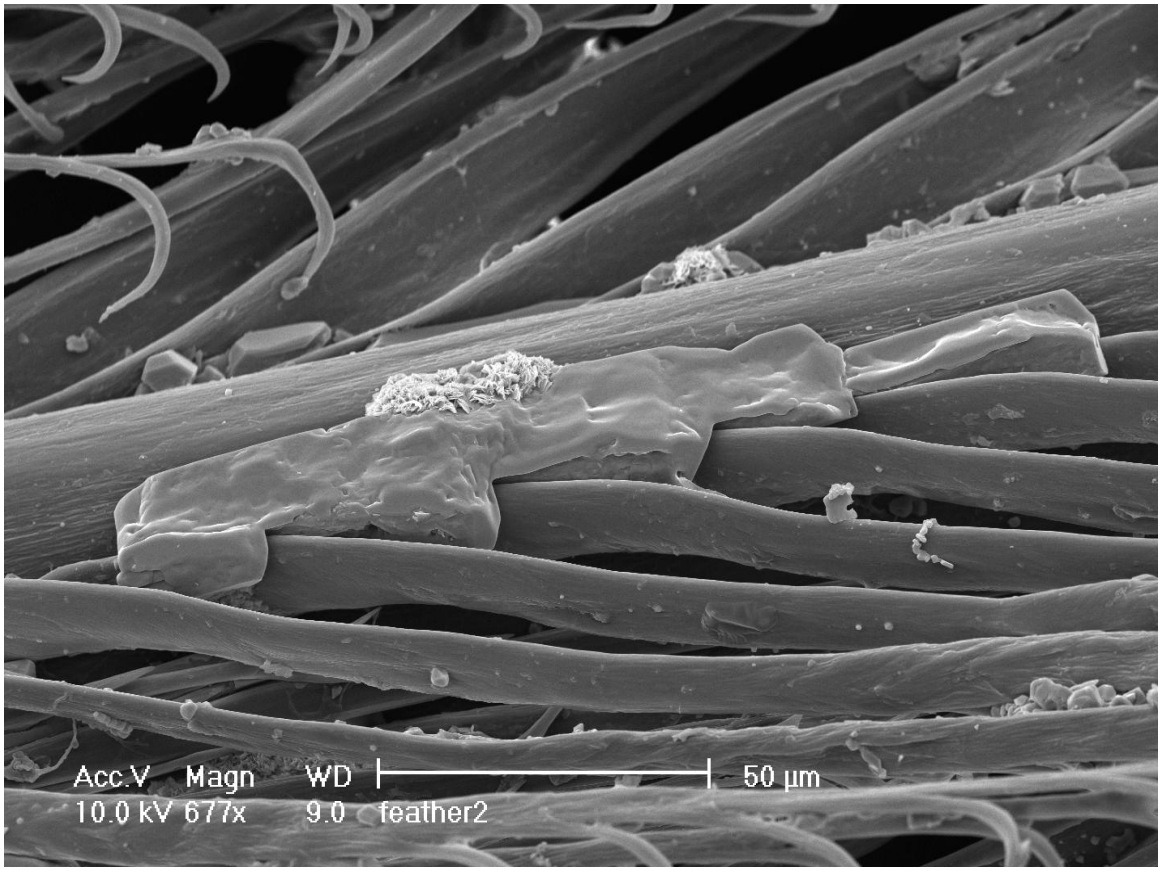
Physical effects of Prudhoe Bay crude oil water accommodated fractions (WAF) and Corexit 9500 chemically enhanced water accommodated fractions (CEWAF) on common murre feathers and California sea otter hair



Final Report May 30, 2009

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INTRODUCTION:

As of November 2002, much of the off-shore waters 3 – 200 miles along the California coast were designated as chemical dispersant pre-approval zones. Zone designation was based on an evaluation process conducted by marine area committees as required by the Region IX Regional Response Team (Addassi et al. 2005). A central part of this decision-making process was use of a modified Ecological Risk Assessment (ERA) known as a Net Environmental Benefit Analysis (NEBA) (Pond et al. 2007; Aurand et al. 2001; Kraly et al. 2001). The NEBA provided a process to identify concerns, prioritize risk, and a framework by which decision-makers could make "trade-off" determinations within a specific ecological context. When appropriate data was not available, participants were required to use "best professional judgment" in making evaluations and conclusions.

A widely held assumption concerning the use of dispersants is that chemical dispersion of oil will dramatically reduce the impacts to seabirds and marine mammal species, primarily by reducing their exposure to petroleum hydrocarbons (NRC 2005) by removing these materials from the water's surface. However, dispersing oil into the water column is known to transfer toxicity to subsurface organisms that would not otherwise have been exposed by a surface slick. Since dispersed oil is known to be highly toxic to invertebrates and fish larvae even in low concentrations, evaluating the validity of this assumption of benefit to surface-dwelling animals is critical because it is often a key factor in the decision on whether or not to use dispersants, and was a key assumption in the California dispersant use evaluation process. Specifically, the NEBA results suggested that "the appropriate and timely use of dispersants could. . . greatly reduce the risk of spilled oil reaching the more abundant and sensitive habitats . . . and species found in the more inshore, coastal areas as well as marine bird and mammal species that rely on water proofing for thermoregulation (Addassi and Faurot-Daniels 2005).

