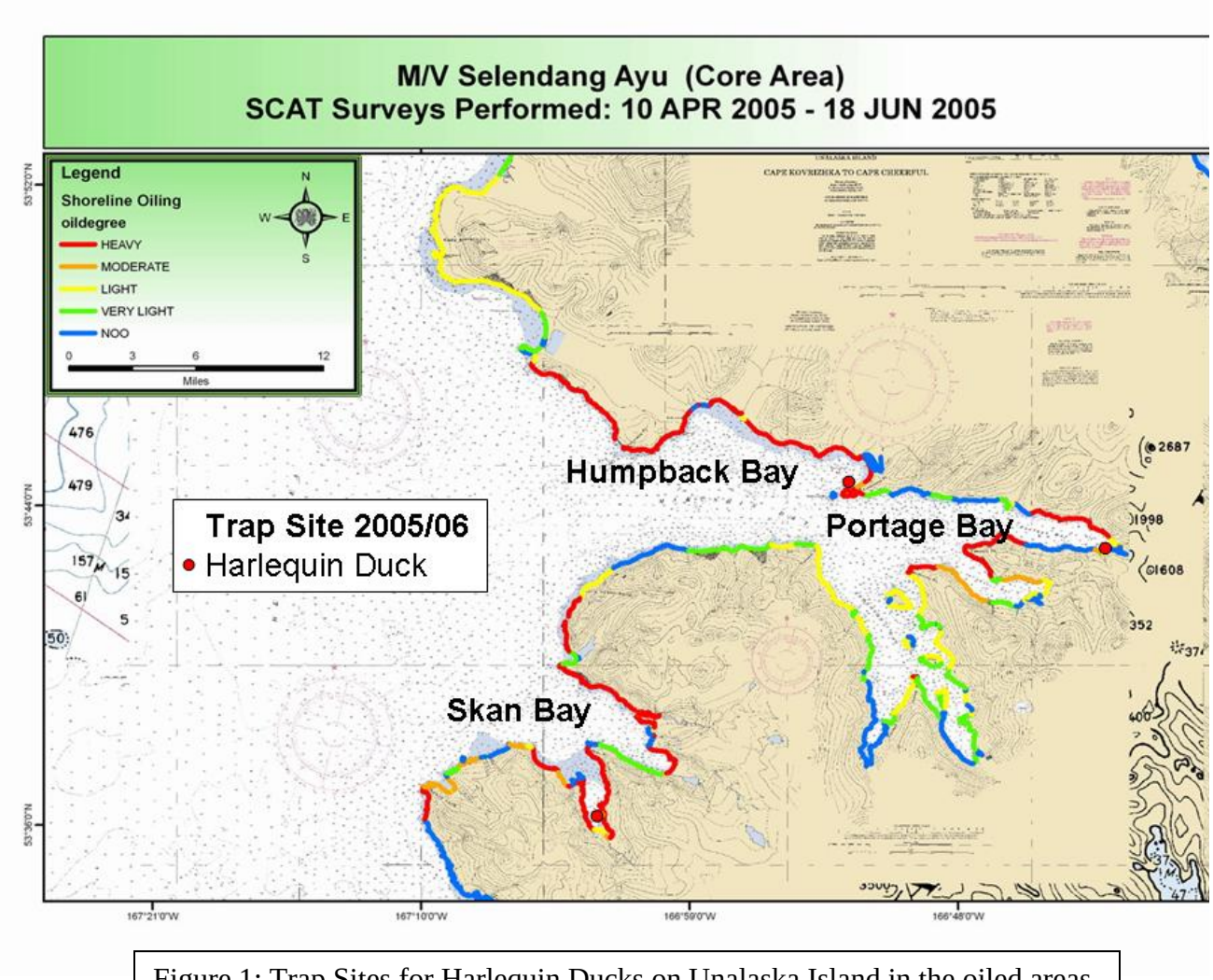

Figure 1: Trap Sites for Harlequin Ducks on Unalaska Island in the oiled areas of the Selendang Ayu spill zone, 2005-2006.

