A Comparison between Chemical and Natural Dispersion of a North Sea Oil-spill

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ABSTRACT

The application of dispersants to an oil-slick is a key remediation tool and thus understanding its effectiveness is vital. Two *in situ* oil slicks were created in the North Sea (off the coast of The Netherlands), one left to natural processes whilst dispersant (Slickgone NS) was applied to the other. GC-MS analysis of seawater from the surface slick, and at 1.5 and 5 m below the slick, revealed only two samples with measurable hydrocarbons (221 ± 92 µg ml^{-1} seawater), from the surface of the "Slickgone Dispersed" oil-slick ~25.5 hours after oil-slick formation, which was likely due to environmental conditions hindering sampling. Additionally, 16S rRNA gene quantitative PCR and amplicon analysis revealed extremely limited growth of obligate hydrocarbonoclastic bacteria (OHCB), detected at a relative abundance of $<1\times10^{-6}$ %. Furthermore, the Ecological Index of Hydrocarbon Exposure (EIHE) score, which quantifies the proportion of the bacterial community with hydrocarbon-biodegradation potential, was extremely low at 0.012 (scale of 0 – 1). This very low abundance of hydrocarbon-degrading bacteria at the time of sampling, even in samples with measurable hydrocarbons, could potentially be attributed to nutrient limitation (~25.5 hours after oil-slick creation total inorganic nitrogen was 3.33 µM and phosphorus was undetectable). The results of this study highlight a limited capacity for the environment, during this relatively short period, to naturally attenuate oil.

INTRODUCTION

The overall goal of oil-spill response is to minimise impact to life as well as natural and economic resources. A balance must be made between potential environmental/economic impacts and "natural recovery" or "recovery through intervention" (National Oceanic and Atmospheric Administration, 2010; IPIECA *et al.*, 2017). Oil-spill response in the marine environment requires a comprehensive knowledge of immediate and surrounding environments, local stakeholders and political legislation, and available remediation tools. One such tool is the application of dispersants. Dispersants transform oil on the surface of the water into droplets (10 – 300 μm, North *et al.*, 2015) in the water column, which increases oil surface area for microbial attachment (Prince *et al.*, 2013), thus allowing hydrocarbon-degrading microbes to expend more energy on growth and less energy producing biosurfactants, thereby expediting hydrocarbon-biodegradation (Prince *et al.*, 2016; Brakstad *et al.*, 2018). Prince *et al*. (2015) found that three commonly applied dispersants (Corexit 9500, Finasol OSR 52, and Slickgone NS) significantly increased the biodegradation of hydrocarbons when compared to a floating oil-slick, with no added dispersants. Several other studies also observe that dispersants increase biodegradation (Brakstad *et al.*, 2015; Prince *et al.*, 2016) and enhance the growth of hydrocarbon-degrading bacteria (HCB) (Hazen *et al.*, 2010; Dubinsky *et al.*, 2013; Ribicic *et al.*, 2018). In contrast, other studies show that dispersants may not enhance biodegradation (Lindstrom and Braddock, 2002; Rahsepar *et al.*, 2016) or may even inhibit the growth of HCB (Hamdan and Fulmer, 2011; Kleindienst *et al.*, 2015); though there are many criticisms of the experimental procedures used in these studies (Gregson *et al.*, 2021).

With such contradictory results from studies investigating the effects of dispersant application on oil spills it is evident that further research is required. Studies must replicate natural environmental conditions as best they can. The optimal way would be to collect samples during the application of a dispersant to a real oil-spill. However, due to logistical, financial, and safety issues this is often not possible. The next best option is to conduct a controlled experiment *in situ*. However, once again there are many legislative, economic, and technical

barriers to such experiments being approved. Due to limitations sampling real and experimental oil spills, most oil/dispersant research is conducted in the laboratory (Buist *et al.*, 2011; Tremblay *et al.*, 2017; Doyle *et al.*, 2018). However, conducting *ex-situ* oil-spill experiments has potentially negative biases due to confinement in bottles or tanks, which would not necessarily occur at sea. Confinement does not allow dispersed oil to rapidly dilute to sub-ppm concentration which would occur *in situ* (Bejarano *et al.*, 2013), and higher concentrations of oil can potentially inhibit hydrocarbon degradation (Lee *et al.*, 2013; Prince *et al.*, 2016). Moreover, dispersion over a wider area, which would occur at sea, may allow access to further inorganic nutrients, which in turn could lead to faster hydrocarbon degradation. Given the potential biases of conducting laboratory oil-spill experiments, obtaining a permit to conduct a controlled oil release at sea, with dispersant application, is highly valuable.