The National Research Council (NRC) in 2005 described that limited available data suggested comparable toxicity of dispersed and untreated oil to seabirds and mammals, but found no conclusive information regarding the impacts of dispersed oil and dispersants in the waterproofing properties of fur and feathers. One of the recommendations of the previous NRC report (NRC 1989) was that studies be undertaken "to assess the ability of fur and feathers to maintain the water-repellency critical for thermal insulation under dispersed oil exposure conditions comparable to those expected in the field." This recommendation was reaffirmed by the NRC in 2005 primarily due to the importance of this assumption in evaluating the environmental trade-offs associated with the use of oil dispersants.

Enabling legislation requires the Administrator of the OSPR to provide for the best achievable protection of coastal and marine resources and Government Code Section 8670.12(b)(6) specifically requires the evaluation of dispersant effects on fish and wildlife. Therefore, this project attempted to address this dearth of scientific data on dispersant effects on wildlife. Specifically, this study was designed to begin evaluating the effect of dispersed oil on fur and feathers by: 1) designing a system to exposure fur and feathers to dispersant and dispersed oil; 2) quantifying TPH levels on individual feathers and hair; 3) assessing structural changes to feathers and hair associated with dispersant and/or oil exposure; and 4) evaluating dose-response relationships in these results with differing levels of dispersant and/or oil.

MATERIALS AND METHODS:

Artificial seawater was created with distilled water plus Instant Ocean (Spectrum Brands, Inc, Atlanta, GA) added to a salinity of 35 PPT. Ten aliquots of 1.5 L were measured into 2 L

tubulated glass aspirator bottles. Experimentation occurred in a cold room maintained at 64 F (17.8 C) for the duration of the experimental stage of the project. Test bottles were allowed to sit in the cold room until temperature equilibrium was achieved. Test solutions were created utilizing modified CROSERF procedures outlined in Oil Spill Dispersants: Efficacy and Effects (NRC 2005). Plain artificial seawater served as the control solution. Nine experimental solutions were created at three different loading concentrations of Prudhoe Bay crude oil (PBCO, supplied by California Department of Fish and Game Petroleum Chemistry Laboratory), 3 concentrations of PBCO plus dispersant (Corexit 9500, Exxon Corp), and 3 concentrations of dispersant only. Corexit was added at 1 part per 20 parts oil in the solutions containing both materials. Loading doses of PRCO and dispersant were selected to generate an expected range of concentrations across each set of solutions (see Table 1).

Table 1. Loading doses vs resultant ppm in test solutions.

Test Solution Loading Dose	Resulting ppm TPH in solution	Resulting ppm Corexit in solution
Instant Ocean (IO) only	0.28	ND
IO + 2 g/L PBCO + 0.1 g/L Corexit	320	39.4
IO + 0.5 g/L PBCO + 0.025 g/L Corexit	110	7.2
IO + 0.125 g/L PBCO + 0.00625g/L Corexit	13	2.4
IO + 25 g/L PBCO	6.7	Not tested
IO + 2.5 g/L PBCO	4.6	Not tested
IO + 0.25 g/L PBCO	2.3	Not tested
IO + 10 ml/L Corexit (9.5 g/L)	ND	400
IO + 1 ml/L Corexit (0.95 g/L)	ND	350
IO + 0.1 ml/L Corexit (0.095 g/L)	ND	12

Figure 1. Common murre contour feather

Common murre ventral contour feathers (see Figure 1) were plucked from a single, non-oiled, adult individual deceased bird obtained through routine rehabilitation efforts at the San Francisco Bay Oiled Wildlife Care and Education Center. One hundred feathers of approximately the same size were chosen and clips numbered 1-100 were affixed to each feather's calamus. Feathers ranged from 3.9-4.2 cm long. The midpoint of each feather was marked to enable later evaluation of feathers for comparable size by measurement of the diameter of each shaft at the midpoint. Feathers were randomly allocated to ten exposure groups, with 10 feathers per group. Ten aliquots of 0.1 g feathers (16-19 feathers) were also collected for exposure to test solutions and subsequent TPH analysis.

One California sea otter pelt was acquired from a deceased individual admitted to the CDFG Marine Wildlife Veterinary Care and Education Center in Santa Cruz, CA. The otter pelt was thawed, closely flensed, and ten 10 x 3 cm pieces plus ten 2 x 1 cm pieces were cut from the dorsal to ventral abdominal area. All cutting was accomplished from the visceral side of the pelt to minimize potential damage to the hairs. Loose hairs from the cut edges were removed by rinsing in distilled water. One piece of each size of pelt was assigned to each exposure solution.

Test solutions were mixed utilizing magnetic stir bars and plates. PBCO and Corexit 9500 were added to the center of each seawater aliquot by direct application to

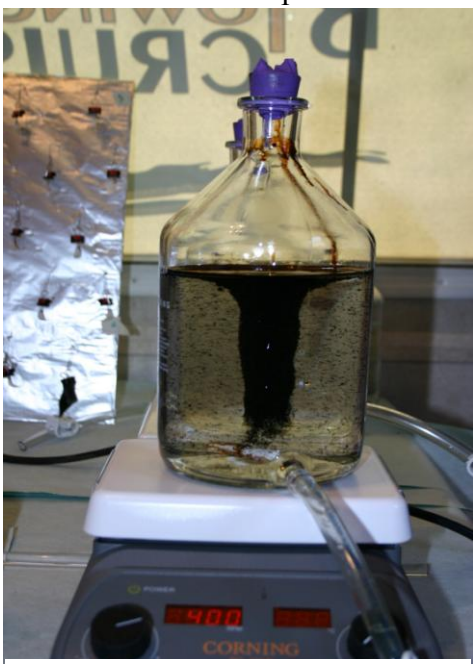

Figure 2. Artificial seawater being mixed with Prudhoe Bay Crude Oil in 2L aspirator

the central vortex created during mixing, with the oil added immediately prior to application of dispersant. Bottles were covered with aluminum foil and capped with nitrile-covered rubber stoppers during mixing. Mixing proceeded at 400 rpm for 18 hours (see Figure 2). This rate of rotation produced an approximate 25% vortex in each bottle. Solutions were allowed to settle for 6 hours. One liter of each exposure solution was subsequently drawn off the bottom of the aspirator bottles into 1 L beakers, with any surface slick material remaining settled on top of the water, thus remaining in the aspirator bottle.

