Report on Recent Lingering Oil Studies

I. Introduction

In the 20 years since the *Exxon Valdez* Oil Spill, the Trustee Council has commissioned numerous scientific studies to evaluate effects of the spill on the environment. At the time of the spill, most scientists believed that, within a few years, the process of weathering would either break down and decompose the oil, or would cause it to turn into a form of asphalt that would have little potential to release toxic components into the environment. Contrary to these expectations, oil persists at some sites in Prince William Sound and the Gulf of Alaska in a relatively unweathered and potentially toxic condition. Therefore, the Trustee Council has undertaken several studies to determine the impact that this lingering oil may have on the environment and the feasibility of measures to address any potential adverse effects.

This report contains summaries of recent studies to address lingering oil. These studies can be divided into three categories: (1) development of a model to identify the geological and hydrological features that are most likely to result in the persistence of unweathered oil; (2) field and laboratory studies of the species most likely to show the effects of exposure to lingering oil; and (3) field and laboratory studies to evaluate the feasibility of treating lingering oil to reduce its potential impact.

II. Extent of Lingering Oil

In 2007 the Trustee Council funded a project to develop a model for predicting the likelihood that lingering subsurface *Exxon Valdez* oil would be found within individual shoreline segments of Prince William Sound and the Gulf of Alaska. The model that was generated used oiling data from 264 shoreline segments randomly sampled in 2001, 2003, and 2007 by NOAA's Auke Bay Laboratory and Research Planning, Inc. and from six oiled sites in the Gulf of Alaska found by representatives of the United States Geological Survey conducting studies there in 1999 and 2006. The model also incorporated various geomorphological characteristics of the nearly 2,000 kilometers of shoreline in Prince William Sound and the Gulf of Alaska that were oiled, including shoreline type, topographic and bathymetric complexity, exposure to wave action, beach slope, grain size of beach materials, and subsurface hydrology. Just as a coastal geomorphologist would integrate all of the factors when evaluating a shoreline segment for the likelihood of having subsurface oil, the model simultaneously evaluates all of the variables to make a similar assessment in a rigorous, unbiased manner. The model was validated with additional field surveys in 2008.

The final model indicates that there are a significant number of unsurveyed locations in Prince William Sound and the Gulf of Alaska that likely contain lingering *Exxon Valdez* oil below the surface. The number of such sites that the model generates differs depending on the confidence threshold and the level-of-oiling criteria. For example, using a 90% positive predictive value cutoff, the model predicts that there are 167 sites with any subsurface oil; however, there are 64 sites with moderately or heavily oiled residues, totaling 3.57 kilometers of shoreline. Using inputs of moderately or heavily oiled residues at greater than 15% frequency of oiled pits, at the 90% positive predictive value threshold, there are 52 sites totaling 2.62 kilometers of shoreline. This information can be used to prioritize beach segments in Prince William Sound and, to a lesser extent, the Gulf of Alaska, for remediation.

III. Environmental Effects

To evaluate potential effects of lingering oil on the environment, field and laboratory studies have focused on vertebrates most likely to be exposed to lingering oil in the intertidal environment, including several species of birds, sea otters, and fish.

A. Birds

Bird studies have focused primarily on harlequin ducks, although other species that feed in intertidal areas (Barrow's goldeneyes, black oystercatchers, and pigeon guillemots) have been examined. Studies have addressed both exposure to oil and its potential effects.

To consider exposure, cytochrome P4501A was measured in all four bird species. P4501A is part of vertebrates' systems for breaking down certain compounds, including those found in oil. For years after the spill, birds were exposed to oil, as shown by higher levels of P4501A in oiled areas compared to unoiled areas in all species measured. The most recent data, however, suggest declines in exposure. In the case of Barrow's goldeneye, 2009 data show little difference in average P4501A values between oiled and unoiled areas. Similarly, 2008 data from pigeon guillemots showed no difference between oiled and unoiled areas. However, black oystercatchers had slightly higher P4501A on oiled areas during 2008, and harlequin ducks in 2009 had considerably elevated P4501A on oiled areas, indicating that at least some bird species continued to be exposed to oil.

A study has been conducted recently that shows that other potential causes of P4501A expression, specifically polychlorinated biphenyls (PCBs), were not higher on oiled areas in harlequin ducks or sea otters. This finding indicates that P4501A results can be confidently interpreted as measures of exposure to lingering oil.

Potential effects of oil exposure in harlequin ducks were addressed by comparing survival of female ducks over the winter in oiled versus unoiled areas. Survival of females is an important factor determining duck population trends. During the mid-1990's, survival rates were significantly reduced in areas affected by the spill. A population model showed that this difference would lead to lack of population recovery in oiled areas. A study conducted during

2000-03, however, indicated that female winter survival rates in oiled areas had returned to normal and the population model indicated that duck populations should be on a trajectory to recover, with full recovery about 24 years after the spill. This appears to be corroborated by the latest surveys of wintering harlequin ducks, which show slight numerical increases in oiled areas. Further data will be needed to determine whether this trend will continue.

Additional studies are being conducted to determine whether there are adverse effects from oil exposure on harlequin ducks at the cellular level. These studies are still in progress.

B. Sea Otters

Sea otters often dig pits underwater in soft sediments to find prey. Digging pits in intertidal areas places them at risk of exposure to lingering oil. Recent otter studies are intended to evaluate whether otters are being exposed and adversely affected by lingering oil.

Exposure studies are designed to determine whether otters from areas affected by the spill continue to show exposure to oil. Recent studies have relied on measures of the induction of P4501A, as well as the expression of other genes that can be caused by oil exposure. These studies are currently underway.

Another exposure study was designed to determine the degree of sea otter use of intertidal zones, through use of time and depth recorders attached to otters. This study confirmed that foraging otters use intertidal habitat for at least some of their foraging, which means that they disturb sediments at tidal heights in which lingering oil remains. Moreover, pits dug by otters during feeding were found on beaches known to contain lingering oil.

Other studies are addressing the potential for adverse physiological effects of oil exposure on otters, through examination of gene expression related to immune response, tumor suppression, cellular stress-response and reproduction. This work is still in progress.

Population models have been used to estimate effects of oil on otter population dynamics. These models used demographic data, such as the age composition of otter carcasses, to conclude that otter populations remained depressed in heavily oiled areas up to at least 2005. The most recent data, collected in 2006-09, have not been incorporated into these models, however.

Recent surveys of otter populations show increasing numbers in the most heavily oiled areas of western Prince William Sound since 2003, in contrast to stable numbers prior to that period. Although current numbers are still below the estimated pre-spill population, this is some evidence of progress towards recovery.

C. Fish

Recent field studies also have examined two species of fish that use intertidal areas: masked greenlings and crescent gunnels, which had both shown evidence of exposure to oil in previous studies. In 2008, gunnels taken from areas that were heavily oiled during the spill continued to show elevated P4501A compared to those from unoiled areas. However, greenling did not show significant differences between oiled and unoiled areas during 2008. This difference between species is probably due to gunnels' greater use of intertidal sediments. As with the birds, reductions in levels of P4501A to background levels in some species suggest that conditions have improved.

IV. Restoration

A. Can the Lingering Oil Be Further Bio-degraded?

A microcosm study was designed to address the extent to which the lingering oil in Prince William Sound (PWS) is biodegradable given varying degrees of weathering. At the 2007AMOP conference, Exxon-Mobil consultants developed and presented an oil bioremediation index (BI) based on the degree of weathering of the oil contamination. They argued that, if the degree of weathering of oil is 70% or more, then further attempts to bioremediate it would be futile and not justified. To test this conclusion, the microcosm study collected samples of beach substrate from representative sites in PWS contaminated with oil residues with BI weathering index values of 76%, 60%, and 30%.

The results of the study demonstrated that the lingering oil is still very much biodegradable, regardless of the degree of weathering. Nutrient addition significantly stimulated biodegradation compared to natural attenuation. However, substantial biodegradation occurred in the natural attenuation microcosms, even without the addition of nutrients. This is likely due to relatively high levels of natural biogenic nitrogen that was found in the sediments.

The primary conclusion was that the reason for most of the observed biodegradation was the presence of excess dissolved oxygen, which was not present in the field. Nitrogen was a limiting factor, but oxygen was the primary one. This strongly indicates two points relating to remediation of lingering oil: first, that bioremediation appears to be a promising technology able to remove the persistent oil present in some locations in PWS, and second, any effective *in-situ* bioremediation treatment needs to introduce oxygen and nutrients in a manner that will increase their contact with the oil.

B. What Factors Are Limiting Further Bio-degradation of the Lingering Oil?

Before remediation methods can be developed to treat the lingering oil, the factors that are limiting the natural degradation of the oil must be understood. Therefore, a "limiting factors study" was designed to identify and compare the hydrogeological processes on gravel beaches with and without the presence of lingering subsurface oil. The field work for the initial study was conducted in 2007 and 2008, with supplemental field work in 2009.

One of the key findings of the 2007 and 2008 studies was that the beaches consisted of two layers: an upper layer with a very high permeability and a lower layer with a very low permeability. The contrast in permeability between the layers was found to be around three orders of magnitude. The dissolved nutrient concentration in the beaches was much smaller than that needed for maximal growth of microorganisms and the subsequent consumption of oil. Modeling suggested that the concentration of dissolved oxygen in the lower layer was most likely too low to sustain aerobic biodegradation.

The 2009 field program gathered important data about groundwater flow in the lower layer of the beaches. In designing effective *in-situ* remedial techniques, it is critical to know the flow rates in the lower layer, the "area of influence" of an injection well under different injection pressures, and the maximum injection pressure that can be reliably applied while maintaining well integrity. The 2009 study determined an allowable pressure range for injection into the lower layer, and showed that the area of influence after one day of pressure injection extended to a radius of 6-feet from the injection well. On Smith Island, introduction of tracer chemicals in the lower beach layer at ambient pressures determined the extent of influence to be 6 feet seaward and 1 foot landward in one day.

The results of the 2009 field work can be used to design pilot studies of the best approaches for *in-situ* remediation. The data on flow rates and area of influence in the lower layer can be used to design injection trenches or injection wells as a means of introducing oxygen and nutrients in the beaches. The data is used to design the distance between injection trenches or wells, the injection pressure for wells, and the amounts of oxygen and nutrients to be injected, whether by well or trench injection. It can also be used to design the distance between wells or injection trenches and monitoring locations.

V. Conclusions and Recommendations

The most significant remaining oil residues constitute approximately 50 beach segments that represent a total shoreline length of about 2.5 kilometers, although not all of the subsurface sediment within these segments would contain lingering oil. We can identify these 50 sites with a high degree of confidence.

The studies of the species most likely to have been affected by lingering oil present a generally consistent picture. Organisms that use the intertidal were severely affected by the spill and continued to show adverse effects from exposure to oil for many years after the spill. These effects manifested themselves in reduced survival rates and diminished populations. In recent years, however, there is evidence of improvement. The extent of oil exposure appears to be diminishing in most species, and there is evidence that the populations of some species are beginning to increase. Further monitoring will be needed to determine whether the environment is truly on a trend to complete recovery. Therefore, existing studies should be completed to verify these conclusions and there should be continued monitoring of sea duck and otter populations to determine whether current population trends continue.

The remaining lingering oil can significantly further bio-degrade under the right conditions, no matter how weathered the oil is. The key factor is to expose the lingering oil to oxygen; lack of exposure to oxygen is the primary reason that lingering oil remains. Techniques for exposing lingering oil to oxygen *in situ* have limitations, but studies to date have shown that these techniques have promise on beaches with certain physical characteristics. Pilot studies to test *in situ* remediation techniques could determine how effective these techniques would be, and whether wider employment of these techniques on beaches with lingering oil is warranted.

Finally, to focus remediation efforts, it would be useful to identify, at a minimum, those beaches that are both highly likely to contain lingering oil and are used by the species exposed to lingering oil. To that end, additional work is recommended to determine the spatial correlations between known and modeled distribution of lingering subsurface oil and data the Trustee Council has gathered on duck and otter populations (abundance, trends, intertidal habitat use and bioindicators of health).

Appendix

Summary of Lingering Oil Studies Funded by the *Exxon Valdez* Oil Spill Trustee Council

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INTRODUCTION

Since 2001, the *Exxon Valdez* Oil Spill Trustee Council (EVOSTC) has funded a number of studies to assess the amount and location of lingering subsurface oil, factors that might be causing its persistence, effects it may be having on natural resources, and feasible methods for remediation.

An early "lingering oil" project was a study conducted in 2001 by Jeff Short of the Auke Bay Laboratory, National Oceanic and Atmospheric Administration (NOAA). The study was conducted because, as of 2001, it was known that relatively unweathered oil remained at some locations that were heavily oiled initially and protected from dispersion by storm-generated waves. The extent of the remaining oil was unknown, and this uncertainty raised concerns about the effects that lingering oil may have had on humans and on fauna that may become exposed to the oil. The project sought to address these concerns by providing a quantitative estimate of the amount of shoreline (length, area, sediment and volume) that remained contaminated. Using a stratified random sampling design, Short et al. (2004) estimated that there were 7.8 hectares (range of 4.06-12.7) of remaining subsurface lingering oil in Prince William Sound (PWS). The mass of remaining subsurface oil was estimated at 55,600 kilograms (kg) (range of 26.1-94.4 kg).

Short et al. (2007) also estimated that the areal extent of oiled beaches in PWS had not changed significantly between 2001 and 2005, indicating that the rate of decline had slowed. No studies had been conducted to estimate the geographic extent of lingering subsurface oil in the Gulf of Alaska, although Irvine et al. (2006) documented the persistence of subsurface oil at specific sites along the Alaska Peninsula.

Following the work describing occurrence and extent of lingering oil, the EVOSTC funded the following studies to better understand the persistence and potential effects of the lingering oil:

- Modeling the distribution of lingering subsurface oil
- Indices of oil exposure in nearshore vertebrates:
    - Sea ducks

    - Other birds (black oystercatchers and pigeon guillemots)
    - Intertidal fish
    - Sea otters
- Consideration of alternative CYP1A inducers
- Sea otter spatial relations with lingering oil
- Potential effects of exposure to lingering oil – demographic:
    - Harlequin duck survival
    - Harlequin duck population model
    - Sea duck population trends
    - Sea otter survival
    - Sea otter population model
    - Sea otter population trends
- Potential effects of exposure to lingering oil – suborganismal effects:
    - Sea otters
    - Harlequin ducks
- Factors responsible for limiting the oil degradation rate on Prince William Sound beaches
- Biodegradability of lingering oil 19 years after the *Exxon Valdez* Oil Spill

This Appendix summarizes the key results of these studies. The objective is to provide a clear, concise explanation of the results of each study, in order to facilitate an understanding of the likely correlations between lingering oil and injuries.