The North Sea, in the north eastern area of the Atlantic Ocean, is located between the United Kingdom and borders continental west Europe. Approximately 13 miles off the coast of Scheveningen harbor, The Hague, Netherlands, a controlled *in situ* experiment was conducted in which oil slicks were created in April 2019. One oil-slick was left to undergo natural attenuation and dispersion whilst the other oil slick was chemically dispersed using the widely applied commercial dispersant Slickgone NS (Dasic International). Samples were taken from both oil slicks approximately 1, 5.5, and 25.5 hours after oil-slick creation, providing a rare and valuable opportunity to evaluate whether dispersant application on oil spills affects HCB growth and hydrocarbon-biodegradation *in situ*.

METHODS

Sampling Campaign: Oil-slick creation took place on the 16th April 2019 approximately 13 miles off the coast of Scheveningen harbour, The Hague, Netherlands. Full sampling and technical details can be found in the ITOPF ExpOS'D technical report (Zeinstra *et al.*, 2020), but in summary, a light-medium Arabian Crude oil was released continuously, using an air membrane pump with a flow rate of 6.7 litres s^{-1}, via a 2-inch hose. This trailed 20 m behind

the vessel, on floatation bladders, travelling at 1.85 knots. The natural dispersion oil slick ("Naturally Dispersed") was created into the wind at 10:25, using ~2.5 m^3 of the crude oil. The oil slick for chemical dispersion ("Slickgone Dispersed") was created into the wind at 11:40, using ~2.5 m^3 of the crude oil. The "Slickgone Dispersed" oil slick was sprayed (by an onboard MARKLEEN Dispersant spray system) with Slickgone NS dispersant 30 minutes after release, for one hour at a ratio of 20:1 oil to dispersant. Triplicate 250 ml seawater samples were taken from the surface and at depths of 1.5 m and 5 m in sterile plastic containers. Surface samples were taken directly by reaching over the side of the rigid inflatable boat (RIB). Samples from 1.5 and 5 m depths were taken by means of a sterile hose, lowered to the required depth, and samples pumped into sterile plastic containers. Sample water (150 ml) was passed through Millipore® Sterivex™ filters (0.22 μm) and flash frozen at -150°C in a Cryogenic Vapour Shipper, to preserve DNA, prior to storage at -20°C. The filtrate from this process was also flash frozen prior to being stored at -20°C for nutrient analysis of ammonium (NH_4^+), phosphate (PO_4^{3-}), nitrate (NO_3^-), and nitrite (NO_2^-), using a SEAL Analytical AA3 HR AutoAnalyzer tandem JASCO FP-2020 Plus fluorescence detector. In addition, triplicate 40 ml seawater samples were collected from the surface as well as at depths of 1.5 m and 5 m (same method as above), in sterile brown-glass 40 ml vials capped with PTFE-lined silicon septa, and immediately frozen at -20°C for hydrocarbon analysis. Sampling of oil slicks occurred ~1.5 hours, ~5 hours (16th April 2019), and ~25.5 hours (17th April 2019) after oil-slick creation.

Environmental Measurements: Temperature (9.06 ± 0.11 °C), salinity (30.9 ± 0.85 psu), and pH (8.41 ± 0.02) were all measured at the time of sampling. Wave height measurements were collected by two stations: 'IJgeul 1' (4,264°E, 52,488°N, located 31 km of sampling site) and 'Q1 platform' (4,150°E, 52,925°N, located 75 km northeast of sampling site). Wind speed/direction measurements were collected by two offshore stations: P11 (3,342°E,

52,359°N, 45 km northwest of sample site) and Europlatform (3,275°E, 51,998°N, 55 km southwest of sample site) (Zeinstra *et al.*, 2020).

Hydrocarbon Degradation (GC-MS): Hydrocarbons were extracted from 40 ml brown-glass vials (collected *in situ*) using a 20 ml solvent extraction of 1:1 hexane : dichloromethane, vigorously shaken for 30 seconds, and placed in an ultrasonic bath for 30 minutes. The 20 ml of solvent extract was then passed through reversed-phase solid-phase extraction tubes (Supelclean™ ENVI™-18 SPE, Sigma), using an method adapted from Risdon et al. (2008), before being eluted in 6 ml of 1:1 hexane : dichloromethane and then concentrated to 1 ml under nitrogen gas. Sample quantification was performed on an Agilent 7890A Gas Chromatography system coupled with a Turbomass Gold Mass Spectrometer with Triple-Axis detector, operating at 70 eV in positive ion mode, using conditions as previously described by Coulon et al. (2007). Only those hydrocarbons detected are shown in Fig. 1 (B).

