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Effects of oil and oil burn residues on seabird feathers

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1 Abstract

It is well known, that in case of oil spill, seabirds are among the groups of animals most vulnerable. Even small amounts of oil can have lethal effects by destroying the waterproofing of their plumage, leading to loss of insulation and buoyancy. In the Arctic these impacts are intensified. To protect seabirds, a rapid removal of oil is crucial and *in situ* burning could be an efficient method. In the present work exposure effects of oil and burn residue in different doses was studied on seabird feathers from legally hunted Common eider (*Somateria mollissima*) by examining changes in total weight of the feather and damages on the microstructure (Amalgamation Index) of the feathers before and after exposure. The results of the

experiments indicate that burn residues from *in situ* burning of an oil spill have similar or larger fouling and damaging effects on seabird feathers, as compared to fresh oil.

2 Keywords

In situ burning; burn residues; oil spill; damage; seabirds; feathers

3 Introduction

Oil spills in Arctic waters are connected with great environmental consequences, and the challenges are more difficult to handle than oil spills in temperate waters. This is primarily due to the ice, as it complicates the accessibility to the spill site, thereby making conventional methods less efficient. The remote location, darkness for many months of the year and lack of infrastructure also add to the challenges of dealing with an oil spill in the Arctic. For removal of oil in ice-infested waters *in situ* burning (ISB) is a response technique with high potential. In short, ISB is to ignite the oil at the spill site and thereby removing large amounts of the oil by converting it into CO_2, water, soot and other combustion products. Burning effectiveness higher than 90 % has been found under the right circumstances (fresh oil, thick oil slick and relatively large spill area; e.g. Fingas et al. 1995). After flame extinction a highly viscous and sticky burn residue that might sink is left behind (Fritt-Rasmussen et al. 2015). The residues, though substantially reduced in amount compared to the original spill, might be difficult and time consuming to collect as the measures for collecting is often done manually through the use of forks and absorption pads. During the Deep Water Horizon incident in the Gulf of Mexico in 2010, hundreds of burn operations were conducted as part of the oil spill response. However, the residue was not collected and hence the fate of the residue remains unknown (Shigenaka et al. 2015).

In spite of its potential environmental risk, little research has been made to gain knowledge about the residue and its environmental effects (Fritt-Rasmussen et al. 2015). A few toxicity studies have been made and the overall conclusion was that the burn residue is not more toxic than what is found from the oil spill itself (Gulec and Holdway 1999; Cohen and Nugegoda 2000; Fingas et al. 1994; Faksness et al. 2012). These studies include only a few aquatic species (including fish, amphipods, copepods, asteroids and snails) and a few oil types. Furthermore, studies on fouling effects from the burn residue on birds and other surface living organisms are also missing (Fritt-Rasmussen et al. 2015).

It is well known that in case of an oil spill, seabirds are among the groups of animals that are most vulnerable (e.g. Piatt 1990). Most seabirds spend their entire non-breeding season at sea, relying on feathers for flight, insulation and buoyancy (Stephenson 1997), and it is well documented that feather fouling from oil is the primary cause of mortality in seabirds exposed to oil pollution (Leighton 1993). Even small amounts of fresh oil can have lethal effects on seabirds by destroying the waterproofing of their plumage, leading to loss of insulation and buoyancy and causing rapid death by hypothermia, starvation or drowning (Leighton 1993). In the Arctic, these impacts are intensified, as the cold water leads more rapidly to hypothermia (O'Hara and Morandin 2010). Thus, the residues remaining on the water surface might represent a potential risk for the pelagic seabirds due to their biology and habits e.g. foraging behaviour.

Although oil pollution and its lethal fouling effects on seabirds is well documented, little research has been conducted on the effect of oil on the microstructure of the feathers (Hartung 1967, O'Hara and Morandin 2010, Morandin and O'Hara 2014) and to our knowledge, no research has been made to investigate the potential effects burn residues might have on seabird feathers. The aims of the study are thus to investigate and compare the effects of fresh oil and of burn residues on seabird feathers.