MODELING THE DISTRIBUTION OF LINGERING SUBSURFACE OIL

Basis for the Study

The *Exxon Valdez* spill oiled approximately 2,000 kilometers (km) of shoreline. Short et al. (2004) estimated that about 0.2% of the spill volume remained stranded on the shoreline, primarily in the subsurface. But, they did not map out specifically the subsurface oil locations. It is not feasible to investigate every potential location for subsurface oil. Therefore, a study was conducted to determine whether a model could be constructed that relates shoreline characteristics that are known everywhere, to the presence of subsurface oil and shoreline characteristics known at a relatively few sampled locations. If so, it was hoped, the model then could be translated into a map predicting where subsurface oil may occur, with different degrees of accuracy. With such a map, it was thought, it would be possible to assess the feasibility of different cleanup methods, better determine potential effects, and inform the public on where it might encounter lingering oil. The draft final report is currently under review.

Study Approach and Methods

The study approach consisted of the following steps:

1. Build a preliminary model based on data from Short et al. (2004). They sampled 124 locations in PWS randomly selected from segments that were described during Shoreline

Cleanup Assessment Team (SCAT) surveys as heavily or moderately oiled at any time during the period from 1990 to 1993 and beaches described as heavily oiled during 1989 but that had only light to no oil impact during subsequent years. It is important to note that ***all*** of these segments would have a high probability of having lingering oil because of their initial degree of oiling. The data for these segments with subsurface oil were related to available geomorphic variables to generate a map predicting the presence of subsurface oil.

2. Use the preliminary map results to randomly select sites for field investigation in 2007 that:
    a. Were located in both PWS and the GOA, to expand the geographic scope
    b. Included all segments that were documented as being oiled so the model could be "trained" to know where the lingering oil did not occur and be able to predict oil locations throughout the oil-impacted areas
3. Conduct field sampling at 108 sites in PWS and 32 segments in the GOA using the same methods as Short et al. (2004).
4. Test the model performance with the new field data.
5. Review field data from all 264 sites that were sampled to identify the geomorphic and hydrologic factors that appear to be controlling the presence and absence of subsurface oil. Use these data to develop new predictor variables.
6. Refine the model using the new predictor variables, better model methodology, and at a higher spatial resolution.
7. Validate the refined model through additional field sampling in 2008, focusing on sites predicted to have the most oiling.
8. Use the refined model to generate maps and statistics for different degrees of oiling at different levels of accuracy.

Results

Geomorphic and Hydrologic Controls

A systematic review of all collected field data was conducted to summarize geomorphic controls on the persistence of subsurface oil. A number of factors were identified as being associated with ***increased*** likelihood of retaining lingering subsurface oiling, including:

- Low topographic slope
- Low exposure to wave action
- Armoring of gravel beaches
- Tombolos/natural breakwaters
- Rubble accumulations
- Subsurface hydrology
- Edge effects (transitions between permeable and impermeable shoreline types)

Similarly, a number of factors and morphologies were identified as being associated with ***reduced*** likelihood of retaining lingering subsurface oiling, including:

- Impermeable bedrock
- Platforms with thin sediment veneer
- Wide fine-grained gravel beaches
- Extensively treated bayhead beaches
- Steep exposed gravel beaches
- Low-permeability raised bay-bottom beaches
- Strong shallow groundwater flow
- Sandy tidal flats
- Proximity to a stream outlet

The report includes detailed discussion of these factors, which would be of particular value when identifying areas within priority segments to search for subsurface oil prior to implementation of treatment methods. It would guide field teams on sites to focus on within segments with a high probability for containing subsurface oil, as well as guide them on sites to avoid.

Subsurface Oil Thickness and Location in the Intertidal Zone

Figure 1 shows the average thickness and depth of burial for the different degrees of oiled sediments, for the 509 oiled pits (out of 12,357 pits dug) from all PWS surveys in 2001, 2003, and 2007. The heavier oiled sediments (HOR and MOR; see definitions in Figure 1) were thicker and nearer to the surface than the lightly oiled sediments (LOR, OF, and SH). Half of the oiled pits contained MOR. Figure 2 shows the number of pits (and by oiling degree) by tidal elevation; 25% of the pits with subsurface oil were in the upper intertidal zone, 70% were in the middle intertidal zone, and 5% were in the lower intertidal zone (zones = tidal range of 4.8 m/3).

Refined Model Results

The refined model included the following data-processing steps and variables:

1. A finer-scale topographic/bathymetry digital elevation model was generated and used to calculate intertidal slope and an index of local topographic complexity, as a measure of the influence of slope and natural breakwaters.
2. The shoreline type (based on the Environmental Sensitivity Index [ESI] data) was used to calculate an index of geomorphic complexity based on distance to an interface between a permeable and impermeable shoreline type (e.g., transition from a gravel beach to a rocky shore), as a measure of the edge effect and local sheltering.

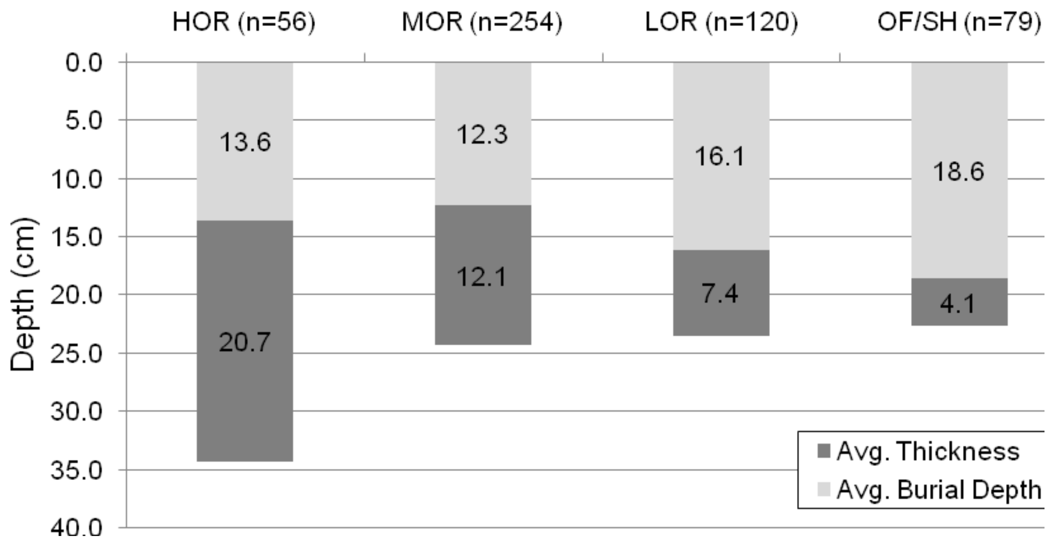

Figure 1. Average thickness of subsurface oiled layers and burial depth in cm below the surface by oiling descriptor based on data from pits in PWS surveyed in 2001, 2003, and 2007 (n=509). SO = surface oiling, SSO = subsurface oiling, OF = oil film, SH = sheen, LOR = lightly oiled residue, MOR = moderately oiled residue, and HOR = heavily oiled residue.

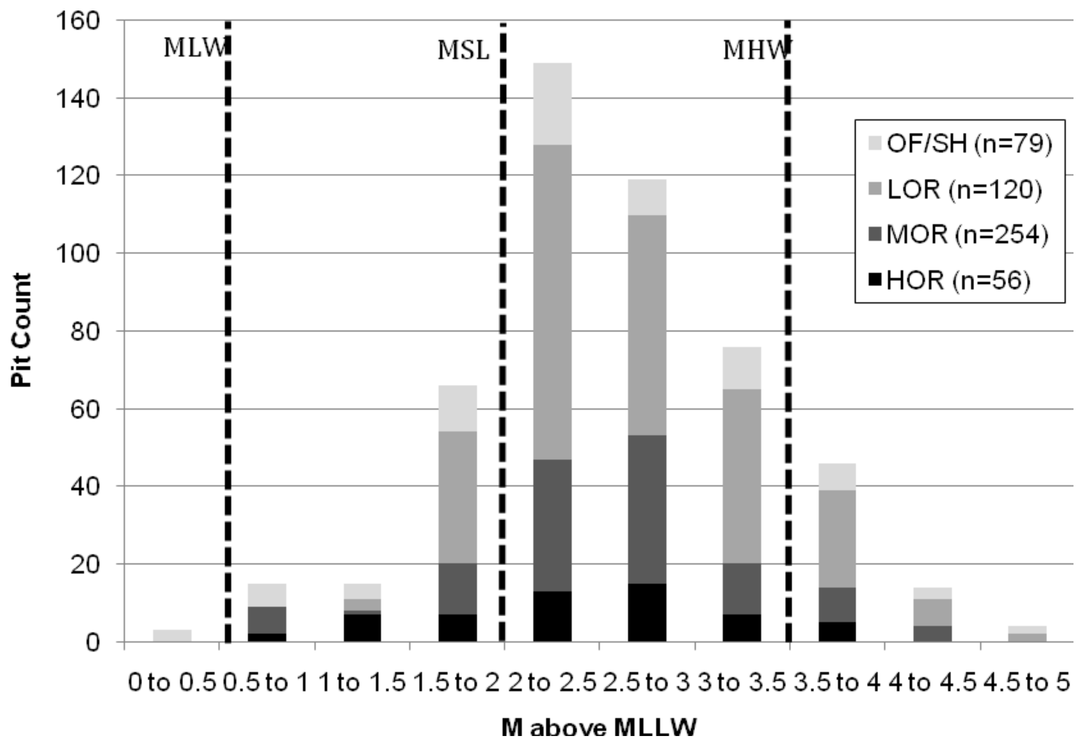

Figure 2. Counts of oiled pits by oiling descriptor by tidal elevation in meters above MLLW based on data from oiled pits in PWS surveyed in 2001, 2003, and 2007 (n=509). The elevations of MHW, MSL, and MLW are indicated on the chart.

3. An index of substrate permeability was developed based on the ESI shoreline types.
4. Distance to stream mouths was calculated, to reflect surface and ground water flow and the tendency for finer-grained sediments and higher sediment reworking.
5. Indices of shoreline convexity were calculated at scales of 50, 100, and 500 m, to reflect small-scale sheltering from wave energy.
6. Overwater distance to the release site was calculated, to reflect a general trend in decreased degree of oiling with distance from the source.
7. An oil-approach angle index was calculated, to reflect the orientation of the shoreline relative to the angle of the approach of floating oil slicks.
8. A quantitative index of oiling history based on SCAT oiling categories (No Oil, Very Light, Light, Moderate, and Heavy) for 1989, 1990, and 1991 was calculated.
9. Exposure to wave action was represented by fetch and an exposure index.
10. Inclusion of all the field data from 2001, 2003, and 2007, and six oiled sites in the GOA from Irvine et al. (1999, 2006).
11. A reduction in the spatial scale for all the variables in the model (from segments that were ~10-100 m in length to cells that were ~10 m in length).

The refined model was constructed using an ensemble of tree-based classifiers via Generalized Boosted Modeling (GBM) that can inherently handle non-linearities and interactions among multiple predictor variables. Model performance statistics showed excellent overall model performance. The oiling histories (e.g., SCAT oiling descriptors) were the most significant drivers, though all of the predictor variables played some role.

Different models were run for the following:

- Any subsurface oil
- Any oil equal to or greater than lightly oiled residues (LOR)
- Any oil equal to or greater than moderately oiled residues (MOR)
- Any oil equal to or greater than heavily oiled residues (HOR)
- Any oil equal to or greater than MOR and greater than 15% cover (representing oil in 1 of 6 pits in the column)
- Any oil equal to or greater than MOR and greater than 30% cover (representing oil in 2 of 6 pits in the column)
- Any oil equal to or greater than MOR and greater than 50% cover (representing oil in 3 of 6 pits in the column)

The results are shown in Tables 1-3 for different degrees of accuracy. The tables show number of cells (about 10 m in shoreline length), the total length in kilometers, and the number of "sites" defined as a group of cells within 100 m of each other. Looking at Table 1, under the "known" category, there are 393 cells, 2.85 km, and 67 sites with any kind of subsurface oil. These statistics were derived from only the actual field-based sampling. Based on the model predictions for the entire oil-impacted area (PWS and GOA), using the 90% positive predictive value threshold, there are 2,668 cells, 19.36 km, and 167 sites with any subsurface oil. Similar statistics are provided in Table 3 for the three different geographic regions: PWS, the Kenai Peninsula (KEN), and Shelikof Strait (SHL). Using a treatment threshold of any oil equal to or greater than MOR and the 90% positive predictive value threshold, there are 492 cells, 3.57 km, and 64 sites. Looking at Table 2, using treatment thresholds of any oil equal to or greater than MOR and greater than 15% frequency of oiled pits, at the 90% positive predictive value threshold, there are 361 cells, 2.62 km, and 52 sites. Using treatment thresholds of any oil equal to or greater than MOR and greater than 50% frequency of oiled pits, at the 90% positive predictive value threshold, there are 114 cells, 0.83 km, and 31 sites. The model could be used to predict the number of cells, kilometers of shoreline, and number of sites for different screening criteria and accuracy thresholds.

Table 4 shows the model-based estimates of area and mass from this study (10.51 ha of oiled area and 82.6 tons). These values are slightly higher but well within the 95% confidence intervals (CI) of the design-based estimates from the stratified-random sampling of Short et al. (2004) who estimated 7.80 hectares (ha) (95% CI: 4.06–12.7) and 55.6 tons (95% CI: 26.1–94.4). These calculations, like those of Short et al., include areas with subsurface oiling descriptors of oil film (OF) in the estimates of area, but not of mass (because oil film does not have significant amounts of measurable oil). The similarity of these two estimates is encouraging when evaluating overall model validity.