qPCR Analysis of Bacterial 16S rRNA genes: DNA was extracted from *in situ* seawater samples from thawed Millipore® Sterivex™ filters with a DNeasy PowerWater Sterivex Kit (Qiagen) according to the manufacturer's instructions. The primers used for quantification of bacterial 16S rRNA genes were 341f - CCTACGGGNGGCWGCAG and 785r – GACTACHVGGGTATCTAATCC (Klindworth *et al.*, 2013). qPCR was performed using a CFX384™ Real-Time PCR Detection System (BioRad) using reagents, cycle conditions, and standards as previously described (McKew and Smith, 2015).

Amplicon Sequencing and Bioinformatics: Amplicon libraries were prepared, as per Illumina instructions. PCR primers were the same as those used for qPCR but flanked with Illumina overhang sequences. PCR products were quantified using Quant-iT PicoGreen dsDNA Assay Kit (ThermoFisher Scientific) and pooled in equimolar concentrations. Quantification of the amplicon libraries was determined via NEBNext® Library Quant Kit for Illumina (New England BioLabs Inc.), prior to sequencing on the Illumina MiSeq® platform, using a MiSeq® 600 cycle v3 reagent kit and 20% PhiX sequencing control standard. Sequence

output from the Illumina MiSeq platform were analysed within BioLinux (Field *et al.*, 2006), using a bioinformatics pipeline as described by Dumbrell *et al.* (2016).

Statistical Analysis: Prior to community analysis, sequence data were rarefied to the lowest library sequence value (5,747). Data were first tested for normality (Shapiro-Wilks test), those data which were normally distributed were tested for significance with ANOVAs or appropriate linear models. Non-normally distributed data were analysed using appropriate GLMs (Generalised Linear Models) as follows. The relative abundance of operational taxonomic units (OTUs) or genera in relation "Uncontaminated Seawater", both oil-slicks, depth, or time were modelled using multivariate negative binomial GLMs (Wang *et al.*, 2010). Here, the number of sequences in each library was accounted for using an offset term, as described previously (Alzarhani *et al.*, 2019). The abundance of bacterial 16S rRNA gene copies was also modelled using negative binomial GLMs (Venables and Ripley, 2002). The significance of model terms was assessed via likelihood ratio tests. The Environmental Index of Hydrocarbon Exposure (Lozada *et al.*, 2014) was calculated using the script available at the ecolFudge GitHub page (https://github.com/Dave-Clark/ecolFudge, Clark, 2019) and EIHE values modelled using poisson GLMs. All statistical analyses were carried out in R3.6.1 (R Development Core Team, 2011) using a variety of packages available through the references (Venables and Ripley, 2002; Csardi and Nepusz, 2006; Hope, 2013; Wilke, 2015, 2020; Becker *et al.*, 2016; Auguie, 2017; Oksanen *et al.*, 2019; Hvitfeldt, 2020; Kassambara, 2020; Lenth, 2020; Pedersen, 2020). All plots were constructed using the "ggplot2" (Bodenhofer *et al.*, 2011) and "patchwork" (Pedersen, 2019) R packages.

RESULTS and DISCUSSION

Hydrocarbon Analysis Reveals Difficulty in Conducting in situ *Oil-spill Experiments*

Analysis of hydrocarbons revealed that only two samples, from the "Slickgone Dispersed" oil-slick ~25.5 hours after oil-slick creation, contained any measurable hydrocarbons; including *n*-alkanes ($C_{14} - C_{31}$), branched alkanes (pristane and phytane), and