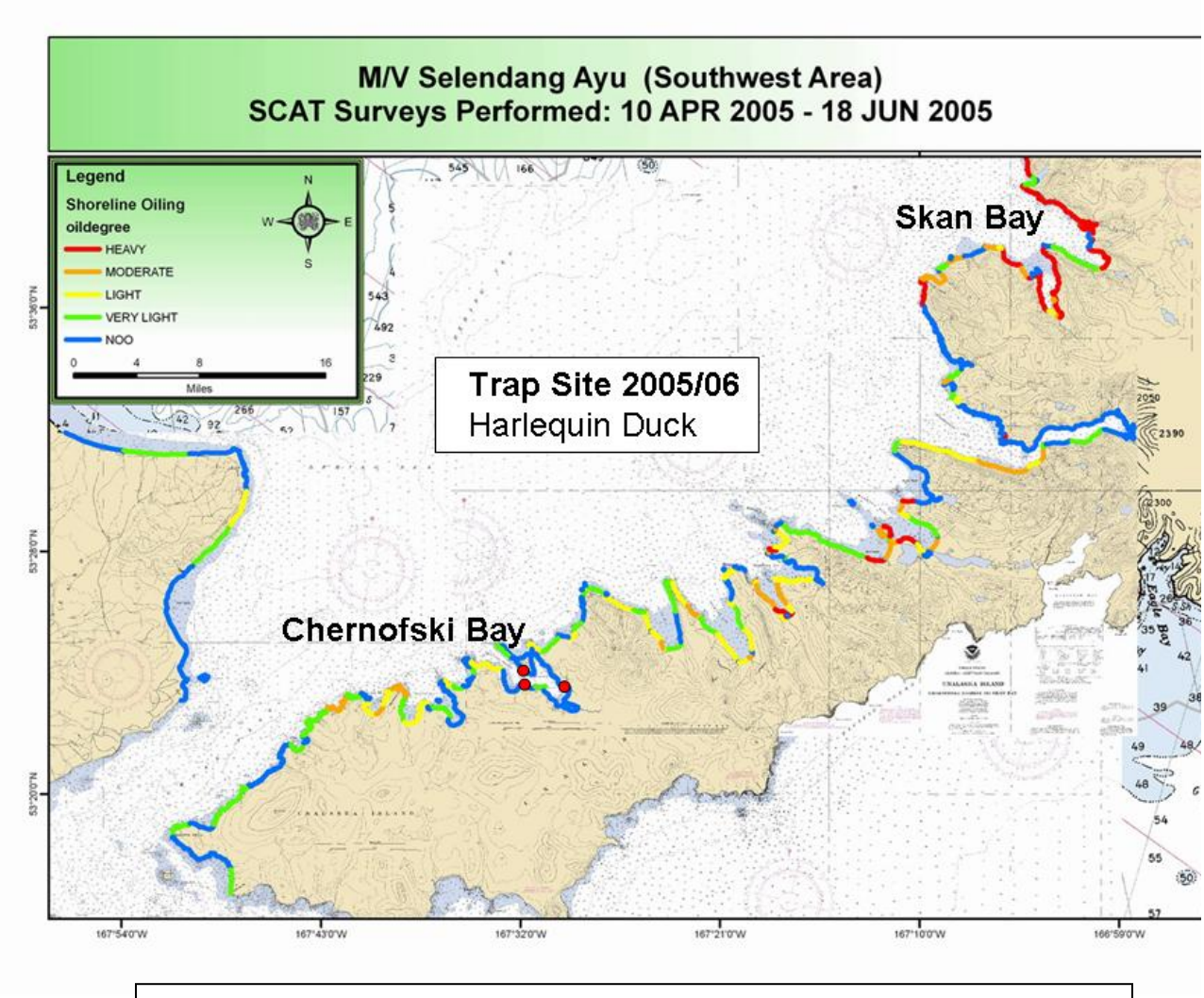

Figure 2: Trap Site for Harlequin Ducks on Unalaska Island in a minimally oiled area near the Selendang Ayu spill zone, 2005-2006.

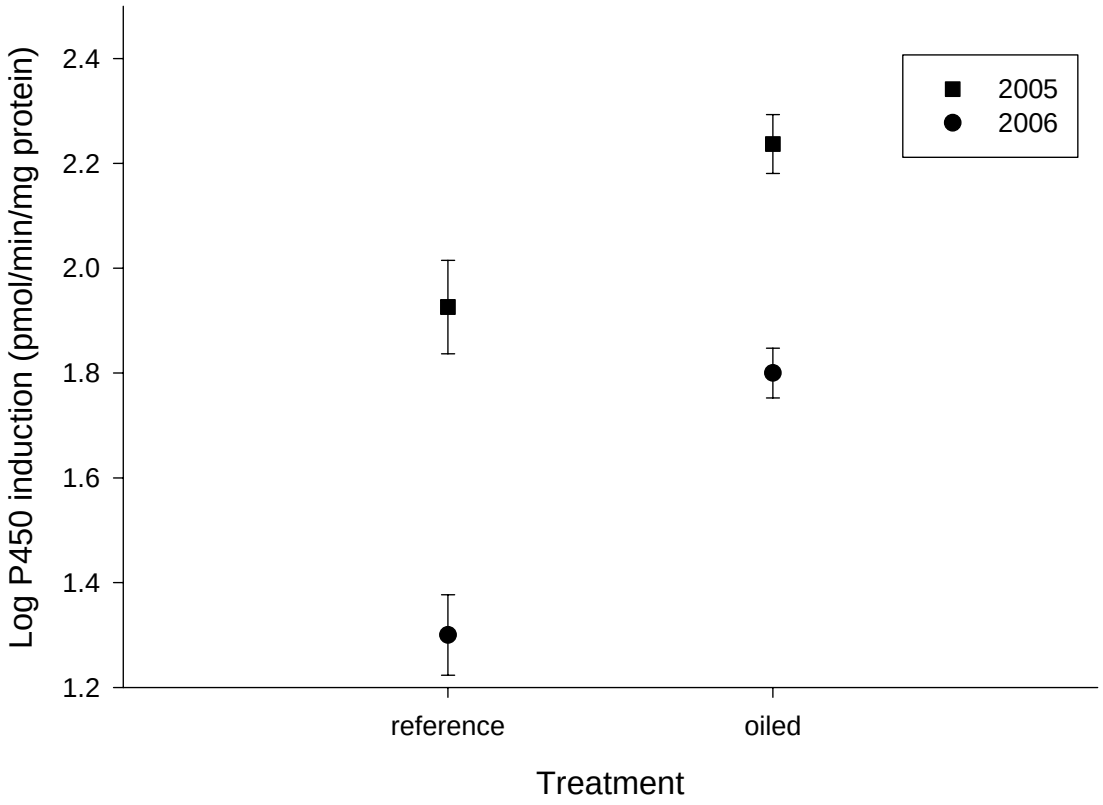

Figure 3: P450 induction was significantly higher in oiled locations than in Chernofski Harbor ("reference") ($P < 0.003$) in both 2005 and 2006. Due to differences in results for laboratory standards, absolute values of P450 inductions are not directly comparable among years.