Effects of Renewable Diesel Exposure on Feather Structure and Waterproofing in Common Murres (*Uria aalge*)

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Prepared by: Weill School of Veterinary Medicine, University of California, Davis
944 Garrod Dr., Davis, CA 95616

Prepared for: California Department of Fish and Wildlife
Office of Spill Prevention and Response
1010 Riverside Pkwy, West Sacramento, CA 95605

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Atharva Kaprekar
Weill School of Veterinary Medicine, UC Davis

April DaSilva
California Department of Fish and Wildlife, Office of Spill Prevention and Response

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Introduction

Marine oil contamination poses a well-documented threat to seabirds (Jenssen, 1994; O'Hara and Morandin, 2010) particularly through disruption of feather structure and waterproofing, which are essential for thermoregulation and buoyancy. Feather function depends on the precise alignment of barbs and barbules that create a cohesive, water- repellent surface (Rijke, 1970). Even subtle disruptions at the microstructural level can compromise this system, leading to water penetration, loss of insulation and increased energetic stress.

Previous research has demonstrated that petroleum-based contaminants, including fresh, naturally weathered, and chemically dispersed crude oil, can impair feather integrity and function (Matcott et al., 2019; O'Hara and Morandin, 2010). Experimental studies have shown that exposure to chemical dispersants can alter feather microstructure, including changes in barb spacing and barbule organization, ultimately leading to feather dysfunction and reduced waterproofing performance.

Despite extensive research on natural (e.g., crude and refined) oil, there is limited understanding of how alternative fuels such as renewable diesel affect seabird feathers. Renewable diesel used in this study refers to commercially available hydrotreated fuel derived from biological feedstocks such as vegetable oils, waste oils, and animal fats, making it chemically distinct from conventional petroleum diesel (Knothe, 2010). Renewable diesel is often promoted as a cleaner alternative fuel due to its reduced lifecycle greenhouse gas emissions, lower Sulphur content, and compatibility with existing diesel infrastructure. Increasing regulatory and industrial shifts towards lower-emission fuels have expanded the production and environmental presence of renewable diesel in marine and transportation systems (Knothe, 2010); however, its ecological impacts, particularly on wildlife, remain largely understudied. Given the increasing production and use of renewable fuels, evaluating their potential biological effects is critical.

The present study focuses on Common Murre (*Uria aalge*) feathers as a model system to investigate the effects of fuel exposure on seabird feather structure and waterproofing. Seabirds in the Auk family are particularly sensitive to oil contamination due to their reliance on feather integrity for survival in cold marine environments (Jensen, 1994; Piatt et al., 1990; Rijke 1970). Common Murres were among the seabird species most heavily affected during the *Exxon Valdez* oil spill and subsequent chronic oil exposure events associated with the SS *Jacob Luckenbach*, contributing substantially to observed seabird mortality (NOAA, 2023; Piatt et al., 1990). Their pursuit-diving behavior, prolonged contact with the water surface, and reliance on feather waterproofing for insulation and thermoregulation make them particularly vulnerable to hydrocarbon contamination (Jensen, 1994; Piatt et al., 1990; Rijke 1970).

The primary objectives of this study were to determine whether exposure to renewable diesel alters feather waterproofing and structure compared to seawater controls, compare the effects of renewable diesel with conventional diesel on feather integrity and function, and explore potential relationships between microstructural changes and functional outcomes. The study integrated complementary analytical approaches, including light microscopy (to assess macroand meso-scale feather structure), waterproofing assays (to evaluate functional performance), and scanning electron microscopy (SEM) (to quantify microstructural changes, particularly barbule clumping). By addressing these objectives, this study provides a multi-scale assessment of how renewable diesel exposure affects seabird feather structure and function and evaluates whether it presents risks comparable to conventional diesel. Based on previous evidence that hydrocarbon contaminants impair feather waterproofing and structure, we hypothesized that renewable diesel exposure would significantly alter feather waterproofing and structural integrity relative to seawater control (H1). We additionally hypothesized that renewable diesel would differ from conventional diesel in its effects on feather function and structure (H2). These hypotheses were evaluated using complementary functional, structural, and microstructural analyses.

Materials and Methods

Study Design and Sample Groups

Feathers were collected from a single Common Murre (*Uria aalge*) carcass obtained through International Bird Rescue (Fairfield, California). The carcass was refrigerated and stored at the One Health Institute (OHI) at the University of California, Davis Weill School of Veterinary Medicine until feather collection. All feathers were collected post-mortem and handled in accordance with institutional guidelines.

A total of sixty-six ventral contour feathers were used for light microscopy and waterproofing analyses. Sample size was determined by the number of comparable ventral contour feathers available from a single Common Murre carcass and by the need to distribute feathers across all treatment groups in a reasonably balanced manner. A one-way Analysis of Variance (ANOVA) power calculation was used during the study planning phase as an approximate guide for the overall experimental design. Sample size was additionally constrained by the decision to use only comparable ventral contour feathers from a consistent body region, which was done to reduce structural heterogeneity across samples and improve comparability among treatment groups. Ventral contour feathers were specifically selected because they play an important role in waterproofing and insulation by contributing to the formation of the feather surface barrier responsible for maintaining hydrophobicity and thermoregulation in seabirds (Muzio et al., 2024; Rijke, 1970). Due to limited sample size of sixty-six feathers, and associated low statistical power, the final analyses were conducted using non-parametric and exact methods.

Feathers were randomly assigned to treatment groups consisting of seawater control and multiple diesel exposure conditions. Diesel treatments included both conventional diesel (CD) and renewable diesel (RD).

Fuel treatments were structured across fuel type, blend percentage, and concentration, as summarized in Table 1. This resulted in a total of eight diesel treatment groups (two CD groups and six RD groups), in addition to one seawater control group.

Table 1. Summary of experimental exposure groups, fuel types, blend percentages, and concentrations used in Common Murre (*Uria aalge*) feather diesel exposure experiments. Blend formulations consisted of 80% renewable diesel/20% conventional diesel (RD80/20) and 95% renewable diesel/5% conventional diesel (RD95/5). Nominal concentration refers to the total fuel concentration with the exposure mixture.

Fuel Type	Group	Blend Formulation	Nominal Conc. (g/L)	n
Seawater	Control	0	0	8
Renewable Diesel	RD100_0.125	100% RD	0.125	7
	RD100_0.5	100 RD	0.5	7
	RD80/20_0.125	80% RD/ 20% CD	0.125	7
	RD80/20_0.5	80% RD/ 20% CD	0.5	7
	RD95/5_0.125	95% RD/5% CD	0.125	7
	RD95/5_0.5	95% RD/5% CD	0.5	7
Conventional Diesel	CD100_0.125	100% CD	0.125	8
	CD100_0.5	100% CD	0.5	8

Experimental Workflow and Facilities

The experimental workflow was conducted across three facilities. Phase 1 was conducted in the OHI Laboratory and involved feather collection, labelling, artificial seawater preparation, and diesel-seawater solution preparation. Phase 2 was conducted in the California Veterinary Emergency Team (CVET) Response Trailer, where feather exposures were performed under cooled (16–18°C), field- relevant conditions. Phase 3 involved feather drying, waterproofing assessment, light microscopy, and SEM imaging and was conducted across the OHI Laboratory and the Advanced Materials Characterization and Testing (AMCaT) Laboratory at UC Davis. The overall experimental workflow is illustrated in Figures 1A and 1B.

Feather Collection and Baseline Handling

Feathers were collected using clean forceps and placed on a clean working surface. Each feather was assigned a unique identifier (Feather_ID) and labelled accordingly. Feathers were stored dry at room temperature prior to exposure. Handling procedures were designed to minimize contamination and preserve native feather structure prior to experimental treatment (Muzio et al., 2024; Rijke, 1970).

Randomization and Blinding

Feathers were randomly assigned to treatment groups prior to exposure to minimize allocation bias. Assignment was conducted using a randomized distribution of feather identifiers across control and diesel treatment conditions. To reduce observational bias, feather identifiers were used throughout data collection and analysis, and measurements were performed without reference to treatment groups where feasible. Light microscopy measurements and semi-quantitative scoring were conducted using coded samples to maintain consistency in evaluation. All scoring was performed by a single observer, and observer consistency was maintained across all samples.