polycyclic aromatic hydrocarbons (PAHs; phenanthrene and methyl-phenanthrene/anthracene) at average concentrations of 188.13 (± 76.91), 27.20 (± 11.91), and 5.84 (± 3.13) µg ml^{-1} seawater, respectively (Fig. 1B). These samples did not contain any measurable C_{11} – C_{13} *n*-alkanes or naphthalenes and fluorene, in comparison to a profile of the oil (Fig. 1A), suggesting these hydrocarbons has partitioned into the air and/or water. Furthermore, the ratio of *n*-C_{17}/pristane and *n*-C_{18}/phytane was 0.95 and 1.63, respectively, with no significant difference to the original oil (*n*-C_{17}/pristane (0.94) and *n*-C_{18}/phytane (1.47)), indicating no biodegradation. A similar *in situ* North Sea oil spill by Gros *et al.* (2014) observed rapid mass transfer of >50% of <C_{17} hydrocarbons, as well as no detectable naphthalene, from surface samples 25 hours after oil-slick creation. The lack of measurable hydrocarbons, in all other surface samples from this field trial, was despite the fact oil was clearly visible to the naked eye and via radar, at all sampling time points. Samples were taken by reaching out of a rigid inflatable boat (RIB) and collecting surface oil/water in sterile vials. However, this proved difficult during the first day (16.04.2019) as increased wind speeds and wave heights, 8.33 ± 0.71 m s^{-1} and 105.26 ± 17.32 cm respectively, bounced the RIB, pushing the oily surface water beyond reach. On the second day, wind speed and wave height reduced to 5.15 ± 0.66 m s^{-1} and 58.52 ± 7.63 cm respectively, and samples were collected by means of a vial attached to a 2 m stick. Whilst the calmer environmental conditions and the new sampling technique meant sampling the oil/water interface was easier, movement of the RIB still made it difficult, resulting in only 2 of the 9 surface samples, collected ~25.5 hours after oil-slick creation, having any measurable hydrocarbons. These results reflect the difficulty in efficiently obtaining *in situ* oil-spill samples from the surface oil/water interface. Samples collected at depths of 1.5 and 5 m would not have been affected as seawater was directly pumped from those depths into sterile vials, suggesting oil either remained on the surface or had dispersed beyond these depths.