4 Materials and Methods

The experiments involved a two-step process, the first including the generation and collection of residue from burning of oil and the second was to study the potential effects of burn residues on seabird feathers.

4.1 Burning experiments

Burn residues tested in the study were collected from laboratory burning experiments conducted in an experimental set-up of the Technical University of Denmark. The laboratory burning set-up consists of a 1 x 1 m water bath that is placed under an exhaust hood. A Pyrex Glass Cylinder (PGC) with a closed bottom was placed in the middle of the bath filled with a 30 ‰ salt water solution. A known amount of oil was carefully placed on top of the salt water surface in the PGC. The oil was ignited with a butane blowtorch, and after flame extinction, the residue was collected by use of absorption pads. The residue was stored in a glass bottle in the freezer (-18 °C) until further analyses. More details regarding the set-up and method can be found in Brogaard et al. (2014) and van Gelderen et al. (2015).

Two types of oils were investigated; Grane (crude oil) and IFO30. Grane is an asphaltenic crude oil, rich in resins and asphaltenes and therefore forms stable water-in-oil emulsions, the density is high and the evaporative loss is low (Fritt-Rasmussen 2010). IFO30 refers to an intermediate fuel oil and is a mixture of gasoil and heavy fuel oils, with a viscosity of 30 centistokes at 50 °C. IFO30 was provided by Trumf Bunker in Aabenraa, Denmark (Fritt-Rasmussen 2010). The physical and chemical properties for Grane and IFO30 are given in Table 1. Refined products even within the same IFO grade can vary in properties depending on the refinery process and type of crude oil (Moldestad and Leirvik 2005), thus the values in Table 1 only gives an indication of the properties of such oil.

Please insert Table 1

The burning efficiencies and other burning related parameters for both oils are given in Table 2. More details and discussions of results from the burning experiments for Grane are reported in Brogaard et al. (2014) and van Gelderen et al. (2015).

Please insert Table 2

As a result of the burning, the residual changed its properties and became almost solid. The buoyancy of the residue was tested for the IFO30, 40 mm experiment. The fresh IFO30 was buoyant but after flame extinction the residue sank slowly (Figure 1). The sinking was not observed for the 10 mm burning experiments

Please insert Figure 1

4.2 Experimental burn residue effects on seabird feathers

The laboratory study included exposure of seabird feathers in different oil and burn residue doses followed by measurements of the feather microstructure disruption following a modified methodology of O'Hara and Morandin (2010). Also, changes in the total weight of the feather due to increased uptake of water or fouling by oil or residue were measured.

Feathers from legally hunted seabirds of Common eider (*Somateria mollissima*) were used in the study. Common eider is widespread in the coastal area in the Arctic. To minimize the impact to the feathers, the feathers from the chest of the birds were removed carefully and at no point were the birds/skin frozen. The feathers were stored carefully to avoid any unwanted disturbances of the feather structure.

The samples for testing were: different dilution of fresh oil samples and burn residues of: Grane crude oil and IFO30 oil. Salt water and the solvent Dichloromethane (DCM) was included as controls.

4.2.1 Sample preparation

4.2.1.1 Burn residue and fresh oil

The burn residue that was sampled on an absorption pad (see Section 4.1) was dissolved in 25 mL DCM and stirred carefully for 30 minutes. The absorption pad was then removed and dried for 24 hours before weighing. Less than 5 % of the burn residue was left on the absorption pad by this extraction method. The dilution series were made from these dissolved burn residue stock solutions diluted in 25 mL DCM to 10, 100, 1000 and 10.000 dilutions. The corresponding oil slick thicknesses can be found in Table 3.

The dilution series were made of fresh Grane crude oil and IFO30 respectively applied to a red-cap bottle and filled with 25 mL DCM. The amounts of oil added correspond relatively to the amount of oil that was removed by ignition. The dilution series were made from this stock solution to 10, 100, 1000 and 10.000 times dilutions.