Figures 3 and 4 show histograms of the cumulative length of oiled shoreline per discrete contiguous site. These plots provide a descriptive summary of the estimated along-shore length of the subsurface oil patches meeting the type and relative amount criteria within these discrete sites. As can be seen in Figure 3, about 80% of all the sites with any subsurface oil are 10 m or greater in length. For sites with MOR or greater and 15% coverage, 32 of the 52 sites (62%) are 10 m or greater. The relatively small sizes of the heavier lingering oil sites is not of particular concern in terms of treatability given the results of the Boufadel et al. studies (discussed below), which indicate there likely are effective treatment methods for remediating small patches of subsurface oil.

Summary

This work sought to develop a suite of geospatial models to: (1) identify potential areas where subsurface oil is still present on the shorelines of PWS and GOA affected by the *Exxon Valdez* oil spill; and (2) differentiate between relative amounts of subsurface oil so that the models could be used as screening tools to prioritize shorelines for different remediation methods. The models were based on data collected at 314 shoreline segments that were surveyed between 2001 and 2008. These data allowed identification of a number of geomorphologic and hydrologic factors that have contributed to the persistence of subsurface oil within PWS and GOA two decades after the spill.

Because detailed data layers for each of these factors were not available, the model used existing data sets as surrogates to represent these factors, such as distance to a stream mouth or shoreline convexity. While the linkages between the data used and the physical phenomena that drive persistence are not clearly understood in all cases, the performance of these models was remarkably good. Just as a coastal geomorphologist would integrate all of the factors when evaluating a shoreline segment for the likelihood of having subsurface oil, the model

Table 1. Known and model-estimated shoreline raster cell counts, lengths, and discrete site counts by model version and model score cutoff for all subsurface oil (SSO), and the three oiling descriptors LOR, MOR, and HOR; discrete site defined as cluster(s) of shoreline locations with a given cutoff for a given model version and contiguous by less than 100 m.

Cutoff	All SSO			LOR or >			MOR or >			HOR or >		
	Cells	Length (km)	# Sites	Cells	Length (km)	# Sites	Cells	Length (km)	# Sites	Cells	Length (km)	# Sites
Known	393	2.85	67	353	2.56	57	200	1.45	37	66	0.48	17
90% PPV	2668	19.36	167	1598	11.59	113	492	3.57	64	304	2.21	47
80% PPV	4829	35.04	276	3176	23.04	194	885	6.42	105	405	2.94	49
70% PPV	6832	49.57	321	4439	32.21	235	1560	11.32	147	807	5.86	88

Table 2. Known and model-estimated shoreline raster cell counts, lengths, and discrete site counts by model version and model score cutoff for all MOR, and three different frequencies of MOR occurrence; discrete site defined as cluster(s) of shoreline locations with a given cutoff for a given model version and contiguous by less than 100 m.

Cutoff	MOR or >			MOR or > and > 15% Cover			MOR or > and > 30% Cover			MOR or > and > 50% Cover		
	Cells	Length (km)	# Sites	Cells	Length (km)	# Sites	Cells	Length (km)	# Sites	Cells	Length (km)	# Sites
Known	200	1.45	37	160	1.16	32	97	0.71	19	35	0.25	6
90% PPV	492	3.57	64	361	2.62	52	122	0.89	22	114	0.83	31
80% PPV	885	6.42	105	702	5.09	75	493	3.58	55	198	1.44	42
70% PPV	1560	11.32	147	1024	7.43	98	1205	8.74	126	221	1.60	53

Table 3. Known and model-estimated shoreline raster cell counts, lengths (km), and discrete site counts by region and model score cutoff for the binary all surface oil model for the entire study area, PWS, Outer Kenai Peninsula (KEN), and Shelikof Strait (SHL); discrete site defined as cluster(s) of shoreline locations with a given cutoff for a given model version and contiguous by less than 100 m.

Cutoff	All SSO - Total			All SSO - PWS			All SSO - KEN			ALL SSO - SHL		
	Cells	Length (km)	# Sites	Cells	Length (km)	# Sites	Cells	Length (km)	# Sites	Cells	Length (km)	# Sites
Known	393	2.85	67	349	2.53	60	3	0.02	2	41	0.30	5
90% PPV	2668	19.36	167	2223	16.13	138	196	1.42	14	249	1.81	15
80% PPV	4829	35.04	276	4040	29.31	227	350	2.54	24	439	3.19	25
70% PPV	6832	49.57	321	5660	41.07	261	497	3.61	30	675	4.90	30

Table 4. Model-estimated intertidal area (ha) and oil mass (metric tons [t]) for PWS. Results also presented for the study area of Short et al. (2004) for comparison. Results are not presented for the GOA regions due to lack of reliable descriptor-specific model version output outside of PWS.

Region	All SSO		LOR or >		MOR or >		HOR or >	
	Area (ha)	Mass (t)	Area (ha)	Mass (t)	Area (ha)	Mass (t)	Area (ha)	Mass (t)
PWS – Heaviest Initial Oiling per Short et al. (2004)	10.51	82.6	5.19	27.00 31.2	24.48 2.4	27.0	1.2	24.5
PWS Entire Oil-Impacted Shoreline (Michel et al. 2009)	12.4	97.2	6.1	36.6	2.9	31.7	2.9	28.8

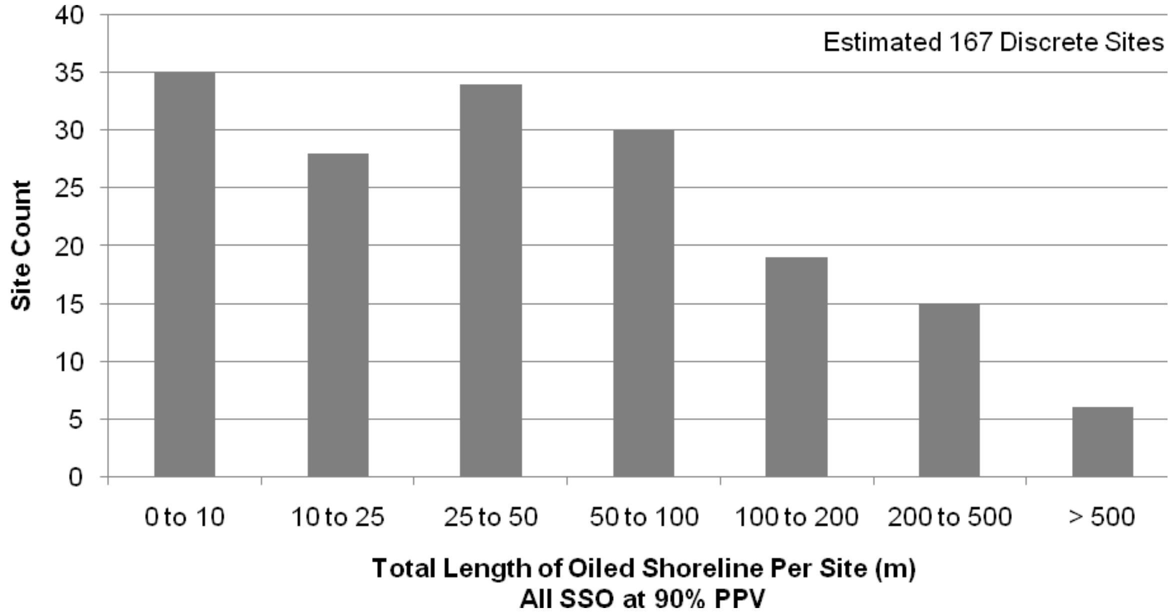

Figure 3. Histogram of total cumulative length of oiled shoreline length per discrete contiguous site for all regions; discrete site defined as cluster(s) of shoreline locations with a cutoff value above the 90% PPV for the binary oiled vs. unoiled model version and contiguous by less than 100 m.

Figure 4. Histogram of total cumulative length of oiled shoreline length per discrete contiguous site for all regions; discrete site defined as cluster(s) of shoreline locations with a cutoff value above the 90% PPV for the MOR or > and > 15% cover and contiguous by less than 100 m.

simultaneously evaluates all identified variables to make a similar assessment in a rigorous, unbiased manner. The model results suggest there are a limited but significant number of as-yet unsurveyed locations in PWS and GOA that are likely to contain lingering subsurface oil. Furthermore, the model results may be used to quantitatively prioritize shoreline locations for investigation with known uncertainty.

It should be noted that regional differences in physical environment and oiling history, and sparser recent data, mean that extra caution should be used in interpreting model results in the GOA region. With understanding of these limitations, these model results can be used by educated field practitioners in concert with the factors that increase or decrease the likelihood of subsurface oil within a given shoreline segment. Used as such, the models presented here are capable of predicting the distribution of the lingering oil across the entire spill area with sufficient accuracy and resolution to perform as useful tools for evaluating potential ongoing impacts to users of these shorelines and prioritizing shoreline locations for potential remediation based on various screening criteria.

GENERAL INTRODUCTION ON BIOLOGICAL EFFECTS OF LINGERING OIL

The studies described in this section were funded to consider the issues of (1) exposure of vertebrates to the lingering oil, and (2) consequences of any exposure, as well as how both of these have changed over time. These studies have focused largely on two species, sea otters and harlequin ducks, which had been shown to have reduced abundance, density, and/or survival in oiled areas during the mid to late 1990s, coincident with evidence of exposure of some vertebrates to oil (Bodkin et al. 2002, Esler et al. 2002). More recently Barrow's goldeneyes, black oystercatchers, pigeon guillemots, crescent gunnels, and masked greenlings have been studied, because they also are vertebrates that inhabit intertidal areas and, thus, might be at risk of exposure to residual oil.

EXPOSURE OF VERTEBRATES TO LINGERING OIL

A number of studies have indicated exposure to, or uptake of, hydrocarbons by animals in areas of PWS oiled by the 1989 *Exxon Valdez* oil spill (Jewett et al. 2002 and citations therein). Some of these indicators declined relatively quickly post-spill, but others persisted for a least a decade (Trust et al. 2000, Jewett et al. 2002). In this section, measures of exposure in several vertebrates are summarized for the second decade post-spill.

One of the most useful tools for evaluating exposure to lingering oil has been the use of bioindicators of induction of cytochrome P4501A (CYP1A). CYP1A is a member of the cytochrome P450 family of genes, which are induced by exposure to certain compounds and play an important role in oxidation of those compounds. In the case of CYP1A, induction results from exposure to specific PAHs, including those found in crude oil, and halogenated aromatic hydrocarbons, including polychlorinated biphenyls (PCB) and dioxins. Because the latter compounds do not occur in high concentrations in PWS and there are no other broad-scale sources of bioavailable PAH contamination (see below), CYP1A has been interpreted as an indicator of spatial and temporal variation of exposure of vertebrates to residual *Exxon Valdez* oil. CYP1A induction may or may not indicate deleterious effects on individuals or populations (see below), but it does serve as a reliable measure of exposure to inducing compounds.

Sea Ducks

Basis for the Studies

Two species of sea ducks, harlequin ducks and Barrow's goldeneyes, have been examined for indicators of elevated induction of CYP1A on oiled areas relative to unoiled areas. Both of these species forage on benthic invertebrates in intertidal and shallow subtidal zones, placing them at risk of exposure to lingering *Exxon Valdez* oil. A study of sea duck CYP1A induction during 1996 to 1998 (Trust et al. 2000) found significantly elevated indicators of CYP1A induction in oiled areas in both species. More recent work has examined CYP1A induction in both species up to 2009, to track the timeline of exposure to lingering oil.

Study Approaches and Methods

For sea ducks, induction of CYP1A was estimated by measurement of hepatic 7-ethoxyresorufin-O-deethylase (EROD) activity, a method that has been widely used in many taxa, including birds. This method required small liver samples, which were surgically removed from anesthetized birds captured in oiled and unoiled areas of PWS. Sea ducks were captured in floating mist nets in a number of areas oiled during the *Exxon Valdez* spill, including Bay of Isles, Herring Bay, Crafton Island, Falls Bay, Green Island, and Foul Pass. Also, birds were captured on nearby northwestern Montague Island and Culross Passage, which were not oiled and thus were considered reference sites.

Since the results reported by Trust et al. (2000), harlequin ducks have been captured in November 2000, November 2001, November 2002, March 2005, November 2006, March 2007, and March 2009. Sample sizes and other details for 2000 through 2002 samples are found in Esler and Iverson (2010); for 2005 through 2009, details are in Esler et al. (in press). For Barrow's goldeneyes, captures following up on the work of Trust et al. (2000) occurred during March 2005 and March 2009.

Measures of EROD activity were conducted at two labs: Woods Hole Oceanographic Institution (WHOI; 1998-2005) and the University of California Davis (UCD; 2005-2009). During one sampling period (March 2005), a comparison of results between labs using paired samples from the same individual harlequin ducks confirmed that the labs were producing similar results. EROD activity is expressed in picomoles per minute per milligram of protein, i.e., pmol/min/mg protein. To contrast or combine EROD activity data across years, a standardizing index of CYP1A values was created, in which average EROD activity in unoiled areas for a given year and species was set to 1, and all values were adjusted accordingly within the same sample year (Esler and Iverson 2010).

Results

Harlequin Ducks

Harlequin ducks consistently had elevated CYP1A induction (indicated by EROD activity) in oiled areas relative to unoiled areas from 2000 through 2009 (Figure 10), similar to the findings of Trust et al. (2000) for samples from 1998. For the years 2000, 2001, and 2002 combined, the index to CYP1A induction averaged significantly higher in oiled areas than in unoiled areas (Esler and Iverson 2010). Similarly, findings from 2005 through 2009 (Esler et al. in press) indicated higher EROD activity on oiled areas than unoiled, with no effects of age, sex, body mass, or season. Figure 10 shows the average EROD activity index across study years on oiled areas, which are all well above the average on unoiled areas by factors between 2 and 5.