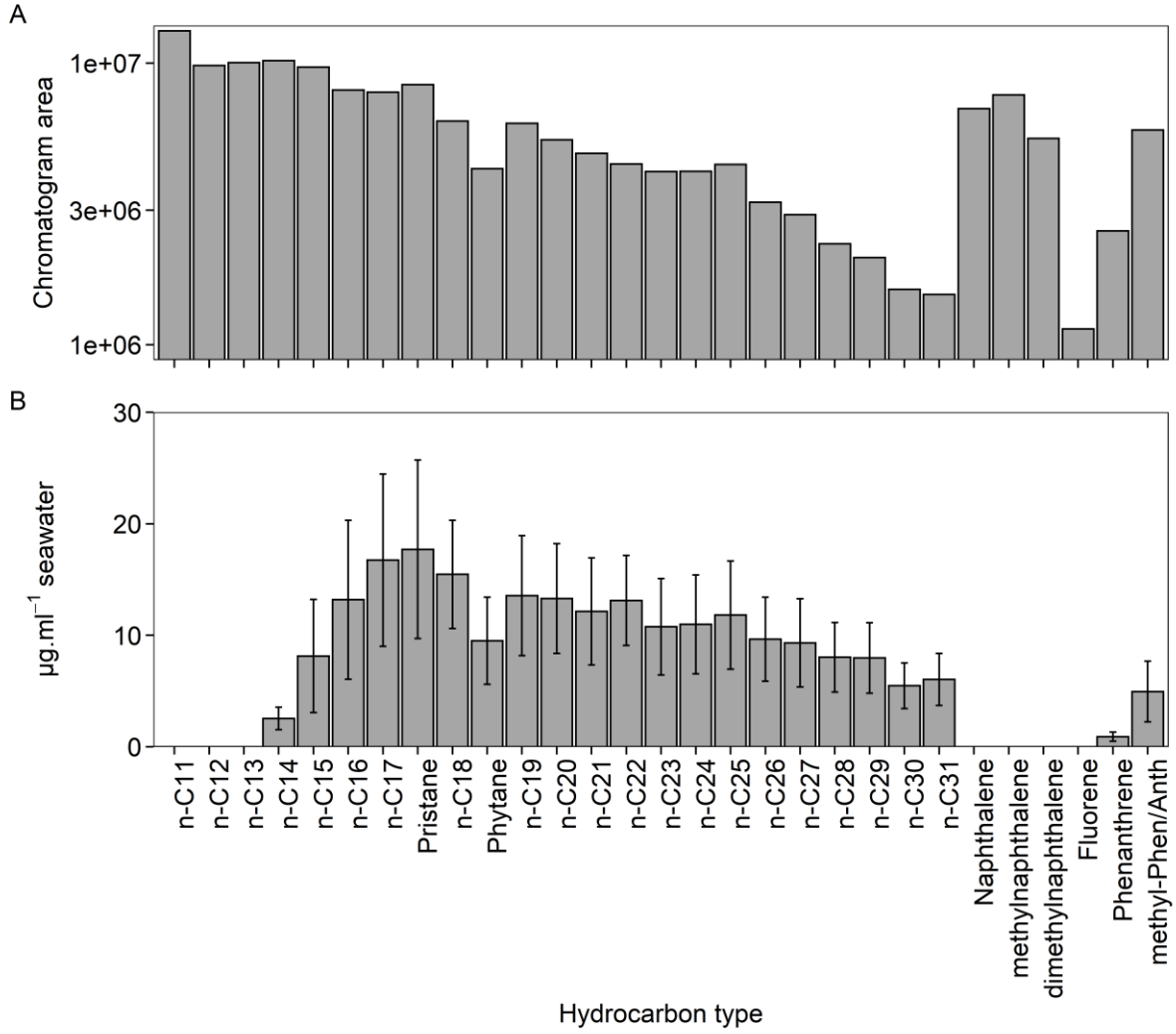

Fig. 1: profile of the light-medium Arabian Crude deposited during oil-slick formation; including *n*-alkanes (C_{11} to C_{31}), branched alkanes pristane and phytane, and PAHs (naphthalene, fluorene, phenanthrene, and any methylated derivatives (naphthalene and phenanthrene/anthracene (Phen/Anth)) (**A**). Concentration of measured hydrocarbons from seawater samples taken from the "Slickgone Dispersed" oil-slick, ~25.5 hours after oil-slick creation (**B**).

The overarching criticism of *ex situ* oil-spill experiments is that the oil spills are enclosed by some form of container, be it a microcosm, mesocosm, or wave tank. This containment is believed to create a number of biases, one of which is that containment decreases oil dispersal and dilution, which would otherwise dilute to sub-ppm concentrations *in situ* within 1 to 4 hours (Nedwed and Coolbaugh, 2008; Bejarano *et al.*, 2013). Therefore, adding oil at greater concentrations than sub-ppm, may inhibit the growth of some hydrocarbon-degrading bacteria (HCB), and thus reduce the rate of hydrocarbon

biodegradation (Prince, *et al.*, 2016). The results of this study could suggest that the concentration of oil from marine oil slicks that have been sprayed with dispersant does not always reduce to sub-ppm immediately, as the two samples with measurable oil (from the surface of the "Slickgone Dispersed" oil-slick ~25.5 hours after oil-slick creation) contained hydrocarbons at ~221 ppm. Moreover, the "Slickgone Dispersed" oil-slick, whilst reduced in size, remained visible by radar at all time-points. The application of dispersants to an oil slick requires suitable environmental conditions, which include wind speeds of 4 – 12 m s^{-1} (ITOPF, 2011) and full salinity seawater at 32-35 psu (Chandrasekar *et al.*, 2006). Additionally, dispersant efficacy is affected by the type of oil, as increasing oil viscosity decreases dispersant effectiveness, and therefore its application is more suited to light-to-medium oils (Trudel *et al.*, 2010). Weathering of oil increases viscosity, and thus the window of opportunity to apply dispersants to oil slicks ranges from a few hours to a few days (Chandrasekar *et al.*, 2005; ITOPF, 2011). These criteria were met during this study, and therefore it is unlikely that environmental conditions (wind speed, wave height, and salinity), oil type (light-medium Arabian Crude), or window of opportunity (one hour after oil-slick creation), inhibited dispersant efficiency. It should be noted, however, that the application of Slickgone NS on the oil slick was below the recommended level to sufficiently coat the oil-slick. Approximately 200 litres of dispersant was applied to the oil-slick, however, this is considerably lower than the 700 litres required to achieve the manufacturer's recommendation of 40 – 50 L per 10,000 m^2 of oiled area (Zeinstra *et al.*, 2020). This was due to time constraints restricting the number of dispersant-spraying passes through the oil-slick, thus not all areas of the slick had dispersant applied. None of the samples pumped directly from 1.5 or 5 m depths contained any measurable hydrocarbons, suggesting that either where the seawater was sampled the dispersant had not been applied to that part of the oil-slick, or that, had the dispersant been applied to that area, the oil had already been dispersed beyond 5 m.

Nutrient Limitation Potentially Inhibited the Growth of Hydrocarbon-degrading Bacteria

Certain microbes can degrade a range of hydrocarbons found in crude oil and its derivatives and thus oil-spills dramatically alter marine microbial community composition, resulting in a decrease in species richness and diversity, in conjunction with selection for HCB (Head *et al.*, 2006; McGenity *et al.*, 2012). However, during this study there was a clear lack of growth of OHCB or those genera with known hydrocarbon-degrading species. The Ecological Index of Hydrocarbon Exposure (EIHE), which quantifies the proportion of the bacterial community with hydrocarbon-biodegradation potential (Lozada *et al.*, 2014), was extremely low, averaging 0.012 (± 0.003; scale of 0 – 1) over all samples.