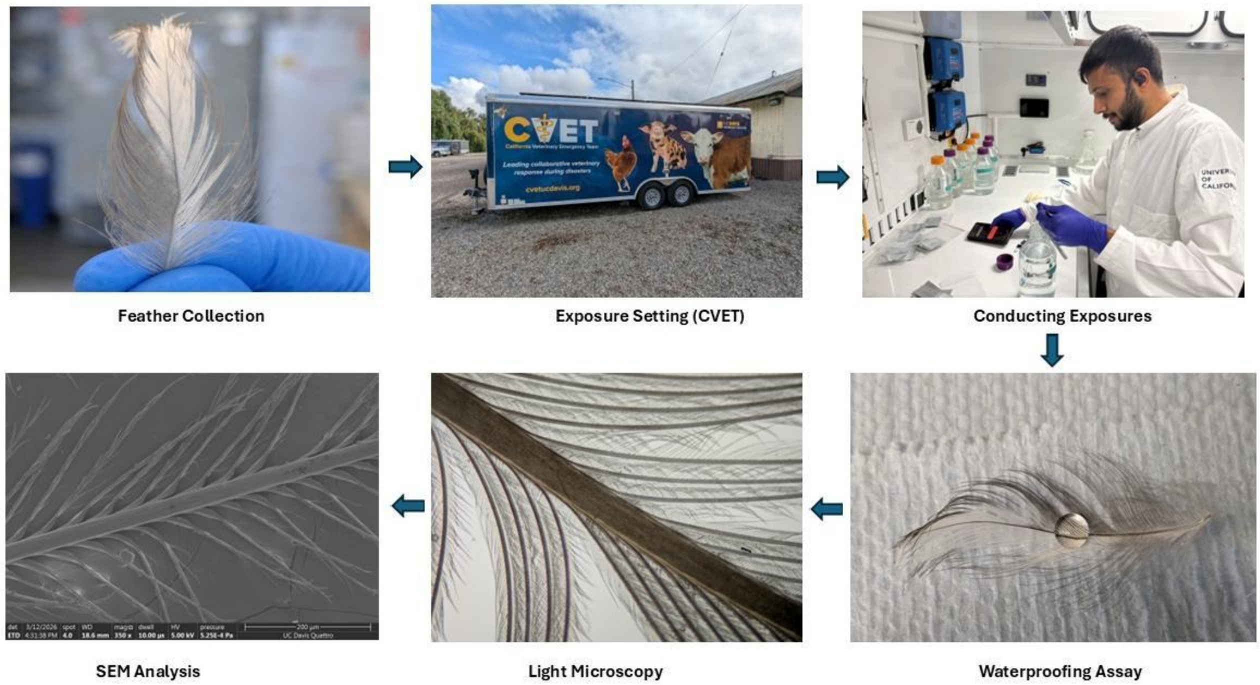

Figure 1A. Experimental workflow for evaluating the effects of diesel exposure on Common Murre (*Uria aalge*) feathers.

Figure 1B. Exposure workflow inside CVET Trailer.

For Scanning Electron Microscopy (SEM) analysis, a subset of feathers was selected from the full dataset to ensure representation across treatment groups and concentrations. Selection was performed to balance group representation while accommodating logistical constraints associated with SEM imaging.

Artificial Seawater Preparation

Artificial seawater was prepared to a target salinity of approximately 35 ppt using commercial sea salt (Instant Ocean®, Blacksburg, VA, USA) dissolved in deionized (DI) water at a ratio of 35 g/L. A total volume of approximately 10 L was prepared and mixed on a magnetic stir plate for approximately 60 minutes to ensure complete dissolution and consistency. Prepared seawater was subsequently stored at 4°C and used within 7 days of preparation. These procedures (Kester et al., 1967) ensured consistent baseline conditions across all exposure groups.

Diesel-Seawater Solution Preparation

Renewable and conventional diesel fuel samples used for exposure preparation were provided by the California Department of Fish and Wildlife, Office of Spill Prevention and Response. Fuel exposure solutions were prepared in the OHI Laboratory under a fume hood using conventional diesel (100%), renewable diesel (100%), and two renewable diesel blend formulations consisting of 80% renewable diesel/20% conventional diesel and 95% renewable diesel/5% conventional diesel. For consistency with project identifiers, the blend formulations are referred to throughout the manuscript as RD80/20 (80% renewable diesel/20% conventional diesel) and RD95/5 (95% renewable diesel/5% conventional diesel). Each fuel type was evaluated at nominal diesel concentrations (0.125 g/L and 0.5 g/L). For each 1 L solution, artificial seawater was added to a glass container, diesel was weighed using an analytical balance and added to the solution, and mixtures stirred on magnetic stir plates for approximately one hour before settling for approximately five hours. Renewable and conventional diesel fuels were not fully miscible with seawater under the experimental conditions used in this study and therefore formed mechanically mixed hydrocarbon-water exposure systems rather than homogenous aqueous solutions. Solutions were subsequently stored at 4°C and used within 48 hours.

To improve consistency across treatments, all preparation procedures, exposure volumes, settling periods, immersion conditions, and handling methods were standardized across all exposure groups. Although the hydrophobic properties of diesel fuels may have resulted in some localized variability in feather coating or hydrocarbon contact during exposure, all feathers were subjected to identical preparation and exposure conditions to maintain consistency for comparative analyses between renewable diesel and conventional diesel treatments. The exposure system was also intended to approximate environmentally relevant marine hydrocarbon-water interactions observed during spill-related contamination events. All preparation steps were standardized to ensure reproducibility across treatments (National Research Council, 2005).

Exposure Procedure (CVET Trailer)

Feather exposures were conducted in the CVET Response Trailer under controlled environmental conditions designed to approximate field-relevant scenarios. The trailer was maintained at a reduced temperature (16 – 18°C) to mimic cold marine environments reflecting ecologically relevant marine conditions for Common Murres (Jenssen, 1994; Rijke, 1970). Samples were handled in a clean enclosed workspace to minimize contamination, and all exposures were performed using standardized procedures and consistent solution volumes across treatments. Lighting and handling conditions were kept consistent throughout the experiment to reduce variability.

Feathers were submerged individually using clean forceps for approximately 60 seconds per feather. Following exposure, feathers were removed, excess solution was allowed to drip off, and samples were labelled and placed individually on a drying rack. All exposures were conducted using standardized handling procedures and consistent exposure durations across treatment groups.

Drying and Storage

Following exposure, feathers were air-dried for approximately 12 - 24 hours while remaining physically separated to avoid contact between samples. Once dry, feathers were stored individually in aluminum foil packets at room temperature. Aluminum foil was used as an inert, non-absorbent barrier to minimize moisture retention and cross-contamination between samples (Rijke, 1970).

Light Microscopy and Image Analysis

Feather samples were imaged using light microscopy at 10x magnification. Digital images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) to quantify structural features (Schneider et al., 2012).

Three primary structural variables were measured from light microscopy images. Barb distance was defined as the distance between adjacent barbs and was measured in pixels using the straight-line tool in ImageJ, as feather barb spacing is closely associated with feather wettability and waterproofing performance (Muzio et al., 2024; Rijke, 1970). Barb angle was measured using the angle tool in ImageJ to evaluate potential alterations in feather geometry associated with waterproofing performance and feather surface organization (Muzio et al., 2024; Rijke, 1970). In addition, several semi-quantitative structural variables were scored on ordinal scales ranging from 0 to 3, including barb alignment, barbule clumping, structural integrity, and residue coverage. Multiple measurements were collected for each feather to capture within-image variability. Pixel units were retained for consistency across images due to the absence of a calibrated scale bar. Representative measurements of barb distance and barb angle performed using ImageJ are illustrated in Figure 2.

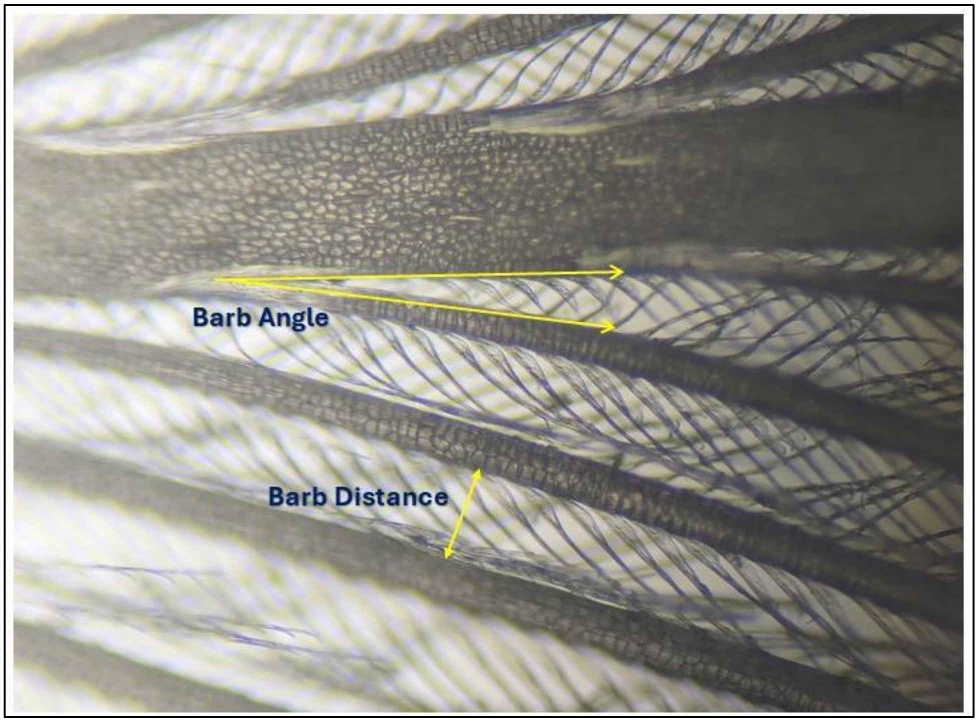

Figure 2. Representative light microscopy image of a Common Murre (*Uria aalge*) ventral contour feather illustrating structural measurements performed using ImageJ. Barb distance was measured between adjacent feather barbs using the straight-line tool, while barb angle was measured using the angle tool to evaluate feather geometry and barb orientation.

