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AN EXPERIMENTAL STUDY OF THE EFFECTS OF CHEMICALLY DISPERSED OIL ON FEATHER STRUCTURE AND WATERPROOFING IN COMMON MURRES (*URIA AALGE*)

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ABSTRACT: Following an oil spill in the marine environment, chemical dispersants, which increase oil droplet formation and distribution into the water column, are assumed to provide a net benefit to seabirds by reducing the risk of exposure to oil on the water surface. However, few data are available regarding acute, external impacts of exposure to dispersed oil. We evaluated the effects of known concentrations of dispersant and crude oil in artificial seawater on live Common Murres (*Uria aalge*). Waterproofing and microscopic feather geometry were evaluated over time and compared to pre-exposure values. Birds exposed to a high concentration of dispersant experienced an immediate, life-threatening loss of waterproofing and buoyancy, both of which resolved within 2 d. Birds exposed to oil, or a dispersant and oil mixture, experienced dose-dependent waterproofing impairment without resolution over 2 d. Alterations in feather geometry were observed in oil-exposed or dispersant- and oil-exposed birds and were associated with increased odds of waterproofing impairment compared to control birds. At a given contaminant concentration, there were no significant differences in waterproofing between oil-exposed and dispersant- and oil-exposed birds. We found that acute, external effects of oil and dispersed oil exposure are comparable and dose-dependent. Our results also indicate that a zero-risk assumption should not be used when seabirds are present within the dispersant application zone.

Key words: Common Murre, Corexit 9500A®, crude oil, dispersant, feather structure, oil spill, seabird, waterproofing.

INTRODUCTION

Many seabirds, including the Common Murre (*Uria aalge*), are exquisitely sensitive to external oil contamination due to their unique reliance on plumage for thermoregulation and buoyancy. Structural properties of feathers establish a water-resistant barrier between the body and the environment, trapping an insulating layer of air against the skin (Jessup and Leighton 1996; Albers 2003). Oil exposure acutely disrupts the plumage barrier in a dose-dependent manner (Hartung 1967; Jenssen and Ekker 1988), allowing water to penetrate to the skin and resulting in loss of insulation and buoyancy, often to lethal effect (Leighton 1991; Newman et al. 2000).

Surface oil slicks present a high exposure risk for seabirds (French-McCay 2004). Spill response measures that reduce surface oil, such as chemical dispersion, have the potential to decrease exposure risk and thereby reduce morbidity and mortality (Peakall et al. 1987; National Resource Council [NRC] 2005). Chemical dispersants are typically applied to the water surface of an oil slick from a boat or airplane. Their detergent-like action increases oil droplet formation and promotes entrainment into the water column. This reduces surface oil, increases availability of petroleum to water-borne bacteria for biodegradation, and decreases shoreline habitat contamination (French-McCay 2004). Therefore, appropriate dispersant use is often considered to provide a net environmental benefit when compared to allowing oil to remain at the surface or to come ashore (Pond et al. 2000; Addassi et al. 2005; McCay and Graham 2014).

Although use of dispersant has a theoretic net benefit to seabirds, there are few data with which to evaluate risks. Lambert et al. (1982) and Jensen and Ecker (1991) documented increased basal metabolic rate and increased heat loss in birds experimentally exposed to oil and dispersant mixtures compared to controls; however, effects on plumage structure, differences in survival between oil and dispersant exposure, and change in effects over time were not explored. The lack of information was highlighted in a 1989 report by the NRC (1989), which called for research into the effects of dispersant and dispersed oil on water repellency of seabirds in realistic exposure conditions. In 2005, the NRC reiterated that the available data were insufficient to evaluate impacts of dispersant on seabirds and recommended additional study (NRC 2005). This knowledge gap remains today (Coastal Response Research Center 2017).

To assess the potential acute effects of oil and dispersant on seabirds, we ran a pilot study that examined impacts of a dispersant, Prudhoe Bay crude oil, or dispersant-treated oil on Common Murre feathers (Duerr et al. 2011). Exposure to dispersant alone, and a dispersant and Prudhoe Bay crude oil mixture, resulted in grossly decreased water repellency, altered microscopic feather geometry, and increased crystalline debris as compared to controls. However, limitations of that study precluded confident extrapolation of its results to effects in live birds. Therefore, we designed a multifactorial study to build on these preliminary data to evaluate the effects of known concentrations of dispersant alone and a dispersant and Prudhoe Bay crude oil mixture on feather geometry and whole-body waterproofing in a live seabird exposed in a single, simulated dive through a plume of contaminated water.

MATERIALS AND METHODS

All procedures were conducted under the University of California Davis Institutional Animal Care and Use Committee Protocol no. 17350. Collection and release were conducted under US Fish and Wildlife Service Scientific Collecting Permit MB-101637-0 and in collaboration with the California Department of Fish and Wildlife. Methods were briefly described in Fiorello et al. (2016).

Capture and husbandry

In December 2013, 40 Common Murre were captured on Monterey Bay, California, US (36°57′38″N, 122°0′7″W) using the Whitworth et al. (1997) technique. Birds were housed indoors in ambient temperatures (15.5–18.3 C) in freshwater pools (diameter 3.0–3.6 m, depth 1–1.3 m). Facility constraints prohibited the use of salt water except in exposure pools. All birds received night smelt (*Spirinchus starski*) ad libitum and were force-fed four to six fish once daily. Force-feeding was discontinued on study day 9 (2 d prior to initial waterproofing evaluation) and reinstituted on days 14 and 17. Itraconazole (Sporanox Oral Suspension, Amerisource Bergan, Chesterbrook, Pennsylvania, USA) was administered orally daily at 20 mg/kg body weight for prevention of aspergillosis. Sodium chloride tablets (Consolidated Midland Corporation, Brewster, New York, USA) were administered orally every other day to mitigate physiologic effects of freshwater housing (Frankfurter et al. 2012). Vitamin supplements (Seatabs, Pacific Research Laboratories, San Diego, California, USA) were administered orally every other day.