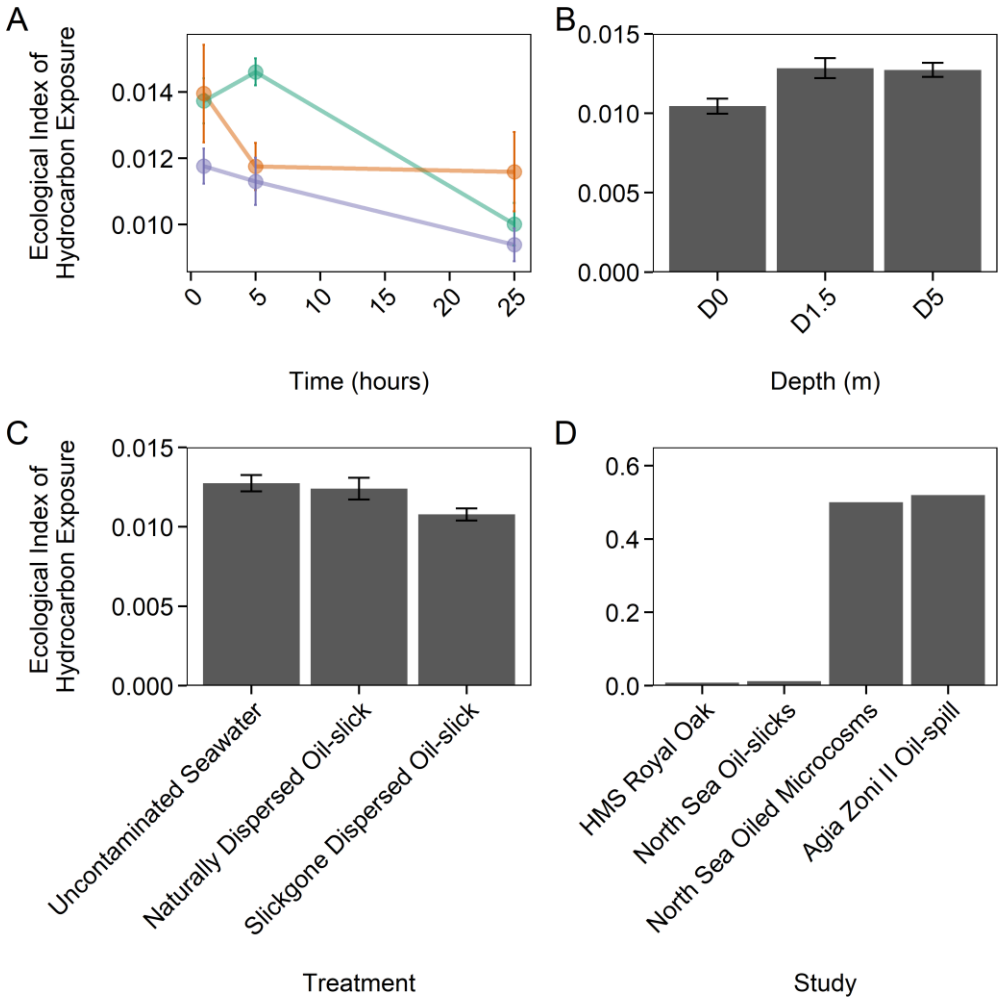

Fig. 2: Ecological Index of Hydrocarbon Exposure (EIHE) scores (± SE, *n* = 3, ratio % up to 1), representing relative abundance of bacteria with hydrocarbon-biodegradation potential (Lozada et al., 2014), from seawater sampled over ~25.5 hours (**A**), and over a 5 m depth profile (**B**), from "Uncontaminated Seawater" and "Naturally Dispersed" and "Slickgone Dispersed" oil-slicks (**C**). Additionally, a comparison between EIHE scores from seawater samples taken

in this study ("North Sea Oil-slicks") and other marine environments (**D**): "HMS *Royal Oak*" (Thomas *et al*., unpublished, average over all samples), "North Sea Oil-slicks" (this study), "North Sea Oiled Microcosms" (Thomas *et al*., unpublished; average over dispersant treatments (which reduced oil/water interfacial tension) after 24 hours), and "Agia Zoni II Oil-spill" (Thomas *et al.*, 2020, average at impacted sites in September 2017).