Waterproofing Assay

Functional performance of feathers was assessed using droplet-based waterproofing assay following previously reported methods (Jenssen, 1994; Matcott et al., 2019; Muzio and Rubega, 2024; O'Hara and Morandin, 2010). A standardized water droplet (5 mm diameter) was applied to each feather using a dropper.

The waterproofing assay evaluated three functional outcomes: shape score, absorption status, and time to collapse. Shape score was assessed using a four-level ordinal scale (0-3) based on droplet stability and spreading on the feather surface. A score of 3 represented a stable spherical droplet with minimal spreading, whereas a score of 0 represented complete droplet collapse and/or absorption into the feather. Intermediate scores (1-2) reflected progressively greater droplet spreading and reduced stability. Shape score was developed as a study-specific ordinal measure of droplet stability informed by published feather waterproofing and wettability assessment approaches (Jenssen, 1994; Muzio and Rubega, 2024; Rijke, 1970). Absorption was recorded as a binary variable indicating whether droplet penetration occurred (Jenssen, 1994; Matcott et al., 2019; O'Hara and Morandin, 2010). Time to collapse was recorded in seconds and represented the duration for which droplets maintained structural integrity on the feather surface, serving as a functional indicator of feather waterproofing persistence following hydrocarbon exposure (Jenssen, 1994; Matcott et al., 2019; O'Hara and Morandin, 2010).

Measurements were collected at standardized time points (0, 30, and 60 seconds) to maintain consistency across samples. Representative waterproofing assay outcomes corresponding to stable and unstable droplet behavior, as well as shape score classification, are illustrated in Figure 3.

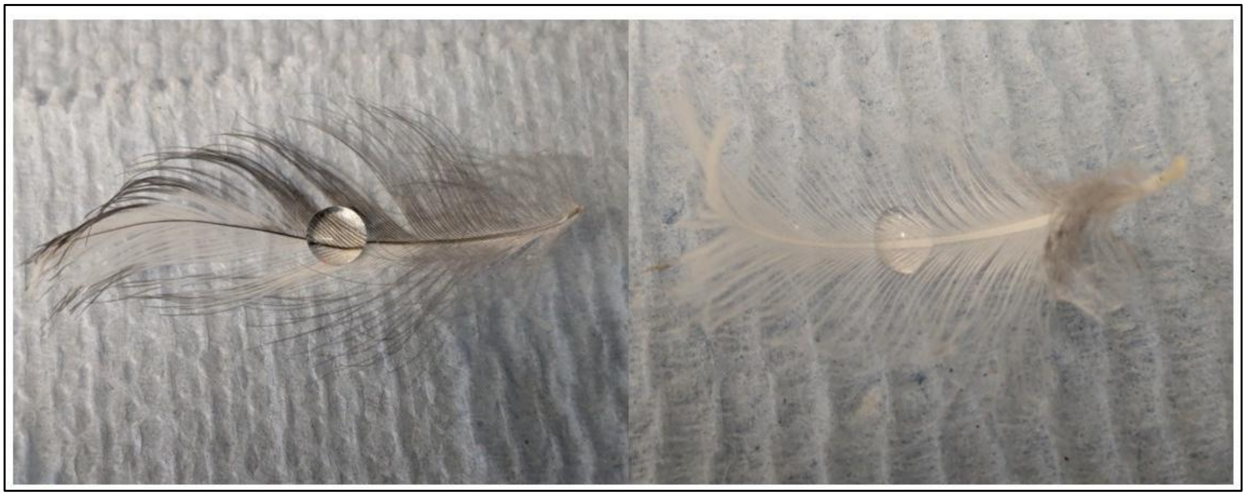

Figure 3. Representative waterproofing assay outcomes observed in Common Murre (*Uria aalge*) ventral contour feathers following droplet application. The left panel illustrates a stable spherical droplet with high retention on the feather surface, representing greater droplet stability and waterproofing performance. The right panel illustrates increased droplet spreading and feather wetting, representing reduced droplet stability and impaired waterproofing performance.

Scanning Electron Microscopy (SEM) and Clumping Index

SEM imaging was conducted at the Advanced Materials Characterization and Testing (AMCaT) Laboratory at UC Davis, and image analysis was performed following previously described digital imaging approaches (Schneider et al., 2012). Briefly, small feather sections were mounted on SEM stubs using carbon adhesive, labelled appropriately, and prepared for imaging. Barbule clumping was subsequently quantified using ImageJ software (Schneider et al., 2012). Multiple annotation was used to identify total and clumped barbule points, and index was calculated as the proportion of clumped points relative to the total number of points assessed. Clumping index values ranged from 0 to 1, with higher values indicating greater microstructural disruption and barbule aggregation, which have previously been associated with hydrocarbon-related feather fouling and impaired waterproofing (Matcott et al., 2019; O'Hara and Morandin, 2010). Clumping index was calculated as the proportion of visibly aggregated or fused barbule regions relative to the total observable barbule field within each SEM image using the following relationship:

Clumping Index = (Number of Clumped Barbule Regions) / (Total Observable Barbule Regions)

Localized areas where adjacent barbules appeared adherent, bundled, or structurally compacted were identified as clumped regions. Higher clumping index values therefore represented greater degrees of barbule aggregation and microstructural cohesion disruption. Clumping assessment was performed using semi-quantitative visual evaluation of SEM micrographs. Representative examples of localized barbule aggregation used for clumping assessment are illustrated in Figure 4.

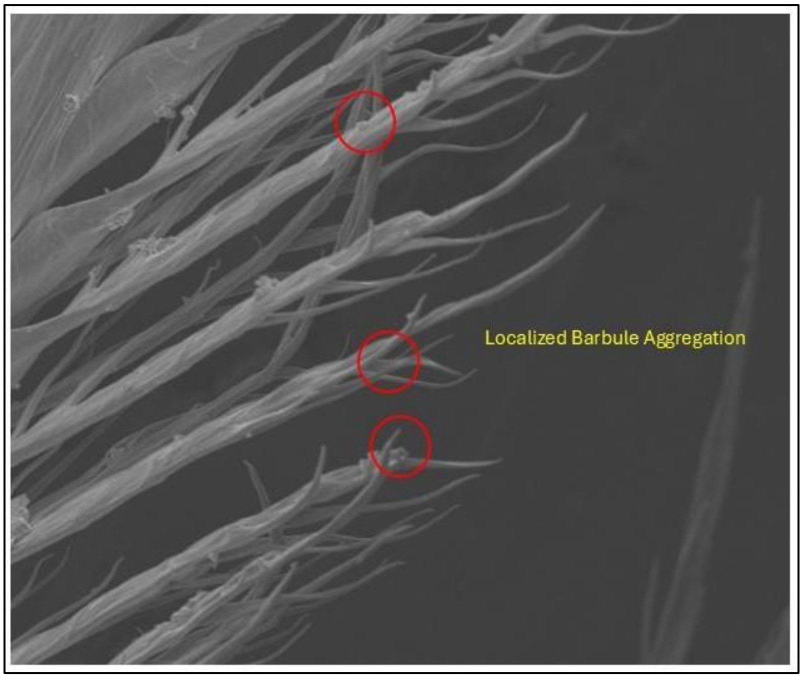

Figure 4. Representative scanning electron microscope (SEM) image of a Common Murre (*Uria aalge*) ventral contour feather illustrating localized barbule aggregation regions evaluated during clumping index assessment. Circled regions indicate representative areas of localized barbule aggregation quantified during semi-quantitative microstructural analysis.

Data Processing and Integration

All datasets (light microscopy, waterproofing and SEM) were cleaned and merged in R (version 4.2.2: R Foundation for Statistical Computing, Vienna, Austria) using a common feather identifier. Variables were standardized, and categorical group variables were harmonized across datasets to ensure consistency in downstream analyses. Derived variables were subsequently created to align with the primary study hypotheses, including fuel type categories (Control, CD, and RD) and treatment classification (Control vs. Treated). Data cleaning procedures additionally included variable type conversion, handling of missing values, and harmonization of labels across datasets.

Statistical Analysis

All statistical analyses were conducted in R (R Core Team, 2024). Due to small sample sizes, non-normal data distributions, and the presence of ordinal variables, non-parametric methods were used throughout. Statistical significance was defined at $\alpha=0.05$. A univariate framework evaluating two primary comparisons: (H1) renewable diesel exposure versus seawater control and (H2) renewable diesel exposure versus conventional diesel. Given the study design and sample size constraints, analyses were conducted using non-parametric methods to ensure valid inference under small-sample conditions and to provide clear and interpretable results while avoiding overfitting. For binary outcomes (absorption), comparisons were performed using Fisher's exact test for pre-specified pairwise contrasts (Control vs. Renewable Diesel and Renewable Diesel vs. Conventional Diesel). Fisher's exact test was selected due to small sample sizes and sparse contingency tables, where chi-squared assumptions were not met. To further evaluate the overall effect of fuel exposure, additional comparisons were conducted between control feathers and all diesel-exposed feathers combined. A supplementary logistic regression model (absorbed ~ fuel_type) was also implemented to assess absorption across all fuel types within a unified framework. The model demonstrated significantly increased odds of absorption in diesel exposed feathers relative to controls since there were no absorption events in controls, leading to complete separation and unstable coefficient estimates. Therefore, logistic regression results were interpreted cautiously and used only as supporting evidence rather than primary inference. Time-to-collapse was additionally evaluated using survival analysis. Kaplan-Meier curves and log-rank tests were used to compare collapse dynamics across fuel types, and Cox proportional hazards models were used to estimate hazard ratios where feasible.