Pre-exposure assessment

Initially, birds received physical examinations and evaluation of complete blood counts and plasma chemistry panels by a veterinarian, and individuals with abnormalities were excluded. Each resultant healthy bird was randomly assigned to a control or treatment groups (Tables 1, 2). Age class was estimated from plumage and supraorbital ridge prominence in a modification of Nevins and Carter (2003). The following baseline data were collected 2 d prior to exposure: body weight and pectoral muscle mass (normal, decreased, severely decreased; scored by palpation of pectoral muscle contour and keel prominence); attitude (alert, quiet, depressed, nonresponsive); hydration status (slight, moderate, or severe dehydration; scored by mucous membrane moisture and eyelid skin turgor); and plumage condition (scored by a single observer as poor, fair, good, or excellent by presence of broken, stripped, worn, or absent contour feathers). Waterproofing status (reflected by depth of wetness) was evaluated by visual and manual inspection and categorized as the estimated percent of body surface area superficially wet (SW; presence of water-logged feathers on the exterior plumage overlying dry skin) or wet-to-skin (WTS; regions of wet skin with or without overlying wet plumage). Categories were selected

TABLE 1. Treatment groups, sample sizes, contaminant loading doses (mL/L) in the water, and measured contaminant concentrations (ppm) used for experimental exposure of Common Murres (*Uria aalge*) to oil (Prudhoe Bay Crude oil), dispersant (Corexit 9500A), and dispersed oil in artificial seawater.[a]

		Prudhoe Bay crude oil			Corexit 9500A		
			TPH concentration (μg/mL)			Concentration (μg/mL)	
Treatment group	*n*	Loading dose mL/L	Tank 1	Tank 2	Loading dose mL/L	Tank 1	Tank 2
Control	5	0	0.71	2.18	0	RL	RL
DISP-L	6	0	RL	RL	0.01	12.1	5.6
DISP-M	5	0	RL	RL	0.1	96	75
DISP-H	5	0	RL	RL	1.0	918	971
OIL-L	4	0.2	99	134	0	RL	RL
OIL-M	3	2.0	1,066	—	0	RL	—
MIX-L	5	0.2	88	99	0.01	6.3	8.4
MIX-M	3	2.0	1,128	—	0.1	78	—

[a] TPH = total petroleum hydrocarbon; RL = below reporting limit; DISP-L = low concentration dispersant; DISP-M = medium concentration dispersant; DISP-H = high concentration dispersant; OIL-L = low concentration oil; OIL-M = medium concentration oil; MIX-L = low concentration dispersed oil; MIX-M = medium concentration dispersed oil; — = a second exposure tank was not used due to small sample size in that group.

to identify birds that were completely unaffected (0%), mildly affected (1–25%), or moderately to severely affected (26–100%) by waterproofing loss, with the physiologic interpretation that greater than 25% of body surface area represents significant impairment which requires intervention for recovery (Stephenson 1997). Two feathers were plucked from the central-most portion of the ventrum for later comparison with postexposure changes. Additional behavioral and physiologic data were collected as part of this study, but will be analyzed separately as they do not directly relate to waterproofing and feather structural abnormalities.

Exposure

The control group was exposed to artificial seawater while treatment groups were exposed to increasing concentrations of the dispersant Corexit (Corexit® 9500, Ecolab, St. Paul, Michigan, USA) alone (DISP) or in combination (MIX) with Prudhoe Bay crude oil (OIL) in artificial seawater (Table 1), with an industry-standard dispersant-to-oil ratio of 1:20 (Lewis and Aurand 1997; International Tanker Owners Pollution Federation 2014). Treatments were classified as low (L), medium (M), or high (H) according to DISP or OIL level (Table 1). To confirm exposure doses, total petroleum hydrocarbon and Corexit concen-

TABLE 2. Study timeline and data collected during experimental exposure of Common Murres (*Uria aalge*) to dispersant, oil, and dispersed oil in artificial seawater.[a]

Study day	Event	Physical exam	Waterproofing	Feather sample
1–6	Capture	✓	—	—
11	Examination, baseline data collection	✓	✓	—
13	Pre-exposure evaluation	—	✓	✓
13	Postexposure evaluation	—	✓	✓
14	Day 1 postexposure evaluation	—	✓	—
15	Day 2 postexposure evaluation	✓	✓	✓
16	Cleaning	—	—	✓
17	Postcleaning evaluation	—	✓	—
21–24	Examination and release	✓	✓	—

[a] ✓ = data collected; — = data not collected.

tration were analyzed in water samples by the Petroleum Chemistry Lab (California Department of Fish and Wildlife, Sacramento, California, USA) using gas chromatography-mass spectrometry and standardized methodology in accordance with US Environmental Protection Agency Method 8015 (US Environmental Protection Agency 2014). Contaminant concentrations were representative of potential exposures in the upper 10 m of the water column shortly after a surface release (e.g., Kim et al. 2013) and within the reported total petroleum hydrocarbon ranges after the Deepwater Horizon spill (Sammarco et al. 2013). The DISP-H treatment was selected to model exposure of a seabird in the direct path of aerial or vessel-based dispersant application.

