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Surveillance of sea surface activities in Canada using earth observation satellites and aerial surveillance sensors

Louis Armstrong[1]

Keywords: Canada, chronic oil pollution, aerial surveillance, satellite

In Canada, the catastrophic release of oil into the marine environment from vessel accidents, especially major tanker accidents, is rare. In recent years, the majority of cases, which have had the most devastating environmental consequences, do not involve a vessel mishap but rather an accidental or illegal deliberate discharge at sea. These incidents are much more frequent despite the efforts of the Government of Canada to curtail the polluting of waters under Canadian jurisdiction. Although individual releases may be small in comparison to catastrophic releases, they are chronic and the damage is devastating. This continues to be an issue in Canada each year as oiled birds continue to be found along Canada's vast coastline.

Transport Canada (TC) is the lead federal department responsible for preventing pollution from ships and the National Aerial Surveillance Program (NASP) is one method by which this is achieved. To combat the issue of illegal discharges in Canada, TC has committed to improving the effectiveness of its surveillance program. To this end, TC is modernizing its surveillance aircraft with state of the art pollution surveillance equipment, which can be used during conditions of reduced visibility such as night or low cloud cover. These surveillance systems will assist TC in the detection, classification and tracking of all targets of potential interest and marine oil spills. To compliment the surveillance, surveillance flights are being timed to coincide with Radarsat passes so that wider area surveillance can be conducted.

TC is confident that this new equipment will enhance our ongoing ability to secure appropriate evidence that will aid with successful prosecutions. Deterrence is the key priority and TC is confident

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that when the international marine community learns of the improved surveillance capability, there will be a reduction in pollution activity off our Coast.

Evaluating the long-term exposure of nearshore vertebrates to lingering oil from the *Exxon Valdez* oil spill

Ballachey, B.E.,[1] Bodkin, J.L.,[1] Esler, D.,[2] Irons, D.,[3] and Snyder, P.[4]

Keywords: *Exxon Valdez*, ***oil spill, cytochrome P450, vertebrates***

In March 1989, the *Exxon Valdez* oil spill (EVOS) released over 40 million liters of crude oil into the waters of Prince William Sound (PWS), Alaska. Acute losses were heavy, with many thousands of birds and mammals dying from oil exposure within weeks of the spill (Spies et al. 1996). Studies to evaluate injury and recovery of affected species in the spill area have been ongoing for almost two decades, and several species inhabiting nearshore areas, including sea otters *(Enhydra lutris)* and harlequin ducks ducks *(Histrionicus histrionicus)*, are not yet considered to have recovered fully from the spill (EVOSTC 2006). As recently as 2003, oil from the EVOS has been found in intertidal sediments of PWS shorelines (Short et al.; 2004, 2006). Since 1996, we have conducted studies on a suite of nearshore vertebrates in PWS to assess the status of recovery, and whether or not recovery has been constrained by chronic exposure to residual oil (Peterson and Holland-Bartels 2002, Peterson et al 2003). Species sampled include sea otters (Bodkin et al., 2002), river otters *(Lutra canadensis)* (Bowyer et al., 2003), harlequin ducks (Esler et al., 2002), and pigeon guillemots *(Cepphus columba)* (Golet et al., 2002). Exposure to oil has been assessed by quantifying the cytochrome P4501A (CYP1A) enzyme biomarker, which is induced by aromatic hydrocarbons (Whitlock, 1999). Over the course of the studies, all species sampled have shown elevated CYP1A in individuals captured within areas that were most heavily oiled in 1989, compared to those from nearby unoiled areas. Species that forage

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for invertebrate prey in intertidal habitats (including sea otters and harlequin ducks) appear to be at greatest risk of continuing exposure, whereas species which consume primarily fish appear to be at less risk. In addition, concurrent studies have demonstrated elevated mortality of sea otters and harlequin ducks in the oiled areas we studied, compared to their counterparts in the unoiled areas that we studied (Monson et al., 2000, Esler et al., 2002). Our findings suggest that chronic exposure to lingering oil remains a concern for some species and may be constraining recovery of these species and the nearshore ecosystem they inhabit.

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Differential gene expression induced by exposure of captive mink to fuel oil: a model for the sea otter

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Keywords: mink, differential gene expression, petroleum, sea otter

ABSTRACT

Free-ranging sea otters are subject to hydrocarbon exposure from a variety of sources, both natural and anthropogenic. Effects of direct exposure to unrefined crude oil, such as that associated with the Exxon Valdez oil spill, are readily apparent; however, the impact of subtle but pathophysiologically relevant concentrations of crude oil on sea otters is difficult to assess. The present study was directed at developing a model for assessing the impact of low concentrations of fuel oil on sea otters. Quantitative PCR was used to identify differential gene expression in American mink that were exposed to low concentrations of bunker C fuel oil. A total of 23 genes, representing 10 different physiological systems, were analyzed for perturbation. Six genes with immunological relevance were differentially expressed in oil-fed mink. Interleukin-18 (IL-18), IL-10, inducible nitric oxide synthase (iNOS), cyclooxygenase 2 (COX-2) and complement cytolysis inhibitor (CLI) were down-regulated while IL-2 was up-regulated. Expression of two additional genes was affected; heat shock protein 70 (HSP70) was up-regulated and thyroid hormone receptor (THR) was down-regulated. While the significance of each perturbation is not immediately evident, we identified differential expression of genes that would be consistent with the presence of immune system-modifying and endocrine-disrupting compounds in fuel oil. Application of this approach to identify effects of petroleum contamination on sea otters should be possible following expansion of this mink model to identify a greater number of affected genes in peripheral blood leukocytes.

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Introduction

The Southern sea otter *(Enhydra lutris neresis)* population has experienced recent dramatic declines (Bodkin et al., 2003; USFWS, 2002). Because their ranges tend to be limited and concentrated near the coast, sea otters are vulnerable to runoff or shipping related contamination with petroleum oil products (VanBlaricom and Jameson, 1982). The acute effects of petroleum oil exposure include disturbances in thermoregulation, respiration, and metabolism (Geraci and Williams, 1990; Rebar et al., 1995; Williams et al., 1995). These pathologies can be detected clinically, by hematological and serum chemical analyses or at necropsy. Since the immediate effects of direct petroleum oil exposure are dramatic, the short-term impacts on individual or populations of sea otters in the spill area are relatively straightforward to record, monitor, or study. A number of studies have documented the long-term impacts of a catastrophic oil spill (Monson et al., 2000; Bodkin et al., 2002). These impacts may be a result of sublethal pathology in individuals exposed to oil at the time of the spill or chronic physiological stresses from continued exposure to oil remaining in the environment. Whatever the mechanism behind these long-term effects, the pathophysiological changes within an individual may be significant but subtle, and consequently undetectable using classical diagnostic methods. In fact, many of the studies investigating low-grade, long-term impacts of oil spills use statistical techniques to identify either changes in population demographics, patterns of mortality, reproductive efficiency, or survivability. While the conclusions from these studies are compelling, the supporting data are incomplete and complicated by confounding factors that also impact population demographics and survival.

Marine mammal toxicology has relied heavily on the identification of chemical contaminants within individual tissues as an indicator of toxic insult. Unfortunately these assays are information-limited because the way xenobiotics affect the health of an individual is not assessed. Therefore methods for measuring sensitive indicators of lingering, low-grade pathophysiological changes in oil-exposed individuals are urgently needed. Contemporary gene expression analysis used to identify an organism's genomic stress response to environmental contamination by individual chemicals or complex mixtures has the potential to transform marine toxicology research (Burczynski, 2000; Bartosiewicz, 2001). The advantage of using gene expression assays in marine mammal toxicology lies in the capability to measure the physiologic responses (acute or chronic) of an individual to toxic insults.

Microarray analysis and gene-specific quantitative realtime polymerase chain reaction (qRT-PCR) yields important information on the physiological mechanisms that orchestrate an integrated response to a variety of stressors (Marrack, 2000). The value of these novel technologies is that the up- or down-regulation of many genes, which provide the transcriptional messages important in mediating toxicological and immunological reactions, can be assayed from a single sample. This is ideal for wildlife researchers where the amount of sample collected can be limiting. Gene expression

analysis can be used to detect transcriptionally active genes that are up- or down-regulated by particular toxicants and this may give insights into changes in an animal's response to toxic insult.

The long-term goal of this study is to develop sensitive and specific markers that can be used to measure long-lasting pathophysiological changes associated with either acute or chronic low-grade exposure to petroleum oil. Since petroleum oil has multiple components, the toxic effects of exposure and ingestion are likely to be diverse and widespread within the body. For this reason, the utility of a single marker of sub-lethal oil-induced pathology would be limited. The development of molecular technique(s) capable of detecting toxin-specific patterns in gene expression would permit examination of animals for subtle alterations in multiple physiological processes. Such an approach would facilitate monitoring long-term effects of oil exposure in individual, free-ranging organisms.

Surrogate species are invaluable for defining immunologic changes associated with exposure to environmentally relevant chemical contaminants. Captive American mink (Mustela vison) have been successfully used as a model for sea otters to study the toxic effects of fuel oil (Schwartz et al., 2004a; Schwartz et al., 2004b; Mazet et al., 2001; Mazet et al., 2000). Petroleum oil-induced perturbations were observed in both immune and endocrine systems. This manuscript describes the further development of mink as a sensitive model for detecting petroleum oil-induced changes in gene expression. Captive ranch mink exposed to fuel oil were analyzed for alterations in gene expression by using a human microarray in combination with quantitative real-time PCR (qRT-PCR). Such an approach would be useful for monitoring the long-term effects of an oil spill on the health of individual as well as populations of animals.

Materials and Methods

Animals and oil exposure protocol

Ranch mink used in this study were part of a large fuel oil exposure experiment examining the chronic toxicological effects of bunker C fuel oil on the immune system. Full details of the exposure have been published elsewhere (Schwartz et al., 2004a and b) and only information pertinent to the present study is described below. Animals (8 month-old males) were divided into two groups: one group (N = 9) was maintained on a ranch feed (150-200 g/day) containing 500 ppm of Bunker C fuel oil for 113-118 days. The control group (N = 5) was maintained on the same feed ration with mineral oil added instead of fuel oil. The concentration of fuel oil fed to the mink corresponded to the petroleum hydrocarbon concentrations measured in invertebrates sampled in the oiled Prince William Sound region one year after the Exxon Valdez spill (Mazet 2001).

At the end of the exposure, venous blood from every animal was collected into cell-separation vacutainer tubes (8 ml) (CPT w/ sodium citrate, Becton Dickinson, Franklin Lakes, NJ). Tubes were centrifuged at 1800 x g for 20 minutes. Isolated mononuclear cells were suspended in sterile PBS (phosphate buffered saline) containing 0.5 M EDTA (pH 7.4), centrifuged (250 x g, 8 minutes), resus-

pended in cryopreservation media (10% Dulbecco's modified eagles medium, 10% DMSO and 80% fetal bovine serum), rate frozen at –80°C and then transferred to liquid nitrogen.

Animals were euthanized by CO2 asphyxiation following blood collection. Cells from the spleen obtained from each animal were flushed out with cell culture media, the mononuclear cells collected by density gradient centrifugation, and then cryopreserved in the same manner as PBMLs (peripheral blood mononuclear leukocytes).

RNA extraction & cDNA synthesis

Total RNA was isolated from splenic mononuclear leukocytes and PBMLs using silica-based gel membranes combined with microspin technology (Qiagen, Santa Clarita, CA) and stored at -70oC. A standard cDNA synthesis was performed on 2 µg of RNA template from each animal. Reaction conditions included 4 units reverse transcriptase (Omniscript®, Qiagen, Valencia, CA), 1 µM random hexamers, 0.5 mM each dNTP, and 10 units RNase inhibitor, in RT buffer (Qiagen, Valencia, CA). Reactions were incubated for 60 minutes at 37oC, followed by an enzyme inactivation step of 5 minutes at 93oC and stored at –20oC until further analysis.

Development of mink-specific quantitative PCR primers

Microarray analysis was performed by Genome Explorations Inc. (711 Jefferson Ave. Suite 415, Memphis TN). Mink-specific quantitative PCR systems were designed for: i) select genes identified by microarray as being differentially expressed between oiled and control mink, ii) genes representing six broad categories of biologically relevant physiological systems (Table 4), and iii) an endogenous control gene, ribosomal subunit S9. Degenerate primers were designed based upon multi-species alignments (GenBank) (Table 1). The six systems were selected based on petroleum oil's or components of petroleum oil's known effect on immune defense (Schwartz et al., 2004a, 2004b), cellular injury (Ghanem et al., 2006), signal transduction (Burchiel et al, 2004), xenobiotic and metal metabolism (Schwartz et al., 2004a, 2004b), tumorigenesis (Ramesh et al., 2004) and reproduction (Mazet et al., 2001). Briefly, degenerate primer pairs were utilized on cDNA generated from three mink spleen samples. PCR amplifications using these primers were performed on 20ng of each cDNA sample in 50µl volumes containing 20 to 60pmol of each primer, 40mM Tris-KOH (pH 8.3), 15mM KOAc, 3.5mM Mg(OAc)2, 3.75µg/ml bovine serum albumin (BSA), 0.005% Tween-20, 0.005% Nonidet-P40, 200µM each dNTP, and 5U of Advantage® 2 Taq polymerase (Clontech, Palo Alto, CA). The PCR was performed on an MJ Research PTC-200 thermal cycler (MJ Research, Watertown, MA) and consisted of 1 cycle at 94 oC for 3 minutes, 40 cycles at 94oC for 30 sec, 60oC for 30 sec and 72oC for 2 minutes, with a final extension step of 72oC for 10 minutes. The products of these reactions were electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining. Bands representing PCR products of the predicted size were excised from the gel, and extracted and purified using a commercially available nucleic acid-binding resin (Qiaex II Gel extraction kit, Qiagen).

Table 1

Gene of interest	Forward primer	Sequence 5' - 3'	Reverse primer	Sequence 5' - 3'
Aryl hydrocarbon receptor	AHRF1	GGACAGAAMAAGAAAGGGAAAGATG	AHRR1	GGTCTCTGAGTTRCAATGATATAATC
Estrogen receptor beta	ERBF1	GGATATCACTATGGAGTCTGGTCG	ERBR1	CATCATCATGGAGGCGTCGGTG
Glutathione-S-trans-ferase	GLUF1	CCTGAATGCCAAGGGAATCCGG	GLUR1	GCCAGATGAGGTAATCAATCATAG
UDP-glucuronyltrans-ferase	UDPF2	GAGGACTCCACTGCAAACCTGC	UDPR2	GGGATCCCATGGTAGATTGCCTC
Heat shock protein 90	HSP90F1	GCCTGAGGAAGTGCACCATGGA	HSP90R1	GATCACAACCACTTTCTCTGCCAC
Heat shock protein 70	HSP70F1	ACCTGGGCACCACCTACTCCTG	HSP70R1	GCTTGTTCTGGCTGATGTCCTTCT
Interleukin-2	IL-2F1	CAAGTGCAGTCATTGCTGCAGGAT	IL-2R1	GTAATCCATTTGTTCAGAANTTCTACAG
Interleukin-12	IL-12F1	CMTCRTGGCCATRTGGGAACTGGAG	IL-12R1	CACTGAATTTCARATCAGTACTGATTGC
Interleukin-18	IL-18F1	GATGAARACCTGGAATCRGATYACT	IL-18R1	CATGTCCWGGRACACTTCTYTGAA
Interleukin-10	IL-10F2	GACTTTAAGGGTTACCTGGGTTGC	IL-10R2	TCCACCGCCTTGCTCTTGTTTTC
iNOS	INOSF1	CAGGAACCTACCAGCTGACGG	INOSR1	GTGATGGCCGACCTGATGTTGC
TGFβ	TGFBF1	CAGTACAGCAAGGTCCTGGCCC	TGFBR1	CTGCTCCACCTTGGGTTGCG
COX-2	COX-2F1	GAGCTCTTCCTCCTGTGSCTGA	COX-2R1	CTTTGRCTGTSGGMGGATACAYCT
S9	S9F1	GTGGCCCGGARCTGGGTTTG	S9R1	GGCGYCTCTCYAARAAATCC
Metallothionein	metF1	AGCCTTCCACGTGCGCCTTATAG	metR1	GCACAGCAGCTGCACTTSTCYG
Complement cytolysis inhibitor	CYTF1	ACGAGCTGCTAAAGTCCTACCAGTG	CYTR1	ACTGAGGTGGTCGTGAAGCTCTTTG
HDCMB21P	HDCF1	ATGTTCTCCGACATCTACAAGATCC	HDCR1	CATGAACCATCACCTGCAGGAAAC
DQA	DQAUD	CCAGTACACCCATGAATTTGATGG	DQALD	GAAACACAGTCACCTCAGGAACC
DRA	DRAU102	ATAAGTGGAGTCCCTGTGCTA	DRAL	CCCAGTGCTCCACCTTGCAGTCATA
DRB	DRBU71	CGGGACSGAGCGGGTKC	ZcDRB4L	CCACTTGGCAGGTGTAGACCTCTCC
Interleukin-6	IL-6F1	CYCTGGGRCTGCTYCTGGTG	IL-6R1	CTGACCAGARRARGGAATGCC
Thyroid hormone receptor	THRBF1	GGACAAACCGAAGCACTGTCCAG	THRBR1	GGAATATYGAGCTAAGTCCAAGTGG
Cold inducible RNA-binding protein	CIRBPF1	TTCTGAGTGTAGTGTGGTAGGACCC	CIRBPR1	AGTGGCTGAGGAATTCTTGTACGCA
FoxP3	FOXP3F1	CAGGCACTCCTCCA GGACAG	FOXP3R1	CTCCCTGGACACCCATTCCAG

Isolated fragments were ligated into a T/A type cloning vector (pGEM®-T Easy vector systems, Promega, Madison, WI). Following transformation, growth, and blue-white selection in competent cells (SE DH5α competent cells, Life Technologies Inc, Rockville, MD), the DNA from positive clones was isolated. Nucleotide sequences of both strands were determined by dideoxy nucleotide methodology using an automated sequencer (Model 373, Applied Biosystems, Foster City, CA). Nucleotide sequences of the PCR products were analyzed using Align™ and Contig™ sequence alignment software programs (Vector NTI™, Informax Inc, North Bethesda, MD) and compared to known sequences

using the NCBI BLAST program (Altshul et al., 1990), and the IMGT/HLA database (Robinson et al., 2001).

Quantitative PCR

Real-time PCR systems for mink S9 and the genes of interest were run in separate wells. cDNA was examined using an intercalating fluorescent dye PCR (Bowen et al., 2006). Each reaction contained 500ng DNA in 25µl volumes with 20pmol SSP, Tris-Cl, KCl, (NH4)2SO4, 2.5mM MgCl2 (pH 8.7), dNTPs, HotStar Taq DNA Polymerase (Quantitect SYBR Green PCR Master Mix, Qiagen, Valencia, CA), and 0.5 units uracil-N-glycosylase (Roche, Indianapolis, IN). Amplifications were performed in an ABI 7300 (Applied Biosystems, California) under the following conditions: two minutes at 50oC, followed by 15 minutes at 95oC, and 35 cycles of 94oC for 30 seconds, 58oC for 30 seconds, and 72oC for 30 seconds, with a final extension step of 72oC for 10 minutes. Reaction specificity was monitored by melting curve analysis using a final data acquisition phase of 60 cycles of 65oC for 30 seconds and verified by direct sequencing of randomly selected amplicons (Bowen et al., 2006).

Gene expression was analyzed by relative quantitation, using the comparative CT (cycle threshold) method; values are expressed relative to a calibrator (weakest signal of the normalized values) (Bowen et al, 2006). Amplification efficiencies of S9 and the other genes of interest (GOI) were determined using six dilutions of cDNA preparations (run in triplicate).

Statistical analysis was performed in NCSS (Number Cruncher Statistical System). Differences between GOI transcription were analyzed with standard t-tests. Differences were considered significant if $p < 0.05$.

Results

Quantitative PCR

Twenty-three genes, representing approximately 10 physiological pathways and one endogenous control gene, were amplified and sequenced (Table 2). Genes were divided into broad functional categories based upon biological relevance, i.e. immunomodulation, inflammation, cellular stress-response, cytoprotection, tumor suppression, reproduction, xenobiotic and metal metabolism, antioxidant metabolism, and cell-cell adhesion.

Table 2

Gene of interest	Mustela vison gene sequence 5'–3'
Aryl hydrocarbon receptor	CGATAAGTGTCTGTAGTATTCAGGCTTCTTACCAAAGATAATCGATGGGCCTGGGTTCAGTCTAATGC ACGCTTAGTGA
Estrogen receptor beta	AATCAGTGTACAATAGATAAGAATCGGCGCAAGAGCTGCCAGGCCTGCCGGCTCCGGAAGTGCTAT GAAGTGGGGATGGTGAAGT

Glutathione-S-transferase	TGATCTACGAATCTGCCATCACCTGTGAGTACCTGGATGATGTATATCCAGGAAAGAAGCTATTGCCA GATGACCCCTATGAGAAAGCTCGTCAGAAGATGGTGTTTGAGTTATTTT
UDP-glucuronyltransferase	AGAGTTTGTCCAGAGCTCTGGAGAAAATGGTATTGTGGTGTTTACACTAGGGTCTATGATCACTAACC TGCCAGAGGAAAAAGCCAATGTGATTGCATCAGCCCTTGCACAGATTCCACAAAAGGTTCTATGGA GATTTGCTGGCAAGAAACCAGACAACTTAGGACCAAATACTCGGCTGTATGACTGGATCCCCCAGA ATGACCTTCTTGGTCATCCAAAAACCAAAGCCTTTGTAACTCATGGTGGAACCAATGGCATCTATGA GGCAATCTACCATGGG
Heat shock protein 90	CCCAGGAACGCACCTTGACTCTAGTGGACACAGGCATTGGCATGACCAAAGCCGATCTCATAAATAA TTTGGGAACAATTGCCAAGTCGGGAACTAAAGCATTCAT
Heat shock protein 70	TCGAGCGTAGCGGTGTCGGCCGCAGTTCGGCGACCCGGTGGTGCAGTCGGACATGAAGCACTGG CCCTTCCAGGTGATCAGCGATGGCGATAAGCCCAAGGTGCAGGTGAGCTACAAGGA
Interleukin-2	CCATAGTACTCAACTTTGGAGGAGTGCTATATTTAGCTCAAGCAAAAACTTTCACTTGACAGACATCA AGGAACTAATGAGCAATATCAATGTAACACTTCTGAAACTAAAGGGATCTGAAACAAGA
Interleukin-12	TGGTGGTCCTCACCTGCCAAACCCCTGAAGAAGATGACATCACCTGGACCTCGGACCAGAGCAGT GACGTCTTAGGCTCCGGTAAAACTCTGACCATTCAAGTCAAAGAATTTGGAGATGCTGGCCGGTATA CCTGTCATAAAGGAGGCAAGGTTCTGAGCCATTCGCTCCTGCTGATTCACAAAAAGGAAGATGGAA TTTGGTCCACTGATATCTTAAAGGAACAGAAAGAATCCAAAAATAAGATCTTTCTAAAATGTGAGGCA AAGAATTATTCTGGACGTTTCACCTGCTGGTGGCTGACAGCAATCAGTACTGATC
Interleukin-18	ATCATACGAAATTTGACGACCAAGTTCTCTTCGTTAACGAGGGCAATCAACCAGTGTTCGAGGATA TGCCTGATTCTGACTGTACAGAAAACGCATCCCATACCATATTTATCATAAATATGTATAAAGATAGC CTCACCAGAGGTCTGGCGGTAACCATCTCTGTGATGTGTAACAGAATGTCTACTCTGTCCTGTAAG AACAAAACTATTTCCTTTAAGGAAATGAGACCTCCAGACAGTATCAGCGACGAAGGGAATGACATCA TATTCTTTCAAAGAAGTGT
Interleukin-10	CAGTTTTACTTGGAGGAGGTGATGCCCCAGGCCGAGAACCACGACCCTGAAGTCAAGGAGCTCGT GAACTCGCTGGGGGAAAAGCTGAAGACCCTGCGGCTGAGGCTGCGGCGCTGTCATCGATTTCTG CCCTGTGAGAACAAGAGCAAGGCCGTGGAA
iNOS	CCCCGCTGCATCGGGAGGATCCAGTGGTCCAACCTGCAGGTCTTCGACGCCCGGAGCTGTTCCAC TGCCAAGGAAATGTTCGAGCACATCTGCAGACACCTGCGTTATGCCACCAACAATGGCAACATCAG GTCGGCCATCAC
TGFβ	AGACATCAGCTGTCTGCTTTCCGGGCAGAGTTGCGCCTGCTGAGGCTCAAGTTAAAAGCGGAGCA GCACGTGGAGCTGTACCAGAAATA
COX-2	TTGCATTCTTTGCCCAGCACTTCACCCATCAATTTTTCAAGACAGATCATAAGCGAGGGCCAGGTTT CACCAAAG
S9	GACTCCATCCTTCTCGTCCGCCTGGATTCCCAGAAGCACATTGACTTCTCCTTGCGGTCTCCGCTAT GGGGGTGGCCGCCCAGGCCGCGTGAAGAGGAAGAATGCCAAGAAGGGCCAGGGA
Metallothionein	AGAGCTGCTGCTCCTGCTGCCCAGTGGGCTGTGCCAAGTGTGCCCAGGGCTGTGTTTGCAAAGG GA
Complement cytolysis inhibitor	CGTCTCCTGCTGAGCAGCTGGACGAGCAGTTTAGCTGGGTGTCCCAGCTGGCGAATCTCACTCAGA CTGAGGACCCGTTCTATCTCCAGGTCACGACGGTGAGTTCCCAGACTTCTGACTCCAGTGCTCCCT CTGGCGTCACTGAGGTGGTCGTGAAGCTCTTTGA
HDCMB21P	CTGTGTCTGGAGGTGGAGGGGAGATGGTCAGTAGGACAGAGGGTAACATTGATGACTCGCTCATTG GTGGAAATGCCTCCGCTGAAGGCCCGGAGGGCGAAGGTACCGAAAGCACAGTCATCACTGGTGTT GATATTGTCATGAACCATCACCTGCAGGAAACA
DQA	CAGCTTGATCACAGGTTCACTGAGAACTTGGCAATAGCGAAACAAAACTTGAACATCCTGACTAAAC GGCTCCAACTATACCGCTGCTA
DRA	ATCACGCCATGTGGACTGGGAGAGCCCAACACCCTCATCTGCTTCATTGATAAGTTCTCCCCACCAC GTGATCAATGTCACGTGGCTTCGAAATGGAAACCCTGTCACCACA

DRB	GGATGACAGCTCTGACATTGATTTTGATGGTGCTGAGTCCCCTCTTGGCTTGGGCCAGGGACACCC CACCACATTTCCTGKTYCTGRYGAMGTCGGAATGCTACTTCACCAACGGCACGGAGCGGGTGCGG TTCCTGGAKAGGTATTTCTATAACGGCGAGGAGTTCSTGCGCTTCGACAGCGACGTGGGGGAGTAC CKGSCGGTGACCGAGCTGGGGCGGCCGGACGCTCCGTACTGGAACAGCCAGAAGGACTTYGTGG AGCRGAMGCGGGCCGAGGTGGACACGTACTGCAGACACAACTACGGGGTGGGTGAGAGCTTCAC GGTGCAGCGGCGAGTGGAGCCTATAGTGACWGTGTATCCTKYGAAGACCCAGCCCCTGAAGCACC ACAACCTCCTGGTCTGCTCTGTGAATGGTTTCTATCCAGGCCACATTGAAGTCAGRTGGTTCCGGAA TGGCCAGGAAGAGGAGTCTGGGGTCGTGTCCACAGGTCTGATCCATAATGGAGACTGGACCTTCCA GACCCTGGTGATGCTGGAGACAGTTCCTCAGAGTGGAGAGGTCTACACCT
Thyroid hormone receptor	ACTGGTAGCTAGTAGGAATGTCTGAAGCCTGCCTCCATAGGAAGAGCCATGCGGAGAGGCGCAGC GCATTGAAAAATGAACAGTCGTCACCACATCTCATCCAGGCCACTTGGACTTAGCTCAATATTCC
Cold inducible RNA-binding protein	AGGCGCTCCGGCTGCCCTGGGCCTGGCGTGAACGGCCCTTCTATGCTATGTATGACCCGCAGTAG ATCGCAGACGTCCCTCGAGAGGTTCTTGAAAATGTTTATTTATATTGTCCTTTTTTACTGGAAGACGT ATGCATATCTTATTGATGTTGTATTTGAAGTGGCTGAGGAATTCTTGTACGCAAA
FoxP3	CCGCTGGGCCATCCTGGAAGCTCCCGAGAAGCAGCGGACACTCAACGAGATCTACCACTGGTTCA CGCGCATGTTTGCCTTCTTCAGAAACCACCCTGCCACCTGGAAAAACGCCATCCGCCACAACCTGA

Quantitative real-time PCR systems were developed and optimized based upon these previously unpublished sequences (Table 3). Gene expression differed between fuel oil-fed vs. control mink;

Table 3. Mink-specific primer pairs

Gene of interest	Derivation	Forward primer	Sequence 5' – 3'	Reverse primer	Sequence 5' - 3'
Aryl hydrocarbon receptor	Alignment	MvAHRF1	GTGCTGAGTACCATATACGGATGA	MvAHRR1	GTTCAGTCTAATGCACGCTTAGTG
Estrogen receptor beta	Alignment	MvERBF1	GCATTCAAGGACATAACGATTACAT	MvERBR1	GTGGCTCCCGGAGGGAAAGGT
Glutathione-S-transferase	Alignment	MvGLUF1	CTGGTGCCAGTTCTGGAAAACAG	MvGLUR1	CTAAGGTCCCATCTTTGGTAAC
UDP-glucuronyltransferase	Microarray	MvUDPF2	GAGCTCTGGAGAAAATGGTATTG TGGTGTTTACA	MvUDPR2	CCGAGTATTTGGTCCTAAGTTGTC TGGTTTCTTG
Heat shock protein 90	Alignment	MvHSP90F1	CCAAGTTGGACAGCGGTAAAGAG	MvHSP90R1	GGAGGCTCTTCAGGCTGGTGC
Heat shock protein 70	Alignment	MvHSP70F1	CCAGGTGGCGCTGAACCCGC	MvHSP70R1	CAAGGTGCAGGTGAGCTACAAGG
Interleukin-2	Alignment	MvIL-2F1	GGCCACAGAATTGACTCATCTTCA	MvIL-2R1	CTGAAACTAAAGGGATCTGAAACAAG
Interleukin-12	Alignment	MvIL-12F1	GCCATTCGCTCCTGCTGATTCAC	MvIL-12R1	GTTTCACCTGCTGGTGGCTGAC
Interleukin-18	Alignment	MvIL-18F1	GTACAGAAAACGCATCCCATACC	MvIL-18R1	CCTTTAAGGAAATGAGACCTCCAG
Interleukin-10	Alignment	MvIL-10F2	GACTTTAAGRGTTACCTGGGTTGC	MvIL-10R2	TCCACSGCCTTGCTCTTRTTYTC
iNOS	Alignment	MvINOSF1	CCGCTGCATCGGGAGGATCC	MvINOSR1	GCAACATCAGGTCGGCCATCAC
TGFβ	Alignment	MvTGFBF1	GCTCTTCAACACATCGGAGCTC	MvTGFBR1	CAGCAACGATTCCTGGCGCTAC
COX-2	Alignment	MvCOX-2F1	CATTCCTGATCCCCAGGGCAC	MvCOX-2R1	GACTGGGCCATGGGGTGGAC
S9	Alignment	MvS9F1	CCAGCGCCACATCAGGGTCCG	MvS9R1	GAATGCCAAGAAGGGCCAGGG
Metallothionein	Microarray	MvmetF1	GAGCTGCTGCTCCTGCTGCCC	MvmetR1	CCAGGGCTGTGTTTGCAAAGGG
Complement cytolysis inhibitor	Microarray	MvCYTF1	GCTGGACGAGCAGTTTAGCTGG	MvCYTR1	CCAGTGCTCCCTCTGGCGTC
HDCMB21P	Microarray	MvHDCF1	CTGTGTCTGGAGGTGGAGGGG	MvHDCR1	CAGTCATCACTGGTGTTGATATTG
DQA	Alignment	MvDQAF1	CTGTCTGGCAGCTGCCTGTGTT	MvDQAR1	CCAATGAGGTTCCTGAGGTGAC
DRA	Alignment	MvDRAF1	CACCAATGTACCTCCGGAGGTG	MvDRAR1	GGAGTGTCGGAGACAGTCTTCC
DRB	Alignment	MvDRBF1	CGGCGAGTGGAGCCTATAGTG	MvDRBR1	CGGAATGGCCAGGAAGAGGAG
Interleukin-6	Alignment	MvIL-6F1	CCTGCAGTTCAGCCTGAGGGC	MvIL-6R1	CATAAGTTATGTGCCCAATGGACAG
Thyroid hormone receptor	Alignment	Mv THRBF1	GGACAAACCGAAGCACTGTCCAG	Mv THRBR1	GGAATATYGAGCTAAGTCCAAGTGG
Cold inducible RNA-binding protein	Microarray	MvCIRBPF1	TTCTGAGTGTAGTGTGGTAGGACCC	MvCIRBPR1	TGCGTACAAGAATTCCTCAGCCACT
FoxP3	Alignment	MvFOXP3F1	CCGCTGGGCCATCCTGG	MvFOXP3R1	CAGGTTGTGGCGGATGGC

these differences were not always evident in cells derived both from blood and spleen (Table 4). Decreased expression of 11 genes was identified in oil-fed mink; results were significant for six of these genes (interleukin-18, P=0.002; interleukin-10, P=0.04; inducible nitric oxide synthase (iNOS), P=0.01; cyclooxygenase-2 (COX-2), P=0.047; complement cytolysis inhibitor (CLI), P=0.05; thyroid hormone receptor, P=0.01). Increased expression of 8 genes was identified in oil-fed mink; results were significant for two of these genes (heat shock protein 70 (HSP70), P=0.02; interleukin-2 (IL-2), P=0.04). Gene expression was virtually identical between fuel oil-fed and control mink in four of the genes (heat shock protein 90 (HSP90); transforming growth factor-beta (TGFβ); and major histocompatibility complex class II DQA and DRB).

Table 4. Differential gene expression in oil-fed mink. Standard t-tests were performed and statistical significance ($P \leq 0.05$) indicated by an *.

Gene of interest	Spleen			CPT			
	Average normalized Ct values			Average normalized Ct values			
	Oiled mink	Control mink	P value	Oiled mink	Control mink	P value	
Aryl hydrocarbon receptor	15.78	14.88	0.17	16.13	15.1	0.19	Responds to classes of environmental toxicants including polycyclic aromatic hydrocarbons, and polyhalogenated hydrocarbons (Oesch-Bartlomowicz et al. 2005)
Estrogen receptor beta	14.83	14.86	0.53	14.29	14.28	0.47	Endocrine-alterations in hormone synthesis, transport, receptor interaction, metabolism, excretion, or feedback regulation (Dahlman-Wright et al. 2006)
Glutathione-S-transferase	7.18	6.52	0.1	7.83	7.88	0.41	Important role in the detoxication and metabolism of many xenobiotic and endobiotic compounds (Armstrong et al. 1993).
UDP-glucuronyltransferase	22.25	21.50	0.35	NA	NA		Catalyzes the glucoronidation of xenobiotic compounds (Daidoji et al. 2005)
Heat shock protein 90	2.81	3.12	0.26	4.03	4.02	0.44	Produced in response to thermal or other cellular stress (Tsan and Gao, 2004)
Heat shock protein 70	2.57	3.8	0.02*	8.13	7.6	0.10	Produced in response to thermal or other cellular stress (Tsan and Gao, 2004)
Interleukin-2	14.66	16.24	0.04*	17.67	18.4	0.16	T-cell growth factor (Kindt et al. 2007)
Interleukin-12	12.7	13.3	0.15	17.93	18.37	0.43	Proinflammatory cytokine (Kindt et al. 2007)
Interleukin-18	5.13	4.08	0.002*	6.91	6.54	0.29	Proinflammatory cytokine (Kindt et al. 2007)
Interleukin-10	16.22	15.26	0.02*	21.08	20.52	0.34	Anti-inflammatory cytokine (Kindt et al. 2007)
iNOS	16.91	15.08	0.01*	18.60	20.00	0.22	Induced upon macrophage activation (Kindt et al. 2007)
TGFβ	5.46	5.22	0.5	5.66	5.8	0.5	Anti-inflammatory cytokine (Kindt et al. 2007)

Gene of interest	Spleen			CPT			
	Average normalized Ct values			Average normalized Ct values			
	Oiled mink	Control mink	P value	Oiled mink	Control mink	P value	
COX-2	7.08	7.18	0.31	9.03	7.42	0.047*	Induced upon macrophage and neutrophil activation (Kindt et al. 2007)
S9	NA	NA		NA	NA		18S ribosomal subunit/housekeeping gene
Metallothionein	7.32	8.04		6.84	7.34	0.21	Modulates the bioavaibility of physiological cations and the toxicity of heavy metals and modulate immune functions (Andrews 2000)
Complement cytolysis inhibitor	8.82	7.86	0.05*	8.68	8.44	0.25	Interferes with cytolytic function of the membrane attack complex in the complement cascade (Jenne and Tschoopp 1989)
HDCMB21P	-0.49	-1.06	0.07	-0.36	-0.54	0.13	Translationally controlled tumor protein is implicated in cell growth, cell cycle progression, malignant transformation and in the protection of cells against various stress conditions and apoptosis (Bommer and Thiele, 2004)
DQA	3.2	3.38	0.47	4.60	4.56	0.28	Binding of pathogens/initiation of immune response (Kindt et al. 2007)
DRA	4.28	3.88	0.16	5.57	5.46	0.31	Binding of pathogens/initiation of immune response (Kindt et al. 2007)
DRB	0.79	1.16	0.45	1.88	1.82	0.27	Binding of pathogens/initiation of immune response (Kindt et al. 2007)
Interleukin-6	9.36	9.26	0.42	12.72	13.16	0.5	Proinflammatory cytokine (Kindt et al. 2007)
Thyroid hormone receptor	15.39	15.02	0.16	17.01	15.10	0.01*	Hormone-activated transcription factors bind DNA in the absence of hormone, usually leading to transcriptional repression (Tsai and O'Malley, 1994)
CIRBP	9.46	9.78	0.33	9.54	9.74	0.44	Cold-shock protein (Nishiyama et al. 1997)
Fox FP3	11.12	11.28	0.32	10.78	10.68	0.32	Selectively expressed in a subpopulation of T-cells with regulatory activity (Ziegler 2007).

Discussion

The pathophysiological effects of oil exposure undoubtedly impact multiple organ systems. Ingestion of low concentrations of petroleum hydrocarbons has been associated with reproductive failure, genotoxicity, hematological changes, or impaired immune function (Mazet et al., 2001; Bickham et al., 1998; Mazet et al., 2000; Schwartz et al., 2004; Burchiel and Luster, 2001). Exposure to xenobiotics also has been implicated in compromised immunological health as well as increased incidence of disease (Harvell et al., 1999).

Quantitative real-time PCR was employed as a sensitive and specific assay for detecting fuel oil-induced changes in gene expression in mink. Our study suggests that animals exposed to petroleum oil have alterations in multiple physiological pathways including immunomodulation, inflammation,

cytoprotection, calcium regulation, cellular stress-response, metal metabolism, xenobiotic metabolizing enzymes, tumor suppression, reproduction, antioxidant enzymes, and cell-cell adhesion. A variety of immunologically relevant genes were differentially expressed in fuel oil exposed versus control mink. The T cell cytokine, IL-2, was up-regulated in mink exposed to fuel oil. This would suggest some level of increased T cell activation as IL-2 is classically considered a T cell growth factor (Kindt et al., 2007). Expression of the regulatory cytokines, IL-10 and IL-18, were decreased in exposed mink. IL-10 is largely of T cell origin and is typically considered an anti-inflammatory mediator due to suppression of macrophage activation while IL- 18 is produced by macrophages and dendritic cells and is considered a pro-inflammatory cytokine by inducing production of interferon gamma by T cells (Kindt et al., 2007). While it is not possible to suggest a specific impact of these perturbations, due to the pleotrophic and often redundant activity of cytokine activity, it is ample evidence of an immunologic insult. Two additional genes of immunologic interest, iNOS and COX-2, were down-regulated in fuel oil-fed mink. Both of these genes are induced upon activation of phagocytic cells, iNOS being associated with production of a bacteriocidal environment and COX-2 being integral in production of pro-inflammatory prostaglandins (Kindt et al., 2007). Thus, taken together, a reduction of the expression of these two genes would support compromised activity of macrophages and neutrophils. The differential expression of complement cytolysis inhibitor was initially observed using the human microarray and verified to be down-regulated by QPCR. The product of this gene has been described as interfering with the cytolytic function of the membrane attack complex in the complement cascade (Jenne and Tschoopp, 1989). Again, while the significance of this perturbation is unknown, it provides additional evidence that ingestion of fuel will impact the immune system.

Two additional genes were identified with altered expression. HSP70 was up-regulated in fuel oil-fed mink. HSP70 is an intracellular molecular chaperone and is typically up-regulated by cells in response to an insult (Tsan and Gao, 2004). Up-regulation of this gene is good evidence that the ingestion of fuel oil resulted in cellular stress. Thyroid hormone receptor, a nuclear membrane receptor tightly associated with chromatin, was down-regulated. This receptor is a DNA-binding protein that regulates gene expression (Tsai and O'Malley, 1994). While the potential impact of this specific perturbation is unknown, its altered expression would be consistent with the presence of endocrine-disrupting compounds in fuel oil.

Results from quantitative PCR differed between samples taken from spleen and CPT tubes. Except for the thyroid hormone receptor and COX-2 genes, significant differential expression was demonstrated in spleen samples only. Assuming these differences are probably due to tissue-specific effects of the oil, this study suggests caution should be taken in gene selection when blood is the only readily available sample; however, newer technology has improved the stabilization of isolated RNA from blood (eg. PaxGene blood collection vacutainer tubes permit "immediate" fixation of leukocyte mRNA upon blood collection). As gene transcripts have variable half-lives, it is reasonable to assume differential expression of additional genes may well be identified in blood with this new technology.

The need to develop molecular tools for evaluating physiological, biochemical, and histopathological effects of chronic exposure to petroleum oil and other xenobiotics in the marine environment is obvious (Peterson et al., 2003). The quantitative real-time PCR assay developed in this study for detecting petroleum oil-induced changes in gene expression in mink provides a framework for monitoring the effects of sublethal levels of contaminants and for facilitating the assessment of ecosystem health.

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Managing emergency responses with multi-organizational involvement

Barbara Callahan[1]

Keywords: emergency management, incident command system, oiled wildlife response, companion animals, disaster relief

Recognizing that there are frequent opportunities to bring many organizations into the management of emergency responses, incorporation of those organizations or their staff can be difficult if not well planned and thought-out. This paper addresses the challenges of blending multi-organizations into the management of an emergency response by evaluating several case studies of oiled wildlife responses in Estonia, Norway, Spain and the companion animal response after hurricane Katrina. This paper will present lessons learned from these experiences and examine strengths that come out of the incorporation of team members from many organizations or cultures.

The International Fund for Animal Welfare Emergency Response Team (IFAW ER Team) has helped manage many massive responses and learned lessons from each one. It is challenging, at best, to jump into a situation where there are many different cultures, work ethics and levels of expertise coming together and acknowledge what is important, while laying the groundwork for understanding these differences.

As with any kind of emergency response, there must be some sort of management structure put in place for the response to be both efficient and cost effective. In situations where there isn't an incident management team overseeing the entire event, the animal response organizations are left to sort this aspect by creating that infrastructure for themselves. This is a critical step, however, and will provide structure, efficiency and cost effectiveness if put in place and agreed upon by key decision makers. The animal response management team is responsible for filling all key positions within a response such as Logistics Manager, Search and Collection Manager, Wildlife Rehabilitation Manager, Cleaning Manager, Veterinary Services Manager, etc. Senior Managers must be placed in positions

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to be able to gather information from each area of the rehabilitation program and be able to use that information to make critical decisions in a timely manner that will provide a way for the animals to move through the process as soon as they are ready. Specific to the response, key positions should be named and job duties assigned to them and these key mid-management positions can also supervise staff in each area. Defining roles and responsibilities through-out the response team will help create a team that can work efficiently and effectively at caring for the animals. One of the most important aspects of putting a management system in place is to be able to limit span of control so no supervisor has more than 5–7 personnel to oversee.

When blending personnel from several organizations, one of the biggest issues to come to terms with at the earliest possible time is what protocols should be used. This often comes into play during oiled wildlife responses, and it is important to agree between all parties on the particular protocols that will be put in place at the onset of a response. If there are disagreements over protocols, senior managers should discuss the differences and make a decision as to what protocol is to be used and why. There are an infinite number of ways to do each step in the process of rehabilitating oiled wildlife so agreement at the onset of a response will prevent lost time later in the response.

During a wildlife response, or any emergency response, it is critical that documentation be kept throughout the process and what kind of documentation is to be done should be one of the protocols agreed to at the beginning of a multi-organizational response.

Another issue that can get in the way of an efficient and successful response is public affairs. While it is important that each organization at the response have an opportunity to fairly represent the work that they are doing there, these are issues that should be worked out at the earliest possible time so each organization understands what the agreement is and can work to maximize the press for their agency, if that's appropriate.

In the recent past there have been many large-scale events such as the Treasure and Prestige oil spills and hurricane Katrina that have required unprecedented responses to animals in crisis. While there are a few animal welfare organizations with the experience, financial support and depth to be able to handle responding to these events alone, it is becoming increasingly likely, both in the fields of oiled wildlife response and disaster relief, that there will be a multitude of organizations responding to the same event. Even when the animal event is relatively localized, there are always opportunities to collaborate with other organizations such as in the 2006 response to a mystery oil spill in the Baltic Sea, off the coast of Estonia.

The IFAW ER Team responded, along with Sea Alarm, to do the initial assessment. Once it was decided that a response was needed, IFAW and Sea Alarm worked with the local non-governmental organization (NGO), Estonian Fund for Nature (ELF), and the Estonian Ministry of Environment. Additionally, responders from the Royal Society for the Prevention of Cruelty to Animals (RSPCA), Project Blue Sea and the Bird Rehabilitation Center at Ostend, Belgium were incorporated into the response. Each organization brought strengths to the response, which were recognized and utilized

appropriately. The IFAW ER Team provided the rehabilitation oversight, along with the Estonian Ministry of the Environment, Sea Alarm provided liaison with industry, NGO's and government, RSPCA, Project Blue Sea and the Bird Rehabilitation Center at Ostend, Belgium provided rehabilitation services in several areas from supportive care to washing, managing the operations center and assisting with field capture. This was an example of an effective multi-organizational response.

Certainly, utilizing the strengths of each responder is an important aspect. Often times, when a regional, national or international team comes to an emergency response, there are local organizations and institutions whose jurisdiction it is to oversee the handling of the animals in crisis. It is imperative to work with these local organizations to assist them in the areas they need help with but letting them manage the overall response on the local level. This has been true in every oiled wildlife response the IFAW ER Team has assisted with including the Prestige spill in Spain, where the Ministry of the Environment was able to provide support to the IFAW ER Team in terms of logistics, volunteer management and communications with other teams. During the Rocknes response in Norway, the IFAW ER Team worked with a local coalition (Action Clean Birds) to respond to oiled wildlife. The local coalition was able to interface with the government to get permission to handle the animals, as well as liaise with local businesses and support the volunteer effort and other logistics.

During the Katrina Companion Animal Response, jurisdiction of the animals was held by the State of Louisiana and they empowered one of the animal rescue groups, the Humane Society of the United States (HSUS), with the management of the overall response. The HSUS, in turn, developed a full incident management team and brought in general command staff from three organizations, IFAW, American Humane Association (AHA) and the HSUS. This multi-organizational management team was unprecedented in the disaster relief field in the U.S. and was a model of effective management in the face of an enormous animal crisis. Teams whose expertise was in sheltering animals were put in charge of the massive animal shelter, organizations who specialize in animal control in large cities were added to the search and collection teams in the field and the experience and expertise of the AHA and IFAW teams was utilized to put an effective operational plan in place for the overall operation of the companion animal rescue. This collaboration paved the way for the development of a new coalition of animal welfare agencies who now work together on preparedness for emergency response to disasters in the U.S. The coalition has agreed upon protocols in documentation to be used, sheltering protocols, minimum responder training and qualifications and a credentialing system. This is a great example of effective collaboration.

Multi-organizational responses can be effective but must be well planned out through the development of a clear management system and the definition of roles and responsibilities. When the framework is well developed, these responses can be wonderful opportunities for organizations to work together to better understand one another, share ideas and strengthen less experienced staff members.

Acknowledgments

The IFAW ER Team wishes to acknowledge the many, many different organizations and individuals who have come together to assist in emergency responses over the years who have helped to provide local knowledge, professional and volunteer support for responses and made a difference in their communities. Without such support, responding internationally would be much more difficult and less effective.

Personnel for oiled wildlife response: challenges in identifying, training and maintaining an effective team

Curt Clumpner[1]

In formulating an effective wildlife response plan there are four basic building blocks: a facility or place to do the work, proper equipment to do the work with, a structure to organize the work in an effective and efficient manner and personnel to perform all of the varied tasks required to successfully respond to each unique incident. While each of these blocks are important, and a case could be made for any as the "most" important, in reality the foundation on which a wildlife response is built is personnel, just as in every other emergency response. Without adequately trained personnel, the best equipment, the finest facility and a brilliant organizational structure will all be wasted, and the failure of the wildlife response is pre-ordained.

Unfortunately, not only are identifying, training and keeping personnel ready the most important factors in maximizing the success of a wildlife response, they are the most challenging. Many of the people introduced to oiled wildlife response during an oil spill are at least initially keen to the possibility of pursuing it as a career. While the enthusiasm of many wanes when they learn the harsh realities and unpredictability of the work, in many cases the problem is that there is not a clear path to follow to receive the training and experience necessary and little hope of being able to support themselves or perhaps even a family once they have achieved the required skill level.

While industry, governmental agencies and non-governmental organizations (NGO's) have all attempted to solve this problem in a variety of ways, there is still no model that provides a real answer to this challenge.

The basic goals of any oiled wildlife response plan are to assess and document the impacts of the oil spill and to mitigate those impacts to the extent possible. To achieve these goals requires a team to not only lead and manage the wildlife response but also sufficiently transfer their knowledge to untrained

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or inexperienced staff and volunteers so the correct assessment and documentation of impacts can be made following the rescue and rehabilitation of oiled wildlife. This requires a team that is able to work well together under extreme circumstances including seemingly overwhelming numbers of affected animals, severe weather conditions in unfamiliar surroundings, and limited resources.

A number of models have been developed since oil spills in the early 1970s to facilitate the containment and clean up of oil spills to minimize environmental and social impacts. These include company spill response teams, industry owned cooperatives, and private spill response corporations. Most oil companies have a number of in-house response teams from site-specific spill response teams (SRTs) to corporate emergency response teams with global responsibilities. Creation of a number of spill response cooperatives owned by oil industry members occurred in the late 70's and early 80's including Oil Spill Response – East Asia Response Limited, a not for profit cooperative wholly owned by thirty-one oil and energy companies. Other similar organizations include, Alaska Clean Sea (ACS) based in Alaska, the Marine Spill Response Corporation (MSRC) based in the US, and the Eastern Canada Response Corporation (ECRC) based in Canada. There are also a large number of for profit spill response companies. These range from small local companies to large corporations such as Seacor Environmental. During a spill response one or more of these types of organizations are activated to assist in the clean-up operations under the incident management system in place. Organizations in each of these model groups are important components of the overall spill response capability.

The timeline of development of wildlife response models is somewhat similar. The first model appeared in the late 1960's and early 1970's. A number of oiled wildlife response organizations were founded in the wake of spills impacting large numbers of wildlife around the world. International Bird Rescue Research Center (IBRRC), Tri-State Bird Rescue & Research, Inc., The Royal Society for Prevention of Cruelty to Animals and the South African Foundation for the Conservation of Coastal Birds (SANNCOB) were all either founded or formalized as oiled wildlife rescue organizations in this period. All of these were organized as nonprofit organizations depending on donations to fund their work. Over the years many of these organizations worked increasingly to secure contracts or retainer agreements with oil and shipping companies as well as governmental agencies to provide a more stable basis for funding.

The Exxon Valdez Oil Spill and the experience gained during the wildlife response brought about many changes both to the existing organizations and subsequent thinking regarding oiled wildlife response. In addition, a series of oil spills in California impacting wildlife "close to home" in the years following the Exxon Valdez compelled California to look for a new model to deal with oiled spills and improve the ability to further minimize impacts of oil spills on wildlife. This provides the second model for this discussion. This was through California's Office of Spill Prevention and Response (OSPR) and the Oiled Wildlife Care Network (OWCN). OWCN is "a statewide collective of wildlife care providers and regional facilities interested in working with oil-affected wildlife". It

is administered by the Wildlife Health Center at the School of Veterinary Medicine, University of California, Davis. The 25 participating organizations provide a wide range of experience as well as potential access to hundreds of staff and volunteers for training and use during a wildlife response. The OWCN is funded through OSPR. It provides oiled wildlife response within the state of California as well as promotes research to increase the knowledge of oil spill impacts and improve the quality of care for oiled wildlife. Members receive equipment and training as well as remuneration for requested services during a response.

The third and final model for this discussion comes from Europe and is the most recent. This model is that of an international NGO network. The example for this is the European Network organized by a Dutch foundation called Sea Alarm which was established in 1999. The European Network consists of associated members with a background in marine wildlife rehabilitation, science, industry, governmental organizations or NGO's.

The purpose of organizations that fall within each of these models is to provide a high level of care to oiled wildlife. To do that they must provide a management structure and the trained personnel to accomplish each task critical to the success of an oiled wildlife response. While the management structure utilized by industry and governmental agencies for spill response and other emergencies has been successfully adapted to oiled wildlife response in many situations around the world, I would argue there has been much less success in providing a well trained pool of personnel of sufficient size to fulfill this need and facilitate the continued improvement needed in this field.

Currently there is no model currently that stands out as truly successful. Each has its own strengths and weaknesses. There is no currently successful business model that has wide geographical, economic and cultural application. None provides an adequate pool of dedicated staff to respond to multiple events. None provides an adequate and stable pool of properly trained wildlife response personnel to meet the current or future needs of the oil and shipping industries, governments and their agencies and NGO's to respond effectively and efficiently to oiled wildlife. Each model has struggled to identify, recruit, train and retain adequate numbers of skilled people to fulfill current obligations and provide assistance in wildlife response planning and the appropriate training of even low-level wildlife responders. Organizations in each model depend on a handful of individuals with the necessary training and experience to manage a wildlife response. In the event of concurrent medium events any of the organizations are stretched to meet personnel requirements and the response may well be compromised. There is a need for a model that can provide the support needed to recruit and retain sufficient numbers of wildlife responders to successfully manage concurrent oiled wildlife incidents while providing "best achievable care".

There are a number of common factors in building and maintaining any team.

These are recruitment, initial training, and acquisition of real experience, retention and replacement of individuals lost through normal attrition. If the model successfully achieves the first four

factors it will be relatively easy to replace the occasional individual who changes jobs or retires from within the team or colleagues.

In recruiting it is important to identify individuals who are dedicated to the work and if properly supported will continue in the field for many years. By minimizing turnover investment in training will be maximized and experience gained will provide the greatest benefit to the organization. There are some characteristics that can be helpful in identifying individuals who are more likely to be successful as wildlife responders. These include the ability to work under extreme pressure; the ability to focus on tasks while maintaining a view of the larger picture; the ability to identify and solve problems and a belief in the importance of wildlife rehabilitation. Rescue and rehabilitation of oiled wildlife utilizes a variety of skill sets. These include impact assessment and monitoring, wildlife deterrence, wildlife capture, veterinary medicine and nursing, wildlife rehabilitation, crisis management, working with hazardous materials and management of staff and volunteers to name a few. Individuals with backgrounds in wildlife biology, animal husbandry, veterinary sciences, wildlife rehabilitation or emergency response may all have skills that can be combined to build a well-rounded team for oiled wildlife response.

Training is a key component in preparing personnel to successfully respond to a spill involving wildlife. While most wildlife response organizations are active in training, little of it focuses on their key personnel. It is most often used to introduce outsiders such as industry, government and local NGO personnel to the basic concepts of wildlife response, or to maintain contact with potential low-level responders or volunteers and also as a revenue source. If management level professional wildlife responders are to maintain or improve their skills and develop needed team relationships, it is critical that they be involved in periodic training that focuses on building common understanding of concepts, trust and an understanding of individual strengths and weaknesses. Yearly scenario based training that allows participants to work together to solve realistic problems will both build strong teams and individual skills.

Retention of wildlife response personnel is an issue that has plagued all of the models presented. For a variety of reasons it has proven difficult to date to keep many individuals in the field. One of the key reasons has been financial stability and compensation. While most "professional wildlife responders" are compensated quite well during an oil spill response, there has never been a structure that provided adequate financial support to more than a handful around the world on an ongoing basis. Most wildlife response organizations' budgets include only a few salaried "responders" with even those personnel having primary daily responsibilities outside of true response. They often function primarily as planners, trainers or administrators, trying to produce income for their organization while waiting for the next call to respond oiled wildlife. As a result, many of the true responders, mainly contractors or volunteers for many of the response organizations eventually must find some other way to support themselves and their families and leave the field. This funding problem, which is connected to the unpredictability of the work, also contributes to the retention challenge in other

ways. Without financial support it becomes doubly important to maintain a feeling of importance, camaraderie and shared purpose within the team. Without frequent, regular, significant communication, bonds dissolve quickly and team members - whether volunteer or staff - lose focus and move on to more practical or achievable goals.

For wildlife response organizations to truly provide a high level of care it is critical that they develop a business model that will allow them to recruit, train and retain quality team members who are dedicated to the work and able to work together as a team to achieve the best results possible in each unique incident. This means providing financial support to an adequate core team of responders, on-going training to build and maintain the efficiency and effectiveness of the organization's team and co-operative training with other organization to increase the joint capabilities for large-scale responses. It also means maintaining a continuing program to identify and nurture individuals throughout the world who can be integrated and eventually replace the core as needed.

Post-release survival of oiled, rehabilitated waterfowl

Dunne, Rebecca[1] and Miller, Erica DVM

Keywords: waterfowl, mallard, Canada goose, annual survival rate, post-release survival, oiled

Abstract

Band returns may provide insight into survival in the wild after rehabilitation for oil contamination. Previous studies on survival of oiled, rehabilitated birds have focused on seabirds. Tri-State Bird Rescue & Research has historically responded primarily to inland oil spills, which occur more frequently than open water spills and can impact waterfowl. We analyzed data from the USGS Bird Banding Laboratory to compare annual survival rates of oiled, rehabilitated waterfowl with non-oiled, free-ranging waterfowl. Results indicate that contact with oil and subsequent rehabilitation is not an indicator of reduced post-release survivability in mallards and Canada geese and that release location has a significant impact on longevity.

Introduction

Many organizations treating wildlife impacted by oil spills band the birds prior to release, using bands registered with a central data base such as the Bird Banding Laboratory (BBL) of the U.S. Geological Survey (USGS) and the Canadian Bird Banding Office (BBO) of the Canadian Wildlife Service. Bird banding, also known as ringing, is done in hopes that it might provide insight into survival in the wild after rehabilitation for oil contamination. Tri-State Bird Rescue & Research (TSBRR, Tri-State) has historically responded primarily to inland oil spills, which occur more frequently than open water spills in the US (Etkin, 2006) and can impact waterfowl. Since 1990, Tri-State has responded to 54 spills resulting in impacted waterfowl (61% of total responses) (TSBRR data).