Results

Overview

A total of sixty-six feathers were analyzed for light microscopy and waterproofing outcomes. A subset of randomly selected samples (n=30) was analyzed for SEM-based clumping index data due to time and funding limitations.

Group Distribution

Feathers were distributed across nine groups (see Table 1), with 8 samples for the first three groups and 7 samples for the remaining 6 groups. Group-specific waterproofing outcomes for all fuel formulations and concentrations are summarized in Table 2.

Waterproofing Outcomes: Absorption (Binary Outcome)

Control feathers exhibited no water absorption (n= 0/8), whereas feathers exposed to diesel fuels showed frequent absorption events. In the comparison between control and renewable diesel groups (H1), 48% (n= 20/42) exhibited absorption compared to 0 out of 8 control feathers, representing a significant difference (Fisher's exact test, p=0.015). Similarly, when all diesel-exposed feathers were combined, 48% (n= 28/58) showed absorption, which also differed significantly from controls (Fisher's exact test, p=0.017). In contrast, no significant difference was observed between renewable and conventional diesel groups (H2), with absorption occurring in 8 out of 16 conventional diesel (50%) and 20 out of 42 renewable diesel feathers (48%) (Fisher's exact test, p=1.00). These functional differences in absorption across fuel types are summarized in Figure 5.

Table 2. Group-specific waterproofing outcomes for Common Murre (*Uria aalge*) ventral contour feathers following seawater control, conventional diesel, renewable diesel and renewable diesel blend exposures. Shape score is presented as the number and percentage of feathers exhibiting droplet absorption within each treatment group. Median shape score is reported as an ordinal measures of droplet stability and waterproofing performance.

Group	Fuel Formulation	Nominal Conc. (g/L)	n	Score 0	Score 1	Score 2	Score 3
Control	Seawater	0	8	0 (0%)	0 (0%)	0 (0%)	8 (100%)
CD100_0.125	Conventional Diesel	0.125	8	4 (50%)	1 (12.5%)	1 (12.5%)	0 (0%)
CD100_0.5	Conventional Diesel	0.500	8	3 (37.5%)	3 (37.5%)	3 (37.5%)	1 (12.5%)
RD100_0.125	Renewable Diesel	0.125	7	3 (42.9%)	2 (28.6%)	2 (28.6%)	0 (0%)
RD100_0.5	Renewable Diesel	0.500	7	2 (28.6%)	1 (14.3%)	1 (14.3%)	0 (0%)
RD80/20_0.125	Renewable Diesel	0.125	7	3 (42.9%)	2 (28.6%)	2 (28.6%)	0 (0%)
RD80/20_0.5	Renewable Diesel	0.5	7	2 (28.6%)	4 (57.1%)	4 (57.1%)	0 (0%)
RD95/5_0.125	Renewable Diesel	0.125	7	2 (28.6%)	2 (28.6%)	2 (28.6%)	2 (28.6%)
RD95/5_0.5	Renewable Diesel	0.5	7	4 (57.1%)	3 (42.9%)	3 (42.9%)	0 (0%)

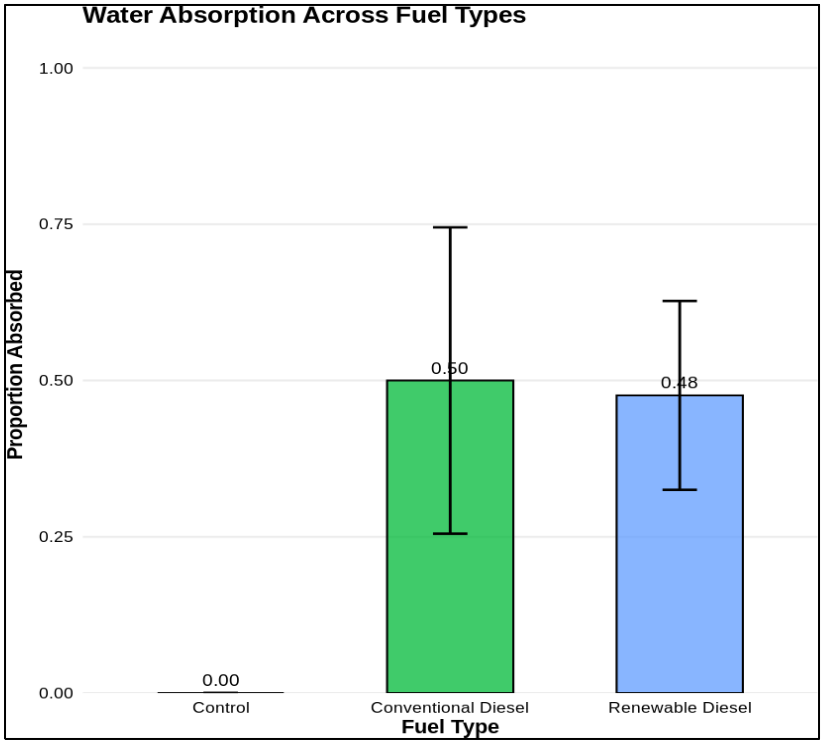

Figure 5. Proportion of Common Murre (*Uria aalge*) ventral contour feathers exhibiting water absorption following exposure to seawater control, conventional diesel (CD), and grouped renewable diesels (RD). Absorption was recorded as a binary waterproofing outcome following droplet assay testing. Error bars represent approximately 95% confidence intervals.

Waterproofing Outcomes: Shape Score (Ordinal Outcome)

Shape scores were consistently high in control feathers and reduced in diesel-exposed groups. Comparison between control and renewable diesel groups (H1) demonstrated a significant reduction in droplet stability following renewable diesel exposure (Kruskal-Wallis test, $\chi^2=19.28$, $p<0.001$). In contrast, no significant differences were observed between renewable and conventional diesel (H2) (Kruskal-Wallis test, $\chi^2=0.18$, $p=0.67$). To evaluate the overall effect of fuel exposure, an additional comparison was conducted between control feathers and all diesel-exposed feathers combined. This analysis also demonstrated a significant reduction in shape score following diesel exposure (Kruskal-Wallis test, $p< 0.001$), reinforcing the consistency of the observed functional impairment across fuel types. Stacked bar plots illustrating shape score distributions across fuel types and individual treatment groups are presented in Figures 6 and 7, respectively. Each bar represents the proportion of feathers within each shape score category, allowing visualization of shifts in droplet stability outcomes across treatment groups. Figure 7 further illustrates shape score distributions for each individual fuel formulation and concentration, allowing comparison across the full set of renewable diesel blends, conventional diesel treatments, and seawater control.

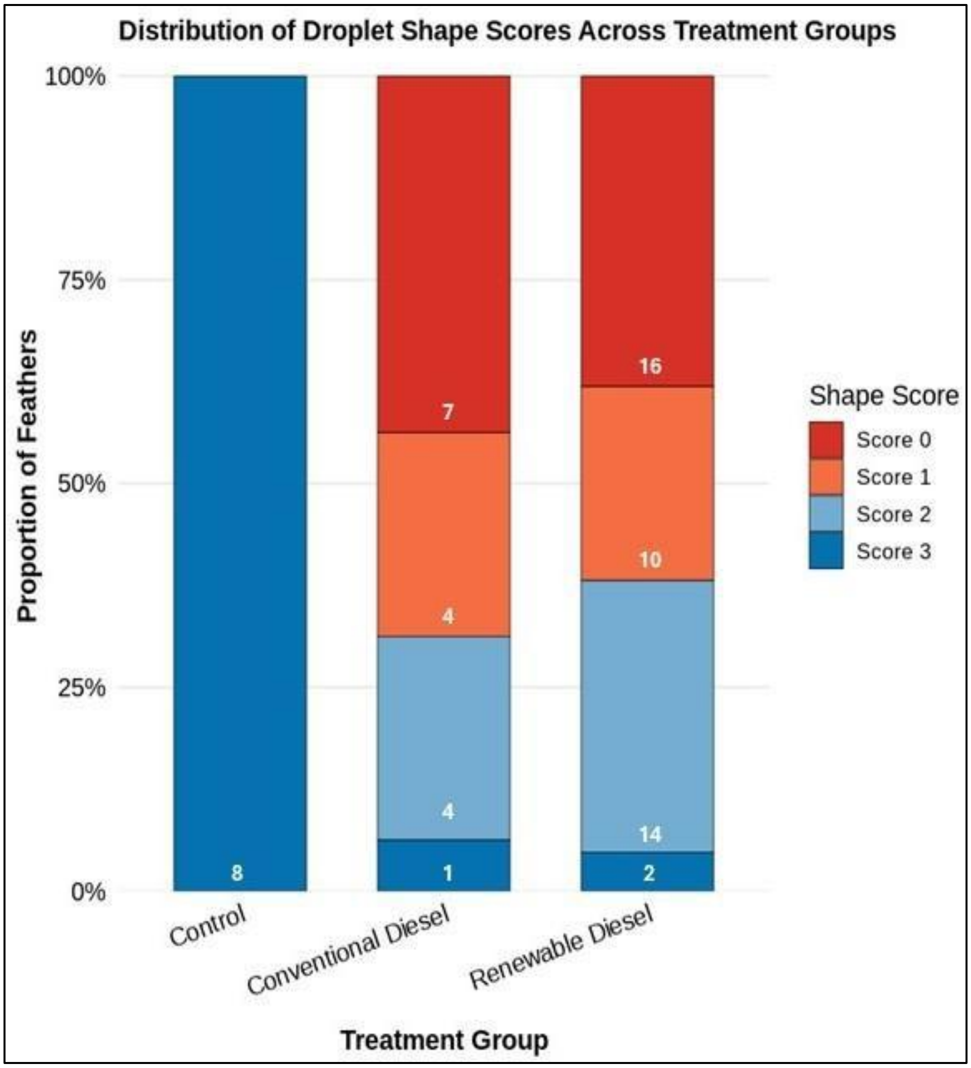

Figure 6. Stacked bar plots showing droplet shape score distributions for Common Murre (*Uria aalge*) ventral contour feathers following exposure to seawater control, conventional diesel, and grouped renewable diesels. Shape score was measured as an ordinal waterproofing outcome representing droplet stability on the feather surface, with higher scores indicating greater droplet retention and waterproofing performance. Numbers within the bar segments indicate the corresponding feather counts for each shape score category.