Two exposure tanks (308 L volume, 96 cm diameter, 45 cm deep) were filled with fresh water and Instant Ocean (Aquarium Sea Salt Mixture, Blacksburg, Virginia, USA) to 3.5% salinity. A circular current involving the entire depth of the water column was established using a 57 L/min aquarium pump. Contaminants were added via the pump intake line and circulated in the tank for 90 s; this interval was selected from pilot testing to allow full mixing of oil through the water column but minimize formation of a surface slick. Water samples were collected from the pump intake line immediately prior to bird exposure. Each exposure pool accommodated up to three birds simultaneously, so treatment groups were split into two groups for exposure in separate pools. Two to three birds in each group were placed simultaneously into exposure tanks and encouraged to dive by waving hands at the water surface. Birds were hand-captured starting at 75 s and held submerged to the neck until simultaneous removal at the 90-s mark. A 90-s exposure was selected to approximate a single dive (60 s; Ainley et al. 1990) with an additional 30 s to account for surfacing multiple times during the exposure dive. After exposure, waterproofing was evaluated and two feathers were plucked from the ventrum. Birds were then placed in a 600-L freshwater rinse pool for 60 min to simulate movement away from the plume and into uncontaminated water. A haul-out platform was introduced into the rinse pool if it appeared that birds were struggling to stay afloat and would not survive without assistance.

Postexposure assessment

After exposure, birds were housed by treatment group in pens custom-designed for out-of-water seabird housing (Oiled Wildlife Care Network 2014). On days 1 and 2 after exposure, each group was placed in a freshwater pool for a 45-min evaluation period. A haul-out was provided at minute five and removed at minute 40, and birds were removed at minute 45. Waterproofing was assessed directly after removal. Two feathers were plucked from the ventrum after the day 2 evaluation.

Cleaning, conditioning, and release

On day 3 after exposure, birds were cleaned, rinsed, and dried in a standardized manner (Oiled Wildlife Care Network 2014). The 2-d interval from exposure to cleaning was selected to allow documentation of effects over time without compromising ability to rehabilitate and release study subjects. Two feathers were plucked from the ventrum after cleaning. The day after cleaning, each group was placed in a freshwater pool for a final 45-min evaluation period followed by waterproofing assessment. Conditioning for release was initiated the following day. Birds were released in Monterey Bay after meeting pre-established criteria (Oiled Wildlife Care Network 2014) or approval by a veterinarian.

Analysis

Collected feathers were suspended by the calamus and air-dried. Each rachis was cut to produce two samples (a 1-cm section centered on midpoint of the rachis and the distal tip) and mounted on glass slides with coverslips secured by Cytoseal (ThermoFisher Scientific, Waltham, Massachusetts, USA) at the margin. Two images were collected from opposite sides of the rachis at 100× magnification, and images were evaluated in QCapture Pro 7 software (QImaging, Surrey, British Columbia, Canada). Three measures were evaluated at three locations (Fig. 1): distance (measurement between barbs at 200 μm from the rachis), angle (measurement between rachis and barb), and clumping (ratio of number of barbules arising from a 0.5-cm section of barb 200 μm from the rachis and the number of clumps formed from those same barbules; modified from O'Hara and Morandin 2010). Angle and distance measures were evaluated in both the center and tip sections while clumping was only evaluated in the tip due to feather morphology. Analyses were performed separately for the tip and center sections due to differing levels of gross contamination and morphologic change.

Differences in distribution of morphologic and physiologic characteristics (e.g., age class, plumage condition) within and between exposure groups were assessed using Kruskal-Wallis H tests (KW) and one-way analysis of variance. The KW was used to investigate for differences in distribution of waterproofing scores across groups at each time period, and post hoc pairwise comparisons were performed with Dunn's (1964) procedure and Bonferroni correction for multiple

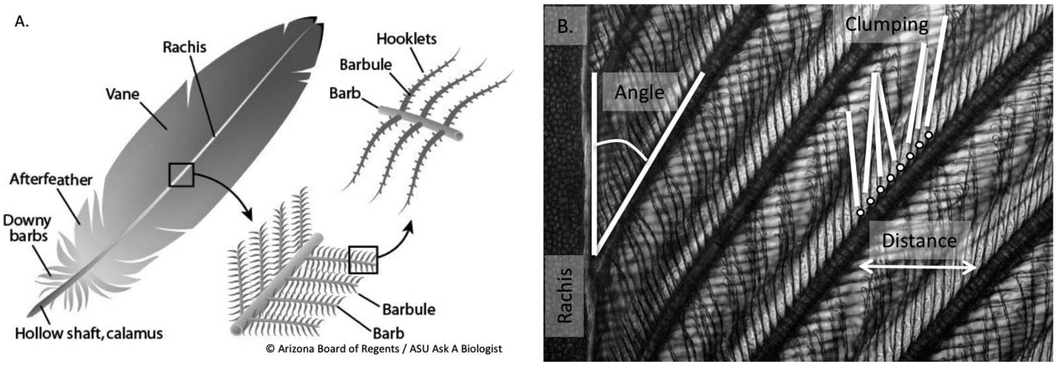

FIGURE 1. (A) Schematic representation of feather structure and (B) light microscopy image of a Common Murre (*Uria aalge*) feather at 100× light magnification. The light microscopy image (B) illustrates the measures used for quantification feather structural change after exposure of live Common Murres to oil (Prudhoe Bay Crude oil), dispersant (Corexit 9500A), and dispersed oil in artificial seawater. Angle is measured between the central rachis and a barb. Distance is measured between adjacent barbs 200 μm from the rachis. Clumping is the ratio of number of barbules arising from a 0.5-cm section of barb 200 μm from the rachis and the number of clumps formed from those same barbules. Schematic used with permission of Arizona Board of Regents, ASU School of Life Sciences, "Ask a Biologist" (https://askabiologist.asu.edu).