Previous studies on survival of oiled, rehabilitated birds have focused on seabirds, including common murres (*Uria aalge*), brown pelicans (*Pelecanus occidentalus*) and several species of pen-

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guins (Sharp, 1996; Wernham et al., 1997; Anderson et al, 1996; Whittington, 1999; Goldsworthy et al., 2000). These studies may influence how industry and agencies consider the value of oiled wildlife rehabilitation during a response or long-term effects of oil on wildlife during damage assessment. Two studies, one in North America and one in Great Britain, indicate that post-release survival of oiled, rehabilitated common murres is much lower than would be expected for non-oiled birds (Sharp, 1996; Wernham et al, 1997). One study tracking cleaned brown pelicans with external radio transmitters came to a similar conclusion (Anderson et al., 1996). These studies ignited controversy about whether cleaning oiled birds was justified, given the poor survival rates. However, researchers examining survival of oiled, rehabilitated penguins of several species have found significantly higher survival rates (Whittington, 1999; Goldsworthy et al., 2000). No longevity studies of oiled, rehabilitated waterfowl have been published, although waterfowl are ubiquitous in inland waters and are often impacted by oil spills.

Band recoveries in the studies of murres ranged from 1% to 4% (Sharp, 1996; Wernham et al., 1997), and an analysis of African penguin returns had a similar low recovery rate (Whittington, 1999), allowing limited information on which to base conclusions. Band recoveries for waterfowl are expected to be higher, due to hunter returns and the partially terrestrial natural history of these birds (Giudice, 2003; Sheaffer et al., 2005). The hypothesis is that higher band returns would lead to a larger sample size and more reliable results.

This study attempts to calculate the annual survival rates of oiled, rehabilitated mallards (*Anas platyrhyncos*) and oiled, rehabilitated Canada geese (*Branta canadensis*) and to compare longevity with that of natural samples. Mallards and Canada geese were chosen as representative of semi-aquatic waterfowl. Both species are commonly impacted by oil spills, both are hunted, and both are common across North America.

Methods

Definitions

Annual survival rate refers to the proportion or percentage of animals alive one year that will be alive the next year.

Natural sample, or *non-oiled sample*, refers to birds coded as status 300 by the USGS Bird Banding Laboratory (normal, wild bird; released in same 10-minute block as captured).

General oiled sample, or *general oiled, rehabilitated sample,* refers to birds coded as status 740 by the USGS Bird Banding Laboratory (rehabilitated and held longer than 24 hours: sick, exhausted, injured or crippled; oiled: cleaned and released). This sample is not controlled for practitioner protocols.

TSBRR sample refers to oiled birds rehabilitated by TSBRR in accordance with specific treatment protocols and release criteria.

All time intervals are inclusive.

Treatment of Oiled Wildlife

Treatment protocols for oiled wildlife may vary between practitioners; the following description is consistent with treatment at TSBRR. Following capture each animal is placed in a well-ventilated container and transported to the oiled wildlife rehabilitation facility. The animal receives a full physical examination including weight, blood values (packed cell volume [PCV], total solids [TS], buffy coat [BC]), body temperature, and a thorough accounting of the extent of oiling. Stabilization includes oral fluids (Pedialyte® [Abbott Laboratories, Abbott Park, IL] at 3% of body weight), intravenous fluids (2.5% dextrose in lactated Ringer's solution at 3% of body weight), flushing of the eyes and nares with 0.9% normal saline and radiant heat if necessary. The individual is also given a small amount of Pepto-bismol® (Proctor and Gamble, Cincinnatii, OH) orally, which coats the gastro-intestinal (GI) tract, counteracts inflammation in the GI mucosa, and may prevent further absorption of ingested oil. Injuries unrelated to oiling may also be treated. Oiled animals are offered drinking water and are nutritionally maintained with a gavaged diet.

When the oiled animal is stable and presents PCV ≥ 30, it is washed in multiple tubs of diluted DAWN® dishwashing liquid (Proctor and Gamble, Cincinnati, OH) in hot water (38 – 40°C). Following the wash, the individual is thoroughly rinsed in hot water under pressure (40-60psi) to remove all soap. The bird is then transferred to a drying area. When it is completely dry it is moved to an indoor pen with drinking water and appropriate food. The animal may be moved to an outside area with pool access when rehabilitators are certain that it is self-feeding. It remains in an outside pen until release.

Animals are released when they are completely waterproof, are at an appropriate weight for that species at that time of year, exhibit normal behavior and present a PCV appropriate for that species. Before animals are released TSBRR consults with state and federal wildlife agencies to determine appropriate or preferred release locations (See Welte and Frink, 1991; Miller and Frink, 1995; or Miller and Welte, 1999 for a more complete protocol).

Samples

Encounter histories were obtained from the USGS Bird Banding Laboratory. The samples included mallards and Canada geese of status 740 (rehabilitated and held longer than 24 hours: sick, exhausted, injured or crippled; oiled: cleaned and released) and status 300 (normal, wild bird; released in same 10-minute block as captured). Birds of status 300 represent the control. Encounter histories were also tabulated from oiled mallards and Canada geese rehabilitated, banded and released by TSBRR.

Data were compiled for mallards banded between 1974 and 2004 and encountered between 1974 and 2006. BBL records for oiled Canada geese were available starting in 1976, so data from oiled and

non-oiled Canada geese were compiled from birds banded between 1976 and 2004 and encountered between 1976 and 2006. Both live and dead encounters were included. Birds with "unknown" encounter status were considered dead. Live birds included individuals with status "Alive – released" and "Alive – in captivity." Data for oiled mallards treated at TSBRR were available for birds banded between 1993 and 2004 and encountered between 1993 and 2006. Data for oiled Canada geese rehabilitated at TSBRR were available for birds banded between 1990 and 2004 and encountered between 1990 and 2006.

Every record from oiled, rehabilitated birds was incorporated into the analysis. However, because the available data for non-oiled birds was significantly greater than that for oiled birds, a random sample was selected from each of the natural samples in order to compare groups of similar sizes.

The sample of non-oiled mallards was distributed across all four North American flyways (Pacific, Central, Mississippi, and Atlantic) (Figure 1). Most oiled, rehabilitated mallards were from the coasts, with a few birds from the Mississippi flyway and none from the Central flyway (Figure 2). The majority of TSBRR mallards came from the Atlantic flyway, with two from the Mississippi flyway.

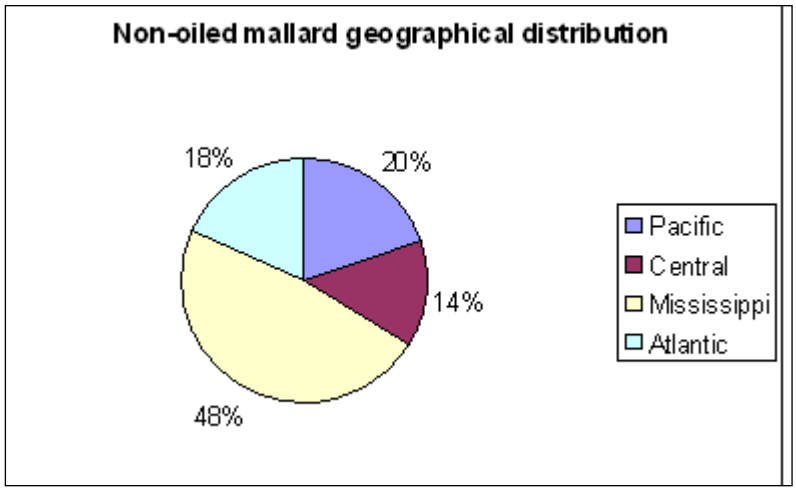

Figure 1. Non-oiled mallard geographical distribution

Similarly, the natural sample of Canada geese came from all four flyways, although the majority was banded and encountered in the Mississippi flyway (Figure 3). Oiled, rehabilitated Canada geese were also distributed across all four flyways; however, a large majority was present in the Atlantic flyway (Figure 4). Canada geese treated at TSBRR were generally from the Atlantic flyway with less than 10% from the Mississippi flyway.

Analysis

Program MARK (White and Burnham, 1999) was used to evaluate annual survival rate from encounter history. The program employs techniques described by Burnham (1993) to model joint live and dead encounter data. Data sets were run with all parameters held constant in order to determine annual survival rate over each time interval. Results were then compared with an unpaired t-test.

Comparing a general sample of oiled, rehabilitated birds to a natural sample assumes that treatment protocols and release criteria for oiled birds are identical between rehabilitation centers or that they do not affect survival. We have attempted to address this assumption by comparing banding data

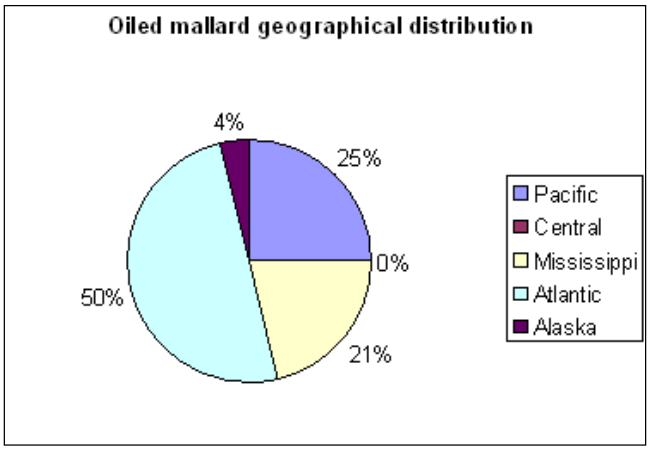

Figure 2. Oiled mallard geographical distribution

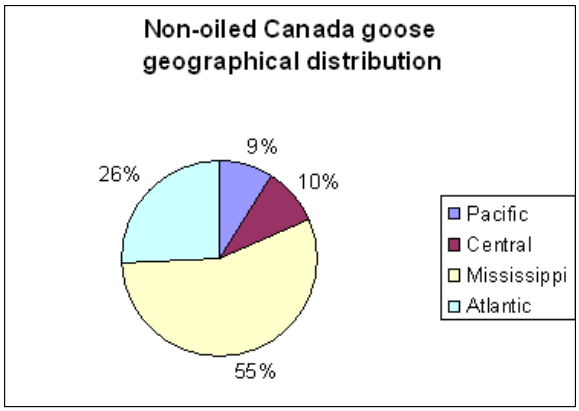

Figure 3. Non-oiled Canada goose geographical distribution

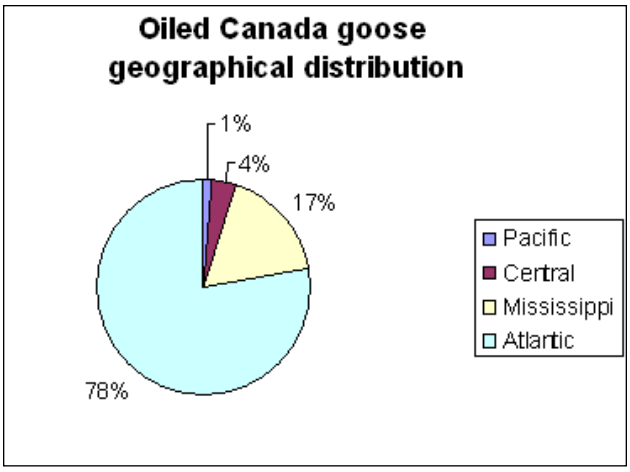

Figure 4. Oiled Canada goose geographical distribution

from oiled birds treated at TSBRR to the general oiled and non-oiled samples. This study also assumes that longevity potential remains constant across all types of oil, geographic locations and times of year. Addressing these assumptions is unfortunately beyond the scope of this study.

Results

Mallards

Mallards were divided into two sets for analysis. The general samples of both non-oiled and oiled, rehabilitated mallards were run as a large sample (1974 – 2004) corresponding to the data available for the general oiled sample. Tri-State data were available from 1993 to 2004, so data from the general samples were also run as a smaller sample (1993 – 2004).

The natural sample of mallards had an annual survival rate of 0.685 between 1974 and 2004 (n=65, SE=0.035). Median years survived for this group was one year, mean years survived was 1.98 years, and the range was 0-13 years.

Annual survival rate for the natural sample between 1993 and 2004 (the time period for which data is available for TSBRR mallards) was 0.679 (n=29, SE=0.052). Median years survived was one year, mean years survived was 1.62 years, and the range was 0-8 years.

Annual survival rate for the general sample of oiled, rehabilitated mallards was 0.694 between 1974 and 2004 (n=64, SE=0.037). Median years survived was one year, mean years survived was 1.75 years, and the range was 0-9 years.

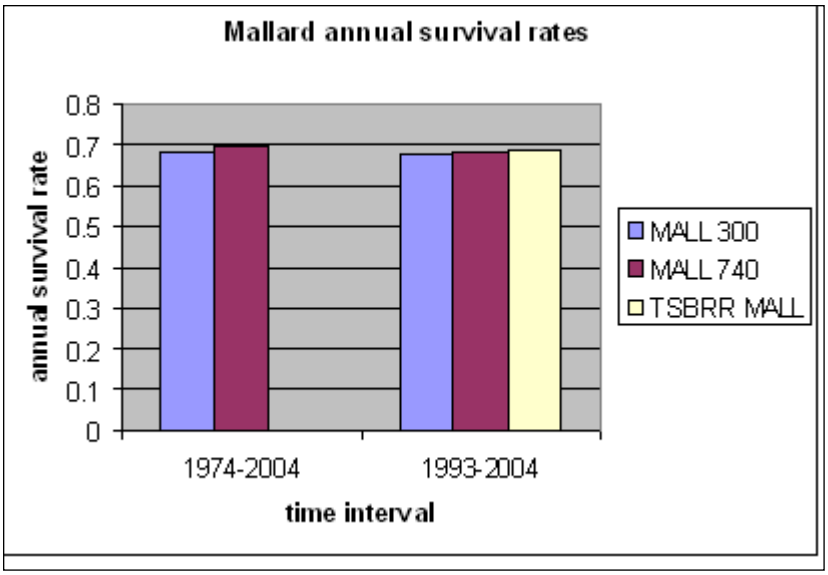

Figure 5. Mallard annual survival rates

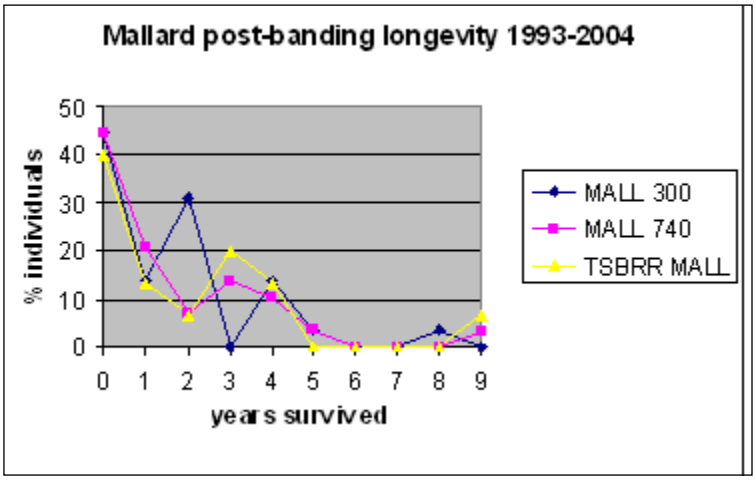

Figure 6. Mallard post-banding longevity 1993 – 2004

The general oiled, rehabilitated sample from 1993 to 2004 had an annual survival rate of 0.685 (n=30, SE=0.063). Median years survived for this group was one year, mean years survived was 1.6 years, and the range was 0-9 years.

Annual survival rate for the general sample excluding TSBRR birds was 0.615 (n=14, SE=0.101). Median years survived was one year, mean years survived was 1.43 years, and the range was 0-5 years.

Mallards treated at TSBRR from 1993 to 2004 had an annual survival rate of 0.689 (n=15, SE=0.069). Median years survived was one year, mean years survived was 2.2 years, and the range was 0-9 years.

Canada Geese

Canada geese were divided into three subsets for analysis. General data for both the natural sample and oiled, rehabilitated Canada geese were run as a large sample (1976 – 2004), as a smaller sample corresponding to the available TSBRR data (1990 – 2004) and as a sample excluding data from the Delaware River Oil Spill (1990 – 2003). Tri-State data were run in the two sets for which data was available (1990 – 2004 and 1990 – 2003).

The natural sample of Canada geese for the years 1976 to 2004 had an annual survival rate of 0.745 (n=254, SE=0.016). Median years survived for this group was two years, mean years survived was 2.4 years, and the range was 0-20 years.

The natural sample for the years 1990 to 2004 had an annual survival rate of 0.738 (n=158, SE = 0.023). The median years survived was two years, mean years survived was 2.18 years, and the range was 0-11 years.

The natural sample from 1990 to 2003 had an annual survival rate of 0.747 (n=151, SE=0.230). The median years survived was two years, mean years survived was 2.23 years, and the range was 0-11 years.

The general oiled, rehabilitated sample of Canada geese had an annual survival rate of 0.717 (n=209, SE=0.187) for the years 1976 – 2004. The median years survived was one year, mean years survived was 2.04 years, and the range was 0-13 years.

Annual survival rate for the general oiled sample between 1990 and 2004 was 0.700 (n=158, SE=0.023). The median years survived was one year, mean years survived was 1.91 years, and the range was 0-13 years.

Annual survival rate for the general oiled sample from 1990 to 2003 was 0.770 (n=107, SE=0.025). The median years survived was one year, mean years survived was 2.49 years, and the range was 0-13 years.

Canada geese treated at TSBRR had an annual survival rate of 0.687 (n=149,

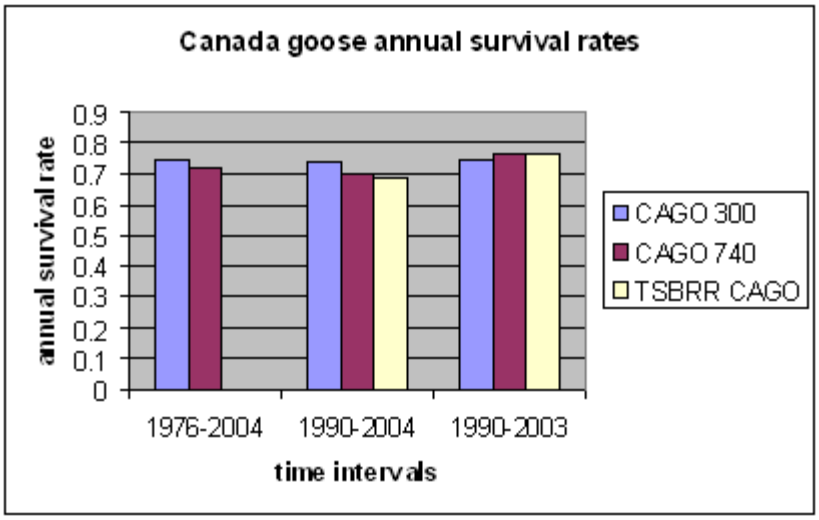

Figure 7. Canada goose annual survival rates

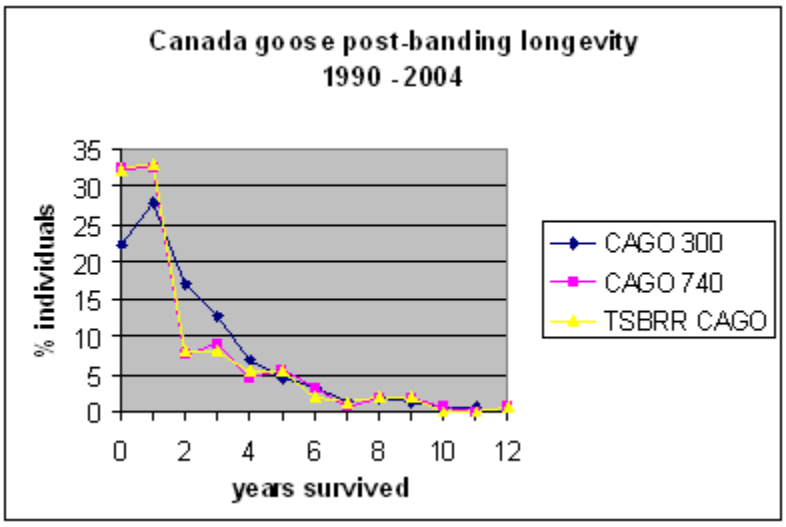

Figure 8. Canada goose post-banding longevity 1990 – 2004

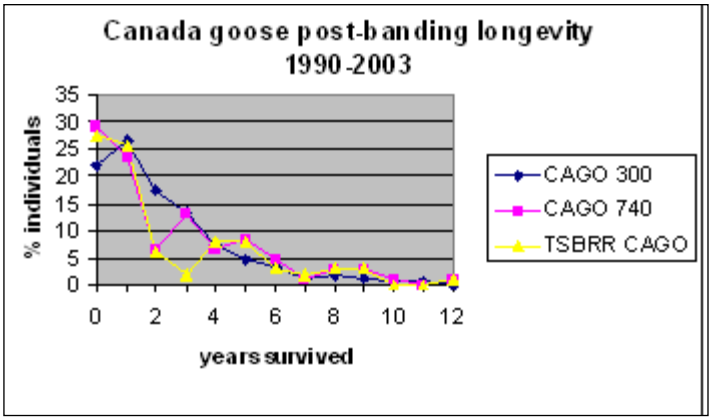

Figure 9. Canada goose post-banding longevity 1990 - 2003

SE=0.024) from 1990 – 2004. The median years survived was one year, mean years survived was 1.85 years, and the range was 0-12 years.

From 1990 – 2003 the TSBRR sample had an annual survival rate of 0.761 (n=97, SE=0.027). Median years survived was one year, mean years survived was 2.44 years, and the range was 0-12 years.

Discussion

Mallards

A comparison of the natural sample and the general oiled sample of mallards shows very little difference during the time period between 1974 and 2004 (p=0.1343) as well as during the more recent time period, 1993 – 2004 (p=0.7159). These results demonstrate that oiling and subsequent rehabilitation does not have an effect on post-release survival of mallards.

Tri-State mallards were compared to the natural sample and the oiled sample for the period that encounter records are available for TSBRR birds. Mallards treated at TSBRR had a similar annual survival rate both to the natural sample (p=0.5961) and to the general oiled, rehabilitated sample (p=0.8320). Since TSBRR birds accounted for fully half of the patients in the general oiled sample, we also analyzed the general sample after removing these data. The difference between TSBRR mallards and those treated by other rehabilitators was significant (p=0.0299), but further study may be warranted due to the small sample size. Nevertheless, this disparity suggests that the non-TSBRR birds may have been exposed to more negative factors (less stringent treatment protocols and release criteria, more toxic oil, more severe weather conditions, release during hunting season, etc.).

Looking at the TSBRR data is important for our center in terms of refining protocols, and it is also a useful way to remove one of the variables inherent in the general oiled, rehabilitated sample. In the birds rehabilitated, released and encountered from the general sample, there is no way to know which rehabilitators treated the birds. Treatment protocols and release criteria may vary widely between practitioners and may influence survival potential of released individuals. Although treatment may vary slightly according to the needs of the individual, birds rehabilitated, released and encountered from the TSBRR sample have undergone essentially the same treatment and are released only when they meet specific criteria (see Methods). The increased survival of TSBRR mallards compared to all other oiled, rehabilitated mallards suggests that treatment protocol and release criteria strongly influence longevity of released mallards.

Canada Geese

The general sample of oiled, rehabilitated Canada geese had a significantly lower annual survival rate than the natural sample in both the 1974 to 2004 sample (p=0.0001) and the 1990 to 2004 sample (p=0.0001). Similarly, TSBRR Canada geese from this latter interval showed a significantly lower survival rate than both the natural sample (p=0.0001) and the general oiled, rehabilitated sample

(p=0.0001). When comparing the same data but deleting the bands from the Delaware River Oil Spill (2004), however, both the general oiled, rehabilitated sample and the TSBRR sample showed a significantly higher survival rate than the natural sample (p=0.0001, p=0.0001). These large changes show the impact that one event, such as the Delaware River Oil Spill, can have on the calculated survival rate of a population, and they underscore the importance of accounting for major events that may confound results.

The Delaware River Oil Spill occurred in November of 2004. TSBRR released 291 Canada geese impacted by crude oil from this spill, (TSBRR data) and most band returns from 2004 came from geese shot within days of their release after this spill. It is TSBRR's policy to consult with wildlife agencies regarding release locations for birds affected by oil spills; in this case agency requested that birds be released to a location an average of 130 miles from their capture location (TSBRR data). In a foreign location birds may have less knowledge of food resources and areas protected from predators. In addition, the geese were released during hunting season on land open to hunting. Birds may have become disoriented, or they may have been more likely to fly over hunting lands in an attempt to return to their home locations. In TSBRR's experience, most oiled, rehabilitated birds are returned to their capture sites. Because of the unique circumstance of this release time and location, the authors felt that removing data from the Delaware River Oil Spill provided more representative information on normal survivability of oiled, rehabilitated Canada geese.

Conclusion

Contact with oil and subsequent rehabilitation is not an indicator of reduced post-release survivability in waterfowl. The results of this study demonstrate that oiled, rehabilitated mallards and Canada geese have a similar or even greater annual survival rate than similar species that have never been oiled. Rehabilitated Canada geese, especially, show a significantly higher survival rate (disregarding one aberrant event) than normal, wild geese. Rehabilitation may even give these birds a slight advantage over their counterparts through consistent access to food, a brief protection from predators and general herd health management practices such as deworming. This data gives strong support to the current practice of cleaning and rehabilitating oiled waterfowl.

Waterfowl present a convenient sample for a study using encounter histories. However, seabirds are also primary victims of oil spills. Past research has calculated survival rates for seabirds using the Brownie method (Brownie et al, 1985), which only accounts for dead recoveries. The Burnham method, which also explains live encounters, may provide more representative information; however, banding data for seabirds are far less complete than for waterfowl. We suggest that calculating survival rates from banding data may be inappropriate for seabirds due to extremely low encounter rates.

Separating the oiled, rehabilitated sample to control for variability in petroleum products or times of year was beyond the scope of this study, but would be a logical next step in determining the effects of these factors on long-term survivability of oiled waterfowl. It may also be of interest to model

annual survival rates for the first several years following release. In addition, oiled wildlife practitioners would benefit from research that correlates annual survival rates to specific treatment protocols and release criteria.

Acknowledgments

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Resources

Bird Banding Laboratory, USGS Patuxent Wildlife Research Center, 12100 Beech Forest Road, Laurel, MD 20708-4037, 301-497-5795, http://www.pwrc.usgs.gov

Program MARK: Mark and Recapture Survival Rate Estimation. Gary White, Department of Fishery and Wildlife, Colorado State University, Fort Collins, CO 80523 http://www.warnercnr.colostate.edu/~gwhite/mark/mark.htm

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New European tools for oiled wildlife preparedness and response

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Keywords: oiled wildlife, Europe

Abstract

In Europe, more information on good practices and tools for oiled wildlife planning and response is needed. This paper describes three European projects co-financed by the Commission in 2006-07, in which Sea Alarm has been involved. As part of these projects, three workshops on Oiled Wildlife Response Planning, Wildlife Impact Assessment and Oiled Wildlife Cleaning and Rehabilitation were organised. A new set of specific tools has been developed aiming to improve the level of oiled wildlife preparedness and response for European Union (EU) Member States, Norway and Iceland. The new European tools will become available in the course of 2007.

Proper preparedness is crucial to effective oiled wildlife response. An integrated oiled wildlife response plan that is regularly exercised and that can mobilize and deploy the right expertise and resources, is the best guarantee that a successful and cost-efficient response in the aftermath of an oiled wildlife incident can be carried out.

It is known that European coastal countries are unequally prepared for oiled wildlife incidents. Although most countries have general oil spill response plans in place, few include explicit reference to oiled wildlife response. Because of communicated experiences with oiled wildlife response during a number of recent European oil incidents (most notably Erika, Prestige, Tricolor), the subject has been generating interest with an increasing number of oil spill response authorities in different countries.

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Recognizing the developing need for more information on good practices and tools for planning and response, Sea Alarm in 2005 initiated the formation of some consortia in order to propose three projects for European funding. All three projects were adopted by the Commission (Community Framework for Cooperation on Accidental and Deliberate Marine Pollution - DG Environment) and began in February 2006. The main element in each of the projects was the organization of a dedicated workshop in which a particular issue of oiled wildlife response and planning would be examined by delegations from EU Member States, Norway and Iceland. The workshops are briefly described in this paper.

All three workshops aimed at exchanging experiences, discussing national and international approaches and exploring cross border co-operation while focusing on developing specific tools. A different perspective was chosen in each project, as is described bellow.

1) European Workshop on Oiled Wildlife Response Planning (12-15 June, Brest, France)

This workshop was organized as part of a project run by Sea Alarm (B – lead partner), Centre de Documentation de Recherche et d'Expérimentations sur les Pollutions Accidentelles des Eaux (CEDRE, F) International Fund for Animal Welfare (IFAW, US/UK), International Tanker Owners Pollution Federation (ITOPF, UK), the Finnish Environment Institute (SYKE, Fin), Oil Spill Response and East Asia Response Limited (OSRLEARL, UK), and Istitutio Centrele per la Ricerca Scientifica e Tecnologica Applicata al Mare (ICRAM, I). Its goal was to bring together authorities from European Coastal States to discuss lessons learnt from past oiled wildlife incidents and to explore national and international solutions for effective preparedness to future incidents in Europe. In total, 18 European countries attended the workshop, including authorities responsible for oil spill management, authorities responsible for animal welfare issues and recognised non-governmental organizations (NGOs). The workshop concluded that European coastal countries should aim for an integrated national wildlife response plan as the overall effectiveness of an oiled wildlife response depends on the preparedness of competent authorities and key stakeholders. Response planning should always be based on best practices. It was also concluded that European countries are not equally prepared, and a number of activities, both at the national and international level were recommended. It was highlighted that developing capacity for an effective response that included mutual assistance throughout Europe could be facilitated through international training courses. It was also stressed that regular communication between agencies and countries would be highly beneficial. The contents of the envisaged www.oiledwildlife.eu were agreed upon.

2) Workshop on Wildlife Impact Assessment (7-9 September, A Coruña, Spain)

This workshop was organized as part of a project run by Royal Netherlands Institute for Sea Research (NL– lead partner), Sea Alarm (B), University of A Coruña (E). It aimed to define best practices with regards to the collection of dead oiled seabirds in the aftermath of an oil spill incident and the subsequent necropsy and data analysis. Also it was discussed which data on (seasonal) seabird distribution would be needed by oil spill managers in the early stages of an incident, to be used in the

decision making process on environmental protection. In total, 38 participants attended the workshop, including mainly scientists, but also regulators and NGOs amongst which were 20 formally appointed delegates from 11 European countries. One of the main conclusions of the workshop was the importance of integrating wildlife impact assessment into the overall oiled wildlife response plan. It was highlighted that certain baseline data collected during and after the event, are essential to be able to assess the impact of an oiled wildlife incident. By having updated sensitivity maps with high quality information on habitats and species it is possible to minimise the wildlife mortality associated with a spill. The contents of the envisaged Handbook were agreed to as well as concrete research recommendations.

3) Workshop on Oiled Wildlife Cleaning and Rehabilitation (21-23 October, Albufeira, Portugal)

This workshop was organised as part of a project run by Zoomarine (P– lead partner), Sea Alarm (B), International Fund for Animal Welfare (IFAW, US/UK), and Istitutio Centrele per la Ricerca Scientifica e Tecnologica Applicata al Mare (ICRAM, I). This workshop aimed to discuss backgrounds and principles of the cleaning and rehabilitation of oiled wildlife in the aftermath of an oil spill incident and tried to identify good practices that could be made available to wildlife responders throughout Europe. In total, 41 participants attended the workshop including regulators, scientists and rehabilitators, amongst which were 29 formally appointed delegates from 18 European countries. The workshop identified a number of solutions in order to link the gaps that exist between what wildlife responders do on a regular basis and the rehabilitation success that is reached elsewhere based on good practices. It was concluded that authorities should demonstrate active leadership in the planning and response to oiled wildlife involving key stakeholders and make sure that wildlife responders can be compensated for their efforts. It was stressed that the principles of animal welfare must be applied under the different circumstances of an oiled wildlife response. A number of international activities and products were identified to improve the success of oiled wildlife rehabilitation, including training modules and opportunities, guidelines and handbooks, websites and databases, newsletters and discussion forums, workshops and conferences. The contents of the Handbook were agreed upon.

As a result of the discussions at the workshops, new European tools have been developed and will become available in the course of 2007, including:

- www.oiledwildlife.eu

This new website (www.oiledwildlife.eu) is hosted by Sea Alarm and aims to provide access to quality information, examples and references regarding various aspects of oiled wildlife response. Eleven main entries (European preparedness, Oiled Wildlife Incidents, Planning, Response, Compensation, Impact assessment, Training, Effects of Oil on Wildlife, Mobile Equipment, Industry and Oiled wildlife Care and Rehabilitation) will provide the visitor with references or detailed information that can be accessed elsewhere on the web. The portal site is under continuous review and new useful information and new links will be added over time. The site includes a Forum and a Blog

to facilitate the exchange of opinions and online discussions. Although developed under a European umbrella, the website is not limited to European information and can be expected to develop into a global resource.

Out of the conclusions and recommendations of the three workshops, a European Oiled Wildlife Response Plan will be developed. It will read as a European strategy that identifies the opportunities and lists a number of objectives and activities that that could be adopted and carried out at different national (including subnational), regional (regional seas) and international (European or global) levels. Although not a formal European strategy, the document will be presented to national governments and at each relevant international platform (e.g. European Commission, Helsinki Convention, Bonn Agreement, Barcelona Convention, Copenhagen Agreement).

The EU Handbook, which will be published in August 07, will be a guide to best practices that can be applied in carrying out a wildlife impact assessment. The Handbook, which will be subject to ongoing review, with the most recent version always available on the worldwide web, describes methods and tools that can be applied even under the most difficult and stressful circumstances, protocols, peer reviewed scientific articles and a myriad number of relevant data on vulnerable seabirds in European waters. The handbook will be a useful tool for scientists, wildlife responders and governmental officers looking for specific information in this field. Anticipated chapters will include: Short History and Case Studies; Questions To Be Answered; Pre-spill Preparedness: vulnerability atlases; Oil Spill Response (I): Providing Biological advice; Oil Spill Response (II): Impact Assessment; Data Analysis and Publications; Post-spill Studies; relevant addresses of key-persons, organisations and institutions involved and several annexes with e.g. health and safety issues, drift experiments using corpses, species manual and search and collecting strategies (see www.nioz.nl/oilspills and www.oiledwildlife.eu).

The EU Handbook, published May 2007 and available through www.oiledwildlife.eu , contains guidance for wildlife rehabilitators and governmental authorities on best practices with regards to the rescue, cleaning and rehabilitation of oiled birds, especially in large incidents. The Handbook, which intentionally was limited to only 20 pages, provides principles rather than detailed methodologies on a number of important issues, including search and rescue, transportation, setting up facilities, health and safety, the involvement of volunteers, the compensation of costs and how to deal with animal welfare issues. Also, contact addresses and reference to other sources of information are provided. The Handbook is available in printed and in electronic formats (See www.oiledwildlife.eu).

Oiled wildlife response is an emerging field of interest in Europe. The three workshops that were held in 2006 offered the first opportunities to raise and discuss the issue at a truly EU level. Almost all EU coastal states have attended one or more of these workshops, creating new international networks that will be instrumental for future activities and progress towards a greater European preparedness for oiled wildlife incidents. The tools that were, or are being developed as part of the described projects will help raise the international perception of what is good practice. The website

www.oiledwildlife.eu will serve as a focus for actors in the different countries to expand upon their knowledge and understanding and it will help to develop the kind of common language that is needed for effective international cooperation.

Challenges of wildlife response in Southeast Alaska

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Keywords: Alaska, wildlife response, SEAPRO, oiled wildlife

Southeast Alaska is a maritime environment with only a few areas of concentrated infrastructure on 13,000 miles of coastline. The wet, cool climate supports temperate rain forests. Thousands of islands, channels, and bays create an environment ideal for wild waterfowl and marine mammals. As a result, at least 107 species of seabirds, waterfowl, and shorebirds migrate through, overwinter or reside in Southeast Alaska (Heinl, 2004; Armstrong, 2001; Gibson, 2007; ADFG) for some portion of the year including sensitive species such as the short-tailed albatross, black oystercatcher, marbled murrelet, and Kittlitz's murrelet. In addition, sea otters, Stellar's sea lions, harbor seals, and many terrestrial species depend upon the unique marine environment for food and habitation. Humans have inhabited the region for thousands of years and today, as then, many depend upon the rich maritime resources for their livelihood.

There is the potential capacity for transport of 1,779,039 barrels (bbls) or 74,719,638 gallons of petroleum products throughout Southeast Alaska each year. In reality, probably about one half of that volume is actually transported (exact figures are proprietary information and not available). Most of this volume is composed of distillates such as diesel and gasoline. In addition, there is a steady flow of boat traffic ranging from fishing vessels and ferries up to cargo vessels and cruise ships, all of which carry some measure of fuel or petroleum products. Fortunately there have been few accidents and fewer large scale petroleum spill disasters in Southeast Alaska.

Following the Prince William Sound Oil Spill in 1989, the U.S. Government passed the Oil Pollution Act of 1990. Around the United States, oil spill response organizations (OSRO) were developed to meet the requirements of the new law. The establishment of Southeast Alaska Petroleum Resource Organization (SEAPRO) in 1990 created an organization that met the federal and state laws governing the transportation, storage and response capabilities for member companies in Southeast

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Alaska. Since that time the organization has grown considerably and now has eight 249-barrel skimmer-equipped aluminum barges with LORI Skimming Systems and two 48-foot custom-built oil spill response vessels (OSRV). SEAPRO maintains a total skimming capacity of 46,661 bbls per day effective recovery rate, 39,060 feet of boom and, with our members, over a million barrels of storage capacity. SEAPRO compensates for the remote nature of Southeast Alaska by staging several caches of supplies and recovery equipment and by maintaining an active volunteer training program for a corps of responders from all communities. Because of the smaller scale of oil transportation activities and the light and volatile nature of the products involved (which quickly disperse or evaporate), most of SEAPRO's response efforts have concentrated on rapid response and containment of product.

In addition to primary responses (containment of petroleum products), SEAPRO also attempts to be prepared for secondary (wildlife hazing) and tertiary (wildlife capture and treatment) response. Many oil spill response co-ops have retainer agreements with oil spill response groups, such as the International Bird Rescue Research Center, to handle their tertiary response efforts in the event of a spill. While this is probably an optimal scenario for many situations, there are several reasons why this arrangement is not as applicable in Southeast Alaska. First, since SEAPRO's member companies (and their budgets) are small, there is inadequate funding to justify retainers for outside response groups. Second, since most of Southeast Alaska is roadless and does not have airports, all materiel must be transported to the scene by boat, helicopter, or small airplane. Third, local knowledge of weather, geography, and local species of birds and wildlife should be vital, especially during the early stages of response when little information is available. Most of SEAPRO's responders are native Alaskans with excellent familiarity with boats, harsh conditions, and field expediency. While an outside team will most likely be called in the event of a large-scale disaster, they will still be reliant upon our wealth of local knowledge and experience.

SEAPRO is still expanding its ability to respond to oiled birds with the ultimate goal of being ready to respond to 150 birds within 48 hours as an interim response until a larger wildlife response group can arrive. To meet this goal, SEAPRO has stationed wildlife support equipment in communities around Southeast Alaska including Ketchikan, Juneau, Kake, Petersburg, and Sitka. This includes two wildlife treatment modules which are constructed from 40-foot insulated cargo transport containers. They were modified from locally-available refrigeration containers. These virtually self-contained units can be trailered or barged to any location in Southeast Alaska on little notice. The washing module features three on-demand propane-powered water heaters, a triage area, net pens for drying, and basic medical equipment and supplies. Currently, the washing module is stationed at the Alaska Raptor Center in Sitka where they maintain it and utilize it as needed.

A second module, the treatment module, is basically a mobile clinic. It is still under development but currently houses three examination tables, medical supplies, basic laboratory equipment, and a room for housing recovering patients. Southeast Alaska Wildlife Center in Ketchikan utilizes this module as their wildlife rehabilitation clinic and has helped immensely in its continued development.

Working with local wildlife rehabilitation centers helps SEAPRO in several ways. First, these organizations can provide input on development and equipping of response facilities. Second, these groups can help in the maintenance and keeping of equipment so that it is always ready. Third, these groups provide a potential workforce which is locally available and already familiar with working with birds and the geography and environment of Southeast Alaska. With additional safety training and specific oil spill response training, the volunteers of these organizations are a great resource. In addition to benefiting SEAPRO, we are also able to support the efforts of local rehabilitators and SEAPRO's employees have the option to gain further experience working with wildlife. The Alaska Department of Fish and Game also utilizes some of these volunteers in their citizen science research programs.

Aside from logistical challenges, another concern is having tactics and tools on-hand for hazing marine waterbirds. Because of the unpredictability of where a response will occur and what the habitat, weather conditions, and season will be, we need to be prepared to respond to a great variety of birds and circumstances. Techniques generally available include pyrotechnics, static devices such as mylar tape, balloons, and effigies, sonic devices such as propane cannons and electronic call generators, and more active methods such as aircraft, boats, falconry, and ground personnel. While these techniques can be successfully deployed in protected waters or close to shore, many of them become less effective (or unsafe) in rough conditions or around exposed islands and rocks. Also, breeding and staging birds can be more resistant to hazing pressures. Many species of birds will also dive underwater in response to surface hazing techniques. It is also commonly recognized that most species of birds will become habituated to hazing attempts that are not multiple and dynamically deployed (Greer, 2000). Therefore, it seems prudent to develop a larger arsenal of techniques that will work in a variety of conditions and that will address our particular area of weakness in hazing diving birds. We have several novel hazing techniques that are being considered for research and development and would welcome the chance to collaborate with other organizations in their development. These techniques will be briefly discussed and illustrated during our presentation at the conference.

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Evolution of penguin rehabilitation at Fundación Mundo Marino, Argentina (1987–2006)

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Keywords: penguin, rehabilitation, chronic oiling, Argentina, South America, protocols, methods

Fundación Mundo Marino, located in San Clemente del Tuyú—Buenos Aires (36°22' S / 56°44' W), has a Marine Fauna Rescue and Rehabilitation Center that has been operating since 1987 as a leader institution in the field, rescuing different species of seabirds, sea turtles and sea mammals. The center not only rescues animals, but also gives the public the possibility to increment their knowledge on the ocean's urgent situation, through education and exposure.

The Atlantic coast of Buenos Aires Province represents a valuable area in terms of biological diversity and is also a place for numerous human-related activities, which place this region in a vulnerable situation because of the environmental impact that these activities can produce. The ocean's contamination by hydrocarbons (oil) is one of the most evident and conspicuous threats for seabirds (Jehl 1975). From 1998 to 2006, numerous spills have occurred along the Argentine coast, which according to the reports of the Argentinean Navy, happened mainly in ports, and have been controlled by trained personnel. Authorities have not reported accidents or illegal discharge maneuvers off shore (Prefectura Naval Argentina, personal communication).

According to different authors, incidental releases of oil by spills or shipwrecks are a possible source of petroleum affecting penguins, but oil contaminated ballast water discharged at sea appears to

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be the main source of pollution (Boersma 1987, Gandini et al. 1994, Ruoppolo et al. 2003, Garcia-Borboroglu et al. 2006). However, the constant beaching of affected penguins evidences the chronic presence of petroleum in our waters.

South America has seven species of penguins; out of the total, three are often found oiled along the coast of Buenos Aires Province: Magellanics *(Spheniscus magellanicus)*, Rockhoppers *(Eudyptes chrysocome)* and Kings (*Aptenodytes patagonica).* Other species of seabirds are affected, but penguins are the most common sight (Ruoppolo et al. 2003). In Argentina, the most important oil spill affecting wildlife occurred in 1991, on the Chubut Province, when 17,000 oiled penguins died (FVSA, 2000). This first large-scale spill has raised the public's preoccupation about the fragility of our coasts and wildlife.

Figure 1- Through weekly beach patrols Fundación Mundo Marino rescues hundreds of oiled penguins every year. Credit: FMM/ S. Rodriguez Heredia

From 1987 to 2006, 2173 penguins of four species have been rescued alive and admitted for rehabilitation at FMM. Of these, 76.57% (1664/2173) were oiled. For detailed information, please refer to Tables 1 and 2. The most common species treated are Magellanic penguins (93.7%, 2037/2173), for which there are growing concerns related to their conservation, due to oiling, overfishing and by-catch. The International Union for Conservation of Nature and Natural Resources (IUCN) status for the species was lowered from Lower Risk in 2000, to Near Threatened in 2004 (BirdLife International, 2004).

Table 1. Total number of penguins treated by Fundación Mundo Marino presented by species and percentage treated due to oiling. San Clemente del Tuyú, 1987-2006

Species	**Total Intake #**	**Total Oiled**	**% Oiled**
Magellanic	2037	1585	77.8 % (1585/2037)
Rockhopper	123	75	60.9 % (75/123)
King	12	4	33.3 % (4/12)
Gentoo	1	0	0
	2173	1664	76.57% (1664/2173)

Table 2. Number of penguins received and treated due to oiling along the years and related rehabilitation percentage. San Clemente del Tuyú 1987–2006

YEARS	TOTAL LIVE INTAKE #	# OILED	% REHABILITATION OF OILED BIRDS
1987	20	18	55% (10/18)
1988	23	22	54.5% (12/22)
1989	108	96	53.3% (51/96)
1990	43	40	50% (20/40)
1991	64	57	32% (18/57)
1992	48	26	84% (22/26)
1993	27	21	80% (17/21)
1994	147	102	80% (82/102)
1995	165	71	80% (57/71)
1996	167	160	69% (111/160)
1997	127	125	62% (78/125)
1998	94	88	59% (52/88)
1999	120	113	76.1% (86/113)
2000	67	62	75.8% (47/62)
2001	300	217	88.6% (192/217)
2002	180	136	94% (128/136)
2003	31	17	100% (17/17)
2004	34	22	90.9% (20/22)
2005	48	48	93.7% (45/48)
2006	360	223	94.6% (211/223)
TOTAL	2173	1664	76.57% (1664/2173)
Average rehab %			76.68% (1276/1664)

Since the early beginnings, in 1987, FMM has experienced a significant increase in survivability of the birds rehabilitated, this due to the development and application of practical and effective rehabilitation protocols for penguins. Our protocols now address the primary issues that oiled birds face on intake, including emaciation, severe dehydration, hypothermia, immune suppression, anemia and secondary problems associated with these conditions (Ruoppolo et al., 2004).

As part of our current treatment protocols, each new penguin is placed in the quarantine area, an evaluation of its general condition is performed, the bird is individually identified with a plastic flipper band, standard morphometrics are taken, a clinical record is prepared, and corresponding treatment is given, according to the condition diagnosed.

Basically, the rehabilitation of oiled birds at FMM consists of:

- Stabilization: Removal of excess oil from the bird's nares, eyes and oral cavity, administration of electrolyte solutions by stomach tube, the bird's temperature and weight are recorded and different biological samples are taken for initial diagnostics, documentation and research purposes (blood and fecal samples, oiled feathers and an individual picture – Ruoppolo et al. 2005).
- Feeding: different species of fish are utilized, depending on season availability, including: Engraulidae *(Engraulis anchoita),* Sciaenidae *(Cynoscion striatus, Micropogonias furnieri),* Stromatidae *(Stromateus brasiliensis)* and Clupeidae *(Brevoortia aurea).*

Methods used for feeding include force-feeding, gavage-feeding, free-feeding, and the penguin feeding box (Callahan 2001, Silva et al. 2003).

- Veterinary supervision
- Removal of feathers' contaminants
- Plumage and physical reconditioning

After this process, if the bird is medically approved, coinciding with the migration season, it is released within the study area. In reverse conditions, the bird stays at the rehabilitation center until the next migratory season. If the birds die during the rehabilitation process a necropsy is performed. In specific cases, complementary techniques are performed to diagnose the animal's condition such as endoscopic examination, microbiological, cytological and histopathological studies.

In the past different treatment techniques have been used to treat oiled penguins, and these have been perfected throughout time. Older techniques are presented along with new methods in Table 3.

Table 3: Past and current techniques used to treat oiled wildlife, that have been changed due to learning experience along 19 years. San Clemente del Tuyú, 1987–2006

	Past technique	Current technique
Facilities	Lack of adequate facilities	Facilities built for marine animal rehabilitation attend seals, seabirds and sea turtles
Personal Protective Equipment (PPE)	Proper PPE not utilized	Nitrile gloves, proper long-sleeve washing gloves and goggles when handling King penguins
Intake	Grouping birds through intake date	Intake exam including blood sampling, initial isolation for observation, later grouping birds through condition= herd health
Record keeping	Individual bird record	Intake log and individual bird record
Feeding	Force feeding and free feeding	Force feeding, free feeding and the Penguin feeding boxes (Callahan 2001, Silva et al. 2003)

	Past technique	Current technique
Washing criteria	Fair body condition, standing	Determined through pre-wash exam: PCV ≥30%; BC ≤2%; TP ≥2,5g/dL Normal body temperature: 39-41°C Body condition: Fair Behavior: Normal, alert, standing Normal hydration status Lungs clear
Cleaning agent	Testing different solutions. Use of dispersants was toxic to people and animals and ineffective	Magistral® dishwhasing detergent (Procter & Gamble) diluted at 1-2%
Bird cleaning	Using brushes, bird not submerged in water	Feather manipulation submerged in soapy hot water, using proper protective gloves (IBBRC SOP)
Number of times the birds were washed and time spent	Birds were washed several times in different occasions. This was stressful and time-consuming	After stabilization, birds are washed once through successive baths in warm water with detergent (IBBRC SOP). Time spent 20-30 minutes
Restraint	Rubber band closing the beak	Proper handling prevents the need for rubber bands
Water hardness	No knowledge of problems related to using hard water for bird washing	Water softener used to decrease carbonates in the water used for washing and rinsing the birds (IBBRC SOP)
Rinsing	Water without any pressure, not necessarily removing all the detergent	Removal of all the detergent with water pressure (IBBRC SOP)
Drying	Outside in the sun	Heat lamps or pet dryers
Access to pools	A week after washing	Next day after the wash in fresh water and the second day into salt water
Waterproofing	Reached after over a month in captivity	"softer-water" for washing and rinsing and knowledge allows waterproofing to be reached within 10-14 days
Release criteria	Good Body Condition History showing improvement Behavior: Normal and feeding well	Determined through pre-release exam: Good body condition Behavior: Normal and feeding well Lungs clear PCV ≥ 38 %; BC ≤2%; TP >3.0 g/dl Grading: 100% waterproof after 1 hour in no haul-out pool
Process length	60 days	20-30 days

Legend:

BC: Buffy Coat; PCV: Packed Cell Volume; TP: Total Protein or Total Solids

IBRRC SOP: International Bird Rescue Research Center Standard Operating Procedure

Throughout the years, along with an increasing caseload of animals, came the difficulties associated with economical resources to be able to collect and treat the animals affected that stranded yearly along our coast. These constraints included funds for gasoline, large amounts of fish to feed

Figure 2- FMM's staff about to start cleaning one of the several affected birds in 2007. Credit: FMM/ K. Alvarez

the animals in care, medication and consumables. Lack of funding turned out to be a limitation to develop this indispensable task.

Since 2001, after joining the IFAW Penguin Network (Ruoppolo et al. 2005), IFAW has collaborated with FMM during the oiled penguin season (May-September) and through emergency calls, helping to increase the amount of animals rescued through: fuel for beach patrols, personnel and equipment during emergency responses, fish and medication for the birds in care. FMM's staff involvement in the IFAW ER Team responses in Argentina (2001 and 2006), Mexico and Chile also helped improving and changing our protocols.

Different techniques have been used to treat oiled birds over the years, which have been perfected throughout 19 years, details presented on Table 3. Applying more efficient protocols have allowed the percentage of rehabilitated birds to reach 94% in the last five years, for detailed information please refer to Table 2.

This paper has intended to show the importance of applying efficient treatment protocols, consequently having the birds in captivity for shorter periods of time, optimizing resources and maximizing survival.

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Volunteer management at a large scale oiled bird incident in San Pedro, California

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Keywords: volunteers, management, volunteer operation center, oiled bird incident, United States Coast Guard (USCG)

Background

After the 1989 *Exxon Valdez* Oil Spill, significant concern was expressed about response to oil spills and the United States federal government passed the Oil Pollution Act (OPA 1990). The OPA expanded the authority of the U.S. Coast Guard (USCG) to respond to marine oil spills and allowed the federal government to direct all oil spill response efforts. The act also created the Oil Spill Liability Trust Fund, required coastal areas to develop contingency plans in the event of an oil spill, and required owner/operators to prepare oil spill response plans. Finally, the act increased penalties for noncompliance and allowed each state to establish their own laws.

At the same time, the California legislature began work to expand and establish more state-specific requirements. In 1990, the Lempert-Keene-Seastrand Oil Spill Prevention and Response Act (SB 2040) was passed and established an additional branch of the California Department of Fish and Game known as the Office of Spill Prevention and Response (OSPR). SB 2040 gave the Administrator of the OSPR the authority to direct oil spill prevention, response and clean up in California. Additionally, OSPR was given the authority to conduct natural resource damage assessments, to direct oil spill response research, and to have the findings incorporated into established protocols and oil spill response plans.

SB 2040 also required the Administrator to develop rehabilitation stations and ensure that these facilities were in a constant state of preparedness to provide the best achievable treatment to wildlife affected by marine oil spills. To further meet these mandates, two additional bills, Senate Bill 775 and

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Assembly Bill 1459 were passed to fund the development of six rehabilitation facilities, provide funds for upkeep and develop a research program. The Oiled Wildlife Care Network (OWCN) was established in 1994 by the Administrator to comply with the federal and state regulations. The OWCN was charged to construct and maintain an infrastructure of rehabilitation centers; educate rehabilitators, biologists and technicians in providing the best treatment to oiled wildlife; develop a research program to continually improve response efforts; and respond to oil spill events in California. Since 1994, the OWCN has trained volunteers and staff from 24 participating organizations across the state to help prepare for and respond to oil spills.

To expedite the integration of volunteers who are interested in assisting with caring for oiled wildlife, volunteers at the OWCN participating organizations are given the opportunity to attend the regular OWCN trainings, which include mandatory health and safety training. Providing training to OWCN participating organizations improves the quality and speed of care that oiled wildlife receives because trained volunteers are familiar with the oiled wildlife protocols and treatments.

In January 2005, seabirds were reported to be oiled and were being brought to established OWCN participating organizations. This incident turned out to be the largest oiled bird incident in California since the inception of the OWCN. Over 700 birds had entered care at the Los Angeles Oiled Bird Care and Education Center (LAOBCEC) in San Pedro, California in the first three days of the incident, and more were expected to arrive. The LAOBCEC was designed to care for a total of 1,000 birds at full operating capacity, therefore proper planning was important to assure an effective oiled wildlife response. Within hours of the reported incident; USCG, OSPR and OWCN were in the field collecting oiled wildlife and locating the source of the contaminant. Treatment was provided to affected wildlife in the field and at the primary care facility, the LAOBCEC.

This large-scale event tested the ability of OWCN participating organizations to lend equipment, volunteers trained in the care oiled wildlife and staff. Due to the size of the incident, numerous volunteers were needed. Initially a large number of trained personnel were brought in to ready the center, establish treatment protocols and set up the facility. As the numbers of oiled wildlife increased, more personnel were needed. On the third day of the response, a media announcement was broadcast requesting volunteer assistance and support from the general public. Of those who responded to the request, approximately 500 volunteers were utilized to provide care for the oiled wildlife. At the pinnacle of the response we had more than 150 volunteers assisting in one day. This number includes both OWCN-trained volunteers and volunteers with no formal oiled wildlife response training. A Volunteer Operation Center (VOC) was established to manage and ensure that volunteers were aware of the potential hazards and working in a safe environment.

When an oil spill is large enough to exceed the capacity of immediate resources available, volunteers are needed to fulfill the requirements for oiled wildlife care. The Unified Command managing the Ventura Oiled Bird Incident decided that volunteers were required in addition to trained responders. On the third day of the declared incident the State Volunteer Coordinator (SVC) and the OWCN

Volunteer Coordinator (VC) established the VOC at the LAOBCEC in three hours. In California, all volunteers are required to complete the Oath of Allegiance and Volunteer Service Agreement forms. Once these forms are signed and submitted, volunteers are entitled to coverage with the State Worker's Compensation Program should an injury or illness occur while working at the responding facility. Additionally, the volunteer coordinators provide mandatory health and safety training for all volunteers.

The VOC established a toll-free telephone number for public calls in response to the oiled wildlife incident. During the incident, volunteer coordinators scheduled volunteers to complete required health and safety training and paperwork at set times to foster improved efficiency. Volunteers were also trained to answer calls from the public and solicit more volunteers.

To aid in response planning, volunteer coordinators worked with the oiled wildlife response staff to establish which facility response areas needed volunteer assistance and the number of volunteers needed to complete the work. Once the volunteer coordinators received that information a shift schedule was outlined and volunteers could schedule themselves for times they were available. Additionally, volunteers could call the 1-800 telephone number and asked to be placed on the shift schedule.

It was essential that volunteers were mentored and given hands-on training before being allowed to work with wildlife. Groups of no more than four volunteers were matched with the lead staff in each of several response areas. Lead staff introduced the areas and communicated the goals and duties of that area. Each volunteer was shown how to do the task then asked to complete the task and finally to teach the task to another volunteer. Only when the volunteers had learned, demonstrated, and perfected a required skill set were they allowed to train other volunteers. The lead staff in each area was able to assess volunteers' abilities in that area and provide feedback to the volunteer coordinators at the end of a work shift or day.

The volunteer coordinators worked directly and daily with the lead staff to address any concerns with volunteer personnel. Volunteers were rotated through the various work areas within the facility. During work breaks, they were provided food and beverages. The VOC served as a communication center where volunteers could ask questions, refer to the status board for current information about the spill response, and provide feedback to the volunteer coordinators about their experience.

The VOC contained telephone and facsimile lines that were used to coordinate with the public and the OWCN's participating organizations. Volunteer coordinators were able to contact other centers to solicit more trained volunteers, who could assist with transportation from the mobile field stabilization unit to the center and to other OWCN primary care facilities.

The established network of participating organizations allowed for the best achievable treatment for the large number of affected wildlife. Personnel who were trained in the OWCN animal care protocols and who had participated in previous deployment drills were able to respond quickly and stabilize oiled wildlife. During this incident, volunteers and SeaWorld staff transported oil-affected

brown pelicans *(Pelecanus occidentalis)* to the SeaWorld Oiled Wildlife Care facility where the pelicans received care from that facility's trained staff and volunteers.

Several avenues of volunteer appreciation existed during and after the spill response incident. Volunteer participation in bird releases was one of the methods for appreciation. During the incident, volunteers who assisted for more than 12 hours became eligible to go on bird releases. The volunteer coordinators worked with the staff to establish who was eligible and could participate on bird releases. OWCN considers it critical that the volunteers are appreciated and coordinators emphasized with staff the importance of politeness, addressing volunteers by name and thanking volunteers routinely. After the response, the OWCN VC and the SVC were able to highlight the contributions of volunteers in a newsletter and at a formal appreciation event. A video summarizing the response and highlighting the work the volunteers provided was shown at the appreciation event.