A Plexiglas framework was constructed to allow each tissue sample to be suspended in the same place in the middle of the 1 L beaker's water column. During exposures, beakers of test solution were magnetically stirred at 200 rpm. Individual feathers were hung from their numbered clips on stainless steel hooks and suspended in the center of the beaker for 60 seconds. Otter pelt pieces were similarly suspended from the central hook and exposed for 90 seconds. These exposure times were selected to approximate wild foraging dive times of these species (Ainley et al. 1990; Yeates et al. 2007). Feathers were exposed to each solution prior to otter pelt to eliminate possible visual contamination of feathers with loose otter hairs. The 100 feathers and small otter pieces targeted for visual examination were individually hung to dry on an aluminum foil lined drying rack. The 1L exposure solutions were transferred to glass bottles and the feather aliquots and larger otter pieces for total petroleum hydrocarbon (TPH) analysis were placed into foil immediately after exposure. All TPH analysis samples were refrigerated until delivery to the Petroleum Chemistry Laboratory at California Department of Fish and Game, Rancho Cordova, CA for analysis.

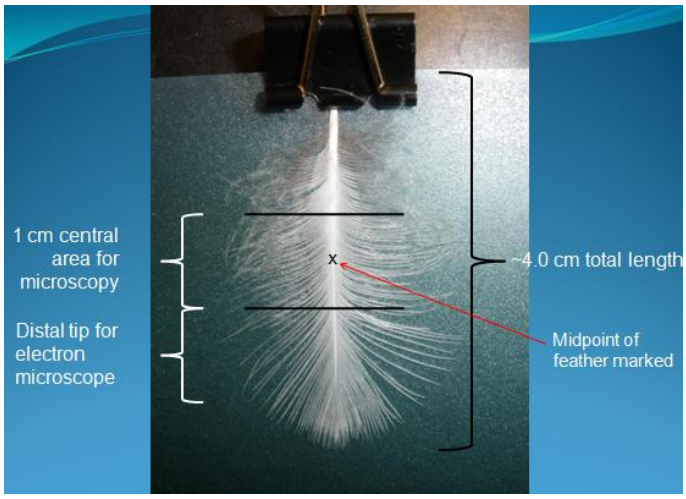

Figure 3. Common murre contour feather, portions prepared for light and electron microscopy

After air drying, the central 1 cm of feather (0.5 cm on either side of each feather's midpoint) was cut and permanently mounted on glass slides (see Figure 3). Due to difficulties arising from the solubility of oil in common mounting media and the hydrophobic nature of feathers, feathers were not embedded in any slide-mounting material, although the edges of the cover slips were affixed in place with judicious application of Cytoseal-60 (Richard-Allen Scientific). The small otter pieces were also allowed to air dry, and then were dissected under magnification to extract portions of skin with attached clusters of hair. Slide preparations were made of each hair sample in the same manner as the feathers. The skin was included in order to retain relative orientation of the hairs.

Photographic analysis was performed (see Figure 4) utilizing Q-Capture Pro software to measure feather structures (QImaging, Surrey, BC, Canada) after calibrating the photographic equipment with a micrometer. Image series were obtained of (A) feather barbs in relation to the centrally located rachis at 100x, (B) same location with focus pulled to the plane of the hooklet tips at 100x, (C) distal barbs at 100x, and (D) barbules in relation to barbs at 400x. For series A, measurements were made to evaluate the angle between each barb and the rachis plus distance between barbs 400 µm from the rachis. Barbule spacing 50 µm from the barb was measured for

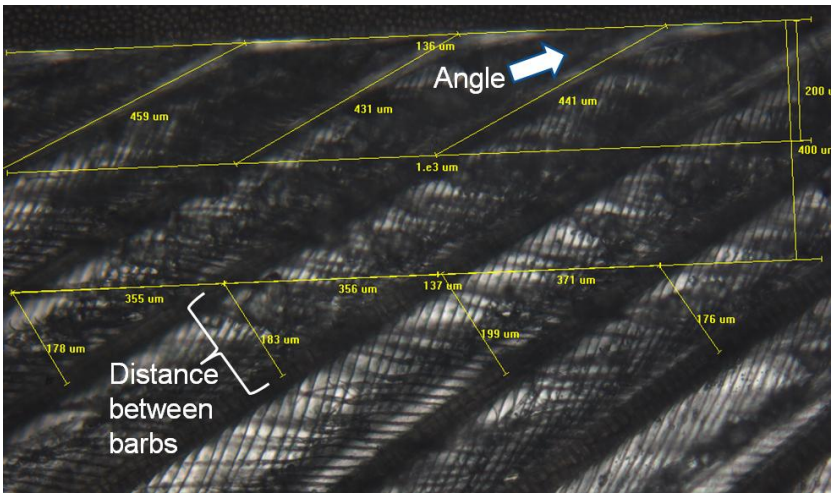

Figure 4. Common murre contour feather exposed to oil plus dispersant solution, under 100x light microscopy with measurements of angle at shaft and distance between barbs at 400 um from shaft.

series D. Feather series B, C, and D were evaluated by a blinded observer who graded each feather's structures as orderly, mildly, moderately or severely disarrayed, and on the presence of zero, small, moderate or large amounts of foreign material seen. The diameter of each feather shaft at the marked center was measured to evaluate relative feather size and thus comparability of the randomly assigned groups.