comparisons. Repeated measure random effects (mixed effects) models were used to evaluate differences in feather measures between treatments and control. Fixed effects for treatment, time period, and the treatment by time period interaction were included in the models, in addition to random effects for bird and feathers nested within birds, to account for the multiple measurements on each feather from each bird. Distance and clumping were log transformed to meet underlying homoscedasticity assumptions of the models. Post hoc comparisons were performed between exposure groups and control at each time point as well as between the three groups at the medium contaminant level. Feather characteristics (distance, angle, clumping) were averaged across feathers at each time point and evaluated as predictors of wetness. Generalized estimating equation approaches for repeated measures ordinal data, in the context of multinomial logistic regression with a cumulative logit link function, were used to assess how feather characteristics were associated with wetness and the difference by groups. Model building began with single feather characteristics, and variables with a *P*-value less than 0.1 were considered together in a joint model (including interactions between feather characteristics). Analyses were conducted in SAS (SAS Institute 2011) and SPSS (IBM 2013), with an alpha level of 0.05.

RESULTS

Of 40 birds captured, four were excluded from the study and transferred to rehabilitative care due to chronic disease (*n*=3) or gross plumage oil contamination (*n*=1). Of the remaining 36 birds, four mortalities (three deaths and one euthanasia) occurred between the exposure and cleaning phases including birds from OIL-M (*n*=2), DISP-H (*n*=1), and MIX-M (*n*=1). Gross necropsy and histopathology revealed multiple abnormalities in each bird including bacterial pneumonia (*n*=3), air sacculitis (*n*=2), suspected viral bronchitis (*n*=2), and coccidial enteritis (*n*=1).

Of 36 birds enrolled in the study and observed during the pre-exposure exam, plumage condition was excellent (*n*=35) or good (*n*=1), with no gross evidence of molt or plumage contamination. There were no significant differences in distribution of age class (KW, *P*=0.663), plumage condition (KW, *P*=0.348), body condition (KW, *P*=0.663), attitude (KW, *P*=1.0), or hydration status (KW, *P*=0.817) across treatment groups or in mean body weight (analysis of variance, $F_{7,28}$=0.680, *P*=0.688). Similarly, the distribution of waterproofing scores was not significantly different between treatment groups before exposure (KW, SW *P*=0.143, WTS *P*=1.0).

Distribution of waterproofing scores (Table 3) was significantly different across treatment groups immediately after and on days 1 and 2

TABLE 3. Waterproofing of Common Murre (*Uria aalge*) plumage as evaluated after exposure to oil (Prudhoe Bay Crude oil), dispersant (Corexit 9500A), and dispersed oil in artificial seawater. Data are presented for each exposure group from five time points: immediately before exposure, immediately after exposure, after 45-min in-water evaluation periods at days 1 and 2 after exposure, and after cleaning. Values are given for the proportion of each treatment group with percent of body surface area (0%, 1–25%, or 26–100%) superficially wet (SW) or wet-to-skin (WTS).

		Proportion of each treatment group with percent of body surface area SW or WTS at time points before and after exposure																			
		Before exposure				Immediately after exposure				Day 1 after exposure				Day 2 after exposure				After cleaning			
Treatment group[a]	Measure[b]	*n*	0%	1–25%	26–100%	*n*	0%	1–25%	26–100%	*n*	0%	1–25%	26–100%	*n*	0%	1–25%	26–100%	*n*	0%	1–25%	26–100%
Control	SW	5	0	1	0	5	0	0.8	0.2	5	0.2	0.8	0	5	0.2	0.8	0	5	0	1	0
	WTS		1	0	0		1	0	0		0.2	0.8	0		0.6	0.4	0		0.4	0.4	0.2
DISP-L	SW	6	0	1	0	6	0	0.83	0.17	6	0.2	0.6	0.2	6	0.17	0.5	0.33	6	0.5	0.5	0
	WTS		1	0	0		0.17	0.83	0		1	0	0		0.5	0.5	0		0	0.5	0.5
DISP-M	SW	5	0	0.4	0.6	5	0	0	1	5	0.4	0.6	0	5	0.4	0.6	0	5	0.4	0.6	0
	WTS		1	0	0		0.25	0.75	0		1	0	0		1	0	0		0.2	0.4	0.4
DISP-H	SW	5	0	0.6	0.4	5	0	0	1	5	0.2	0.2	0.6	4	0.25	0.75	0	4	0.25	0.75	0
	WTS		1	0	0		0	0	1		0.2	0.4	0.4		0.5	0.5	0		0.75	0.25	0
OIL-L	SW	4	0	1	0	4	0	0	1	4	0.25	0	0.75	4	0	0.25	0.75	4	0.25	0.5	0.25
	WTS		1	0	0		0	1	0		0.5	0.5	0		0	1	0		0.5	0.25	0.25
OIL-M	SW	3	0	1	0	3	0	0	1	3	0	0	1	3	0	0	1	2	0	0.5	0.5
	WTS		1	0	0		0	0.33	0.67		0	0	1		0	0	1		0	1	0
MIX-L	SW	5	0.2	0.6	0.2	5	0	0	1	5	0	0	1	5	0	0.2	0.8	4	0.5	0.25	0.25
	WTS		1	0	0		0	1	0		0	0.8	0.2		0	1	0		0.25	0.25	0.5
MIX-M	SW	3	0	1	0	3	0	0	1	3	0	0	1	3	0	0	1	3	0	0.33	0.67
	WTS		1	0	0		0	0.67	0.33		0	0	1		0	0	1		0	0.33	0.67

[a] DISP-L = low concentration dispersant; DISP-M = medium concentration dispersant; DISP-H = high concentration dispersant; OIL-L = low concentration oil; OIL-M = medium concentration oil; MIX-L = low concentration dispersed oil; MIX-M = medium concentration dispersed oil.