0.5 mL of sample (burn residue or fresh oil) was carefully transferred to the 30 ‰ salt water layer in a Petri dish with a glass micropipette on the inner side of the dish to make sure that the sample positioned on the salt water surface. The set-up was left for at least 5 minutes to allow for the DCM to evaporate completely. The exposure procedure is described in Section 4.2.2.

Based on an assumption that oil / residue were homogeneously distributed over the salt water surface in the petri dish, the doses applied have been converted to an estimated minimum oil slick thickness for the different dilutions (Table 3). The initial amount of residue was smaller compared to the fresh oils to simulate a burning situation where the oil amount is considerably reduced as a result of the burning. The burning efficiencies found in the burning experiments (Section 4.1) were used to calculate the amount of oil/residue used in the experiments. This is also reflected in the slick thickness of the burn residues that are thinner (Table 3).

Please insert Table 3

4.2.1.2 Control experiments

Control experiments with only 30‰ salt water were made. The exposure procedure is described in Section 4.2.2. In addition, control experiments with 0.5 mL DCM carefully transferred to the Petri dish with a glass micropipette on the inner side of the dish were made.

4.2.2 Exposure experiments on seabird feathers

The following procedure was followed during all the exposure experiments:

1. A glass Petri dish (11 cm D) was filled with salt water (30 ‰).
2. The test sample was applied to the surface of the salt water with a micropipette.
3. The feather was weighed and subsequently placed on the surface film in the Petri dish for 15 s using tweezers and picked up by the calamus. Hereafter, the feather was drawn three times over the surface to simulate mechanical stress. The surface could either be salt water alone or added DCM (the control experiments) or salt water added the fresh oil samples or oil burn residue samples. Finally, the feather was placed on the surface for 15 s.
4. The feather was then weighed.
5. The feather was placed on a glass plate, with the convex surface upwards. A smaller glass plate was place over the tip of calamus to fix the feather.
6. The feather was photographed four places (see Figure 2), with two places on each side of the middle and one overall reference photo for the quantifying of the damages on the feather microstructure (see Barbule Amalgamation Index 4.2.3).

Please insert Figure 2

4.2.3 Barbule Amalgamation Index

O'Hara and Morandin (2010) developed the 'barbule Amalgamation Index (AI)' to quantify the clumping of barbules as a result from the exposure to oil and the method has been used for the evaluation of the effect of the exposure tests. The clumping of the barbules relates to the capacity of the feather to repel water, which, among other things, is dependent on the ratio of barb thickness and distance between barbs (Stephenson 1997).

Sections of approximately 25 proximal barbules were measured on each feather (see Figure 3 for terminology details). In each section the numbers of proximal barbules in each clump were counted and the AI was calculated for each section as mean barbules per clump. This was done for three barbs on each photo resulting in 12 AI for each feather. The number of holes between barbules clumps is also noted.

Please insert Figure 3

Barbules, projecting from the barb (see Figure 3), are fitted with hooks and notches, forming an interlocking rigid surface that prevents the barbs from being drawn together by the surface tension forces of the water that secures a high contact angle with water droplets, contributing to water repellence (Rijke 1968, Rijke and Jesser 2011). Thus, the water repellence of the feather is dependent on the ratio of barb thickness and distance between barbs and the surface tension of water (Stephenson 1997). Oil have a much lower surface tension than water and oil is therefore absorbed in large quantities by the feathers instead of being repelled (Leighton 1993). Thus, oil disrupts the feather microstructure as a result of the collapse of hooks, barbs and barbules (Hartung 1967; Jenssen 1994) and thereby the ratio of the barbules and barbs. As a result, the surface tension no longer prevents water penetration. As little as 12.5 mL crude oil has been found to cause a significant high increase in the metabolic heat production of eiders (Hartung 1964, in Leighton 1993).