All volunteers were awarded a certificate of appreciation for their assistance during this incident. Since the volunteers signed in and out, we were able to assess the number of hours each volunteer contributed and provided them with pins for 20, 50 and 100 hours of service. We also designed and dedicated a T-shirt for the volunteers to further commemorate their contributions and efforts.

Data documentation of dead and debilitated oiled wildlife: California's approach

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Keywords: documentation, mortality, oiled wildlife, oiled birds, evidence, preparedness

A critical but sometimes neglected and underappreciated component of oil spill response is the collection of data on all dead and debilitated wildlife affected by a spill. Such documentation is the basis for assessing an oil spill's overall impact on seabird and other wildlife populations (Carter et al., 2003), and can also link oiled wildlife to a specific oiling event. With much focus on containment, cleanup, and rehabilitation, in some regions documenting dead oiled wildlife receives almost no attention. This is even true despite the higher frequency with which dead birds are often found compared to live debilitated wildlife, and the fact that birds that wash ashore dead can represent different species than those found alive. In addition, non-medical data (e.g., morphometrics, collection location, extent of oiling) on live animals can be overlooked in the face of rehabilitation needs.

Documentation of both dead and debilitated wildlife is the basis for assessing an oil spill's impact on wildlife populations (Heubeck et al., 2003), and without it inaccurate estimations will be made regarding mortality in impacted populations. It can also play a critical role in determining which species or populations to focus on for restoration and monitoring efforts following a spill event. Through evidence collection, such documentation can also link oiled wildlife to a specific oiling event: this can aid in the identification of the responsible party liable for payment of wildlife damages to mitigate the effects of the spill on impacted wildlife.

Having standardized protocols for these elements of oil spill response incorporated into overall response plans, as well as having individuals identified to conduct this work, will ensure that such documentation is conducted during an actual spill response. California Department of Fish and

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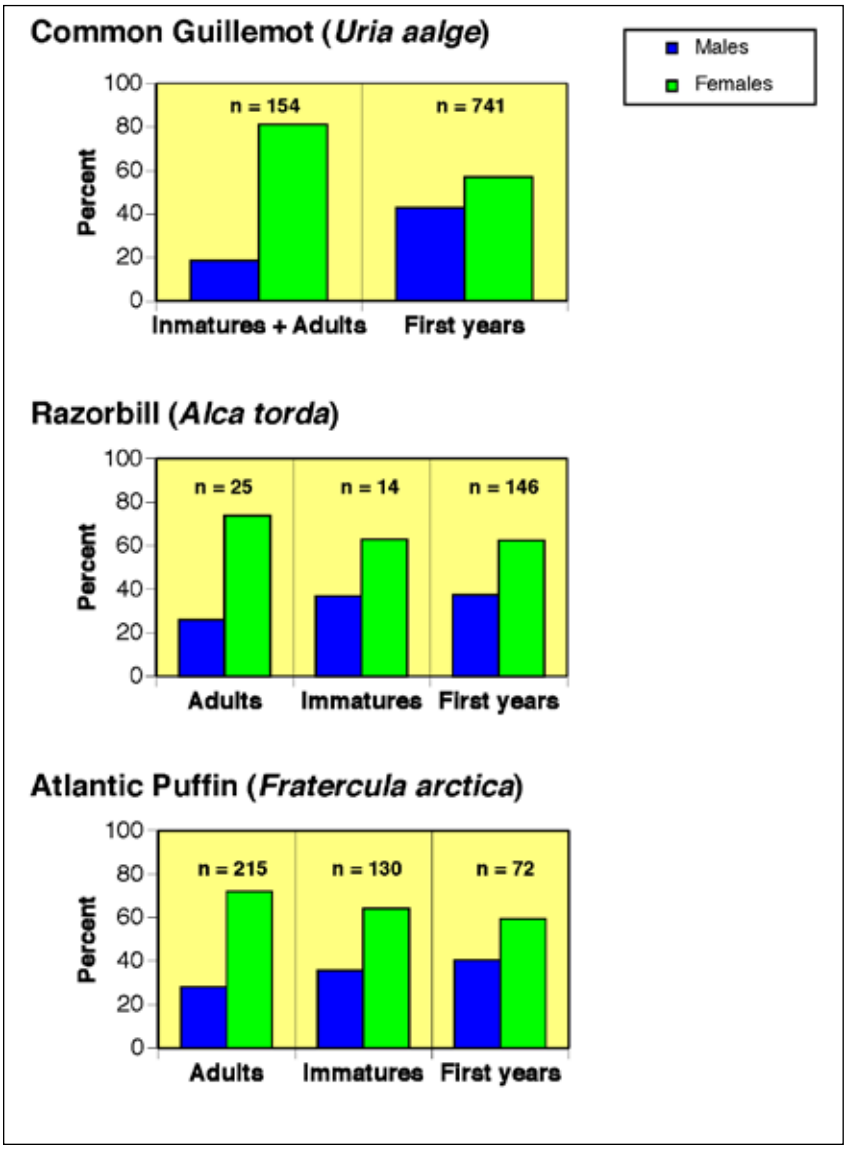
Game's Office of Spill Prevention and Response (OSPR) includes such data documentation protocols for oiled wildlife as an integral component of their wildlife response plan (OSPR, 2005). Standardized beach search effort is obviously a necessary counterpart to these efforts (Heubeck et al., 2003; OSPR, 2005). In practice, deployment of methods for data documentation is closely linked with rehabilitation, and in California the process is made possible through a partnership between OSPR, rehabilitation and response experts from the Oiled Wildlife Care Network (OWCN), and biologists from PRBO Conservation Science. A Memorandum of Understanding was created between the state of California and PRBO to define the roles of all parties.

PRBO Conservation Science, a scientific private non-profit organization, is officially deployed to conduct wildlife processing in all sizeable events in California, working under the Oiled Wildlife Care Network during these responses. PRBO maintains a team (called the Wildlife Processing Unit; WPU) of over 50 biologists from both within and outside the organization who can be called upon to respond in the event of a spill. Biologists are from various wildlife agencies and organizations throughout the state in an effort to increase response capabilities to spills in different geographic regions. The WPU is part of the incident command structure in California. Annual training courses identify and train new members of the WPU in data documentation protocols for oiled wildlife and provide Hazard Communication (HAZCOM) training necessary to work with oiled wildlife in a rehabilitation facility during oil spills. PRBO's WPU team leaders conduct the annual training courses, participate in annual oil spill drills and are permanently on call in the event of a spill in California.



Elements of data documentation for every animal collected during a spill event include: 1) information specific to the oil spill (e.g., collection date, collection location); 2) data reflecting the condition of the bird (oiling status, percent of body oiled, where oiling occurs, freshness of carcass, evidence of scavenging), 3) information to help understand which populations are affected (species, identification, age, sex, morphometrics, federal band recoveries), and 4) evidence collection (oiled feather or fur samples, photographs, carcass storage).

We recommend that all countries, states and provinces vulnerable to oil spill events that have not already done so develop response plans that not only include wildlife components, but also specifically include standardized data documentation protocols for both live and dead wildlife. We recommend that regional response plans also involve steps to ensure preparedness (e.g., trainings and development of protocols) in case of such an event. Similar steps are important to prepare for conducting beach surveys for dead or debilitated wildlife, which is also an integral component of the response. Preparedness includes: (1) identifying individuals or organizations to conduct data documentation during a response, and maintaining agreements to do so; (2) maintaining a network of potential responders from the local scientific community who have the level of expertise required for such elements of the response; (3) developing partnerships between pertinent organizations, individuals, and agencies, including engagement between the scientific community and appropriate members of the rehabilitation community and government agencies; and (4) readying equipment to ensure immediate response capabilities, including both specialty items (e.g., scientific supplies) and an initial amount of other necessary supplies.

Our recommendations stem directly from the collective experience of decades of oil spill response and population impact determination in California, and from comparing the effectiveness of responses before and after initiation of oiled wildlife data documentation protocols.

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Age and sex structure of auk (*alcidae*) mortality during the *Prestige* oil spill in Galicia, NW Spain, November 2002–April 2003

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Keywords: oil spills, Prestige, skewed mortality, common guillemot, razorbill, Atlantic puffin

The Galician coast (NW Spain) has been affected by several oil tanker accidents over the course of the last 30 years. The last one, on November 19th, 2002, took place when the tanker Prestige, operated by a Greek shipping company under a Bahamanian flag, sank 130 miles off the coast leaking 77,000 tonnes of fuel in the succeeding months. The generated oil spill extended from northern Portugal to the southern French coast killing between 115,000 to 250,000 seabirds according to various estimates (García et al., 2003; Arcos et al., 2004). Wintering auks made up 84.2% of the casualties, and the species most affected were the common guillemot or murre *(Uria aalge,* 50.9%), razorbill *(Alca torda,* 16.7%) and Atlantic puffin *(Fratercula arctica,* 16.6%) (Camphuysen et al., 2002; García et al., 2003).

From the start of the oil spill a team of biology students from the University of A Coruña (Universidade da Coruña, UDC) were trained in seabird necropsies by expert marine ornithologists from Scotland (MH) and The Netherlands (KC). From November 2002 to April 2003, external aging

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and sexing of the corpses by experienced ringers, as well as necropsies carried out by supervised volunteer students, were done routinely. Priority was given to obtaining age and sex data, since age- and sex-skewed mortality during oil spills can have implications for the demography of breeding seabirds (Martínez-Abraín et al., 2006). It is therefore imperative for any proper oil spill impact assessment to record data on the age and sex structure of the birds affected during these events (Heubeck et al., 2003).

A total of 6,183 seabirds collected from A Coruña province, in Galicia, were processed. This figure represented 27% of the total number of birds (n = 23,181; García et al., 2003) recovered during the beached bird surveys performed by volunteers of the Spanish Society of Ornithology (Sociedad Española de Ornitología, SEO) from the coast of northern Portugal to southwestern France.

Post-mortem analyses followed standard procedures (Jones et al., 1982; van Franeker, 1983; Camphuysen, 1995) and included full biometrics, sex and age determination (externally and/or by gonad and *bursa Fabricii* inspection when possible), oil coverage estimation, and assessment of the general physical condition of the birds according to the degree of damage to internal organs. The necropsy component was not done on carcasses in poor condition, and was not done for every carcass on days when bird numbers were excessive.

A total of 2,175 common guillemots were processed, of which 1,768 could be aged. The presence of white tips on underwing coverts were used as the criteria for the identification of hatch-year birds (Camphuysen, 1995; Cadiou et al., 2003). Most (82.2% ; n = 1,768) belonged to this age category.

The overall sex ratio (1 : 1.6; M : F) in common guillemots differed significantly from equality in those birds that could be sexed ($\chi 21 = 46.03$; $P < 0.001$; n = 922). Sex ratios also differed significantly from equality in hatch-year birds ($\chi 21 = 14.09$; $P < 0.001$; n = 741) and after-hatch-year birds ($\chi 21 = 59.8$; $P < 0.001$; n = 154).

Post-mortem analyses of razorbills were carried out in 999 individuals. External aging of the birds, based on the development of bill grooves (Camphuysen, 1995), revealed that most were hatch-years (88.7% ; n = 925), followed by immatures (7.0%) and adults (5.2%). Sex ratio (1 : 1.6) was significantly different from equality ($\chi 21 = 9.48$; $P < 0.05$; n =186). This significant difference was also found in the sex ratio of hatch-years ($\chi 2\ 1 = 5.37$; $P < 0.05$; n = 146) and adults ($\chi 2\ 1 = 4.84$; $P < 0.05$; n = 25), but not of immatures ($\chi 2\ 1 = 1.14$; P >0,05; n = 14).

A total of 1,814 Atlantic puffins were processed., of which 1,588 could be aged by the number of bill grooves (Petersen, 1976; Harris, 1981). Adults were the main casualties (53.1%; n = 1,588), followed by subadult (31%) and hatch-years (16%). The overall sex ratio (1 : 2.2; M:F) was significantly different from equality ($\chi\ 21 = 60.38$; $P < 0.001$; n = 424). Significant differences were also found in adults ($\chi\ 21 = 60.38$; $P < 0.001$; n = 215), subadults ($\chi\ 21 = 60.38$; $P < 0.001$; n = 130) and hatch-year birds ($\chi\ 21 = 60.38$; $P < 0.001$; n = 62).

The results on age and sex structure for the auks affected by the *Prestige* oil spill indicate a strong bias in the region towards females in the three species, confirming previous results found for this

oil spill in the common guillemot (Álvarez and Pajuelo, 2004). This strong sex segregation towards females was not found in previous oil spills occurring in more northern European coastal waters, suggesting that female auks move further away from their breeding colonies during winter. Hatch-year birds were the main casualties for common guillemots and razorbills, but adults were the age group mostly affected in the Atlantic puffin. This suggests a potentially delayed population impact at breeding colonies of the former two species, and an immediate effect in the 2003 breeding season for Atlantic puffin. The fact that adults rather than immatures were the age class more affected by the oil spill in this species suggests that the direct impact on the breeding populations of origin might be greater than in common guillemot and razorbill, despite the larger number of guillemots killed.

Ringing recoveries of auks killed during the spill were mostly of birds ringed at colonies on the west coasts of Britain and in Ireland (Moreno-Opo et al., 2003; Grantham, 2004), and wing lengths of most adults of the three species (Table 1) agreed with those recorded from birds at colonies within this latitudinal range (Cramp 1985; Jones 1988). Both the results obtained on sex and age structure of wintering auks in Galician shelf waters, as well as of their origin, can also be of interest for the definition of Important Seabird Areas in the Northeastern Atlantic. Additionally, knowing the origin of most of the auks allows biologists to do post-spill monitoring in appropriate regions where potentially impacted populations occur.

Table 1: Wing measurements for adult auks collected during the Prestige oil spill (November, 2002 – April, 2003) according to sex

	Number in sample	Mean	Standard error	range
Common Guillemot				
Males (After-Hatch-Year Birds)	28	196.2	3.9	189-204
Females (After-Hatch-Year Birds)	110	198.5	4.7	187-210
Razorbill				
Males (adults)	5	195.0	3.5	191-199
Females (adults)	12	198.3	6.6	181-206
Atlantic Puffin				
Males (adults)	45	159.3	0.6	149-167
Females (adults)	116	156.4	0.4	143-168

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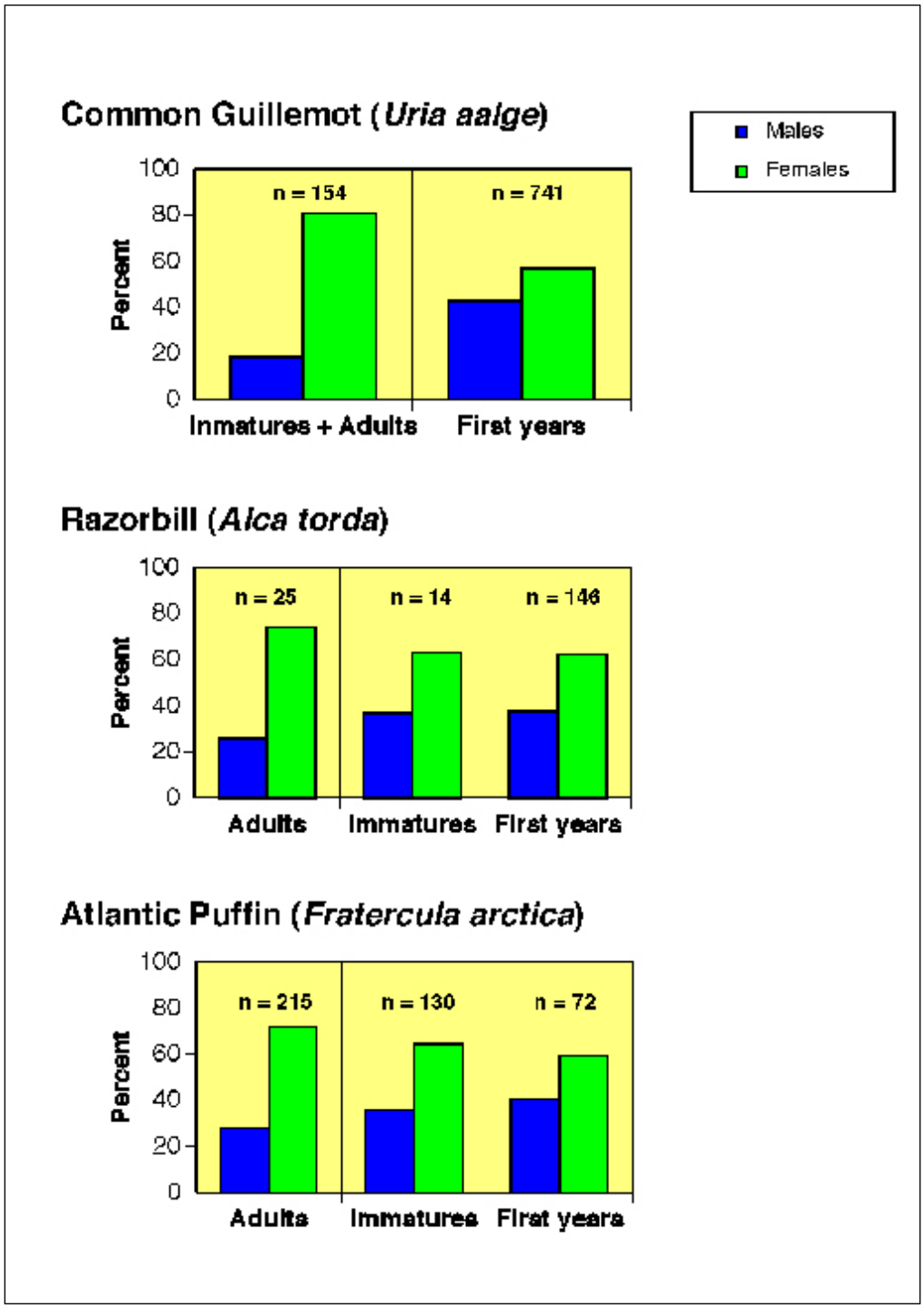

Figure 1. Age and sex composition of auks collected during the Prestige oil spill (November, 2002 – April, 2003)

Baseline health parameters and polycyclic aromatic hydrocarbon (PAH) exposure levels for clinically normal harbor seals *(Phoca vitulina)* in California, USA

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Introduction

When disasters like oil spills occur, the determination of injury suffered by animals and their populations, and the legal case for restoration depends on ability to demonstrate impacts, often by comparisons with established baselines. Regional baseline population levels may be established by periodic counts or census and similar techniques have been used for local populations. If baseline values are established for hematologic and serum biochemical values, immune system function, reproductive hormone levels, pathogen exposure and background exposure to toxic fractions of petroleum like the polycyclic aromatic hydrocarbons (PAHs), the extent to which catastrophic exposure to petroleum is responsible for changes in health and normal physiologic function of animals can be inferred. Alterations in health parameters and levels of toxic fractions can also help guide clinical care of animals affected by petroleum exposure (Loughlin 1994). As part of a larger project to define baseline health for three common species of marine mammal in California, we tested plasma from 20 clinically normal harbor seals *(Phoca vitulina)* with no known exposure to petroleum for presence of PAHs as well as a standard suite of serum biochemical and hematologic tests, serologic tests for selected pathogens, radioimmunoassays for reproductive hormone levels, and immune function tests.

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Materials and Methods

We collected blood samples from 20 (7 adults, 13 juveniles) harbor seals at San Nicolas Island (33°14'N, 119°27'W). Apparently healthy seals were observed for several minutes while resting prior to initiation of capture and physical restraint. Respiratory rate was obtained by counting chest excursions for 2–5 minutes; heart rate was obtained where possible by observing the left lateral chest wall just behind the foreflipper for the slight movement associated with each heartbeat. The body was scanned for obvious abnormalities such as fresh wounds or nasal discharge.

Harbor seals were approached on the beach, captured in hoop nets and manually restrained. Blood samples were collected as soon after capture as possible (generally within 5 minutes). Blood was collected from the extradural vein (Geraci and Smith, 1975) using a syringe and either a 1.5 inch or 3.5 inch, 18 gauge, spinal needle or a 2.0 inch, 16 gauge, spinal needle. Whole blood was placed into serum separator Vacutainer® tubes (SST; Becton, Dickinson & Co., Rutherford, New Jersey, USA) and Vacutainer® tubes containing anticoagulant (ethylenediaminetetraacetic acid disodium salt [Na-EDTA] and lithium heparin). Blood samples were kept cool and transported to a field laboratory within four hours for preliminary processing and preparation for transport. Serum and plasma were frozen (-20°F) and whole blood was kept refrigerated until hematologic and biochemical analyses were completed within 48 hrs. Details of sample handling and analysis for immunologic assays and polycyclic aromatic hydrocarbon (PAH) assays are described below.

Seals were examined by a veterinarian and were measured (standard length and axillary girth). Physical exams included an evaluation of the musculoskeletal system (symmetry, lesions) and cardiopulmonary system (heart rate, mucous membrane colour, respiratory rate and character). The integument was examined for evidence of molt, wounds or other lesions. Eyes, ears, nares and oral cavity were inspected for discharges or lesions such as corneal ulcers and broken teeth. Hydration was estimated by visual inspection of mucous membranes, skin turgor and tears. The urogenital and gastrointestinal systems were evaluated opportunistically by examining urine or feces and by visual inspection for lesions at urogenital openings. Examination of the nervous system consisted of a limited evaluation of animals' vision, hearing, cranial nerve function (e.g. blink reflex) and peripheral nerve function (e.g. flinching when rear flippers were touched) in response to the activities of handlers.

Hematologic and serum biochemical assays were performed by the SeaWorld San Diego Animal Care Laboratory (San Diego, California). Hematocrit, erythrocyte counts, leukocyte counts, platelet counts and mean corpuscular volume (MCV) were measured with a Coulter particle counter (model ZBI, Coulter Electronics, Hialeah, Florida). A Coulter hemoglobinometer was used to determine hemoglobin concentration. Mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) were calculated. Microscopic blood smear evaluation was used to make differential leukocyte counts, to rule out the presence of platelet clumps and to evaluate cell morphology.

Biochemical assays were performed on a Ciba-Corning 550 Express Plus. Eighteen serum chemistry parameters were measured, including biomarkers for liver, kidney and muscle abnormalities (alanine aminotransferase, ALT; aspartate aminotransferase, AST; lactate dehydrogenase, LDH; total bilirubin, gamma glutamyltransferase, GGT; blood urea nitrogen, BUN; creatinine, CR; creatine kinase, CK), body condition and nutritional status (glucose, cholesterol, triglycerides, total protein), inflammation/infection (albumin, globulin, alkaline phosphatase), electrolytes (sodium, potassium, chloride) and other ions (calcium, phosphorus).

Blood samples for immunologic assays were collected in CPT (leukoprep) tubes, which are designed to separate all of the mononuclear cells (lymphocytes and monocytes) from polymorphonuclear cells and red blood cells. The tubes were spun within 24 h of collection and mononuclear cells pulled off, placed into cryovials and frozen for later analysis. Cell viability (% of cells surviving the freezing process) was excellent (>80%) when samples were handled in this manner. Immunologic assays were performed by the University of California, Davis, Laboratory for Marine Mammal Immunology (cf. DiMolfetto-Landon et al. 1995). The Cell Proliferation ELISA was used to measure lymphocyte proliferation in vitro in response to two concentrations of each of three mitogens (concavalin A, pokeweed mitogen and phytohemagglutinin). Run to run variability was evaluated using banked equine cells from a single animal processed in the same manner as the seal samples (with minor changes due to interspecies differences). Cells were cultured in the presence of mitogen for 72 h, and then labeled with the pyrimidine analogue BrdU for 18 h which is taken up into the DNA of proliferating cells. Cells were then fixed, the DNA was denatured, and cells were incubated in the presence of anti-BrdU POD. The anti-BrdU POD binds to the BrdU incorporated in newly synthesized DNA and these immune complexes were washed and then incubated in the presence of TMB substrate. The substrate reaction was quantified by measuring absorbance with a scanning multiwell spectrophotometer. The Stimulation Index (SI) values reported in Table X are the difference between the optical density of a treated sample and the optical density of the media-control. These indices provide a 'yes-no' evaluation of lymphocyte responsiveness, rather than a quantitative evaluation of immunocompetence. That is, animals with SIs higher than 2 are generally regarded as responsive but animals with higher SIs cannot be considered more immunocompetent than those with lower SIs (J. Stott, pers. comm.).

Interleukin-6-like activity was measured as biological activity using murine B9 cell line (King et al. 1993) and evaluated with respect to recombinant IL-6 standard. This assay was performed by the Laboratory for Marine Mammal Immunology at the University of California, Davis.

Serologic tests for canine distemper virus (ELISA, ref) and phocine herpesvirus-1 (Goldstein et al. 2003) were performed by the Laboratory for Marine Mammal Immunology, University of California, Davis. Herpesvirus ELISA values <1 were considered negative and animals with ELISA values >5 were considered seropositive. Equivocal samples were those falling between these two values, and paired samples would be required to determine the status of these individuals. *Brucella* sp. and

Chlamydophila sp. assays were performed by the California Animal Health & Food Safety Laboratory System, Davis, California.

Reproductive hormone (testosterone, estrogen, progesterone) levels in thawed heparinized plasma were evaluated via radioimmunoassay by Dr. Shannon Atkinson (Hawaii Institute of Marine Biology).

The EDTA plasma samples used for polycyclic aromatic hydrocarbon assays were hemolyzed and therefore a single sample was used for a preliminary extraction evaluation. The sample could not be accommodated by the 1g/6cc SPE and modifications were made to the method to extract the remaining samples.

Plasma samples were removed from frozen storage (- 20 +/- 5 º C) and allowed to thaw. Samples were gently stirred and a 100 uL aliquot removed and refrozen for later lipid analysis. Approximately 1 to 2 grams of plasma were accurately weighted into a pre-cleaned culture tube, spiked with deuterated extraction surrogates, and covered with a Teflon lined cap. A 4 mL aliquot of formic acid is introduced to the sample in order to denature proteins which interfere with extraction. Digested samples were vortexed and allowed to degas for 10 minutes.

The denatured samples were extracted using Varian Mega Bond Elut® 10g/60cc C-18 solid phase extraction (SPE) columns, which were prepared with one 50 mL rinse of 50:50 n-hexane:methylene chloride, one 50 mL rinse of methanol, and one 50 mL rinse of pre-extracted water. The serum/formic acid mixtures were loaded directly onto the wetted columns at ~5 psi vacuum pressure. The columns were dried under vacuum for 15 min. The analytes were eluted from each column with one 50 mL aliquot of 1:1 hexane:methylene chloride. The extract was then eluted through a silica/alumina column using 100% methylene chloride. The PAH fraction was concentrated to 250 uL using rotary evaporation followed by N2 gas evaporation.

PAHs were quantified by analysis on a Hewlett Packard 5890 Series II capillary gas chromatograph equipped with a 5971A mass spectral detector (GC/MSD). A 2-uL splitless injection was chromatographed on a 60m x 0.25 mm i.d., 0.25um film DB-5ms column and analyzed in the single ion monitoring (SIM) mode. Six deuterated PAH extraction surrogates were used to provide internal standard quantitation. Quality control measures included instrument calibration verification, documentation of surrogate recoveries, the dilution of samples which exceed the instrument's calibrated range, and the tracking of accuracy and precision as performance indices.

Samples were tested for 12 low molecular weight PAHs (naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, biphenyl, 2,6-dimethlynaphthalene, acenaphthylene, acenaphthene, 2,3,5-trimethylnaphthalene, fluorine, phenanthrene, anthracene, 1-methylphenanthrene) and 12 high molecular weight PAHs (fluoranthrene, pyrene, benz[a]anthracene, chrysene, benzo[b/j]fluoranthrene, benzo[k]fluoranthrene, benzo[e]pyrene, benzo[a]pyrene, perylene, indo[1,2,3-c,d]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene).

Instrumental calibration was verified with continuing calibration check (CCC) solutions every 10-16 hours. Solutions purchased from the National Institute of Standards and Technology (NIST) were used to prepare all CCC solutions.

All surrogates were inspected for acceptable recoveries prior to sample analysis. Samples with recoveries exceeding the criterion of 50% -150% were subjected to re-analysis or re-extraction. Marginal recoveries which were in control yet exceeded the range of 60% - 120% were closely inspected and corrective action was taken as appropriate.

The instrument was initially calibrated with a 5 point curve ranging from 0.025 to 25.0 ng/uL. Samples which exceeded the calibrated range were subjected to a standard 1:20 dilution. All samples were spiked prior to GC analysis with a dilution internal standard (DIL-ISTD) to facilitate this treatment while allowing for internal standard quantitation.

Tracking of analytical precision and accuracy was accomplished through the use of method duplicates, matrix spikes, and standard reference materials (SRMs). Since SRMs for PAHs in blood were unavailable, matrix spikes were relied upon to assess methodological accuracy. Method blanks were analyzed within each analytical batch to assess contamination levels allowing for corrective action if necessary.

Hard copies of all chromatograms, area percent reports and internal standard reports were generated and archived for each sample analyzed. Additionally, all phases of the analysis were magnetically archived in duplicate on mini data-cartridge tapes to ensure that the data remain retrievable.

Methodological precision was assessed through the analysis of one plasma sample in duplicate. Duplicates are scheduled in such a manner that they are not included in the same extraction or analytical set when feasible. The method duplicate samples were extracted in a separate set and analyzed in the same analytical run. Since only two residues were detected in the duplicate samples, the matrix spike sample was analyzed in duplicate to provide an assessment of analytical precision for all analytes at ~1.5x – 2.5x MDL (Method Detection Limit; MDL for this study = 2 ng/g wet weight).

Samples of saline were provided in two different collection tubes to test for possible contamination from sample vials. No PAH residues were detected in saline samples stored in either soft top or hard top blood vials.

Dilutions of certified NIST solutions were prepared and analyzed with each set of samples to verify instrumental calibration stability over the length of the analytical run. The PAH calibration solution was prepared from NIST SRM 2260.

All samples met the holding time criterion of 40 days from extraction to analysis. Analyses were performed within 10 days.

All surrogate recoveries met the QC criterion of 5 to 150% with the exception of sample RDUF227 which was used in the preliminary evaluation of method performance for the hemolyzed samples. The low recoveries associated with this sample were the result of clogging on the C18 SPE column.

Results and Discussion

Table 1. Hematologic and serum biochemical parameters for 20 harbor seals sampled at San Nicolas Island, California.

	Adult			Juvenile		
Parameter	N	Mean	SD	N	Mean	SD
Hemogram						
RBC (M/microliter)	7	5.1	0.4	13	5.4	0.5
HCT (%)	7	60.9	3.4	13	60.2	3.3
HGB (g/dL)	7	21.7	1.2	13	21.3	1.4
MCV (fL)	7	120.4	5.8	13	112.9	5.5
MCH (pg)	7	43.0	2.5	13	39.9	2.1
MCHC (g/dL)	7	35.6	0.5	13	35.5	0.5
Platelets (K/microliter)	7	478.6	114.5	13	513.9	85.0
WBC (K/microliter)	7	13000.0	3716.2	13	13584.6	3056.4
BAND (%)	7	0.1	0.4	13	0.0	0.0
NEUT (%)	7	36.9	11.9	13	46.1	10.5
LYMPH (%)	7	40.3	12.5	13	32.8	9.9
MONO (%)	7	9.6	3.9	13	8.4	2.1
EOS (%)	7	11.0	2.8	13	11.9	5.6
BASO (%)	7	2.0	2.0	13	0.9	1.3
Serum Biochemistry						
T. PROT (gm/dL)	7	7.1	0.7	12	7.1	0.6
ALB (g/dL)	7	2.4	0.2	12	2.4	0.2
GLOB (g/dL)	7	5.1	0.6	12	4.7	0.6
BUN (mg/dL)	7	49.6	20.5	13	44.2	16.0
CREAT (mg/dL)	7	0.9	0.2	12	0.8	0.2
GLU (mg/dL)	7	94.1	31.5	13	109.8	27.5
CHOL (mg/dL)	7	208.0	28.8	12	186.1	24.1
T. BILI (mg/dL)	7	0.2	0.1	13	0.3	0.2
ALK PHOS (U/L)	7	128.7	103.3	13	75.0	68.0
AST (U/L)	7	68.1	21.3	13	94.5	47.8
ALT (U/L)	7	36.3	14.0	12	39.1	14.9
LDH (U/L)	7	764	255.6	12	793	277.2
CK (U/L)	7	244.0	174.0	13	359.0	247.4
NA (mEq/L)	7	155.7	4.5	10	150.3	7.4
K (mEq/L)	7	4.8	0.5	10	5.1	0.4
CL (mEq/L)	7	107.7	4.3	10	105.4	3.0
CA (mg/dL)	7	9.9	0.2	10	10.4	0.5
PHOS (mg/dL)	7	7.5	1.4	10	7.7	1.4

Table 2a. Seasonal reproductive hormone levels from 3 adult female harbor seals at San Nicolas Island, California.

	Estrogen (EIS; ng/ml)		Progesterone (ng/ml)	
Season	Mean	S.D.	Mean	S.D.
Pupping (Spring)	0.1	0.1	18.0	1.7
Mating/Molting (Summer)	0.3	0.2	8.0	0.7

Table 2b. Seasonal reproductive hormone levels from 4 adult male harbor seals at San Nicolas Island, California.

	Testosterone (ng/ml)	
Season	Mean	S.D.
Winter	0.0	0.1
Pupping (Spring)	2.5	1.2
Mating/Molting (Summer)	5.1	1.4

Table 3. Assays for selected pathogens for 20 harbor seals sampled at San Nicolas Island, California (N/A = Not Available).

Animal I.D.	Canine distemper ELISA	Brucella (USDA card test, buffered Brucella Rose Bengal Antigen)	*Chlamydophila* sp.	Phocine herpesvirus type-1 (PhHV-1)
RDUF 226	Negative	Negative	Positive	Positive
RDUF 227	Negative	Negative	N/A	Positive
RDUF 228	Negative	Negative	N/A	Positive
RDUF 236	Negative	Negative	Negative	Positive
RDUF 237	Negative	Negative	N/A	Positive
RDUF 341	Negative	Negative	N/A	Positive
RDUF 342	Negative	Negative	N/A	Positive
RDUF 343	Negative	Negative	N/A	Positive
RDUF 358	Negative	Negative	N/A	Positive
RDUF 360	Negative	Negative	N/A	Positive
RDUF 369	Negative	Negative	Negative	Positive
RDUF 370	Negative	Negative	Negative	Positive
RDUF 371	Negative	Negative	N/A	Positive
RDUF 374	Negative	Negative	Negative	Positive
RDUF 388	Negative	Negative	N/A	Positive
RDUF 389	Negative	Negative	Negative	Positive
RDUF 390	Negative	Negative	Negative	Positive
RDUF 392	Negative	Negative	Negative	Positive
RDUF 397	Negative	Suspect Positive	N/A	Positive
RDUF 400	Negative	Negative	Negative	Positive

Hematologic and serum biochemical (Table 1) and reproductive hormone values (Table 2) were within established reference ranges and all physical examination findings were within normal limits for these 20 harbor seals. Assays for selected pathogens are presented in Table 3. One adult female had antibodies to a Clamydophila sp. and one adult male had a suspect positive reaction to the Brucella card agglutination test. All seals were negative for canine distemper virus and positive for phocine herpesvirus-1 (purified PhHV-1, Pacific Isolate HS950). Morbillivirus has not yet been detected in free-ranging harbor seals by any investigator in the North Pacific but surveillance continues because the virus has caused epizootics elsewhere (e.g., Harkonen et al. 2006). Goldstein et al. (2003) concluded that phocine herpesvirus-1 is endemic in North American harbor seals after finding that 37.5% of pre-weaned pups, 87.6% of weaned pups, and 99.0% of subadults and adults were seropositive. The 20 seropositive adult and juvenile seals reported here were among the 866 serum samples evaluated by Goldstein et al. (2003). Interleukin-6-like activity was negative for all seals tested and immunologic function tests (lymphocyte blastogenesis, Table 4) were within normal limits for 19 of the 20 seals.

Table 4. Quantitative lymphocyte blastogenesis results from 20 harbor seals sampled at San Nicolas Island, California.

One seal, RDUF 369, did not respond effectively (Simulation Index < 2.0) to 3 of 6 mitogen challenges, which suggests that this animal may be immunocompromised.

Blastogenesis Assay Results – Simulation Index (= Treatment O.D./Media O.D.)						
Animal I.D.	ConA 2.5 ug/ml	ConA 0.5 ug/ml	PWM 2.5 ug/ml	PWM 0.5 ug/ml	PHA 10 ug/ml	PHA 2 ug/ml
RDUF 226	10.6	11.4	7.1	10.0	5.2	10.3
RDUF 227	7.0	8.9	4.5	7.0	4.5	7.8
RDUF 228	13.8	14.8	8.5	10.2	5.9	10.1
RDUF 236	10.2	12.0	5.8	7.2	5.9	10.2
RDUF 237	4.6	7.4	4.2	6.2	3.0	6.8
RDUF 341	6.6	9.4	3.9	6.3	2.4	4.8
RDUF 342	9.6	13.3	4.4	5.5	5.1	9.1
RDUF 343	6.1	8.4	5.4	6.5	3.7	6.5
RDUF 358	15.2	13.4	4.1	6.4	6.8	10.2
RDUF 360	9.7	9.4	2.0	3.1	4.2	6.5
RDUF 369	2.4	3.5	1.7	1.6	1.4	2.8
RDUF 370	6.0	6.9	3.0	4.0	2.5	4.8
RDUF 371	5.1	3.3	4.2	3.4	7.5	7.3
RDUF 374	5.9	4.3	3.6	2.7	7.0	7.5
RDUF 388	3.9	2.4	2.4	2.5	4.6	4.6
RDUF 389	5.6	2.6	3.9	3.6	10.0	9.2
RDUF 390	12.0	10.4	5.6	7.5	5.5	7.6
RDUF 392	9.0	8.7	3.0	3.2	3.0	4.2
RDUF 397	11.4	11.8	4.7	5.5	5.5	8.4
RDUF 400	9.0	8.3	2.3	2.3	3.6	6.1

King et al. (1993) reported elevated IL-6-like activity in two species of phocid seals with evidence of systemic infection, but detected no activity in healthy seals. DiMolfetto-Landon et al. (1995) established baseline values for blastogenesis expression assays for harbor seals and suggested that using multiple assays provides a useful indication of lymphocyte competence (as a measure of health) in this species. In aggregate, the results suggest that these 20 harbor seals were indeed appropriate for establishing a normal baseline set of values.

Detectable low levels of naphthalene, 2-methylnaphthalene, or 1-methylnaphthalene were found in 11 plasma samples after correcting for laboratory background levels (Table 5). The levels detected probably represent levels present in most environments as a result of widespread low levels petroleum contamination, thus a baseline against which higher levels would suggest more acute, recent or potentially toxic exposure.

Table 5. Polycyclic aromatic hydrocarbons (plasma wet weight) detected in harbor seal plasma samples. All residues were blank corrected by the amount reported for the method blank in the same analytical batch. (ND = Not Detected).

Animal I.D.	Naphthalene (ng/g)	2-methylnaphthalene (ng/g)	1-methynaphthalene (ng/g)
RDUF236	4.91	5.29	3.22
RDUF358	3.15	4.03	2.07
RDUF370	2.33	2.99	ND
RDUF388	2.19	2.7	ND
RDUF389	2.36	3.2	ND
RDUF390	2.31	3.82	ND
RDUF226	ND	2.4	ND
RDUF392	ND	2.26	ND
RDUF369	ND	2.44	ND
RDUF371	2.21	2.81	ND
RDUF374	2.19	3.15	ND

Ocular lesions, skin irritation and brain and liver lesions were reported in 27 harbor seals collected following the *Exxon Valdez* oil spill (Spraker et al. 1994) and an estimated 300 harbor seals were believed to have succumbed to oil exposure subsequent to the spill (Loughlin et al. ,1996). The care and management of oil exposed harbor seals is somewhat controversial. Gales and St. Aubin (1995) noted that congregations of seal pups in tidal pools may lead to those age cohorts being oiled at much higher rates than adults. Eighteen oiled harbor seals were rehabilitated and released in Alaska subsequent to the *Exxon Valdez* spill (Williams et al., 1994). Three biochemical values (aspartate aminotransferase, alanine aminotransferase and lactate dehyrogenase) were abnormally high in these pups, and two pups showed a marked decrease in packed cell volume during rehabilitation. However, Williams et al. (1994) concluded that factors other than oil contamination may have been responsible for these changes, including the stresses of capture, handling and transport. Although they were

covered with crude oil, care and medical management were fairly routine and successful and most were released. Questions subsequently arose as to whether breaking maternal bonds by placing seal pups in rehabilitation centers for care was relatively more beneficial than no treatment (Williams et al., 1994). Harbor seals and other marine pinnipeds that have thin hair coats and insulating blubber are unlikely to experience the hypothermia and hypoglycemia experienced by oiled sea otters, which rely on the insulation and buoyancy provided by air trapped in their underfur. Indeed, some agencies question the need to routinely rehabilitate oiled cetaceans or pinnipeds (J. Cordaro, pers. comm..) and agreements between California Department of Fish and Game and the U.S. National Marine Fisheries Service specify that pinnipeds and cetaceans will not be routinely or preemptively captured for washing and care, but rather handled on a case-by-case basis as incident specific factors and symptoms may indicate.

For seals that show clinical signs of petroleum intoxication, a return to background levels of petroleum exposure, as well as health parameters, might help guide treatment and release criteria. For legal injury determination it is important to recognize that low levels of some constituents of petroleum are relatively common in marine environments and represent a baseline against which more serious exposures resulting from illegal or catastrophic events can be measured.

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Dietary Exposure to Napthalene in the Japanese Quail *(Coturnix coturnix japonica)*

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Introduction

Petroleum products contain alkylated naphthalene and phenanthrene (both polycyclic aromatic hydrocarbons: PAHs), which are persistent environmental contaminants from oil spills. Unfortunately, their toxicity profile in birds is not characterized and this severely limits oil toxicity assessment. To begin to characterize the toxicity of PAHs in birds, Japanese Quail were fed naphthalene during their maturation and reproductive phases and signs of toxicity were examined in adults and their chicks.

Japanese Quail were used as a target species because they are a standard bioassay model species for birds. This is because they have well known husbandry parameters, a quick life cycle, and their genetics have been minimally changed during domestication.

Methods

Animals and experimental design: Quail from the UC Davis Avian Science colony were hatched on September 28, 2005. The quail used in the experiment were selected at four weeks of age from a two-fold larger population so that initial body weights were as similar as possible. Hatchlings were housed in brooder batteries with woven wire floors and fed turkey starter (Purina Mills, St. Louis, MO) and water for *ad libitum* consumption. They were provided 8 hrs of light each day and cages were cleaned weekly.

When quail were 6 weeks of age, they were paired with mates and moved to the experimental room that contained racks holding quail breeding cages. The room was kept at a constant temperature of 25°C and day length was set at 18 hrs to promote breeding. Each reproductive pair was housed

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in individual cages that had a shared feeder, automatic waterer and sloped floor to facilitate egg collection. There were four dietary treatment groups, each with 12 replicate pens of breeding pairs: Control, 25 mg/kg naphthalene, 50 mg/kg naphthalene, and 200 mg/kg naphthalene. The naphthalene was added to a nutritionally complete diet (game bird breeder; Purina Mills) to give the indicated levels and mixed in a stainless steel mixer (Hobart; Troy, Ohio) for 15 minutes. Diets were stored in tightly sealed containers and refrigerated. Fresh diet was provided to birds daily. New diets were mixed every 4 weeks. Diet samples were taken over a 30-day period to determine actual naphthalene levels.

At the time that diets were introduced (day 1 of the experimental period) the average body weights were 107 and 123 g for male and females, respectively. Every 4 weeks subsequently, birds were weighed and bled from the vena cava into heparinized syringes. Birds were observed for abnormal behavior or pathological signs on a daily basis. Egg production was recorded daily. During the 11th week, all eggs were collected and saved for measuring egg weight and shell thickness. After 14 weeks, breeding birds were killed by CO2 asphyxia, posted for pathology scoring, and tissues collected and weighed.

Half of the eggs collected during week 11 and all of those during weeks 12-14 were incubated (41° C) until hatching (18d). Eggs that did not hatch were examined to estimate fertility and embryonic mortality. Hatched chicks were raised as described above. Body weight was determined on days 7 and 14. On day 14, chicks were euthanized and tissue samples taken.

Eggshells, including membranes, were air-dried before measurement of shell thickness. Measurements were taken at 3 points around the circumference of the shell that had the widest diameter using a micrometer.

Hematology. Packed cell volume was determined by the microhematocrit method. Total erythrocyte count and total leukocytes were determined using a hemocytometer. For differential leukocyte count, freshly prepared blood smears were stained with Wright's stain and cell types were identified based on morphology. Hemoglobin concentrations were determined by the cynamethemoglobin method, using Drabkin's solution.

Clinical chemistry. Clinical chemistry parameters were determined using an autoanalyzer (Beckman Instruments, Fullerton , CA), according to the instructions of the manufacturer.

Acute phase proteins. Plasma haptoglobin was measured according to manufacturer instructions, using a commercial kit (Phase Haptoglobin kit, Tridelta Diagnostics, TP801). Plasma α-1 glycoprotein was determined by rocket gel electrophoresis as previously described (Adler et al., 2001).

Histopathology. Intestinal segments from the mid-point of the duodenum (1.5 cm in length) were excised from hens, flushed with saline to remove digesta, and fixed in 100 g/L buffered formalin (pH 7.0). Formalin fixed intestinal samples were embedded in paraffin sectioned and stained with hematoxylin-eosin by a commercial laboratory (Idexx Laboratories, West Sacramento, CA) and evaluated for the following: thickness of the lamina propria; villous height from the base of the lamina

propria to the apex of the villus; villous width at its midpoint; and crypt depth between adjacent villi. Morphometric data were collected on 10 different villi per bird on each of two different serial sections. Measurements were made and analyzed by computer-aided light microscopic analysis at magnifications between 10 and 100X using Image-Pro-Plus analysis software for the PC (Media Cybernetics, Del Mar, CA). The number of leukocytes in 10 villi per slide and the number of leukocytes in the lamina propria underneath and within these 10 villi were enumerated. Assessments were made only on cleanly sectioned and perpendicular villi.

Statistics. Data were checked for homogeneity of variance and then analyzed by ANOVA with Tukey's means comparisons.

Results

There was no pattern of treatment related mortality. Two hens in the control group suffered mechanical injuries due to cage malfunctions during week 11 and were replaced with other birds that had not originally been included in the study but were treated identically. One male on the 25 mg/kg treatment died of undetermined causes during the termination of the experiment.

There was no detectable naphthalene in the control diet. Analyzed naphthalene levels in the diet averaged 29.5, 47.9, and 200.5 for the 25, 50, and 200 mg/kg doses, respectively. There was no evident loss in naphthalene concentrations over 30 days of storage of the 25 and 200 mg/kg diets. However, naphthalene decreased from 70.7 to 28.5 mg/kg between day 0 and day 30 for the 50 mg/kg diet. The reason for this disparity is not known.

After feeding the experimental diets for 14 weeks, the body weights and the rate of body weight gain of both male and female quail were significantly lower for those fed the 200 mg/kg diet than those fed any of the other diets (Tables 1-4). Feed consumption was decreased in groups fed either 200 or 50 mg/kg compared to controls (Table 5). Feed consumption is reported for the combined consumption of the males and the females because the two sexes ate from the same feeder; it was not possible to measure the consumption of the sexes independently.

Table 1. ANOVA for Effect of Naphthalene on Body Weights (P values)

Source	Week 6	Week 10	Week 14
Treatment	0.75	0.34	0.01
Sex	0.00	0.00	0.00
Treatment x sex	0.38	0.48	0.43

Table 2. Effect of Naphthalene on Body Weight at Week 14 (g)

	Male				Female				All		
Treatment	Ave		SD		Ave		SD		Ave		SD
0	117.6	+	2.1		146.4	+	2.4		132a	+	1.6

25	120.2	+	2.2		143.9	+	2.1		132a	+	1.6
50	115.6	+	2.1		145.9	+	2.1		131a	+	1.5
200	112.7	+	2.1		138.6	+	2.1		125b	+	1.5

Table 3. ANOVA for Effect of Naphthalene on Change in Body Weight (P values)

Source	Week 6 - 10	Week 6 - 14
Treatment	0.34	0.02
Sex	0.00	0.00
Treatment x sex	0.24	0.69

Table 4. Effect of Naphthalene on Change in Body Weight between Weeks 6-14 (g)

	Male				Female				All		
Treatment	Ave		SD		Ave		SD		Ave		SD
0	10.9	+	2.3		23.4	+	2.5		17.2a	+	1.6
25	7.8	+	2.4		10.7	+	2.3		14.4a	+	1.6
50	8.5	+	2.3		18.9	+	2.3		13.7a	+	1.5
200	5.7	+	2.3		13.6	+	2.3		9.6b	+	1.5

Table 5. Effect of Naphthalene on Feed Consumption (g)

Week 11-14 (both sexes combined)

Treatment	Ave		SD
0	1247ab	+	126
25	1372a	+	67
50	1231b	+	79
200	1161c	+	87

P = 0.00

Table 6. Effect of Naphthalene on Egg Production

(# eggs laid in 4 wks)

Treatment	Ave		SD
0	24.5	+	4.7
25	24.6	+	8.5
50	24.6	+	4.3
200	21.2	+	7.8

P = 0.51

The number of eggs laid (Table 6), egg weights, and the thickness of egg shells (Table 14) were not affected by naphthalene level. Naphthalene also did not affect the fertility of the breeding pairs,

hatchability of the eggs, or the incidence of embryonic mortality (Table 7); although the pairs fed 50 mg/kg or above tended (P=0.07) to have decreased fertility.

Table 7. Effect of Naphthalene on Fertility (%) and Embryonic Mortality

Week 11-14

	Fertility				Mortality		
Treatment	Ave		SD		Ave		SD
0	92.8	+	9.9		3.3	+	6.2
25	88.9	+	10.7		8.4	+	8.4
50	72.9	+	24.4		9.1	+	8.7
200	74.6	+	25.4		4.1	+	4.1
P value	0.07				0.21		

Table 8. ANOVA for Effect of Naphthalene on Organ Weights of Adults at Week 14
(P values)

Source	Liver	Spleen	Testes	Kidney
Treatment	0.32	0.67	0.11	0.13
Sex	0.00	0.00	0.00	0.00
Treatment x sex	0.50	0.31		0.01

Post mortem examination of the quail did not reveal any gross pathology associated with treatment groups. Liver, spleen, testes, and ovary weights were unaffected by dietary naphthalene (Tables 8 & 10). However, there was a significant treatment by sex interaction for kidney weight (Tables 8, 9 & 11), indicating that naphthalene at 50 mg/kg and above increased kidney weights in females, but not in males.

Table 9. Effect of Naphthalene on Kidney Weights of Adults at Week 14 (g)

	Male				Female		
Treatment	Ave		SD		Ave		SD
0	0.08	+	0.05		0.31	+	0.05
25	0.21	+	0.05		0.30	+	0.05
50	0.26	+	0.05		0.15	+	0.05
200	0.11	+	0.05		0.18	+	0.05

Table 10. ANOVA for Effect of Naphthalene on Organ Weights as % Body Weights

(P values)

Source	Liver	Spleen	Kidney	Testes	Ovary
Treatment	0.58	0.76	0.13	0.29	0.87
Sex	0.00	0.04	0.41	-	-
Treatment x sex	0.53	0.33	.01	-	-

Table 11. Effect of Naphthalene on Relative Kidney Weights (g/100 g BW)

	Male				Female		
Treatment	Ave		SD		Ave		SD
0	0.17b	+	0.33		0.21a	+	0.39
25	0.18b	+	0.35		0.21a	+	0.33
50	0.22a	+	0.34		0.10b	+	0.34
200	0.09b	+	0.34		0.13b	+	0.34

Few of the hematological and clinical chemistry endpoints examined were affected by naphthalene exposure (Tables 12, 13, & 15). The exception was hematocrit level, which was decreased by the highest level of naphthalene.

Table 12. ANOVA for Effect of Naphthalene on Blood Hematocrits (P values)

Source	Week 10	Week 14
Treatment	0.72	0.03
Sex	0.00	0.17
Treatment x sex	0.19	0.78

Table 13. Effect of Naphthalene on Hematocrit at Week 14 (% RBCs)

	Male & Female		
Treatment	Ave		SD
0	52.3b	+	3.1
25	45.0ab	+	3.0
50	44.8ab	+	3.1
200	41.6a	+	2.0

Table 14. Effect of Naphthalene on Intestinal Histology of Hens (week 14)

Treatment	lamina propria (µm)	villus height(µm)	villus width (µm)	crypt depth (µm)	intra-epithelial lymphocytes (#/villi)	lamina propria leukocytes (#/villi)
0	69ab	458b	77	81	15a	27
25	55a	477b	75	75	17a	15
50	68ab	451ab	75	85	22ab	32
200	94b	411a	79	88	29b	39
Pooled SD	14	15	4	6	5	6
P value	0.04	0.03	0.51	0.63	0.04	0.06

Table 15. Parameters for which there were no Significant Differences (P>0.10) due to Naphthalene in Adults at 14 Weeks

Hemoglobin (g/dl)	6.3*	+	0.8
Lymphocytes (%)	64	+	7
Heterophils (%)	29	+	9
Serum Protein (g/dl)	3.3	+	0.3
Triglyceride (mg/L)	98*	+	11
ALT (IU/L)	17.2	+	3.8
LD (IU/L)	611*	+	29
AST (mg/L)	459	+	35
Uric acid(mg/L)	6.9*	+	0.5
Albumin (mg/dl)	1.8*	+	0.1
Haptoglobin (ug/dl)	3.2	+	0.6
α1-glycoprotein (U/L)	12.2	+	2.5
Shell thickness (µm)	213	+	30

*Significant effect due to sex

Ingestion of feed containing 200 mg/kg naphthalene caused duodenal villi to be shorter with increased numbers of intra-epithelial lymphocytes and a tendency (P=0.06) to have higher numbers of leukocytes in the lamina propria.

Exposure of hens to naphthalene did not have an effect on the growth rate, mortality, hemoglobin concentration, or white blood cell numbers of chicks hatched from their eggs. However, it should be noted that all chicks were fed diets that did not contain naphthalene.

Table 16. Effect of Naphthalene on Chicks (day 14)

Hatching weight (g)	6.3	+	0.8
Weight gain (g)	64	+	7
Mortality (%)	29	+	9

Hemoglobin (g/dl)	459	+	35
Lymphocytes (%)	6.9	+	0.5
Heterophils (%)	1.8	+	0.1

No significant treatment effects (P>0.10)

Table 17. Summary of Parameters Significantly affected by Naphthalene in females.

Parameter	Lowest diet level giving effect (µg/kg diet)	Lowest dose giving effect (mg/kg BW/day)
Final weight	200	31.5
Weight gain	200	31.5
Feed intake	50	7.7
Kidney weight (female)	50	7.7
Hematocrit	200	31.5
Intestinal inflammation	200	31.5

Discussion

Based on measured average daily intakes of the diet, analyzed naphthalene concentrations in the diet and average body weight, the daily naphthalene consumption was 6.2, 9.2, and 37.6 mg per kg body weight for male quail consuming diets with 0, 25, 50, and 200 mg/kg respectively. Calculated daily naphthalene intake for females was 5.2, 7.7, and 31.5 mg per kg body weight, respectively.

Female quail fed naphthalene at 50 mg/kg or above had decreased food consumption. However, the hens did not significantly decrease egg production. The combination of decreased feed intake and continued egg production apparently led to diminished deposition of body weight. Males fed naphthalene at 50 mg/kg or above also decrease food consumption and diminished their deposition of body weight. It is not clear if the decrease in feed consumption was due to systemic toxicity caused by naphthalene (e.g. metabolic derangements or inflammatory disturbances in the digestive tract) or if it was secondary to the volatile nature of this compound. Birds are highly reliant on organoleptic cues in their regulation of food intake and naphthalene's effect may not have been the result of systemic toxicity. Future experiments should equalize food consumption between control and treated groups in order to make this distinction.

Naphthalene did not affect the quantity of eggs or the quality of those eggs as indicated by size and shell thickness. The reproductive capacity of the quail was also not affected by naphthalene as indicated by fertility, hatchability, and incidence of chick mortality. There also were no indications of diminished chick size at hatching, birth defects, or growth rates. Although naphthalene is transferred to the egg, especially the yolk (Eisele, 1985), reproduction does not appear to be a sensitive indication of naphthalene toxicity compared to direct effects on the adult (see below).

Of the organs weights examined, only kidney weights were affected by naphthalene. In laying chickens chronically fed naphthalene, the kidney accumulates the greatest amount of naphthalene and its metabolites (Eisele, 1985). Naphthalene metabolites also accumulate in the kidneys of redhead ducks *(Aytha americana)* fed crayfish contaminated with naphthalene (Tarshis and Rattner, 1982). Naphthalene is metabolically activated to the reactive intermediates, naphthalene oxide (NO) and naphthoquinones, which cause oxidative stress (Stohs et al., 2002). Naphthalene also forms protein sulfhydryl adducts in several tissues, including the kidney (Tsuruda et al., 1995). In mice, but not rats, the kidney is especially sensitive to naphthalene, with damage to the cells in the proximal tubules (O'Brien et al., 1985). In humans, naphthalene toxicity causes renal failure (Choudri et al., 1995). Thus, it is likely that the enlarged kidneys observed in quail were due to hypertrophic compensation of kidney damage. Further histological studies are needed to verify this.

Naphthalene caused signs of intestinal inflammation when fed at 200 mg/kg diet, including shortening of the villi and infiltration of leukocytes into the epithelium and lamina propria. Given that the digestive epithelium is the first tissue exposed to ingested naphthalene, this tissue would likely be exposed to the highest effective dose. Evidently the highest concentration of naphthaline examined was sufficiently high to cause local inflammation. However, systemic inflammation was not observed as indicated by normal levels of the acute phase proteins haptoglobin and α-1 glycoprotein. Inflammation of the nasal epithelium has also been observed when rats were chronically exposed to naphthalene by inhalation (Long et al., 2003).

Conclusion

A reduction in feed intake was the most sensitive indicator of oral naphthalene exposure when males and females are considered together. The no observed adverse effect level (NOAEL) and lowest observed adverse effect level (LOAEL) using feed intake as the criterion were 25 and 50 mg/kg diet, respectively. However, we cannot be certain that this is a true toxicological effect rather than an organoleptic effect. In females, but not males, increased kidney weight gave the same LOAEL or NOAEL as did decreased feed consumption. In males the most sensitive tissue change was intestinal inflammation, which occurred at a LOAEL and NOAEL of 50 and 200 mg/kg diet, respectively.

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Modeling oil spill impacts on seabirds, with special reference to cassin's auklets

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Keywords: Cassin's auklets, Channel Islands, Chukchi Sea, eiders, indirect effects of oil spills, modeling oil-spill impacts, oil-spill effects on birds, prioritizing oil-spill cleanup

The Oiled Wildlife Care Network was set up mainly to mitigate impacts of direct oiling of birds and mammals, by cleaning them and releasing them to the wild. Rehabilitation of birds is steadily improving, but their survival after release is not yet as high as desired (Sharp, 1996; Mead, 1997; Anderson et al., 2000). So in addition to improving treatment of oiled birds, it is also important to try to decrease the number of birds that are exposed to oil, and to minimize indirect effects on the remaining population that is not directly exposed (Carter, 2003). Indeed, it is those birds that remain in the wild that have the most effect on population recovery after a spill.