Otter samples were photographed at 100x and 400x at the base of the hairs and mid-shaft, and the mid-shaft area at 1000x. Images were evaluated by a blinded observer for the presence of zero, small, moderate or large amounts of foreign material seen.

Distal portions of four test feathers exposed to the three most concentrated test solutions and plain IO were examined under scanning electron microscopy to evaluate the nature of encrusting material noted on light microscopy. These samples were further evaluated with back scatter electron x-ray microprobe at the University of California Davis X-ray Analytical Laboratory to obtain crude qualitative identification of targeted material. High resolution x-ray mapping was performed to highlight locations of the most commonly identified elements Na (Cl), K, Ca, Mg, and S.

RESULTS:

Observations during experimentation

All 9 test solutions with any combination of PBCO and Corexit loading doses formed a visible slick-like layer on the water surface after settling. Feathers exposed to either plain IO or to the water from the oil-only solutions retained their fluffy appearance even when under water, with ample air bubbles trapped amongst the barbs, and immediately shed water to appear dry when removed from the solution. Feathers exposed to either the oil + dispersant or dispersant only solutions quickly lost their bubbles and the feathers appeared folded in on themselves when removed from the solutions. When dry, the feathers from these solutions appeared to have the distal tips of each barb firmly stuck in this folded up position (see Figure 5). Test

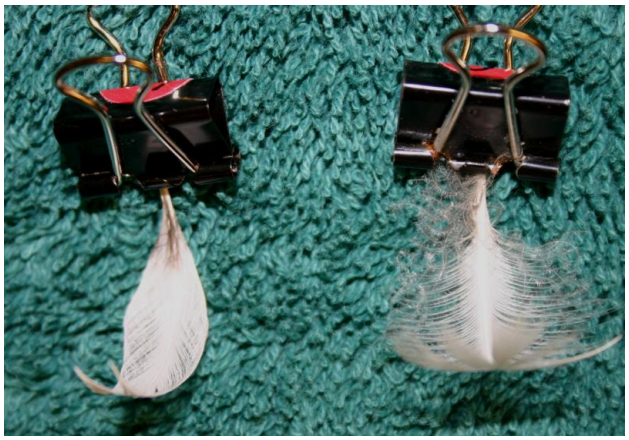

Figure 5. Common murre feather on left was exposed to artificial seawater with dispersant and Prudhoe Bay crude oil (PBCO). Feather on right was exposed to artificial seawater with just PRBO. Note fluffy appearance of feather on right in contrast to left feather, which is folded in on itself.

solutions from beneath slicks of oil at 6.7, 4.6 and 2.3 ppm TPH appeared as clean water but had a distinct petroleum smell.

Test Solutions:

A range of concentrations of PBCO was successfully created, as shown in Table 1. As expected, ppm PBCO in solution was much higher in the solutions with dispersant than in the oil-only groups. In the Corexit-only group, the middle concentration solution was very similar to the maximum concentration obtained (350 vs 400 ppm). Although Coprexit was added at

Feather morphometrics:

With the exception of a single outlier that was excluded from statistical analyses, feather diameter was not significantly different among test groups ($p = 0.799$). Barb angles at the rachis and mean distances between adjacent barbs 400 um from the rachis were significantly different ($p < 0.0005$) (see Figure 6) among types of treatment but there were no significant dose-dependent effects in any set of 3 ranges of concentrations. Feathers exposed any concentration of the oil-only solutions did not display significant differences from the control feathers for mean barb angle at shaft, mean distance between barbs 400 um from shaft, or mean distance between barbules 50 um from barbs ($p = 0.386$, 0.921 and 0.071 respectively). Feathers from any of the groups exposed to solutions with either oil and dispersant or dispersant-only had more acute angles between the rachis and barbs, and reduced distances between adjacent barbs than feathers exposed to plain IO or PBCO solutions ($p < 0.0005$). Mean spacing between barbules 40 µm from the barb was not significantly different among any treatment groups ($p = 0.521$) or when examined by dispersant vs no dispersant in the test solution ($p = 0.551$). No significant dose dependent effects were noted. Many of the non-control feathers had crystalline material present that complicated imaging under light microscopy.

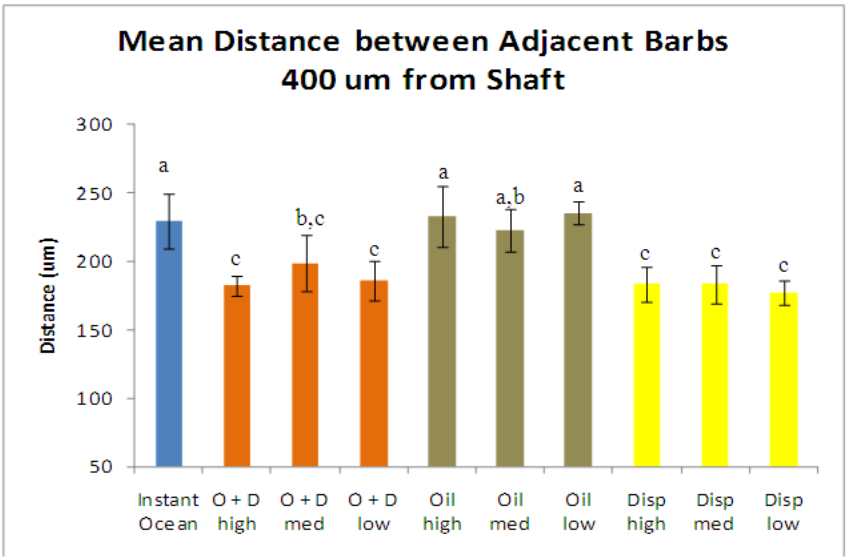

Figure 6. Distance between feather barbs 400 um from the shaft. Test groups with a letter in common may not be significantly different by Tukey's multiple comparison test at a level of significance of $\alpha = 0.05$. Error bars are standard deviations.