4.2.4 Data treatment

The AI of each feather was calculated as the mean of the AI count on the three photos on which four locations were counted. One of the assumptions of an analysis of variance (ANOVA) is variance homogeneity. Bartlett test showed that this assumption was not fulfilled ($p<0.001$). However, performing an ANOVA on rank transformed data is a useful approach when dealing with results of an experimental design (Conover 1999), and therefore the mean AI index of each feather were rank transformed prior to performing an ANOVA. Tukey's *post hoc* test with a significant level of 5% was applied to test of pairwise differences in mean AI among treatments.

In case of the control results for the weight data, the Bartlett test showed a homogeneous variance (p=0.91) and therefore no transformation was performed.

The effects of dilutions degree on AI were estimated by weighted (1/SD) linear regression of mean feather AI index on log-10 transformed dilution factor. Ordinary linear regressions were performed when evaluating the effect of dilutions degree on feather weight increase. Statistical analyses were done by using the software R version 3.1.3 (R Core Team 2015).

5 Results

5.1 Changes in total weight of the feather

For the three control exposure experiments with i) DCM, ii) distilled water and iii) salt water, no significant difference was found (ANOVA, p=0.32) in the changes in feather weight before and after exposure. This shows that DCM will not affect the feather structure or weight and thus is suited as a solvent. It should be noted that it is important that enough time for the DCM to evaporate is allowed before the actual exposure experiments.

The weight differences decrease with increasing dilution, which is evident for both oil types and burn residues (Figure 4). Note that the dilution factor is based on log-10 transformed dilution, e.g. 1 on the x-axis corresponds to 10 times dilution. At 100 times dilution, the weight differences are in the same range as the salt water control indicated by the red lines.

Please insert Figure 4

5.2 Amalgamation Index – effects on microstructure

The mean AI index of the three controls experiments with i) DCM, ii) distilled water and iii) salt water were significantly different (Figure 5, ANOVA, p=0.008). No significant difference was found between distilled water and DMC (*Tukey's post hoc* test, p=0.97), whereas salt water had significantly lower mean AI index than DMC (Tukey’s post hoc test, p=0.03) and it was also close to being significantly lower than the index for distilled water (*Tukey’s post hoc* test, p=0.05). However, the magnitude of the effect of DMC on the AI index was minor (Figure 5).

Please insert Figure 5

In Figure 6 the undiluted samples have not been included because they in most cases had a maximum AI of 25 as the feathers were completely smeared with oil/residue and the barbules could not be distinguished and thus counted.

Please insert Figure 6

As for the feather weight changes, a decrease in AI with increasing dilution factor was found. The results also indicate that effects on the feather microstructure can be measured for higher dilutions (e.g. lower doses) for the burn residues than for the corresponding oil. Grane oil effects on the feather microstructure were also higher than those of IFO30, which is assumed to be due to the difference in chemical composition of two oil types.

6 Discussion

Based on the weight changes, a measurable transfer of oil and oil residue to the feather could be observed for the exposure to dilutions greater than or equal to 10 times. However, the effects from lower oil/residue doses were not measurable. The weight difference measurements are considered a useful way to quantify effects of exposure to high doses of oil and / or burn residues on seabird feathers. However, potential impacts on the microstructure of the feather caused by exposure to lower doses are only detectably by means of Amalgamation Index. The correct feather structure is of great importance as it contributes actively to the water repellence (Rijke 1968). The rough and porous surface of the feather causes air to be entrapped in the hollows and interstices. This results in the formation of air-liquid interfaces that will increase the water repellence (Rijke 1968). The AI has been used to quantify effects on the microstructure (Figure 5 and Figure

6). Intersects of the AI mean of the salt water control (corresponding to no exposure) and the weighted regression lines for average AI results for exposure of fresh oil or burn residue are shown in Table 4. Based on an assumption that oil / residue were homogeneously distributed over the salt water surface in the petri dish, the dilution intersects have been converted to a “no or minor microstructure effects film slick thickness” (see Table 4).