[b] SW = presence of water-logged feathers on the exterior plumage overlying dry skin; WTS = regions of wet skin with or without overlying wet plumage.

TABLE 4. Results of a Kruskal-Wallis H test (KW) for differences in distribution of plumage waterproofing scores of Common Murres (*Uria aalge*) exposed to varying levels of oil (Prudhoe Bay Crude oil), dispersant (Corexit 9500A), and dispersed oil in artificial seawater. Plumage waterproofing was evaluated by estimating percent of body surface area superficially wet (SW) and wet-to-skin (WTS). At each time point after exposure, the distribution of waterproofing scores across treatment groups was compared to the scores of the control group at that time point and compared to the scores of all birds before exposure. Distribution of waterproofing scores across-treatment groups was significantly different from the control group immediately after exposure and on days 1 and 2 after exposure. Distribution of scores was not significantly different from control after cleaning. For all time points after exposure, distribution of scores was significantly different from the pooled scores of all birds before exposure.

Comparison group	Waterproofing measure[a]	*P*-values for KW of distribution of waterproofing scores across treatment groups			
		Immediately after exposure	Day 1 after exposure	Day 2 after exposure	After cleaning
Control group at each time point	SW	<0.001	0.006	0.003	0.084
	WTS	<0.001	<0.001	0.001	0.278
All birds before exposure	SW	<0.001	<0.001	<0.001	0.004
	WTS	<0.001	<0.001	<0.001	<0.001

[a] SW = presence of water-logged feathers on the exterior plumage overlying dry skin; WTS = regions of wet skin with or without overlying wet plumage.

after exposure, both when compared to control and to pooled pre-exposure scores for all birds (KW, all $P<0.05$; Table 4). Most striking of these differences was a catastrophic loss of waterproofing in DISP-H, which was evident immediately after exposure and lessened over the subsequent 2 d (Figs. 2C, 3C). We observed negative effects on waterproofing from exposure to lower dispersant concentrations, but they were not as severe as in DISP-H (Figs. 2, 3). Waterproofing scores of all oil-exposed groups (OIL or MIX) worsened after exposure in a dose-dependent manner and did not resolve over time (Figs. 2, 3). There were no significant differences in distribution of scores between OIL and MIX groups at the same contamination level at each time period (KW, all $P=1.000$). The distribution of waterproofing scores was not significantly different across treatment groups after cleaning when compared to control (KW $P=0.278$; Tables 3, 4).

The log transformed distance in the tip section of feathers differed across groups over time. Values for each group were not different from control before exposure, apart from DISP-L, which had a smaller distance on average ($P=0.037$; Fig. 4). The magnitude of change in distance from pre-exposure to each time point after exposure varied significantly compared to the control ($P<0.001$; Fig. 4). Immediately after exposure, there was a significantly greater decrease in distance in OIL-L ($P=0.003$), OIL-M ($P<0.001$), MIX-L ($P=0.028$), and MIX-M ($P=0.002$) compared to control. At day 2 after exposure, there was a significantly greater decrease in distance in OIL-L ($P=0.043$), OIL-M ($P<0.001$), and MIX-M ($P<0.001$) compared to control. After cleaning, there were no significant differences from the control in the magnitude of the change in distance from pre-exposure to postcleaning. There were no significant differences between groups in distance at the center section of feathers.

In a similar fashion, clumping in the tip section of feathers varied significantly between exposure groups over time ($P<0.001$; Fig. 5). Before exposure, values for each treatment group were not different from