Blinded Observer:

The blinded observer did not detect any differences between exposure groups in the otter hair sample SEMs or light micrographs. All samples were found to have comparable amounts of foreign material present on hairs and no geometric arrangements were able to be evaluated.

In the feather images, the observer was able to successfully differentiate between feathers exposed to solutions containing dispersant vs those exposed to solutions with no dispersant ($p <$

0.0005), but could not distinguish between feathers exposed to plain seawater and those exposed to solutions from below a PBCO slick (see Figure 7), or between feathers exposed to PBCO + Corexit vs Corexit-only. The observer produced similarly significant results evaluating all sets of feather images. No significant dose dependent effects were noted.

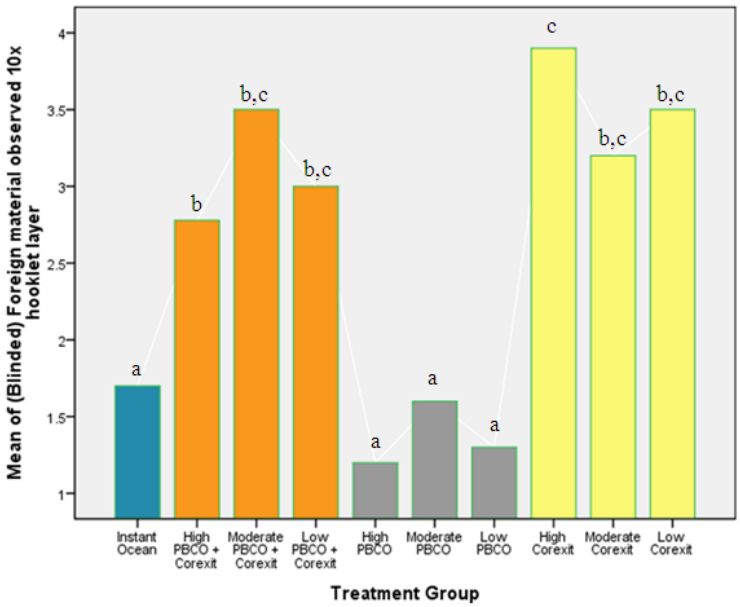

Figure 7. Blinded observer scoring of foreign material at level of hooklets at 100X. 1 = zero, 2 = small amount, 3= moderate amount, 4 = large amount of foreign material seen. Test groups with a letter in common may not be significantly different by Tukey's multiple comparison test at a level of significance of $\alpha = 0.05$.

Total Petroleum Hydrocarbons (TPH) in exposed feathers and fur:

Results obtained from the CDFG Petroleum Chemistry Laboratory are shown in Table 2.

Table 2. Total Extractable hydrocarbons reported as Total Petroleum Hydrocarbons (ppm) from tissues exposed to each test solution.

	Feathers (0.1 g) ppm	Otter pelt(10 g) ppm
Unexposed	3100-4000	600-1000
Instant Ocean	4300	2300
High PBCO + Corexit	2400	1300
Med PBCO + Corexit	4000	1500
Low PBCO + Corexit	1600	640
High PBCO	3400	1100
Med PBCO	3400	1200
Low PBCO	3400	1300
High Corexit	NA	NA
Med Corexit	NA	NA
Low Corexit	NA	NA

Scanning electron microscopy and x-ray microprobe results:

When viewed under SEM, the crystalline material that made feather imaging under light microscopy difficult was visualized as a literal crystalline encrustation. The crusting material was noted to clump the feather hooklets together and appeared as discrete crystals or as an amorphous crust. Feathers exposed to plain Instant Ocean were indistinguishable from feathers exposed to feathers from the PBCO-only group and feathers exposed to any solution containing dispersant were observed to have more crusting material present (see Figure 8), although neither of these observations were quantifiable. Under SEM, the otter hair again did not appear significantly different among treatment groups (see Figure 9).

When these crusts were examined by x-ray microprobe, Na, Cl, K, Ca, S and Mg were noted to be abundant. Discrete crystals of presumptive $CaSO_4$, NaCl, and $CaCO_3$ were identified based on emission spectra in conjunction with crystal geometry (see Figures 10 and 11). Unfortunately, atoms smaller than nitrogen cannot be identified using this imaging technique, thus the presence of carbon and oxygen in crystals is presumptive. Other crystalline material was found to be more complicated mixtures of elements such as NaMgSCl. Much of the amorphous crust was identified as simple NaCl. Chloride was found to very closely match the location of