There were no significant differences in the EIHE score between "Uncontaminated Seawater" and each oil-slick at all time points and depths (Fig. 2A-C). Compared to some other marine environments (Fig. 2D) it can be observed that the EIHE score of 0.012 is similar to that found in sediments around the WWII shipwreck HMS *Royal Oak*, where the EIHE score was 0.008 and PAH levels were 229.2 ± 126.5 µg kg^{-1} of dry sediment (Thomas *et al*., unpublished). Furthermore, the EIHE score of 0.012 observed in this study is much lower than the EIHE scores observed in contaminated sediments (EIHE 0.52; TPH 1,093 – 3,773 µg g^{-1} dry sediment) sampled five-days after the Agia Zoni II oil-spill (Thomas *et al.*, 2020) and in oil/dispersant North Sea seawater samples taken after 24 hours (EIHE 0.50; TPH 54.95 µg ml^{-1}) from an oil/dispersant microcosm experiment (Thomas *et al*., unpublished). The relative abundance (%) of genera assigned to obligate hydrocarbonclastic bacteria (OHCB), a group of widely distributed marine bacteria that are specifically adapted to using hydrocarbons as an almost exclusive source of carbon and energy (Yakimov *et al.*, 2007), was less than 1×10^{-6}.

Potentially, the growth of HCB was inhibited by the absence of nutrients, where the level of total inorganic nitrogen (TIN; sum of ammonia, nitrate, and nitrite) significantly decreased in all samples (23.74 to 3.33 µM) ~25.5 hours after oil-slick creation, as well as phosphate being undetected (Fig. 3); the limit of detection for nutrients was 0.02 µM. Both nitrogen (N) and phosphorous (P) are vital for microbial growth, for example, N is required for the synthesis of proteins and nitrogenous bases whilst P is required for the synthesis of nucleic acids and phospholipids (Bristow *et al.*, 2017). N and P are especially important during hydrocarbon degradation of an oil slick (Atlas, 1981), and therefore the availability of these nutrients in the presence of hydrocarbons is vital (Ron and Rosenberg, 2014). Certain HCB, such *Alcanivorax* and *Cycloclasticus*, have specific systems for scavenging nutrients in

oligotrophic environments (Wang *et al.*, 1996; Cappello and Yakimov, 2010). However, the lack of growth of microbes such as *Alcanivorax* and *Cycloclasticus* species and other OHCB, suggests P limitation, or that growth was limited in some other way. The concentration of nutrients in the North Sea is primarily driven by a seasonal cycle, with higher levels of N and P in the winter months compared to the summer months (Tett and Walne, 1995). It is likely that the rapid decline of TIN, over ~1 day, was due to a decrease in vertical mixing as wave energy declined. Phytoplankton blooms, which take place during times of increased sunlight and nutrients in the euphotic zone, often occur in the spring and last until summer when nutrients become depleted (Mann and Lazier, 2013). Satellite images captured by MODIS (Moderate Resolution Imaging Spectroradiometer) suggest a phytoplankton bloom in the North Sea began on March 29th, 2019 (NASA, 2019). Sampling of this study occurred on the 16th and 17th April 2019 and therefore the high abundance of phytoplankton could have depleted phosphorous.