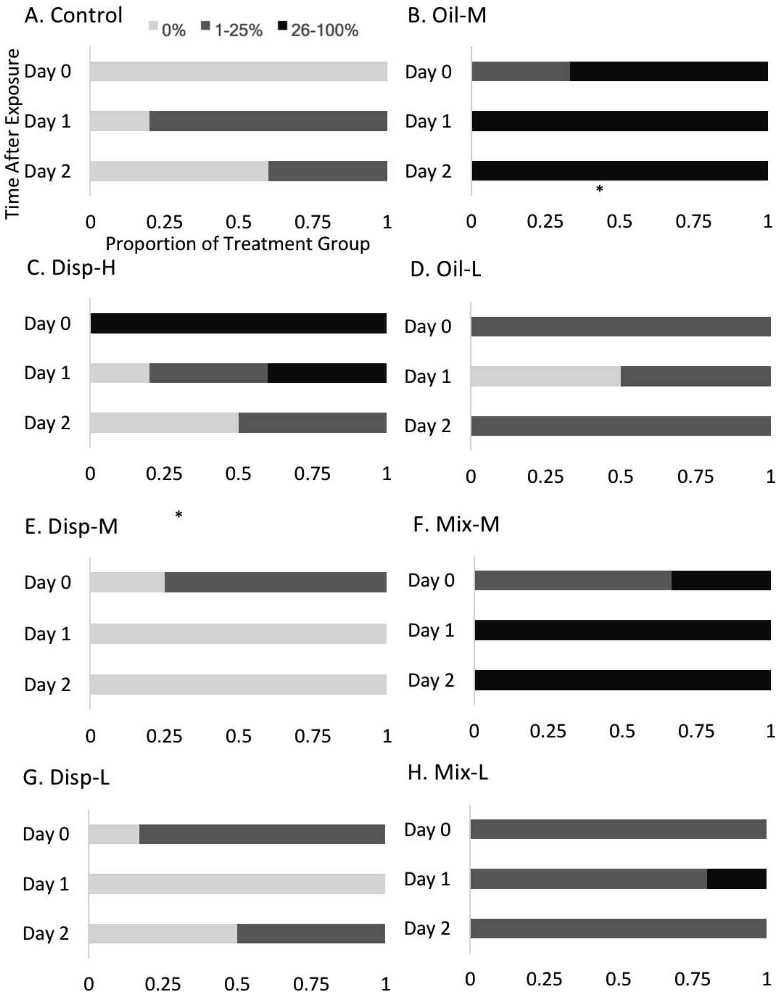

FIGURE 2. Waterproofing of Common Murre (*Uria aalge*) plumage after exposure to oil (Prudhoe Bay Crude oil), dispersant (Corexit 9500A), and dispersed oil in artificial seawater. Data are presented from three times: immediately after exposure and after 45-min in-water evaluation periods 1 and 2 d after exposure. Plumage waterproofing was quantified by estimating percent of body surface area wet-to-skin. An asterisk indicates significant difference in distribution of waterproofing scores from control at that time point (Kruskal-Wallis H test with post hoc pairwise comparisons using Dunn's [1964] procedure and a Bonferroni correction for multiple comparisons, alpha level 0.05). Oil-M=medium concentration oil; Disp-H=high concentration dispersant; Oil-L=low concentration oil; Disp-M=medium concentration dispersant; Mix-M=medium concentration dispersed oil; Disp-L=low concentration dispersant; Mix-L=low concentration dispersed oil.

control with the exception of a lower score in MIX-M ($P=0.004$). In the control, clumping was increased at day 2 after exposure ($P<0.001$) and after cleaning ($P<0.001$) compared to before exposure. For the majority of treatment groups, clumping increased from before to immediately after exposure and decreased from day 2 after exposure to after cleaning (Fig. 5). Immediately after exposure, the magnitude of increase in clumping from pre-exposure was significantly greater compared to control for MIX-M ($P<0.001$), OIL-M ($P<0.001$), and OIL-L ($P=0.016$) immediately after exposure. On day 2 after exposure, MIX-M and OIL-M had a further significant increase in clumping

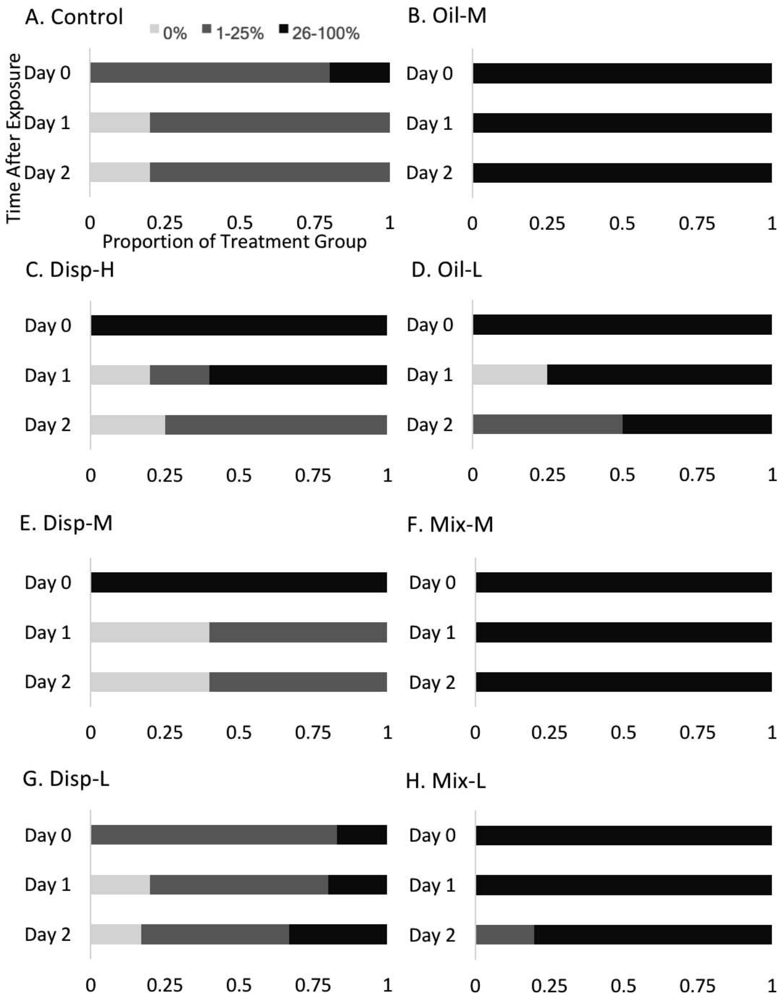

FIGURE 3. Waterproofing of Common Murre (*Uria aalge*) plumage after exposure to oil (Prudhoe Bay Crude oil), dispersant (Corexit 9500A), and dispersed oil in artificial seawater. Plumage waterproofing was quantified by estimating percent of body surface area superficially wet. Data are presented from three times: immediately after exposure and after 45-min in-water evaluation periods 1 and 2 d after exposure. In parts C, E, F, and H, on Day 0 there was a significant difference in distribution of waterproofing scores compared to control (Kruskal-Wallis H test with post hoc pairwise comparisons using Dunn's [1964] procedure and a Bonferroni correction for multiple comparisons, alpha level 0.05). Oil-M=medium concentration oil; Disp-H=high concentration dispersant; Oil-L=low concentration oil; Disp-M=medium concentration dispersant; Mix-M=medium concentration dispersed oil; Disp-L=low concentration dispersant; Mix-L=low concentration dispersed oil.

compared to control ($P<0.001$) while changes in clumping for other groups were similar to control. After cleaning, there were no significant differences between any exposure group and control.

To further elucidate the impacts of oil versus dispersant on clumping, differences for OIL-M, DISP-M, and MIX-M were compared. Immediately after exposure, clumping in both OIL-M and MIX-M was significantly higher than in DISP-M ($P<0.001$). Clumping in OIL-M was also significantly higher than in MIX-M ($P=0.022$). On day 2 after exposure, clumping in OIL-M and MIX-M was still significantly higher than in DISP-M ($P<0.001$), but there was no significant difference in clumping between OIL-M and MIX-M.