As another metric of changes in exposure to oil over the years, proportions of captured individuals with elevated EROD activity were calculated. Elevated EROD activity was defined as any individual with ≥ 2 times the average EROD activity on unoiled areas within that year (or group of years in the case of 2000-02). The proportions of harlequin ducks from oiled areas with

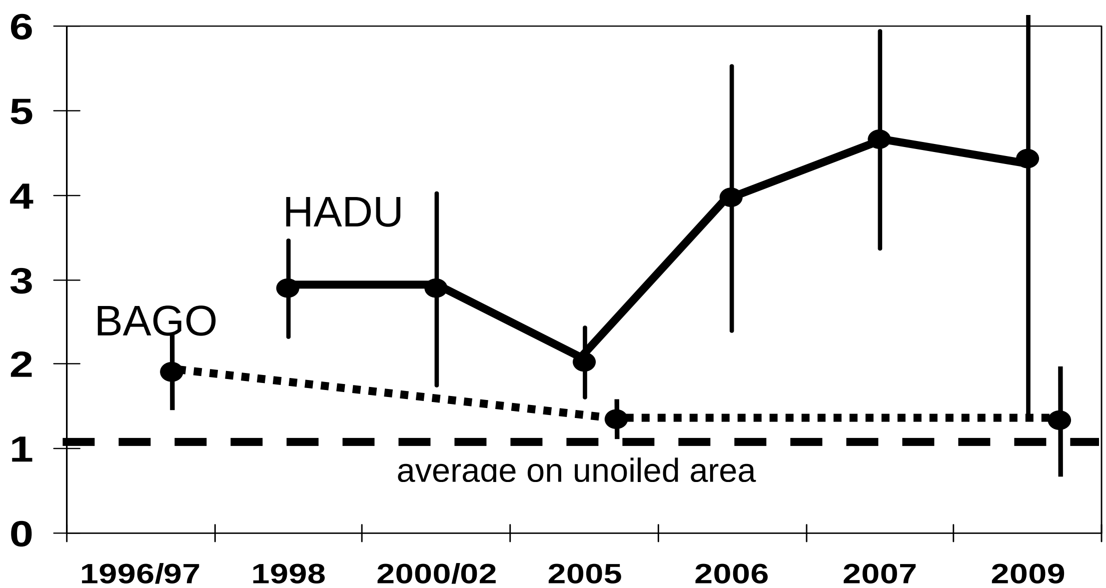

Figure 10. Average (± 95% C.L.) index of CYP1A induction (measured as EROD activity) of harlequin ducks (HADU) and Barrow's Goldeneyes (BAGO) captured on areas oiled during the *Exxon Valdez* spill, relative to unoiled areas, where the average is standardized to 1.0.

elevated EROD activity (Figure 11) were consistently above 0.20, and above 0.40 in every year except 2009, whereas the proportions from unoiled areas were always below 0.20. The drop in the proportion of harlequin ducks with elevated EROD activity in 2009 is an encouraging sign of potential progress towards recovery. Recent lab studies (see below) indicate that harlequin ducks have particularly sensitive CYP1A responses. This may result in higher and more variable levels of endogenous expression (i.e., in unoiled areas) and stronger responses when exposed to an inducing compound.

Barrow's Goldeneyes

Patterns of indicators of CYP1A in Barrow's goldeneyes differed from those of harlequin ducks. Average hepatic EROD activity of Barrow's goldeneyes differed between oiled and unoiled areas during 2005, after accounting for effects of sex, age, and body mass, although the magnitude of the difference between areas was reduced relative to the Trust et al. (2000) findings from winter 1996-97. By 2009, average EROD activity was similar between oiled and unoiled areas, with subtle relationships between EROD and sex and mass. The patterns are illustrated in Figure 10, which shows the decline in the average CYP1A index on oiled areas from 1996-97 through 2009, and the point estimate in 2009 with confidence intervals that overlap the average from the unoiled area.

Consistent with comparisons of averages, the proportion of Barrow's goldeneye individuals captured on oiled areas with elevated EROD activity declined from 41% in winter 1996/97 to 10% and 15% in 2005 and 2009, respectively (Figure 11). No individuals captured in unoiled areas showed elevated EROD in any year.

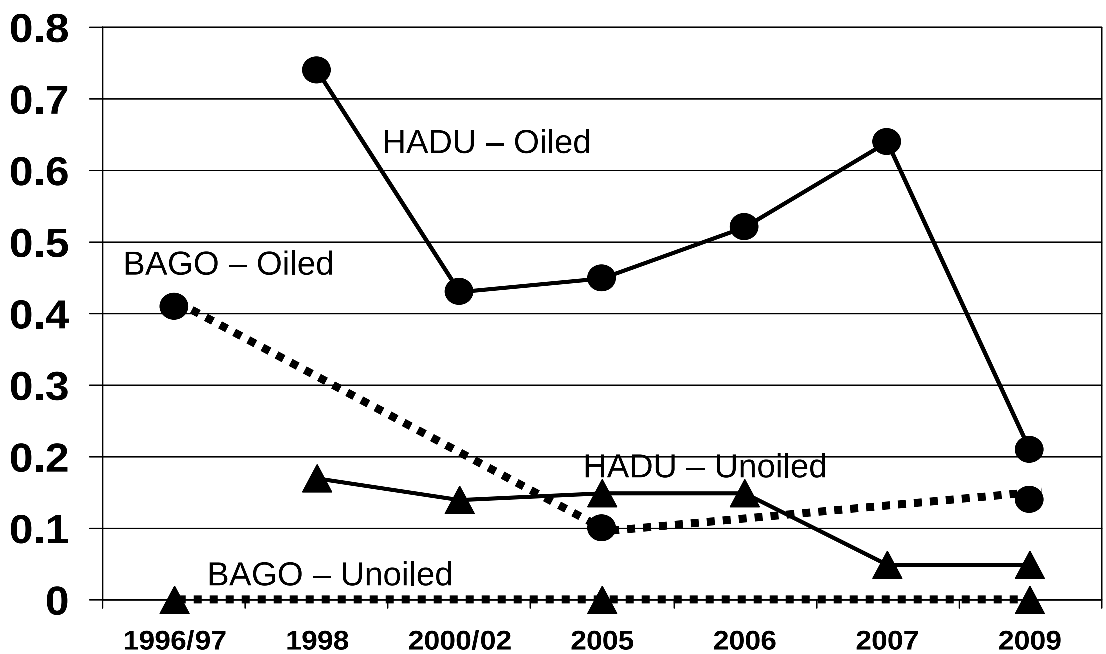

Figure 11. Proportion (y-axis) of individuals with elevated EROD activity, defined as ≥ 2 times the average on unoiled areas for that species and time period. HADU = harlequin duck and BAGO = Barrow's goldeneye.

Summary of the Sea Ducks Exposure Studies

Indicators of CYP1A induction (EROD activity) showed that harlequin ducks from areas of PWS oiled by the *Exxon Valdez* spill were exposed to inducing compounds through 2009. Given the low likelihood of other potential inducers (see below), this is interpreted as evidence of continued exposure to lingering oil. Barrow's goldeneyes also had higher EROD activity in oiled areas during 2005, but not 2009. These patterns are corroborated by examination of the proportion of individuals with elevated EROD activity. The reduction in EROD responses to near reference levels in Barrow's goldeneyes by 2009, as well as the decline in the proportion of harlequin ducks with elevated EROD, suggest that exposure to lingering oil is declining over time in these sea duck species that are highly susceptible to exposure. The timeframe over which exposure occurred (e.g., at least 2 decades in harlequin ducks) was unanticipated at the time of the spill, but the evidence of progress towards cessation of exposure is encouraging.

Exposure Studies of Other Birds

Basis for the Study

Two other bird species, black oystercatchers and pigeon guillemots, were examined for EROD activity indication of elevated CYP1A induction in oiled areas of PWS relative to unoiled areas during 2004. Both of these species feed in intertidal zones where lingering oil occurs, although oystercatchers feed largely on benthic invertebrates, whereas guillemots feed on both fish and invertebrates. Oystercatcher CYP1A induction had not been examined previously, but they were thought to be at risk of exposure due to their habitat and prey preferences. Guillemot adults, but not pre-fledging chicks, showed elevated EROD activity on oiled areas in 1998/99. Guillemot adults may have been exposed to oil during foraging activities in intertidal zones or through consumption of invertebrates, which (unlike fish, see below) tend to accumulate rather than metabolize hydrocarbons.

Study Approach and Methods

Liver biopsies were surgically collected from adult pigeon guillemots captured on nests in the Naked Island group (oiled; n = 25) and Jackpot and Icy Bays (unoiled; n = 19) during June 2004. Samples were immediately frozen in liquid nitrogen and shipped to Woods Hole Oceanographic Institution for analysis of EROD activity.

Similarly, adult black oystercatchers were captured on nests on Knight and Green Islands (oiled; n= 24) and Montague Island (unoiled; n = 20). Surgeries, sample collection, and lab analysis followed those of sea ducks and guillemots.

Results and Summary of Exposure Studies of Other Birds

Adult pigeon guillemot EROD activity did not differ between oiled and unoiled areas of PWS. In contrast, average EROD activity of adult black oystercatchers was slightly (but statistically significantly) higher on oiled areas than unoiled, by a factor of 1.5 (Golet et al. 2002).

By 2004, pigeon guillemots did not show evidence of exposure to lingering Exxon Valdez oil, suggesting that conditions had improved since the previous measure in 1999. However, black oystercatchers had higher EROD activity in oiled areas of PWS, suggestive of exposure to lingering oil. This is consistent with findings from several other species that occur in intertidal zones and consume benthic invertebrates (see above and below).

Exposure Study of Intertidal Fish

Basis for the Study

Jewett et al. (2002) examined several indicators of hydrocarbon exposure in two species of intertidal fish during 1998 and 1999. All of their measures, including hepatic EROD activity as an indicator of CYP1A induction, were elevated in either masked greenlings or crescent gunnels collected from oiled areas, relative to those collected in unoiled areas. These results were interpreted to indicate exposure of these benthic-feeding, intertidal fish to lingering *Exxon Valdez* oil for at least a decade following the spill. Recent studies funded by the Trustee Council were designed to replicate this work and evaluate exposure of benthic fish to lingering oil during summer 2008, nearly two decades after the spill.

Study Approach and Methods

Both species of intertidal fish were collected in areas considered previously to be heavily oiled, moderately oiled, and unoiled; these were northern Knight Island, Prince of Wales Passage and, for gunnels, northwestern Montague Island, whereas for greenlings, the unoiled area was Parry Island. Greenlings were caught by hook and line, and gunnels were collected from the intertidal during minus tides. Fish were anesthetized, and livers removed and frozen immediately in liquid nitrogen. Lab analysis of hepatic EROD activity was conducted at the University of California Davis following standard procedures.

Results and Summary of the Exposure Study of Intertidal Fish

A one-way analysis of variance for gunnels ($n = 43$) indicated that fish from heavily oiled areas had significantly higher hepatic EROD activity than those from unoiled areas in 2008. Average EROD activity from heavily oiled sites was 2.8 times that on the unoiled site. Gunnels from the moderately oiled area did not differ from those from unoiled sites.

In contrast, results for greenling ($n = 42$) indicated that average EROD activities of individuals from both heavily and moderately oiled areas did not differ from the average from unoiled areas.

Similar to results described for sea ducks, above, EROD activity indicated significant exposure to CYP1A inducing compounds on oiled areas for one intertidal fish (gunnels) but not another (greenling). EROD is a sensitive and specific measure of exposure, and significantly higher activity in gunnels from oiled areas is interpreted as evidence of continued exposure to lingering *Exxon Valdez* oil nearly two decades after the spill. Lack of elevated EROD in greenlings suggests that exposure to oil in that species has diminished to reference levels. Gunnels are more closely associated with intertidal sediments than greenling, which may explain observed interspecific differences.

Sea Otters Exposure Study

Basis for the Study

As benthic foragers that excavate large numbers of pits in soft sediments, including in intertidal zones where lingering oil occurs (see below), sea otters are at risk of exposure to residual *Exxon Valdez* oil. Sea otters have been sampled since the mid-1990's for biomarker studies; however, the approach used to measure CYP1A in otters has proven to be methodologically challenging and, unfortunately, data that were generated are not considered to be reliable. However, efforts funded by the Trustee Council are underway to test alternative, newer methods for reliably measuring biomarkers of oil exposure that can be applied to recent and archived samples.

Study Approach and Methods

A method for measuring differential gene expression of sea otters in response to hydrocarbon exposure has been developed; this approach is based on research that was conducted with captive mink dosed with hydrocarbons (Bowen et al. 2007). The array of genes that is evaluated includes CYP1A1 and aryl hydrocarbon receptor (AhR), which are both measures of hydrocarbon exposure, as well as other genes that may vary in expression following hydrocarbon exposure, and which may play roles in various aspects of physiological responses and consequences of that exposure (see below).

Results and Summary of the Sea Otter Exposure Study

This work is in progress, and results of comparisons of gene expression, including those that would indicate oil exposure, are not yet available to report. However, to date, methods have been successful and are being applied to recently collected samples. These same approaches will be tested for samples that have been archived from as far back as the mid-1990s, with the intent of providing a longer timeline of variation in exposure to oil.

POTENTIAL CONFOUNDING CYP1A INDUCERS

Basis for the Study

Several studies have contrasted CYP1A induction in vertebrates between areas oiled by the *Exxon Valdez* spill and unoiled areas. In many of these studies, indicators of CYP1A induction have been elevated in animals from oiled areas; this pattern has been interpreted as evidence of continued exposure to lingering oil (e.g., Trust et al. 2000, Jewett et al. 2002, Esler et al. 2010). Although CYP1A is induced by a limited number of compounds, there is the potential that vertebrates are being exposed to inducing chemicals other than *Exxon Valdez* oil.

Hydrocarbons from sources other than the *Exxon Valdez* oil spill occur in PWS. These include local-scale contamination associated with boat harbors, residual oil in the form of tar balls remaining from releases caused by the 1964 earthquake, and natural hydrocarbons. However, Short et al. (2004) estimated that the vast majority of hydrocarbons on beaches in oiled areas originated from the *Exxon Valdez* spill. Therefore, observed patterns in temporal and spatial distribution of CYP1A induction are not reasonably explained by other hydrocarbon sources.

Another family of compounds known to induce CYP1A is polychlorinated biphenyls (PCB). A recent Trustee Council-funded study analyzed data on PCB concentrations in blood plasma of sea otters and harlequin ducks to determine whether the distribution and concentrations of PCB were a plausible explanation for observed induction of CYP1A in vertebrates from areas of PWS that were oiled during the *Exxon Valdez* spill. Objectives were to determine: 1) concentrations and composition of PCBs in blood plasma of sea otters and harlequin ducks inhabiting oiled and unoiled areas in PWS; 2) PCB composition relative to recent or point source exposure; and 3) PCB concentration relative to reproductive status and age in sea otters.