Please insert Table 4

According to ERIN and OCL (2003) in O’Hara and Morandin (2010) an oil film greater than 3 µm is referred to as a slick and 25 µm is used as a conservative thickness for simulating oil slicks in O’Hara and Morandin (2010). Our oil slick thicknesses are found to be within that range for the 1 times dilution and for the 10 times dilution doses (see Table 3). According to the Oil Spill Observation Glossary (Hazmat 1996) different oil sheen thicknesses can be categorized as follows: (A) 0.04 µm barely visible sheen, (B) 0.07 µm silver sheen, (C) 0.1µm first colour trace sheen , (D) 0.3 µm bright colours sheen, (E)1 µm dull colours sheen, (F) 3 µm dark colour or thick sheen. The 100, 1,000 and 10,000 times dilutions doses results in thicknesses that all represent different types of oil sheens (Table 3). The exposure results (Table 4 – the no-effect film slick thicknesses) indicate that feather microstructure effects are observed for barely sheens and even thinner sheens (number A according to the Oil Spill Observation Glossary).

The largest alterations in feather microstructure are seen for the burn residue samples when compared to the corresponding fresh sample. Grane crude oil in general has a thinner no-effect film slick thickness compared to IFO30. This is expected to be due to the nature of the oils, where Grane is considered more viscous with a high number of asphaltenes. Similarly, microstructure effects were found after exposure to 0.1 and 3.0 µm crude oil sheens for Common Murre feathers and 3.0 µm crude oil sheens for Dovekie feathers (O’Hara and Morandin 2010). The differences in results are expected to be due to different oil types used in the experiments.

It is well known, that in case of oil spill, seabirds are among the groups of animals most vulnerable. The damaging effects of oil on the feather plumage are well documented in the literature as seabirds rely on an intact feather plumage for insulation, buoyancy and 'aqua' and aero dynamics. An oiled plumage will let seawater into the insulating airspace leading to hypothermia, and reduced swimming, diving and flying abilities (Clark 1984; Schreiber and Burger 2002). Most oiled seabirds will quickly die, especially in cold environments (Piatt and Ford 1996). Thus, a fast and efficient oil spill response operation for an overall benefit to the environment is required. To assess which oil spill combat technique(s) to use in a specific situation a Net Environmental Benefit Analysis (NEBA) must be conducted. Through the NEBA the environmental pros and cons between different or combinations of oil spill response techniques (e.g. *in situ* burning, chemical dispersants or mechanical recovery) and a doing nothing scenario is assessed in the actual environmental frame of organisms present and in risk of being impacted by the oil slick as well as potential side effects of the response techniques. According to Fritt-Rasmussen et al. (2013) the results of a NEBA for a response option might 1) be positive effects for the environment, 2) cause no effects (or alternatively ineffective) or 3) cause unwanted additional environmental impact compared to those already expected from the spill.

In situ burning of oil spills has been suggested as an effective method for combating oil spill in the sea particular in remote Arctic waters, where the low temperatures and presence of ice might extent the window of opportunity for the method (Fritt-Rasmussen and Brandvik 2011). Combining the findings of the experiments and the knowledge about the seabird behaviour, it seems likely that exposure to burn residues will lead to the same effects as seen for oil exposure (Culik et al. 1991). However, in this perspective and when conducting a NEBA it should be added that the amount of residue is largely reduced by the combustion process compared to the initial amount of oil (Fritt-Rasmussen and Brandvik 2011) and that in some cases the burn residue is submerged or even sunken (Fritt-Rasmussen et al. 2015), and, as a result, the risk for exposure of the birds is reduced. Therefore, this technique is considered as beneficial for birds on the sea surface, but the potential negative effects of burn residues on organisms below the surface must be taken into consideration in the specific situations.