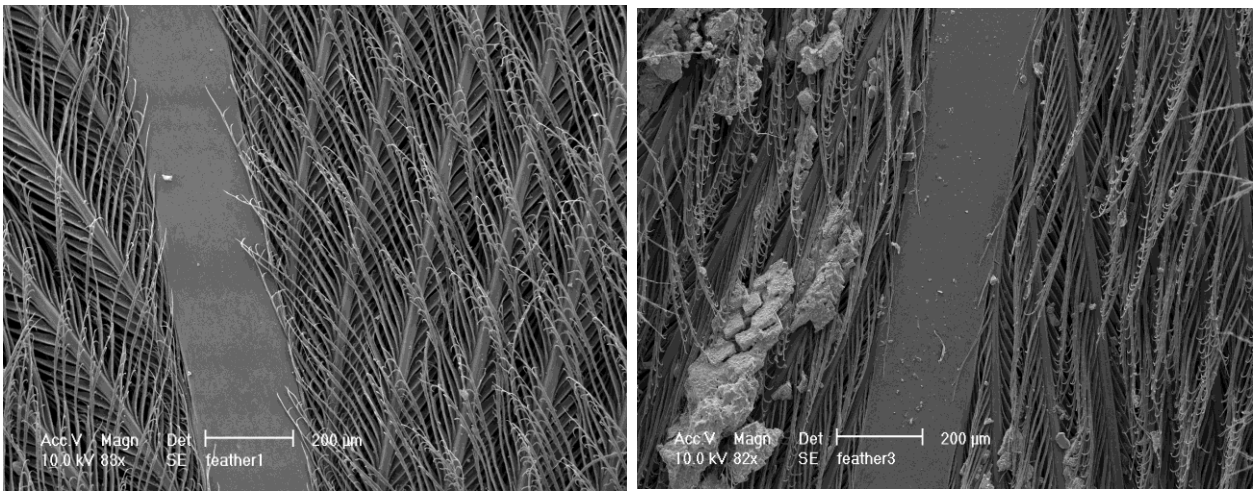

Figure 8. Scanning electron micrographs of common murre contour feathers exposed to plain artificial seawater (left) and the most concentrated solution of Prudhoe Bay crude oil and Corexit 9500 (right).

sodium so was deleted from the set of scanned elements since only five elements could be scanned in detail simultaneously and biological samples degrade quickly under x-ray bombardment.

With x-ray microprobe mapping (see Figure 12), if an element is present at all in the sample, it shows as an outline of the object. As many components of feathers include the elements imaged, this is largely responsible for the background blue image of each feather; for example, cysteine in feather keratin contains sulfur. The focal bright areas in the images indicate areas of concentration of the element, with the color scale along the right side of each image giving relative concentration (black = absent to red = high concentration). Each element is imaged at a different scale, so comparisons cannot be made between elemental sets. In set A, each feather is imaged in back scatter SEM to show the overall conformation of the feather and foreign material. Set B shows the same pieces of feather with sodium (and chloride by proxy) shown to be the majority of the long thin amorphous crusts along the feather barbs in the two feathers on the right which were exposed to oil + dispersant or dispersant-only. A small number of NaCl crystals are present on the feather exposed to oil-only, but very little is seen on the control feather. Set C

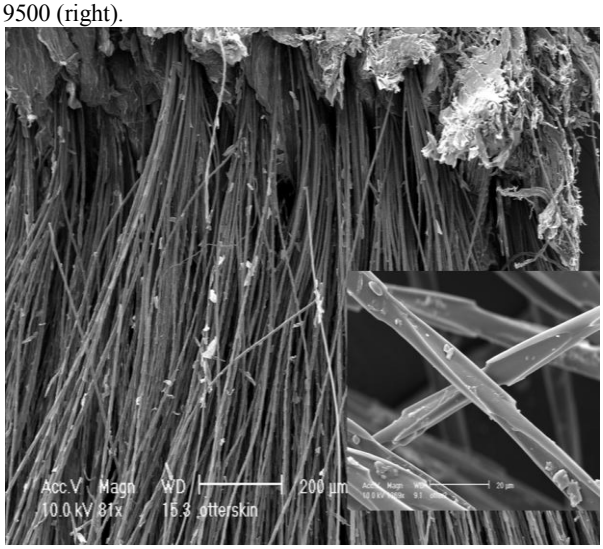

Figure 9. Scanning electron micrographs of CA sea otter hair at its emergence from the skin. No differences were seen among treatment groups.

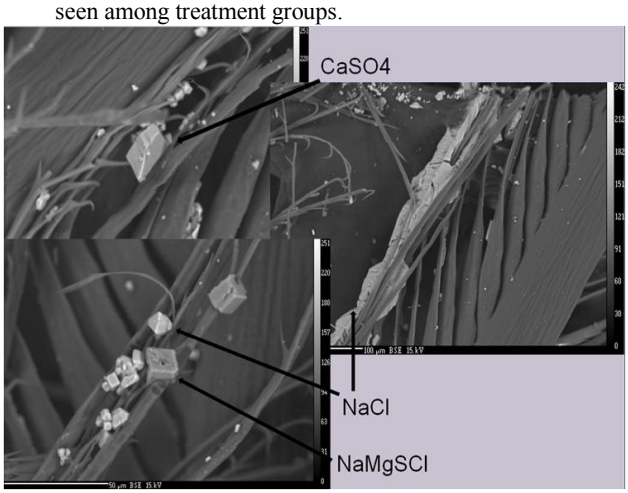

Figure 10. Back scatter electron microscopy view of crystals showing variety of crystals identified from feathers exposed to PBCO and Corexit 9500. Black dot in NaMgSCl crystal is a 1 µm laser probe hole. Scale varies among images.