Study Approach and Methods

Blood plasma was collected from sea otters and harlequin ducks that were captured during 1998; samples included 18 otters and 10 harlequin ducks from oiled areas and 10 of each species from unoiled areas. Plasma samples were analyzed for total PCBs, as well as concentrations of more than 90 specific congeners. Data were analyzed to evaluate differences between areas for four groupings of congeners: 1) Total PCBs comprised all congeners analyzed; 2) the sum of several congeners known to strongly induce CYP1A; 3) a panel of congeners recommended for monitoring in marine environments as indicators of bioaccumulative PCB pollution by the International Council for the Exploration of the Sea (ICES); and 4) congener 138 because of its recalcitrant nature and because it was the only congener detected in all samples.

Results

PCB concentrations in harlequin duck blood plasma were similar between oiled and unoiled areas for all groupings of congeners (Figure 12). For sea otters, concentrations of total PCB and CYP1A-inducing PCB differed between areas, although not in a direction that would explain elevated CYP1A induction observed in many vertebrates in oiled areas of PWS. In this case, PCB concentrations were higher for sea otters from unoiled areas (Figure 12).

Most measures of PCB concentrations did not differ by sea otter age or pup dependency status. However, concentrations of ICES congeners were significantly lower in females with dependent pups than those without pups. Also, PCB concentrations were not correlated with any plasma chemistry factors.

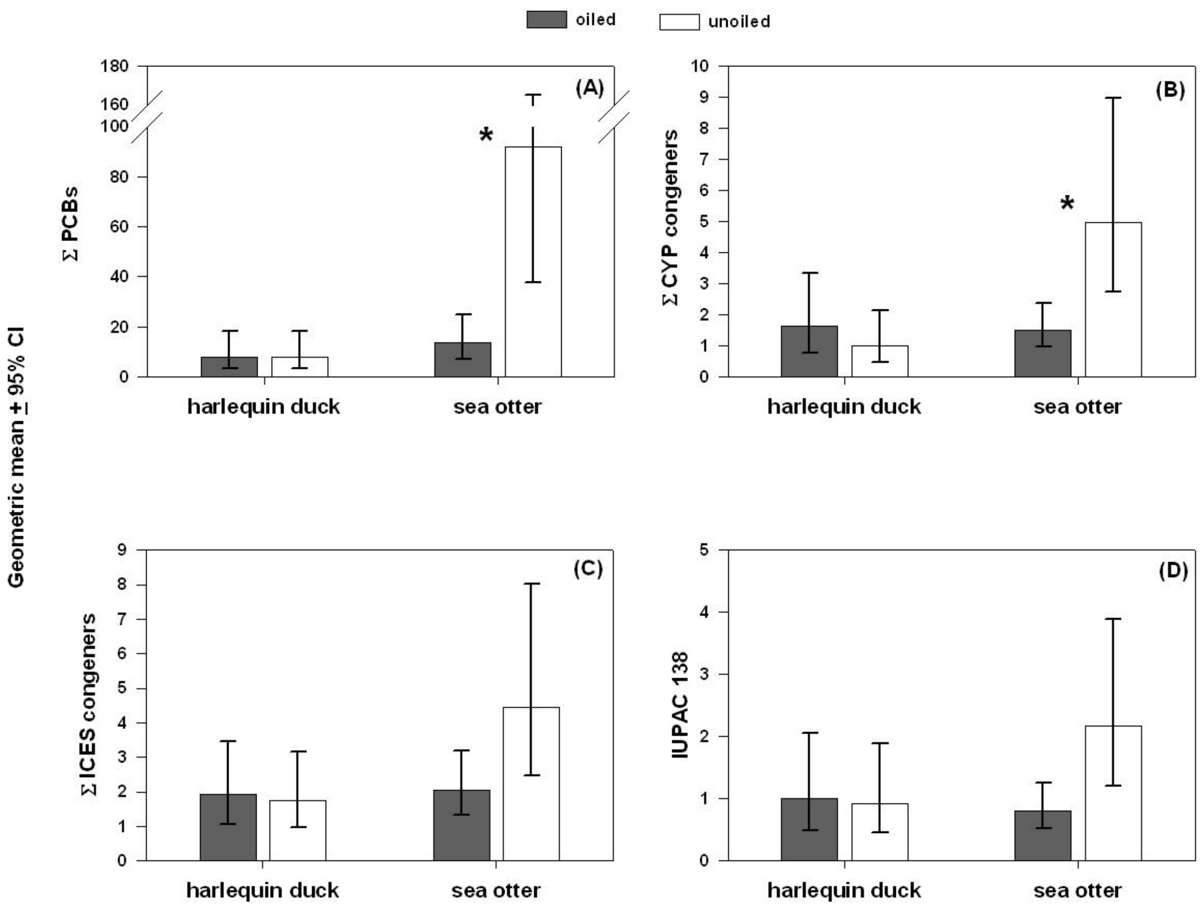

Figure 12. Back-transformed geometric mean concentrations (ng/g, wet weight) and 95% confidence intervals for PCBs in blood plasma samples from harlequin ducks and sea otters inhabiting oiled and unoiled areas of PWS, 1998. PCBs are grouped according to the sum of (A) 93 congeners, (B) non-, mono, and di-*ortho* congeners capable of inducing the cytochrome p450 (CYP) mixed function oxygenase system, (C) IUPACs 28, 52, 101, 118, 138, 153, 180 recommended for monitoring in marine environments by the International Council for the Exploration of the Sea, and (D) IUPAC 138. Asterisks above groups denote significant ($P < 0.05$) differences determined by ANOVA. Note variable y-axis scales.

Summary

Overall, concentrations of PCBs were low in harlequin ducks and sea otters from PWS. It is unlikely that PCBs exerted immunotoxic or physiological effects on the species in the study. Further, the patterns of PCB composition in sea otters and harlequin ducks did not suggest recent exposure to persistent PCBs but rather exposure to distantly derived, weathered PCBs.

Because PCB concentrations were similar between oiled and unoiled areas of PWS or higher in unoiled areas for some groupings of PCBs in sea otters, PCBs do not explain observed patterns of CYP1A induction described above for some species, namely higher induction in areas oiled during the *Exxon Valdez* spill. Combined with considerations of sources of hydrocarbons on beaches (Short et al. 2004), it is unlikely that there are any compounds confounding interpretation of CYP1A induction as an indicator of exposure to lingering *Exxon Valdez* oil.

SEA OTTER SPATIAL RELATIONS WITH LINGERING OIL

Basis for the Study

Much of the lingering oil from the *Exxon Valdez* spill is found in sediments in the middle and lower intertidal zones (see above). The degree of risk of exposure to that oil incurred by sea otters depends on their degree of use of the intertidal zone for foraging. This issue was addressed by measuring dive depth distributions and foraging effort using time-depth recorders, surveying beaches for evidence of sea otter foraging (pits), and an experiment to evaluate how long pits persist on beaches to help interpret findings from the pit survey.

Study Approach and Methods

In 2003 and 2004, sea otters at northern Knight Island were captured and instrumented with archival time-depth recorders (TDRs), which were programmed to record depth at 2-second intervals. In 2004 and 2005, instrumented otters were targeted for recapture to retrieve the devices and access archived information of numbers and depths of dives. Of the 28 devices deployed, 16 were recovered from 12 adult females and 4 adult males. Numbers of dives, depth of dives, and the proportions of dives that occurred in the intertidal zone were calculated from the TDR data.

Surveys for sea otter foraging pits were conducted during negative tides in summer 2008 at soft sediment beaches of the northern Knight Island archipelago. At each beach segment, the entire area of exposed intertidal soft sediments was searched for evidence of sea otter foraging; areas with foraging pits were mapped using GPS and the density of pits and tidal elevation were recorded.

Experimental pits were dug to simulate those created by foraging sea otters, to evaluate their persistence over time. Pits were dug in soft sediment beaches within the northern Knight Island archipelago during April – June 2008, using an experimental design that allowed consideration of different exposures and intertidal elevations. Sites were revisited regularly during negative tide cycles and the status of each pit was documented.

Results

Based on the TDR data, individual sea otters averaged 197 foraging dives per day, of which an average of 13% were in the intertidal zone; taken together, roughly 25 foraging dives are estimated to occur in the intertidal zone per sea otter per day, on average. Females tended to dive more often and in shallower water, including intertidal zones, than males. Season also had an effect, with both sexes foraging in intertidal areas most frequently during spring.

Natural sea otter pits were detected in the intertidal areas of most of the soft sediment beaches (71%) surveyed in the northern Knight Island archipelago, including some that were known to have lingering *Exxon Valdez* oil. Further, the proportion of the entire length of soft sediment beaches that had evidence of sea otter pits was 54%. Most of these were in the lower intertidal (-0.5 to 0.5 m), although some pits were found at all tidal elevations.

Pit persistence was related to intertidal elevation; by September (3 to 5 months after pits were excavated), 56%, 42%, and 20% of pits were detectable at 0 m, 1 m, and 2 m, respectively. By March 2009, almost all (97%) of the pits at all tidal elevations were gone, supporting a conclusion that natural pits observed on beaches are indicative of sea otter foraging activity that has occurred recently, on a scale of months.

Summary

Results of these studies demonstrate that sea otters definitely forage in intertidal zones, including the tidal elevations and specific beaches that harbor lingering *Exxon Valdez* oil. Given the average number of daily intertidal dives estimated for foraging sea otters, observations of extensive foraging in soft sediment beaches of northern Knight Island, and the apparent lack of avoidance of beaches known to contain lingering oil, exposure of some individuals to residual *Exxon Valdez* oil is likely, corroborating estimates by Short et al. (2006). As a related note, the large amount of sediment turnover caused by sea otter foraging likely serves as a form of bioremediation, exposing residual oil to weathering and dispersal.

CONSEQUENCES OF EXPOSURE OF VERTEBRATES TO LINGERING OIL

As described above, a number of vertebrates have been shown to have been exposed to lingering oil during the second decade post-spill, with some species continuing to show evidence of exposure through 2009. In this section, studies funded by the Trustee Council that consider potential effects of exposure to lingering oil are summarized. These include studies of population-level demography (e.g., survival rates and population trends), as well as studies examining potential deleterious effects at the individual or sub-organismal levels.

DEMOGRAPHIC RELATIONSHIPS WITH LINGERING OIL

HARLEQUIN DUCKS: SURVIVAL

Basis for the Study

In the mid- to late-1990s, nearly a decade after the 1989 *Exxon Valdez* oil spill, winter survival of female harlequin ducks was lower in oiled areas of PWS relative to unoiled areas (Esler et al. 2000). This observation was coincident with evidence of exposure to lingering oil, and the survival differential was determined to be sufficient to cause different population trajectories and lack of recovery in oiled areas. A more recent study evaluated female survival during winters 2000-01 to 2002-03 to determine whether differential survival persisted and to evaluate whether individual-level indices of oil exposure were related to survival (Esler and Iverson 2010).

Study Approach and Methods

Female harlequin duck winter survival was measured using radio telemetry. In the most recent studies, females were captured on oiled and unoiled areas during Novembers of 2000, 2001, and 2002 and equipped with implanted VHF radio transmitters. While under anesthesia, a liver biopsy was removed from each individual to be used to evaluate CYP1A induction (see above). Radioed individuals were tracked by airplane through March. Variation in survival was analyzed in relation to area (oiled versus unoiled), age class (hatch-year versus after-hatch-year), season (mid-winter [December and January] versus late-winter [February and March]), and a measure of CYP1A induction.

Results

In contrast to earlier studies (Esler et al. 2000), survival during winters 2000/01 through 2002/03 did not differ by area. Also, individual measures of oil exposure (CYP1A) were not related to survival probability. These results were interpreted to indicate that exposure to lingering oil was no longer affecting gross demographic attributes of the harlequin duck population in oiled areas of PWS. Effects of age and season were evident (Figure 13) and consistent with previous studies, with younger females having poorer survival and mid-winter survival lower than late-winter.

Summary

Despite evidence of continued exposure to lingering *Exxon Valdez* oil in harlequin ducks (see above), winter survival rates were similar between oiled and unoiled areas within PWS during winters 2000//01 – 2002/03. These findings indicate that direct, gross demographic consequences of chronic oil exposure had abated by the early 2000s. Potential subtle and suborganismal effects of continued exposure are discussed below.

HARLEQUIN DUCKS: POPULATION MODEL

Basis for the Study

Harlequin ducks have been well-studied in PWS following the *Exxon Valdez* oil spill and this body of work has generated a considerable amount of population demographic data, including results on female winter survival (Esler et al. 2000, Esler and Iverson 2010), dispersal (Iverson and Esler 2006), and population abundance and age structure (McKnight et al. 2006, Rosenberg et al. 2005). These data were assembled in a population model to (1) compare the relative magnitudes of acute and chronic oil spill mortality, (2) assess the current recovery status of harlequin ducks in PWS, and (3) project a timeline to recovery.