Enforcing regulations to prevent small releases of oil (Hampton et al., 2003), and maintaining the trained personnel and infrastructure to contain large spills if they occur (McCrary et al., 2003), is expensive. Thus, to sustain such efforts continuously in the long term, we should focus priorities on seasons and sites that are most critical to bird populations. Simple counts of birds are not always the best indicator of an area's importance, because high turnover of individuals might obscure much greater use of an area than counts suggest. Also, counts alone, or even radiotelemetry, do not indicate whether a heavily used site is the only place where the birds can obtain enough food, or rather just the best place among a number of viable alternatives. Knowing this difference is important to prioritizing both preparations for and responses to spilled oil.

Understanding such aspects of foraging ecology can help in mitigating the long-term, indirect effects of spills (Golet et al., 2002). In some cases, indirect effects of oil on behavior of adults have been blamed for chick mortality due to reduced feeding of young by the adults (Eppley and Rubega,

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1989; Eppley, 1992). In particular, reduced feeding rates and increased chick mortality might result if adults have reduced foraging efficiency at alternative sites outside the main spill area (Ford et al., 1982). This effect might be increased if the adults spend more time preening, or use more energy thermoregulating, due to minor exposure to oil (Jenssen et al., 1985; Eppley and Rubega, 1989).

Although such indirect effects may be quite important to the wild population that remains after a spill, they can be difficult to detect in field studies because of high environmental variability. For example, the Exxon Valdez oil spill was estimated to have killed about 250,000 birds, of which 74% were murres. But when these numbers were compared to variations in murre populations outside the spill area, researchers were unable to distinguish between long-term effects of the Exxon Valdez spill and the response of murres to natural variations in their environment (Piatt and Anderson, 1996). In the same area, lack of recovery of pigeon guillemots (Cepphus columba) has also been difficult to ascribe to either continued exposure of the birds to oil, indirect effects via oil impacts on their prey, or natural variability unrelated to spilled oil (Golet et al., 2002). Does this mean that we should not be concerned about the impacts of a spill of that magnitude, because our current survey methods cannot discriminate its effects from natural variations?

We clearly need the capability to evaluate effects of oil spills in the context of environmental variations, so that those variations can be accounted for in both planning for oil spills and responding to them. A major challenge is to assess and predict the cumulative, significant impacts of combinations of stressors that by themselves would be statistically undetectable in field studies. One important way to do this is by developing computer simulation models that allow us to evaluate the relative contributions of different factors to energy balance (Ford et al., 1982; Lovvorn and Gillingham, 1996). Such models can help in (1) setting regulations to decrease risk, (2) prioritizing cleanup operations, and (3) predicting population effects, especially from low-level exposure to oil or altered foraging patterns.

An example of the value of modeling is provided by concerns over an array of threatened seaducks in Alaska that migrate through an area that has just been opened for oil and gas exploration. A number of seaducks, including 5 eider species and the long-tailed duck (Clangula hyemalis), winter in various places throughout the Bering Sea and Gulf of Alaska, and migrate along the eastern coast of the Chukchi Sea to nesting areas on the Arctic coasts of Alaska and Canada. Across the board, numbers of these seaduck species on various breeding areas are down by 50–95%, and two of the eider species are federally (United States) listed as threatened. These seaducks, as well as most of the bowhead whales (Balaena mysticetus) in the region, migrate to the Beaufort Sea along a narrow corridor of open water that develops between moving pack ice and landfast ice in the spring.

In the draft environmental impact statement for oil and gas exploration in this area (Minerals Management Service, 2006), it is projected that pipelines to service offshore oil rigs will extend across the migration corridor at various points along the coast. Over a decade ago, as part of their listing under the United States Endangered Species Act, some of this area was designated critical habitat for Steller's and spectacled eiders (Polysticta stelleri, Somateria fischeri). Designation as critical habitat

does not mean that all extractive activities in the area must stop. All it means is that such activities must be regulated if there is a reasonable probability that they will jeopardize the current status or recovery of the listed population. Because this region is so remote, we have little information on how the birds use the area, making it difficult to establish the probability of damage to the listed species. It is also hard to determine the location and extent of areas that are truly critical to the birds, and should be protected from potential impacts of oil spills. How can we meet these information needs, so that both conservationists and the oil industry have some confidence that protected areas are no smaller – or larger – than they need to be?

In constructing a legal argument for protecting the eiders, the U.S. Fish and Wildlife Service is using models to develop a chain of probabilities:

a. Probability of spills at different areas and times
b. Probable trajectory of spills according to local winds and currents
c. Probable location of feeding concentrations of the birds
d. Probable adult mortality or breeding failure depending on these factors
e. Probable population consequences of these effects

The main model used by the Minerals Management Service to derive probabilities of oil spill trajectories is the Oil Spill Risk Assessment Model (McCrary et al., 2003). However, this model does not include algorithms to predict where the birds will be according to foraging conditions, and their actual dependencies on different areas given the prey communities and the birds' energy needs. My students and I focus on the latter aspects, and over the last 8 years we have developed a detailed model of the foraging energetics of eiders that should help address these issues.

The point in describing this situation for seaducks in Alaska is to show that this very formal, legally-mandated process for federally threatened species offers a valuable template for other situations where sensitive species are susceptible to oil spills. As an example, I'll move now to our work on Cassin's auklets *(Ptychoramphus aleuticus)* in California, which is partly funded by the Oiled Wildlife Care Network.

As late as the 1980s, Cassin's auklets were the second most abundant seabird breeding in California (Briggs et al., 1987). However, from 1985 to 1994, the breeding population on the central California coast declined by 87% from 62,000 to 8,000 birds (Oedekoven et al., 2001). They have not recovered since, and reached the lowest point in a 35-year record with nearly total breeding failure in 2005 and 2006 (Sydeman et al., 2006). This decline has apparently resulted from effects of ocean conditions on foraging energetics, by altering prey abundance and the distance of feeding areas from colonies (Ainley et al., 1996; Sydeman et al., 2001). About 20% of this population now nests in the Channel Islands, where they feed in a limited area mostly in and near a shipping lane for oil tankers (Adams et al., 2004a,b).

From a population perspective, an oil spill in this key foraging area might have at least two effects:

1. The birds might have reduced access to their main feeding area during the oil spill and cleanup, which might impact their provisioning of chicks.
2. If alternative feeding areas are inadequate, the birds might refuse to feed elsewhere and continue exposing themselves to oil.

If these effects are likely, then high priority should be given to cleaning oil from key feeding areas as quickly as possible. Thus, to ensure prompt and effective responses after a spill, a firm ecological basis for cleanup priorities should be established before a spill occurs. Our development of a model for Cassin's auklets in the Channel Islands is not yet complete, but here I will review some components of the work.

First, what determines the location and quality of feeding areas? Cassin's auklets in this region feed mainly on krill *(Thysanoessa spinifera* and *Euphausia pacifica).* Sometimes krill form surface swarms during the day that are accessible to shallow-diving birds like Cassin's auklets (Smith and Adams, 1988), but these surface swarms appear to be too rare to support routine foraging. Also, a model of underwater visual feeding suggested that the krill do not have to be as dense as they are in surface swarms to support profitable foraging (Lovvorn et al., 2001).

Comprehensive surveys with acoustic instruments and net sampling have shown that krill in this region tend to be focused in areas of upwelling along the shelf break (Fiedler et al., 1998). The intensity of upwelling and availability of krill vary greatly between years, being strongly affected by large-scale climate patterns such as El Niño events (Marinovic et al., 2002). If upwelling fails, it can mean that krill densities will be low, or that adequate feeding sites will be farther away from colonies. For small seabird populations, the predicted increase in frequency of El Niño events can greatly increase extinction probabilities due to other catastrophes such as oil spills (Vargas et al., 2007).

What determines how near the water surface and how dense the krill must be for the auklets to feed on them profitably? A major determinant is the cost of diving. At the University of Wyoming we have built an experimental dive tank, in which captive Cassin's auklets are trained to feed at the end opposite a respirometry chamber where we measure energy expenditure as oxygen consumption. We can vary the temperature of water in this tank from about 6°C to 25°C, to account for effects of temperature on dive costs.

To measure patterns of diving in the field to which we can apply these measured costs, Josh Adams of USGS has been putting time-depth recorders on Cassin's auklets in the Channel Islands. Breeding failures over the last 2 years have hampered this effort; however, almost all dives by the two birds instrumented so far were to <20 m, and about half to less than 10 m (Adams et al., 2005).

Unfortunately, there are no ongoing studies of the 3-dimensional dispersion of krill in the Santa Barbara Channel. However, regular research cruises in Monterey Bay (Croll et al., 2005) are yielding important insights into a similar system that help in developing models for the Channel Islands.

Finally, the National Park Service and USGS have established nest boxes for Cassin's auklets in breeding colonies on the Channel Islands. Work on birds using these boxes has provided important data for initial models (Adams et al., 2004 a,b). When the auklets start nesting again after breeding failure in 2005 and 2006, these boxes should allow excellent monitoring of the breeding responses of auklets to changing oceanographic conditions. They will also provide easy access for deploying time-depth recorders, and for using doubly-labeled water to measure total energy expenditure (Obst et al., 1995; Hodum et al., 1998).

I emphasize that we cannot gather all the information needed to develop detailed simulation models for every species. Instead, we must focus on representative sentinel species that have sensitive population status, and heightened vulnerability to a combination of oil spills and natural environmental variations. For the Channel Islands, Cassin's auklets appear to be a good choice, and we will continue work toward developing a useful simulation model.

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A comparison of bird drying pens covered by shade cloth, sheet and blanket

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Keywords: drying pen, drying time, airflow, temperature, humidity, rehabilitation, seabird, oiled wildlife

Introduction

The U.S. Fish and Wildlife Service (USFWS) document *Best Practices for Migratory Bird Care During an Oiled Wildlife Response* (2002) states that a pet dryer is the most effective means of drying most birds species that have been washed to remove contaminants from their feather coat. Net-bottom pens are recommended to improve air circulation to the ventral feathers, but the document does not specify the type of covering that should be placed over the net-bottom pen. The Oiled Wildlife Care Network (OWCN) protocol suggests placing a light colored sheet over the top of the pen to retain heat and maintain a temperature of 90 – 95°F (OWCN, 2000). Heavier coverings (e.g., blankets) have been used in situations where the ambient room temperature was too cold for the pet dryer to maintain the desired temperature range (San Giacomo, pers. comm.).

Captive seabirds are extremely sensitive to fungal infections, especially *Aspergillus* spp. (Burco, 2007). Adequate air circulation may play a role in preventing development of this opportunistic disease. Ten to 15 air exchanges per hour has been recommended for animal holding areas (USFWS, 2002). This standard was implemented during the construction of the OWCN oiled wildlife response facilities; however, covering drying pens with a sheet disrupts airflow and effectively decreases air exchange within the drying pen.

Leaving drying pens uncovered is not an option. The covering prevents birds from escaping and provides a visual barrier to decrease captivity-related stress. Consequently, this study sought to compare three types of covering (shade cloth, sheet and blanket) to determine which is most appropriate.

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Materials and Methods

Drying Pen Configuration

Wooden handled natural feather dusters were used in place of live birds for the experimental model. Three feather dusters were hung vertically in a diagonal line across a 2' x 4' painted plywood net-bottom pen. The pen was covered with shade cloth (SC), a cotton sheet (SH), or a lightweight polyester blanket (BL). A pet dryer (Oster® Hi-velocity Table and Cage Dryer; McMinnville, Tennessee, USA) was hung over the side of one end of the pen with the nozzle directed downward. A digital combination thermometer/hygrometer (Fisher Scientific Traceable® Thermometer/Clock/Humidity Meter, Model No. 06-662-4, Friendswood, TX, USA) was placed horizontally on the net floor at the opposite end. All three trials were conducted simultaneously to eliminate variation in ambient environmental conditions as a confounding factor. Identical models of pet dryer and thermometer/hygrometer were used for each treatment. The experimental trial was conducted at the San Francisco Bay Oiled Wildlife Care and Education Center with the ventilation system engaged to provide 10-15 air exchanges per hour.

Drying Time, Temperature, Humidity

Each group of feather dusters were held in a cluster and rinsed for five minutes with water at 105°F, 40-60 psi, and 2-5 grains of hardness. Excess water was allowed to drain for 5 minutes and each feather duster was weighed to determine initial wet weight (Time 0, T_0). Temperature, humidity and weight of each feather duster were measure every 15 minutes for three hours.

Airflow

Airflow was subjectively evaluated using 45-second tracer smoke (Smoke Emitters, Marysville, Ohio, USA). Tracer smoke was video taped for two minutes to document observations of the covering, the inside of the pen and the air space below the netting. At the end of two minutes the covering was raised and the amount of smoke remaining in the drying pen was graded from 0-4+.

Data Analysis

Percentage of original wet weight was calculated for each feather duster at each time point using the following formula:

$$\% \text{ Weight Loss} = \frac{T_n - T_0}{T_0}$$

T_0 represents the original wet weight of each feather duster, and Tn represents the weight measured at each 15-minute interval. Percentage of wet weight lost, temperature, and humidity measurements were compared via repeated measures ANOVA.

Results

Weight loss did not differ significantly between treatment groups (F=1.54, P=2.19). Temperature and humidity were both significantly different (F=11.88, P<0.001 and F=10.31, P<0.001 respectively). Pair-wise comparisons (paired t-Test) of temperature and humidity showed significant differences between all treatments for temperature. Humidity values for the SC pen differed significantly from both the SH and BL pens, but the SH and BL pen measurements did not differ significantly.

Smoke appeared to flow freely through the shade cloth covering. It was seen to escape only around the edges and corners of the sheet and blanket. Within 30 seconds of ignition, the smoke emitter was obscured by smoke in the pens covered by the sheet and blanket. Visibility was much better in the pen covered with shade cloth. When the covering was raised after two minutes, the smoke remaining in the SC pen was scored 1+. The smoke remaining in the SH and BL pens was scored 3+.

Discussion

The type of drying pen covering did not affect drying time as measured in this study. Feather dusters were used as the experimental model because the rate at which a bird's feathers dry is largely dependent upon the amount of preening. I chose to eliminate this variable to better model the impact of environmental conditions on drying time.

Temperature within the drying pen was significantly different for all three treatments. The sheet covering maintained the highest internal temperature, followed by the blanket. It is unlikely that the sheet provided greater insulation against heat loss than the blanket; therefore, this difference may be explained by variation in the individual pet dryers. Whether or not this is the case, the biological implication of these temperature differences is minimal. The temperature in the SH pen averaged 3.1°F (SD=1.49) higher than the SC covered pen and 1.8°F (SD=1.37) than the BL pen. The temperature in the BL pen averaged 1.3°F (SD=0.42) higher than the SC pen. These temperature variations are within the 5°F range specified in the USFWS document, and increasing the ambient room temperature during an oiled wildlife response could raise the internal temperature of the pen.

The humidity was significantly higher in the SC pen. It is possible the increased air circulation allowed ambient moisture to enter the pen from the surrounding air. Like temperature, the difference in humidity was minimal (SC>SH, mean=1.8% (SD=0.90); SC>BL, mean=1.5% (SD=0.66)); however, in a rehabilitation facility, the increased humidity might have a beneficial effect on birds' respiratory health and lessen the dehydrating effect of the forced air from the pet dryers pen.

Tracer smoke confirmed the airflow in the SC pen was superior to the other two treatments. The lack of difference in drying time, combined with minimal impact on temperature and humidity would suggest that the SC covering is the better of the three. Although the SC covering is less visually opaque than the other two, a recent experiment involving captive Western Grebes *(Aechmophorus*

occidentalis) used shade cloth exclusively to cover net-bottom holding pens. No adverse effect on the birds' behavior was observed (Gaydos, pers. comm.).

Future experiments could include repeatedly measuring temperature and humidity in the same pen while varying the three coverings and a using single pet dryer. This would eliminate a bias from the pet dryer or pen. Also, behavioral and physiological studies testing the impact of visual stress on birds occupying pens covered by the various materials might determine whether the covering is a factor.

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An emergency station for oiled penguin rehabilitation in the Strait of Magellan, Chile

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Keywords: Rehabilitation, penguins, oil, Strait of Magellan, Chile, mystery spill.

Introduction

Currently, Chile does not have a contingency plan for wildlife affected by oil spills. Between May 5th until July 28th of 2006, treatment and rehabilitation was accomplished on 76 oiled Magellanic penguins *(Spheniscus magellanicus),* rescued from the Natural Monument "Los Pingüinos", Isla Magdalena.

A temporary station was enabled in Punta Arenas, requested by Corporación Nacional Forestal (CONAF, National Parks Bureau), where the oiled birds were transferred. For approximately two weeks, all the penguins were washed to remove the oil. Veterinary attention was provided when required.

Penguins were released in small groups, according to response to treatment. Forty days after the rescue the first group of birds was released with a total of 54 birds released in total. The release percentage achieved was 71% (54/76).

A total of 22 birds died during treatment, some of them showing aspergillosis on necropsy and others signs of intoxication by hydrocarbons. Histopathology studies from all of the birds deceased are in process, but out of three cases histopathological lesions confirm aspergillosis infection in one, which is the first case ever reported for this region.

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Case Report

On May 5th 2006 CONAF reported oiled penguins showing up on the shores of Isla Magdalena, in the Strait of Magellan. In order to elaborate a plan for penguin evacuation and rehabilitation, the CONAF's staff consulted the authors and an improvised plan led to the creation of a temporary rehabilitation station using resources available at the time.

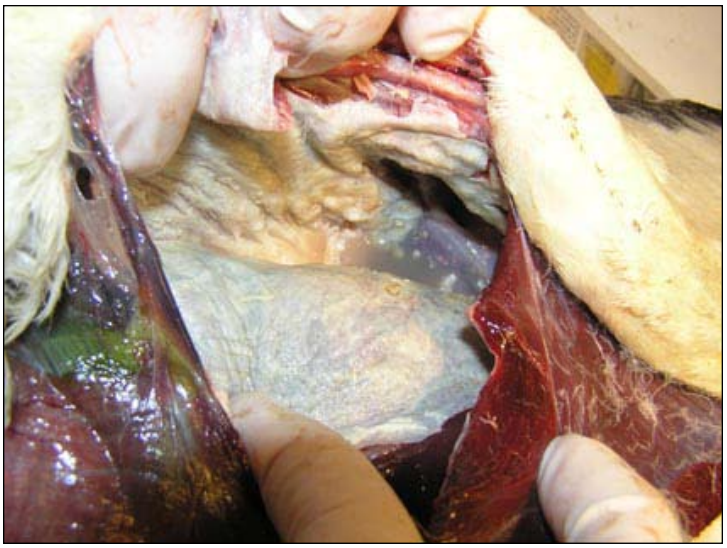
Since in Chile there is no formal governmental contingency plan related to oil spills affecting wildlife, no facilities are available when birds or mammals are oiled. The authors have been working on bird rehabilitation since 1980, and responded to an oil spill in May 2004, when rock cormorants *(Phalacrocorax magellanicus)* and Magellanic penguins *(Spheniscus magellanicus)* were affected in large numbers after the Berge Nice episode in the Strait of Magellan.

During the event in 2006 at Isla Magdalena, only Magellanic penguins were affected, and a total of 76 birds were captured and transported in 5 May, using a RIB (Rigid Inflatable Boat), to bring them into town, Punta Arenas. Once at the terminal, the birds were transferred to empty fiberglass containers and later taken to the rehabilitation station, located ten kilometers away.

The birds were kept in a makeshift facility consisting of a basic roofed structure with sand flooring, while waiting for the washing process. For the rehabilitation process we followed Callahan (2001), the protocols of Centro de Recuperação de Animais Marinhos (Ruoppolo et al., 2004) and previous personal experience in this region.

Washing all the birds took two weeks and the water used for both the washing and feather reconditioning was obtained from a stream using water pumps. Stainless steel bands were used to individually identify each bird (IFAW, 2006). After the cleaning process, the birds were transferred to the drying room for about five to six hours. Once dry, the penguins were kept in a different section of the facility, with outdoor access, where water was available for them to swim as they chose. A different tank was used for forced swims. The amount of time of the forced swim was increased daily; grading, blood sampling (hematocrit values) and weight were evaluated weekly until the birds reached satisfactory criteria to be released. The fish used to feed the birds was sardine *(Sardinops ocellatus* and *Sardina pilchardus),* obtained frozen from the local fishing companies.

Out of the total 76 birds treated, 22 died; 45% (10/22) showing signs of aspergillosis at necropsy and others intoxication by hydrocarbons and miscellaneous other findings. Histopathology studies were carried out on three birds, one of which confirmed aspergillosis infection for the first time in this region. The histopathology studies for another 18 birds are in process.



The lack of a contingency plan is a necessary issue to be discussed in Chile, as the event of another oil spill in the Strait of Magellan is highly probable, considering the traffic of vessels in an area with high diversity and abundant wildlife year round.

Acknowledgments

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Long-term survival, behavior and reproductive success of stranded southern sea otter *(Enhydra lutris nereis)* pups reared for release with a surrogate mother

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Keywords: Southern sea otter,* Enhydra lutris nereis, *rehabilitation, captive rearing, surrogate, survival, foraging ecology

The southern sea otter *(Enhydra lutris nereis)* is currently listed as threatened under the U.S. Endangered Species Act of 1973 due to limited range (extending 600 kilometers from Pillar Pt. to Pt. Conception along the California coastline), vulnerability to catastrophic oil spills, and slow recovery from commercial exploitation (USFWS, 2003). In the Final Revised Recovery Plan for the Southern Sea Otter, the U.S. Fish and Wildlife Service (USFWS, 2003) identified actions necessary for protection and recovery of the species including, population monitoring, assessment and elimination of fisheries-related incidental deaths, evaluation of delisting thresholds for the population, and improvement of captive management techniques, including rehabilitation and reintroduction of stranded or oiled otters to mitigate effects of a catastrophic oil spill if such an event occurs (Nicholson et al., In Press).

The Monterey Bay Aquarium Sea Otter Research and Conservation (SORAC) program responds to or coordinates response for all live-stranded southern sea otters (n=395 sea otters since 1984). Live stranded southern sea otters contribute to research studies designed to support population recovery as identified by the USFWS (2003), including refining techniques to release stranded sea otters to the wild after medical treatment and rehabilitation in captivity.

Currently, 25% (41 of 165, 2002-07) of live-stranded southern sea otters admitted to the Monterey Bay Aquarium (MBA) are neonate (<8 weeks of age) pups. Neonate sea otter pups strand following

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premature separation from their mothers, possibly resulting from mother's death, poor health, inexperience, or inadvertent loss of pup during stormy seas (Nicholson et al., IN PRESS). Neonate pups may suffer mild dehydration, hypoglycemia, and/or hypothermia, but are otherwise clinically healthy (38 of 41, 93%, 2002-07). Historically, pups rehabilitated for release were raised by methods that relied heavily on human care (Williams and Hymer, 1992), a factor that may have contributed to release failures and low survival in the wild (27% survival per year, Nicholson et al., In Press).

To address failures of sea otter pups to re-integrate with the wild population and avoid interactions with humans, SORAC initiated a surrogate program in 2001, pairing stranded pups with non-releasable adult female sea otters that adopt the pups as their own (Nicholson et al., In Press). In contrast with other strategies, surrogates provided species-specific mentoring, tactile stimulation while grooming and nurturing the pup, nourishment through food-sharing, and demonstration of feeding methods, such as dismembering crabs and cracking open hard-shelled bivalves using rocks as tools (Nicholson et al., In Press).

Ultimately, success of the SORAC surrogate program depends upon demonstrating that rehabilitated sea otter pups integrate into the wild sea otter population after release, behave normally, and contribute reproductively to the wild population. This manuscript summarizes rehabilitation methods developed for rearing wild-born sea otter pups with a captive surrogate, outlines a study designed to assess the effectiveness of these methods through long-term post-release monitoring of individual sea otters, and presents preliminary findings on foraging behavior, and post-release survival of individuals released since 2005. If successful, these methods would be applicable in the event of an oil spill or other catastrophe affecting the wild sea otter population.

Animal care protocols developed for rearing sea otter pups with a surrogate sea otter mother were approved by the MBA Institutional Animal Care and Use Committee (IACUC) on 15 March 2005. An age-dependent timeline is presented (Fig. 1) outlining the rehabilitation process.

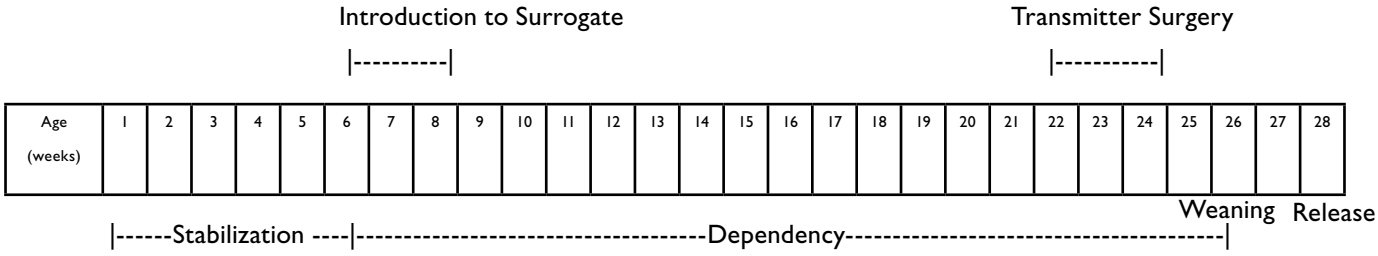

Figure 1: Timeline (weeks) outlining critical periods during rehabilitation of neonate sea otter pup reared for release with surrogate mother at MBA. Once a stranded neonate sea otter pup has been admitted to MBA, body temperature, hydration, and nutrition of pup are stabilized prior to introduction to surrogate (0-6 weeks of age). Acceptance of pup by surrogate may occur immediately, or may take several introductions (6-8 weeks of age). During dependency, pup remains with surrogate continuously except for brief separations one or two times per week to assess health of pup and record length, weight and dental development (6-26 weeks of age). In preparation for release, the pup is surgically implanted with a VHF radio transmitter equipped with temperature-sensitive mortality switch and time-depth recorder (TDR) in the peritoneal cavity (22-24 weeks of age; minimum body weight for transmitter surgery=8.2kg). Approximately two weeks prior to release, the pup is permanently separated (weaned) from the surrogate mother, and housed alone or preferably with cohorts (26 weeks of age). Pups are released at 28 weeks of age.

Surrogate-reared juveniles are released by boat in Elkhorn Slough, California (36° 49'N, 121° 45'W). The slough is inhabited by variable numbers of southern sea otters, and is protected from heavy surf and large swells, with relatively abundant sea otter prey, including: Washington clams *(Saxidomus nuttali),* gaper clams *(Tresus nuttali),* cancer crabs *(Cancer* spp.), fat-innkeeper worms *(Urechis caupo),* shore crabs *(Hemigrapsus oregonensis* and *Pachygrapsus crassipes)*, and green crabs *(Carcinus maenas).*

During the first two weeks following release, daily re-sights by shore, boat and/or plane take place to determine 1) prey selection and foraging success, 2) travel time and distance (along shore or off-shore), and 3) whether the juvenile interacts with humans. Attempts are made to recapture juveniles that do not appear to be foraging successfully, that travel far offshore, or that interact with humans. Re-release may occur following failure during this period depending on health of the individual, or nature of human interaction.

After two weeks, juvenile sea otters are considered independent, and re-sights (via shore or boat) and foraging observations are made semi-weekly, with missing animal surveys (via airplane) taking place opportunistically based on budget and availability of the pilot. To track rehabilitated juvenile sea otters from release through prime-age adulthood (3-10 years of age), surviving individuals will be re-captured and transmitters replaced at two-year intervals (minimum battery life of VHF transmitters) up to a maximum of four surgeries (Mike Murray, pers. comm.).

Survival rate estimates based on re-sight data are calculated from the Kaplan-Meier equation (Pollock et al., 1989), except that in this study, survival of each individual is calculated from date of release (age-specific survival). Surrogate-reared juveniles are released sequentially over the course of several years, rather than as a single cohort (Table 1); therefore, inter-annual effects or effects of season on survival are not accounted for in study design. Diet and foraging success of rehabilitated juveniles will be compared with recent data from Tinker et al. (2006), and on-going studies of the wild population (M. Staedler, pers. comm.).

Table 1. Summary of sea otter pups admitted to research study since formal MBA IACUC approval on 3-12-05.

Addition of time depth recorders (TDR) to expand data collection capabilities was approved 7 January 2007 by MBA IACUC. Sea otters 379 and 386 are currently (as of 5-17-07) housed with surrogates at Monterey Bay Aquarium and scheduled to receive VHF transmitter and TDR prior to releases in summer 2007.

Sea Otter	Sex	Stranding Date	Release Date	Transmitter (Frequency)	TDR
315	M	12-18-04	7-5-05	VHF (5.385)	no
324	F	2-25-05	8-1-05	VHF (4.931)	no
327	F	3-24-05	12-3-05	VHF (5.258)	no
339	F	10-25-05	7-5-06	VHF (5.272)	no
344	M	12-11-05	6-19-06	VHF (4.931)	no
353	F	4-9-06	10-7-06	VHF (5.091)	no
371	F	8-22-06	3-29-07	VHF (4.693)	yes
379	F	12-20-06	6-20-07a		
386	M	2-22-07	8-15-07 a		

a Proposed release dates.

Between 2005 and 2007, SORAC has released seven surrogate-reared juveniles (sex-based differences were not considered in this preliminary analysis). Five of seven (71%) have survived a minimum of eight months, and continue to be monitored. Both mortalities occurred within two weeks of release due to starvation (sea otters #324 and #371). Failed releases displayed similar patterns: 1) high percentage of very small (<4 cm) or unidentified prey on observed foraging dives (Table 2), 2) difficulty or inability to track (including by plane) due to movement offshore and continuous pattern of long dives and short surface intervals for extended periods, and 3) dispersal >20 km from release site within first week following release. Evidence from a time/depth recorder (TDR) recovered from one of the females that died (#371) showed that after exiting Elkhorn Slough and traveling offshore, this individual had multiple foraging bouts lasting longer than 24 hours (data analysis in progress). The short surface intervals during this extended period of diving, suggested that most dives were either unsuccessful, or resulted in very small prey items that did not contain enough calories to offset energy expenditure. In addition, because of the high level of activity and proportion of time underwater, shore-, boat- and plane-based searches were unsuccessful in locating this individual during much of the post-release period.

In contrast, among the five survivors, small or unidentified prey, while initially prevalent in diets, were replaced within the first week following release by larger (6-10 cm) prey items, specifically cancer crabs (*Cancer* spp.) among juveniles remaining in Elkhorn Slough/ Moss Landing area, and large mussels (*Mytilus* spp.) in the case of one individual who re-located to Santa Cruz. Foraging success rate on larger prey items increased with time during first two weeks, suggesting that recently

Table 2. Percent successful dives and prey items consumed by surrogate-reared juveniles during the first two weeks after release.

Recorded prey items included: Washington clam (was), gaper clam (gap), other clam (oth), sand dollar (sdollar), purple urchin (urch), mussels (muss), cancer crab (can), green crab (green), shore crab (shore), and unknown prey (unk).

			Diet (%)											
				Clam						Crab				
Sea Otter #	Days Post-Release	# Dives	% Success	was	gap	oth	sdollar	urch	muss	can	green	shore	unk	status
315	0-7	37	32.4	8						33		33	25	Alive
	8-14	82	60.2							82	6	6	3	
324	0-7	63	46.0									41	59	Died
	8-14	No Data*												
327	0-7	137	33.6							83		9	9	Alive
	8-14	27	81.5							100				
339	0-7	114	44.8	2						37		45	16	Alive
	8-14	260	70.4							66		18	24	
344	0-7	120	23.3	11	9	26					22	26	39	Alive
	8-14	76	77.6	2						58		8	12	
353	0-7†	149	14.2						100					Alive
	8-14	33	91.3						100					
371	0-7	162	22.8		14	5	11	14	5				57	Died
	8-14	No Data*												

* Sea otters 324 and 371 had no data for second week after release due to disappearance and death.

released juveniles must learn or acquire foraging skills necessary to survive very rapidly in an unfamiliar environment. In addition to rapid development of successful foraging, four of five (80%) survivors remained within 5 km of Elkhorn Slough during the first two weeks following release. Decreased dispersal distances following release may have benefited juveniles by conserving energy while foraging skills were acquired.

Post-release survival of surrogate-reared sea otter pups (Table 3) compares favorably with annual post-weaning survival of wild juvenile southern sea otters (both males and females) in the north-central portion of the range (0.776 – 0.883, Tinker et al., 2006).

Table 3. Kaplan-Meier post-release survival estimates for surrogate-reared southern sea otters released between 2002 and 2007 by Monterey Bay Aquarium Sea Otter Research and Conservation program (n=13).

Sea otters released between 2002 and 2004 (n=6) were part of a pilot study to develop rehabilitation methods incorporated in the present study. Number of sea otters at risk decreased between intervals of time without losses due to death or censorship because individuals have been released sequentially over a 5-year period rather than as a single cohort. For the lower survival estimate, censored animals were assumed to have died, for the upper estimate, censored animals were presumed to have survived. Confidence limits were calculated based on lower survival estimate.

				Survival		
Days Post-Release	# at Risk	# of Deaths	#Censored	lower	upper	95% C.I.
0 – 14	13	2 [a]	1	0.769	0.846	0.569 – 0.970
15 – 60	10	1[b]	0	0.692	0.761	0.454 – 0.930
60 – 210	9	0	0	0.692	0.761	0.441 – 0.942
211 - 304	8	0	0	0.692	0.761	0.426 – 0.958
305 - 320	7	0	0	0.692	0.761	0.408 – 0.976
321 - 518	6	0	0	0.692	0.761	0.386 – 0.998
519 – 567	5	0	1	0.554	0.761	0.230 – 0.878
568 – 669	4	0	0	0.554	0.761	0.192 – 0.916
670 – 1218	3	0	1	0.369	0.761	0.038 – 0.700
1219 – 1298	2	0	0	0.369	0.761	0.000 – 0.775
1299 – 1773	1	0	0	0.369	0.761	0.000 – 0.943

a Cause of death for both individuals was starvation.

b Cause of death was trauma due to shark bites.

Preliminary evidence suggests that during the first two weeks following release, critical determinants for survival of juvenile sea otters appear to be 1) establishment of successful foraging on relatively large (6-10 cm) prey, and 2) lack of long-distance (>5-10 km) dispersal from release site in Elkhorn Slough. Heisey and Fuller (1985) noted that the time needed after marking for adjustment to the transmitter package, recovery from capture, stress or injury, or resumption of normal social bonds should not be included in survival calculations. Clearly, juvenile sea otters reared in captivity with a surrogate mother must rapidly adjust to unfamiliar conditions after release to the wild. Probability of failed release or mortality during the first two weeks is relatively high, but appears to approach zero after two weeks. To avoid underestimating survival of surrogate-reared pups, mortalities that occur during the first two weeks could be censored rather than considered as deaths, or the first two weeks not included in calculation of post-release survival, in which case, only sea otters surviving that period would be included in the sample.

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Case report of 2006 mystery oil spill in Estonia

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Keywords: case report, Estonia, Baltic Sea, mystery spill, oil, wildlife

Introduction

The mystery spill, which took place in the northwest of Estonia in the beginning of 2006, was the largest oiled wildlife incident in Estonian history. The oil slick came to shore in an area which is not only a protected landscape reserve, but also Natura 2000 bird area and contains habitats of protected species. At the time of the spill, there were about 50,000 wintering waterfowl in the region. During the first days, a few thousand oiled birds came to the beach and more than ten thousand remained on the open sea.

Before this spill, there had been only small-scale incidents in Estonia, where the wildlife response involved between 10 and 20 birds (mostly swans). These cases were managed without wider public attention by Nigula wildlife rehabilitation centre. The 2006 mystery spill was clearly too large an incident for an unequipped and unprepared Estonia.

The initial response attempt began without any prior experience at handling such an incident. The responsibilities of different agencies were not clear, and necessary facilities and equipment were unavailable. More time was spent discussing who should be handling the situation than actually responding. An unfortunate side-effect of this was that many of the actions undertaken had more negative impacts than good results.

Fortunately international interest brought about a change to the response. International intervention provided expertise and protocols and helped make decisions to bring the logistics and responsibilities of managing the response under control.

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Case Report

The Estonian 2006 mystery spill took place in the northwest of Estonia, in the Baltic Sea. The area where the oil came ashore is designated as an Important Bird Area, Natura 2000 bird directive area, and is protected by the regimes of landscape reserves and special protection area with the aim to maintain the favourable status of the coastal habitats. The coastline is also famous as a recreation area, due to the sandy beaches and coastal pine forests.

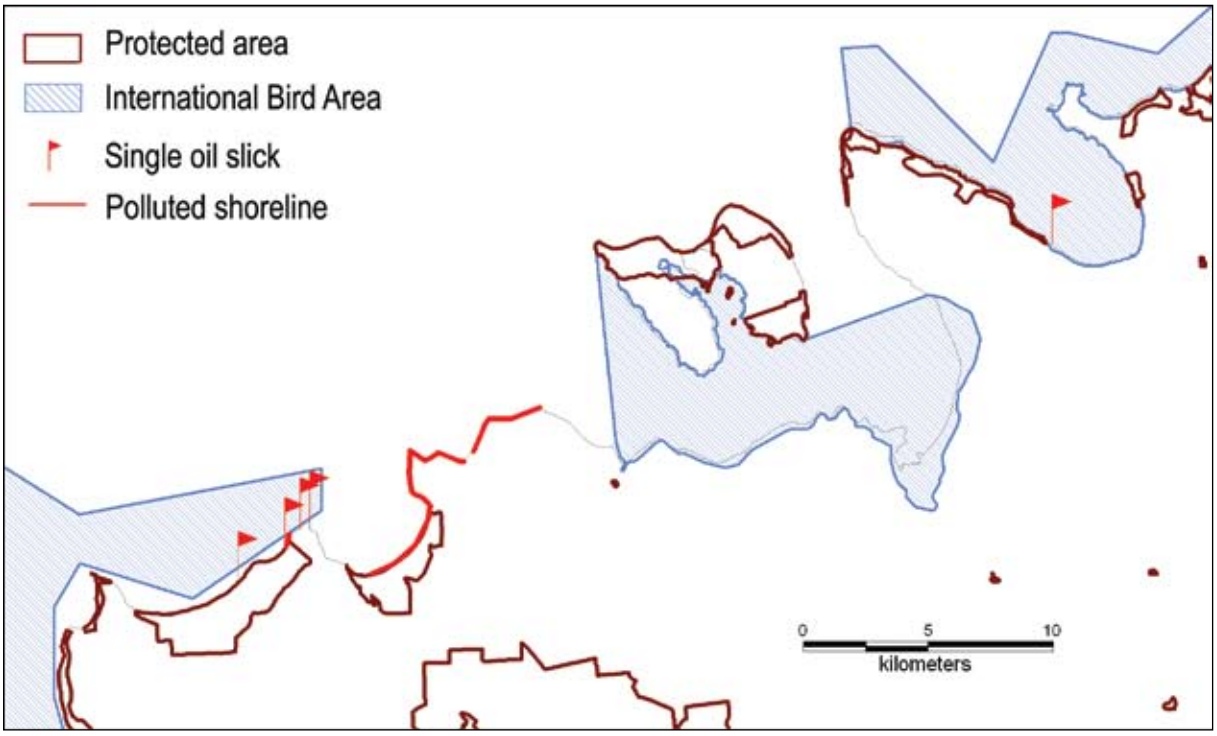

Figure 1: Oil pollution discovered during the mystery spill of 2006 in NW of Estonia (Raudsepp, U. et al., 2006)

The spill was discovered in the end of January. Most of the response took place in February. Though Estonian winters are normally quite difficult, 2006 was colder than normal. During the first weeks of February the temperature ranged between -20 and -30 ° C. These temperatures, combined with ice, snow and strong winds made conditions unpleasant for spill response.

Figure 2 shows the findings of oil spills (red spots), areas of decreased water-transparency (brown) and potentially sunken oil (purple). The shoreline affected was around 35 km in total length and it was estimated to be polluted with 20 tonnes of residual marine fuel oil.

In the beginning of the response there was some doubt that the polluter was a ship named Flawless, which had reported a spill of fuel oil to the ship quarterdeck a week before. The ship was later proven not guilty.

Figure 2: Analysing the LDI images proved that the affected shoreline was larger than estimated in the beginning (Raudsepp, U. et al, 2006)

The total number of birds captured during the response was 3443 +/- 100 (species seen in Table 1). The majority of birds affected by the oil were wintering seabirds and diving ducks—the species which are most difficult to rescue and rehabilitate.

Table 1:Birds captured during the oil spill

Bird	***Latin***	**Number**
Long-tailed Duck	*Clangula hyemalis*	2472
Goldeneye	*Bucephala clangula*	397
Velvet Scoter	*Melanitta fusca*	49
Mute Swan	*Cygnus olor*	15
Great Cormorant	*Phalacrocorax carbo*	4
Common Teal	*Anas crecca*	2
Common Merganser	*Mergus merganser*	13
Great-crested Crebe	*Podiceps cristatus*	3
Red-breasted Merganser	*Mergus serrator*	4
Tufted Duck	*Aythya fuligula*	
Mallard	*Anas platyrhynchos*	
Common Gull	*Larus canus*	2
Red-throaded Diver	*Gavia stellata*	6
Smew	*Mergus albellus*	1
Herring Gull	*Larus argentatus*	1
Undetermined*		469
TOTAL		3443

* before SNCC ornithologists took over the corpse management, the staff of RB and BG were not trained to determine the species.

The general opinion of the Estonian people about wildlife rescue and rehabilitation is mostly positive. Although there is no long term history of such activities in Estonia, the wider public demands that wildlife should be helped by "someone." In the mystery spill in 2006, the wider public immediately demanded a response. During the incident, the response demand grew into political arguments about who was to blame for the incident and the initial poor response, with the Estonian minister for environment being blamed.

The first week after the pollution was discovered, an initial attempt to respond was carried out by the Border Guard, Rescue Board, State Nature Conservation Centre and Estonian Fund for Nature. Efforts to rescue the oiled birds were uncoordinated, carried out by inexperienced people in unsuitable facilities sometimes lacking even common sense. As a result, the initiation of an adequate response was delayed.

During the first week of the spill response, it was possible to catch a lot of birds. Most of the birds were caught by people cleaning the beach, though these birds were often clearly not suitable for rehabilitation any more. Still, a number of birds in rather good physical condition were captured, but slowly killed with the wrong care. From the 3443 birds, less than 1000 were captured alive, with around 700 reaching rehabilitation. By the time international help arrived, about 200 were alive.

Although many mistakes were made, there were also some positive actions undertaken as well. It was quickly understood that preventive measures also should be undertaken, for instance, to protect animals that started to feed on the oiled birds arriving on the beach. Feed stands with fresh fish were established on the beach for the Sea Eagles and other raptor species.

International help arrived one week after the discovery of the spill and the initial local response attempt. Their arrival brought relief to all the people already working without proper protocols, training, equipment or coordination. The response attempt was assessed and failures were explained carefully if not tentatively to initial responders. Changes were immediate: a temporary facility was established in a public sauna followed by the set-up of a proper bird hospital a few days later. Also the local responders received training.

Changing the response to follow more widely accepted protocols had quick and visible results:

- Birds that were slowly being killed by inexperienced care were helped with either ending their suffering or successful rehabilitation;
- People trying to do something for the oiled birds were trained to rehabilitate properly;
- When it was understood that the response to the spill was not only an internal Estonian matter, things started to change. This included concern from the ministries, but more importantly changes to state funding.

The Baltic Sea borders Estonia to the North and West and the total length of the coastline is 3793 km (including islands). In addition, about 40% of the Estonian coastal sea is designated as different kinds of protected areas. The Baltic Sea has a high level of shipping traffic, an estimated 2000

ships per day, of which about 200 are oil tankers. The Environmental Inspectorate has analyzed the probability of oil pollutions and the result is that every 10 years there will be 3-5 larger incidents (Merereostusjuhtumite, 2004). The density of the previous spills can be seen in Figure 3.

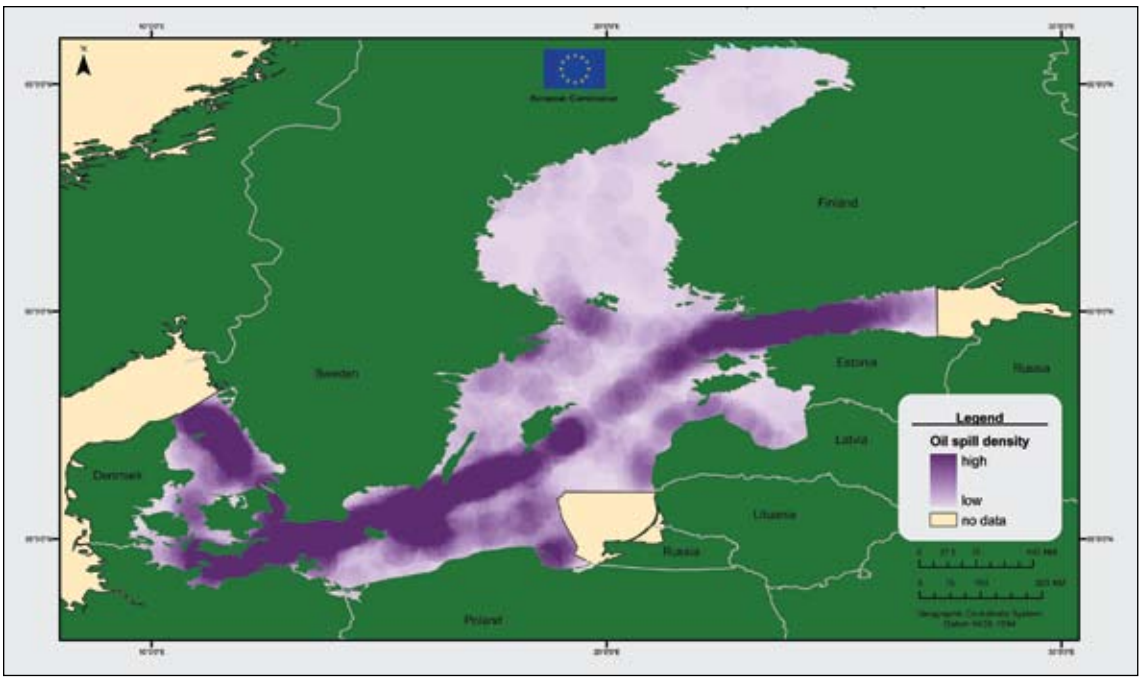

Figure 3: Oil spill density – Helcom – Baltic Sea – Years 1998-2004 (Helcom, 2007)

When we take all these facts into account, we can see that there is serious risk of oiled wildlife incidents in the future, and with experiences from the 2006 mystery spill, Estonia is aware of the requirements for an effective oiled wildlife response. But the actual readiness of the country and its facilities does not meet these requirements. For instance, the bird hospital building is being sold by the ministry.

Still there are some improvements compared to January 2006:

- Estonian Fund for Nature has a network of 300 volunteers who have gone through training, about 20 of them offered their help in the case of cleaning a beach area polluted in spring 2007.
- 3 people (2 from EFN, 1 from SNCC) went through a training in a bird centre in Belgium in the winter 2007;
- SNCC has developed mobile rehabilitation units, the purchase of the mobile units is awaiting funding decisions;
- SNCC coordinates the process of oiled wildlife response planning in cooperation with EFN, RB and BG;

- SNCC is developing a new hospital in Nigula, which should meet the demands of an initial oiled wildlife response until mobile units are established, the building of the hospital (depending on funding) will start in 2008;
- SNCC staff has organized meetings for Latvian decision makers about oiled wildlife response issues, more practical training for Latvian Ornithological Society (the agreed responder of oiled wildlife incident) is going to take place in summer 2007.

Discussion

The shocking 2006 Mystery spill was a wake-up call for Estonia. People became aware of the oiled wildlife issues and understood that hiding your head in the sand does not help in the long term. Still the initiative of building capacity on the oiled wildlife response is too slow, and it seems that one year has been long enough to forget, even though the "axe of next pollutions" is hanging over our heads.

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Natural history, daily work, and frequent sightings are the keys to good husbandry

Monte Merrick[1]

Keywords: natural history, husbandry, daily rehabilitation, observation

"We need another and a wiser and perhaps a more mystical concept of animals. Remote form universal nature, and living by complicate artifice, man in civilization surveys the creature through the glass of his knowledge and sees thereby a feather magnified andthe whole image in distortion. We patronize them for their incompleteness, for their tragic fate of having taken a form so far below ourselves. And therein we err, and greatly err. For the animal shall not be measured by man. In a world older and more complete than ours they move finished and complete, gifted with extensions of the senses we have lost or never attained, living by voices we shall never hear. They are not brethren, they are not underlings; they are other nations, caught with ourselves in the net of life and time, fellow beings of the splendour and travail of the earth."

—Henry Beston, *The Outermost House,* 1928

Using my own experiences as a rehabilitator, as an oil spill responder, and speaking of my own affections and aspirations, I will both plea that experience in day-to-day wildlife rehabilitation is indispensable if we are to provide the "best achievable care" during an oil spill, and that rehabilitators and our patients are served immeasurably by intimate and immediate witness of the various species for which we care in their autonomous and wild lives. I will start with a short glance at the idea of husbandry.

Husbandry, as I found in a brief Google search, may be defined any number of ways. From the perspective of raising livestock and crops, to the care and maintenance of captive animals, to general stewardship of resources, whether those are household finances or the system of natural parks. Generally, however, good husbandry means that what is in our care shall thrive. In wildlife rehabilitation, and specifically in a spill situation, husbandry can mean keeping alive three hundred baby Brown Pelicans through the process of getting them clean, or 20,000 African Penguins, or forty-three Mallards and Canada Geese.

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Good husbandry needs no defense—at least not here. We all know that a clean environment, an appropriate diet, and housing that acknowledges the needs of the species housed, with as many of the inherent stressors of both injury and recovery as reduced as possible, are critical to the rehabilitation and eventual release of our patients. While not my point, I think it is very hard to overstate the importance of husbandry in this process. Good husbandry is nearly identical to good wildlife rehabilitation; we must always refine our methods, always be ready to accommodate our latest observations, and always look for new ways to increase the quality of the care that we provide during oil spills and other catastrophic events that impact wildlife so adversely.

Husbandry of wildlife brings its own questions, both of theory and practice—and we learn, of course, from our patients in these matters. We capture and we care for them because we believe that we must, though the theory remains unproved. We capture and we try to keep them alive when the ways of the world had agreed they were dead, as dead as the many more we never get to treat. To keep them alive until they are strong enough to wash, strong enough to decide that they will live after all—and it is always the hardest to lay to rest the victims whose fierce gaze is only strengthened by their ordeals though their bodies are utterly broken.

To rehabilitate an oiled bird is something we decide to do before we know how to do it.

We learn on the job.

Some of us started learning on the job thirty or forty years ago, some of us are just beginning.

One of the joys of caring for injured wildlife is its kinship to those things that are very old and very common—cooking, child-rearing, hunting, art, craft. These human engagements, although varied, are similar in that all are simple and accessible and require a lifetime to master.

Any of us can follow the protocols that have been established through what is now decades of trial and error in the effort to rehabilitate oiled wildlife. We have a body of knowledge to lean on, documented and accessible. But who it is that can really make that body of knowledge come to life is the rehabilitator who brings a set of experiences, especially those coveted moments of inspiration, in which sudden and permanent learning occur. Ask any rehabilitator and she or he will tell you that these moments happen daily. Each day we are schooled in what a sick or injured seabird needs.

Surely a Western Grebe and a Common Loon have similar lives and therefore quite similar needs—neither can tolerate a long period of time off their water home—keel and hock and foot lesions will develop quickly as we all know—but what does it mean when a Common Loon stops evading the net? Anyone who has treated a few loons will know at least this: it isn't anything good.

What we learn as rehabilitators is incredibly specific—to the species, and to the individual. Think of a Common Loon, say, a big one, in breeding plumage, who nearly takes off your finger in one lunging bite and you know that this bird needs only a few days and out she'll go—yet a gull might do the same, flapping his left wing while his right wing is shredded. Consider how all cormorants will bite but a Brant's Cormorant who seems more aggressive than usual probably has a fish hook somewhere

in his guts,—just as a Western Grebe who cries in his hospital pool all day should be x-rayed or palpated for a featherball impaction. These are the things you learn when you do this work daily. What a particular bird needs is learned, and in a manner that endures, from daily care for birds in general.

And there is no limit to the intricacies one might learn.

Each day hundreds of people are engaged in the work of nursing wildlife back to health. In the day to day care of the animals routinely injured or sickened by their contact with the machinery of humanity we learn to care for the large numbers of wildlife affected during a oil spill. And as the oceans deteriorate, and more species become threatened with decline and extinction, and saving as many individuals as we can becomes the world's work, wildlife rehabilitators will be there, with skills and knowledge to help ensure that the victims of the altered environment are given the best possible care.

Like many rehabilitators, I came into this work with a desire to help wildlife which had been whetted by some reading. I was anxious to be of use, and hungry for something elemental, un-mediated—what we may call reality. I became a volunteer before I became a true student of natural history. I held baby house sparrows and fed them baby bird slurry long before I understood the life of a sparrow who is no orphan, if I ever have. I became one of the relatively small number of people in this world who knows a Mallard's tongue the way a child knows a cat's. It was another year before I saw truly wild Mallards living in open seclusion, on a pond high in the Cascades, and began to understand how the integrity a sick duck presents at the center is but a shadow of its true nature. If I hadn't become a volunteer at a local wildlife rehabilitation center I may have not seen them at all. Every Steller's Jay I see today is the gift of the first cat-mauled jay whose bandage I changed—every nest I've searched for depends from the first baby bird basket I cleaned. And this is true for every species—even those who are with us each day—robins and crows, gulls and pigeons.

An interest in animals, so common, leads us to wildlife rehabilitation and that leads us back to the literature of nature, which leads us to nature itself.

Natural history can lead us everywhere. The short history of the Common Murre that is found in the guide book will tell us where we might find him or her, and at what time of year, what sort of plumage we might expect either to wear—maybe how its voice might sound were we to try to transcribe the song into words. Another text may explain discovered facts about how the alcids breed, and where they feed, and what any of us have seen—and so on, until at last we are driven from our house to the field, from the book to the sea.

And here I plead that we plunge into this sea. What is true about the world, about life, about our lives is manifest in the lives of our patients. We are in the unparalleled position of holding wildness in our hands and restoring its autonomy. We muck around in oil a foot deep, pulling dead loons, mergansers, otters, muskrats from its clutches—we see wings blasted at the shoulder and ravens shot from the skies by children who are ignorant of their meaning and their worth. What I am trying to say is that the reasons for getting out to where our patients are at home are manifold—we learn who they truly are—we restore our own sensibilities—we give our affections a chance to grow—we

preserve what we love. It is not enough to know that Northern Fulmars are pelagic birds who breed in the Arctic—although we may still provide good care for them with little more knowledge than this—but what kind of care might be possible after seeing these birds asleep on the slopes and the crests of twenty-foot waves in the Bering Sea.

The first Magnificent Frigatebird I saw was in a pet carrier retrieved from the airport. The aptly-named bird had been found far off course in British Columbia and sent by jet to our center in Los Angeles. He was juvenile and very thin. I fed him fish and marveled at how merely spreading his nine feet span of wing sent him aloft. The next one I saw was on the Gulf of Mexico, floating far above me, far above the achingly blue sea. They seemed more like a dream. Now the first one's effortless lift from the perch in the large aviary made beautiful sense. I began to understand what the patient longed to do. Now I would be a better husband to that bird.

We may teach or be taught to scatter a few leaves in the bottom of a Spotted Towhee's cage—a good thing to do—but what happens when we see for the first time, and each time after, one of these creaking birds rattling around beneath a blackberry vine, kicking up dead alder leaves, searching for insects is immeasurable—a true sympathy begins. Now we can begin to imagine what will make the towhee more at ease while recuperating. Now we are more able to reduce stress.

A few years ago I had occasion to be on the central California coast—I camped overnight at Big Sur. The campground is primitive but accessible. As easy to use as a motel—but with the sky, the surf, the fog, the trees, the birds, the easy camaraderie of fellow campers, and a wood fire as the finest amenities and all so affordably priced—I paid seven dollars for the privilege.

On my way to the beach, a pair of Swainson's Thrushes flew in circles through a thicket of young trees, singing their spiraling flute of song and calling their liquid drops in a bucket. The guide book calls them drab little birds but I prefer to think of them as subtle. It had been two years since I'd last heard this song, and I've never seen with such clarity, unaided. Always they'd give just a glimpse here, a flash of tail there. But these two put on a show—calling their hearts out and chasing each other through the branches—a regular song and dance number.

Just past these trees the trail splits—one branch to the beach the other to the headlands. I took the headland trail. Out at its point, the ocean is perhaps seventy-five feet below. An orphaned piece of the land sits about one-hundred and fifty feet out. On this sea stack, facing the setting sun and the onshore wind, were thirty or so adult Western Gulls. Their plumage was pristine in the slanted light. They looked like a million bucks. Rats with wings, they are called—but out here they are truly home—a broken-off chip of continent, stained with generations of droppings—and they are beautiful and they are perfect, perfectly matched to this place in the sea and the sky. And as I watched them cavort in the wind, pivot on a wingtip like the universe around Polaris, suddenly a gull chick, grey and speckled and until this moment neatly hid by his plumage and the rocks, stretched his young wings and stood facing the wind—the air sliding through his feathers not yet ready to bear him up—but he faced the wind and lifted his wings and his dream of flight was no pipe dream. He

watched his parent swoop and dive and everything stretched out before this young bird. Just now becoming acquainted with the wind, it would be a lifelong romance and here was the very start.

I watched for another hour, eventually counting ten chicks, some maybe a week old others nearly ready to fledge. I wanted to stay to see them off. I wanted to put a small stove and rocking chair there on the edge of this bluff and make coffee and sit and do nothing more than see what happened next out on the rock of the gull. I looked about some more—on the same rock, in the cavities etched into its steep sides, were a handful of nesting Brant's Cormorants, with a few nestlings. An osprey made several trips to sea and back, on each return a fish realizing its old dreams of flight clutched in its talons. Single file, fifteen Brown Pelicans brushed soundlessly past me as they banked toward the surf. Bank Swallows and Cliff Swallows were acrobats flying up and down the face of the bluff. Loosened feathers raced in the wind and it was and it is a bird's world. The sun got fat and red and then sank.

Reluctantly I took my heavy body—solid and without feathers—ungainly and oafish—back down the trail to my sleeping bag.

Two days later I was back at the center in San Pedro. We had a Western Gull who'd been covered in cooking oil. When I'd last seen him he had yet to be washed, but now he was clean and standing and looking much better. But still, against the birds I'd just seen teaching and learning to fly, I could see that his fierce and wild nature was dimmed. He stood in the aviary, facing east, eyes half closed, warming himself in the morning sun. He'd begun to preen his feathers back to shape; his body was responding to the medicine—soon he would begin to fly again, perching higher, nearer to the sky. Soon catching him would be possible only because he was captive. This is the gift that all of our patients give to us—they bring us into a world that we forget is ours and teach us to see by its lights. People outside of this field often wonder if our patients ever express anything like gratitude. Of course they do not, I say. And besides, we are the ones who are indebted.