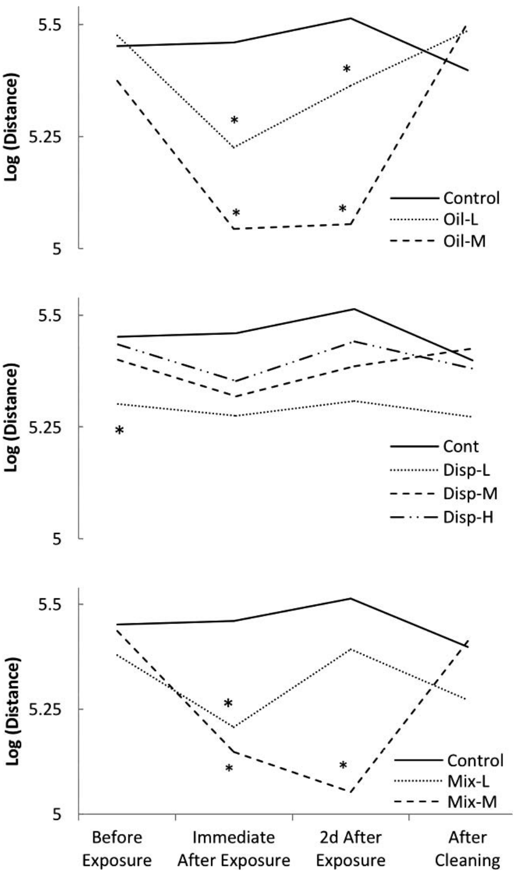

FIGURE 4. Microscopic structure of feathers collected from Common Murres (*Uria aalge*) after exposure to oil (Prudhoe Bay Crude oil), dispersant (Corexit 9500A), and dispersed oil in artificial seawater. Structure was quantified by measuring the distance between adjacent barbules at the distal tip of each feather. The log transformed data are presented as estimated from a fitted mixed-effects model from four times: before exposure, immediately after exposure, after a 45-min in-water evaluation period 2 d after exposure, and after cleaning. An asterisk indicates the magnitude of change in tip distance from pre-exposure through that time point is significantly different from the control (alpha level 0.05). Oil-L=low concentration oil; Oil-M=medium concentration oil; Cont=control; Disp-L=low concentration dispersant; Disp-M=medium concentration dispersant; Disp-H=high concentration dispersant; Mix-L=low concentration dispersed oil; Mix-M=medium concentration dispersed oil.

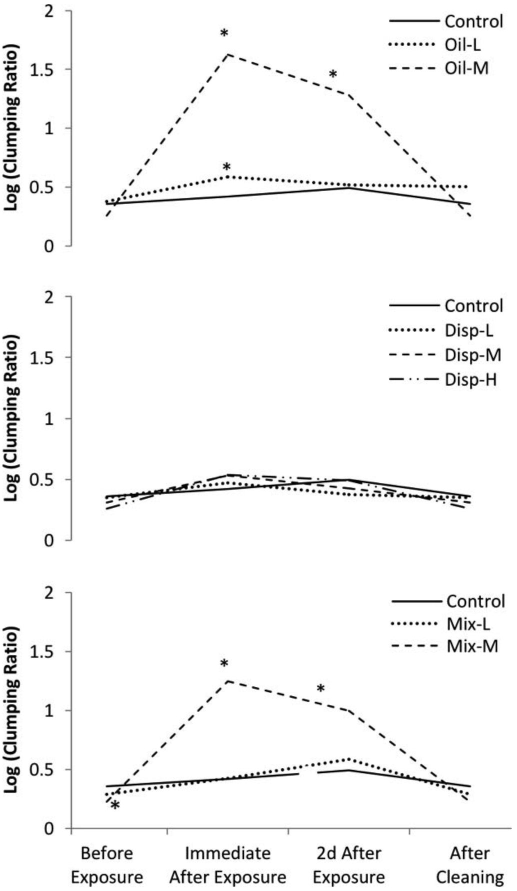

FIGURE 5. Microscopic structure of feathers collected from Common Murres (*Uria aalge*) after exposure to oil (Prudhoe Bay Crude oil), dispersant (Corexit 9500A), and dispersed oil in artificial seawater. Structure was quantified from the ratio of barbules to barbule-clumps along a 0.5-cm section of feather barb at 200 µm from the rachis in the central section of each feather. The log transformed data are presented as estimated from a fitted mixed-effects model from four time points: before exposure, immediately after exposure, after a 45-min in-water evaluation period 2 d after exposure, and after cleaning. Higher values indicate greater clumping of barbules. An asterisk indicates significant difference from control at that time point (alpha level 0.05). Oil-L=low concentration oil; Oil-M=medium concentration oil; Disp-L=low concentration dispersant; Disp-M=medium concentration dispersant; Disp-H=high concentration dispersant; Mix-L=low concentration dispersed oil; Mix-M=medium concentration dispersed oil.

To evaluate associations of feather structural changes with qualitative waterproofing scores, univariate and multivariate models were designed incorporating distance, clumping, time, and exposure. In the simple models including only one feather measure, smaller mean distance (P=0.025) and higher mean clumping (P=0.010) in the tip section were the only measures that had a marginal or significant association with increased SW. In the multivariate model including all factors, there were significant differences in SW by time period (P<0.001) and by clumping (P=0.047). Compared to before exposure, all groups had increased odds of higher SW scores immediately after exposure (P<0.001) and all groups had decreased odds of higher SW scores after cleaning (P=0.048). Smaller mean tip distance (P=0.039) and higher mean clumping (P=0.029) were associated with greater odds of higher WTS scores in univariate models. In the multivariate assessment, there was a significant interaction between clumping and time period (P=0.031). On day 2 after exposure, an increase in clumping was associated with greater odds of high WTS score (P=0.004) while an increase in clumping was marginally significantly associated with lower odds of a high WTS score after cleaning (P=0.050).