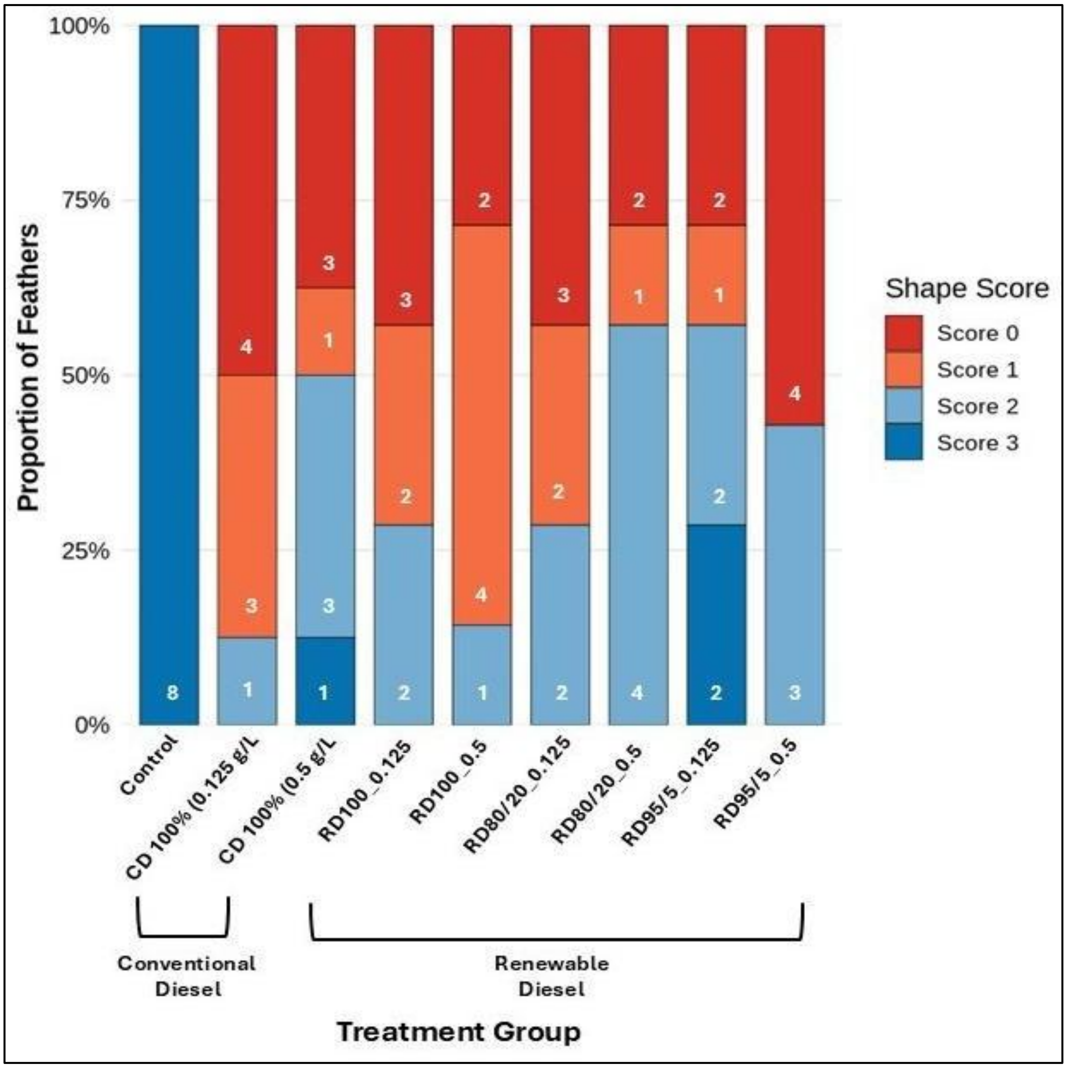

Figure 7. Stacked bar plots showing droplet shape score distributions across experimental treatment groups for Common Murre (*Uria aalge*) ventral contour feathers following diesel exposure. Treatment labels represent fuel type and nominal exposure concentration. Shape score was measured as an ordinal waterproofing outcome reflecting droplet stability on the feather surface, with higher scores indicating greater droplet stability and waterproofing performance. Numbers within bar segments indicate the corresponding feather counts within each treatment group.

Structural Measurements (Light Microscopy)

No statistically detectable differences were observed in inter-barb distance (Kruskal–Wallis χ^2=0.14, p=0.711) or barb angle (Kruskal- Wallis χ^2=1.48, p=0.224) between control and renewable diesel groups. Representative light microscopy images for all fuel formulations are provided in Figure 8.

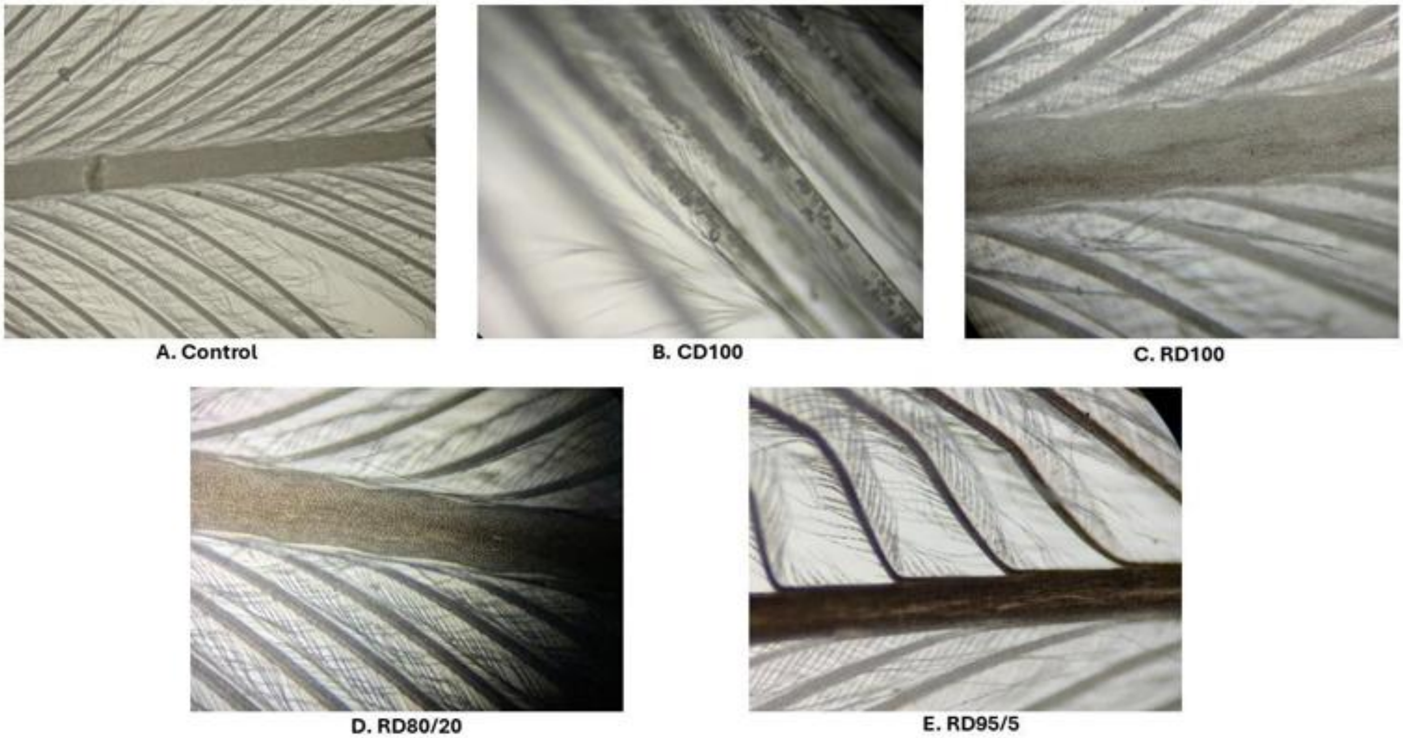

Figure 8. Representative light microscopy images of Common Murre (*Uria aalge*) ventral contour feathers following exposure to (A) seawater control, (B) conventional diesel (CD100), (C) renewable diesel (RD100), (D) 80% renewable diesel/20% conventional diesel blend (RD80/20) and (E) 95% renewable diesel/5% conventional diesel blend (RD95/5). Images are presented for qualitative comparison of feather microstructure across exposure groups.