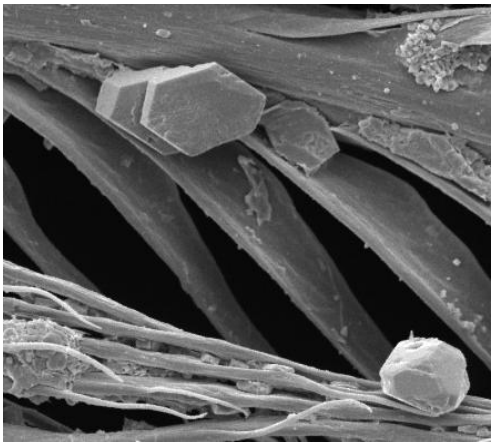

Figure 11. Hexagonal calcite crystals on feathers, as determined by crystal geometry and x-ray emission spectra.

shows that magnesium is also abundant in the NaCl crusts in the same two feathers on the right, with minimal amounts seen on the feathers not exposed to either of the dispersant solutions. Set D shows the abundance of sulfur; note the small number of sizable crystals seen, particularly at the lower left corner of the oil + dispersant exposed feather. This same crystal also lights up with a high concentration of calcium in set F. There is another individual crystal at the middle top of the oil + dispersant feather that lights up with magnesium, potassium and sulfur. Set E also shows several other crystalline areas illuminated with potassium.

Set F representing calcium shows a less distinct difference between the feathers exposed to dispersant vs not exposed to dispersant, but the smallest amount of calcium is seen on the feather exposed to IO only. A similar effect is seen in set A with sodium present in large amount in the two feathers exposed to the two dispersant solutions. Many more small NaCl crystals were seen in the oil-only solution as compared to the IO-only solution.

DISCUSSION

Under the mixing conditions selected, the investigators were able to generate small volumes of mechanically and chemically dispersed oil with a diversity of concentrations for use as test solutions. As seen in Table 1, the loading dose of PBCO and/or Corexit added to the mixing vessel did not have a linear effect on the resultant ppm, but rather drove the water accommodated fraction toward maximum amounts in solution.

As discussed with the CDFG PCL chemist, the TPH results presented in Table 2 may be the result of the assay measuring all extractable hydrocarbons including cell membranes, waxes, skin oils and other materials. These results are preliminary and are undergoing further analysis.

Results of this study showed a distinct effect of the presence of dispersant in the geometry and orderliness of structures and an increased presence of foreign material, including a variety of crystalline or amorphous matrix salts, whether imaged by light microscopy, scanning electron microscopy, x-ray mapping, and subjective observation by the blinded observer. These effects were seen even at the lowest concentrations of PBCO + Corexit (13 and 2.7 ppm respectively) or Corexit-only (12 ppm). This may be due to a direct disruptive effect on the waterproofing characteristics of feathers leading to a larger amount of water remaining on each feather to crystallize out as solids during evaporation. As the crystals condense they may literally pull the feather barbs closer together, functionally collapsing the feather plume. However, considering that the feathers from these solutions appeared grossly to have an immediate alteration in conformation while freshly wet suggests another mechanism altering the feather structure. Considering that oiled birds that are washed routinely regain lost waterproofing without replacing oiled feathers with new ones, this change must be due to a reversible process. As discussed in Stevenson and Andrews (1997), "…the wetting phenomenon and its physiologic cost to the animal, is a non-specific effect of surface chemistry rather than a characteristic of oil per se."