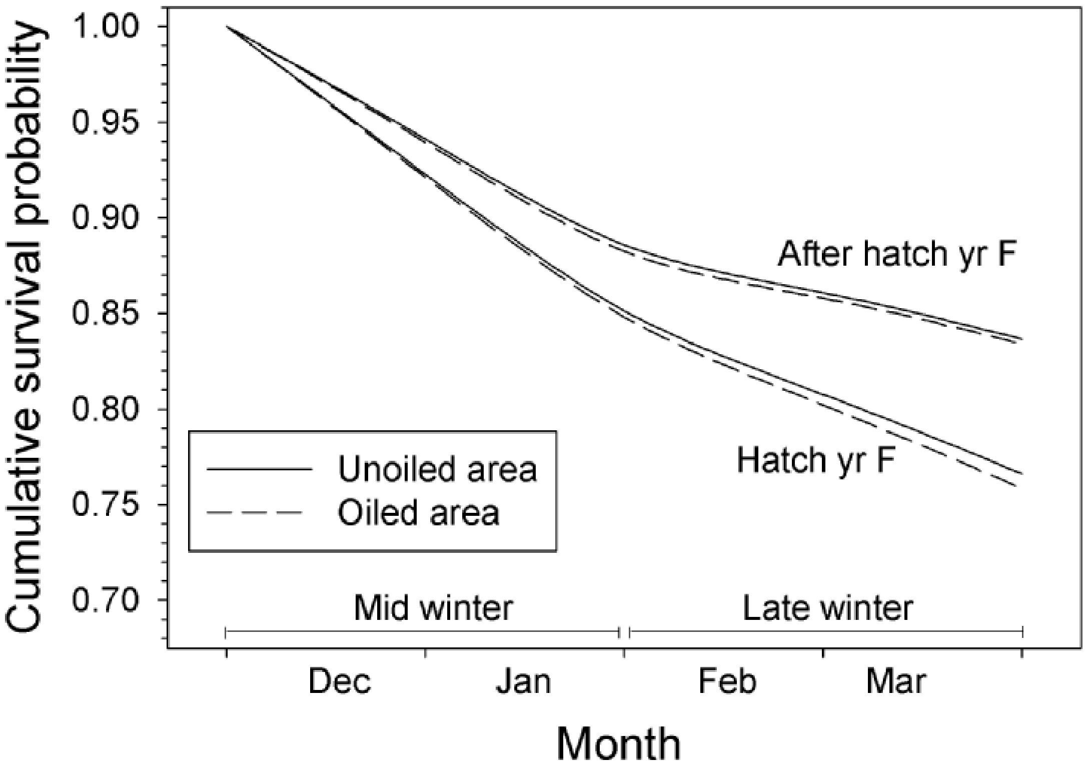

Figure 13. Cumulative winter survival probabilities of radio-marked female harlequin ducks (n = 138) from PWS during winters 2000-01 through 2002-03. Lines represent model-averaged estimates across models allowing variation in relation to season, age class, and area.

Study Approach and Methods

A deterministic, age-structured matrix model was constructed for female harlequin ducks, with measured estimates of winter survival, population abundance, and dispersal, and estimates of fecundity from the literature, as model inputs. A number of scenarios and permutations were run to accommodate variability and uncertainty in model inputs and formulations. Results are presented as the findings from the most likely scenario, bounded by the best-case and worst-case scenarios.

Results

As expected based on the life history of harlequin ducks, variation in survival rates had the strongest effect on population dynamics. Estimated acute mortality of female harlequin ducks during the weeks and months after the spill was approximately 400; this was exceeded by model-generated estimates of females dying during the chronic phase of the spill (772), based on survival rates that were depressed on oiled areas for at least 9 years after the spill.

The most likely model scenario generated estimates of a declining population trend that was predicted to persist for at least 6 years after the spill across PWS, with a mean population growth rate of $\lambda = 0.976$ during this period of decline. At their lowest point, female numbers were predicted to be reduced 14.7% below pre-spill abundance overall and by 55.3% in oiled areas. After the declining trend was reversed, models projected a mean annual population growth rate at

1.008 for PWS as a whole and a timeline to recovery to pre-spill numbers of 24 [range: 16-32] years across all areas (Figure 14).

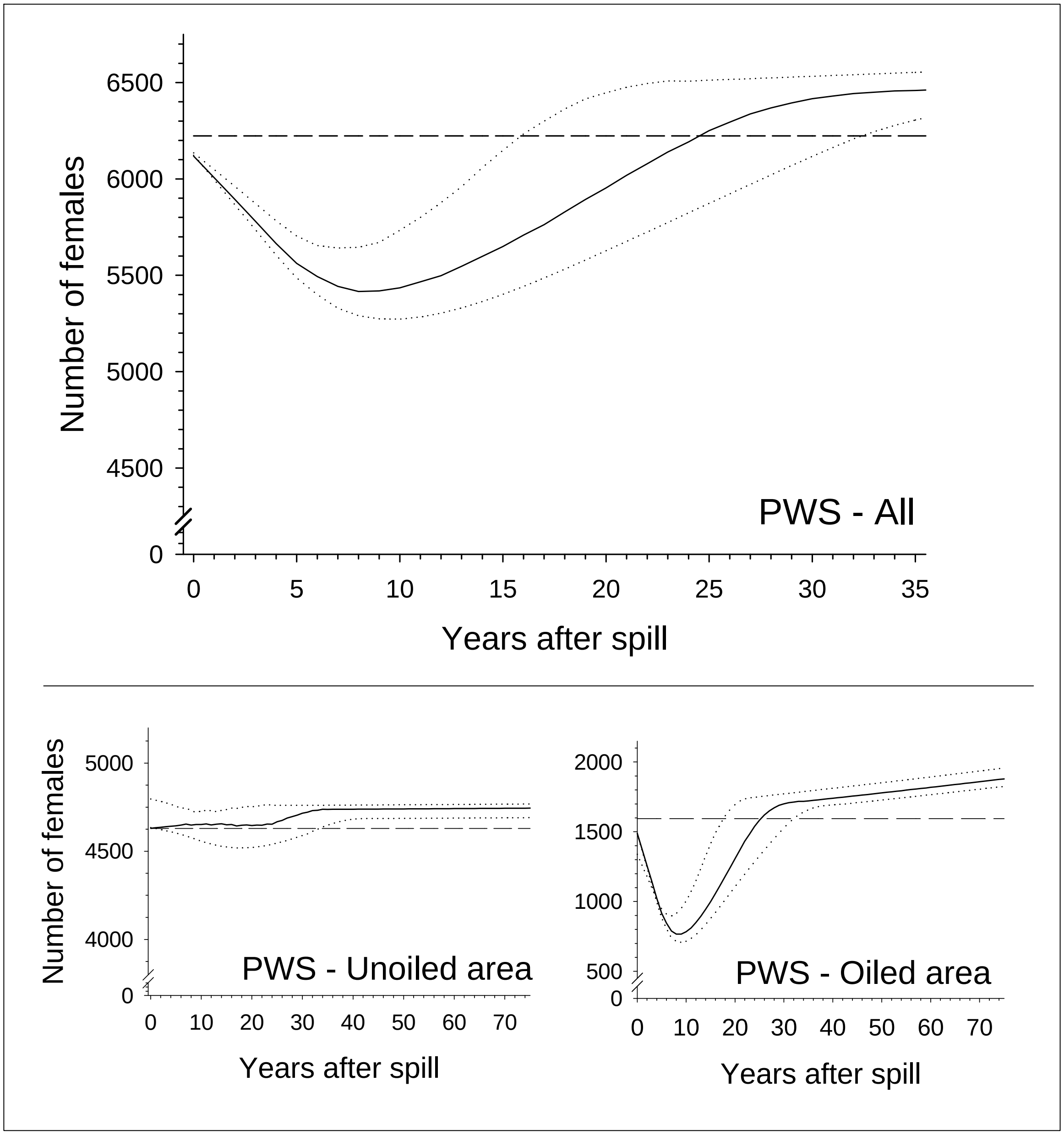

Figure 14. Model projected population recovery for harlequin ducks in relation to variation in oiling history, productivity, and dispersal among oiled and unoiled areas of PWS derived using the most likely combination of model inputs (solid line), with confidence intervals derived using worst- and best-case scenario models (dotted lines).

Summary

The population model confirmed that chronic mortality associated with lingering oil was a major driver of population dynamics and lack of recovery for the first decade following the *Exxon Valdez* oil spill. Estimated mortality during the chronic phase was roughly double that estimated during the acute phase. Equilibration of survival rates between oiled and unoiled areas after the first decade following the spill allowed the population in the oiled area, and throughout PWS, to begin to recover, with the most likely estimate of full recovery requiring two and a half decades.

SEA DUCKS – POPULATION TRENDS

Basis for the Study

Two sea ducks, harlequin ducks and Barrow's goldeneyes, have close associations with intertidal habitats and are fairly common and widespread through PWS during winter. In addition, these species have been shown to have elevated indicators of oil exposure through 2005, in the case of Barrow's goldeneyes, and 2009 for harlequin ducks (see above). Also, for harlequin ducks, demographic injury (reduced survival) was detected up to a decade post-spill, and population recovery was projected to take more than two decades (see above). Surveys of abundance are useful for gauging recovery status of these species.

Study Approach and Methods

Population trends in harlequin ducks and Barrow's goldeneyes were evaluated based on two series of boat surveys. First, the U.S. Fish and Wildlife Service (USFWS) boat surveys are designed to provide PWS-wide population estimates and trends of all marine birds (and some marine mammals), as well as estimates and trends at the scale of oiled and unoiled portions of PWS. These surveys have been conducted during March in 1990, 1991, 1993, 1994, 1996, 1998, 2000, 2004, 2005, and 2007.

Second, the Alaska Department of Fish and Game (ADFG) has conducted boat surveys specifically designed to monitor harlequin ducks. These have been conducted in March, nearly annually from 1997 to 2009. In addition to quantifying numbers, age and sex information is recorded for all harlequin duck observations. In addition to their regular surveys, ADFG conducted a series of surveys in 2007 and 2008 that replicated work conducted during pre-spill years of 1972 to 1973.

Results

USFWS surveys describe stable winter densities of both Barrow's goldeneyes and harlequin ducks since the oil spill through 2007, in both oiled and unoiled parts of PWS (McKnight et al. 2007). The authors interpret this as evidence of lack of recovery, under the expectation that recovery would require significantly increasing abundance of injured species on oiled areas.

ADFG surveys of harlequin ducks describe fairly stable numbers, densities, and distributions during the period 1997 through 2009. Slight positive increases were detected on oiled areas, particularly for females, which is consistent with a recovering population.

As expected for harlequin ducks, like many waterfowl, sex ratios were male-biased. Sex ratios did not vary over years, although there was a slight but significant difference between oiled and unoiled areas. Over all years, 40.7 (95% CI: 40.2 – 41.2) females per 100 birds were observed in oiled areas, compared to 41.9 (95% CI: 41.3 – 42.5) females per 100 birds in unoiled areas. Over the study period, recruitment has increased slightly, based on an increasing proportion of juveniles observed in both oiled and unoiled areas.

ADFG comparisons of 2007 and 2008 data with surveys conducted during March of 1972 and 1973 revealed no overall difference in densities between time periods, suggesting that current harlequin abundance is similar to pre-spill abundance.

Summary

Based on both USFWS and ADFG surveys, winter abundances of Barrow's goldeneyes and harlequin ducks have been generally stable since the *Exxon Valdez* spill. Although lack of recovery was inferred for both species due to lack of evidence of increasing trends in oiled areas during USFWS surveys, indication of increasing trends in harlequin ducks on oiled areas based on ADFG surveys is an encouraging sign. The specialized ADFG surveys have greater power to detect subtle trends. Also, ADFG work indicated that contemporary densities of harlequin ducks are similar to pre-spill densities, also suggestive of recovery and consistent with expectations of the population model (above). ADFG collected data on Barrow's goldeneyes during their harlequin duck surveys, but summaries of these data through 2009 have not yet been prepared.

SEA OTTERS – SURVIVAL

Basis for the Study

Based on data from age-at-death information from carcasses collected systematically in western PWS, Monson et al. (2000) determined that survival of sea otters was depressed in the oiled region during the *Exxon Valdez* spill through at least 1998. This was interpreted as a demographic consequence of injuries suffered during both acute and chronic phases of the spill. More recent work has updated the survival consideration through 2005.

Study Approach and Methods

Sea otter carcasses have been collected from 1976 through 2005 from shorelines of western PWS during beach surveys conducted in April or May, after snow melt but prior to the growth of beach grasses that can conceal carcass remains. From each carcass, a tooth was recovered for age estimation, providing data on ages at death of sea otters over approximately a 30-year period. Survival analyses were conducted both as simple demographic models, such as those used in previous studies (Monson et al. 2000), and as part of more complex and presumably more realistic source-sink population models (see below).

Results

In the most recent modeling effort, the simplest analyses were consistent with the previous findings of Monson et al. (2000); namely, that survival continued to be depressed in oiled areas relative to baseline conditions up to 16 years after the *Exxon Valdez* spill. In the more complex (and better supported) source-sink models, survival of juveniles was most strongly reduced during the immediate post-spill years, whereas survival of older age classes was more strongly depressed starting 4-5 years after the spill. However, survival of all age classes was estimated to be lower than baseline levels through 2005.

Summary

The findings using data collected through 2005 suggest continued depression of survival rates for all age classes of sea otters in oiled areas of PWS. Note that these analyses were not conducted using the most recent data (2006-2009) when sea otter abundance has increased at northern Knight Island (see below, Population Trends). Possibly, incorporating the more recent data will reveal different patterns of survival.

It is also worth noting that alternate estimates of sea otter survival rates, obtained from radiotelemetry studies of sea otters in western PWS from 2002-2005, are consistent with rates estimated from the modeling work and suggestive of adverse effects on the growth rate of the western PWS otter population.

SEA OTTERS – POPULATION MODEL

Basis for the Study

Population models were constructed to evaluate underlying explanations for observed sea otter population trends and age structure (both living otters and carcasses) in western PWS.

Study Approach and Methods

Various sets of model inputs representing different hypotheses about factors affecting population dynamics of sea otters in western PWS were contrasted, using data on population abundance, age composition of dead otters recovered during beach surveys (see above), and age composition of live otters captured in the field. The model structures allowed consideration of source-sink dynamics, wherein a portion of the western PWS population is constrained to have a stable or declining population trajectory (the “sink” population, which is that portion of the population with deleterious oil spill effects and declining numbers), and the remaining western PWS population is considered to be the “source.”

Results

Models incorporating source-sink dynamics were most strongly supported by the available data. The best supported of these models indicated reduced survival in all age classes in the sink population, with different temporal patterns of mortality for young otters and older animals (see above). Also, the best-supported model indicated stable numbers in the sink portion of the population. This model generated an estimate of mortalities exceeding baseline conditions of approximately 540 individuals from 1990 through 2005.

A second model received modest support. In this case, the size of the sink population was allowed to change over time. Under this scenario, the proportion of the population suffering oil spill effects declined over time, i.e., effects were constrained to progressively fewer otters (<10 by 2005), and total chronic mortality above baseline was estimated to be 245 animals.

Summary

Based on these models, sea otter population dynamics in western PWS are best explained in the context of differential processes occurring in source and sink subpopulations and survival patterns that varied through time according to age class. However, these models were built using data collected through 2005, prior to the observed increase in numbers at northern Knight Island in 2007-2009 (see below). Revisiting this exercise including data during this apparent recovery period might elucidate the factors driving more recent population dynamics and recovery.