Chemical dispersants is a method used to enhance the natural dispersion and thereby transferring the oil from the water surface and into the water column for subsequent degradation (Lewis and Daling 2001). This will also reduce the potential impacts on e.g. birds (Tamis et al. 2011). In the NEBA of a potential chemical dispersant operation important things to consider are e.g. water depth and mixing energy for the application to be effective, but also knowledge about impacts from the chemicals itself is needed. From laboratory experiments where living Mallards (*Anas platyrhynchos*) were exposed to dispersants on a water surface it was found that the birds swimming in these dispersants “sank to a much lower level than normal” and that “the birds could not shake or preen the water off their plumage as usual” (Lambert et al. 1982). Other studies, also including dispersants, showed enhanced effect of plumage contamination seen from oil/dispersant mixtures exposure (Jenssen and Ekker 1991) and the reason for this is suggested to be a result of the surfactants (a substantial amount of the dispersants) binding to the hydrophobic waxes in the plumage thereby more easily adhere to the feather structure (Jenssen 1994). Also surfactants are found to reduce the surface tension of the water, thereby allowing the passage of water into the plumage (Stephenson 1997) which likely causes the observed effects. Overall chemical dispersants are assessed as beneficial for birds on the sea surface (Peakall et al. 1987), however, these side effects must be considered, especially with regard to precision of spreading dispersants on an oil slick, the amount to be used and efficiency of the method. It should be noted that to the knowledge of the authors, no studies have been conducted by use of AI to assess the feather changes from chemical dispersant exposure.

Mechanical recovery is, in general, environmental beneficial as oil is removed from the environment. However, for birds located on the sea surface in the trajectory of the oil slick, the technique may prove to be too slow and inefficient to protect these birds; this should be taken into considered in the NEBA process. Also doing nothing, which may be the only option if certain weather conditions make it impossible to bring any measures in use, will, in general have a negative effect on the environment as the oil is left on the sea surface for drifting and spreading.

The potential environmental positive or negative effects of the different techniques must be considered carefully against presence of sensitive organisms, e.g., birds on the sea surface as well as other organisms together with the different side effects of the combat techniques. Hence, the results from present study and similar, such as Jenssen and Ekker (1991), is valuable knowledge to be included in a NEBA prior to a decision on which oil spill response methods to be used in a specific oil spill response situation. It is not possible to draw any clear conclusions on when and where *in situ* burning or chemical dispersants should be used as it is, as discussed above, an assembly of many factors, e.g., operational conditions, environmental effects and presence of species sensitive to oil, etc.

7 Conclusion

The results of our experiments indicate that burn residues have similar or larger damaging effects on the seabird feathers, compared to the corresponding amount of fresh oil. Damages on the feathers microstructure were detected for very thin films not even visible to the eye. It was found that Grane crude oil in general (both residues and fresh samples) appeared somewhat more damaging, i.e. seen as effects from very thin oil sheens, than IFO30 probably due to the relatively high content of asphaltenes in Grane and hence the more viscous appearance.

From the evaluation of the two methods that was used to measure the exposure effects it was found that to be able to assess microstructure effects from very thin sheens measurements of the AI is essential, whereas the weight different measurements is a useful tool to measure the impact from bird feather exposure to crude oil or burn residues in high doses (slicks and thicker sheens).

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Figures and Figure captions

Figure 1: Illustration of the sinking of the residue after flame extinction for the burning of IF30, 40 mm initial thickness. Each picture illustrates approximately a time interval of 2 s.