Chronic, oral exposure to bunker C fuel oil causes the development of adrenal hypertrophy with decreased responses to a model stressor in ranch mink *(Mustela vison)*

F. Charles Mohr,[1] Bill Lasley,[2] Steven Bursian[3]

Keywords: Mink,* Mustela vison, *bunker C fuel oil, adrenal gland, glucocorticoids

Contamination of the coastal marine environment with petroleum hydrocarbons can cause environmental damage to this sensitive ecosystem. While the acute effects of petroleum oil intoxication in mammals are known (Geraci and Williams, 1990; Lipscomb et al., 1993), the sublethal effects of chronic exposure to petroleum hydrocarbons from natural or anthropogenic activities are less understood. It is important to identify the sublethal effects of petroleum hydrocarbon contamination on animals because they can influence survival and reproductive success, and this can eventually have an impact on population growth. Bunker C fuel oil (fuel oil No. 6) is a refined petroleum product that can accidentally enter the marine environment (Irwin et al., 1998). This petroleum product is known to persist in the environment, essentially unchanged for many years (Vandermeulen and Singh, 1994). We have been studying the effects of bunker C fuel oil on ranch mink *(Mustela vison),* a mustelid surrogate for sea otters *(Enhydra lutris).* Sea otters are particularly vulnerable to the effects of petroleum oil contamination because of their limited range, near-shore residence, and prey items in their diet can serve as a source of petroleum hydrocarbons.

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In the course of studying the effects of fuel oil on the immune system of mink we discovered that the adrenal glands were larger (hypertrophied) in fuel oil-exposed animals than in control animals (Schwartz et al., 2004). We were interested in determining if fuel oil-induced adrenal hypertrophy has physiological relevance because under conditions of hypertrophy, these glands usually release more glucocorticoids into the blood. Our objective was to explore the relationship between chronic, oral, low concentration fuel oil exposure and adrenal hypertrophy by performing dose-response exposures and measuring changes in serum and fecal adrenal steroid concentrations.

The adrenal gland is a stress response organ that is part of the hypothalamic-pituitary-adrenal (HPA) axis. This axis is not only important in mediating stress responses but is also important is maintaining normal physiological homeostasis. All elements of the HPA axis are important in regulating the normal physiological responses of this system, and interference of normal function in any element of the axis can lead to dysfunction and altered responses to stressors. The major steroid mediators released from adrenal glands upon stimulation are the glucocorticoids. Release of glucocorticoids occurs after stimulation of the gland with adrenocorticotropic hormone (ACTH), a peptide is released from the pituitary gland. Glucocorticoids have important roles in energy metabolism, reproduction, immunity, development, and cardiovascular regulation (Sapolsky et al., 2000).

Two fuel oil exposure studies were performed with funding from the Oiled Wildlife Care Network. Both exposures took place on the Experimental Fur Farm at Michigan State University, a facility that is operated under USDA regulations and a protocol approved by the MSU Institutional Animal Care and Committee. In the first exposure, 48, 8-month-old, male mink, were fed bunker C fuel oil in the diet for 60-62 days over January-March. The animals were randomly divided into groups of twelve and fed 0 ppm (mineral oil), 48 ppm, 520 ppm or 908 ppm fuel oil mixed into the ranch feed. To minimize the exposure of these animals to any stressor but fuel oil, the animals were not handled for the entire course of the exposure. At the end of the exposure, the animals were anesthetized with the combination of ketamine and xylazine and bled by cardiac puncture. After the bleeding the animals were necropsied and major organs were weighted and sampled for microscopic examination. Throughout the exposure period, feces from individual animals were collected and frozen for assay of fecal glucocorticoid concentrations.

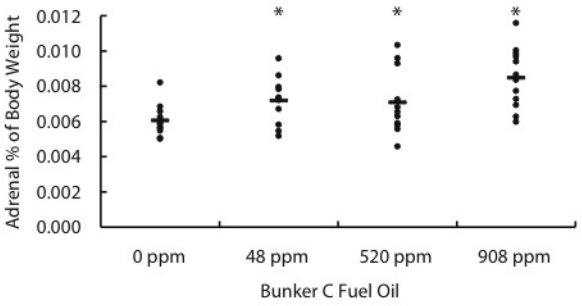

Fig. 1. Effect of bunker C fuel oil on adrenal gland relative weight. Circles represent relative weights of the combined adrenal glands from individual animals. Bars indicate mean relative weights for each group. Asterisks indicate significant differences ($p < 0.05$) between control and fuel oil groups.

The relative weights (% of body weight) of the adrenal glands from fuel oil-fed mink were greater than the controls. Among the fuel oil-exposed groups there were no significant differences in relative weights. Microscopic

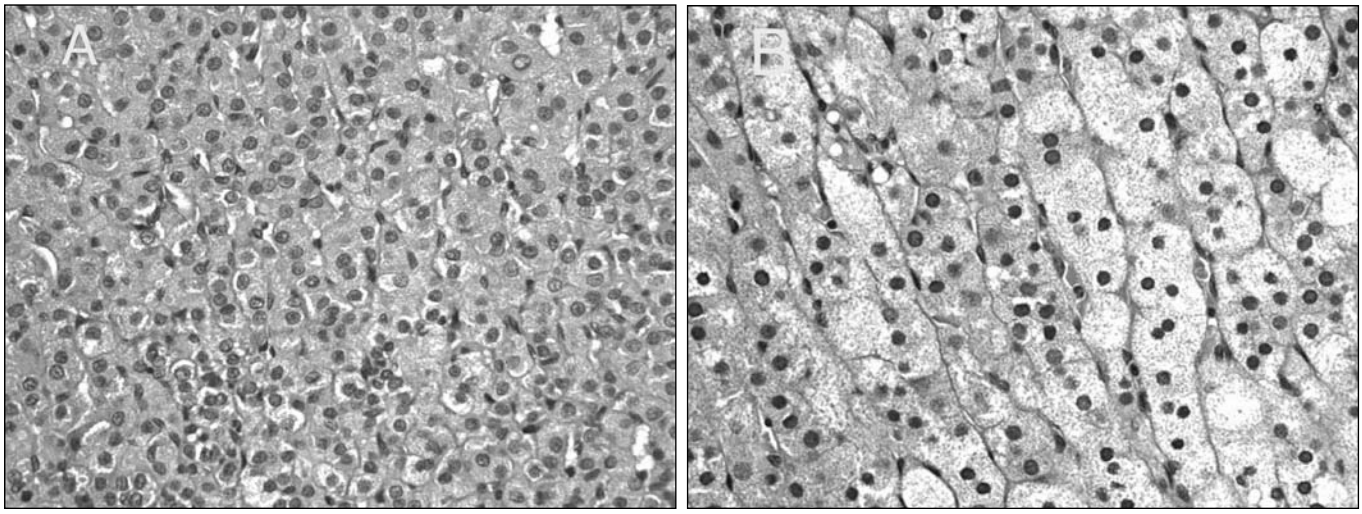

Fig. 2. Histological appearance of cortical cells in the zona fasciculata. (A) Normal vacuolation of cortical cells. (B) Heavy vacuolation of cortical cells. (400x)

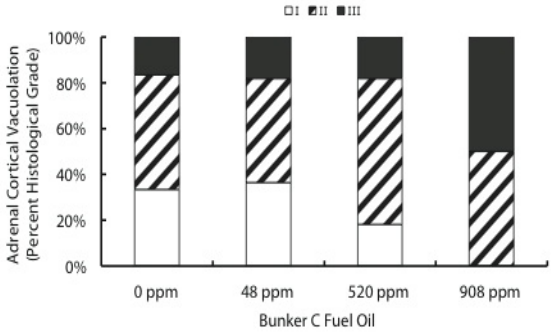

Fig. 3. Effect of bunker C fuel oil on adrenal cortical vacuolation. Bars represent the percent contribution of each of the three grading classifications for each concentration of bunker C fuel oil and the control. Grade I is for no-to-mild, II is for moderate, and III is for marked increases in the number of cells swollen with cytoplasmic vacuoles in the zona fasciculata and zona reticularis.

examination of the adrenal glands showed that in the 520 and 908 ppm fuel oil-exposed groups there were more cortical cells with heavily vacuolation, due to increase in cytoplasmic lipid droplets, than in the control and 48 ppm fuel oil groups. The 908 ppm fuel oil group had the greatest number of vacuolated cells. Interestingly, serum glucocorticoid and progesterone concentrations were not significantly

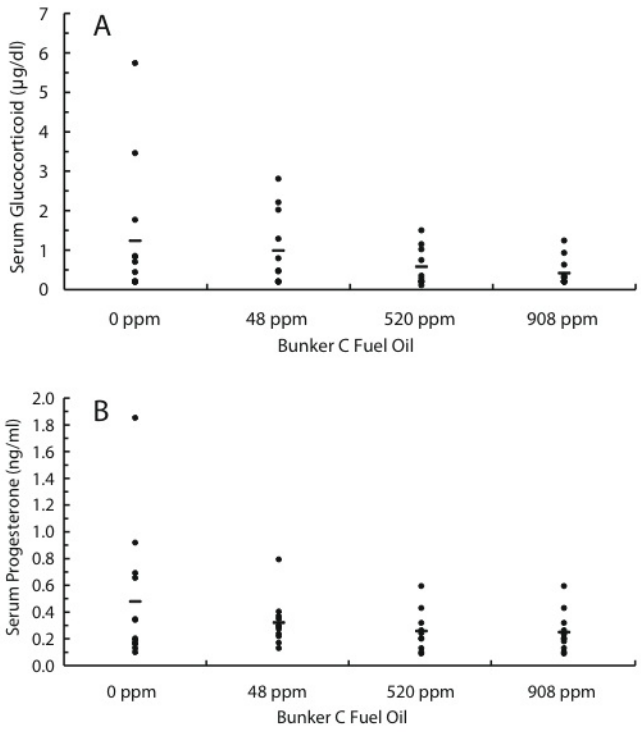

Fig. 4. Effect of bunker C fuel oil on serum glucocorticoid and progesterone concentration. Circles represent the serum concentrations of glucocorticoids (A) or progesterone (B) from individual animals. Bars indicate mean concentrations for each group. There were no significant differences among the four groups ($p > 0.05$)

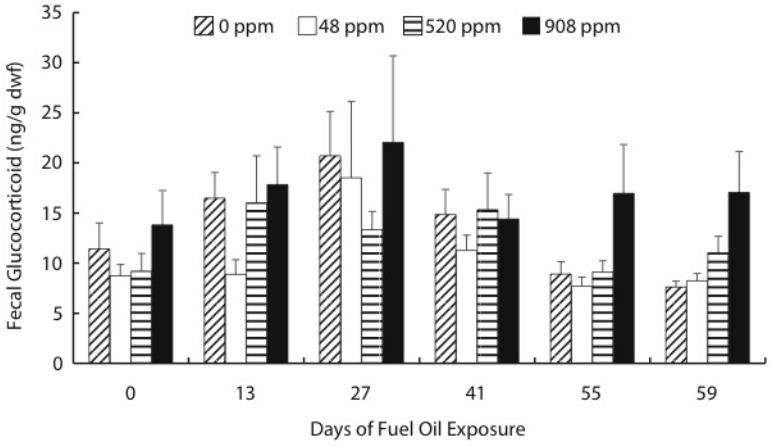

Fig. 5. Effect of bunker C fuel oil on fecal glucocorticoid concentration. Fecal glucocorticoid concentrations in ng/g dwf (dry weight feces) are expressed as mean ± SE.

different among any of the groups (fuel oil or control). In addition, over the time course of the exposure the concentrations of glucocorticoids in the feces were not different among the three fuel oil groups or between the two highest fuel oil groups and the controls. However, over the time course of the exposure, fecal glucocorticoid concentrations were lower in the 48 ppm fuel oil group compared to the control group.

Because adrenal hypertrophy was not associated with a change in serum or fecal glucocorticoids, we hypothesized that under conditions of stress, fuel oil-exposed adrenal glands would release less glucocorticoids than adrenal glands from non-exposed animals. We decided to use ACTH stimulation tests to test this hypothesis by determining if adrenal responsiveness to this model stressor was different between fuel oil-exposed and control animals. A second exposure study was performed at the same facility with thirty-six male mink of similar age as the first exposure study. Equal numbers of animals were fed either 500 ppm bunker C fuel oil or mineral oil in the diet. After sixty days of exposure, twelve animals within each treatment group were anesthetized, had blood samples taken, and were injected with 50µg/ml of cortrosyn (Amphastar Pharmaceuticals Rancho Cucamonga, CA), an active synthetic subunit of ACTH. The other six animals in each group were injected with a 0.9% NaCl solution and served as controls. After ninety minutes, blood samples were collected for assay of serum glucocorticoids. We found that the increase in serum glucocorticoid concentration in fuel oil-exposed mink after injection with ACTH was diminished by 22 % when compared to ACTH-injected control (mineral oil-fed) animals.

Our findings demonstrate that chronic, oral exposure to bunker C fuel oil causes adrenal hypertrophy in mink. The glandular enlargement is most likely an adaptation to adrenal insufficiency caused by exposure to petroleum hydrocarbons. This adaptive change allows the adrenal gland to maintain its basal output of adrenal steroids during non-stimulating conditions. A model explaining our findings is that components of fuel oil act as endocrine disruptors by inhibiting adrenal steroidogenesis. Consequently, the output of glucocorticoids from the adrenal glands is decreased, which leads to a decline in serum concentrations. This releases the hypothalamus and pituitary gland from negative feedback inhibition caused by glucocorticoids. The outcome is an increase in the release of ACTH from the pituitary. ACTH acts as a growth factor and stimulates adrenal hypertrophy. The enlarged gland can now maintain basal concentrations of glucocorticoids in the blood. The results of

the ACTH stimulation test support this hypothesis because inhibition of adrenal steroidogenesis by components of fuel oil would decrease adrenal responsiveness to this model stressor.

Our research suggests that sea otters and other marine foraging mammals living in areas of lingering petroleum hydrocarbon contamination might have compromised stress response systems. If these animals are exposure to additional stresses either from natural causes or in a rehabilitation setting (handling stress, prolonged time in captivity), a reduction in the amount of glucocorticoids released from the adrenal glands may compromise the animal's ability to return to a normal physiological homeostasis. This could affect the success of rehabilitation and also of adaptation to changing or unfavorable conditions in the environment.

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Chemical sedation of the sea otter, *Enhydra lutris*

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Keywords: sedation, anesthesia, sea otter, Enhydra lutris, fentanyl, midazolam

Sea otters hate Mr. Bubbles. Yet, when their pelage is contaminated with oil, detergents are needed to wash off the oil, and chemical sedation is necessary. Sedation of the sea otter is typically employed using one of several combinations of injectable agents, including butorphanol, diazepam, midazolam, fentanyl, and medetomidine, and/or inhalants, such as isoflurane. Each agent or combination of agents is associated with both advantages and disadvantages, depending upon the patient and circumstances surrounding its application.

As in any chemical immobilization procedure, pre-sedation preparation is critically important. Basic emergency and resuscitative drugs and equipment should always be at hand. It is recommended that a prepared table of emergency drug doses based upon drug concentrations and sea otter weight be readily available (Table 1). In addition to the protocols considered "standard" for chemical immobilization, there are some idiosyncrasies of the sea otter which should be recognized.

The exquisitely dense fur of the sea otter is ideal for mitigating the effects of convective and conductive heat loss from animals in the cold waters of the northern Pacific Ocean. The insulating capability of the fur may be problematic when the animal is removed from the water and sedated. Sea otters tend to over-heat relatively easily when denied access to water. Therefore, whenever sea otters are sedated, their body temperature should be monitored closely, typically with a rectal thermometer. Ice packs should be available for application should hyperthermia become problematic.

A second idiosyncrasy of the sea otter involves the relatively caudally situated and surprisingly small orifice of the larynx. As a result, use of a laryngoscope is advised. A selection of endotracheal tubes varying in size from 4.0 mm ID to 7.0 mm ID is generally adequate, should intubation be necessary.

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Table 1

					SEA OTTER WEIGHT (kg)								
DRUG	INDICATION	DOSAGE	STRENGTH	COMMENTS	1	3	5	10	15	20	25	30	35
Activated Charcoal (Toxiban)	Poison injestion	10ml slurry/kg PO slurry= 1g/ 5ml H2O			10ml	30ml	50ml	100ml	150ml	200ml	250ml	300ml	350ml
Aminophylline	Bronchodilator	5mg/kg	25mg/ml		0.2ml	0.6ml	1.0ml	2.0ml	3.0ml	4.0ml	5.0ml	6.0ml	7.0ml
Atropine	bradycardia	0.02mg/kg IV or IM	0.54mg/ml		0.03ml	0.11ml	0.19ml	0.37ml	0.56ml	0.74ml	0.93ml	1.1ml	1.3ml
Calcium Gluconate	Hypocalcemia	94mg/kg IV or IP SLOWLY!	98mg/ml	montior cardiac rate/ rhythm	0.95ml	2.85ml	4.75ml	9.5ml	14.25ml	19.0ml	23.75ml	28.5ml	33.25ml
Dexamethasone SP	shock	5mg/kg IV or IM	4mg/ml		1.25ml	3.75ml	6.25ml	12.5ml	18.75ml	25.0ml	31.25ml	37.5ml	43.75ml
2.5% Dextrose in normal saline	Hypoglycemia, dehydration	20ml/kg SQ- initial dose			20ml	60ml	100ml	200ml	300ml	400ml	500ml	600ml	700ml
50% Dextrose	profound hypoglycemia	0.5- 2.0ml/kg IV or IP slowly	500mg/ml	0.5ml/kg dose	0.5ml	1.5ml	2.5ml	5.0ml	7.5ml	10.0ml	12.5ml	15.0ml	17.5ml
				2.0ml/kg dose	2.0ml	6.0ml	10.0ml	20.0ml	30.0ml	40.0ml	50.0ml	60.0ml	35.0ml
Diazepam (Valium) (controlled drug)	status epilepticus	0.5-1.0mg/kg IV or IM 0.5-2mg/kg per rectum or intranasally	5mg/ml in safe	0.5mg/kg dose	0.1ml	0.3ml	0.5ml	1.0ml	1.5ml	2.0ml	2.5ml	3.0ml	3.5ml
				1.0mg/kg dose	0.2ml	0.6ml	1.0ml	2.0ml	3.0ml	4.0ml	5.0ml	6.0ml	7.0ml
				2.0mg/kg dose	0.4ml	1.2ml	2.0ml	4.0ml	6.0ml	8.0ml	10.0ml	12.0ml	17.0ml
Diphenhydramine (Benedryl)	uticaria, anaphylaxis	2mg/ml IM PRN	50mg/ml		0.04ml	0.12ml	0.2ml	0.4ml	0.6ml	0.8ml	1.0ml	1.2ml	1.4ml
Doxapram (Dopram)	respiratory depression	4mg/kg IV or IM PRN; 1-2 drops under tongue for a newborn	20mg/ml		0.2ml	0.6ml	1.0ml	2.0ml	3.0ml	4.0ml	5.0ml	6.0ml	7.0ml
Epinephrine	cardiac stimulant, anaphylactic shock	0.1mg/kg IV or intratracheally	1mg/ml (1: 1000)	in refrigerator	0.1ml	0.3ml	0.5ml	1.0ml	1.5ml	2.0ml	2.5ml	3.0ml	3.5ml
Furosemide (Lasix)	cardiogenic/ pulmonary edema	4mg/kg IV or IM	50mg/ml		0.08ml	0.24ml	0.4ml	0.8ml	1.2ml	1.6ml	2.0ml	2.4ml	2.8ml
Glycopyrrolate (Robinul)	bradycardia	0.011mg/kg IV or IM	0.2mg/ml		0.05ml	0.165ml	0.27ml	0.55ml	0.825ml	1.1ml	1.3ml	1.6ml	1.9ml
Lidocaine	ventricular arrhythmias	3mg/kg SLOW IV q 10-15min PRN max 8mg/kg over 10min.	20mg/ml	3mg/kg dose	0.15ml	0.45ml	0.75ml	1.5ml	2.25ml	3.0ml	3.75ml	4.5ml	5.25ml
				max dose	0.4ml	1.2ml	2.0ml	4.0ml	6.0ml	8.0ml	10.0ml	12.0ml	14.0ml
Lorazepam (controlled drug)	sedative, seizures	0.04mg/kg IM	2mg/ml	in refrigerator	0.02ml	0.06ml	0.1ml	0.2ml	0.3ml	0.4ml	0.5ml	0.6ml	0.7ml
LRS	Dehydration, shock	66ml/kg SQ p/d PUPS 500-1000ml SQ p/d ADULT											
Mannitol	reduce intracerebral pressures, diuretic	1500mg/kg IV ONCE	200mg/ml	20% solution	7.5ml	22.5ml	37.5ml	75.0ml	112.5ml	150.0ml	187.5ml	225.0ml	262.5ml
Phenobarbital (controlled drug)	status epilepticus	2-4mg/kg IM or IV q30min., NTE 20mg/kg	130mg/ml in safe/ med kit	2mg/kg dose	0.01ml	0.03ml	0.05ml	0.1ml	0.15ml	0.2ml	0.25ml	0.3ml	0.35ml
				4mg/kg dose	0.03ml	0.09ml	0.15ml	0.3ml	0.45ml	0.6ml	0.75ml	0.9ml	1.05ml
				NTE this dose	0.15ml	0.45ml	0.75ml	1.5ml	2.25ml	3.0ml	3.75ml	4.5ml	5.25ml
Prednisolone Sodium	shock	1mg/kg	100mg btl= 10mg/ml		1.1ml	3.3ml	5.5ml	11.0ml	16.5ml	22.0ml	27.5ml	33.0ml	38.5ml
Succinate (Solu- Delta-Cortef)		IV or IM	500mg btl= 50mg/ml										
Sodium Bicarbonate	Metabolic acidosis	1 mEq/kg IV then 0.5mEq/kg q 10-15min during CPR	84mg/ml (1mEq/ml)	initial dose	1.0ml	3.0ml	5.0ml	10.0ml	15.0ml	20.0ml	25.0ml	30.0ml	35.0ml
				0.5mEq dose	0.5ml	1.5ml	2.5ml	5.0ml	7.5ml	10.0ml	12.5ml	15.0ml	17.5ml

Given the thermoregulatory strategy of the sea otter, it is not surprising that there are few superficially located blood vessels capable of supporting an indwelling venous catheter. Additionally, shaving the fur is not recommended, therefore use of water soluble gels with disinfectant are generally used to "part" the fur for vascular access. Catheterization is generally limited to the jugular vein, therefore, emergency supplies should include catheters of at least 2-4" length for use within this vessel.

A number of drug combinations have been suggested for chemical immobilization of sea otters (Table 2) (Haulena, 2001). Rarely is a single agent utilized, and in most cases, an opiate and a sedative are given in combination. Several characteristics of these combinations are shared: they can be administered intramuscularly; the volume administered is relatively small; and specific reversal agents are available. The "reversibility" of the drug is of critical importance, as thermoregulatory concerns mandate that sea otters be returned to the water as soon as possible, and incomplete recovery risks drowning. For that reason, there seems to be little indication for dissociative agents, such as ketamine, despite its wide use in a variety of terrestrial carnivores.

Table 2

Drug	Conc (mg/ml)	Dose (mg/kg)	Reversal	Rev Dose (mg/kg)	Comment
Oxymorphone / Diazepam					
Oxymorphone	1.0 - 1.5	0.3	Naltrexone	2.0 - 6.0	Inadequate sedation alone
Diazepam	5	0.5	n/a	n/a	
Medetomidine / Butorphanol					
Medetomidine	1	0.01 - 0.02	Atipamezole	0.05 - 0.1	Inadequate with excited otter
Butorphanol	10	0.2	Naltrexone	0.4 - 0.8	Startle response preserved
Fentanyl / Diazepam					
Fentanyl	10	0.22 - 0.33	Naltrexone	0.44 - 1.32	Resp depression
Diazepam	5	0.07 - 0.11	n/a	n/a	Absorption ?'s;
Fentanyl / Midazolam					
Fentanyl	10	0.22 - 0.33	Naltrexone	0.44 - 1.32	Resp depression
Midazolam	5	0.07 - 0.11	n/a	n/a	Preferred method
Isoflurane					
Isoflurane	n/a	1.0 - 3.0 %	Off gas		Slow recovery; Precision vaporizer required

Oxymorphone / Diazepam. This combination of a semi-synthetic opiate analgesic and a benzodiazepine does not provide adequate immobilization. While the sea otter is significantly sedated, they remain intolerant of handling, and remain dangerously responsive. Typically, this combination is followed with mask-induction with an inhalant, such as isoflurane. As described in other species, the absorption of diazepam administered intramuscularly is inconsistent and unpredictable, making this combination somewhat inconsistent (Plumb, 2002). And, oxymorphone's commercial availability as an injectable agent is unreliable, and when available, expensive. These disadvantages make this combination somewhat undesirable.

Medetomidine / Butorphanol. This combination of an alpha-2 adrenergic sedative analgesic (medetomidine) and a synthetic opiate partial agonist (butorphanol) has been widely used in a variety of domestic and exotic carnivores (Muir, 1999). This combination has been utilized on approximately fifty occasions in sea otters of a variety of ages, sexes, and physical conditions at the Monterey Bay Aquarium without any significant side effects. The drugs may be administered intramuscularly within a single syringe; they are both readily available and relatively inexpensive; and both agents have reversal agents, atipamezole (medetomidine) and naltrexone (butorphanol).

Several disadvantages to this combination have been observed. A significant bradycardia associated with the alpha-2 agonist is typically noted (Ko, 2000). While this does not appear to have a great deal of clinical significance, this has not been validated in sea otters with subclinical cardiomyopathy. Incomplete reversal and even apparent "re-narcing" have been noted on occasion, making field use of the combination potentially dangerous. When animals are reversed, this phenomenon may occur quite suddenly and without warning, a potentially dangerous situation for the clinical staff. The most common disadvantage of this combination is incomplete sedation and the preservation of a significant "startle" response. Highly agitated sea otters or those that seem to be under the influence of catecholamines, seem not to be adequately sedated, or at best partially sedated. It is likely that this incomplete sedation is the result of a failure to respond to the medetomidine. A recent report in Grevy's zebra suggests that the alpha-2 agonist should be administered approximately 10 minutes before the opiate (Hoyer, 2007). This has not yet been evaluated in sea otters.

Fentanyl / Diazepam. This combination of a powerful opiate agonist and a benzodiazepine has been widely used in sea otters (Monson, 2001). Fentanyl citrate is available as an injectable compound for human use, however its low concentration (0.05 mg/ml) makes its use impractical. The drug is readily available through compounding pharmacies, and is typically prepared at a concentration of 10 mg/ml. Both drugs may be administered intramuscularly at the same time, and the typical time to effect is 8-10 minutes. As previously stated, the inconsistent absorption of diazepam may be problematic, and significant muscle rigidity and even seizures may be noted. Fentanyl may cause significant respiratory depression, therefore supplemental oxygen may be indicated. In the author's experience, supplementation via a face mask is typically adequate. The use of the synthetic opiate antagonist, naltrexone, results in a nearly complete recovery, with minimal observable remnant sedation.

Fentanyl / Midazolam. Replacement of the diazepam with the water soluble benzodiazepine, midazolam, eliminates much of the concern for inconsistent absorption and subsequent expression of the fentanyl side effects of muscle rigidity and seizures. It results in a more smooth induction and recovery and has been reported to be amnesic in humans. While the drug is more expensive than diazepam, its benefits outweigh that negative, and its use is becoming more widespread. The effects of midazolam may be reversed with the benzodiazepine antagonist, flumazenil; however, this is rarely indicated.

Isoflurane. The inhalant general anesthetic, isoflurane, may be utilized as a primary chemical immobilizing agent, or as an adjunct to injectable agents. Induction via face mask in the awake sea otter is potentially dangerous, and should not be recommended. When used as an adjunct to injectable agents, an endotracheal tube should be in place. Typically, vaporizer settings of 1-3% are adequate for anesthesia. The relatively large lung volume of the sea otter (345 ml/kg) results in a comparatively slow recovery from inhalant agents. As a result, vaporizer settings should be reduced to zero several minutes earlier than would be done in a terrestrial carnivore.

Monitoring. Several parameters should be monitored whenever sea otters are chemically immobilized. As suggested earlier, body temperature is a critically important parameter, as the sea otter has limited capacity to eliminate heat when removed from the water. Ice should be applied to flippers, axillae, inguinal areas, and the neck, as indicated, whenever core body temperature exceeds 101 F (38.3 C) (normal = 99.5/37.5 – 100.6/38.1).

Other physiologic parameters, such as heart rate, respiratory rate, ECG, pulse oximetry, and blood pressure should also be monitored. End tidal CO2 is a parameter often evaluated by many of the commercially available anesthetic monitoring devices. Clinical experience with this parameter does not seem to directly follow dog/cat paradigms, and it should therefore be interpreted with caution. Further work is indicated to better validate its use in the sea otter.

Based upon the available anesthetic/sedative agents to date, it seems that the best protocol is a combination of fentanyl and midazolam administered intramuscularly and reversed with naltrexone. Its effects are predictable, reproducible, and safe, and the recovery following reversal is appropriate and adequate for return to the aquatic environment.

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Laparoscopic liver biopsy in the sea otter, *Enhydra lutris*

Michael J. Murray DVM[1]

Keywords: laparoscopy, sea otter, Enhydra lutris, liver biopsy, minimally invasive surgery

Over the past ten years, there has been an explosion in the use of minimally invasive surgical techniques in veterinary medicine. This increase in the application of rigid endoscopic technology has naturally spilled over into a number of aspects of wildlife medicine (Cook, 1999). Methods by which the effects of long term or persistent oil exposure can be documented include measurement of serum and liver levels of cytochrome p450 and evaluation of liver pathology (Ben-David, 2001). Laparoscopic visualization of sea otter livers coupled with minimally invasive methods of targeted liver biopsy have proven to be safe and effective means of sampling livers in this species.

Laparoscopic evaluation and biopsy of the liver in the sea otter has numerous advantages. Collection of diagnostic specimens can be performed through relatively small (< 1 cm) incisions, thereby decreasing the potential for catastrophic post operative dehiscence.

The laparoscopic liver biopsy is minimally invasive; therefore postoperative recovery is dramatically shortened, as is the incidence of postoperative pain. The procedure is relatively short in duration, typically requiring less than 15 minutes. The resultant decreased anesthetic time results in less opportunity for anesthetic-related morbidity, such as hyperthermia, cardiac arrhythmias, etc. Use of rigid endoscopic equipment provides excellent imaging of the surface of the liver; therefore, specific sites of concern may be targeted for biopsy, rather than the potential inaccuracy of non-targeted blind samples, such as those collected with ultrasound-guided biopsy needles (Wang, 2004; Cole, 2002; Cardi, 1997). Rigid endoscopic equipment provides direct focal illumination with magnification facilitating a more thorough examination of the liver. Current technologies permit image collection through one of several media, such as videotape or still photo. As the liver biopsy is performed under visual control, the degree of post-biopsy hemorrhage can be monitored. The specimen size typically collected is small (< 5mm), and hemorrhage associated with the procedure is minimal. And, the

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technologies necessary to perform the procedure are currently available and can be "pulled from the manufacturer's shelf."

The surgical procedure for laparoscopy and subsequent liver biopsy in the sea otter is very similar to that described in companion animals (McCarthy, 2005). Improvements in endoscopic technology now permit use of a single piece of electronic equipment (Medi-Pak, Karl Storz Veterinary Endoscopy, America, Goleta, CA) for the endoscopic light source, camera, and digital imaging. This has facilitated use of this procedure in field settings.

While laparoscopic liver biopsies can be collected by a single endo-surgeon, a surgical assistant is quite useful in instrument handling and support. The actual protocol is as follows:

- The otter is anesthetized appropriately. Current data suggests use of the combination of fentanyl citrate and midazolam.
- The entry points are identified and prepared for entry by parting the pelage with a combination of water soluble lubricating jelly and povidone solution. The first incision site is located approximately 1/4 to 1/3 of the distance caudally between the xiphoid cartilage and the pubis, just to the right of the midline. The second, approximately 2-4 cm cranial to the first and 1/2 way between the ventral midline and the lateral aspect of the ventral abdomen. In this way the more medial incision can be used for the camera insertion first, permitting visualization of the second port placement more cranially and laterally. The second port is utilized for the biopsy forceps. This placement facilitates "triangulation" for adequate exam and sample collection.
- The ventral body wall is draped for surgery.
- The medial, first incision is made approximately 1 cm in length. Using blunt dissection, the subcutaneous body fat is parted and the sheath of the rectus abdominus muscle exposed.
- A Veress needle is then inserted through the incision and through the body wall into the peritoneal cavity. A distinctive "popping" is felt, as the needle violates the peritoneum. Care must be taken to assure the needle enters at a shallow enough angle to assure that viscera are not damaged.
- A "visual space" is created within the abdominal cavity by insufflation with gas. In the clinical setting, an appropriate insufflator with medical grade carbon dioxide is used. Distension pressures not to exceed 10 - 15 mm Hg are recommended.
- In a field setting, ambient air appears to be safe and effective, although, there is a greater potential for the development of gas emboli if excessive pressure is used. With an air hose and modified resuscitation (Ambu) bag, the abdominal cavity is insufflated enough to create adequate visual space for exam and biopsy of the liver. It is important to avoid over-distension of the abdomen. Adequate space is typically acquired when the body cavity has a slightly rounded appearance, but is neither taught nor tympanic.

- The Veress needle is removed and replaced by a 6 mm trocar and cannula. This is gently directed with a controlled force into the distended abdomen again assuring no damage to viscera.
- The trocar is removed and the 5 mm telescope advanced through the cannula. Visualization occurs via the CCD camera attached to the eyepiece of the telescope.
- A second skin incision is then made in the location previously described. As the blunt dissection proceeds, the telescope is directed in a manner to permit location of the second entry site.
- A second trocar/cannula is then introduced under the visual control offered by the camera.
- The trocar is removed and biopsy forceps introduced. There are 2 types of forceps that may be used. The first is a 9 Fr (3mm) elliptical cup forceps. The second a 15 Fr cup biopsy forceps. On average, the weights of liver samples collected are 2.5 mg and 10.0 mg, respectively.
- An inspection of the surfaces of the liver is conducted. Appropriate images are collected. Specific sites of pathology are typically selected for biopsy. If the liver has a homogenous appearance, a site on the margin is selected. The margin is elevated by the opened biopsy forceps to permit visualization of the underside of the liver. Sites with minimal blood supply should be selected. Typically, a series of 3 liver biopsies are collected
- Following biopsy, the sites are observed for evidence of excessive hemorrhage. It should be noted that the magnification afforded by the telescope will make bleeding look more significant than it really is.
- Once the surgeon is convinced there is no evidence of active bleeding, the instrument and telescope are removed. Excess air is manually massaged out of the abdomen through the cannulas.
- The body wall may then be closed with a single cruciate mattress suture using 2-0 polydioxanone (PDS) absorbable suture material. The subcutis and skin are similarly closed.
- The remainder of the external examination and sampling may then proceed. In those cases in which an abdominal VHF radio is to be implanted, the laparoscopic biopsy should occur first, followed by the laparotomy.

While the potential untoward effects of laparoscopy are uncommon, they do exist. The most obvious one is iatrogenic puncture of abdominal viscera. Should this occur, a traditional laparotomy can be performed to repair the damage. An advantage of laparoscopy is the ability to visualize injuries, should they occur.

A second side effect of laparoscopy is air embolism associated with insufflation. This is a theoretical concern, but is highly unlikely. The pressures necessary to perform liver exam and biopsy are not much greater than atmospheric. Air remaining within the peritoneal cavity is no greater than that which remains following a traditional celiotomy. Room air is then absorbed over the following several days. Studies evaluating a series of insufflation gases found that carbon dioxide gas is the safest with minimal potential for embolism (Lacey, 1998).

Excessive hemorrhage is a possibility, but again would occur under visual control. Hemostatic materials such as gelatin sponges (Gel Foam, Pharmacia Corp, Kalamazoo, MI) can be delivered to biopsy sites and used to enhance clotting. Again, a celiotomy may be utilized should hemorrhage be of significant concern.

Entrapment of the endoscope or biopsy forceps within the falciform ligament may occur as a result of excessive fat deposition in the ligament and placement of the initial entry point too close to the midline. While this is certainly an annoyance to the surgeon, it is of no consequence to the patient. The entrapment is corrected by removal of the cannula and replacing it more laterally.

Obviously, the potential negative sequelae are far outweighed by the benefits of laparoscopic liver biopsy. To date, the author has performed over 150 laparoscopic procedures. The only iatrogenic injury was puncture of the urinary bladder, which was repaired uneventfully. Of this study set, several animals have been subjected to multiple liver biopsies in subsequent capture events, and there was no evidence of post biopsy infection, adhesion, or liver pathology.

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European oiled wildlife response planning

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Keywords: Europe, oiled wildlife, response, planning, international

Maritime traffic, including oil transport, is abundant in the coastal waters of Europe. If the Baltic Sea, North Sea, the wider North East Atlantic, the Mediterranean and Black Seas are considered, about 30 European maritime states are potentially at risk of oil spill pollution, and risks are significantly increasing in some areas. Most of these states have an oil spill response plan in place. Few plans however have a dedicated section on how to deal with oiled wildlife should animals like seabirds, marine mammals or sea turtles become affected by a marine spill. It is no surprise that the few available oiled wildlife response plans have been developed in countries in which one or more significant oiled wildlife incidents have taken place.

Within the framework of three projects funded by the European Commission, the shortcomings of European oiled wildlife preparedness have been discussed by delegates from different European countries (see Gasol and Nijkamp, this conference). One of these projects is expected to develop a European Oiled Wildlife Response Plan. This is basically a strategy to create a structurally higher level of preparedness at national levels, but also in the region as a whole. The Plan, which will be published August 2007, is strongly based on the concept of tiered response (see White and White, this conference) and the international sharing of resources such as expertise through training, (see Nijkamp, this conference) and equipment (mobile units). This paper will provide an overview of European oiled wildlife response preparedness, explain the various challenges and opportunities inherent to bringing this preparedness to a higher level, and describe a number of recent developments that demonstrate a burgeoning interest in this matter in an increasing number of countries.

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A training program for oiled wildlife responders

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Keywords: oiled wildlife, training, Europe, tiered response

A country's capability to deal effectively with an oiled wildlife incident is dependent on many factors, but the immediate availability of experienced and trained oiled wildlife responders is key to a successful response, especially in cases when large numbers of live animals arrive on shores.

In an increasing number of European countries, wildlife responders and their government authorities are making assessments of their national response capacities, especially with regards to rehabilitation expertise and facilities. Although many countries do have permanent rehabilitation centres with trained staff, their expertise with regards to the rehabilitation of oiled animals may not be well developed. Increasingly, wildlife responders having little acquaintance with oiled wildlife rehabilitation methodologies are asking and searching for training opportunities.

The cooperation between Sea Alarm and Oil Spill and East Asia Response Limited (OSRL/EARL) aims, amongst other things (see also the paper by R. Holland, this Conference), to increase the international response capacity for oiled wildlife response. Included in this capacity building program is the development of training for so called tier-3 responders - experts that may be sent to assist in an oiled wildlife incident abroad – and the development of preparedness in individual countries.

Together with a number of leading organizations Sea Alarm has started to explore the contents of a responder training program. In a recent meeting, a number of challenges and opportunities have been identified with regards to the concept of an internationally organised tier-3 response service and the kind of special training modules that building such a system would require. Meanwhile, a group of experienced European oiled wildlife responders have been selected, and their collective training has recently started. This pragmatic approach allows useful discussions between responders from different backgrounds and with various levels of expertise on what is good practice and how this can be trained as part of an international program.

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This paper will elaborate on the concept of the tiered response to oiled wildlife (see also White and White, this Conference) and use the example of Europe to explain which kind of trained responder is needed to fulfil the response requirements in each of the response tiers. The results from recent discussions will be presented as well as the agreed steps that will be taken in the near future in the further development of the international training program.

Update on the IFAW Penguin Network: presenting goals and achievements since 2001

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Keywords: penguin, South America, chronic oiling, rehabilitation, protocols, IFAW.

In South America, penguins' feeding and migrating grounds overlap with heavy maritime traffic and oil exploration areas (Garcia-Borboruglu et al., 2006). Oil affecting penguins along the Atlantic coast of South America has been documented since the early seventies (Jehl 1975) and every year, during the austral winter, oiled and debilitated penguins are found on shore along their migration range and are rescued by different rehabilitation organizations in Argentina, Uruguay, Brazil and Chile (Ruoppolo et al. 2005).

Through providing emergency response, management and animal care protocols; and funding in South America during the past six years, the International Fund for Animal Welfare - Emergency Relief Team (IFAW – ER Team), co-managed by International Bird Rescue Research Center (IBRRC) and IFAW, has developed the IFAW Penguin Rehabilitation and Research Network. The team's main purpose is to bring together organizations working with penguins in South America and help them to increase the number of rescued animals as well as standardize rehabilitation protocols and data collection. The overall objective is to use rehabilitation, research, prevention and publicity to bring attention to the plight of these unknown victims of chronic oil pollution and the effects that this type of pollution has on the environment. Through these practices we may be able to achieve our goal of effecting international policy to prevent chronic oiling in the penguins' range.

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For more information on specifics of the project, please refer to Ruoppolo et al. 2005.

Since 2001, eleven institutions have joined the IFAW Penguin Network. These organizations are distributed between Argentina, Brazil, Chile and Uruguay and fall into three primary categories: Rehabilitation Center; Research Center; and Reporting Institution (shown below from North to South [Figure 1]):

Brazil

Rio de Janeiro State:

- GEMM-Lagos/ENSP/FIOCRUZ (Research group);

São Paulo State:

- Aquário de Ubatuba (Reporting institution) – http://www.aquariodeubatuba.com.br;
- Aquário Municipal de Santos (Reporting institution);

Paraná State:

- Centro de Estudos do Mar (CEM), Universidade Federal do Paraná (Rehabilitation center) - http://www.cem.ufpr.br;

Rio Grande do Sul State:

- Centro de Recuperação de Animais Marinhos (CRAM), Museu Oceanográfico Prof. Eliézer de C. Rios, Fundação Universidade Federal do Rio Grande (Rehabilitation center) - http://www.furg.br/museu

Figure 1 – Distribution of the IFAW Penguin Network member institutions along South America

Uruguay

- Protección de Fauna Marina (PROFAUMA) (Rehabilitation center) – http://www.profauma.org
- Sociedad para la Conservación de la Biodiversidad de Maldonado (SO.CO.BIO.MA) (Rehabilitation center)

Argentina

- Fundación Mundo Marino (FMM) (Rehabilitation center) – http://www.fundmundomarino.org.ar

- Fundación Mar del Plata Aquarium (MDP Aq) (Rehabilitation center) - http://www.mdpaquarium.com.ar/fundacion
- Fundación Patagonia Natural (FPN) (Rehabilitation center) - http://www.patagonianatural.org

Chile

- Natura Patagonia (Research group) – http://www.naturapatagonia.cl

Feather sampling and individual pictures of the birds are the major ongoing objectives of the participant institutions. The goal is to build a databank of evidence that can be used in future oil fingerprinting efforts. This information can t hen be used to determine the source of the oil affecting the animals on a yearly basis (Figures 2 and 3).

Figures 2, and 3 – Examples of the IFAW Penguin Network image databank. Credits: IFAW PN – FMM and CRAM

In 2006, the IFAW Penguin Band program was developed to be able to identify penguins rehabilitated and released by members of the Network; which is a step forward in the development of our own post-release monitoring program. These bands were first used after the Cabo Vírgenes event in Argentina and Chile, and are now being used by Fundación Mundo Marino as well. More institutions will use the bands in the near future. More information can be found at www.ifaw.org/penguinband.

The number of birds rehabilitated by the IFAW Penguin Network from 2001 to 2006, including IFAW emergency responses with penguins in South America, can be seen in Table 1.

Table 1 – Numbers of penguins rehabilitated by some of the IFAW Penguin Network member institutions and IFAW Emergency responses involving penguins.

IFAW 2001-2006

Institution	2001	2002	2003	2004	2005	2006	TOTAL
CRAM (Brazil)	0% (0/2)	81.7% (94/115)	88.8% (16/18)	70% (7/10)	82.3% (14/17)	66.6 % (30/45)*	77.7% (161/207)
ZooNit birds sent to CRAM (Brazil)	xx	xx	xx	xx	xx	66.0% (33/50)*	66.0% (33/50)
IFAW ER Response	Uruguay 91.4% (64/70)	Uruguay 93.3% (126/135)	xx	xx	xx	Cabo Vírgenes spill 65.1% (146/224)	78.3% (336/429)
FMM (Argentina)	88.4% (192/217)	94% (128/136)	100% (17/17)	90.9% (20/22)	93.7% (45/48)	94.6% (211/223)	92.4%% (613/663)
MdP Aquarium (Argentina)	61.7% (165/267)	79.1% (91/115)	80% (16/20)	69% (38/55)	76.9% (40/52)	37% (84/227)*	58.9% (434/736)
Natura Patagonia (Chile)	xx	xx	xx	Berge Nice spill 92.8% (13/14)	xx	Mystery spill, Punta Arenas 71% (54/76)	74.4% (67/90)
Total % released	75.7% (421/556)	87.6% (439/501)	89% (49/55)	77.2% (78/101)	84.6% (99/117)	66% (558/845)	75.5% (1644/2175)

*Includes oiled and un-oiled birds treated

Most of the institutions rehabilitating penguins as part of this Network also rescue other kinds of marine animals. One of the Network's goals is to continue helping these groups to further develop their protocols for other species. In this last year we have grown from IFAW Penguin Network to truly embody all of the IFAW Emergency Relief.

Acknowledgments

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Case study of the Eider spill, Antofagasta, Chile

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Keywords: case report, pelican, Chile, South America, desert, ship grounding, Eider spill, IFAW.

Introduction

On November 1st, 2005, a Hong Kong-flagged cargo ship ran aground off the northern coast of Chile near the city of Antofagasta. Around 7 km of coastline was oiled with bunker fuel IFO 180 (Intermediate Fuel Oil).

The northern coast of Chile is rich in marine fauna where many species of seabirds are found such as the vulnerable Humboldt penguin, Peruvian pelican and different species of boobies, cormorants and gulls. Marine mammals range from sea otters, sea lions and fur seals to many species of whales and dolphins. The presence of so many birds and mammals is directly related to the amount of fish available on the Chilean coast, one of the most important fishing grounds, worldwide.

At the request of Dr. Carlos Guerra, Director of the Wildlife Rescue Center at the University of Antofagasta (Centro de Rescate y Rehabilitación de Fauna Silvestre - CRRFS http://www.uantof.cl/rescate/), the IFAW ER Team – Oiled Wildlife Division, assisted with the wildlife rescue efforts.

Case Report

Arriving on November 10th, the IFAW ER Team assisted CRRFS staff in managing all aspects of the wildlife response. IFAW worked along with CRRFS from November 10th until the 26th, when most birds were released.

When IFAW arrived there were 13 oiled Peruvian pelicans (Pelecanus thagus), 1 oiled Humboldt penguin (Spheniscus humboldti) and two gulls (Larus pipixcan) in care. The Eider oil spill

1 International Fund for Animal Welfare (IFAW), Emergency Relief Team - www.ifaw.org

2 International Bird Rescue Research Center - www.ibrrc.org

3 Centro de Recuperação de Animais Marinhos - www.furg.br/museu

4 Fundación Mundo Marino - www.fundmundomarino.org.ar

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clean-up operations were overseen by the P&I Club in Chile, Cave & Cia (www.cave.cl), who hired Litoral and were advised by the International Tanker Owners Pollution Federation Limited (ITOPF – www.itopf.com).

After dedicating the first day on the ground to finding the best place available to care for the affected birds, it was decided that CRRFS would be adapted. Litoral was asked to supply the necessary supplies for setting up the water system and adapting the aviary to be used for pelicans. IFAW purchased any equipment that would remain at CRRFS after the emergency response, which included 3 pools, 3 large plastic flight kennels, and supplies to build temporary cages and cover the aviary.

Four temporary cages and perches were built to house oiled pelicans. Plywood and shade cloth were fixed around a pre-existing metal structure with a roof. Each cage measured approximately 6.5m2 and held 5 birds at a time.

Waterproofing aquatic birds requires large quantities of water and Antofagasta is on the Atacama Desert, therefore limited water is available. A water system was developed for washing birds that included a total of 3,000 liters of clean water, which was contained outside the washroom designated for washing and for rinsing of oiled birds. The water was pumped from the containers into the washroom through the use of 1 Hp pressure pumps. Inside, 200L containers were used to store hot water for filling the tubs to wash the birds. The wastewater was kept in 200L containers and pumped out of the washroom whenever necessary to be stored in containers outside. When the response was over Litoral removed the contaminated waste water for appropriate disposal. The clean water containers were filled whenever necessary by a water-truck, sent in by Litoral. Around 500L of water were used to wash and rinse each of the pelicans.

A pre-existing aviary used for raptors was adapted for waterproofing and reconditioning clean pelicans. The aviary measured approximately 12m x 4m and was divided into two areas with shade cloth. Two pools were added so the aviary could comfortably house 25 pelicans. The pool dimensions were 4.5m x 2.2m x 0.85m (14.8' x 7.2' x 2.8') and these were filled by the water-truck with 7,570L (2,000 gal) each. To control overflow, with the aim of keeping the surface water moving to remove feces and fish oil, a water-meter and hose were connected to the main water container that supplied the University. The P&I Club agreed to pay for the water expenses. The flooring in the aviary was covered with gravel to absorb the pool overflow. The outside of the cage was covered with shade cloth to provide a visual barrier and prevent birds from trying to fly out. It also provided shade and reduced the indoor temperature.

The facility set up was developed to care for 50 birds, but could have been easily expanded (Figure 1).

<<Figure 1 HERE>>

Figure 1- CRRFS's facilities adapted for treating oiled wildlife, in Antofagasta. Photo: IFAW – S. R. Heredia

While facilities were being set up, and birds in care were being stabilized, search and collection began, with teams focusing on the fish market (Caleta de Pescadores). It was clear from the early days of the spill that pelicans were the species most affected. Each day the pelicans gathered at the fish market as the fishermen came in to clean their catch. During the hours of 8 am to 10 am, the pelicans would congregate in the area. This allowed the IFAW ER Team and CRRFS volunteers and staff to look for oiled pelicans and try and capture them. Since most of the pelicans were habituated and often were given handouts at the fish market, the main method utilized for capturing the oiled pelicans was by baiting the birds around the fishermen and individually capturing the oiled ones by hand or using dip-nets. Some birds were also captured by the fishermen and kept in a cage until we were called to pick them up.

From November 2nd to November 21st a total of 24 Peruvian pelicans; 3 Franklin's gulls and 1 Humboldt penguin were treated. For more information on numbers and species of animals treated, please review Table 1.

Individual examinations were performed and intake records were created for all birds in care on November 12th. Only the cases related to the oil spill were recorded on the Intake Log. The normal influx of animals at the rehabilitation center (e.g. fractured wings, emaciation etc.) were NOT recorded on the IFAW Intake Log.

Intake procedures were based on IBRRC protocols and included:

- Registering capture information on the Live Intake Log;
- Individual identification with temporary colored bands;
- Creating an individual animal care record;
- Physical exam, including body weight, temperature and condition; checking for wounds, burns and injuries; lung auscultation and degree of oiling;
- Blood sampling to evaluate Packed Cell Volume (PCV), Buffy Coat (BC) and Total Protein (TP).

- Oral administration of a re-hydrating solution (NaCl 0.9% or bottled water).

Upon the IFAW Team's arrival, the pelicans already in care were diagnosed as being dehydrated, so in the first days the Team's efforts were concentrated on correcting this condition and stimulating self-feeding. Changes of the husbandry routine included gavage tubing twice a day and intravenous fluids to the worst cases. Serving fish in shallow tubs with water also stimulated the birds to re-hydrate themselves. While in care, the pelicans were fed between two and three times daily in water bowls and by throwing fish for the birds that would feed in that manner.

As soon as the temporary cages were ready, all the pelicans were moved outside on November 14th. Once outside, the birds were divided into smaller groups, which presented less competition for shier birds and better individual performance. The outside cages provided better ventilation and allowed the birds' feathers to dry unlike indoors, where plastic flooring would certainly cause damage due to excessive moisture. Two pelicans were diagnosed with fractured wings and several had swollen leg joints due to administration of sub-cutaneous fluids prior to IFAW's arrival.

Other species such as gulls and penguins were housed at the multi-purpose cages near the food preparation area. Two gulls were treated for probable intoxication due to the dispersant utilized during the environmental clean-up. Except for the third gull, these birds didn't have oil on their feathers.

A pre-wash evaluation was performed on every bird and included a physical examination, body weight and a blood sample. Birds were approved for washing when they reached the following criteria: normal body temperature (≥39˚C), PCV >30%, BC <2%, TP ≥3g/dl, fair body condition, no wounds and normal behavior.

Washing and rinsing took place in the area set up for this purpose (please see Figure 1). IBRRC protocols were again followed for washing, rinsing and drying.

The animals were cleaned in a 1-2% solution of Dawn® dishwashing detergent in warm water (39-41ºC / 106-108ºF), until no traces of oil could be found. In most cases it took between 20 and 30 minutes to wash each bird. Wastewater was kept in barrels and pumped out of the washroom as necessary. The oily/soapy water was then stored in large water containers outside and removed by Litoral, once the response was over, to be treated off-site.

Birds were then meticulously rinsed with warm water at an appropriate pressure through a special nozzle, until all the detergent could be removed. The rinsing process took around another 30 to 45 minutes. Once rinsed, the birds were placed in a drying cage, where pet dryers were set up for blowing warm air towards the birds' perches.

Temporary band numbers were recorded as the birds were directed to the drying cage. There, each bird received an intramuscular injection of vitamin B (20mL/kg), to prevent cases of hypovitaminosis, due to their time in captivity. All birds received 120mL of oral fluids an hour before the wash and before entering the drying cage.

Figure 2- IFAW Team members demonstrating to CRRFS staff how to wash an oiled pelican. Photo: IFAW – V. Ruoppolo

Once dry, the birds were moved to the outside pens, with pools and surface water overflow. All birds received 180mL of oral fluids before entering the aviary cage. Once outside, the pelicans were still fed two to three times daily in water bowls and monitored at all times to ensure they were feeding, preening and behaving normally. During this phase, stress and handling were kept to a minimum, allowing the birds to feed and swim as much as possible.

Pre-release evaluations were performed on 22 pelicans and 2 gulls on November 25th, when 21 pelicans and 2 gulls were approved, according to the following release criteria:

Peruvian pelicans and Humboldt penguins:

PCV ≥ 38 % BC < 2% TP ≥ 3.0 g/dl

Feathers waterproof

Body Condition: Fair to good

Behavior: Normal and feeding well

Lungs clear

Other non-diving birds: gulls

PCV ≥ 32 % BC < 2% TP ≥ 3.0 g/dl

Feathers waterproof

Figures 3 and 4- 21 Peruvian pelicans were released at Islote Lagarto on November 26th, 2005. Photos: IFAW – J. M. Barredo

Body Condition: Fair to good

Behavior: Normal and feeding well

Lungs clear

The 21 pelicans approved were released on November 26th at Islote Lagarto, Mejillones (S23°03' – W70°27') (Figures 4 and 5). The remaining pelican was released on December 3rd by CRRFS staff. The 2 gulls were released on November 27th in Coloso, South of the University's Campus. The Humboldt penguin was released sometime in December 2005, also by CRRFS staff.

The 2 pelicans with fractured wings remained at CRRFS after the IFAW Team demobilized. One was euthanized in December, due to a fracture that could not be resolved, and the second one was determined unsuitable for release and was kept permanently at CRRFS.

Discussion

The IFAW ER Team remained in Antofagasta from November 10th through the 26th, 2005 - when most birds were completely rehabilitated and released. Rehabilitation percentage was 92.8% (26/28), including the bird permanently kept at CRRFS. The overall results, recorded by the IFAW ER Team while on the ground and through post-spill contacts with CRRFS' veterinarian, Dr. Ariel Cortez, can be seen in detail on Table 1.

Table 1. Final results presented by species treated

Species	# Treated	# Released	Captive	Died
Peruvian pelican *(Pelecanus thagus)*	24	22	1	1
Franklin's gull *(Larus pipixcan)*	3	2	0	1
Humboldt penguin *(Spheniscus humboldti)*	1	1	0	0
TOTAL	28	25		
(89.2%)	1	2		

Numerous factors influence the final results of every oiled wildlife response and some strengths and challenges related to the Eider spill are outlined below:

Strengths

1) Having a Spanish-speaking and Latin American team experienced in oiled wildlife rehabilitation and emergency response afforded a certain level of acceptance and trust as well as allowed the IFAW Team a clearer appreciation of the situation.
2) IFAW's ability to liaise directly with the P&I Club and ITOPF ensured their full support of our Team, the animal rehabilitation operation, and all work associated with the wildlife response.

3) Water availability and the ability to setup a water system for washing and waterproofing birds in the desert. The P&I Club was crucial in this matter, as they worked fast to get the equipment we required, gave us all the water we asked for in water trucks, and quickly agreed to pay for the water meter bill at the University.
4) Species affected: pelicans are fairly hardy birds and easier to care for than some seabirds.
5) The birds had a quick turn around once the water system was in place.
6) Good weather conditions and high temperatures.
7) Birds being fed fish scraps at the fish market preserved the birds' body condition for a longer period of time, thus the birds were in decent body condition upon admission.
8) Assistance of local veterinarians' expertise and equipment (pet driers and the blood centrifuge).
9) Ability to be in constant contact with team members not on the ground (Jay Holcomb, Barbara Callahan and Senta da Conceição) to discuss issues on bird rehabilitation, logistical support, politics and moral support.
10) Having financial support readily available, remotely, from IFAW ER and Finance was crucial to start setting up the facilities to care for the animals appropriately.

Challenges

1) While CRRFS was proficient at wildlife rehabilitation, lack of experience in dealing with oiled wildlife and a large number of animals at one time presented challenges, which took additional time to work out in the face of an emergent situation.
2) Late arrival on site. This is frequently an issue in responses where there are local individuals who routinely care for injured wildlife, as trustees and or Responsible Parties assume the local group can care for oiled wildlife and manage an oiled wildlife response. Once it becomes evident that the animals die very quickly without very specific care, our team is called in. Through our continued good relationships with the P & I Clubs and ITOPF we hope to be called into events such as the Eider spill at the earliest possible time.
3) Inability to release the birds at the earliest possible time. Due to CRRFS demand the birds were held after being approved for release for up to a week, which gave us great concern for their welfare. IBRRC/IFAW ER protocols ensure release at the earliest possible time to prevent undue stress to the animals, risking feather waterproofing and health problems. All birds survived the extra week without presenting any captive care problems.
4) The IFAW ER Team did not have access to data on dead bird numbers prior to their arrival on-site. Documentation on oiled and non-oiled birds was unclear, as all birds admitted during the response were identified as being affected by the spill, even when they were not.
5) Having volunteers exclusively from the University made it difficult to organize and schedule the daily activities for the animals as the students still had class schedules to keep, which prevented them from being available to the rehabilitation staff. Consequently, volunteers were not available for an early start of crucial activities like bird washing.

Acknowledgments

We wish to thank Dr. Carlos Guerra for inviting the IFAW ER Team to work in Antofagasta and for allowing the development of activities at CRRFS. We are grateful for the support of CRRFS staff and volunteers, the University of Antofagasta and Servicio Agrícola y Ganadero. Dr. Ariel Cortez was always ready to help; his support was crucial for finding and lending essential equipment to work with the animals. We thank Cave & Cia., Litoral and ITOPF for their receptiveness and for promptly attending the animal's needs during our stay in Antofagasta. Without everyone's commitment and support this response would not have been so successful.