SEA OTTERS – POPULATION TRENDS

Basis for the Study

Sea otter abundance has been monitored nearly annually since 1993, to gauge the status and progress of population recovery. Surveys have focused on western PWS, where oil spilled during the *Exxon Valdez* accident accumulated, resulting in high acute mortality of sea otters, and where lingering oil persists, leading to concerns about chronic effects. Additional emphasis was placed on the northern Knight Island archipelago, which was particularly hard hit during the spill and had not appeared to recover through the early 2000s.

Study Approach and Methods

The survey methodology and experimental design are described in detail by Bodkin and Udevitz (1999). In brief, aerial surveys are conducted as strip transects, stratified into high- and low-density transects, with more effort allocated to high-density strata. Detection probabilities are calculated and applied to each survey to estimate abundance of sea otters in western PWS. Additionally, up to five replicate surveys are conducted annually for the northern Knight Island archipelago, an area where sea otters have been intensively studied.

Results

At the scale of western PWS, the sea otter population in 2009 was estimated (± SE) to be 3,958 (± 653). This represents an increase of nearly 2,000 individuals since surveys were initiated in 1993. Numbers have increased by approximately 2.6% annually over this period (Figure 15).

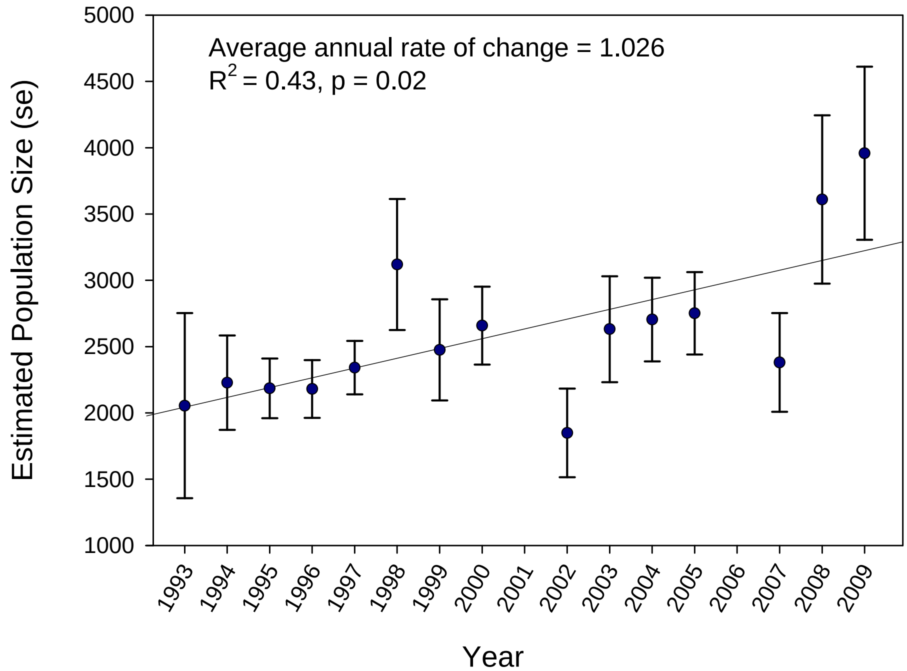

Figure 15. Sea otter population trend in western Prince William Sound, 1993-2009. Line is linear regression fitted to all points, bars indicate ±1 SE.

At northern Knight Island, numbers of sea otters were fairly constant between 1993 and 2001, averaging 78 individuals, which was well below the number estimated to occur in that area before the Exxon Valdez spill (Figure 16). However, from 2003 through 2009, numbers increased markedly, with an average annual increase of about 25%. Although numbers in 2009 were still 30% below the pre-spill estimate, the increasing trend is encouraging.

Summary

The overall increasing trend in number of sea otters in western PWS, and more specifically the recent increasing trend in numbers at northern Knight Island, indicate progress toward recovery. The northern Knight Island area suffered severe oiling and sea otter mortality approached 0.90 in some subareas (Bodkin and Udevitz 1994). Although the 2009 estimate of abundance at Knight Island remains below the best estimate of pre-spill abundance, if similar rates of increase continue, northern Knight Island may achieve pre-spill otter abundance within the next 2-3 years.

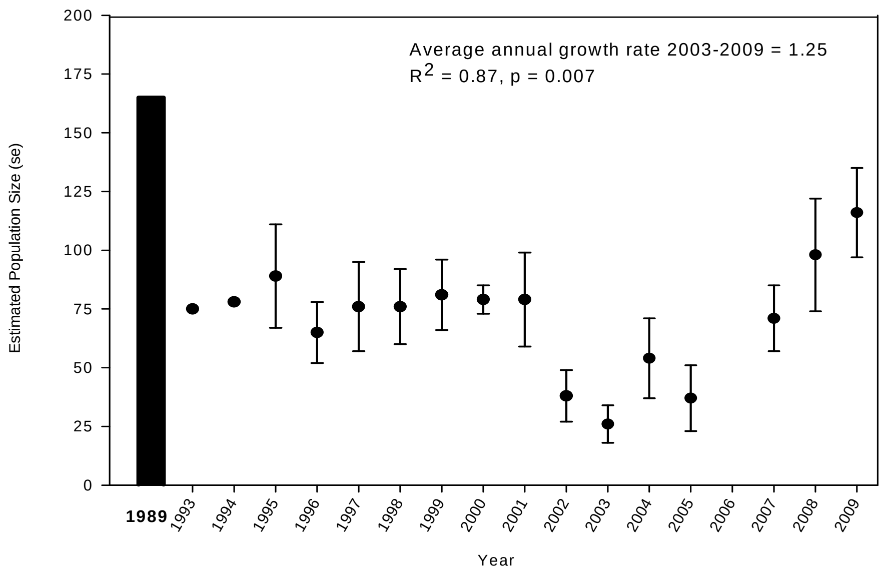

Figure 16. Sea otter population trends at the northern Knight Island study area, 1993-2009. The 1989 estimate (165) is the number of sea otters that were captured live (and taken to rescue centers) or recovered as carcasses during March and April of 1989 from the northern Knight Island area where aerial surveys were conducted from 1993-2009. This number does not include animals that survived or those that died but were not recovered in this area. It may include animals that died elsewhere but were recovered here. The only comparable pre-spill sea otter survey counted about 250 sea otters in this area in 1973.

INDIVIDUAL OR SUB-ORGANISMAL CONSEQUENCES OF EXPOSURE

HARLEQUIN DUCKS

Basis for the Study

Harlequin ducks in PWS continue to be exposed to residual *Exxon Valdez* oil through 2009 (see above). Demographic consequences of exposure were evident as differences in female winter survival between oiled and unoiled areas through 1998, but survival rates were similar between areas in the early 2000s (see above). Despite cessation of gross, population-level effects, subtle and potentially sublethal injury may still occur as a result of exposure to lingering oil. To evaluate this issue, laboratory studies are underway, in which species-specific cell lines are developed, exposed to hydrocarbons, and analyzed with a battery of bioassays to evaluate injury at the cellular level.

Study Approach and Methods

Cell lines were developed for harlequin ducks (and mallards, as a potential surrogate) from embryonic fibroblasts and livers. Cell cultures will be confronted with varying doses of several different hydrocarbon compounds (including hydrocarbons from the field where lingering oil is known to occur). Assays to evaluate cellular injury include several measures of cytotoxicity, CYP1A induction, and measures of chromosome damage and genotoxicity.

Results and Summary

This work is in progress, so definitive results about occurrence and degree of cellular injury are not yet available. However, cell lines of harlequin ducks and mallards have been successfully developed, and a standard, commercially-available rainbow trout cell line has been acquired to assist with bioassay development and interpretation. These cell lines will be exposed to site-specific complex mixtures of bioavailable hydrophobic compounds. This will be accomplished using extracts from semi-permeable membrane devices (SPMD) that were deployed at PWS sites where harlequin ducks continue to be exposed to lingering oil (see above). The battery of bioassays is in development. For greater relevance of dosing with SPMD extracts during bioassay development, reference material has been prepared from SPMDs constructed with Alaska North Slope crude (ANS) and synthetically-weathered ANS crude. During this effort, harlequin duck CYP1A response was demonstrated to be much higher than that of mallards or rainbow trout to a similar dose of chrysene, a reference material used in assay development and validation. Mallard duck response has been shown to be highly responsive to SPMD-ANS extract in spite of its lower sensitivity to CYP1A agonists. Assay validation continues and application of all assays and reporting of results will occur over the next 1.5 years.

SEA OTTERS

Basis for the Study

Sea otters, due to their high foraging rates, occurrence of intertidal foraging, and use of known oiled beaches for foraging (see above) are susceptible to oil exposure and any corresponding injury. To evaluate subtle and perhaps sublethal injury, a gene panel has been developed to evaluate expression of a number of genes that may indicate physiological stress or injury. This panel is based on work with oil-dosed, captive mink (Bowen et al. 2007).

Study Approach and Methods

Bowen et al. (2007) identified a number of genes that showed differential expression between mink that were dosed with oil relative to those that were not dosed. Genes showing differential expression in the mink study and whose functions are understood are being used to evaluate whether sea otters are showing higher expression of genes in oiled areas that may indicate injury. The array includes genes that play roles in immuno-modulation, inflammation, cyto-protection, tumor suppression, reproduction, cellular stress-response, metal metabolism, xenobiotic metabolizing enzymes, antioxidant enzymes, and cell-cell adhesion.

These methods will first be applied to Peripheral Blood Mononuclear Cells (PBMC) and liver samples collected from individual sea otters in 2003-2008. If these PBMC samples produce meaningful analytic results, gene expression will be analyzed in archived PBMC samples from 1996 through 2002.

Results and Summary

Thirteen genes have been successfully sequenced in sea otters that were the same as those expressed in mink experimentally exposed to oil (Bowen et al. 2007), as well as two additional genes that aid in interpretation of stress levels in animals exposed to xenobiotics that include aromatic hydrocarbons found in crude oil. The lab work for the initial analyses of PBMC and liver from 2003-2006 is underway, but results are not available to report at this time. However, this method holds promise, including as an opportunity to evaluate archived samples to see if expression of genes indicating injury has declined over time, as would be expected in a scenario of declining amounts and frequency of oil exposure.

FACTORS RESPONSIBLE FOR LIMITING THE DEGRADATION RATE OF *EXXON VALDEZ* OIL IN PRINCE WILLIAM SOUND BEACHES

Before remediation methods can be developed to treat the lingering oil, the factors that are limiting the natural degradation of the oil must be understood. Therefore, this study, informally called the "limiting factors study," was designed to identify and compare the hydrogeological processes on gravel beaches with and without the presence of lingering subsurface oil.

Study Approach and Methods

Studies were conducted at six gravel beaches in PWS: EL056C and EL058B (Eleanor Island) in 2007; and KN109 and KN114A (Knight Island) and SM006B and SM006C (Smith Island) in 2008. These sites were selected based on two main criteria: sediments thick enough to allow installation of injection and monitoring wells; and presence of patches of lingering subsurface oil adjacent to areas that were free of subsurface oil, allowing comparison of the hydrogeological properties of each area.

The 2007 and 2008 field study methods consisted of the installation of wells with sampling ports at multiple depths in both oiled and unoiled transects on each beach. A tracer was injected into the beach and the rate of change in the tracer concentrations monitored over time and space. Salinity, temperature, dissolved oxygen, nutrients, and pressure were also measured. Sediment samples were collected for grain size analysis, hydrocarbon analysis, TKN, and chemical oxygen demand. The field data were used to develop models of groundwater flow patterns in the beach sediments.

Key findings from the studies in 2007 and 2008 were:

1. The beaches can be viewed as consisting of two layers, an upper layer with a very high permeability and a lower layer with a very low permeability. Surprisingly, the contrast in permeability between the layers was found to be around three orders of magnitude.
2. The dissolved nutrient concentration in the beaches was much smaller than that needed for maximal growth of microorganisms and the subsequent consumption of oil.
3. The oil persisted at locations where the freshwater groundwater flow (moving seaward) was small.
4. Temperature of pore water within the beaches was higher than 12°C in the summer months, suggesting that biodegradation likely is unhindered by temperature.

5. Modeling studies using the software SUTRA (USGS) and MARUN (Boufadel et al. 1999) confirmed the two layers' configuration and suggested that the concentration of dissolved oxygen in the lower layer was most likely too low to sustain aerobic biodegradation.

Many of these findings, along with the results from the microcosm study (discussed above), motivated additional field work that was conducted in 2009.

The 2009 field program gathered important data, previously lacking, about groundwater flow in the lower layer of the beaches. In designing effective *in-situ* remedial techniques, it is critical to know the flow rates in the lower layer, the "area of influence" of an injection well under different injection pressures, and the maximum injection pressure that can be reliably applied while maintaining well integrity. Similarly, where slow-release application at beaches with a shallow oil layer may be proposed, information on the rate of flow within the oil layer and the area of influence from the point of application of slow release material was critical. It was also important to measure oxygen and nutrient levels in the lower layer.

Therefore in 2009, studies were conducted on two beaches, EL056C and SM006C, during two periods. From June 18-25, various sensors were placed into the beach along with systems for high-pressure injection (to determine the pressure at which the injection system "blows out" the well even with a bentonite layer immediately around the well) and systems for low-pressure tracer injection into the lower, less permeable layer (to determine the area of influence of the slow-release injection of bioremediation amendments into the oiled layer). All measurements were conducted during 19-27 August, to allow for the beach sediments to recover from the disturbances during well installation.

Results

The preliminary findings from the 2009 field studies are:

1. It takes approximately one month for the two-layer configuration to be reinstated after excavation and filling of pits.
2. The concentration of dissolved oxygen in the lower layer was less than 1.0 mg/L at oiled locations (Figure 8 for the beach on Eleanor Island and Figure 9 for the beach on Smith Island). It was in general higher than 3.0 mg/L in the clean transects.
3. The blowout of the high-pressure injection well on Eleanor Island (EL056C) occurred at a flow rate of 1.3 gallons per minute.
4. The low-pressure injection studies indicated that a design injection flow could be around 0.3 to 0.4 gallons per minute.
5. On the Smith Island study site (SM006C) where the tracer was released under low pressure, two horizontal manifolds, each around 3 feet long were placed approximately 3 feet deep. The tracer migrated approximately 6 feet seaward and 1.0 foot landward in a 24-hour period.