Microstructural Analysis (SEM)

Clumping index values were obtained from a randomly selected subset of feathers (n= 30, 3 feathers per group) due to time and funding limitations for the study. No significant association was observed between clumping index and absorption status (Wilcoxon test, $p = 0.675$) or between clumping index and shape score (Spearman correlation, $\rho = -0.22$, $p = 0.281$). Although clumping index differed overall across groups, this effect was driven by a general shift between control and diesel-exposed feathers, with control samples showing consistently lower clumping values. No significant differences were observed between renewable and conventional diesel groups, and no clear dose-response pattern was detected. Representative SEM images for all fuel formulations are provided in Figure 9.

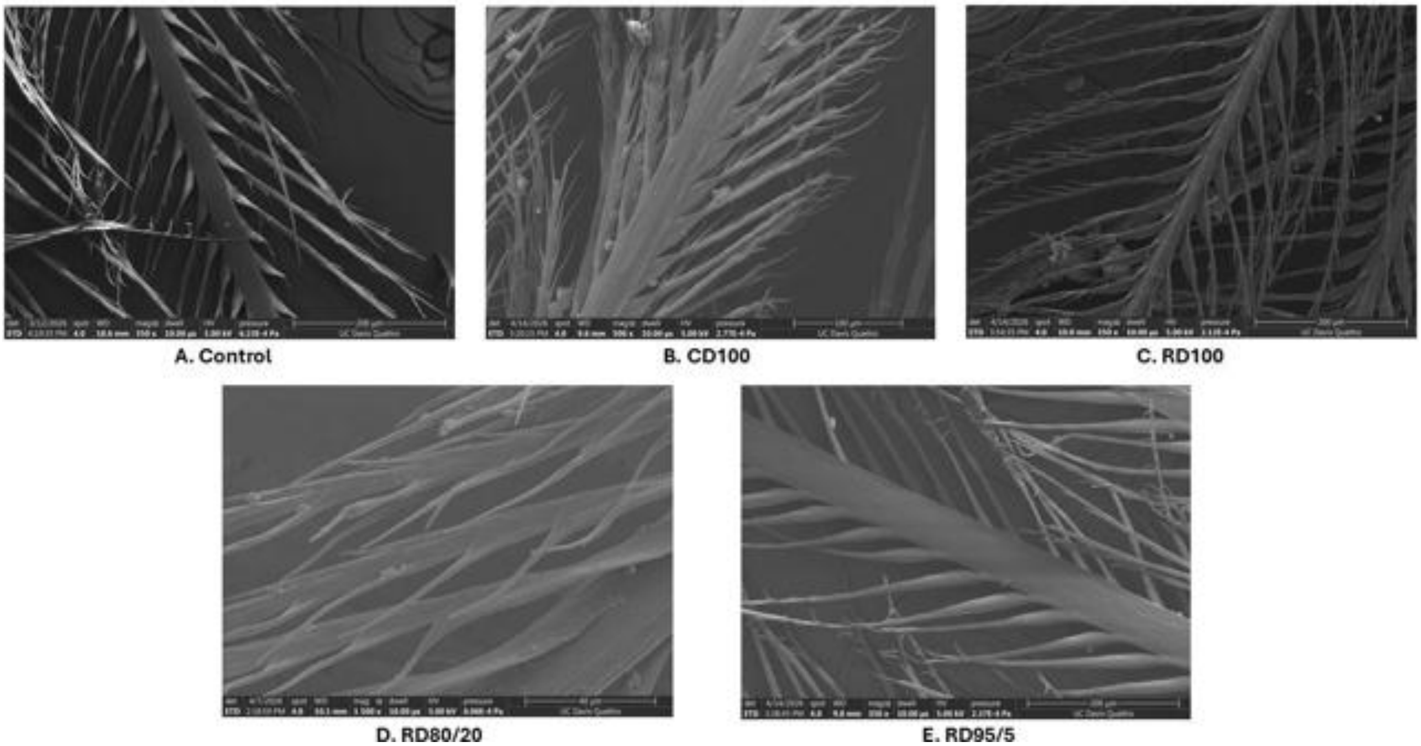

Figure 9. Representative scanning electron microscopy (SEM) images of Common Murre (*Uria aalge*) ventral contour feathers following exposure to (A) seawater control, (B) conventional diesel blend (CD100), (C) renewable diesel (RD100), (D) 80% renewable diesel/20% conventional diesel blend (RD80/20) and (E) 95% renewable diesel/5% conventional diesel blend (RD95/5; E). Images are presented for qualitative comparison of feather microstructure and barbule organization across exposure groups.

Structure-Function Relationships

To evaluate potential mechanisms linking feather structure and waterproofing function, relationships between macrostructural variables and absorption outcomes were assessed. No significant association was observed between barb distance and absorption (p=0.324) or between barb angle and absorption (p=0.604). Although large-scale structural disruption was not detected quantitatively, representative light microscopy and SEM images (Figure 10A and 10B) demonstrated visible surface associated diesel residues and localized coating of feather structures in exposed samples compared to controls.

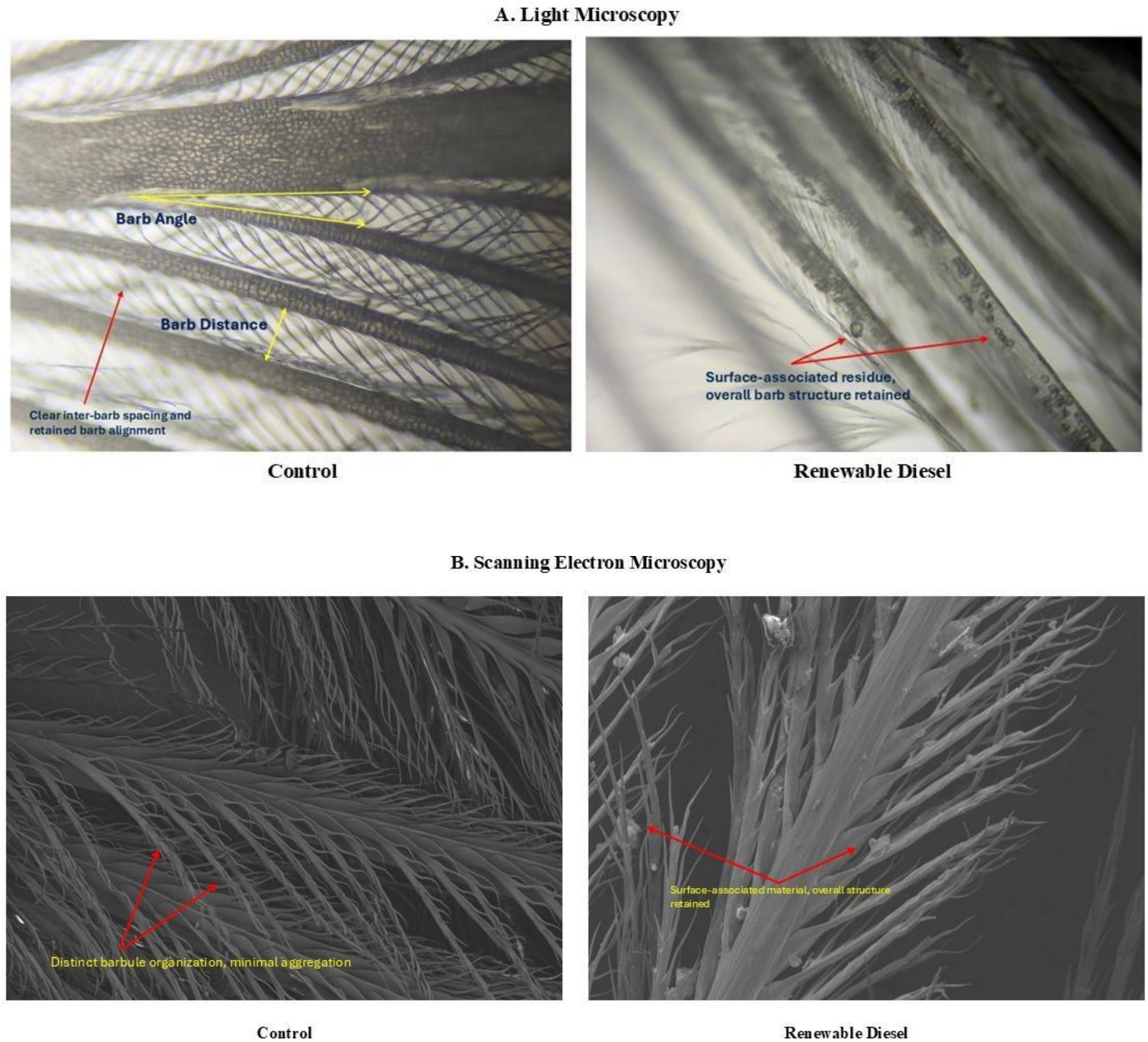

Figure 10. Representative light microscopy and scanning electron microscopy (SEM) images of Common Murre (*Uria aalge*) ventral contour feathers following seawater control and diesel exposure conditions. (A) Light microscopy showing feather barbs, barbules, and overall architecture at 10x magnification. (B) SEM images illustrating barbule organization and feather microstructural arrangement at higher resolution. Arrows indicate representative regions of surface-associated material and localized barbule aggregation in diesel-exposed feathers.