Net environmental benefit analysis (NEBA) of dispersed oil versus non-dispersed oil on coastal ecosystems & wildlife utilizing data derived from the 20-year TROPICS study

Paul A. Schuler[1] and Bart Baca[2]

Introduction

It is axiomatic that the best approach to oil spill response is prevention. This axiom applies as well to the effects of oil after a spill, as plans and response strategies and methods under consideration will determine the extent to which responders can prevent (or minimize) spilled oil from impacting sensitive ecosystems and wildlife. Advocates of the use of dispersants have traditionally viewed the application of dispersants as a means of minimizing environmental damage through the construct of Net Environmental Benefit Analysis (NEBA), trading off lower value resources in favor of those identified as higher value.

This paper draws on empirical data and observations collected over 20 years of the Tropical Oil Pollution Investigations in Coastal Systems (TROPICS) field study in Bahia de Almirante, Panama. The study began in November 1984, when non-treated and dispersed Alaska North Slope (ANS) crude oil were intentionally released into two separate sites representative of mangrove, seagrass and coral ecosystems. Data on the relative physical presence and biological effects of non-treated and dispersed oil in these ecosystems (as well as a third reference site) were acquired and analyzed over various periods (30 days, 20 months, and 3, 10, 17, 18 and 20 years). The short and long-term physical and biological effects of non-treated and dispersed oil are summarized in numerous reports and proceedings (e.g., RPI, 1987; Dodge et al., 1995, Ward et al., 2003, and Baca et al., 2005). The primary goal of this study is to examine the Net Environmental Benefit Analysis (NEBA) for the use or non-use of dispersants in nearshore tropical ecosystems.

1 Clean Caribbean & Americas, Inc., Ft. Lauderdale, Florida

2 CSA South, Inc., Dania Beach, Florida

Twenty-year observations and mangrove substrate core samples reveal the continued presence of oil and diminished mangrove repopulation, as well as substrate erosion, at the non-treated oil site. There was no oil detected and no long-term impacts observed at the dispersed oil and reference sites (Baca et al., 2005). Associated coral reef and seagrass communities were also more adversely affected in the long-term than at the non-dispersed crude oil site. The observed difference in the presence of oil and its long-term effects can be attributed to well-known and understood mechanisms associated with the use of dispersant:

1. The surfactant in dispersants facilitates the formation of stabilized oil droplets that do not as readily adhere as non-dispersed oil to impacted surfaces, such as non-organic and organic substrate, mangrove roots, seagrass blades, sediments, rocks, etc. This allowed the dispersed oil to be flushed from the study site with diurnal tidal floods. The resident time of oil and consequent exposure time of flora and fauna to the oil at the dispersed oil site was therefore significantly shorter than at the non-dispersed oil site. At the non-treated oil site, oil adhered to mangrove roots and became trapped in subsurface crab holes up to 30 cm into the mangrove substrate. Evidence of oil oozing from the substrate could be visually observed 17 and 18 years after the intentional release of oil. The persistence of the non-dispersed oil represents longer exposure time for flora and fauna at the non-treated oil site.
2. At the dispersed oil site, the mixing of the dispersed oil droplets in the water column presented an overall lower concentration of total hydrocarbon exposure to the flora and fauna due to physical dilution. At the non-treated oil site, aside from soluble fragments, the non-dispersed crude oil that impacted flora and fauna was at full concentration. This oil coated mangrove roots at high tide and mangrove substrate and some seagrass at low tide.

TROPICS Methodology & Results

It is important to remember that this spill was a worst case scenario, releasing 4.5-6 barrels of oil into a 900m2, shallow ecosystem. Dispersed crude oil was pre-mixed with Corexit® 9527 in drums and 4.5 barrels of this mix were released by multiple hoses into a boomed, 900m2 site on November 28-29, 1984. A total of 6 barrels of crude oil were likewise applied via multiple hoses to a very similar boomed site on December 1-2, 1984. Chemical analyses of water, sediment, and organism tissues began with pre-spill baseline studies and was ongoing for 20 months after the spills.

In the spring of 2004 mangrove sediment samples at the non-dispersed site showed a petroleum hydrocarbon high of 22ppm; however, this number was much reduced in fall samples. A summary of fall chemistry results is given in Table 1. The 20-year results for the fall samples were provided by U.S. National Oceanic and Atmospheric Administration from the laboratory at Louisiana State University. Samples were collected at various depths (0-17 cm) in the mangrove substrate and results were separated into alkanes and aromatics. Total petroleum hydrocarbon (TPH) is a combination of the two. Data were highly variable due to the patchiness of the oil distribution. Importantly for year

20, aromatics represented only 1.5% of total hydrocarbons. Hydrocarbons were at insignificant levels at all three sites by the end of the 20th year from the date of the spills.

Table 1. Total Petroleum Hydrocarbons (TPH) from Sediment Collections at the TROPICS Sites

Sample Dates	Non-Treated ppm	Dispersed Oil ppm	Reference Site ppm
Pre-dosing (11/84)	1.44+0.51	2.04+0.53	0.97+0.39
1 Year (12/85)	552+713	125+66	Not Analyzed
10 Years (94-95)	19.4+27.5	30.8+36.9	4.1+1.0
20 Years (9/04)	2.00+0.93	1.04+0.14	1.57+0.24

A summary of mangrove results is given in Table 2. Much more data on tree parameters were collected but tree and seedling counts were indicative of the overall trends in effects. Mangroves suffered a serious and unexpected die-off by year 10. At the end of the first phase of the study, researchers assumed that mortalities had stabilized and the sites were essentially ignored for 7 years during which time mortality increased at the non-treated oil site from 17% to 46%. This mortality resulted in a large void and erosional depression in the center of site. This void became a magnet for seedlings to establish, and in the 20th year these seedlings had grown large enough to be classified as trees (i.e., >2 meters tall), effectively re-populating the site. Today they form a dense thicket in the center of the mangrove section of the non-treated oil site. However, in the last five or so years a second mangrove die-off began occurring offsite north of the non-treated oil site and appears to be spreading to that portion of the island. An offsite area similar in size to the original die-off zone now exists north of the site. In stark contrast to non-treated oil results, tree counts and condition at the dispersed oil site remained constant, although seedlings experienced a reduction which rebounded in 20 years. The Reference Site experienced a 24% reduction in trees and a 31% increase in seedlings by year 20.

Table 2. Summary of mangrove counts between treatments

Numbers represent percent of original population; original population size is given in brackets. Notable effects were detected at the non-treated oil site: significant declines in trees and increases in seedlings (later to become trees) were seen by year 10.

Sample Dates	Parameter	Non-Treated	Dispersed Oil	Reference Site
Pre-dose (84)	Mature Trees	100 (149)	100 (72)	100 (108)
	Seedlings	100 (13)	100 (33)	100 (26)
1 Year (85)	Mature Trees	83	100	100
	Seedlings	100	100	100
10 Years (94)	Mature Trees	54	97	100
	Seedlings	685	58	81
20 Years (04)	Mature Trees	98	94	76
	Seedlings	838	100	112

A summary of seagrass results is given in Table 3. Turtle grass, *Thalassia testudinum,* was the only species encountered at the sites. At the start of the study turtle grass occupied dense, wide beds seaward of the mangrove zone. By the end of the 20-year study seagrass had virtually disappeared from the non-treated oil site, being replaced by finger coral, *Porites porites.* Seagrass density increased after one year but also continued to decrease after that. However, the reference site also experienced decreases during that time.

Table 3. Summary of seagrass results during the first 20 years of the TROPICS project.

Numbers represent percent of original measures; original population size (per m2) and growth rates (cm/da) are given in brackets.

Sample Dates	Parameter	Non-Treated	Dispersed Oil	Reference Site
Pre-dose (84)	Density	100 (356)	100 (423)	100 (379)
	Growth rate	100 (0.49)	100	100
1 Year (85)	Density	105	135	113
	Growth rate	90	111	115
10 Years (94)	Density	73	83	86
	Growth rate	111	106	107
20 Years (04)	Density	38	66	113
	Growth rate	89	111	87

A summary of coral results is given in Table 4. Only coral cover is presented because this provides a good representation of overall trends. As shown, percent coral cover increased across all sites, significantly at the non-treated oil site. This increase was due to the increase of *Porites porites* into the seagrass bed at the non-treated oil site whereas coral cover increased primarily on the reef edge at the dispersed oil site. Further investigations were conducted on the spread of Porites coral into the seagrass bed at the non-treated oil site as reported in Ward et al. (2003). A study of other locations on the treatment island revealed that no other location existed where 30m of intertidal seagrass bed was so densely populated by *Porites*.

Table 4. Summary of coral cover results during the first 18 years of the TROPICS project.

Numbers represent percent of original measures; original percent cover data are given in brackets.

Sample Dates	Parameter	Non-Treated	Dispersed Oil	Reference Site
Pre-dose (84)	Total Coral Cover	100 (27.6)	100 (21.6)	100 (15.3)
1 Year (85)	Total Coral Cover	84	75	92
10 Years (94)	Total Coral Cover	129	124	84
18 Years (02)	Total Coral Cover	244	211	110

Dispersant Mechanisms

Spilled crude oil degrades in a typical fashion through a series of physical and chemical steps, depending on type and composition of the spilled oil, weather conditions, evaporation, photo-oxidation, sediment characteristics, and biodegradation (Munoz et al., 1997). In general the lightest hydrocarbons degrade quickest, and among compound classes alkanes degrade quickest followed by naphthenes and lastly aromatics (Andrett et al., 1997). Dispersants generally enhance these processes when applied correctly, although some preferential degradation occurs (Lindstrom and Braddock, 2002). Dispersants in the laboratory have produced a 50-fold increase in solubilized hydrocarbons in the water column (Baca and Getter, 1984). Dispersant at the TROPICS site was pre-mixed in drumss containing crude oil prior to release throughout the water column. The dispersant was observed to form a cloud of the oft-reported discreet, stabilized droplets that do not adhere as readily as non-dispersed oil to surfaces such as organic substrate, roots, sands, rocks, etc. They penetrated the substrate but were more easily removed and degraded. They also mimicked food particle sizes and thus were ingested by invertebrates which in turn suffered high mortality. However, the short-term loss of invertebrates was compensated for by rapid re-colonization. Importantly, the ecological home for these organisms, the mangrove and seagrass ecosystem, remained intact at the dispersed oil site. Being filter-feeding invertebrates, the corals also suffered short-term impacts, but then rebounded to high coral cover.

Application for other Habitats and Wildlife

The study and the dispersant mechanisms have application to other climate zones, ecological communities and their attendant flora and fauna. In fact, due to a large number of sensitivities, it is unlikely that field studies would ever be envisioned that intentionally oil higher order fauna, such as seabirds and marine mammals. Therefore, interpolation from this and other existing studies, while imperfect, may be the only limited scientific inputs available for NEBA for decision-making for the use of dispersants. For example, In the 1997 "San Jorge" spill in Uruguay, dispersants were used to prevent the impact of approximately 2,000 tonnes of spilled Canadon Seco crude oil on an extensive population (200,000-300,000) of sea lions at Isla de Lobos Wildlife Reserve. A number of newborn sea lions were oiled by the spill and at least 200 of these may have died as a direct result of the spill. There are no clear figures, either, on the number of sea lions that died as a direct result of the spill nor the number that may have been saved by the use of dispersants.

The TROPICS sites were habitats for a variety of wildlife which feed on the invertebrates and juvenile fish that dwell among the protective mangrove roots including numerous wading birds, diving birds, and waterfowl. Migratory songbirds were also common. These organisms are not permanently affected by short-term food base loss, but they are affected by loss of tropical ecosystems. The dispersant mechanism observed at the TROPICS site and elsewhere has application in other marine

environments for preventing/minimizing the impact of oil spills on seabirds and marine mammals, including reduced adherence of dispersed oil on feathers, fur, and nesting areas and beaches.

NEBA and Conclusions

The goal of this paper is to use these data to determine a Net Environmental Benefit Analysis (NEBA) for the use or non-use of dispersants in nearshore tropical ecosystems. The first step is to define mangrove, seagrass and coral habitat parameters, as follows:

Habitat (Refer back to Tables 1-4)	Component	Parameters available	Parameters used herein
Sediment	Hydrocarbons	1	TPH
Mangrove	Trees Seedlings	14	# Live # Live
Seagrass	Seagrass Seagrass	4	Density Growth
Coral	Coral	4	% Cover

Next, an assessment can be made of whether the 10-year data indicate a positive or negative benefit for dispersant use. A resulting summary is as follows:

Habitat (Refer back to Tables 1-4)	Parameter	NEB?
Sediment	Hydrocarbons	No
Mangrove	# Live Trees Seedlings	Yes No
Seagrass	Density Growth	Yes Yes
Coral	% Cover	Yes

Hydrocarbon levels were still high at the dispersed oil site so the NEB was negative. Clearly, the benefit for mangroves at the dispersed oil site is positive, although seedlings suffered a decline but returned later. The NEBA is moderately positive for seagrass and corals also, and coral cover increased over reference in both treatments.

Ultimately 20-year data would be evaluated as follows:

Habitat (Refer back to Tables 1-4)	Parameter	NEB?
Sediment	Hydrocarbons	Yes
Mangrove (7)	# Live Seedlings	Yes Yes
Seagrass (3)	Density Growth	? Yes
Coral (4)	% Cover	?

In this case dispersed oil was beneficial for hydrocarbon content over the long term. Likewise the benefit of dispersant use to mangroves is clearly shown. Seagrass density continued to decline such that further studies or additional parameters should be looked at for a final NEBA. However, seagrass density was still nearly twice as high at the dispersed oil site than at the non-treated oil site and seagrass growth rates have remained higher than baseline at the dispersed oil site. Likewise for corals further studies or additional parameters should be looked at for a NEBA although the increase in corals at the dispersed site did not result in an invasion of the seagrass bed, nor was it as high as at the non-treated oil site.

Weighting of Resources

The NEBA analysis did not weigh any of the resources studied over others in the same community. Such a weighting would be relative sensitivity, ability to recover, place in the food chain or role in the ecosystem, and ability to repopulate. Relative value and weighting of respective resources at risk.

The yes/no answers should attempt to use quantitative, statistically validated data for proper assignation. Also, when more parameters are added for a NEBA then the value of each parameter can be taken into account to fine tune the answers. Overall, nearshore dispersant use at the TROPICS site continues to show benefits for mangroves, and to a lesser but positive extent, for seagrass.

The TROPICS sites continue to be active locations for research as many questions remain unanswered, such as:

- What are the causes of the takeover of the non-dispersed oil site by finger coral?
- What is the extent of offsite contamination and damages at the non-dispersed oil site?
- Will the emerging forest on the non-dispersed oil site become productive and permanent?
- When will the decline of seagrasses at both treatment sites stabilize?
- What is happening to the invertebrate fauna at the treatment sites in the twelve years since they were last surveyed?

Scientific Design Issues

There is a lack of publications on this study in peer-reviewed literature, primarily due to the fact that the study sites were non-replicated. Hurlbert published on the error of pseudoreplication in 1984 and this started a reaction where all non-replicated studies, regardless of scale, were disdained. Also followed were back-and-forth arguments about the value of large-scale, non-replicated studies (Oksanen 2001 and others). During the early years of planning the TROPICS study (1982-1983) it was determined that large, controlled-spill sites (>900m2) were advantageous over laboratory or microcosm studies. Smaller, replicated studies had been performed but these were criticized for not representing real world conditions, a criticism still levied today (e.g. Schindler, 1998). In addition, finding a location where one can intentionally release crude oil and/or dispersed oil on a large site

containing mangroves, seagrass and coral is nearly impossible (even 20 years ago), and replication of these large spills was out of the question. Research such as the TROPICS experiment needed to be done because oil spill studies continue to rely on post-hoc data with little or no pre-spill information. There remains a need for adequate pre-spill, baseline data as well as pre-measured and metered dosing, to accurately determine the fate and effects of oil spills and to evaluate treatments options. Opportunities exist for research at the Reference Site, especially since replicate reference sites exist on that island for future use.

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Development and testing of a subcutaneous radio transmitter for sea otters

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Keywords: sea otter,* Enhydra lutris, *subcutaneous, radio telemetry, VHF transmitter

Introduction

The VHF radio transmitters implanted by scientists into the intraperitoneal cavity of sea otters *(Enhydra lutris)* have not undergone significant changes in size or technology for more than 20 years (Williams & Siniff, 1983). Although previous investigators have tried alternative tagging systems—subcutaneous implants (Garshelis & Siniff, 1983), flipper-mounted transmitters (Hatfield & Rathbun, 1996), and external attachments (Garshelis & Siniff, 1983)—all of these systems proved faulty or detrimental to the sea otters. Unfortunately, although the surgical technique for installing abdominal implants, though fairly invasive, has proven uncomplicated and safe throughout hundreds of procedures, and the abdominal implants have yielded satisfactory telemetry results for research purposes, the cost of these units has remained high when used to monitor rehabilitated sea otters, and the associated costs to implant and track the progress of large numbers of otters—for instance, following a major oil spill—may preclude effective post-release monitoring (Monnett et al., 2000; Johnson et al., 2002). Ultimately, investigators agreed that a smaller, less-expensive, less-invasive transmitter package that delivers improved telemetry results would enhance the capability of researchers to monitor sea otters following rehabilitation. Therefore, the objective of this project involved working with engineers to design and test a smaller, less-invasive, subcutaneous VHF radio transmitter in

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live-stranded animals slated for release to the wild. Based on project discussions, the subcutaneous transmitter package had to equal or exceed the performance and reliability of existing transmitters; operate on battery power for at least two years; produce a detectable signal over at least an eight-kilometer range from the air; fit into a smaller package (e.g., < 200 g) to facilitate subcutaneous installation; and cost less than the standard transmitters.

Materials and Methods

Initial work using sea otter carcasses with transmitters installed in subcutaneous spaces and cast adrift in the ocean confirmed that this type of implant approach could yield improved telemetry results over the abdominal transmitters. In some cases, the gain in signal strength and distance in these carcass trials was dramatic. Additional carcass testing permitted refinements to the subcutaneous transmitter design and provided additional information on signal reception. In the first study animal, the transmitter produced a signal of high strength, the implant site maintained its integrity over several months, and the animal did not appear to experience adverse effects from the implant. Based on this preliminary case, investigators established the following project goals:

1. Refine and test additional transmitter designs to improve implantation technique and animal comfort.
2. Implant additional animals and conduct detailed post-release monitoring of transmitter effectiveness.
3. Decide upon an ideal subcutaneous transmitter design, implant up to 10 additional animals, and conduct detailed monitoring to confirm transmitter effectiveness.

The project commenced in March 2000. The initial research protocol called for implanting 20 animals and evaluating telemetry results and animal health over extended periods to ascertain the effectiveness of this transmitter concept.

The first group of prototypes contained a traditional arrangement of transmitter components, but they featured an antenna that exited the resin-encased transmitter package and extended cranially beneath the skin. The second group of prototypes used antennas coiled within the resin-encased transmitter package. No prototypes in the study involved antennas that exited the skin of the animal.

Project veterinarians chose the implantation site based on the following criteria:

- The site must be readily reproducible anatomically.
- The site must not interfere with locomotion, grooming, reproduction, or any other natural sea otter behavior.
- The site must not limit the otter's range of motion, cause irritation to the otter, or predispose the animal to the formation of seromas.
- The site must not be adjacent to any vital structures or large blood vessels.

Based upon several dissections performed on sea otter carcasses, a site in the ventro-lateral femoral region appeared suitable as an implant site. In all but the most emaciated individuals—a nutritional state that would preclude instrumentation and release—an accumulation of adipose tissue develops cranial to the quadriceps femoris muscle group in the area at the front of the thigh that is adjacent to the ventral-lateral body wall, and veterinary personnel decided that this site met the criteria for the subcutaneous implants. Over the course of this study, investigators tried several different orientations of the implant to determine which position caused the least site morbidity and yielded the strongest radio signal.

Veterinary personnel provided chemical sedation and analgesia for each implantation procedure using a variety of anesthetic protocols: oxymorphone/diazepam/isoflurane (Huff, pers. comm.); fentanyl/diazepam (Williams, Williams, & Siniff, 1981); medetomidine/butorphanol with a lidocaine local anesthetic (Murray & Johnson, 2001); fentanyl/midazolam (Murray, pers. comm.). For each procedure, a veterinary technician saturated the surgical site with a 50:50 mixture of a sterile, water-soluble lubricating jelly (e.g., KY Jelly™) and a povidone-iodine solution. This mixture helped separate the fur down to the skin; the resulting part served as the incision site.

Following preparation of the surgical site, the veterinarian would make a skin incision of approximately 4 cm, and, using a combination of blunt and sharp dissection, would identify and free the pad of adipose tissue from its loose attachments to the body wall, reflect the fat pad caudally, and insert the gas-sterilized transmitter underneath the fat pad. The veterinarian would then suture the margins of the fat pad to the body wall and close the incision.

For Generation I transmitters, investigators wanted to test the efficacy of having the antenna emerge from the body of the transmitter and extend under the skin. (In theory, an uncoiled antenna would deliver increased signal range.) The veterinarian would make a small incision further up the body across to the opposite side of the abdomen from the transmitter implant site and would use an endoscopic tool to tunnel through the subcutis, grasp the antenna wire (or suture-material leaders attached to the antenna), and thread the antenna wire under the skin. When satisfied with the positioning of the transmitter and antenna, the veterinarian would close openings to the subcutaneous spaces and the skin with polydioxanone suture material. Upon completion of each procedure, a member of the medical team would administer a prophylactic dose of benzathine and procaine penicillin via intramuscular injection and reverse the narcotic with an appropriate antagonist.

The subcutaneous transmitter implant contained the same components as the traditional abdominal package; however, by using the batteries more efficiently—for instance, by duty cycling the transmitters on and off according to a defined schedule—designers hoped to deliver comparable or better telemetry results in a reduced size while conserving battery power.

An essential component of this project involved detailed field tracking to verify the condition of instrumented animals and to evaluate the performance (i.e., transmitter signal strength and reception) of the transmitter (Monnett, Rotterman, Stack, & Monson, 1990). Comparing telemetry results

between the subcutaneous transmitter prototypes and the abdominal implants relied upon these detailed field data. The Monterey Bay Aquarium, the California Department of Fish and Game, and the U.S. Geological Survey committed staffing, field support, and other resources to this project, although external funding covered the purchase of additional receivers and equipment, the employment of technical staff to help monitor implanted animals post-release, and the cost of some aerial tracking of instrumented animals.

Advanced Telemetry Systems (ATS, P.O. Box 398, 470 First Avenue N., Isanti, MN 55040) manufactured all transmitters used in the study. Following release to the wild of instrumented sea otters, project personnel used ATS R4000 scientific receivers or ATS R4500S receiver/datalogger units connected to three-element folding Yagi antennas to locate and monitor project animals.

Results

Generation I subcutaneous transmitters contained a single small lithium battery, and the antenna wire trailed from the main body of the battery and electronic-components package, external to the acrylic resin (Figure 1). Antenna lengths varied between 13 cm and 50 cm; designers introduced waves or loose coils to the longest antenna wires so that they might flex or spring as the otter moved and remain extended under the skin (Figure 2). Four adult southern sea otters (three males, one female) received Generation

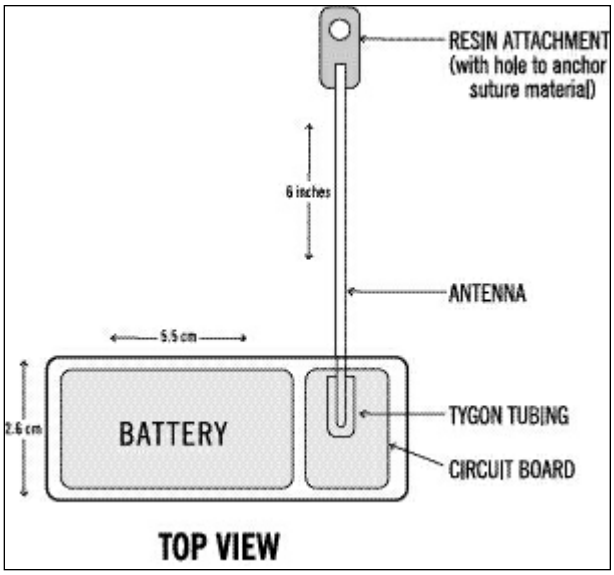

Figure 1. Sketch of subcutaneous transmitter prototype 1 (Generation I).

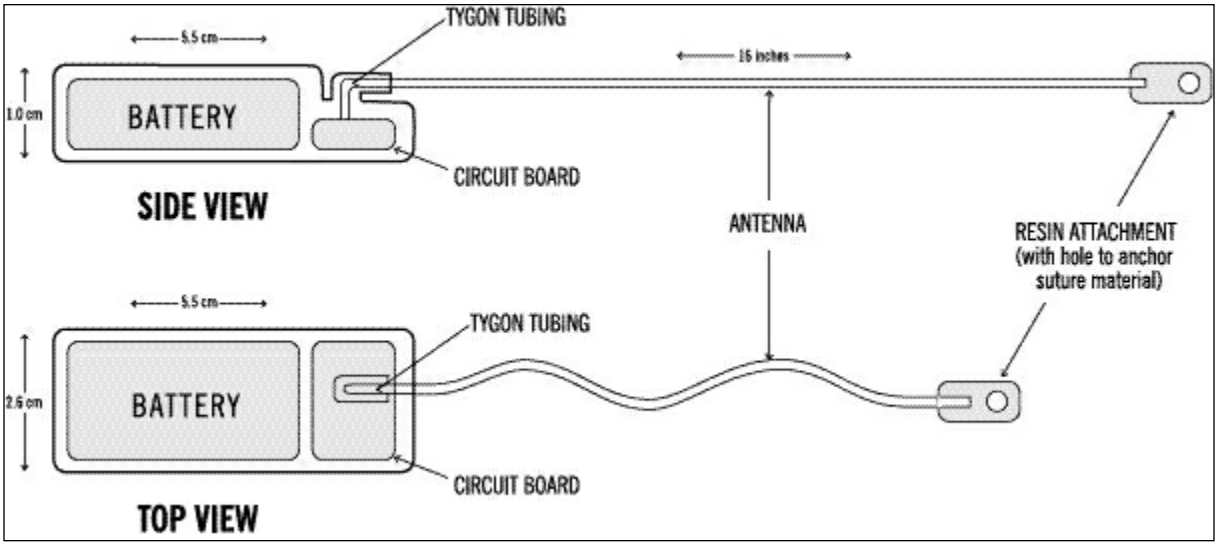

Figure 2. Sketch of subcutaneous transmitter prototypes 5 and 6 (Generation I).

I transmitters with straight antenna wires. Although all of these transmitters had good tracking ranges (up to 1.5 km at sea level), problems developed with the antenna wires—detachment from the transmitter, breakage, and some tissue irritation. The length of time these prototype transmitters functioned in the otters varied between 21 days (premature battery failure) and 182 days (severe damage to the antenna). Two of the study animals died from causes unrelated to the implant: one died of shark-inflicted injuries; the other died from peritonitis. None of the Generation I transmitters reached the guaranteed battery life of 275 days; however, in five of the cases, the transmitters or antennas failed or the animals died before the batteries expired. One of these otters, known as "Judge," received both an intraperitoneal implant and a subcutaneous implant to provide a direct comparison between the two systems. The subcutaneous implant delivered far better telemetry results.

Generation II prototypes involved two major design changes: encasing a tightly-coiled antenna within the acrylic resin and adding a second Keeper® lithium battery (EaglePicher, 13136 82A Avenue, Surrey, BC Canada V3W 9Y6) to extend the range and life of the transmitter. An additional change, using a new circuit board that yielded a stronger signal and increased range, placed more drain on the battery and resulted in a guaranteed battery life of just 212 days. This generation consisted of six prototypes, which differed in the orientation and placement of the batteries and thus affected the length, width, and thickness of the transmitter. This group of transmitters performed the best of all the prototypes. Six different otters (three adult females and three adult males) received these prototypes, and field personnel tested all transmitters for range. Interestingly, even though this transmitter

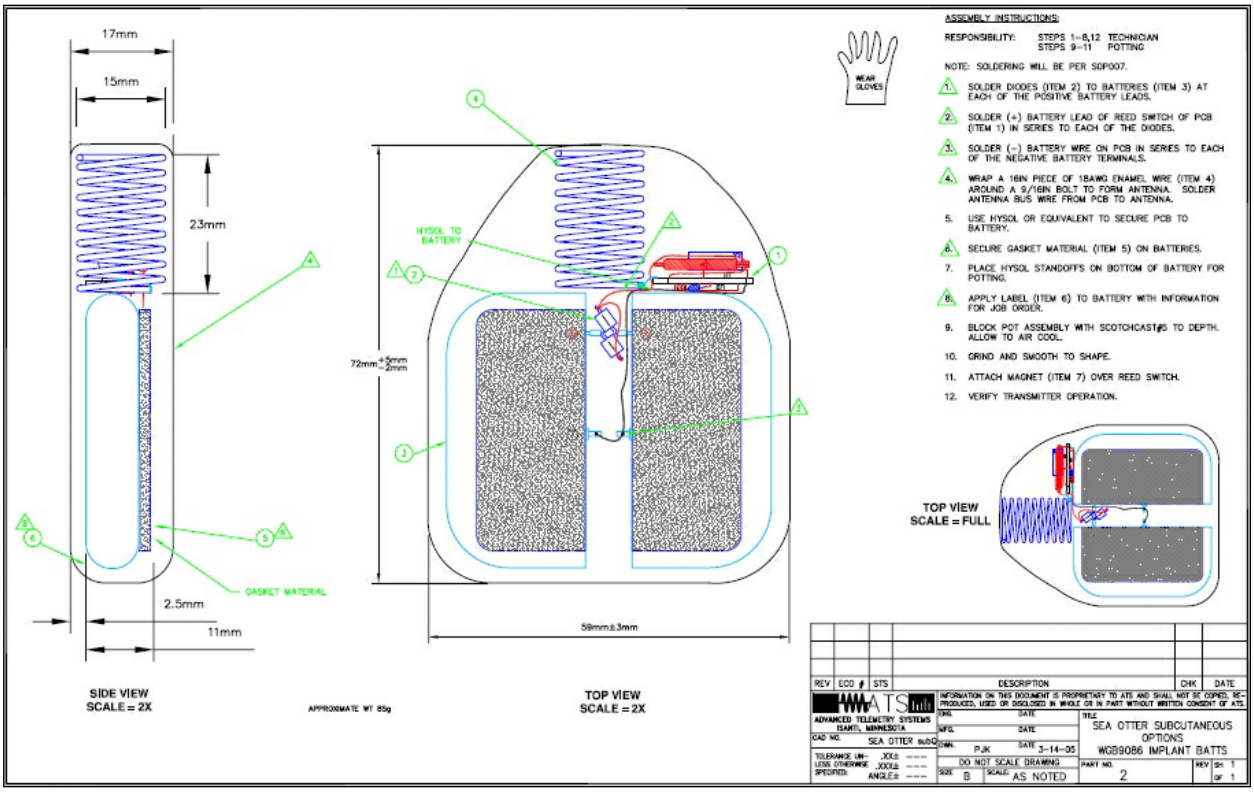

Figure 3. Diagram of prototype 13 (Generation III).

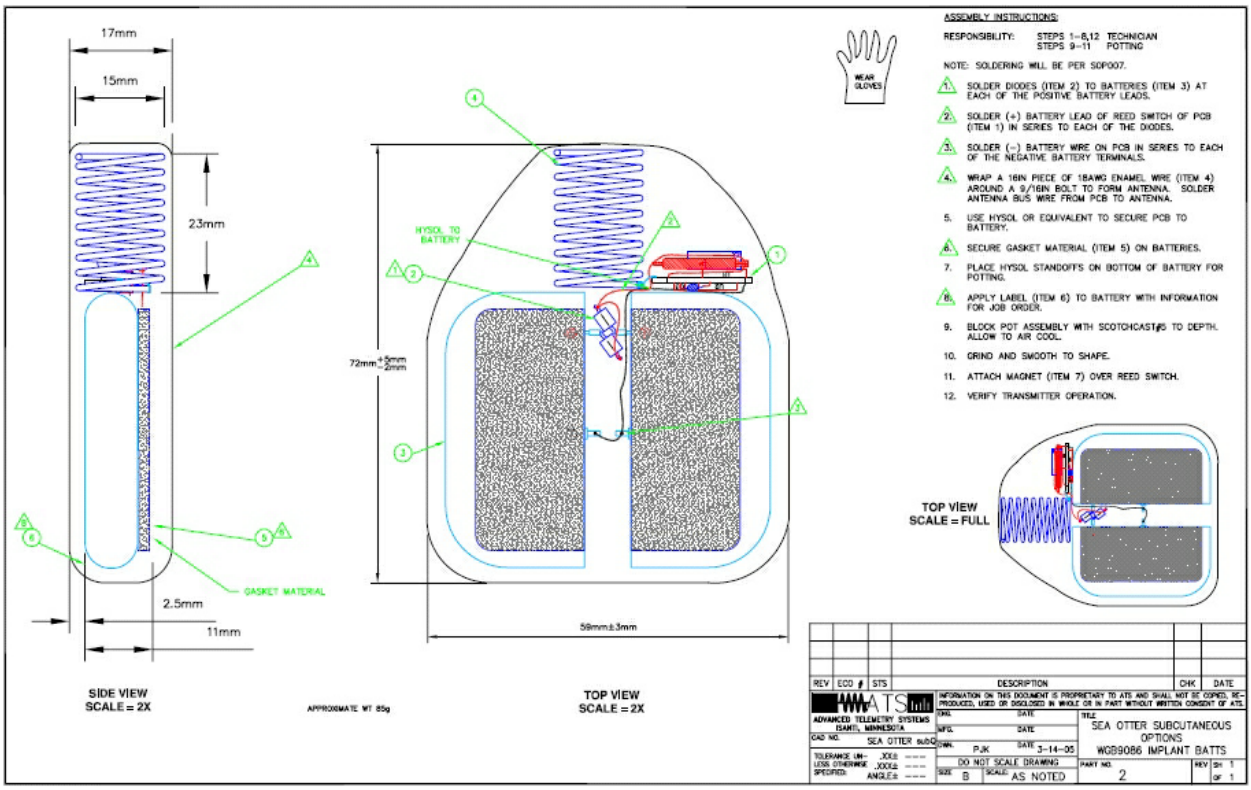

Figure 4. Diagram of prototype 14 (Generation III).

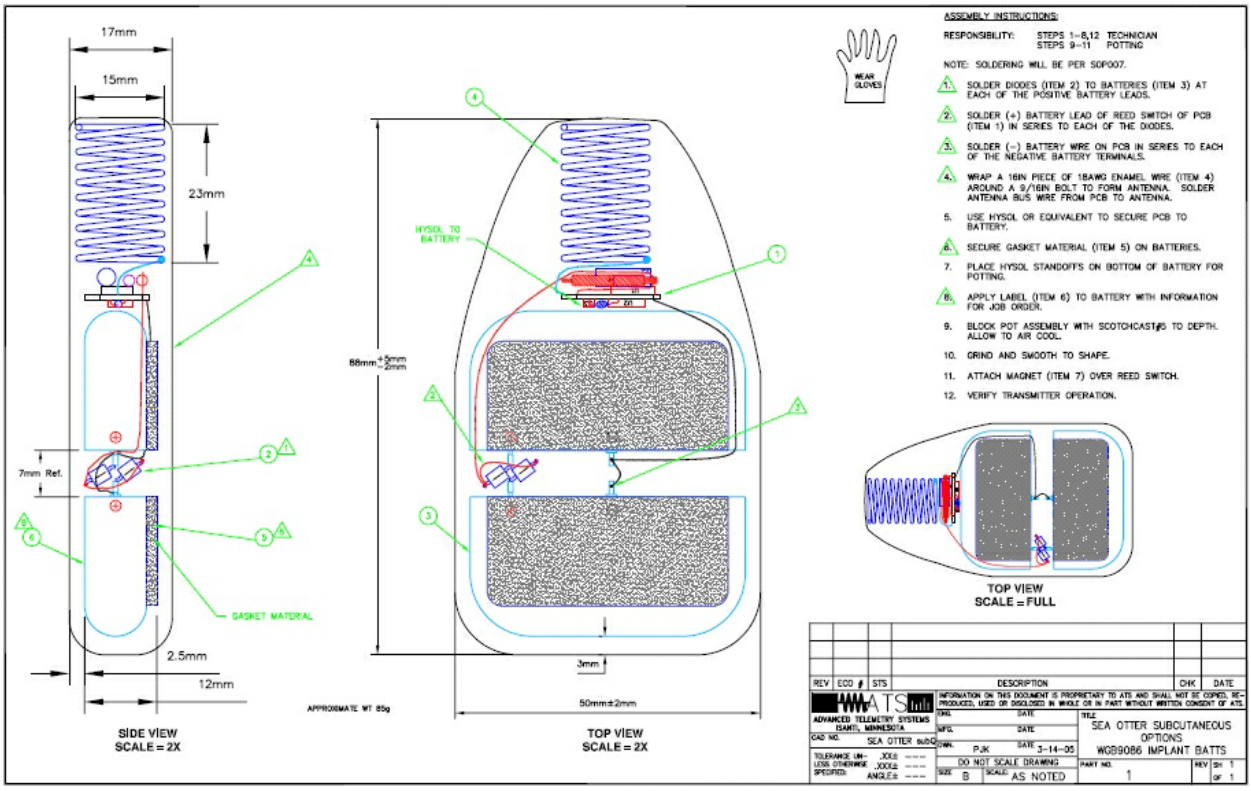

Figure 5. Diagram of prototype 15 (Generation III).

produced a greater drain on the battery, it yielded the longest battery life of any prototype—454 days. During the time of these prototype trials, one otter died from injuries due to shark trauma, and a second otter, 161-98, survived one shark attack but did not survive a second.

Generation III transmitter prototypes incorporated a switch in battery type from the off-the-shelf, less-expensive (and perhaps less-safe) lithium batteries to lithium carbon monofluoride (Li/CFx) pacemaker batteries (Greatbatch, Inc., 9645 Wehrle Drive, Clarence, NY 14031). ATS configured three prototypes for this group (Figures 3, 4, and 5), using different battery and transmitter orientations. The Greatbatch® batteries exceeded the thickness of the Keeper® batteries and increased the size of the transmitter package. (ATS added a thermister to collect real-time body temperatures, although this addition did not make the package larger.) Generation III units incorporated the two-stage circuit boards; therefore, they did not produce as strong a signal over a greater range, but they had a guaranteed battery life of 730 days. Four otters received these instruments; two of them had surgery twice.

This group of prototypes presented the most problems after surgery. The otters seemed inclined to play with the sutures or manipulate the larger, more palpable subcutaneous instrument. Project veterinarians decided to adjust the placement of the transmitter within the first muscle layer but still within the inguinal area.

After the staff veterinarian instrumented sea otter 351-06, an immature male, with subcutaneous transmitter prototype #14 (Figure 4) over his left thigh, caregivers observed the otter "pawing" at the surgical site. During a routine exam nine days after surgery, the veterinarian noted dehiscence of and discharge from the surgical site. Four days later, the veterinarian extracted the instrument and placed the otter on a regime of antibiotics for four weeks. Three months after his first subcutaneous transmitter surgery, 351-06 received transmitter prototype #13 (Figure 3), which had a slightly different battery configuration than prototype #14. This time, the veterinarian positioned the transmitter on the right side of the body over the right thigh. After three weeks of recuperation, caregivers released the otter to the ocean. The transmitter posed no apparent problems following release; however, the otter disappeared or the transmitter failed after 161 days.

Adult female otter, 356-06, received her first subcutaneous transmitter (prototype #13) on May 11, 2006. On May 15, 2006, caregivers noticed dehiscence of her surgical site similar to that of otter 351-06. Over the next several weeks, animal care personnel examined the site for infection, and the otter received a course of antibiotics. Although the veterinarian found no signs of infection at the site, he elected to remove the transmitter and replace it with prototype # 14, which he implanted over the animal's right thigh approximately six weeks after the first surgery. The otter recovered well, caregivers detected no further problems with the transmitter placement, and a tracking crew released the otter to the wild just over seven weeks following the second surgery. Trackers have observed this animal on a regular basis in the Moss Landing area, functioning well with the prototype #14 transmitter, for more than a year.

An adult male (360-06) that had received prototype #15 (Figure 5), the thickest of all the subcutaneous packages, stranded about seven months after his release with an open wound at the approximate location of his implant site. Upon examination, caregivers determined that his transmitter had exited through the skin at the surgical site. After a treatment period, this otter received a Generation II transmitter, and caregivers released him again. Another otter in this group, an immature male, had a recrudescence of peritonitis caused by acanthocephalan parasites and died nine days after his surgery.

Generation IV transmitter prototypes incorporated a three-stage circuit board, which transmitted over a greater range but yielded a shorter guaranteed battery life (i.e., 433 days). As of May 2007, study personnel had tracked three subadult otters—two females and one male—with the Generation IV prototypes installed for at least three months with good results. As expected, the three-stage circuit board model produced a booming signal and achieved reception over a greater range than the two-stage board. The two-stage circuit board model allowed for longer battery life while still producing a strong signal.

Table 1 summarizes the results for all prototypes.

Table 1. Results from 18 subcutaneous transmitter prototypes installed in southern sea otters

Prototype Number	Otter ID	Implant Date	Battery Brand/Type	Battery Number	Circuit Board	Antenna Type/ Outcome	Surgical Site	Battery Days	Comments
1	Louie	3/29/2000	Keeper/ lithium	1	2 stage	Straight, loose wire: single break	NAE*	154	antenna coiled around TX; still functioning; otter released & monitored then recaptured; one break in antenna (antenna slipped and coiled around battery) but still audible in field
2	Morgan	5/2/2001	Keeper/ lithium	1	2 stage	Straight, loose wire: no breakage	NAE*	21	premature battery failure, instrument removed; no other problems seen at removal; otter never released

Prototype Number	Otter ID	Implant Date	Battery Brand/Type	Battery Number	Circuit Board	Antenna Type/ Outcome	Surgical Site	Battery Days	Comments
3	202-01	8/1/2001	Keeper/ lithium	1	2 stage	Straight, loose wire: antenna frayed at TX	NAE*	168	otter released & monitored; stranded and died from peritonitis unrelated to transmitter; antenna frayed at base near exit point from instrument
4	211-01	10/24/2001	Keeper/ lithium	1	2 stage	Straight, loose wire: no breakage	NAE*	56	otter released & monitored; died from shark bites; antenna wire in good shape—no breaks
5	Judge	9/19/2002	Keeper/ lithium	1	2 stage	Coiled, loose wire: broke into three segments	NAE*	182	otter received abdominal implant for range comparison testing; otter released & monitored; recaptured, antenna broken into 3 segments; TX removed without incident
6	240-02	1/12/2003	Keeper/ lithium	1	2 stage	Coiled, loose wire: broke 15 cm from TX	NAE*	35	otter released & monitored; recaptured, antenna broken 15 cm from base, but still heard from 1.2 km away
7	277-04	3/3/2004	Keeper/ lithium	2	3 stage	Coiled in acrylic: no problems	NAE*	51	otter released & monitored; transmitter had good range in field, died from shark injury

Prototype Number	Otter ID	Implant Date	Battery Brand/Type	Battery Number	Circuit Board	Antenna Type/ Outcome	Surgical Site	Battery Days	Comments
8	293-04	6/1/2004	Keeper/ lithium	2	3 stage	Coiled in acrylic: no problems	NAE*	290	otter released & monitored; battery expired after 290 +/-11 days; otter recaptured 2007 and euthanized for health issues; TX removed, no problems observed
9	300-04	10/25/2004	Keeper/ lithium	2		Coiled in acrylic: no problems	NAE*	53	otter released & monitored; tested briefly in field; worked well; otter died from acantho-cephalan peritonitis
10	302-04	10/27/2004	Keeper/ lithium	2	3 stage	Coiled in acrylic: no problems	NAE*	54	otter released & monitored; tested briefly in field; worked well; otter recaptured, died of enteritis
11	314-04	12/30/2004	Keeper/ lithium	2	3 stage	Coiled in acrylic: no problems	NAE*	454	otter released & monitored; tested fully in field; excellent range—almost too strong.; battery lasted 454 days; otter reported dead on 4/29/2007; unable to retrieve carcass
12	Pirate	5/5/2005	Keeper/ lithium	2	2 stage	Coiled in acrylic: no problems	NAE*	33	tested in tank; swelling observed over surgical site; TX removed 6/7/2005

Prototype Number	Otter ID	Implant Date	Battery Brand/Type	Battery Number	Circuit Board	Antenna Type/ Outcome	Surgical Site	Battery Days	Comments
12	Pirate	5/12/2005	Keeper/ lithium	2	3 stage	Coiled in acrylic: no problems	NAE*	403	otter released & monitored;
12	262-03	6/7/2005	Keeper/ lithium	2	2 stage	Coiled in acrylic: no problems	NAE*	341	otter released & monitored; tested fully in field; good range (3 stage better); otter died after second shark attack; total battery time 374 days; still running at removal; tested in field; excellent signal
14	336-05	11/9/2005	Greatbatch/ lithium carbon monofluoride	2	2 stage	Coiled in acrylic: no problems	closing suture pulled loose	9	never tested; died of severe acanthocephalan peritonitis
14	351-06	5/4/2006	Greatbatch/ lithium carbon monofluoride	2	2 stage	Coiled in acrylic: no problems	played with sutures		TX removed 5/15/2006; replaced several months later
13	356-06	5/11/2006	Greatbatch/ lithium carbon monofluoride	2	2 stage	Coiled in acrylic: no problems	played with sutures	21	not released at this time; TX removed; different prototype placed on opposite side of body
15	360-06	6/1/2006	Greatbatch/ lithium carbon monofluoride	2	2 stage	Coiled in acrylic: no problems	NAE*	258	otter released & monitored; stranded on 2/14/2007; TX had ejected from body through small opening
14	356-06	6/29/2006	Greatbatch/ lithium carbon monofluoride	2	2 stage	Coiled in acrylic: no problems	NAE*		otter released & monitored; observed weekly; radio functioning well

Prototype Number	Otter ID	Implant Date	Battery Brand/Type	Battery Number	Circuit Board	Antenna Type/ Outcome	Surgical Site	Battery Days	Comments
13	351-06	8/17/2006	Greatbatch/ lithium carbon monofluoride	2	2 stage	Coiled in acrylic: no problems	NAE*	161	otter released & monitored; missing, not heard after February 2007
17	357-06	12/21/2006	Greatbatch/ lithium carbon monofluoride	2	3 stage	Coiled in acrylic: no problems	NAE*	131	otter released & monitored;
18	352-06	12/21/2006	Greatbatch/ lithium carbon monofluoride	2	3 stage	Coiled in acrylic: no problems	NAE*	131	otter released & monitored; thermister tested in field
16	374-06	1/30/2007	Greatbatch/ lithium carbon monofluoride	2	3 stage	Coiled in acrylic: no problems	NAE*	91	otter released & monitored; thermister tested in field
12	360-06	3/1/2007	Keeper/ lithium	2	3 stage	Coiled in acrylic: no problems	NAE*		otter's second release; different prototype TX; new placement between muscles
*NAE = no adverse effects									

Discussion

In general, progress lagged behind anticipated timelines because suitable animals for the study did not become available as often as expected; however, over a seven-year period, veterinary personnel conducted twenty-four surgical procedures with twenty-one different animals and evaluated eighteen subcutaneous transmitter prototypes. Generation II prototypes produced the best results.

Investigators abandoned the Generation I concept (i.e., with the emerging antenna) after all prototypes evinced malformation, detachment, or breakage of the antenna. The first sea otter slated for a subcutaneous prototype—an adult male sea otter known as "Louie"—received his implant on March 19, 2000. A radiograph at the time of the surgery confirmed the accurate placement of both the transmitter package and the antenna in the targeted sites. On April 13, 2000, before releasing the animal, veterinary personnel obtained another radiograph, which showed that the antenna had retracted and formed a small loop near the insertion into the transmitter package (Figure 6); however, the animal appeared comfortable with the implant in place. On June 1, 2000, about seven weeks following the otter's release, project personnel recaptured the animal from the wild based on observations that an abscess or seroma might have formed near the implant site. As it turned out, veterinary staff found no infection or compromise of the surgery site, but a radiograph showed that the antenna had recoiled

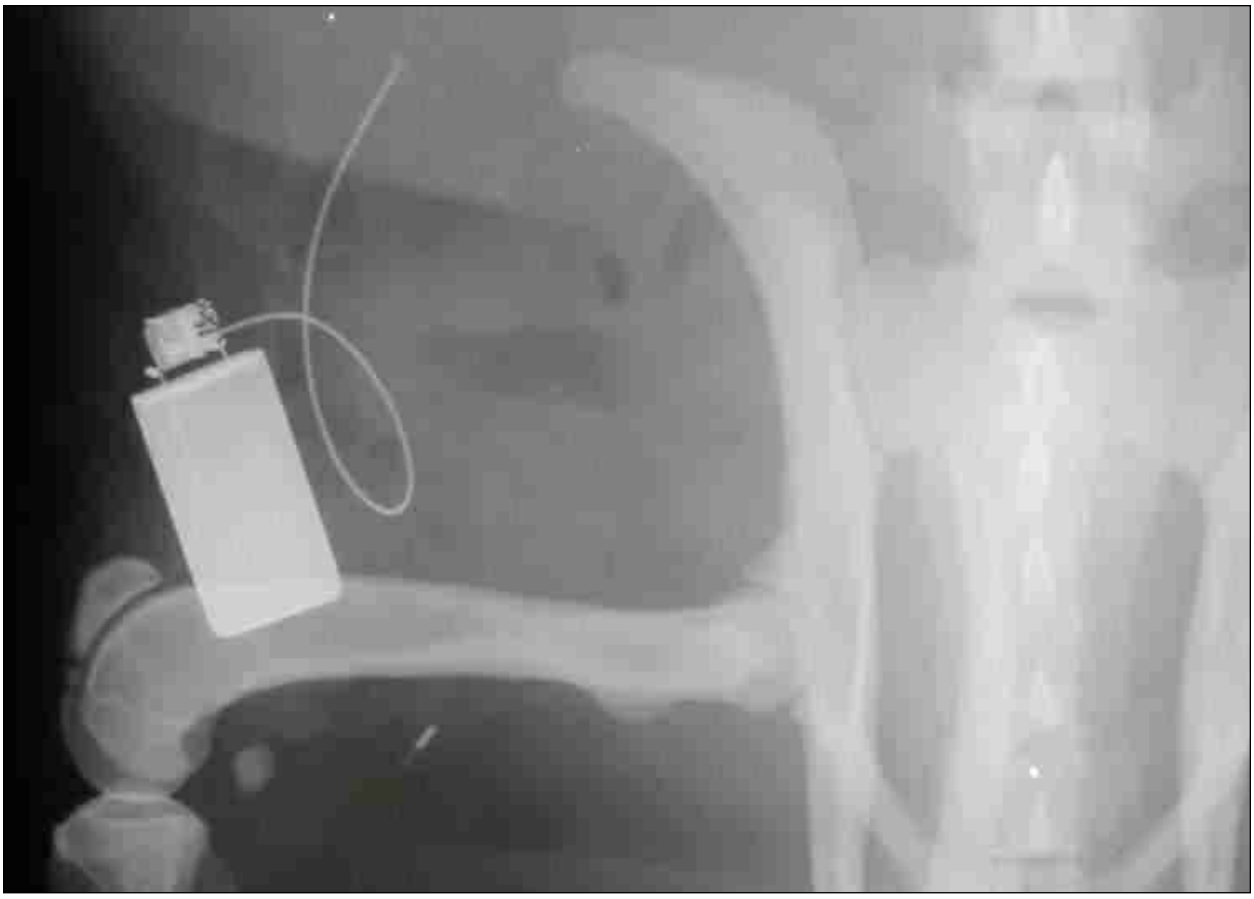

Figure 6. Radiograph of Generation I prototype showing retracted and looped antenna.

even farther toward the implant. (Inexplicably, the quality of the VHF signal from the transmitter had remained high.) In August, after a second release, trackers noticed a decrease in the quality of the transmitter signal and suspected that the antenna might have detached from the transmitter package. On November 13, 2000, project personnel recaptured the otter, and radiographs confirmed that the antenna had broken away from the transmitter. The staff veterinarian removed the transmitter, and caregivers returned the animal to the wild a few days later.

Based upon this initial field trial in a live sea otter, investigators refined the Generation I design and undertook more carcass testing and live-animal testing with the resulting prototypes to confirm that the signal characteristics still equaled or exceeded the abdominally implanted transmitters. Unfortunately, each unit, once implanted, developed problems with either the antenna or the battery. Of particular concern, the antenna wires tended to fray, detach, or break into pieces. It seemed that the extreme flexibility of sea otters—their continual twisting, turning, and contorting during grooming and diving behavior—contributed to antenna wire damage, and no configuration or positioning of the antenna could counteract this reality of the sea otter's natural history.

Using Greatbatch® implantable heart pacemaker batteries, investigators planned to conduct comparisons between subcutaneous transmitters with two-stage and three-stage circuit boards. The differences between two-stage and three-stage transmitters included battery life, signal strength, and

signal range. Transmitters manufactured with a three-stage circuit board produced a much stronger sound with greater range, but they placed a greater drain on the battery. Conversely, transmitters manufactured with a two-stage circuit board emitted a weaker signal with reduced range, but they yielded a slightly longer battery life of 275 days. Field experience with units containing a three-stage circuit board led to the general impression that a subcutaneous transmitter does not require the stronger signal produced by a three-stage circuit board. In theory, being closer to the exterior of the body, the signal would not need to penetrate the body cavity, as it does in the abdominal implant. In practice, particularly in areas around docks and piers, the three-stage transmitter delivered an overpowering signal, and trackers had a difficult time determining the directionality of the signal.

With one animal, project personnel tested transmitter range and strength in the field using a dB attenuator and received a decent signal when the animal moved up to two kilometers offshore—a considerable improvement over the abdominal implants, which generated an acceptable signal at a range of only a kilometer or less.

Project investigators expected that the data emerging from this instrumentation and long-term monitoring project would determine whether subcutaneous implants could replace abdominal implants as the tracking system of choice for sea otters. As it turned out, investigators achieved better telemetry results with the subcutaneous implants than with the abdominal implants; however, the larger package required to accommodate larger or additional batteries, and thus sustain longer tracking periods, introduced moderate complications at the implant site, and the project as a whole has achieved equivocal results. With respect to future applications, investigators have met with engineers to discuss new designs and technologies that can build upon the knowledge obtained through the subcutaneous transmitter study.

Acknowledgments

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A subjective evaluation of suggested products to facilitate contaminant removal from feathers

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Keywords: pre-treatments, cleaning, contaminated feathers, silicone, roofing tar, Tanglefoot®, Orimulsion®, crude oil, cooking oil

ABSTRACT

There are several contaminants that are particularly difficult to remove from wildlife. This study subjectively evaluates 15 different products (pre-treatment agents) that have been suggested as agents to facilitate dish detergent in removal of six different contaminants. The purpose of this project was to prioritize products to be used in objective tests performed by DuPont Chemical Solutions Enterprise. Results showed that six of the fifteen pre-treatment agents have potential to assist in cleaning contaminated feathers.

Introduction

Feathers play an important role in the natural history of birds: they allow many of these animals to fly, thermoregulate, and remain waterproof to varying degrees. A bird's ability to keep its feathers clean, aligned, and supple, combined with the innate structure of the feather, help to keep the bird waterproof. During a contamination event the feather structure is disrupted. Whether a bird comes in contact with crude oil due to a spill or lands on a building freshly coated in roofing tar, the contaminant causes the feathers to lose the ability to provide insulation and repel water.

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In order to restore the contaminated feather to its natural condition, the feather must be completely freed of both the contaminant and cleaning agent(s) (Dein & Frink, 1986; Miller & Welte, 1999). During the cleaning process natural preen oils or waxes are also removed, but are quickly restored during the course of post-washing rehabilitation. Several studies show preen oils to be less important in establishing water repellency and act more directly to condition the feathers, keeping them supple and aligned (Clark & Gregory, 1971; Mahoney, 1984). The most important components to thorough cleaning are the effectiveness of a product at removing the contaminant at physiological temperatures and the ease of rinsing away the cleaning products (Rijke, 1987).

In 1990, Tri-State Bird Rescue & Research, Inc. (Tri-State), along with DuPont Central Research and Development Department (DuPont), developed a method to subjectively and objectively evaluate surfactant efficacy for removing petrochemicals from contaminated feathers (Bryndza et al., 1991 & 1995). The results of that study demonstrated that Dawn® dishwashing liquid detergent (Dawn®) (Procter & Gamble, Cincinnati, OH) was the surfactant of choice among those products tested. Dawn® was more effective at 2% v/v concentration than any of the other cleaning agents, even when the other agents were tested at concentrations as high as 5% v/v.

As new products have been introduced into the field of oil spill response, Tri-State and DuPont have continued to examine different products for their efficacy in cleaning contaminated feathers. In 2000, 86 potential surfactants were evaluated as possible products to remove petrochemicals from wildlife (Miller et al., 2003). Then in 2005, the evaluation process was repeated on fifteen products (Miller et al., 2005). Dawn® was again shown to be the most effective product (Miller et al., 2003; Miller et al., 2005).

Although Dawn® is preferred for the removal of many petrochemicals from wildlife, there are many contaminants that are not easily removed with Dawn® alone. The previous studies have used standard oils (crude, vegetable and synthetic oil) in a one-step cleaning process, which is not always effective with these more difficult products. Many wildlife rehabilitators and oiled wildlife response professionals have found they need multiple steps during the wash procedure to remove certain contaminants. Furthermore, many individuals have begun to suggest using untested agents to facilitate the dish detergent in removal of such contaminants. These "pre-treatments" may assist in the original contaminant removal; by using these agents, an additional foreign substance is added to the feathers that now also needs to be removed effectively.

Many pre-treatments are already being used by wildlife professionals, although their efficacy has never been formally tested. This subjective project is the first step in determining the effectiveness of different pre-treatments on known contaminants that are difficult to remove with Dawn® alone. A total of fifteen different pre-treatment agents were tested on six different contaminants. The pre-treatments that proved to be most effective subjectively will then be used in an objective test to be performed by DuPont.

Materials and Methods

Fifteen potential pre-treatment agents and six contaminants were obtained for this subjective evaluation. Most of the pre-treatment agents were products that have been used by Tri-State and/or recommended by other wildlife rehabilitators; the remaining agents were tested at the request of the manufacturers. The contaminants were chosen as products representative of the contaminants commonly encountered on wildlife presenting for rehabilitation.

All pre-treatment agents were placed into uniform spray bottles and assigned an identification letter by an outside participant in order to reduce bias on the part of the evaluators. The entire list of pre-treatment agents and manufacturers is presented in Appendix A. The list of contaminants and manufacturers is presented in Appendix B. Methods followed were based on the previous surfactant studies developed and modified by Tri-State and DuPont in an effort to keep data comparable and repeatable (Miller et al., 2003).

All fifteen pre-treatments were tested on each of the six contaminants. Within each of these tests, two or three feathers were contaminated and removal was attempted with the pre-treatment and a standardized cleaning process. The contaminants were tested at two different time intervals: 1) after being contaminated for at least one hour (short), and 2) after being contaminated for at least three weeks (long). The long trial was especially important since birds are often not captured and/or treated immediately after becoming contaminated. Many contaminants change properties as they age and are exposed to different weather conditions. By allowing contaminated feathers to sit for specific lengths of time, the experiment simulated "weathering" of the products. Five of the contaminants (crude oil, used cooking oil, silicone, roofing tar and Tanglefoot®) were tested by three different people to evaluate the efficacy of the pre-treatment agents for both the short and long time intervals; only one person examined removing the sixth contaminant, Orimulsion®, from feathers for both the short and long time intervals.