DISCUSSION

Results demonstrated that seabird waterproofing is negatively affected in a similar, dose-dependent manner by both crude oil and chemically dispersed crude oil. Dispersant alone also has negative waterproofing effects, with catastrophic consequences at high concentrations. Impacts of dispersant improved with the time birds spent out of water whereas the impacts of oil and dispersed oil did not improve over time. Before exposure, measures of demographics, plumage quality, waterproofing, and feather structure were largely comparable across treatment groups. Therefore, results reflect effects of treatment rather than of previous condition.

The control group exhibited mild impairment of waterproofing over the course of the study, likely due to the effects of handling and of housing out of water. This established a baseline from which treatment impacts at each time point could be compared. However, minor petroleum product contamination present in control tanks (likely originating from pilot testing) may have slightly contributed to baseline waterproofing impairment.

Birds exposed to oil were affected in a dose-dependent fashion across all measures throughout the duration of the study. Immediately after exposure, OIL-L and OIL-M had decreased distance and increased clumping in feather tips relative to control, indicating collapse of normal architecture. Both decreased distance and increased clumping were associated with increased SW and WTS, indicating these structural changes may affect plumage waterproofing. On day 2 after exposure, OIL-L and OIL-M had persistent but slightly improved decrease in distance at feather tips and OIL-M had continued significant clumping relative to control. There was no evidence of recovery from oil-associated feather structural change and waterproofing impairment 2 days after exposure, suggesting that recovery from contamination without human intervention is unlikely.

Effects of dipsersant-treated oil were similar to those of oil alone. Groups exposed to dispersant and oil had decreased distance at feather tips immediately after exposure relative to control, with this decrease persisting in MIX-M on day 2. The MIX-M feathers also had increased clumping relative to control immediately after and on day 2 after exposure. There was a nonsignificant trend of increased SW and WTS scores in MIX-L and MIX-M. These findings suggest that chemical dispersant does not notably alter the impact of oil exposure on waterproofing, nor does it improve the likelihood of recovering functional waterproofing after exposure, and thus findings are comparable to those few in the literature. Lambert et al. (1982) measured the basal metabolic rate of adult mallards (*Anas platyrhynchos*) experimentally exposed to

OIL, DISP, or MIX and found basal metabolic rates of oil- and oil and dispersant-exposed mallards did not differ, but both increased significantly relative to controls.

Three phenomena were observed in birds exposed to dispersant only. First, observed impacts of DISP-H exposure were immediate and life-threatening. The SW and WTS scores increased significantly after exposure, and birds experienced complete loss of buoyancy; intervention in the rinse pool was deemed necessary to prevent drowning. Lambert et al. (1982) described similar findings in mallards exposed to Corexit 9527 alone. Second, loss of waterproofing in dispersant-exposed groups was distinctly improved after 1 day. Third, dispersant-only exposure did not impact distance or clumping, indicating that, in contrast to oil, the observed impacts to waterproofing do not arise from feather structural change. This finding held true for all time points, including immediately after exposure, prior to the rinse pool.

Collectively, these results indicate that accidental exposure of birds to pure, high-concentration dispersant, such as during aerial or boat-based application, may result in high morbidity and mortality. However, affected birds that are off water for at least 1 day (e.g., that make landfall or are collected from the water) may survive to recover functional waterproofing without further intervention. Feather structural changes that appear to be associated with waterproofing loss after oil exposure are not found after dispersant exposure alone. Several hypotheses are available to explain these findings. Lambert et al. (1982) hypothesized that loss of waterproofing in dispersant-exposed birds was due to infiltration of a hydrophilic surfactant component of dispersant into the plumage. Stephenson and Andrews (1997) measured water penetration due to reduced surface tension (resulting in penetration of water through gaps between feather barbs and barbules) in a variety of waterbird species and estimated that ducks and geese would experience total loss of waterproofing if exposed to surface tension 50–55% lower than normal. In our study, the immediate waterproofing impact may have been due to evaporation of a volatile component of the dispersant over the interval between exposure and evaluation the following day or due to rinsing of the water-soluble dispersant off the feathers during the 60-min rinse period in clean water after exposure. Further work is required to investigate these hypotheses.

Overall, our results suggest that chemical dispersants such as Corexit can have immediate external impacts on seabirds, with possible life-threatening consequences. Further, this study shows that oil-dispersant mixes have similar waterproofing impacts to oil alone; therefore, exposure within a water column could have comparable impacts to that seen swimming through a surface slick. It is important to note that the impact of dispersed oil might vary based on the dispersant to oil ratio. The 1:20 ratio used here reflects US industry recommendations; the actual ratio applied to a spill and that encountered by a bird at sea may be both spatially and temporally variable (Bejarano et al. 2013). Therefore, in net environmental benefit analyses, a "zero risk" assumption associated with dispersant application should not be used when seabirds are present. However, it is clearly understood and acknowledged that surface oiling constitutes a great risk to seabirds in a spill, and effective chemical dispersion of a surface slick (resulting in distribution of oil into the water column) can lead to decreasing the overall concentration of oil to which a given bird might be exposed. These advantages and disadvantages must be weighed carefully when faced with chemical dispersant use in seabird habitats. Additionally, further work is necessary to elucidate broader impacts of dispersed oil on seabirds, including data on effects of internal and chronic exposure and the role of surface tension and volatile components on waterproofing.

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