Survival Analysis (Time-to-Collapse)

Time-to-collapse was analyzed using Kaplan-Meier survival curves to evaluate droplet stability over time. Control feathers exhibited complete droplet stability throughout the observation period, with no collapse events recorded, whereas both diesel-exposed groups demonstrated progressive reductions in droplet stability over time. The overall comparison across all three groups approached but did not reach statistical significance (log-rank test, $p=0.07$). Because no collapse events occurred in the control group, Cox proportional hazards estimate relative to control was unstable and were therefore not used for primary inference (Knothe, 2010). When analysis was restricted to diesel-exposed groups, Kaplan-Meier curves for renewable and conventional diesel showed substantial overlap throughout the observation period, with both groups exhibiting similar collapse trajectories and rates of droplet destabilization over time (Figure 11). A Kaplan-Meier analysis examining individual fuel formulations (Control, CD100, RD100, RD80/20 and RD95/5) is provided in Figure 12. No statistically significant difference was observed between renewable and conventional diesel (log-rank test, $p=0.9$). Cox proportional hazards modelling yielded a hazard ratio of 1.08 (95% CI: 0.48-2.46), indicating no detectable difference in collapse between the two fuel types. Assessment of Cox proportional hazards assumptions demonstrated no evidence of proportional violation based on Schoenfeld residual testing (global test: $p=0.93$. Residual diagnostics did not demonstrate major systematic deviations or influential observations, supporting the appropriateness of the fitted survival model for interpretation under the conditions tested. Similar patterns were observed when renewable diesel formulations were evaluated separately, with no statistically significant differences detected among individual fuel formulations (Figure 12).

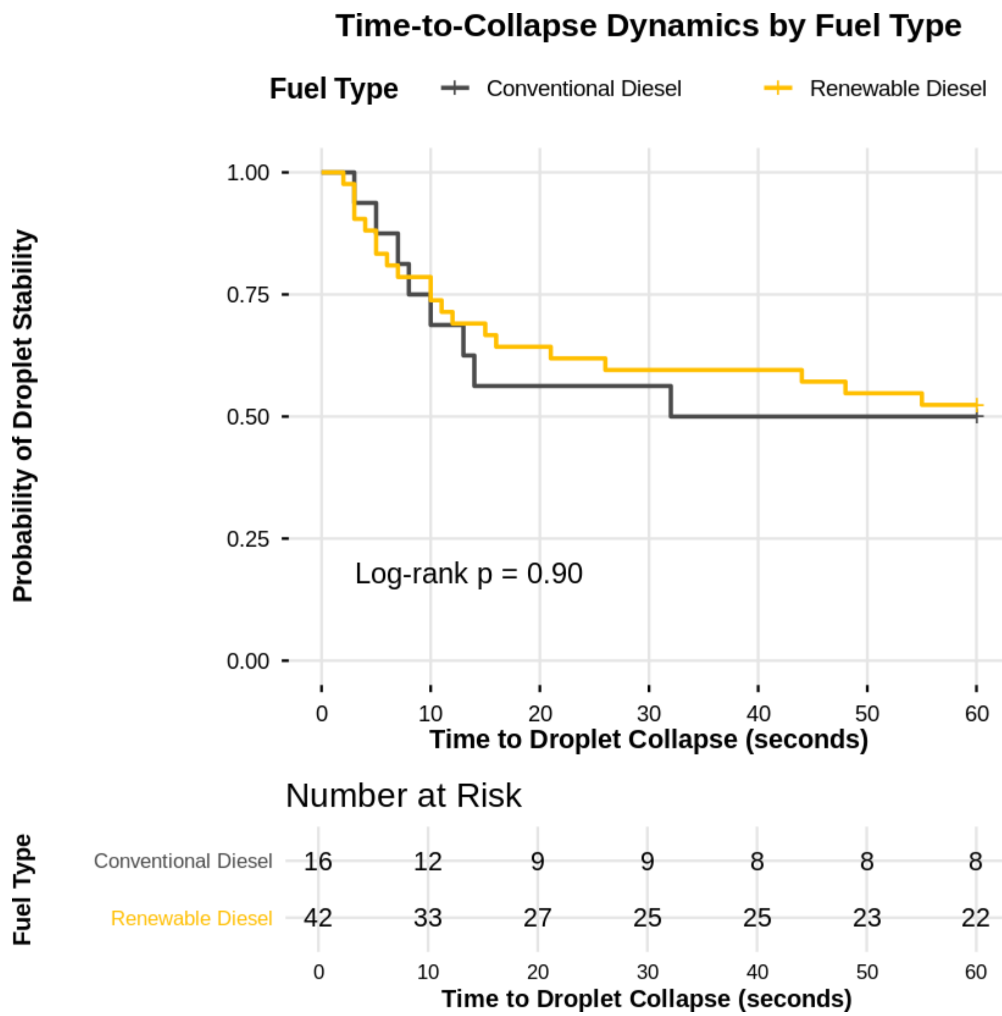

Figure 11. Kaplan–Meier curves showing the estimated probability of droplet stability over time in Common Murre (*Uria aalge*) ventral contour feathers following exposure to conventional diesel (CD) and grouped renewable diesel (RD) fuels.

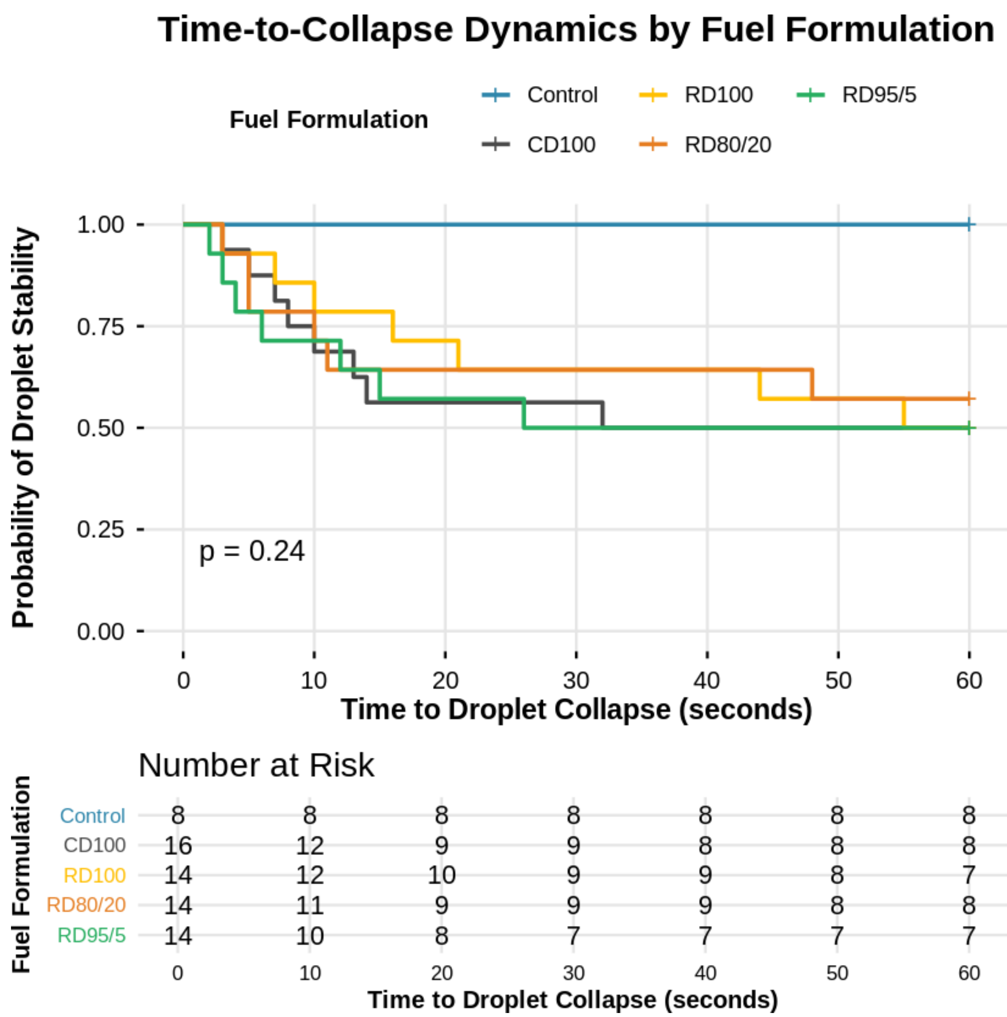

Figure 12. Time-to-droplet collapse dynamics by fuel formulation. Kaplan-Meier survival curves showing droplet stability following exposure to seawater control, conventional diesel (CD 100), renewable diesel (RD 100) and renewable diesel blend formulations (RD 80/20 and RD95/5). The overall log-rank test indicated no significant differences among fuel formulations (p=0.24).

Discussion

Overview of Findings

This study evaluated the effects of renewable diesel exposure on feather structure and waterproofing in Common Murres and compared these effects to conventional diesel and seawater controls. The results demonstrate a consistent pattern: diesel exposure, including renewable diesel, significantly impaired feather waterproofing, while large-scale structural metrics such as barb distance and barb angle remained largely unchanged.