Original Scent Dawn® Ultra Concentrated was used as the surfactant for all of the trials. Although Original Scent Dawn® (non-Ultra) is preferred for cleaning, it is more difficult to obtain, and Tri-State has begun using the ultra concentrated formula. The ultra concentrated formula was previously proven effective in removing standard petrochemicals from feathers (Miller et al., 2003, Miller et al., 2005).

Appropriate safety measures were taken by all evaluators when handling the different contaminants and pre-treatment agents (Appendix C). All Material Data Safety Sheets were kept on file.

Feather collection

All feathers used in this study were body feathers from the carcasses of snow geese *(Chen caerulescens)* that were admitted to Tri-State's wild bird clinic. These birds either arrived dead or where

euthanized due to the severity of their injuries. The feathers were collected by hand and separated from the down. All feathers appeared clean and not previously oiled.

Contaminating feathers

Plastic trays covered with aluminum foil were prepared containing 32-48 feathers (two to three for each pre-treatment and two to three for a control). There were a total of 32 trays of feathers; these were divided into different categories based on the type of contaminant and the length of time the contaminant was on the feather (Figure 1).

Figure 1. An example of contaminated feathers on an aluminum-foil covered tray. Feathers would remain like this for one hour to three weeks depending on which time trial was being tested for the contaminant.

Feathers were oiled individually by hand in an attempt to keep the contamination uniform between feathers. A total of 0.3 ml of contaminant was applied to each feather using a 1-ml syringe, with 0.15 ml applied to each side of the feather. When contaminating a feather with a new contaminant, a sterile syringe was used, but the same syringe was used when doing multiple feathers with the same contaminant. Once a feather was contaminated, it was placed on the foil-covered plastic tray and left undisturbed at room temperature until testing began.

Pre-Treatment Application

All pre-treatment agents were kept in a warm water bath ranging from 32°C to 44°C (90°F to 110°F) before and during the experimental period. When a pre-treatment agent was selected for testing, it was shaken three times before applying it to a feather. This helped to mix the contents of the bottle. The evaluator then applied the agent by spraying it directly onto the feather just prior to the cleaning process.

One full spray was applied to both sides of the feather and then rubbed with the gloved thumb and forefinger for three strokes in the direction of the feather growth. The pre-treated feather was then

placed on a different plastic tray covered with clean aluminum foil, where pre-treatment was again applied with one full spray of the bottle. Then the feather was allowed to react with the pre-treatment for one minute without disturbance.

At this time the evaluator also noted if the pre-treatment agent had an obvious odor, which was then recorded on the observation form.

Cleaning Procedure

At the beginning of each testing period the water hardness was tested using the Hardness (Total) Test Kit (Hach Company, Loveland, CO). The pH was tested using colorpHast® Indicator Strips (EMD Chemicals, Inc., Gibbstown, NJ). All information was recorded on the test observation form.

After the pre-treatment agent was applied and left for one minute, each feather was cleaned by submerging it in a 7% Dawn® in water solution (360 ml water to 25 ml of Dawn®). The feather was agitated back and forth in the tub for 15 seconds and then allowed to remain in the solution for 45 seconds without agitation. While remaining in the 7% solution, the feather was gently stroked by sliding the gloved thumb and forefinger down the length of the feather for ten strokes. The feather was removed and allowed to drip for five seconds. It was then submerged in a 5% Dawn® in water solution (360 ml water to 18 ml of Dawn®) for one minute without agitation. After the minute, the feather was again stroked 10 times while still submerged in the solution. The feather was removed and again allowed to drip for five seconds before placing it in a tub containing 360 ml of clean water. In this tub the feather was agitated by moving it back and forth continually for 30 seconds. The feather was rinsed for 40 seconds (20 seconds/side) under high pressure water (~40-60 psi). Finally, the feather was placed on a clean plastic try for evaluation.

A high concentration of Dawn® and multiple tubs were used since Tri-State has found that the combination of contaminant and pre-treatment agents can be more difficult to remove from feathers than a single substance.

All water temperature was maintained in the range of 39oC to 40oC (102oF to 104oF).

Subjective Evaluation

Immediately after rinsing, each "clean" feather was subjectively ranked by the evaluator into categories:

- Excellent = feather appears perfectly clean and dry (fluffy)
- Good = most of the feather appears clean and dry, only small spots or edges still appeared to "stick" together
- Fair = feather appears mostly clean and dry during the rinse but is wet or out of shape when laid onto the tray

- Poor= feather does not rinse well, appears contaminated and/or wet after the cleaning process;the feather loses shape when placed onto the tray.

Analyzing Results

A value was assigned to each of the subjective rankings given by the evaluators in order to compile a numerical comparison (excellent = 4, good = 3, fair = 2, poor =1). An average score for each of the fifteen pre-treatments was complied for each of the six contaminants (separately for both short and long tests) by using the value of each ranking assigned by the three evaluators.

Results

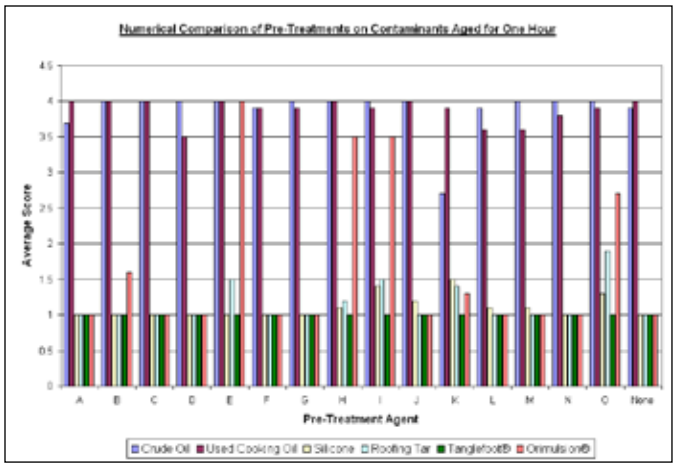

Figure 2. A comparison of the subjective evaluations of all 15 pre-treatment agents used on the six different contaminants after feathers had been contaminated for at least one hour. See Appendix A for index of pre-treatment agents.

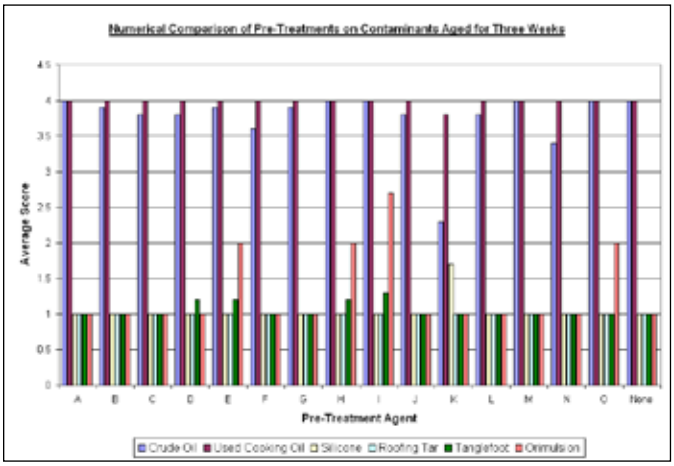

Figure 3. A comparison of the subjective evaluations of all 15 pre-treatment agents used on the six different contaminants after feathers had been contaminated for at least three weeks. See Appendix A for index of pre-treatment agents.

Overall, several pre-treatments consistently worked better then others in both the short and long tests on all of the contaminants (Figure 2 and Figure 3). These were methyl soyate, ArtWash!TM, ethyl oleate, and ethyl lactate. Elastol also proved effective on specific contaminants but did not work well on others. Several contaminants came off easily without the use of a pre-treatment agent in all tests (Figure 2 and Figure 3). Most pre-treatments came off the feathers successfully after being applied to a contaminated feather.

Control

A control was performed by placing each pre-treatment on to an uncontaminated feather; most pre-treatment trials on clean feathers received a score of Excellent; Elastol and Dawn® Power Dissolver received scores of Good.

Crude

The crude oil came off the feathers fairly successfully in all trials (Figure 4). Overall, it was slightly easier to remove the crude oil from feathers that had only

been contaminated for at least an hour than those that had been allowed to "weather". All pre-treatments except Elastol received a scoring of Good to Excellent in both the short and long tests; Elastol received scoring of Fair to Good. Contaminated feathers washed without any use of pre-treatment received scores of mostly Excellent in tests of both time intervals (14 out of 15 feathers).

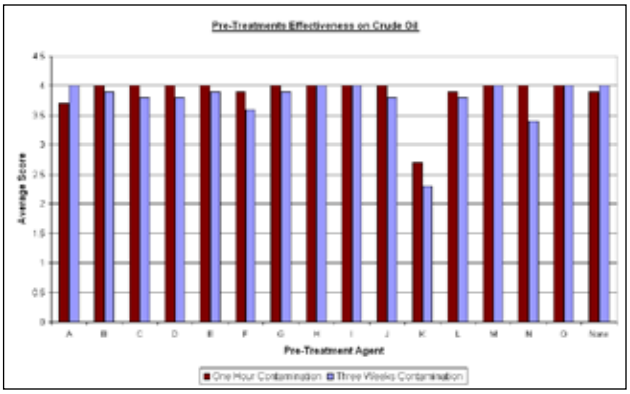

Figure 4. A comparison of the subjective efficacy of the 15 pre-treatment agents on removing crude oil when feathers had been contaminated for different lengths of time. See Appendix A for index of pre-treatment agents.

Used Cooking Oil

Used cooking oil washed off the feathers fairly successfully in all trials (Figure 5). Overall, it was slightly easier to remove the used cooking oil from feathers that had been contaminated for at least 3 weeks. All pre-treatments received a scoring of Good to Excellent in tests of both time intervals. Contaminated feathers washed without any use of pre-treatment received scores of all Excellent.

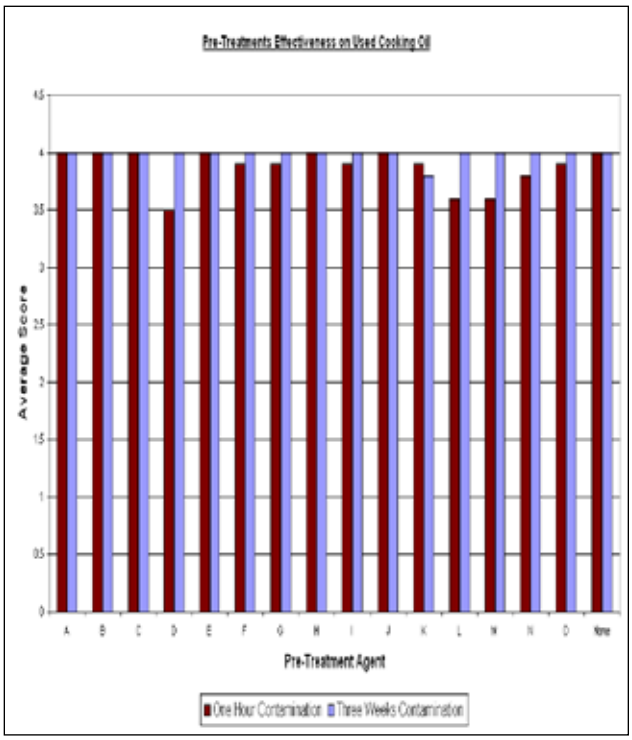

Figure 5. A comparison of the subjective efficacy of the 15 pre-treatment agents on removing used cooking oil when feathers had been contaminated for different lengths of time. See Appendix A for index of pre-treatment agents.

Silicone

Silicone was very difficult to remove from the feathers. Feathers that had only been contaminated for the short time interval had slightly better results then feathers that had silicone on them for 3 weeks prior to wash (Figure 6). Several evaluators noted that the silicone peeled off slightly after the rinse when using certain pre-treatments on the long-exposure trial set. Elastol received the highest scoring, especially when the silicone had been on the feather for 3 weeks; one evaluator got all of the silicone to come off completely using Elastol.

When attempting to remove silicone without any pre-treatment in both the short and long tests, the feathers received a scoring of Poor.

Roofing Tar

Roofing tar was also very difficult to remove from feathers. Feathers that had only been contaminated for the short time interval had slightly better results then feathers contaminated for the longer interval (Figure 7). No pre-treatments scored higher then a Poor if the roofing tar had been left on the feather for at least 3 weeks. Methyl soyate, ArtWash!TM, ethyl oleate, ethyl lactate and Elastol did receive some scores of Fair and Good during the short trial. Ethyl lactate received the highest scoring during these trials.

Figure 7. A comparison of the subjective efficacy of the 15 pre-treatment agents on removing roofing tar when feathers had been contaminated for different lengths of time. See Appendix A for index of pre-treatment agents. NOTE the scale in this figure is expanded to accentuate minor differences in results.

for at least 3 weeks had slightly better results (Figure 8). Ethyl oleate received scores of Fair, and Simple Green All Purpose Cleaner, methyl soyate and ArtWash!TM were given a score of Poor/Fair by one evaluator for feathers contaminated for 3 weeks. Both the short and long intervals for all other pre-treatment agents and for no pre-treatment received a score of Poor.

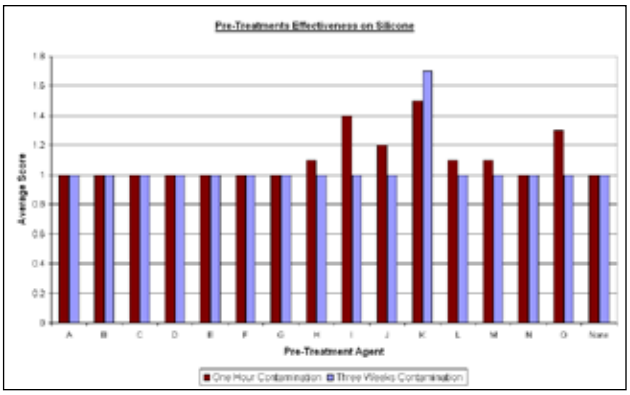

Figure 6. A comparison of the subjective efficacy of the 15 pre-treatment agents on removing silicone when feathers had been contaminated for different lengths of time. See Appendix A for index of pre-treatment agents. NOTE the scale in this figure is expanded to accentuate minor differences in results.

When attempting to remove roofing tar without any pre-treatment in both the short and long interval tests, the feathers received a consistent scoring of Poor.

Tanglefoot®

Tanglefoot® was very difficult to remove from feathers. Feathers that had been contaminated

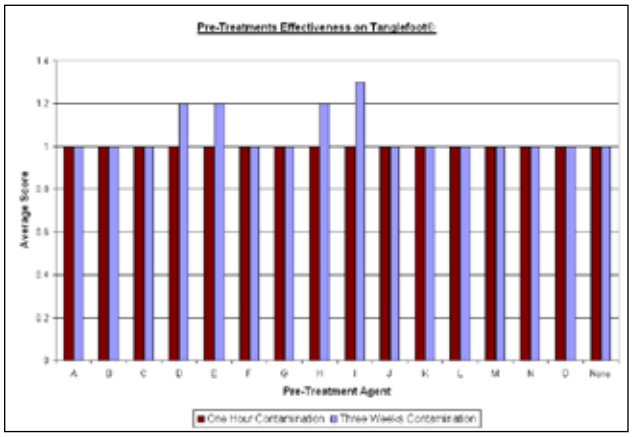

Figure 8. A comparison of the subjective efficacy of the 15 pre-treatment agents on removing Tanglefoot® when feathers had been contaminated for different lengths of time. See Appendix A for index of pre-treatment agents. NOTE the scale in this figure figure is expanded to accentuate minor differences in results.

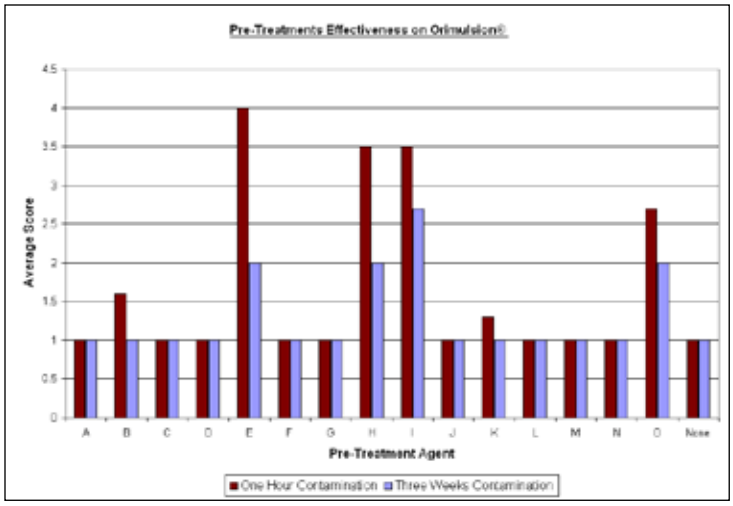

Figure 9. A comparison of the subjective efficacy of the 15 pre-treatment agents on removing Orimulsion® when feathers had been contaminated for different lengths of time. See Appendix A for index of pre-treatment agents.

Orimulsion®

The removal of Orimulsion® was much more successful when using methyl soyate, ArtWash!TM, ethyl oleate, and ethyl lactate after the short contamination interval than after the long contamination interval (Figure 9). Methyl soyate received scores of Excellent, while ArtWash!TM, ethyl oleate, and ethyl lactate received mostly scores of Good. Canola oil and Elastol showed some potential for removing this contaminant, receiving scores of at least Fair by the sole evaluator. All other pre-treatments scored Poor.

Orimulsion® became more difficult to remove after it remained on the feathers for 3 weeks. Methyl soyate, ArtWash!TM, ethyl oleate, and ethyl lactate received the highest scores. Ethyl oleate scored mostly Good (2 of 3) while methyl soyate and ArtWash!TM received all Fair scores, and ethyl lactate was highly variable, receiving a Good, Fair and Poor respectively for the three different feathers it was tested on. All other pre-treatments agents scored Poor.

When attempting to remove Orimulsion® without any pre-treatment in both the short and long tests the feathers received a scoring of Poor.

Discussion

Before discussing the contaminants and the efficacy of the pre-treatment products, there are several additional factors to be examined: the pre-treatment's odor, the reaction of the pre-treatment agent with the contaminant, and the hardness and pH of the water used to wash and rinse the feathers. These are all factors that may influence the results of the tests and the ability to use a certain pre-treatment on contaminated feathers.

Odor

Odor of the pre-treatment agent was important for two main reasons: 1) people working with the product, and 2) animals to which the product might be applied. If a pre-treatment agent worked very well but had an offensive or strong odor this might not be the best choice for incidents involving many animals. These types of odor could affect the volunteers and staff working with the product for long periods of time. Also, the odor could remain with the animals after the wash process was

finished. None of the evaluators noted a strong or offensive odor to the pre-treatments that worked best on the contaminants tested in this study.

Reaction with Contaminant

Each evaluator was asked to make comments on whether the pre-treatment agent seemed to react with the contaminant. "React" was defined as whether or not the contaminant seemed to change (i.e., contaminant started dripping off or was softened, and dissolved off the feather while resting) once the pre-treatment was sprayed onto the feather and while the feather was allowed to rest for one minute after pre-treatment application. Many pre-treatments had a strong reaction with the contaminant, helping to remove much of the contaminant; however, not all the product was removed, so they still received scores of Fair or Poor. Using this type of information, one evaluator was prompted to do some additional tests including lengthening the period of time a pre-treatment was allowed to remain on the feathers and increasing the amount of agitation given to the contaminated feathers before starting the wash procedure. By altering the procedure in this manner, agents that initially received scores of Fair or Poor, now had their scores changed to Excellent or Good.

Water Hardness and pH

Water hardness and pH were checked before testing began on each set of contaminants. Water hardness has been shown to affect the wash process (Bryndza et al., 1990), so it was important to monitor these levels to insure that the hardness was not affecting the results. There were four days when the water hardness was over the current acceptable range of 3.5 ppm, but it never reached higher then 4.1 ppm. When comparing the results of the tests run on these days with the results from the same variables (contaminant type, time contaminant was on feather) but different evaluators, there was no obvious difference in scoring.

The pH remained fairly consistent throughout the experimental time period. The small variation in pH did not seem to make a difference in the scoring between tests with the same variables but different evaluators.

The Contaminants

The used cooking oil applied to the feathers during this experiment was obtained from a local elementary school cafeteria, which had used the vegetable oil for preparing food. Tri-State has seen many different species of birds that have been contaminated by cooking oils due to open waste barrels or to dumping of waste oil down storm drains. Although the vegetable oil used in this experiment did not need any pre-treatment product to assist in removal from the feather, this does not mean all used cooking oils act in the same manner. Knowing what type of cooking oil has contaminated the bird is essential in deciding on cleaning procedures.

Crude oil was obtained from a local oil refinery in Marcus Hook, Pennsylvania. It was an Escravos crude oil and was chosen as a representative of the crude oils commonly refined in the areas (Pennsylvania, New Jersey and Delaware) neighboring Tri-State. This type of crude oil did not need any pre-treatment to assist in removal from the feather. There are many different types of crude oil, and their unique properties may make certain types harder to remove. However, Elastol performed the worst and could be exempted from any further experiments using crude oils. This pre-treatment, although reacted with the contaminant was difficult to remove from the feather; after the wash and rinse process there was still Elastol on feather, decreasing the feather's ability to regain waterproofing.

Tanglefoot® Bird Repellent (The Tanglefoot Company, Grand Rapids, MI) was obtained from a local agricultural feed store. According to the manufacturer, "Tanglefoot Bird Repellent adheres to all surfaces while retaining its soft, sticky elasticity. It simply deters nuisance birds from their resting places by making roosting areas undesirable" (www.tanglefoot.com/products/birdrepel.htm). Songbirds are frequently found with this material adhering to their body and wing feathers. Removal of Tanglefoot® worked better after the product remained on the feathers for 3 weeks. This suggests that birds arriving at a rehabilitation facility with this product should potentially not be washed right away, especially if the contamination occurred recently. During the experiment feathers freshly contaminated and then washed seemed to have the Tanglefoot® distributed to other parts of the feather when agitated by hand. On a whole bird this might cause further contamination of previously clean feathers.

Pre-treatments Simple Green All Purpose Cleaner, methyl soyate, ArtWash!TM, ethyl oleate and Elastol, all had either notable reaction with Tanglefoot® when first applied or received higher scores during the trial with feathers contaminated for three weeks. These products might need to stay on the feathers for a longer period of time before starting the wash process. Additional subjective tests should be performed using these pre-treatment agents before the objective tests begin.

The roofing tar (Gardner Asphalt Corporation, Tampa, FL) was obtained from a local hardware store. Birds often become contaminated with roofing tar during the spring and summer months when new sealant is being applied to building tops. There are many different types of roofing tars, and this study only tested one, so pre-treatment results may vary.

Roofing tar did not fully wash off of the feathers in any of the tests, although a few pre-treatments did have some strong reaction with the contaminant. During the short contamination period, evaluators noted that the tar started to drip off of the feather with the application of the pre-treatments that received a score higher then Poor. Canola oil also had a strong reaction with the tar, but still received an overall score of Poor. The same reaction with the contaminant was observed with these pre-treatments on the feathers that had been contaminated for at least 3 weeks. However,, in the long interval trials, all the pre-treatments received scores of Poor by all evaluators.

These pre-treatments should be tested again and the pre-treatment agents allowed to stay on the feathers for longer then one minute. The results may receive higher scores, and a pre-treatment agent may be found that will remove roofing tar with greater ease.

Since roofing tar was slightly easier to remove when a feather was freshly contaminated, all efforts should be made to remove this contaminant as soon as the bird is stabilized.

The silicone used in this study was GE Silicone II* 100% Silicone Sealant for Window & Door (General Electric Company, Huntersville, NC), obtained from a local hardware store. Over the past few years Tri-State has received birds and taken calls concerning birds getting caught in sealants such as silicone. This product is used as caulk around the outside of windows and swimming pools. The birds most impacted by these contaminants are songbirds which land on surfaces that have recently been caulked but the silicone has not yet cured.

The silicone was very sticky and wet when first applied to the feathers; this may be why it seemed a little easier to remove during the short tests. Some of the product probably stuck to the gloves of the evaluators and small amounts could be rubbed off when agitating by hand. Still, much of the fresh silicone remained on the feather and the agitation seemed to spread the remaining product to other parts of the feather. The scoring for all the pre-treatments and non pre-treated feathers remained low, with only one pre-treatment receiving a score of Fair by at least two of the evaluators. Once the silicone had fully dried on the feathers, several evaluators noted that it seemed to peel away from the feathers. Elastol did remove some of the silicone.

Silicone appears to be a contaminant that comes off easier after it has dried fully, especially since when it is fresh it can easily be re-distributed to other parts of the feather. If attempting to remove silicone when fresh, it might inadvertently contaminate feathers that were previously clean.

Orimulsion® (Bitor America Corporation, Boca Raton, FL) was obtained from the manufacturer. Orimulsion® is used as a commercial fuel for power plant boilers worldwide; a spill involving this product could easily contaminate seabirds and waterfowl. Only one evaluator attempted to remove Orimulsion® after one hour and after three weeks of contamination time. This was decided because past studies have shown Orimulsion® very difficult to remove (Miller et al., 2005).

Methyl soyate might be a useful pre-treatment to remove Orimulsion when a bird has recently been contaminated. ArtWash!TM, ethyl oleate and ethyl lactate did not perform quite as well, but may be viable substitutes. Since ArtWash!TM, ethyl oleate and ethyl lactate did not perform as well after one minute on the feather, allowing them to remain on the feather for longer periods of time before washing may help to increase their ability to facilitate in contaminant removal.

After Orimulsion® remained on the feathers for three weeks it was more difficult to remove. Ethyl oleate performed better then methyl soyate, ArtWash!TM, and ethyl lactate; however, if these pre-treatments remained on the contaminated feather for longer than a minute they may receive higher scorings.

Additional Testing

When evaluators noted that a pre-treatment agent was reacting with a particular contaminant, a few further tests were performed resulting in higher scoring of the feather condition. When a pre-treatment that appeared to be "working" or strongly interacting with several of the harder contaminants to remove, those pre-treatment agents were allowed to remain on the feathers longer before washing; the pre-treatment agent remained on contaminated feathers for five, ten, or fifteen minutes to see if there was a difference in the feather after the wash processes. There was also additional hand agitation on the feather when the pre-treatment was applied, by stroking the feather three to five times half-way through the longer resting period.

The results from these changes in the procedure increased the scoring of some of the pre-treatment agents. Other pre-treatment agents had no difference as compared to the standard protocol. The subjective evaluators noted that there was a higher success at removal of the contaminant when the consistency of the pre-treatment agent was similar to that of the contaminant. An example of this was Elastol; Elastol seems to have potential to remove thicker products such as silicone and Tanglefoot®. This product seemed to bind with these contaminants and allow them to fully come off the feather. When Elastol was used with used cooking oil, crude oil, roofing tar and Orimulsion®, it reacted with the contaminant but could not easily be removed from the feathers.

When Elastol was left for 15 minutes on feathers contaminated with Tanglefoot® for at least 3 weeks, all of the Tanglefoot® was removed, and the Elastol was given a score of Excellent. Further testing is needed to confirm this result, as it was only performed on one feather. Tests are also needed to see whether or not Elastol would have the same results with feathers that had been contaminated for at least one hour, and if other pre-treatment agents would perform better when left on the feather for 15 minutes.

Methyl soyate, ArtWash!TM, ethyl oleate, Elastol and ethyl lactate were allowed to remain for five minutes on feathers that had been contaminated with roofing tar for three weeks, and then were cleaned following the standardized protocol. There were slight to no differences noted with ethyl oleate, Elastol and ethyl lactate; only methyl soyate, and ArtWash!TM received a score of Fair. The results changed when the pre-treatments stayed on for ten minutes: Ethyl oleate and methyl soyate worked the best. Ethyl oleate received a score of Excellent, and methyl soyate received a Good; in these two trials both feathers had only a small amount of staining remaining on their tips. These tests were only performed on one feather each by one evaluator, so further tests are needed to confirm these results.

Silicone was removed from feathers contaminated for at least 3 weeks when Elastol was left on for five minutes; the cleaned feather was given a score of Excellent. This test was performed by three different evaluators on one feather each. One noted that the feather did seem a little damaged, but it was not determined if the damage occurred from the silicone or was pre-existing.

When the pre-treatments were initially chosen, the harmful effects on skin exposed for a short time seemed negligible as deduced from reading the material data safety sheets. Increasing the time exposure to remove the contaminant from the feathers may lead to more skin problems for the animals. This possibility should be addressed, and consultation with a veterinarian and/or toxicologist should always occur before choosing any pre-treatment option.

Potential Sources of Error

As mentioned earlier, pH, water hardness, and water temperature were considered as possible sources of inconsistency/error in the procedure. However, the results demonstrated that variations in these factors played little if any role in affecting the results. Other variables that may have affected the results include true time of contaminant exposure, the malfunction of one spray bottle, and variation in evaluators' techniques.

The goal at the beginning of the experimental period was to have the short contamination period be only one hour. The total time to run a trial of one contaminant testing all fifteen pre-treatment agents was between four and six hours, depending on the number of feathers tested and the individual evaluator. It proved difficult to efficiently run the short tests and also keep all the feathers' contact time with the contaminant close to an hour. All feathers ended up being contaminated at the same time and allowed to sit for an hour before the cleaning began. As a result, by the time the last feather was tested it may have been contaminated for up to seven hours. Consequently, products tested last may not have worked as well as the products tested first. The contaminant may have "weathered" onto the feather and caused a decrease in the scoring result. This potential for error was minimized by having each evaluator test the pre-treatment agents in a different order, so that no one agent was always tested last.

All of the feathers that needed to be contaminated for three weeks were contaminated at the initial set-up of the experiment. Schedules changed for the evaluators who were volunteering their time to run the tests. Consequently, several of the long-term contaminated products did not get tested for an additional one to two weeks.

The initial spray bottle containing pre-treatment ArtWash!TM malfunctioned, and an identical spray bottle could not be found to replace it. ArtWash!TM was then placed in a different spray bottle, so the amount sprayed onto the contaminated feathers may have been different. This might have affected the results for this pre-treatment product, causing it to score either higher or lower then it would have in a bottle identical to the others.

Since this experiment was a subjective evaluation, individual judgment was used to score and evaluate the effectiveness of each of the pre-treatment agents on the different contaminants. Human error or differences in personal technique can play a role in experimental error. Because of this, every effort was made to minimize these potential errors: each evaluator repeated the process two to three times,

several different evaluators conducted identical tests to remove potential bias, and each step and the evaluation scoring were standardized.

Due to some unexpected limitations of the volunteers' availability, two additional Tri-State staff members assisted in the project. As a result, all pre-treatment agent testing on long-exposure to Tanglefoot® was conducted by staff members, while testing on short-exposure to Tanglefoot® was conducted by one staff member and two volunteers. One complete set of all the three-week trials (crude oil, used cooking oil, silicone, Tanglefoot® and roofing tar) was performed by a staff member, while the corresponding one-hour trials were conducted by volunteers. Only one evaluator tested all of the pre-treatment agents on all six of the contaminants for both the short- and long-exposure tests. Due to the variation in evaluators, there many have been slight subjective differences, especially for the results on Tanglefoot®.

Changes for Future Experiments

The scoring used was similar to that used in the experiments testing surfactants. This was done to keep consistency between projects, but it often proved difficult during this experiment. Several times a feather would receive a lower score because of a small amount of contaminant remaining on the feather. The feather would appear clean and waterproof everywhere else, but due to that small amount of contaminant, that pre-treatment would be scored Poor. When attempting to remove the same contaminant without a pre-treatment agent, often nothing would come off, and an evaluator would give it the same score of Poor. There was an obvious difference between the pre-treated feather and the non pre-treated feather's overall clean status, but this was not reflected in the four scoring categories.

Involving Tri-State's volunteers was an important part of this process; the two individuals who participated in this study were part of the Core Team, a group of very skilled and dedicated volunteers. Having volunteers involved in the study helped to reduce bias, and allowed for a more realistic evaluation of how the pre-treatment agent might work when used by different individuals washing a whole bird. As volunteers, however, they had a limited amount of time to assist with testing, and only tested two feathers with each pre-treatment agent, while staff evaluators tested three feathers on every trial. In future tests of this nature, the same evaluators should perform all the tests with all of the different variables (contaminant type and length of contaminant on feathers). This would ensure that the subjective evaluations remained constant throughout the whole experiment.

Wildlife professionals do not usually receive a bird directly following a contamination event, which is the reason for a test performed after three weeks. One hour is a very short period of contaminant exposure, so changing this to 24 hours might be more realistic given the normal treatment of oiled wildlife. Even if brought to a rehabilitation center directly following the contamination event, most animals need to be stabilized and given a rest period before cleaning can be attempted. This paper is

not suggesting that these practices be changed. A bird's overall health must be taken into consideration before addressing the removal of the contaminant.

Next Steps

There are two additional steps planned to complete this project: the objective testing by DuPont, and further subjective testing. Based on the results of this subjective screening test, DuPont will be testing specific pre-treatments on specific contaminants (Table 1).

Table 1. Pre-treatment agents that subjectively worked the best on four of the contaminants tested.

DuPont will objectively test the pre-treatment agents with the contaminants listed in this chart. Used cooking oil and crude oil came off of feathers and received a high score when no pre-treatment agents were applied.

Contaminant	Pre-Treatment
Tanglefoot®	Simple Green All Purpose Cleaner
	Methyl Soyate
	ArtWash!™
	Ethyl Oleate
	Elastol
Orimulsion®	Methyl Soyate
	Ethyl Oleate
	ArtWash!™
	Ethyl Lactate
Roofing tar	Methyl Soyate
	ArtWash!™
	Ethyl Oleate
	Elastol
	Ethyl Lactate
Silicone	Elastol

Since the used vegetable cooking oil and Escravos crude oil in this trial were both easily removed, and we see different types of these oils on a regular basis, testing different types of these oils might prove useful. Used and unused cooking oils derived from animal fats are traditionally more difficult to remove from birds, so these products will be examined in future trials.

There are several other petrochemicals that can be difficult to remove depending on their stage of refinement and length of time remaining on the birds, such as #6 oil. The pre-treatment agents that consistently performed the best during this experiment should be evaluated on other oils to see if they might be effective on these products as well.

Conclusions

This project was highly subjective; however, the tests did reveal some usable information:

- The results show that the pre-treatments methyl soyate, ArtWash!TM, ethyl oleate, Elastol and ethyl lactate have the potential to remove a variety of contaminants from feathers.
- Choosing a pre-treatment agent specific to the type of contaminant and how long it has been on the feathers is important. At this time the authors have not found one pre-treatment agent that removes all contaminants with equal efficacy.
- These results might not be true for mammals; additional testing is needed to understand what works best on fur and hair.

With the information gathered from this project, additional testing is still needed. Further subjective testing will help refine the procedure and continue the evaluation of these products as well as several pre-treatment agents that arrived at Tri-State during or after the testing period. Objective testing by DuPont is needed to confirm and quantify all of these results.

Acknowledgments

Thank you to Maureen Barrett, Anne Kisielewski and Katie Battaglia for all the hours spent in assisting with the subjective testing. Their added enthusiasm to the project was greatly appreciated; to Cindy Naylor and Vera Lee Rao for volunteering to aid the evaluators to ensure the experimental materials were available and to the staff and volunteers at Tri-State Bird Rescue & Research, Inc. for their patience during this project.

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Appendix A: Index of Pre-Treatment Agents Tested Subjectively

A	Vapor Remed (Sarva Bio Remed, Trenton, NJ, www.sarvabioremed.com)
B	Canola Oil
C	Botanic Gold (PureTec International, Milpitas, CA, www.symmetrydirect.com)
D	Simple Green All Purpose Cleaner (Simple Green, Huntington Harbour, CA, www.simplegreen.com)
E	VertecBio Gold (Methyl Soyate) (VertecBio, Downers Grove, IL, www.vertecbiosolvents.com)
F	Spill Remed (Marine) (Sarva Bio Remed, Trenton, NJ, www.sarvabioremed.com)
G	Murphy's® Oil Soap (Colgate-Palmolive Company, New York, NY, www.colgate.com)
H	ArtWash!TM (Rembrandt Graphic Arts, Rosemont, NJ www.rembrandtgraphicarts.com)
I	Ethyl Oleate (Spectrum Laboratory Products, Inc., Gardena, CA, www.spectrumchemical.com)
J	Asphalt Stain Remover (DuPont Chemical Solutions Enterprises, Wilmington, DE)
K	Elastol (Design Engineering Systems Analysis, LLC, Alexandria, VA, www.elastol.com)
L	Extreme Simple Green Aircraft & Precision Cleaner (Simple Green, Huntington Harbour, CA, www.simplegreen.com)
M	Spill Remed (Freshwater) (Sarva Bio Remed, Trenton, NJ, www.sarvabioremed.com)
N	Dawn® Power Dissolver (Procter & Gamble, Cinicnnati, OH, www.pg.com)
O	VertecBio EL (Ethyl Lactate) (VertecBio, Downers Grove, IL, www.vertecbiosolvents.com)

Appendix B: List of Contaminants

Crude oil: Escravos crude obtained from a local refinery in Marcus Hook, Pennsylvania

Used cooking oil: Clear vegetable frying oil obtained from a local elementary school in Mount Laurel, New Jersey

Silicone: GE Silicone II* 100% Silicone Sealant for Window & Door (General Electric Company, Huntersville, North Carolina)

Roofing tar: Fiber Roof Coating (Gardner Asphalt Corporation, Tampa, Florida)

Tanglefoot®: Tanglefoot® Bird Repellant (The Tanglefoot Company, Grand Rapids, Michigan)

Orimulsion®: Orimulsion® (Bitor America Corporation, Boca Raton, Florida)

Appendix C: Safety Protocols

Required PPE:

Nitrile Gloves

Tyvek Sleeves

Apron

- There will be a specific labeled trash can for any items that have contaminant or pre-treatment agents on them (i.e., syringes used to apply contaminant, nitrile gloves, dirty Tyvek sleeves, feathers).
- All syringes should be separated into two parts before placing in the trash.
- Contaminated water needs to go in to the waste tanks.
- When contaminating, applying pre-treatment and washing/rinsing feathers, proper PPE must be worn at all times.
- A new pair of nitrile gloves should be put on before applying a different pre-treatment agent to a contaminant. When repeating a test with the same pre-treatment/contaminant combination, it is ok to reuse nitrile gloves. A new pair of nitrile gloves should be put on before working with a new contaminant.
- Keep nitrile gloves on during the rinse procedure.

DAWN®: A partner in saving oiled wildlife for more than twenty years

C. Tibazarwa,[1] G. Williams,[1] A. McConnell[2]

From the shores of Alaska to the Galapagos Islands, the use of Dawn® dishwashing liquid to gently clean aquatic birds such as ducks, pelicans, gulls, and egrets has found wide application by wildlife rescuers, worldwide. An overview will be provided of work done by Dawn® with wildlife partners in the United States, Canada, and Western Europe. One example took place at the 8th International Effects of Oil on Wildlife Conference: on this occasion, Dawn® and the Tri-State Bird Rescue and Research, Inc. (TSBRR) announced a three-year partnership to create the Canadian Oiled Wildlife Academy (COWRA). The mandate of COWRA is to educate and prepare wildlife rescue teams in the event of oil spills. We will report on the first two years of COWRA, results to date, and future opportunities to continue making a meaningful contribution to wildlife rescue programs. Through Frequently Asked Questions (FAQs) handouts, we will address technical product questions important to wildlife rescuers, and most frequently asked by international wildlife rescue groups.

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Effects of oil on the smolts of salmon *(oncorhynchus tshawytscha)* – preliminary muscle results

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Introduction

Currently all salmon species are classified as "threatened" under the U.S. Federal Endangered Species Act. While they are struggling to recover from the combined effects of over-fishing, habitat decline and pollution, there is serious concern that marine oil spills and associated response activities near rivers of spawning importance may impact smolts entering the ocean.

A number of studies have investigated the toxicity of oil to salmon, particularly using embryos, alevins, and fry of the Alaskan pink salmon *Oncorhynchus gorbuscha* (Rice et al., 1975; Swartz, 1985; Heintz et al., 2000; Rice et al., 2001) and coho salmon *Oncorhynchus kisutch* (Stickle et al., 1982; Thomas et al., 1987, 1989). However, there is currently little information in the scientific literature comparing the lethal and sublethal impacts of oil and dispersed oil on salmon smolts. Due to extensive maritime transport of crude oil from Alaska to California, there is significant potential for a catastrophic spill, which could seriously impact salmon populations during key periods of their migration, particularly when smolts are entering the ocean from native streams and rivers. Information on the relative toxicity of dispersed and un-dispersed oil is therefore needed by resource agencies responsible for coastal spill response activities. Therefore, this investigation compared the toxic actions of the water-accommodated fraction (a naturally dispersed fraction; WAF) and chemically dispersed fraction (a chemically enhanced water-accommodated fraction; CEWAF) of Prudhoe Bay Crude Oil (PBCO) to the smolts of Chinook salmon *(Onchorhyncus tshawytscha).*

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Materials and Methods

Toxicity Tests

All methods followed Singer et al. (1998), and recent standardized methods recommended in Singer et al. (2000). All testing was conducted using Prudhoe Bay crude oil (PBCO) obtained from Resource Technology Corporation (Laramie, WY), and Corexit 9500, obtained *gratis* from Nalco/Exxon Energy Chemicals, L.P. (Sugar Land, TX). Chemical dispersion of oil was carried out at a nominal oil:dispersant ratio of 10:1 (v:v). Untreated oil testing was performed using the water-accommodated fraction (WAF) of unweathered PBCO, and untreated oil WAFs were prepared using a standardized low-energy mixing method (Singer et al., 2000).

CEWAF tests were performed with solutions prepared in much the same way as WAFs. Mixing energies used to prepare CEWAFs were increased to create a vortex 20–25% of water depth to provide sufficient mixing energy for dispersion. Once the vortex was established, known volumes of oil and dispersant were delivered in sequence into the center of the vortex using gas-tight Hamilton syringes (Singer et al., 1998). Exact masses of oil and dispersant delivered were calculated by difference. Mixing lasted 18 h, followed by 6 h of settling time to allow the largest oil droplets to resurface (Singer et al., 2000). The 18-h mix:6-h CEWAF settle regimen was used to match the 24-h total preparation time used for WAFs.

Spiked-exposure 96-h toxicity tests were completed with salmon smolts *(Oncorhynchus tshawytscha)* using established test procedures (Singer et al. 2000), modified to accommodate larger organisms. Chinook salmon smolts (~6 cm) were obtained from the CDFG American River Hatchery and acclimated to seawater culture conditions prior to testing. At the end of each 96-h test, two surviving fish from each of three replicates were dissected for metabolomic analysis (described below).

Water temperature, DO, and pH were monitored daily during testing. Diluent was natural seawater filtered to ~20 μm at ambient salinity (~33‰). Spiked exposures were conducted in sealed, 18-L polycarbonate flow-through exposure chambers. Tests involved six treatments: five WAF or CEWAF treatments and a seawater control, with each treatment having three replicates (eight salmon per replicate). After test initiation, concentrations in all chambers were monitored hourly for 7 h using total carbon (TC) analysis, accomplished by high temperature combustion on a Teledyne Apollo 9000 TOC analyzer (Teledyne, Santa Clara, CA). In order to minimize loss of the lowest boiling-point fractions, TC samples were collected by gas-tight syringe directly from each chamber through the Teflon septum and analyzed immediately. TC data were used to assess acceptability of oil decline rates (Singer et al., 1998).

Variation within and among test populations was assessed by using three replicate exposure chambers within each test treatment and by running three replicate tests for each species/toxicant (WAF

or CEWAF) combination. Median-effect concentrations (LC50) were estimated using the trimmed Spearman-Karber procedure (Hamilton et al., 1977).

Hydrocarbon Analysis

Measurement of hydrocarbons (TPH: C_{10}–C_{36}) was accomplished using a Hewlett-Packard 6890 gas chromatograph fitted with a flame ionization detector (FID), while concentrations of volatile benzene, toluene, ethylbenzene and xylenes (BTEX: C_6–C_9) were analyzed using a Hewlett-Packard 6890-5973 GC-MS equipped with a purge & trap concentrator (US EPA Method 8260). One liter water samples were extracted three times with dichloromethane (DCM) by liquid-liquid extraction. After each extraction, the solvent phase was collected and combined, with the final extract made up to 1-10 ml volume depending on the color in the extract. Quantitation was performed against a set of PBCO standards to better represent the number and relative proportions of the various DCM-soluble compounds contained in the oil (Payne, 1994). Samples were measured by summation of the total resolved chromatogram peak area, after subtraction of dispersant peaks when appropriate (Payne, 1994). They were then quantified using the average response factor of the similarly integrated whole oil standards. While unresolved or non-chromatographable compounds were not directly measured, their inclusion in the mass of oil used to prepare standards allowed them to be accounted for in response factor calculations (Payne, 1994). This technique did not allow for direct quantitation of individual hydrocarbons; however, it produced concentrations based on the total response of samples (corrected for background response of the seawater). Designated total hydrocarbon content (THC = BTEX + TPH), it was not biased by quantifying a specific set of target analytes (Girling et al., 1994).

Metabolomics Analysis

A total of 4 WAF and 4 CEWAF tests were processed via metabolic analysis. After 96-h exposure, two surviving fish from each replicate tank were sacrificed. Muscles were immediately dissected, flash frozen in liquid N2, and stored at -80°C. They were pulverized in a liquid N2-cooled mortar and lyophilized overnight. The homogenous dry tissue powder was weighed, and then extracted with 30 mL/g (dry mass) of methanol/water (2/1). Samples were vortexed for 15 sec three times and put on ice in between. Following centrifugation (10,000 x g, 10 min, 4°C), 0.46 mL of supernatant was removed and then lyophilized prior to NMR analyses.

Metabolomic analysis was performed as previously described, with slight modifications (Viant, 2003). Lyophilized extracts were resuspended with sodium phosphate buffer in D_2O (0.1 M, pH 7.4) containing sodium 3-trimethylsilyl-2,2,3,3-d4-propionate (TMSP) as an internal chemical shift standard. All spectra were measured at 500.11 MHz using Avance DRX-500 spectrometers (Bruker, Fremont, CA). Acquisition parameters for 1D NMR consisted of a 9-µs (60˚) pulse, 6-kHz spectral width, 2.5-s relaxation delay with presaturation of the residual water resonance, and 100 transients collected into 32k data points, requiring a 9-min total acquisition time. All data sets were zero-filled

to 64k points, exponential line-broadenings of 0.5 Hz were applied before Fourier transformation, the spectra were phase and baseline corrected and then calibrated (TMSP, 0.0 ppm) using XWINNMR software (Version 3.1; Bruker).

2D J-resolved NMR spectra were acquired using 4 transients per increment for a total of 32 increments, which were collected into 16k data points using spectral widths of 6 kHz in F2 (chemical shift axis) and 40 Hz in F1 (spin-spin coupling constant axis). A 3.0-s relaxation delay was employed, giving a total acquisition time of 11 min. Datasets were zero-filled to 128 points in F1, and both dimensions multiplied by sine-bell window functions prior to Fourier transformation. Spectra were tilted by 45º, symmetrized about F1, calibrated (TMSP, 0.0 ppm), and the proton-decoupled skyline projections (p-JRES) obtained, all using XWINNMR.

Each spectrum was segmented into 1960 chemical shift bins between 0.2 and 10.0 ppm, corresponding to a bin width of 0.005 ppm (2.5 Hz), using custom-written ProMetab software (Version 1; Viant, 2003) in MATLAB (The MathWorks, Natick, MA). The area within each spectral bin was integrated to yield a 1 x 1960 vector containing intensity-based descriptors of the original spectrum. Bins representing the residual water peak (from 4.60 to 5.20 ppm) were removed. Some groups of bins were compressed into a single bin in order to capture peaks with variable chemical shifts into one bin. The total spectral area of the remaining bins was normalized to unity to facilitate comparison between the spectra. The binned data was subject to generalized log transformation and the columns mean-centered before multivariate analysis. Principal component analysis (PCA) of the pre-processed NMR data was conducted using the PLS_Toolbox (Version 3.5; Eigenvector Research, Manson, WA) within MATLAB.

Results and Discussion

Comparative Toxicity of WAF and CEWAF

Hydrocarbons were measured three times: at test initiation, after 8 h of exposure, and after 24 h of exposure; THCs attained background levels within 8 h. The initial THCs were used to calculate 96-h LC_{50}s, and salmon smolts were found to be 20-fold more sensitive to the WAF of PBCO than to its CEWAF: the mean 96-h LC_{50} for the WAF was 7.46 mg/L, while for the CEWAF it was 155.93 mg/L. This suggests that although there were higher hydrocarbon concentrations in the CEWAFs, their bioavailability was reduced. In comparing the toxicity of PBCO WAF and CEWAF using topsmelt larvae *(Atherinops affinis)*, Singer et al. (1998) also found WAF to be more toxic. Also similar to this study, they found that the WAFs were composed of an average of 96% volatiles (compounds chromatographing earlier than naphthalene), whereas the CEWAFs contained only 67% volatiles.

NMR Metabolomics of Muscle

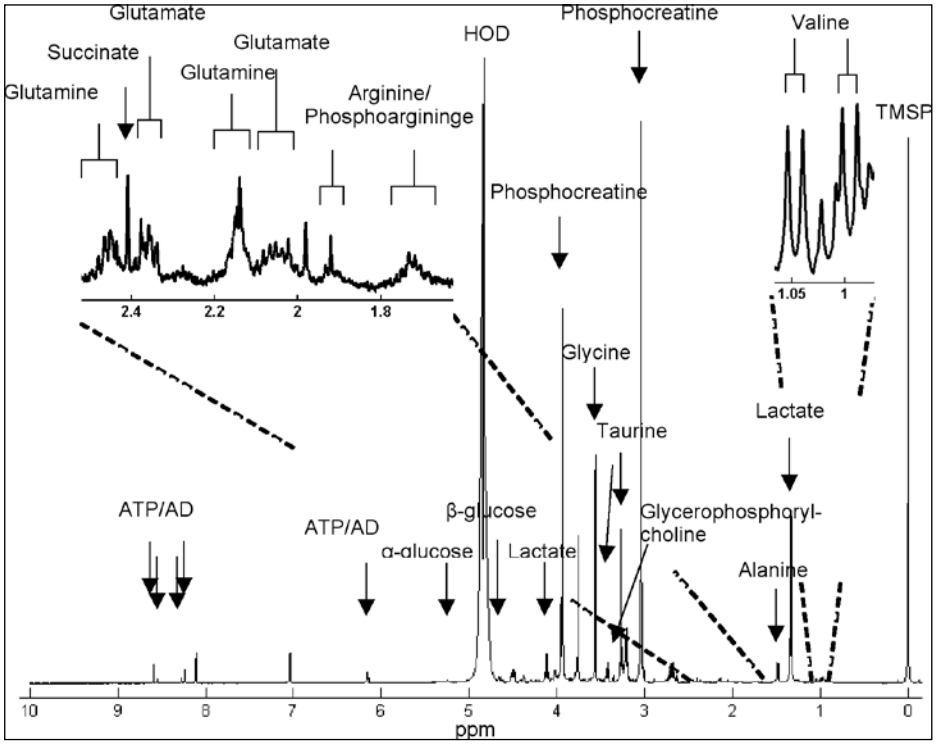

Figure 1. Representative 1D 1H NMR spectrum of a smolt muscle extract.

A representative 1H spectrum showing the metabolic fingerprint of a muscle extract is presented in Figure 1. Major metabolites in both the 1D ^{1}H and p-JRES NMR spectra were assigned by comparison to tabulated chemical shifts and (for the 1D data) from the peak multiplicity (Fan, 1996; Table 1). Some metabolites were further confirmed by ^{1}H-^{13}C heteronuclear single quantum coherence (HSQC). Phosphocreatine and several amino acids (e.g. alanine and glycine) were dominant in the spectra. Other observed metabolites included carbohydrates (e.g. glucose), nucleotides (e.g. ATP and ADP), glycolytic products (e.g. lactate), and citric acid cycle intermediates (e.g. succinate).

Although the 1D ^{1}H spectra of the muscle extracts exhibited some degree of peak overlap, especially between chemical shifts 3 to 4 ppm, the metabolic fingerprints provided valuable information for differentiating metabolic effects. The 1D ^{1}H spectra of 3 WAF tests and 2 CEWAF tests which used the same cohort of fish were analyzed by PCA. Loadings plots (Figures 2 and 3) demonstrate peaks in the spectra contributing to the variation in corresponding principal components. Peaks with positive loadings (e.g. glycerophosphorylcholine in Figure 3) correspond to metabolites that have higher levels in the controls. Several peaks that show the greatest change in the loadings plots in response to exposure have been identified.

Table 1. Metabolites identified from muscle of salmon smolts by NMRa.

Metabolites	1H NMR assignment (ppm)c
Valine	0.995 (d), 1.047* (d)
Lactateb	1.328* (d), 4.115* (q)
Alanineb	1.488* (d), 3.785 (m)
Arginine/Phosphoarginine	1.725 (m), 1.918* (m)
Glutamate	2.070 (m), 2.355 (t)
Glutamineb	2.138 (m), 2.455* (m)
Succinate	2.410* (s)
Phosphocreatineb	3.039* (s), 3.934 (s)
Taurineb	3.268* (t), 3.423* (t)
Glycerophosphoryl-choline	3.358* (s)
Glycineb	3.563* (s)
β-glucoseb	4.650 (d)
α-glucose	5.238* (d)
ATP/ADPb	6.158 (d), 8.273* (s), 8.546* (s)
IMP	8.238* (s)
AMP	8.593* (s)

* Peaks are taken to quantify and compared between treatments.

a Representative metabolites have been selected to illustrate the wide range of metabolite classes detectable with NMR. All peaks are confirmed by p-JRES spectra.

b Metabolite assignment is confirmed by 2D NMR (HSQC).

c Peaks observed as a singlet (s), doublet (d), triplet (t), quartet (q), or multiplet (m).

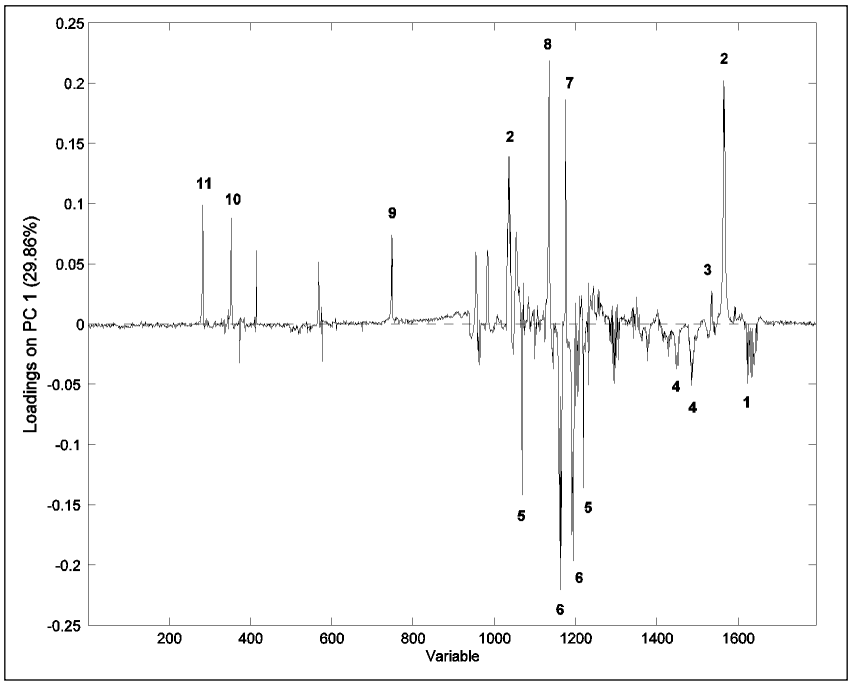

Figure 2. PC1 loadings plot from the analysis of the 1D 1H NMR spectra of the muscle of smolts exposed to WAF. Metabolite assignments: 1. valine, 2. lactate, 3. alanine, 4. arginine/phosphoarginine, 5. phosphocreatine, 6.taurine, 7. glycerophosphoryl choline, 8. glycine, 9. ATP/ADP, 10. IMP, 11. AMP.

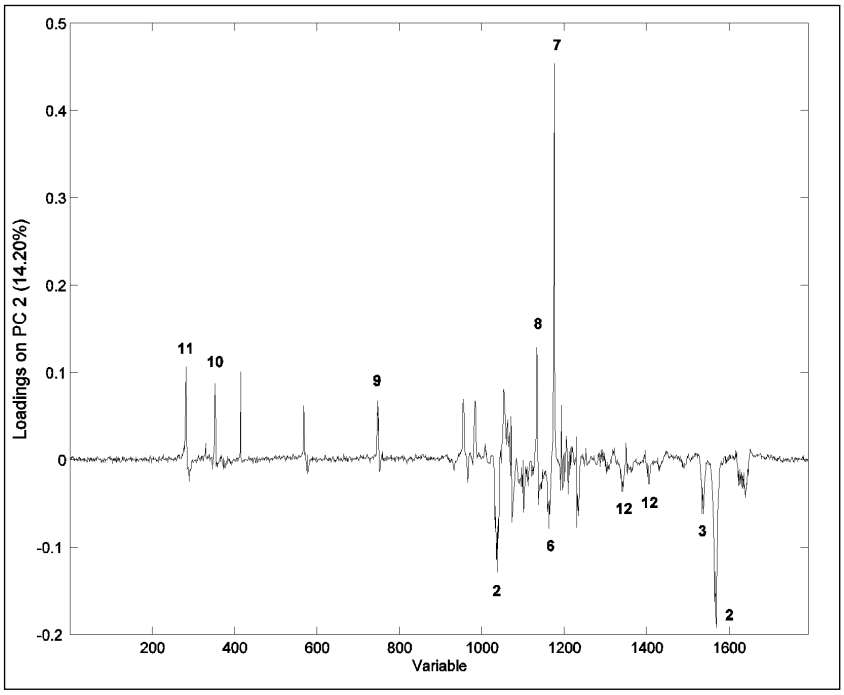

Figure 3. PC2 loadings plot from the analysis of the 1D 1H NMR spectra of the muscle of smolts exposed to CEWAF. Metabolite assignments: 2. lactate, 3. alanine, 6. taurine, 7. glycerophosphorylcholine, 8. glycine, 9. ATP/ADP, 10. IMP, 11. AMP, 12. glutamine.

In summary, amino acids including valine, alanine, arginine/phosphoarginine, glutamate, and glutamine generally increased after WAF or CEWAF exposure. Statistically-significant increases were observed at low doses of WAF (i.e. glutamine; $p<0.05$), intermediate doses of WAF (i.e. alanine; $p <0.01$), and high doses of WAF (i.e. arginine/phosphoarginine; $p <0.05$). Significant metabolite increases were also observed at both low and high doses of CEWAF (i.e. valine; $p <0.05$) but not intermediate doses. The increase of taurine was significant at a high dose of WAF, and at the lowest dose of CEWAF ($p <0.05$); glycerophosphorylcholine decreased in all doses of WAF or CEWAF ($p <0.05$).