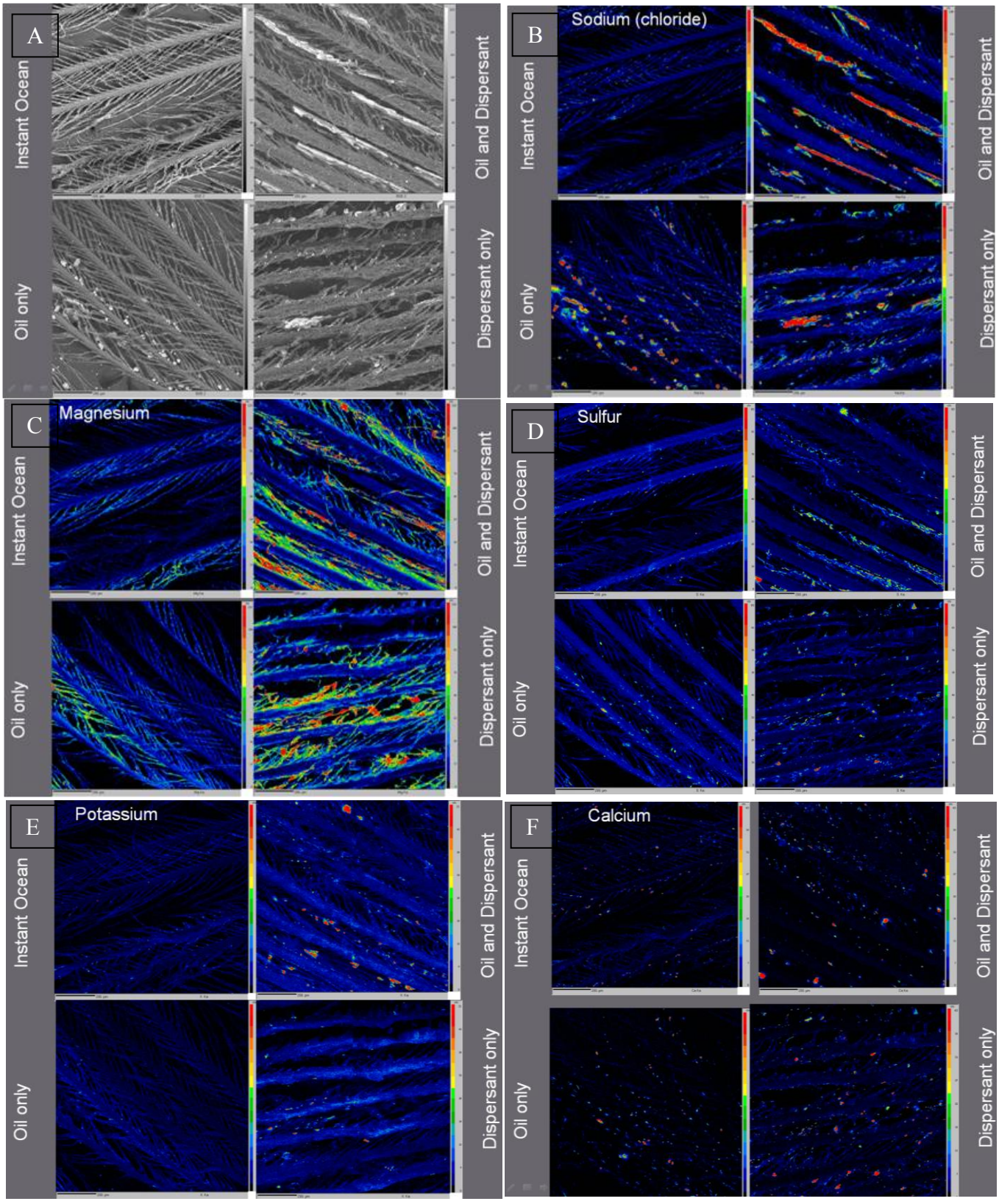

Figure 12. X-ray maps of each element of interest, as identified on common murre feathers exposed to the indicated test solutions. Each set of four images A-F for each element is not directly comparable to other element's images as the scale is different in each set. Set A is of the same pieces of feather in the other sets seen under back scatter electron imaging. Set B images sodium, which also acts as proxy for chloride.

Although no differences in the amount of foreign material were detected among the plain IO group and the various concentrations of PBCO-only exposed feathers by either the blinded observer or the morphometrics analyses, on x-ray mapping there was a mildly increased amount of very small crystals showing the presence of Na, Mg, Ca, and S. With further study it may be determined that sulfur from PBCO may be forming mineral complexes on feathers even when at low ppm and that exposure to low ppm petroleum solutions may have a discernable effect on waterproofing not appreciable here due to low numbers of feathers evaluated.

Weisel et al. (2005) discusses how the three dimensional shape of individual river otter hairs and the geometric relationships of each hair with its neighboring hairs may serve to trap air in the spaces between and among hairs. Sea otter hairs observed under light microscopy in this study were seen to spiral around each other in a manner not mentioned in that paper, although the general morphology of the hairs appeared subjectively quite similar. Further exploration of the nature of sea otter hair's ability to trap air is needed in order to fully understand functional changes due to exposure to petroleum products or dispersants. The photographic preparations made of otter hair in this study did not detect differences due to exposure. If each otter hair's relationships to its neighbors forms the primary basis of the ability of the coat to trap air, then the act of removing hairs from a pelt to examine them destroys the unit of interest even if the hairs remain attached at the skin, due to edge effects at the observable portion of the hairs. Feathers possess an inherent geometric structure that forms the primary basis of air trapping, but similar to otter hairs, each feather also has three dimensional relationships to neighboring feathers that may be altered by exposure to petroleum products or dispersants. These dynamic geometric relationships indubitably contribute to overall resistance to water penetration. Sophisticated three dimensional modeling or scanning may be required to study these relationships in both hair and feathers.

In this study we attempted to model exposure to petroleum product and dispersant as might be encountered by a bird or mammal swimming into a subsurface plume, thus separating the effects of this type of exposure from that of a surface slick. This study visually examined feathers and fur exposed to test solutions in a dry, post-exposure state. However, in a more realistic scenario, an animal might swim through a plume and then perhaps swim through an adjacent mass of pristine water that may serve to rinse the contaminating materials away. It is less likely that an animal would swim through a contaminating plume then quickly beach itself and allow its coat to dry, unless a plume was adjacent to a haul-out area. Since all the test solutions containing any amount of dispersant had an immediate and grossly observable effect on the ability of the feathers to maintain normal water repellency when submerged, it is possible that an animal swimming through a submerged plume of dispersed oil may feel immediate effects of loss of insulation and alterations in buoyancy due to a sudden loss of plumage air, regardless of whether the animal subsequently rinsed itself by swimming through adjacent uncontaminated water. Thorough preening would be expected to be required to restore normal plumage aeration after exposure.

One of the main characteristics of dispersants is their detergent properties, and it has been subjectively noticed by Oiled Wildlife Care Network staff washing oiled seabirds that waterproofing is not regained until feathers have been rinsed of all detergents. The end point of the rinse stage of washing is when rinse water sprayed on feathers is observed to immediately bead up and roll off the cleaned feathers. Further experiments could include a series of test feathers rinsed in clean water subsequent to test solutions exposure. Considering that strong effects on functional morphology were seen even in the most dilute solutions of dispersant or

dispersant + oil, a series of exposures in increasingly dilute solutions is needed to identify a lower boundary of effect. Stephenson and Andrews (1997) and Bakken et al. (2006) discuss methods to evaluate the water pressure required to penetrate single feather vanes and sections of feathered skin, respectively, which suggest methods that may be relevant to future investigations.

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