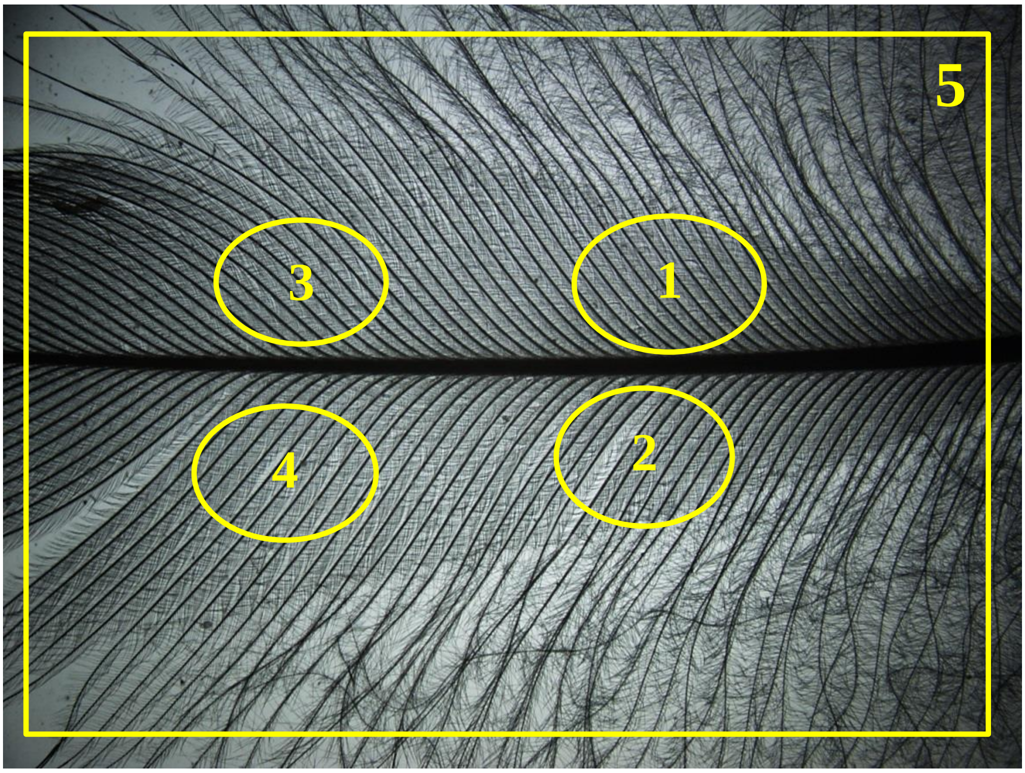

Figure 2: The numbers 1-5 indicate where the five pictures were taken. For the detailed pictures (no. 1-4) a magnification of 1x11.25 was used. One of the detailed pictures represents a width of 1.7 mm.

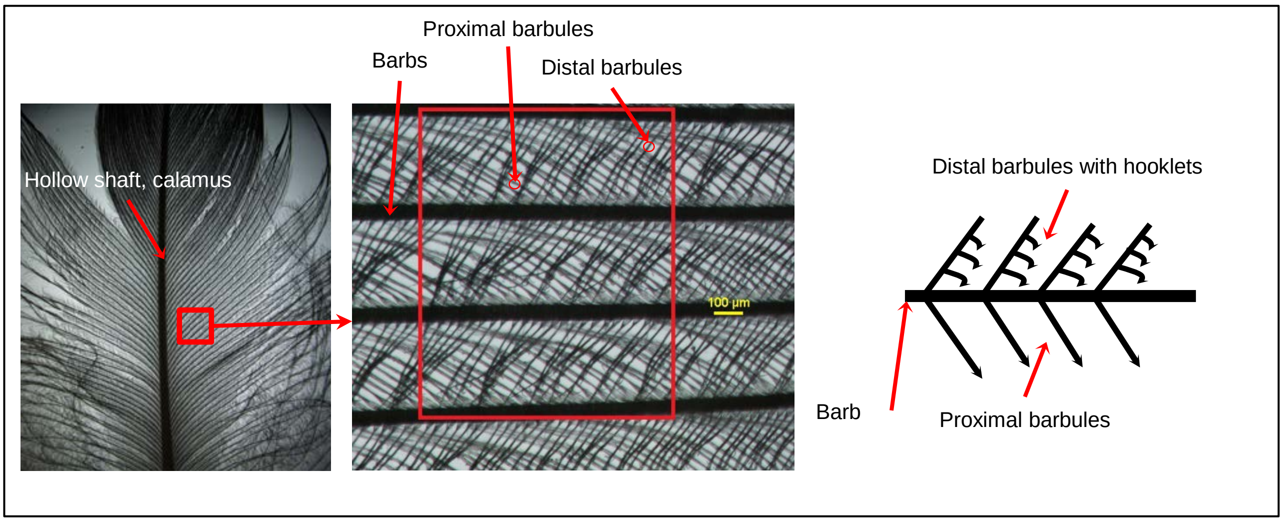

Figure 3: Conceptual bird feather terminology.