Summary

Based on both the field studies and the microcosm work described above, Boufadel et al. have concluded that lack of oxygen availability to the oil in the lower layer is the key factor limiting decomposition of the lingering oil, with nutrient availability in the lower layer as a lesser but measurable factor. The key issue is to increase the amount of oxygen and nutrients in the low permeability layer that contains the oil. The results of the 2009 field work can be used to design pilot studies of the best approaches for *in-situ* remediation. The data on flow rates and area of influence in the lower layer can be used to make design the distance between surface injection points or wells, the injection pressure for wells, and the amounts of oxygen and nutrients to be injected, whether by well or surface injection. It can also be used to design the distance between wells or injection points and monitoring locations.

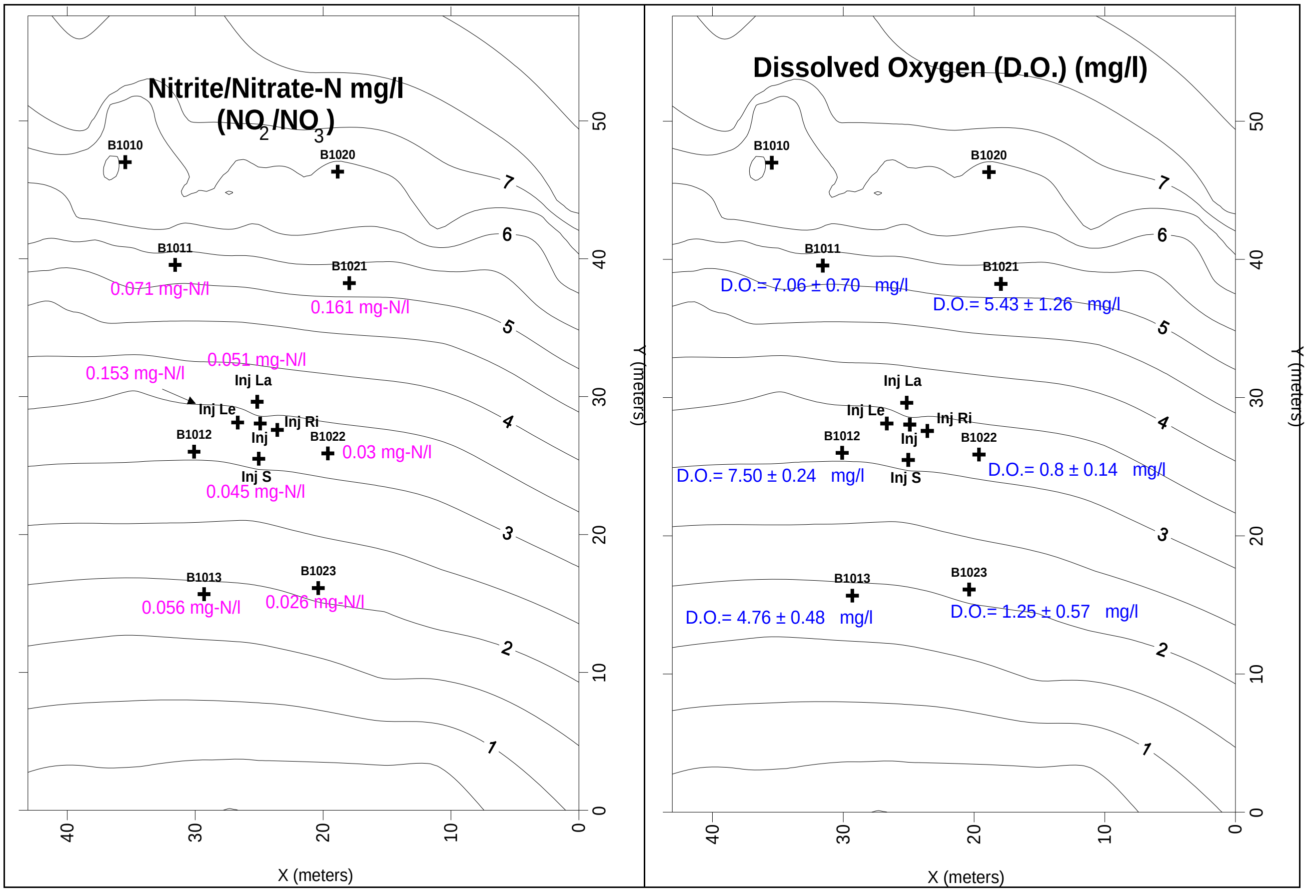

Figure 8. Plan view of beach EL056C, Northwest Bay, Eleanor Island. The landward direction is upward. The wells labeled B1010-1013 (Left) are in the unoiled transect; wells labeled B1020-1023 (Right) are in the oiled transect. Heavily oiled residue (HOR) was present at B1022 and B1023. The contours represent the topography in meters. The cluster in between the transects represents the injection well (in the middle) and the monitoring wells around it.

(Left) Average pore-water nitrate concentration, as N, measured deep into the sediments. The values are low, at least 10-fold smaller than required for optimal biodegradation of oil, which is 2 to 10 mg/L of nitrate-N.

(Right) Average pore-water dissolved oxygen concentration. The depth of the sensors was the same for corresponding Right and Left wells. However, the upper layer of the left transect was deeper, which explains the high dissolved oxygen values at B1012 and B1013 in comparison with B10122 and B1023, respectively.

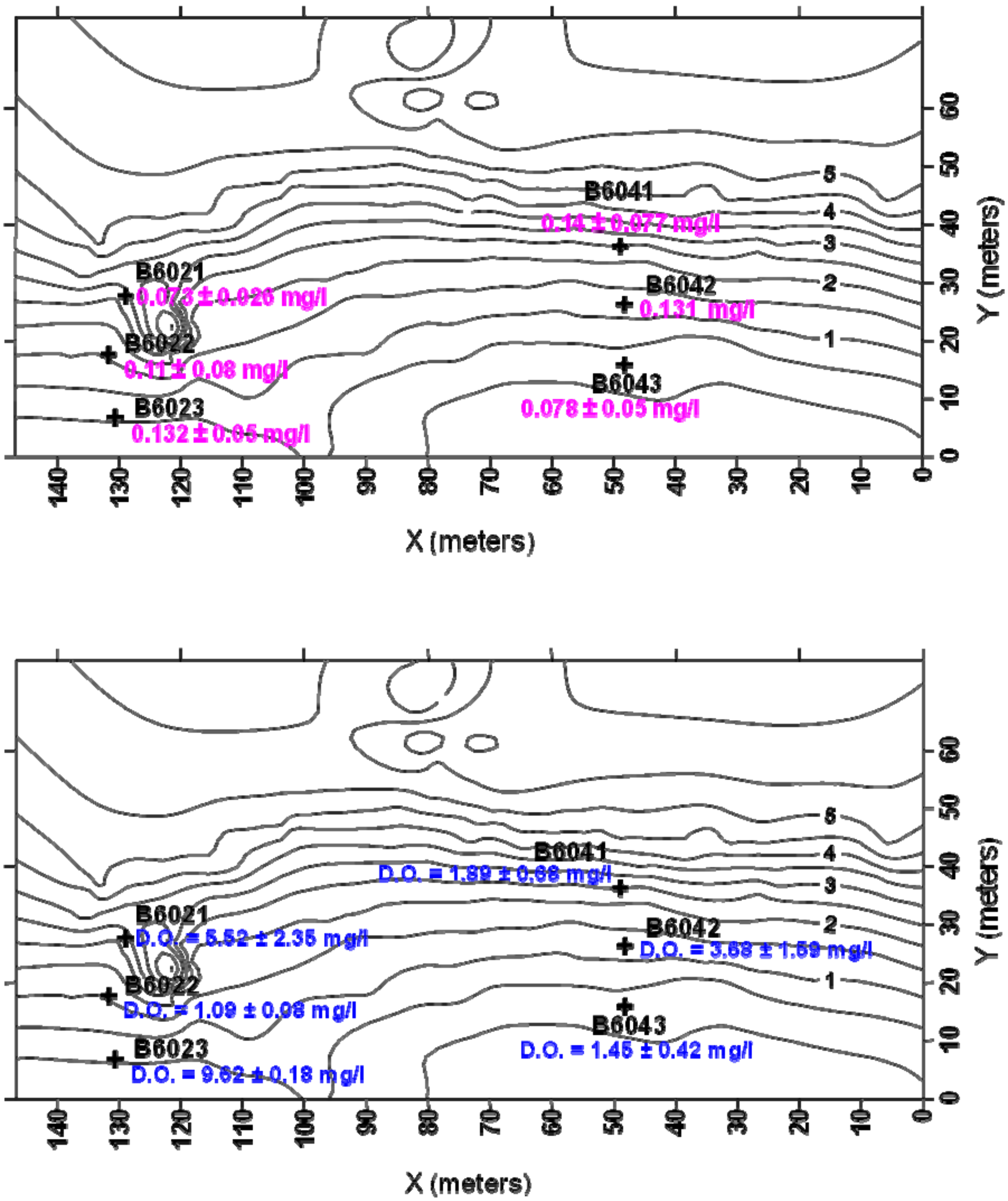

Figure 9. Plan view of beach SM006C, Smith Island. The landward direction is upward. Oil was present at well 6022. (Top) Average nitrate concentration (mg/L of N). Note that the optimal nitrate-N concentration for biodegradation of oil is 2 to 10 mg/L. (Bottom) Dissolved oxygen concentration at an average depth of 2.5 feet.

BIODEGRADABILITY OF LINGERING OIL 19 YEARS AFTER THE *EXXON VALDEZ* OIL SPILL

Basis for the Study

At the 2007Arctic and Marine Oilspill Program (AMOP) conference, Atlas and Bragg (2007) reported a new index that they called the Bioremediation Index suggesting that if the PAH fraction were > 70% weathered, further attempts at bioremediation would be futile. However, Short et al. (2004) used a different index that showed that the Bioremediation Index did not seem to fully account for the PAH content of the lingering oil. Therefore, a lab microcosm study was designed to address the extent to which the lingering oil is biodegradable given varying degrees of weathering. The objective was to measure the biodegradability of the 19-year lingering oil under conditions of excess nutrients and oxygen.

Study Approach and Methods

Samples of beach substrate were collected in the summer of 2008 from representative sites in PWS contaminated with oil residues of varying weathering states according to the Atlas-Bragg model. The three sites were KN114A at the entrance to Herring Bay (BI of 74% - highly weathered), SM006B on the north shore of Smith Island (BI of 58% - moderately weathered), and PWS3A4 on the eastern, outer shore of Eleanor Island (BI of 47% - slightly weathered). The sediments were collected and sieved in the field to meet the requirements for a grain size of less than 2-4 centimeters. Seawater from each site was also collected. Triplicate sacrificial microcosms were set up for each treatment from each site for each sampling event, as follows:

- Six sampling events (Day 0, 14, 28, 56, 112, and 168).
- Two treatments per site:
  - Natural attenuation controls consisting of sediments from each site containing seawater but no further amendments
  - Nutrient treatments where excess nitrogen and phosphorus were added for biostimulation.

The feed reservoir, microcosm, and piping were made of stainless steel. Peristaltic pumps were used to provide a low recirculating fixed flow rate from the reservoir. This recirculation mechanism was intended to simulate intermittent submergence in the intertidal zone and to provide sufficient reoxygenation to prevent anoxic conditions.

Every three days, nitrogen was measured in 2 randomly selected microcosms in each treatment. If the concentration declined to < 5 mg-N per liter (L), potassium nitrate was added to bring the water concentration back up to 10 mg/L. This concentration was increased to 50 mg/L after it was discovered that the nitrogen demand was extremely high. On a designated sampling day, specified microcosms were "sacrificed" by extracting the oil from the sediments, seawater, and all tubing and pipes. The extracts were processed and analyzed for polycyclic aromatic hydrocarbons (PAHs) including 2-4 ring PAHs and alkylated homologs (no alkanes were detected in any of the samples).

Results

KN114A was the most weathered, with loss of most of the naphthalenes, about half of the phrenanthrenes, and the parent and C_1-alkylated fluorenes and dibenziothiophenes. There were little changes in the napthbenzothiophenes and chrysenes. SM006B was moderately weathered, whereas PWS3A4 was the least weathered. The biodegradation curves for the PAHs from the three sites showed typical first-order rate behavior, with the nutrient treatments always giving about 2 to 2.5-fold higher rate coefficients than the corresponding natural attenuation treatments. Thus, the lingering oil as of 2008 was easily biodegradable regardless of the extent of weathering. After 168 days, the % degradation of PAHs was > 80% at all three sites.

Although the nutrient treatments degraded faster, it was unexpected that the natural attenuation microcosms degraded as much as they did. Total Kjehldahl nitrogen (TKN) was found to be between 450-540 mg/kg in the sediments from the three sites, which was interpreted as representing a large amount of biogenic nitrogen from decaying plant material that occurred naturally in the sediments. The significant biodegradation that occurred in the natural attenuation microcosms suggests that, although nitrogen was a contributing limiting factor, the major limiting factor in the field has been lack of oxygen. The major preliminary conclusions were:

- Excavation and removal of sediment perturbed the integrity of the low permeability zone where the oil accumulated over the years.
    - This caused exposure of new surfaces that were not previously exposed to dissolved oxygen *in situ*.
- When the sediments were then exposed to oxygen in the lab microcosms, biodegradation ensued unimpeded.
- The excess nitrogen and phosphorus caused enhanced biodegradation, but the majority of the biodegradation was due to the presence of oxygen.

Bioremediation appears to be a promising technology able to remove the persistent oil present in some locations in Prince William Sound. However, to be successful, *in-situ* treatment needs to introduce oxygen and nutrients in a manner that will increase the contact with these amendments with the oil.

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ACKNOWLEDGEMENTS

The authors would like to thank the original Principal Investigators of the studies summarized here for providing information and interpretation; these include: Al Venosa, Michel Boufadel, Brenda Ballachey, Jim Bodkin, Dan Monson, Liz Bowen, Keith Miles, Tuula Holleman, Katie Springman, Mark Ricca, Sam Iverson, Dan Rosenberg, and David Irons. Also, thanks to the Exxon Valdez Oil Spill Trustee Council for funding the original studies and preparation of this summary.