Importantly, no significant differences were observed between renewable and conventional diesel cross primary functional outcomes, including absorption, shape score, and time-to-collapse. Although diesel exposure consistently reduced droplet stability relative to controls, renewable and conventional diesel produced highly comparable functional impairment under the conditions tested. These findings suggest that, despite chemical differences between fuel types, their effects on feather waterproofing are broadly similar in this experimental system. These findings should additionally be interpreted within the context of a pilot-scale experimental design intended to support future larger-scale investigations of renewable diesel effects in marine seabirds. Notably, comparable waterproofing impairment was observed across renewable diesel treatments spanning a broad range of effective fuel concentrations, suggesting that even relatively low renewable diesel fractions may be sufficient to alter feather surface properties under the exposure conditions evaluated.

Functional Impairment Without Structural Collapse

A key finding of this study is the disconnect between functional and structural outcomes of exposed feathers. While waterproofing performance (absorption and shape score) showed clean and statistically significant degradation following fuel exposure, macrostructural variables did not exhibit corresponding changes. These findings suggest that impairment of feather waterproofing does not necessarily require detectable disruption of overall feather geometry or macrostructural organization. Despite significant functional impairment, barb spacing and feather architecture remained largely intact across treatment groups, indicating that subtle surface-level physicochemical alterations may be sufficient to compromise feather function even in the absence of measurable structural collapse. This interpretation is supported by the observed lack of association between barb distance or angle and waterproofing outcomes. These findings contribute to a growing body of evidence that macrostructural variation was not primary evidence of waterproofing impairment and that feather function is highly sensitive to small scale disruptions that may not be captured by traditional morphometric measurements.

Potential Mechanisms: Role of Microstructural Clumping

Observed Scanning Electron Microscopy-based clumping was not associated with absorption or droplet stability outcomes. Although microstructural clumping was initially considered a plausible microstructural mechanism for waterproofing loss, the final SEM analysis did not support a measurable relationship between clumping index and functional impairment. These findings suggest that the observed loss of waterproofing was not explained by detectable barbule aggregation as quantified in this study. Although diesel-exposed feathers generally exhibited higher clumping values than controls, this pattern appeared to reflect hydrocarbon exposure rather than a direct driver of functional waterproofing loss. Similar observations of increased feather fouling without consistent large-scale structural disruption have been reported in previous experimental oil exposure studies (Matcott et al., 2019). Together with the absence of significant difference in barb distance and barb angle, this indicates that diesel exposure impaired feather waterproofing without detectable structural or microstructural alterations at the resolutions measured here. These findings strongly suggest that functional impairment is driven by physicochemical mechanisms rather than structural disruption. Diesel exposure, whether it is conventional or renewable, likely alters feather surface properties, including hydrophobicity and surface tension interactions, reducing water repellency and promoting droplet spreading and penetration despite intact feather architecture. The observed reduction in droplet stability across diesel-exposed feathers is consistent with impaired feather surface cohesion and altered wettability following hydrocarbon exposure. Together, these effects are likely to contribute to earlier collapse dynamics and reduced waterproofing performance despite largely intact feather architecture. Overall, the findings support the interpretation that diesel exposure primarily disrupts feather surface interactions and wettability rather than causing substantial structural collapse, consistent with previous studies of hydrocarbon-related feather fouling and waterproofing impairment (Jensen, 1994; Matcott et al., 2019).

Comparison with Previous Studies

Previous work on crude oil and chemical dispersant exposure has reported both structural and functional changes in feathers (Matcott et al., 2019; O'Hara and Morandin, 2020), including reductions in barb spacing and alterations in feather geometry. In contrast, the present study did not detect significant changes in these macrostructural metrics. One possible explanation for this difference is that the present study evaluated refined (e.g., diesel) fuels, whereas many previous studies have focused on crude oil and chemically dispersed oil mixtures. Dispersants act as surfactants, actively reducing surface tension and facilitating the penetration of oil into feather microstructure (National Research Council, 2005). This can result in widespread disruption across multiple levels, including barb spacing, barbule organization, and surface cohesion.

Despite these differences, both the present study and prior research converge on the conclusion that hydrocarbon exposure compromises feather waterproofing. The present findings extend this understanding by demonstrating that such impairment can occur even in the absence of detectable large- scale structural changes. Taken together, the results demonstrate a consistent pattern in which diesel exposure alters feather function without measurable changes in structure, supporting a mechanism driven by surface-level physicochemical interactions rather than physical damage.

Implications for Renewable Diesel as an Alternative Fuel

Renewable diesel is often promoted as a more environmentally friendly alternative to conventional petroleum fuels. While this may be true from an emission reduction perspective, the results of this study suggest that, at least in the context of feather structure and waterproofing, renewable diesel does not confer reduced biological impact. From an ecological perspective, these findings are important because seabird morbidity and mortality after hydrocarbon exposure may occur through functional failure of plumage before major structural damage is visually present. Previous work by Duerr et al. (2011) demonstrated that even relatively limited oil contamination can substantially impair seabird survival and complicate rehabilitation outcomes, highlighting the biological importance of maintaining feather waterproofing and insulation following hydrocarbon exposure. Loss of waterproofing can increase water penetration, reduce insulation, increase energetic demand, and shorten the window for effective rescue and rehabilitation. In this context, even subtle surface-level contamination may have population- relevant consequences when exposure occurs during spill events affecting large numbers of diving seabirds (Duerr et al., 2011; Jenssen, 1994; Matcott et al., 2019; Muzio et al., 2024; O'Hara and Morandin, 2010).

These findings highlight the need to evaluate alternative fuels not only in terms of emissions and production sustainability, but also in terms of direct ecological effects on wildlife. The inclusion of lower renewable diesel blend treatments is ecologically relevant because seabirds are often exposed not only to bulk oil, but also to thin surface films, sheens, diluted residues, and weathered fuel mixtures in real spill environments. Interpretation of renewable diesel blend groups should consider that RD80/20 and RD95/5 represented mixed-fuel formulations containing both renewable and conventional diesel components rather than pure renewable diesel exposures. Previous work has shown that small amounts of oil can impair feather structure and waterproofing, and wildlife response studies emphasize that even limited plumage contamination can compromise survival by disrupting insulation and hermoregulation. Therefore, the effects observed at reduced blend percentages in this study may be relevant to field scenarios involving partial or heterogenous fuel exposure rather than only direct exposure to undiluted diesel (Duerr et al., 2011; Jenssen, 1994; Matcott et al., 2019; Muzio et al., 2024; National Research Council, 2005; O'Hara and Morandin, 2010).

Study Considerations

Several considerations should be considered while interpreting these results. All feathers were derived from a single bird, which reduced inter-individual variability and allowed for controlled comparisons across treatment groups, although broader population-level variation was not assessed. In addition, exposure experiments were conducted under controlled laboratory conditions to ensure consistency across treatments; however, natural environmental factors such as exposure to more extreme environmental variables, wave action, and rinsing effects were not incorporated. Structural measurements were conducted in pixel units due to the absence of scale calibration, which limits direct physical interpretation, although measurements were performed consistently across all samples to allow relative comparisons among treatment groups. Finally, the study was primarily powered to detect moderate to large effects, particularly for functional outcomes, and smaller differences in structural or microstructural variables may require larger sample sizes for detection. Despite these considerations, the study design-controlled comparisons across treatment groups provided valuable insight into the relative effects of renewable and conventional diesel exposure on feather structure and waterproofing.

Future Directions

Future work should focus on expanding SEM data to strengthen mechanistic conclusions, increasing sample size and incorporating multiple individuals, evaluating dose-response relationships more explicitly, and investigating recovery processes such as rinsing and preening. Additional studies across multiple seabird species and feather types would further improve understanding of species-specific vulnerability to fuel exposure. Future research should also evaluate whether differences in collapse dynamics, waterproofing persistence, or post-exposure recovery influence survival window and response timelines relevant to seabird rescue and rehabilitation efforts. Such studies would further clarify the mechanisms of feather impairment, improve ecological risk assessment for emerging fuel types, and help evaluate the practical implications of alternative fuel exposure for wildlife response strategies. These findings may therefore have practical implications for wildlife response and seabird rehabilitation efforts following renewable diesel contamination events.

Conclusion

This study demonstrates that exposure to renewable diesel significantly impairs feather waterproofing in Common Murres, as evidenced by increased water absorption and reduced droplet stability, and altered collapse dynamics These functional changes occurred consistently across treated groups and were comparable in magnitude to those observed with conventional diesel. Notably, no significant differences were detected in macrostructural variables such as barb distance and barb angle, and SEM-based clumping index was not associated with functional outcomes. These findings indicate that diesel exposure impaired feather waterproofing without detectable structural or microstructural changes at the scales measured in this study. The lack of significant differences between renewable and conventional diesel suggests that renewable diesel does not confer reduced risk to seabirds in the context of feather integrity and waterproofing. These findings highlight the importance of evaluating alternative fuels not only for their environmental benefits but also for their direct biological impacts.

Overall, this study provides a comprehensive, multi-scale assessment of feather response to diesel exposure and contributes to a growing understanding of how hydrocarbon contaminants affect avian species.

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