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Figure 4: Weight change of each feather (three feathers for each dilution) versus log_{10} of the dilution. The red lines represent the mean of the salt water control and broken red lines 95% confidence limits of the mean. Note that the dilution factor is based on log-10 transformed dilution, e.g. 1 on the x-axis corresponds to 10 times dilution.

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Figure 5. Boxplot of feather Al. Red lines represent medians, the bottom and top of the box show the 25th and 75th percentiles, the vertical dashed lines show the smallest of maximum and minimum or 1.5 times the interquartile range (roughly 2 SD) and the points showed are defined as outliers (more than 2 SD).

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Figure 6: Average of AI of each feather (each feather having 3 photos on which 4 locations are counted) versus log_{10} of the dilution. Black lines represent weighted (1/SD) regression lines and black broken lines the 95% confidence limits for the regression. Red lines represent the mean of the salt water control and broken red lines 95% confidence limits of the mean. The dilution factor is based on log-10 transformed dilution, e.g. 1 on the x-axis corresponds to 10 times dilution. One extreme high value is indicated by a cross and the specific value.

Tables and table captions

Table 1: Physical and chemical properties for the fresh oils used in the experiments. Data from Brandvik et al. (2010). Data for IFO30 from SINTEF Oil Weathering Model (Johansen et al. 2010). Viscosity data for Grane from Faksness (2008).

Oil type	Density (kg/m^3)	Pour point (°C)	Wax (wt. %)	Asphaltenes (wt. %)	Viscosity (cP)
Grane	0.941	-24	3.2	1.4	22 at 5 °C
IFO30	0.936	6	not available	not available	236 a 13 °C

Table 2: Results from the burning experiments. The numbers are average from triplicate experiments. Note that during the 10 mm oil slick experiments boil-over occurred causing vigorous burning, but also a more abrupt flame-out resulting in the lower burning efficiencies.

Sample ID	**Initial oil slick thickness** [mm]	**Burning efficiency** [%]	**Burning rate** [g/cm3]	**Regression rate** [mm/s]	**Burning time** [s]
Grane 10 mm	10	54	0.23	1.25	430
Grane 40 mm	40	62	0.13	0.70	3555
IFO30 10 mm	10	34	0.13	0.82	407
IFO30 40 mm	40	61	0.12	0.77	3149

Table 3: Minimum oil slick thickness [µm] for the different oil types and burn residues as a function of dilution and dilution factor.

Dilution factor	Dilution	Fresh Grane	Grane burn residue	Fresh IFO30	IFO30 burn residue
0	1	32.4	14.2	8.77	6.20
1	10	3.24	1.42	$8.77*10^{-1}$	$6.20*10^{-1}$
2	100	$3.24*10^{-1}$	$1.42*10^{-1}$	$8.77*10^{-2}$	$6.20*10^{-2}$
3	1000	$3.24*10^{-2}$	$1.42*10^{-2}$	$8.77*10^{-3}$	$6.20*10^{-3}$
4	10000	$3.24*10^{-3}$	$1.42*10^{-3}$	$8.77*10^{-4}$	$6.20*10^{-4}$

Table 4: Intersects, rounded off to the nearest 100, between mean salt water control and regression lines for average AI of each feather and the corresponding no-effect film slick thicknesses.

Sample type	Dilution at intersect	No-effect film slick thickness [μm]
Fresh Grane	11,000	< 0.00029
Grane burn residue	175,200	< 0.000081
Fresh IFO30	8,100	< 0.0011
IFO30 burn residue	41,900	< 0.00015