Concentrations of some amino acids (i.e. valine, alanine, arginine) decreased significantly at high doses of WAF and CEWAF. In protein catabolism, proteins are hydrolyzed via proteases into their constituent amino acids. An increase in amino acids may therefore result from protein degradation in addition to cell repair. The imbalance of amino acid supply and usage may lead to a delay in development, reproduction, or ability to adjust to stress. Phosphocreatine decreased significantly in muscle tissues of smolts exposed to high doses of CEWAF. It also decreased in smolt liver tissues exposed to WAF. Phosphocreatine's primary role is as a spatial and temporal buffer of ATP. It donates a phosphate to ADP (ATP + creatine ↔ ADP + phosphocreatine), which is regulated by creatine kinase. Once phosphocreatine decreases, the energy supply to the smolt development or movement may potentially be affected. Decrease in phosphocreatine was also observed in medaka embryos exposed to trichloroethylene (Viant et al., 2005), and hypoxic challenge (Pincetich et al., 2005). Phosphocreatine decreased in eyed eggs of Chinook salmon exposed to pesticides (Viant et al., 2006), and in juvenile

steelhead trout subjected to heat stress (Viant et al., 2003). These earlier metabolomic studies also suggested that the decrease of phosphocreatine co-occurred with the decrease of ATP.

In our experiments, both WAF and CEWAF appear to cause similar metabolic effects (albeit at different concentrations) in muscle. Exposure to WAF and CEWAF resulted in the shunting of the production of energetic substrates (ATP or phosphocreatine) and organic osmolytes (glycerophosphorylcholine) towards increased amino acid synthesis. Increased amino acid production is necessary for protein synthesis required for cellular repair. The imbalance of amino acid supply and usage to generate energy may lead to a delay in development, reproduction, or ability to adjust to additional stress. Elevated amino acids in muscle may also have resulted from protein degradation and potential cell injury.

Oil Spill Response

Response decisions regarding the use of dispersants depend on numerous factors that are unique to each spill (NRC, 2005). Our preliminary results with salmon smolts provide important toxicological information for responders, and suggest that dispersant use may not result in increased toxicity to migrating salmon. Because this study used unweathered PBCO, the results may represent a worse-case scenario where dispersant is applied shortly after a spill. In most spill situations considerable weathering would be expected before dispersant application. Because lighter hydrocarbons would be the first to volatilize, they would rapidly decrease in most spill situations. Therefore, under field conditions we might expect less of a disparity between the toxicities of treated and untreated oil.

Acknowledgements

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The implementation of the OWCN-protocol on oiled birds in Belgium, Europe

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Keywords: OWCN, oiled birds, Europe, protocol, rehabilitation, Belgium, volunteers.

Can a small rehabilitation center with one to two staff members, lots of volunteers, poor accommodations and a small budget acheieve high standards for successful rehabilitation of oiled birds? Our rehabilitation center was founded in the 1980s, but over time knowledge and experience were lost due to the lack of staff and the departure of volunteers. With about 2000 animals per year entering the facility, the 100-200 oiled birds were just a small part of our rehabilitation effort. Lack off financial resources, knowledge, proper accommodations and staff resulted in poor rehabilitation success when it came to oiled birds: only 5 to 15%!

February 2003 was the eye-opener. The Tricolorspill brought us 5000 oiled birds in only a few weeks, along with European expertise contacted by Sea-Alarm. By the end of the winter our volunteer group had learned the basics of oiled bird rehabilitation. In the next year, money was found for one staff member (invited by IFAW) to attend training at the International Bird Rescue and Research Center (IBRRC). As a result of this training we decided to implement the OWCN (Oiled Wildlife Care Network) protocols for the care of oiled birds in our center. This was done over the next two to three winter seasons – the only times we get oiled birds in.

Gradually, it was possible to train our group of volunteers and improve the accommodations for oiled birds. By the winter of 2005–2006, the OWCN protocols were totally implemented into our own protocols, resulting in a 67% release rate of oiled guillemots and razorbills! The following winter (2006–2007) showed us that this was not just good luck. Sixty-one percent of the oiled guillemots and razorbills were successfully released.

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Is the oil industry's tiered preparedness and response approach applicable to oiled wildlife response planning?

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Keywords: oil industry, tiered oil spill preparedness and response, risk assessment, contingency planning, animal welfare, tiered oiled wildlife preparedness and response.

Abstract

The oil industry has long used a three-tiered approach to designing and establishing oil spill preparedness and response capabilities. This paper describes how this has been applied in practice to establish levels of oil spill preparedness commensurate with the risk faced, without proliferating expensive, rarely used response resources. The application of this approach in the field of oiled wildlife preparedness and response is discussed in order to evaluate if it would be practical and lead to an improvement in animal welfare and rehabilitation.

Introduction

Since the 1980s the international oil industry has used a three-tiered approach to designing and building oil spill preparedness and response capabilities. It was introduced as a means of ensuring that an appropriate operational response capability was available to deal with oil spills, whilst attempting to prevent the unnecessary proliferation of response resources around the world. The principles of Tiered Preparedness and Response fully support and promote the framework of international cooperation towards oil spill preparedness and response as stipulated in the now widely

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ratified and adopted IMO Convention on Oil Pollution Preparedness, Response and Co-operation 1990 (OPRC).

Despite images of oiled wildlife often being the focus of public and media attention during an oil spill incident, it is one of the areas of oil spill preparedness that is the least developed in many countries, often due to limited funding and a lack of governmental support. This is surprising as oil spills of any size have the potential to significantly impact wildlife, especially in highly sensitive areas.

This paper describes the principles of Tiered Preparedness and Response, in accordance with IPIECA 2007, and illustrates how it is applied by the oil industry in preparing for and responding to oil spills. Consideration is then given to the feasibility of applying the same approach to oiled wildlife planning and response.

Tiered Oil Spill Preparedness and Response

The principles of Tiered Preparedness and Response provide a structured approach to designing and building levels of oil spill preparedness and actually responding to an incident in such a manner that additional resources can be called upon and integrated into a response operation as an incident grows in severity. The definitions of the three tier levels, and consequently their boundaries, have been focused conventionally and simply around volume of oil spilled and the location of an incident in relation to the respective operational activity. Thus:

- **Tier 1** incidents are likely to be relatively small in terms of spill volume, with the incident only affecting the immediate or local area. Tier 1 response resources would normally consist of modest amounts of equipment held on-site, with responders commonly not being dedicated oil spill specialists but having the responsibility in addition to their normal roles. A Tier 1 response would generally be managed by the operator responsible and dealt with appropriately without external assistance.
- **Tier 2** incidents are larger in scale and are of a more regional level. As a result the potential impact is greater, with a more diverse range of response resources being required and a greater number of stakeholders involved. Possible Tier 2 resources may include industry collectively sharing Tier 1 resources on a mutual aid basis; industry funded regional cooperatives or response centres; specialised regional services for a particular risk; and cooperation with governmental or port authorities to draw on neighbouring resources. Depending on the exact situation, Tier 2 responses would usually be managed cooperatively.
- **Tier 3** incidents are much rarer than Tier 2 incidents, however are on a significantly larger scale and consequently likely to impact a much larger area, perhaps on an international level. Tier 3 response resources are held in a relatively few locations around the world, and normally consist of large amounts of specialised and diverse equipment stored in such a manner that it can be mobilised rapidly by dedicated oil spill specialists. Tier 3 resources can be funded directly by industry, or held and managed by governments, often because they have experienced a major

oil spill incident or because they have a significant risk profile. A number of inter-governmental agreements, as well as the OPRC Convention, exist to facilitate international cooperation in the event of a Tier 3 incident.

In reality the boundaries between tiers is far less easy to define than just considering spill volume and incident location. Only by undertaking a risk assessment for a particular oil handling activity will it become apparent that a range of other influencing factors need to be considered in the design of appropriate levels of preparedness.

Risk Assessment Process

In the risk assessment process, potential oil spill scenarios are described and consideration given to their likelihood to occur and potential impact. In determining the risk profile, it will soon become clear that there is a range of factors, beyond potential spill volume and location, which will influence and control the potential severity of the various scenarios. These factors will therefore also need to be considered when designing levels of preparedness. Examples of such factors include oil type, operating environment (including climatic extremes), proximity to sensitive environments or socio-economic resources, and health and safety issues in responding. These factors will inevitably vary greatly between different types of oil handling activity and different locations.

Designing Tiered Capabilities

The risk assessment process will identify the nature and extent of the oil spill risk being faced, which allows boundaries between tiers to begin to be defined and appropriate response capabilities designed. Whilst factors related to the type of oil handling activity and geographic location will be of paramount importance, at this stage another suite of factors may well influence, or even dictate, what is ultimately required in a tiered capability. Examples of such factors include legislative controls; the level of expertise, experience and attitude of regulatory agencies; political stability; and the robustness and availability of Tier 2 and Tier 3 resources.

In some parts of the world governments may have their own priorities when it comes to defining response strategies and these may not be based solely on technical considerations. Similarly, governments may impose excessive minimal levels of required response capability that are not commensurate with the risk profile. Legislative and regulatory controls may also exist, forcing industry to subscribe to certain Tier 2 or Tier 3 response providers. In other regions, political stability can strongly influence the design of response capabilities, for example where reliance on external support may be restricted as a result of difficulties in crossing international borders.

The availability of response options may affect the design of tiered capabilities in other ways. For example, in some remote, lesser developed parts of the world there may be insufficient, or even an absence of any, Tier 2 resources. As a result Tier 1 capabilities would have to be bolstered and greater reliance placed on accessibility to Tier 3 resources. Conversely, in regions where accessibility to in-

ternational Tier 3 resources cannot be relied upon at all times, for example due to distance or lengthy response times, difficult transport links, extreme climatic conditions or known customs or immigration delays, a greater reliance would have to be placed on Tier 1 and Tier 2 options.

There are no prescriptive rules for defining the exact nature of the response capabilities required by any particular oil handling activity. Only by determining the risk profile and taking into account all other influencing factors can tiered capabilities be designed and built with confidence. In some situations this process may identify surprising scenarios. For example, a large spill might be considered a potential Tier 1 incident because a significant amount of oil can be safely contained within a bunded area, or because it can be dealt with effectively using a larger on-site capability. Conversely, a small spill might be regarded as a potential Tier 3 incident because of the difficulty of responding to the particular oil type and the potential severity of the impact on local socio-economic or natural resources.

As a result of all the influencing factors pertaining to a particular situation, it is entirely logical that similar oil handling activities in different locations or different oil handling activities in the same location may require contrasting capabilities. The challenge is to build a manageable, cost-effective solution for those mainly minor, on-site incidents deemed as Tier 1, whilst being fully organised to call in additional robust resources at the Tier 2 and Tier 3 levels if an incident escalates.

Successful Tiered Oil Spill Preparedness and Response

Although response capabilities at each tier level differ in both the scale and diversity of response techniques that they offer, there are a number of essential elements required so that they can be implemented successfully and integrated during an incident:

- **An oil spill contingency plan** is the basis of any successful response. The fundamental components of a comprehensive oil spill contingency plan, including all the points listed below, are described in numerous other publications, for example IPIECA 2000. In terms of tiered response it is vital that due consideration is given to the processes for managing the integration of local, regional, national and international resources, as appropriate.
- **A management framework** needs to be established at the planning stage that clearly defines the roles and responsibilities of the various stakeholders potentially involved in the range of different oil spill scenarios. There will be variations between countries and in many cases the command structure is likely to change in line with the severity of a spill, passing for example from the operator of the facility from which the oil originated for a Tier 1 spill, to a government agency once the incident is regarded as of national or international importance (Tier 3).
- **Response strategies** need to be described in generic terms for the various areas of operation and in detail for particular areas of high environmental or socio-economic importance. On a wider, Tier 2 or Tier 3 scale of incident, strategies need to be more flexible as a greater and more diverse range of response situations may prevail.

- **On-site equipment** stocks should be established that are commensurate with the Tier 1 risk and other influencing factors. Arrangements should be in place to ensure that the equipment and other response resources are well maintained and regularly tested to ensure they are available for rapid deployment at all times.
- **Arrangements for the integration of additional support** at all tier levels need to be established and pre-agreed at the planning stage by both the potential receivers and providers of manpower and equipment. Failure to consider all the required administrative, technical, financial and legal issues in advance of an incident can result in the mobilisation of additional response resources being delayed during an actual spill, with potentially serious consequences.
- **Logistical arrangements** to support response operations across all tier levels need to be defined at the planning stage. These will include ready access to suitable airfields, land transport, boats, fuel supplies and all the other ancillary equipment and materials needed to move additional resources to the site of operations and deploy them promptly and effectively. Arrangements to ensure the welfare and health and safety of the responders themselves will also be a high priority.
- **Trained practitioners** in oil spill response are vital, both on-site and also at the Tier 2 and Tier 3 levels, if all the resources (equipment and manpower) are to be deployed to maximum effect. On occasion the supply of trained and experienced spill practitioners to help coordinate and direct operations will be more important than additional response equipment.
- **A programme of training, including simulation exercises,** involving all potential stakeholders and all tiers of response resource providers is essential to test different aspects of preparedness, build familiarity and ensure competence.

Tiered Oiled Wildlife Preparedness and Response

There are three principal options when oiled animals arrive on the shore: chose not to intervene and leave the animals to survive or die; seek to euthanize the animals so that unnecessary suffering is avoided; or try to capture the animals in order to clean and rehabilitate them so that they can be released back into the wild. Each of these options will be valid, either individually or in combination, depending upon the circumstances, including government policy. The ethical and practical considerations that will need to be taken into account in the decision-making process are described in other publications, including the EU Handbook on Good Practice (see www.oiledwildlife.eu). In this paper the term 'oiled wildlife response' is meant to encompass any appropriate measure to deal with animals affected by oil spills, although much of the consideration relates to rescue and rehabilitation since this will require the greatest amount of planning and largest response capability.

Levels of both oil spill preparedness and oil wildlife preparedness vary greatly around the world. Some countries are well prepared and equipped to deal with both, while many more are far better prepared to respond to oil spills than to oiled wildlife incidents. Some countries (including some that have a good government and industry oil spill response capability) may regard oiled wildlife, and the

great media and public attention it commands, as an unwelcome distraction or even in extreme cases, an irrelevancy. There are then quite a large number of countries where both oil spill and oiled wildlife preparedness is practically non-existent, despite in some cases there being significant oil spill risks and highly vulnerable habitats and species.

In simplistic terms, three categories of country with contrasting relationships between oil spill preparedness and oiled wildlife preparedness can be recognised:

	Sufficient Capability?		Remarks
	Oil Spill Preparedness	Oiled Wildlife Preparedness	
Category 1	☑	☑	Countries with experience of major oil spills and/or a significant oil spill risk are generally well prepared, often through both government planning and industry commitment. Many such countries with sensitive resources have also developed oiled wildlife preparedness as a result of animal welfare charities and voluntary environmental groups working with government agencies and industry. In some cases there is good cooperation between all these stakeholders whereas elsewhere there is considerable room for improvement.
Category 2	☑	☒	Despite the government and industry of some countries being well prepared for oil spill incidents, little or no investment may have been made in oiled wildlife preparedness. This may be a direct result of a perceived low risk of a spill affecting animals; a lack of sensitive resources; or government and/or industry not considering wildlife as a priority, despite in some cases the existence of highly vulnerable resources. Some governments may realise the value of their wildlife at risk, but are unable to invest in their protection, perhaps due to a lack of expertise or available finances. This may be exacerbated by a lack of influential local animal welfare charities or voluntary environmental groups.
Category 3	☒	☒	There are some lesser developed countries that, despite an inherent risk from oil handling activities, do not regard oil spill preparedness as a government priority. This may be due to oil exploration and other oil handling activities being in their infancy or beyond the government's control. In such cases the onus will probably fall on industry to ensure suitable capabilities for oil spill response are available, although in some extreme cases the local operators may remain ill-prepared and unwilling to invest beyond a very basic level. Political instability and civil unrest, as well as conflicting priorities for limited financial resources, may also result in a lack of government investment in oil spill preparedness and response. In all such cases it is highly unlikely that any serious consideration will be given within the country to oiled wildlife, regardless of any highly sensitive areas at risk. If local animal welfare charities or voluntary environmental groups exist they are likely to face a daunting task in the event of an oiled wildlife incident.

This type of categorisation could be broken down even further as the level of both oil spill and oiled wildlife preparedness can vary significantly even within an individual country. It might also be instructive to chart on a global scale the inter-relation between oil spill risk, vulnerable wildlife and

oiled wildlife preparedness since this would highlight where the most serious gaps exist. However, such studies are too detailed for the purpose of this paper, but might form interesting future projects.

Oiled wildlife response is currently evolving rapidly in a number of countries that are experienced in oil spill response. However, it remains the case in most that the rescue and rehabilitation of oiled wildlife relies on voluntary groups, including wildlife, environmental or animal welfare charities, rather than on government agencies. Whilst most such voluntary groups do an excellent job, they all generally lack the financial and other resources (including trained manpower) to carry out detailed pre-spill planning, including the establishment of Tier 1 equipment stockpiles and related facilities in high risk areas. Cooperation between groups within a country or between countries can also be poor and levels of expertise and manpower highly variable, thus hampering Tier 2 and Tier 3 responses. Sea Alarm, now with the active support of the oil industry through OSRL/EARL, continues to be successful in improving this situation (see other papers at this Conference by Hugo Nijkamp of Sea Alarm and Dr Rob Holland of OSRL/EARL). However, it is suggested that fundamental improvements will only come about when wildlife response planning becomes a fully integrated part of government-led oil spill planning and response, with those involved in rescue and rehabilitation efforts having defined roles and responsibilities within the overall planning and response framework. This is now happening in a greater number of those countries in the first category of the table. However, even in some of these countries wildlife response remains a peripheral activity, and sometimes ignored when oil spill contingency plans are prepared, resources allocated and decisions made during actual incidents.

In principle there seems no reason why a tiered approach, as previously described, should not be followed in preparing for and responding to the oiled wildlife component of spill incidents. The approach is flexible and would allow varying capabilities to be established for all the situations described in the above table, thereby enabling oiled wildlife response to be conducted in a uniform and compatible way for small casualty numbers to more severe incidents that require the integration of additional, international support.

In a number of past spills around the world, an escalation from purely local resources to those available in other parts of the same country or from further afield has been necessary in order to rescue and rehabilitate oiled birds (and to a lesser extent marine mammals, reptiles and other animals). However, in most cases this pseudo-tiered response has occurred on an ad hoc basis during an actual incident and has required coordination by organisations such as Sea Alarm, as well as the direct intervention of international experts provided by groups such as the International Fund for Animal Welfare's Emergency Response Team. In general such required escalation has not been pre-planned, crucially resulting in delays and adverse consequences for the welfare and rehabilitation of affected wildlife.

Risk Assessment Process

Many different oil spill scenarios have the potential to impact wildlife. Small spills, which might otherwise be classified as Tier 1 from a general oil spill response perspective, may result in a large number of animal casualties and therefore qualify as a Tier 2 or 3 oiled wildlife incident. The reverse is also possible when a major Tier 3 spill occurs well away from concentrations of vulnerable wildlife. A risk assessment focussed on wildlife concerns should therefore form the basis for developing suitable levels of oiled wildlife preparedness. This will be analogous to, but distinct from, any related risk assessment for oil spill preparedness.

Potential oil spill scenarios that may impact birds, mammals, reptiles and other animals within defined geographic limits should be identified, along with their likelihood to occur. A wide range of factors will need to be taken into account to gain a realistic appreciation of the risk profile that needs to be planned for. Many of these factors, such as type of oil, will be the same as for the oil spill risk assessment process described previously. However, for oiled wildlife preparedness it will be important to give far greater attention to factors such as the location of key habitats, populations or colonies; the different species of animals at risk; their vulnerability to oiling and potential impacts; their ability to recover from these impacts; their rarity and conservation status; seasonality of breeding or migration patterns.

Sensitivity maps can be employed to help display and compare such information in a clear and accessible way. Such sensitivity information is often based on data prepared by government conservation agencies or specialist non-governmental organisations (NGOs), and is usually available to some degree for even the most remote and least developed areas. In countries with established oil spill preparedness, wildlife information is often available as part of the more general sensitivity maps in national and local oil spill contingency plans prepared by government agencies, port and harbour authorities and industry. However, it is extremely rare that the groups that would be expected to rescue and rehabilitate oiled wildlife during an actual incident are consulted in advance or even given access to the finished plans. This means that valuable input may be missing and, crucially, such groups may be unaware of the risk profile that might help them design and establish a Tiered Response capability.

Designing Tiered Capabilities for Oiled Wildlife

The risk assessment process will identify the nature and extent of the oil spill risk to wildlife, which, as in the case of oil spill response generally, will allow boundaries between tiers to begin to be defined and appropriate response capabilities designed. The basic definition of tiers for oiled wildlife incidents is likely to be best achieved in terms of the numbers of birds, mammals, reptiles or other animals that can be handled effectively by a specific capability. The point at which it will be necessary to escalate to another level may, however, need to be adjusted for rare species or those that are harder to clean and rehabilitate and therefore require more intensive treatment. As in the case of oil spill response, other factors, not all of which will be technical in nature, will ultimately influence the

design of tiered wildlife response capabilities. Perhaps the most pertinent factors are the existing level of oil spill preparedness and the commitment of government agencies, industry and wildlife groups to oiled wildlife response within a particular country.

Experience shows that in countries where there is well developed oil spill preparedness and a commitment to animal welfare (**Category 1**), wildlife response capabilities can be successfully established, with such initiatives being led by charities and voluntary environmental groups, often with the active support of government agencies and industry. In order to ensure the necessary speedy response to a wildlife incident in such countries, dedicated Tier 1 wildlife response centres have been established near areas of high risk from which the capture of any oiled animals can be coordinated and triage and treatment organised. In some cases such Tier 1 centres exist as a permanent resource, often primarily to deal with regular chronic oiling of birds. In countries with long coastlines mobile units may be seen as a more effective solution for treating relatively small numbers of oiled animals.

In a more serious spill when larger numbers of oiled birds or other animals arrive on the shore, possibly over an extensive area, a single local centre, mobile or permanent, is likely to quickly become overwhelmed and arrangements therefore need to be in place for assistance to be provided by other nearby local centres (Tier 2). At this stage additional hands-on help and equipment may also be mobilised from other national centres or even neighbouring countries. In addition, governmental bodies (e.g. police, fire brigade and the military) may be able to offer practical assistance, for example in controlling access to shorelines and helping with the coordination of those involved in the rescue of oiled wildlife. In this way a flexible response can be mounted to an incident that is greater than Tier 1 but falls short of a full Tier 3.

In a major spill involving, for example, many hundreds or even thousands of birds (Tier 3), the local centres may need to change their role by becoming temporary forward holding centres where triage and stabilisation can be carried out. The birds can then be subsequently transported to a more distant washing facility when it is ready to receive them. At this stage it will probably be necessary in even the best prepared countries to call upon international experts to assist in the overall coordination of the wildlife response operation and to help establish and manage a wildlife hospital capable of washing and rehabilitating large numbers of oiled animals humanely and safely.

Whilst the tiered response model described above is viable in a Category 1 country with an existing commitment to animal welfare, it will be much harder to build suitable capabilities if a government does not actively support the treatment of oiled wildlife, despite having suitable levels of oil spill preparedness (**Category 2**). The same will be true if a country is interested in the plight of animals impacted by spills but has neither the expertise nor financial resources to invest in planning for oiled wildlife. In all such cases any local animal welfare charities or environmental groups would be well advised to still seek to utilise and tap into the national and local oil spill preparedness and response framework in order to try to design a Tier 1 and, through cooperation with other wildlife groups, a Tier 2 oiled wildlife capability. A proactive and close working relationship with the local oil industry

may be a productive route to follow in such countries since they may be able to offer general advice, assistance and funding; some resources; and, in the event of an incident, logistical support. When local and regional resources are over-whelmed, reliance would invariably have to be placed on international experts to support the local wildlife groups and veterinarians in directing the response and designing and managing a wildlife hospital.

In lesser developed countries lacking both a credible oil spill response capability and a commitment to animal welfare (**Category 3**), the likelihood is that there will also be very limited, if any, local animal welfare charities, environmental groups or veterinarians with an interest in planning for an oiled wildlife incident. If one or more such bodies do exist then again they should seek to work with industry, especially any international oil companies, to establish a rudimentary Tier 1 oiled wildlife capability. Such companies may also be able to help facilitate the involvement of international wildlife groups and the movement of equipment in the event of a spill having a serious (Tier 2 or 3) impact on wildlife. It must however be understood that in some lesser developed countries the operating companies may, like the government, have no interest in supporting the concept of oiled wildlife response even though highly sensitive habitats and species may exist near oil activities. The consequential total lack of pre-planning, or even the most basic consideration of animal welfare, would severely restrict oiled wildlife response. The infrastructure in such countries and supporting logistics may also be limited, further hampering any oil spill response, let alone oiled wildlife response. In such cases almost total reliance would have to be placed on international wildlife groups to respond to any serious oiled wildlife incident, so long as the welfare and health and safety of the responders can be guaranteed.

Successful Tiered Oiled Wildlife Preparedness and Response

The same basic elements as described earlier in this paper also apply to oiled wildlife preparedness and response, aimed at ensuring high standards of animal welfare and, where appropriate, effective rescue and rehabilitation in the event of an incident.

- **An oiled wildlife contingency plan,** that is an integrated part of the national oil spill contingency planning process involving all stakeholders, is essential for a successful response to an incident involving oiled wildlife, and should include all the points listed below. Detailed advice on such planning, as well as the strategic decisions on treatment that will determine the most appropriate response in various circumstances, can be found in various publications, for example IPIECA 2004 and EU Handbook on Good Practice (see www.oiledwildlife.eu). What is important from the viewpoint of a tiered wildlife response is that due attention is given to the prompt and effective integration of local, regional, national and international resources as appropriate.
- **A management framework** that defines the roles and responsibilities of the various wildlife groups potentially involved in the range of different oiled wildlife scenarios, and their

coordination with other stakeholders involved in oil spill response generally. Depending on the location of a potential incident, management frameworks will vary in design, size and structure.

- **Response strategies** should be defined for the different bird, mammal and other species likely to be encountered, based on international best practices and protocols, taking into account any legal requirements in the country concerned.
- **On-site or nearby equipment stocks** for euthanizing, capturing, stabilising, cleaning and rehabilitating oiled wildlife should be established, commensurate with the Tier 1 risk and agreed response strategies. Sources of both specialist and non-specialist additional resources should be identified and contact made with potential providers and commercial suppliers to establish the extent to which they would be able to assist in an incident.
- **Arrangements for the integration of additional suppor**t (specialist equipment and expertise) from elsewhere in the country or further afield should be established and agreed at the planning stage by all groups and individuals potentially involved. It will be necessary to pre-define precise terms and conditions to cover administrative, legal and financial issues.
- **Logistical arrangements** to support oiled wildlife response operations across all tier levels need to be pre-determined in order to minimise any delays during an actual incident due to problems in importing equipment and deploying it to site. The import of veterinary supplies may pose particular problems in some countries. High priority should also be given at the planning stage to the welfare and health and safety of wildlife responders (including volunteers).
- **Trained practitioners** are vital at all tier levels if the rescue and rehabilitation of oiled animals is to be successful. Specialists in a range of disciplines who have extensive previous experience in oiled wildlife response will be vital to ensure that internationals standards are met and all responders (including volunteers) are suitably trained and managed.
- **A programme of training, including simulation exercises** should be established so that all national and international responders potentially involved in an oiled wildlife incident are competent, working to agreed common principles and/or protocols, and are familiar with the other potential stakeholders likely to be encountered. Only in this way will it be possible to ensure a seamless scaling up of the response during an actual incident.

Conclusions

The principles of Tiered Preparedness and Response have been successfully applied by the oil industry for many years and have been integrated into the plans of other stakeholders, most notably governments. It provides a structured approach to designing and building levels of oil spill preparedness and actually responding to an incident in such a manner that additional resources can be called upon and integrated into a response operation as an incident grows in severity. The design of tiered capabilities needs to take into account the risk faced and a range of influencing factors. Tiered Preparedness and Response is now widely accepted and regarded as international best practice.

Oiled wildlife and the welfare of individual birds, mammals and other animals commands enormous public and media attention during an incident. However, planning to deal with such wildlife events is still a young and evolving component of oil spill response, even in many countries that have well-developed oil spill preparedness. This is largely because the government agencies that are responsible for oil spill planning and response, as well as for protecting a country's natural resources, normally have no remit for animal welfare. Reliance is therefore usually placed during an incident on animal welfare charities and voluntary environmental groups to resolve the difficult ethical and practical issues surrounding oiled wildlife in a speedy manner. However, such bodies lack the resources (financial and other) to undertake their own detailed pre-spill planning and to invest in the level of dedicated facilities and other resources appropriate for the perceived level of risk.

This situation will only change when the important role played by animal welfare charities and voluntary environmental groups is more widely recognised, and oiled wildlife response planning is more generally embraced by governments and industry as an integral part of national, regional and local oil spill contingency planning. This is beginning to happen in many countries but elsewhere there is still much to be done, especially in lesser developed parts of the world. The wildlife response community need to work in close collaboration with both governments and the oil industry in designing and building oiled wildlife response capabilities. The oil industry is potentially a key partner for wildlife responders in many parts of the world, as oil companies will also generally seek to provide the best protection for wildlife at risk from their operations.

Applying the proven principles of Tiered Preparedness and Response will provide a uniform approach to oiled wildlife preparedness that will allow response capabilities to be built in a flexible manner, without the proliferation of expensive and rarely-used resources, whereby additional support from Tier 2 and Tier 3 levels can be called upon and integrated in more severe incidents. In the view of the authors this would lead to a considerable improvement in oiled wildlife planning and response, crucially benefiting the welfare of any affected animals.

Acknowledgments

Thanks are due to Hugo Nijkamp, Director, Sea Alarm Foundation, for his input to this paper.

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An integrated approach to monitor year-round chronic oil pollution in southeastern Newfoundland waters

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Keywords: Beached bird surveys, Canada, Newfoundland, oil pollution, radar, satellite imagery, ISTOP

The Canadian Government has stepped up efforts to reduce oil pollution in territorial waters through increased surveillance and legislative changes enacted in 2005. Beached bird surveys along the southeast coast of Newfoundland carried out since 1984 provide an important baseline of information on the extent of oil pollution in the region prior to these changes. Surveys were conducted on 20 beaches located along the southeastern shores of the Avalon Peninsula (Fig. 1). Annual trends of oiling rates (oiled birds/total birds) reveal that winter (October to March) rates continue to be among the highest in the world, with no significant change over time ($\beta = 0.001 \pm 0.019$, df = 1, Wald $\chi 2 = 0.030$, P = 0.980; Fig. 2). Summer (April to September) oiling rates increased between 1984 and 2006 ($\beta = 0.037 \pm 0.017$, df = 1, Wald $\chi 2 = 2.20$, P = 0.028; Fig. 2). However, the oiling rate is only a reliable index to monitor long-term trends in oil pollution if other mortality factors remain constant within a region over time, as the oiling rate is a function of the total number of dead birds found. This is probably not the case for coastal Newfoundland due to changes in hunting and fishing practices.

In Newfoundland, hundreds of thousands of murres (*Uria* spp.) are legally taken annually during the traditional fall and winter hunt. However, estimates of total harvest have declined significantly since 1977 due to a combination of hunting restrictions implemented in 1993 (Chardine et al., 1999)

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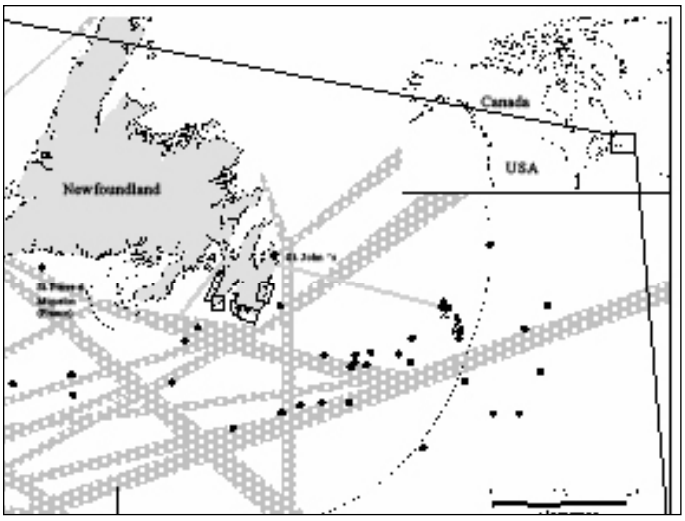

Figure 1. Location of beached bird surveys (rectangular areas) and possible oil-related events (black circles; categories 1 and 2 only) detected during the ISTOP surveillance program during summer (April to September) 2003-2006 off the east coast of Newfoundland, Canada. Black structures represent oil drilling platforms, grey barsrepresent major shipping routes, and black solid line represents the 200 nautical mile limit.

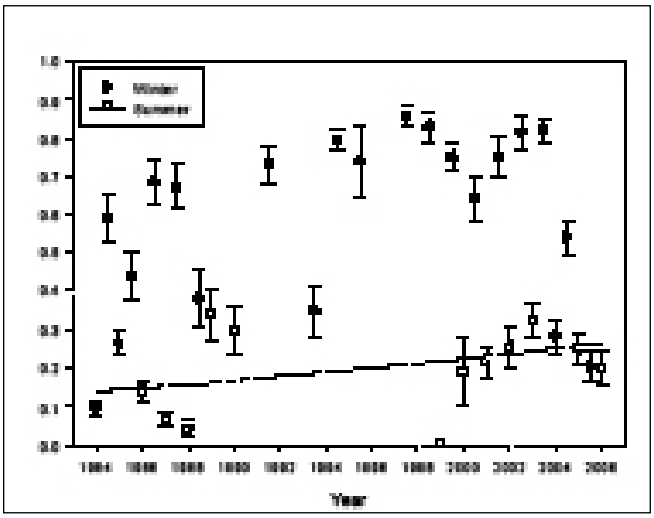

Figure 2. Annual trends in oiling rates of birds found on beaches in southeastern Newfoundland, Canada, during winter (October to March) and summer (April to September).

and a decline in number of hunters (CWS, unpublished data). Changes in hunting effort have been shown to influence the winter oiling rate in Newfoundland because of the hunt's effect on the number of unoiled murres (i.e., murres shot but not retrieved during the hunt) found on the beaches (Wiese and Ryan, 2003). This decrease in hunting effort is reflected in the significant decrease in number of unoiled murres found per km of beach surveyed (birds/km) during winter (Fig. 3).

Similarly, when examining the linear densities of unoiled and oiled murres in summer, it was found that the observed increase in summer oiling rate is largely due to fewer unoiled murres washing up on beaches since the fishing moratorium imposed in 1992 rather than an increase in oiled birds (Fig. 3). Prior to the moratorium, tens of thousands of seabirds, mostly common urres *(Uria aalge)*, drowned in gillnets every summer off eastern Newfoundland (Piatt and Nettleship, 1987). Although bycatch rates have remained similar, fishing effort has decreased significantly since 1992 (Troke, 2004). Consequently, the number of seabirds drowning in gillnets has dramatically declined since 1992, but occasional mass mortalities (hundreds of murres in one net) do still occur (Wilhelm et al., 2003; CWS, unpublished data).

Given these confounds, the linear density (birds/km) of oiled birds appears to be a more representative measure to assess long-term changes in oil pollution for the Newfoundland region. Oiled murres and oiled other seabirds in both winter and summer all showed significant declines when controlling for changes in weather patterns (onshore winds), suggesting that the amount of oil in the marine environment off the southeastern coast of Newfoundland has declined over the past 20 years (Fig. 4 and 5). A baseline for an oiling index

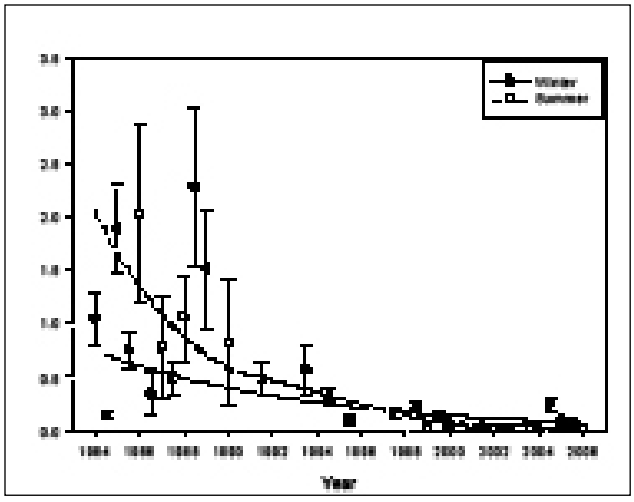

Figure 3. Annual trends in linear density (birds/km) of unoiled murres found on beaches in southeastern Newfoundland, Canada, during winter (October to March) and summer (April to September).

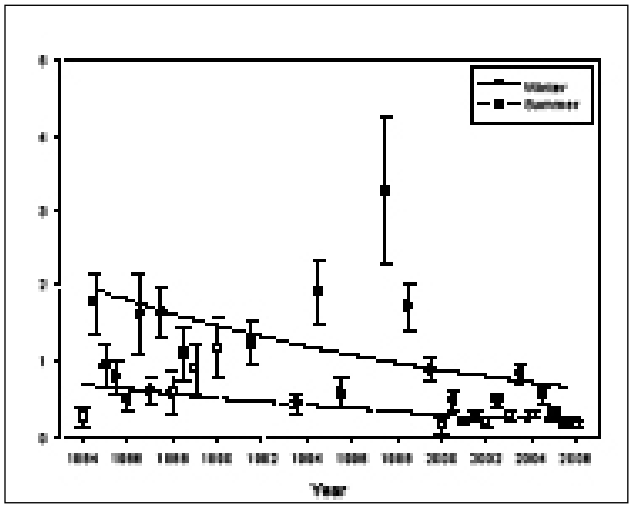

Figure 4. The linear density (birds/km) of oiled murres found on beaches in southeastern Newfoundland has declined since 1984 in both winter (β = -0.049 ± 0.010, df = 1, Wald $\chi 2$ = 5.47, P = 0.001) and summer (β = -0.049 ± 0.015, df = 1, Wald $\chi 2$ = 5.90, P = 0.015). Slope and Wald $\chi 2$ estimates were generated using a repeated measures generalized linear model which included weather covariates.

prior to the implementation of recent legislative changes was generated from the mean (SE) annual linear density of oiled birds (murres and other birds combined) since the imposed hunting restrictions and fishing moratorium and yielded 1.221 (0.322) birds/km (N = 12) and 0.226 (0.038) birds/km (N = 8) for winter and summer respectively.

Radar satellite technology is more commonly being used as an oil pollution monitoring tool (e.g., Espedal and Johannessen, 2000; Ferraro et al., 2007) because it scans large areas of the ocean at a relatively low operational cost. The ISTOP (Integrated Satellite Tracking of Oil Polluters) project is a national surveillance program which uses satellite imagery to detect possible oil slicks on both the Atlantic and Pacific coasts of Canada. Processed satellite images can be used to visually detect anomalies, or dark areas, which are inferred as being oil on the sea surface as oil dampens the normally present wind-generated wavelets on the sea surface, which appear brighter on the image. When an anomaly is observed, it is coded as one of the following categories: 1 (oil detected with high confidence with potential source attached or within 50 km radius), 2 (oil detected with high confidence with no potential source within 50 km), or 3 (oil detected with low confidence). Between June 2003 and March 2007, 63 possible oil-related events (categories 1 and 2 only) were detected through the ISTOP program for the Newfoundland region. Fifteen of these events were associated with the offshore oil extraction production platforms. Drilling and production platforms were contacted immediately after an event was observed and anomalies were usually attributed to the discharging of produced waters which can result in a thin oil sheen visible on the water's surface. However, one of these events was associated with the accidental release of 1,000 barrels of

crude oil from the Terra Nova floating production, storage and offloading platform (Wilhelm et al., 2007).

Although satellite images were acquired year-round (mean of 26 images per month), 55 of the 63 possible oil-related events (87%) were detected during the summer months (April to September, inclusively). However, the number of oiled birds found during monthly beached bird surveys peaked during the winter months, suggesting that oil is being spilled but is not being detected from October to March by the radar. Images acquired when winds are greater than 35 knots, which is not uncommon during the winter off the coast of Newfoundland, have a lower likelihood of detecting oil on the sea surface (J. Hurley, unpublished data).

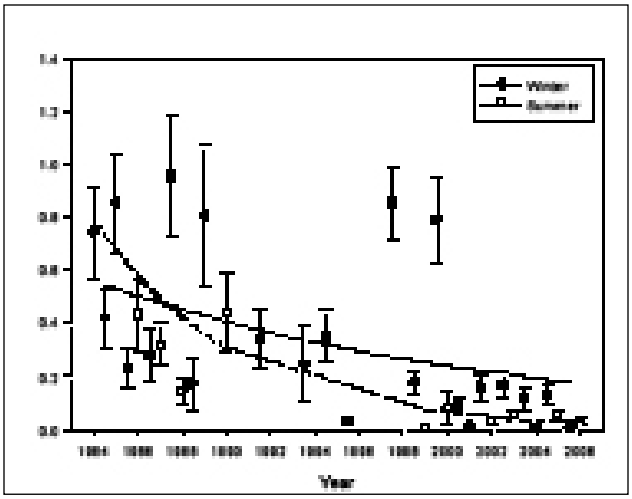

Figure 5. The linear density (birds/km) of oiled other birds found on beaches in southeastern Newfoundland has declined since 1984 in both winter (β = -0.050 ± 0.011, df = 1, Wald $\chi 2$ = 8.96, P = 0.001) and summer (β = -0.150 ± 0.012, df = 1, Wald $\chi 2$ = 9.90, P = 0.002). Slope and Wald $\chi 2$ estimates were generated using a repeated measures generalized linear model which included weather covariates.

The probability that an image acquired from April to September will contain a possible oil-related event (excluding events associated from oil platforms) for each year separately ranged from 4 to 18%, with the majority of spills extending from the southwest coast of Newfoundland to the eastern edge of the Grand Banks along the major shipping routes (Fig. 1), suggesting that these possible oil-related events are likely ship-source based.

The linear densities of oiled birds, controlled for shifts in favorable carcass deposition weather, may be a reliable index to monitor oil pollution for both the winter and summer. Murres, which make up 65% of all species found on southeastern Newfoundland beaches (Wiese and Ryan, 2003), are potentially a more sensitive measure given their particular vulnerability to oil (Wiese and Ryan, 2003) and their widespread distribution along the coast of Newfoundland (Brown, 1986; CWS, unpublished data). Summer surveys do have limitations, as they yield only a fraction of the number of oiled birds found compared to winter surveys, thereby decreasing the power of the analyses. The lower density of oiled seabirds during the summer is likely linked to the fact that most seabirds are breeding during this time of year, thereby spending less time on the sea surface and are more patchily distributed. Therefore, we recommend using ISTOP to supplement the beached bird data to monitor oil pollution during the summer months.

There are limitations with respect to generating an oil pollution index using ISTOP acquired data. Images provide a snapshot in time rather than a continuous monitoring of potential oil slicks and accurate detection rates are dependent on favorable weather. A first step to overcome some of these problems is to increase the monthly image acquisition rates, particularly between April and

September when ISTOP data is most informative. The current ISTOP acquisition regime is below the desired 40-50 images acquired per month as recommended in Wiese (2004) which would ensure that data collected are representative and provide sufficient statistical power to detect annual differences. Furthermore, annual trends in possible oil spills should be expressed as a possible slick/coverage unit (e.g., km2) and exclude images acquired when winds exceeded 35 knots, to obtain a more precise and sensitive measure. Together, beached bird surveys and radar satellite technology have the potential to complement each other as a year-round monitoring tool to document chronic oil pollution activities along the coast of Newfoundland and thereby verify the effectiveness of recent legislative changes to allow for more effective enforcement against marine polluters in Canadian waters.

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Cabo Vírgenes Mystery Spill: Challenges and Lessons Learned

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Keywords: penguin, chronic oiling, Argentina, South America, waterproofing, cold weather challenge, airlifting, remote site.

Introduction

Cabo Vírgenes is located in the northern tip of the Strait of Magellan, in southern Argentinean Patagonia (52°22'S – 68°24'W). It is the second largest continental breeding colony of Magellanic penguins (Spheniscus magellanicus), with approximately 90,000 breeding pairs (Schiavini et al., 2005). There are growing concerns related to the conservation of this species, as in 2004 the International Union for Conservation of Nature and Natural Resources' (IUCN) status of Magellanic penguins was changed from Lower Risk in 2000, to Near Threatened in 2004. The main threats to these birds are chronic oil pollution, over-fishing, and incidental captures in fishing nets (BirdLife International, 2004).

In early May 2006, several hundred oiled Magellanic penguins began washing ashore in the Cabo Vírgenes Provincial Reserve, in southern Argentina. Staff from Consejo Agrario Provincial de Santa Cruz (CAP) in charge of overseeing and monitoring tourism and other activities related to Protected Areas in the Santa Cruz Province, started capturing and stabilizing the birds in the field, utilizing improvised facilities. At the same time, on the southern part of the Strait of Magellan, in Chile, another 76 oiled birds had washed ashore at the colony in Isla Magdalena and were transferred for

1 International Fund for Animal Welfare (IFAW), Emergency Relief Team - www.ifaw.org

2 International Bird Rescue Research Center - www.ibrrc.org

3 Fundación Mundo Marino - www.fundmundomarino.org.ar

4 Centro de Recuperação de Animais Marinhos - www.furg.br/museu

5 Fundación Patagonia Natural - www.patagonianatural.org.ar

6 Dirección de Fauna/Consejo Agrário Provincial (CAP) de Santa Cruz - www.consejoagrario.santacruz.gov.ar

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Figure 1 – Map of the southern portion of South America. Downloaded and adapted from http://www.guiageo-americas.com/mapas.htm

rehabilitation to Punta Arenas (Ricardo Matus pers. comm.). To assess geographical locations please see Figure 1.

Four International Fund for Animal Welfare (IFAW) Team members from Brazil and Argentina arrived in Rio Gallegos through an invitation from CAP to work along with local authorities and assist in the rescue and rehabilitation of the effected animals. Animals affected by chronic oil pollution in Argentina have been reported since the early seventies (Jehl, 1975), and in this case, as in most of them, the source of the oil remains unknown (Garcia-Borboroglu et al., 2006).

Case Report

Simultaneously while capturing and stabilizing birds in the field, CAP's staff, Fundación Patagonia Natural, IFAW and other collaborating institutions started adapting the local sailing club facility (Centro Marítimo Austral - CeMA), in Rio Gallegos, to be able to transfer the animals. Rio Gallegos is the capital of the Santa Cruz Province, located 139 km from the Cabo Vírgenes colony. CeMA's facilities were chosen to be adapted to treat oiled wildlife due to the following characteristics: availability of a warehouse and three smaller rooms to hold the birds while oiled; a second warehouse could be turned into washroom and drying room; a heated room available for meals and daily morning meetings for staff and volunteers. Also a pre-existing pool to teach kayaking could be used to pump water into pools set to waterproofing the birds after they were cleaned (see Figures 2, 3, 4 and 5).

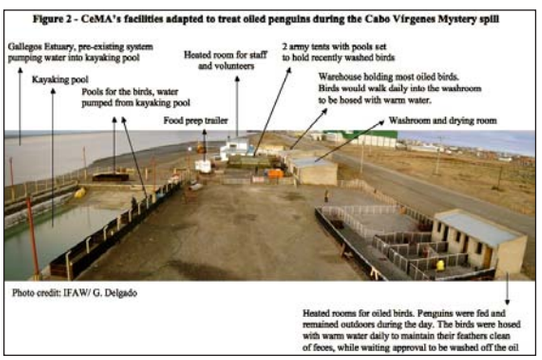

Figure 2 – A view from the makeshift facilities in Rio Gallegos, at the local sailing club Centro Marítimo Austral. Photo credit: IFAW/ G. Delgado

Figure 3 – The warehouse adapted to hold the oiled birds transferred from Cabo Vírgenes. Photo credit: IFAW/ V. Ruoppolo

The water system developed for bird washing included a total of 5,000 liters of clean water, which was contained in two Fastanks® (Fast Engineering Ltd., Antrim, Northern Ireland), outside the washroom. The water was pumped from the Fastank into the water heaters through the use of pressure pumps of 1 Hp. Four water heaters were installed to provide two washing and two rinsing stations, but as the water was kept outside, it reached very low temperatures and an adaptation had to made. The water had to be put through two heaters to get to a warm enough temperature for washing birds. This fact reduced the washing and rinsing stations to only one of each. Inside the washroom, 200L containers were used to store hot water for filling the tubs while washing birds. The wastewater was kept in 200L barrels and pumped out of the washroom, whenever necessary, to be stored in containers outside. The municipality removed the contaminated waste-water for appropriate disposal, when the response was over. The clean water containers were filled daily by a water-truck, sent in by the municipality.

Figures 4 and 5 – Warehouse adapted into a washroom. Before and after. Photo credits: IFAW/ R. Pinho da Silva and IFAW/ V. Ruoppolo

On May 18, the first 182 oiled penguins were transferred from Cabo Vírgenes to the makeshift facilities at CeMA, in Rio Gallegos, using an enclosed truck. Two were juvenile Rockhopper penguins (Eudyptes chrysocome chrysocome), and 180 Magellanics, all adults. CAP's field team remained on site, capturing more birds, as the weather and tides allowed. In the following days another 42 birds were brought to CeMA, totaling 224 birds for treatment. The first two days with the birds in Rio Gallegos were dedicated to performing individual examinations and creating intake records for all birds in care.

Intake procedures were based on International Bird Rescue Research Center (IBRRC) protocols and included:

- Registering capture information on the Live Intake Log;
- individual identification with temporary colored bands;
- physical examination, including body weight, temperature and condition; checking for wounds, burns and injuries; lung auscultation and degree of oiling;
- blood sampling to evaluate packed cell volume (PCV), buffy coat (BC) and total protein (TP);
- oral administration of re-hydrating solution - NaCl 0,9%.

This opportunity was used to train CAP's personnel and volunteers to handle and evaluate the birds individually. The birds were separated into groups, keeping weaker animals under heat lamps and increased nutritional needs were addressed to help them gain weight faster. Fish formula, with added vitamins and Ensure® (Abbott Laboratories) was tube fed to weaker animals two to three times daily. Veterinary attention was available at all times and specific cases were treated accordingly. All oiled birds were re-hydrated once a day and given a fish with vitamins supplementation, before feeding them non-supplemented fish. The birds were taught to eat using "penguin feeding boxes" (Callahan, 2001) since the beginning of the response, aiming to minimize handling after they were clean and regaining waterproofing. All birds were fed fish twice daily.

Obtaining high quality fish was incredibly challenging in Rio Gallegos due to the fact that it was not fishing season, and fish had to be brought from different localities. Different kinds of fish were used and these included silverside - *Odonthestes bonariensis,* Patagonian blennie - *Eleginops maclovinus,* menhaden - Brevoortia aurea cut into smaller edible pieces and Spanish sardine - *Sardina pilchardus*.

Birds were considered approved for washing when presenting all of the following: normal body temperature (39-41˚C), PCV >30%, BC <2%, TP ≥3g/dl, fair body condition, no wounds and normal behavior. Most birds were washed between May 20th and June 16th. IBRRC protocols were again followed for washing, rinsing and drying. The animals were cleaned in a 1-2% solution of Magistral® (Procter & Gamble) dishwashing detergent in warm water (39-43ºC), until no traces of oil could be found. In most cases it took between 20 and 30 minutes to wash each bird. Birds were then meticulously rinsed with warm water at high pressure through a special nozzle (Oxygenics® Skincare Series, Modesto, CA, USA), until all the detergent could be removed. The rinsing process took another 20 to 30 minutes. Once rinsed, the birds were tubed with 120 mL of re-hydrating solution and placed in the drying room where infrared lamps were set up for the purpose. The penguins remained in the drying room until the next day. On the day after washing, they were fed and moved to the tents, where they had access to pools.

The IFAW team worked for six weeks in Rio Gallegos, but due to very cold weather and low water temperatures, the birds had difficulty regaining their natural waterproofing. With outside temperatures ranging from -4 to -14ºC, birds were spending little time in the outdoor pools, drastically hindering the waterproofing process. Additional indoor pools were constructed and heat sources were brought in to encourage the birds to swim and preen more. Unfortunately, the birds were not able to get completely dry with the limited heat in the buildings and the ambient temperature. This forced

Figure 6 – Unloading the birds off the C-130, in Mar del Plata. Photo credit: IFAW/ V. Ruoppolo

Figure 7 – Part of Fundación Mundo Marino's facilities for rehabilitation of marine fauna. Photo credit: IFAW/ V. Ruoppolo

the team to decide to move the birds somewhere with better facilities and weather conditions.

In an unprecedented response, on July 11th the government of Santa Cruz Province and the Argentine Air Force (Fuerza Aérea Argentina) relocated 195 penguins to warmer weather to San Clemente del Tuyú, where Fundación Mundo Marino's (FMM) permanent rehabilitation facilities are located (36º20'S - 56º44'W). The birds were relocated using large plastic totes that each held approximately 20 birds, and the totes were then placed in a C-130 aircraft for flying for three and a half hours into Mar del Plata (Figure 6), and then into an enclosed (well ventilated) truck for another two hours. Three days before transportation and five days after the arrival, a prophylactic treatment with Itraconazole (50 mg/bird, once a day) was given to all birds in care, aiming to prevent Aspergillosis due to the stress imposed by transport (Penguin TAG, 2003). Once in San Clemente (Figure 7), teams from IFAW, FMM and CAP helped the birds finish their recovery. The birds reacted quickly to the warmer weather, preening and swimming non-stop. The combination of warmer weather, better facilities, unlimited clean water for the pools and better fish for feeding the animals was successful.

Figure 8 – Magellanic penguins released in Punta Rasa, San Clemente del Tuyú, on July 31st 2006. Photo credit: IFAW/ V. Ruoppolo

The first birds were released on July 31st with a great deal of

media coverage (Figure 8). A succession of smaller releases followed with the final results of 65.1% (146/224) release rate.

Penguins were approved for release according to the following criteria (IBRRC SOP) determined during the pre-release evaluation: PCV ≥ 38 %; BC < 2%; TP ≥ 3.0 g/dl; normal behavior and feeding well: good body condition; absence of wounds or any obvious infectious diseases; lungs clear; feathers 100% waterproof after swimming for one hour in a pool with no haul out.

Apart from adequate facilities, warmer weather and fish availability, San Clemente del Tuyú was decided to be an appropriate release site due to being within the geographic distribution for the species. The decision to move the birds north was made in accordance with local authorities and with the support from different Argentinean researchers working with Magellanic penguins.

Discussion

The successful rehabilitation of oiled birds is difficult in any circumstance and it was apparent, once again, that there must be a certain level of infrastructure at the rehabilitation location to be able to support an oiled wildlife response. In this case, low water temperatures, hard water, combined with cold weather meant the birds were hesitant to spend any time in the water, struggling to regain their waterproofing because they weren't swimming voluntarily. While the team was able to overcome many of the obstacles of being in a remote location, ultimately, the fact that there wasn't adequate heated space for the pools meant that the birds were not able to get waterproof.

It is also of note that since these animals were properly stabilized, they could successfully be transported some 2,500 km via plane with little impact on their health.

Due to the IFAW Penguin Network (Ruoppolo et al., 2005) and IFAW's extensive work with penguins in South America, our own stainless steel bands were purchased and a website for band returns was developed in Spanish, Portuguese and English (IFAW, 2006). Our goal is to mark penguin species rehabilitated during oiled wildlife responses, always following species limitations for banding (Petersen et al., 2005) and working under local banding permits. Having our banding program gives us the ability to develop our own post-release monitoring program, not depending on third parties to obtain band returns and stimulating the IFAW Penguin Network member institutions to band their rehabilitated animals for release.

All of the birds released during the Cabo Vírgenes spill response were banded with IFAW's stainless steel bands. Another 54 birds rehabilitated and released simultaneously in Punta Arenas, Chile, were banded with these flipper bands. Currently, Fundación Mundo Marino, in northern Argentina, is also using these bands on their rehabilitated birds released.

Some of the strengths and challenges of this response are discussed below.

Strengths:

1) CAP's positive attitude and determination to rehabilitate these birds made this response a success;

2) IFAW was previously known by name through the Environmental Crisis Action Plan (FMM & FPN, 2005), which allowed CAP's invitation and IFAW's personnel filling managing positions on the charts;

Figure 9 – IFAW band returns from two of the Cabo Vírgenes spill birds, as of June 1st 2007. Downloaded and adapted from http://www.guiageo-americas.com/mapas.htm

3) CAP's political positioning to obtain the Air Force's support to airlift the birds to the Buenos Aires Province;

4) Volunteers from the Rio Gallegos community were available to assist with the rehabilitation process;

5) Better facilities were available at Fundación Mundo Marino and the birds could be transferred there;

6) IFAW's support to the ER Team on the ground for an extended period of time, which allowed for oversight throughout the entire process of rehabilitation;

7) Penguins are very sensitive to Aspergillosis (Penguin TAG, 2003). The prophylactic treatment given to all birds in care before and after transportation has prevented the disease from occurring, as no cases were diagnosed during the necropsies of all deceased animals during the rehabilitation process;

8) Two band returns were reported (Figure 9):

- IF-0085 was captured oiled around 2 May 2006, in Cabo Vírgenes. It was released in San Clemente del Tuyú (Playa Norte), 2,500 km north from the capture location, on 10 August 2006. It was spotted alive on two different occasions, at Isla Magdalena in Chile, even further south from the Cabo Vírgenes colony, on 16 January and 29 February 2007. The bird was in very good body condition in both occasions and in pre-molt on February 29th. Figure 10 shows the bird on 16 January. These returns were reported by Corporación Nacional Forestal (CONAF), the Chilean authority in charge of the penguin colony at Isla Magdalena, and Mr. Ricardo Matus, an ornithologist based in Punta Arenas. This report indicates that other birds could have returned to southern Argentina and Chile. Working close with authorities and researchers monitoring the colonies will promote more band returns of live birds during the breeding and migrating seasons;

Figure 10 – Re-sighting of IF-0085 on Isla Magdalena, Chile, on 16 January 2007. Photo credit: CONAF/ R. Fernandez

- IF-0022 was released on July 31st 2006, in San Clemente del Tuyú. This bird was found dead in Necochea on 26th August, among a group of 54 dead Magellanic penguins, all freshly dead and in good body condition. These animals were most probably caught in a fishing net, as they were reported washing ashore all together and in good body condition. This information was reported through a phone call, and the collector mentioned another two IFAW banded birds, but the numbers weren't recorded. The collector, Mr. Rodrigo Sierra, hasn't gone back to the site and the information was lost. Apart from the sad news that the animal was found dead, this band return has shown important background information, including that the bird was alive for almost a month after it was released, that it was able to find a larger group of birds for foraging and migrating, and that it died of other causes.

Challenges:

1) Inclement cold weather created insurmountable problems for both the birds and the staff and necessitated the moving of the birds north;
2) Less daylight due to the time of year meant that the staff had limited daylight hours to care for birds;
3) The team was dependent on third parties to obtain water to fill out the pools and containers (Fastanks) resulting in the loss of precious time, meaning oiled birds waited longer for cleaning;
4) Even when hoses were left running to fill the tanks overnight to provide enough water to wash birds the next day, the water stored outdoors froze overnight, which caused the team to lose time as well;
5) Water heaters had to be installed in-line, to be able to heat the water to the necessary temperature to wash the birds;
6) It was difficult to obtain good quality fish of appropriate type due to the time of year and the remoteness of the region;
7) Pools and other equipment had to be purchased in Buenos Aires and flown to Rio Gallegos, which meant waiting several days for appropriate equipment.

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