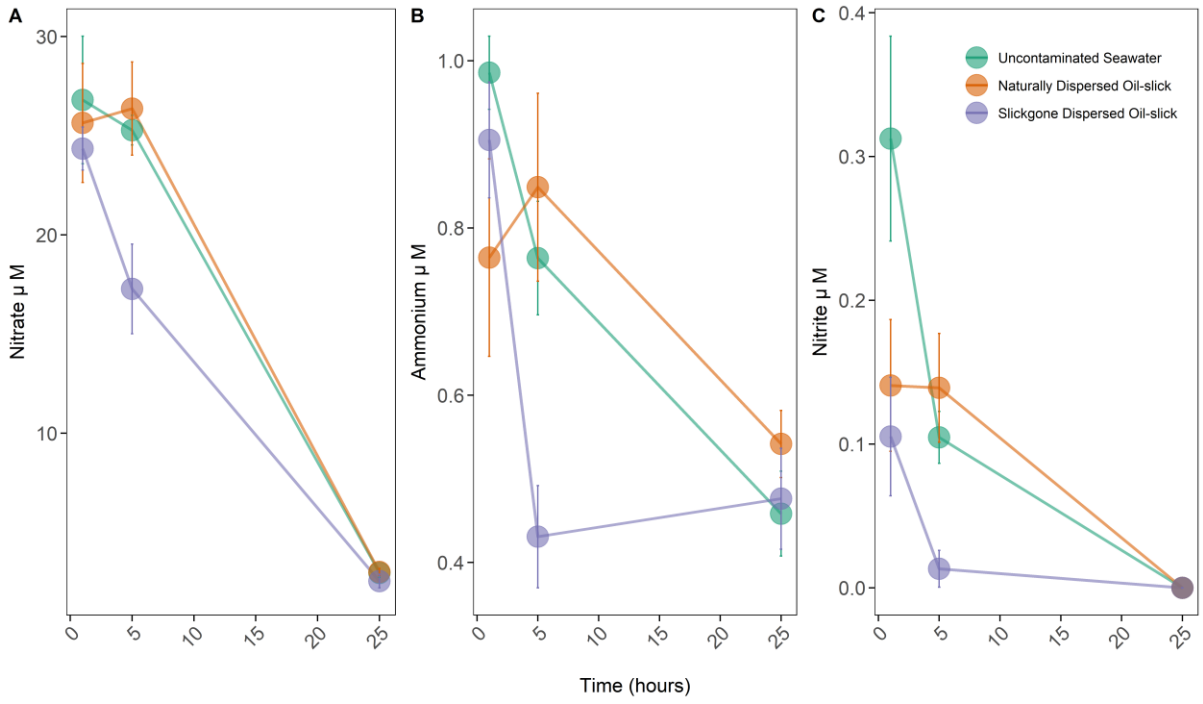

Fig. 3: Nitrate (**A**), ammonium (**B**), and nitrite (**C**) (mean ± SE, *n* = 3) from seawater samples taken from "Uncontaminated Seawater" as well as "Naturally Dispersed" and "Slickgone Dispersed" oil-slicks, ~1.5, ~5, and ~ 25.5 hours after oil-slicks were created. Phosphate was undetected in all samples; the limit of detection was 0.02 µM.

An EIHE score of 0.012 in this study reveals an exceptionally low level of HCB within seawater samples, this includes the two samples which contained hydrocarbons; though the microbial community samples were not truly paired to the samples collected for hydrocarbon analysis as they were collected in separate bottles (although at the same time and area of the slick) and therefore may not have contained oil. However, this demonstrates, at the time of sampling, the environment's ability to naturally attenuate oil was limited. Potentially low levels of phosphorous limited HCB growth, but it cannot be said for certain that this was the limiting factor. Regardless of what is limiting the growth of HCB, such low levels can inform oil-spill response operations. In this short-term study a limited ability for the environment to naturally attenuate oil would highlight a requirement for intervention measures, such as dispersal or physical removal of oil.

Developing in situ *Experimental Oil-spill Methodologies*

The results of this study have highlighted challenges in obtaining meaningful and reproducible seawater surface samples that capture the oil/water interface, with only 2 of the 18 surface samples from the oil-slicks containing any measurable hydrocarbons. The two samples with measurable hydrocarbons were taken from the "Slickgone Dispersed" oil-slick ~25.5 hours after oil-slick creation, though the third of the replicates contained no measurable hydrocarbons. Moreover, microbial community samples are not truly paired to the hydrocarbon samples as these were collected in separate vials for either DNA or hydrocarbon extraction. The primary challenge was the collection of surface samples from the sampling RIB, which would push the oily surface water beyond reach, even in relatively calm waters. One potential solution could be to use a remotely operated surface vehicle (ROSV), which could be remotely piloted (or done autonomously via GPS way-points) into the oil slick with minimal disturbance, collect a surface sample before returning to the crew for downstream processing. A ROSV designed and built for the purpose of oil-spill detection and sampling (e.g. Al Maawali *et al.*, 2019) could be adapted further. Given a large enough capacity, the ROSV could even be

adapted to apply dispersant at a specific location, which could then immediately be sampled, avoiding any doubt as to the efficiency of dispersant application. The efficacy of dispersant application was another limitation observed during this *in situ* oil-spill trial. This was primarily driven by time constraints, resulting in only 200 L, of the recommended 700 L, of dispersant actually being applied to the oil-slick. Technical recommendations advise more spraying passes through the oil-slick and that the dispersant spraying arms should be attached as far to the front of the ship as possible to ensure contact with oil, before it is pushed away by the ship's bow (Zeinstra *et al.*, 2020). Ideally more time would be allocated in such trials, which would allow sufficient and effective dispersant application and sampling to occur. Moreover, longer field trials would allow biodegradation to be measured over more realistic timescales. Whilst rapid growth of hydrocarbon-degrading bacteria can be observed within laboratory seawater-oil microcosms within 24 hours (Thomas *et al.*, unpublished), there is limited evidence that there would be significant growth *in situ* in many open water environments, and degradation would typically be very limited in the first day, particularly in low nutrient systems where a significant lag phase may be observed. However, due to permit restrictions requiring all oil to be removed from the sea surface after one day, high financial costs of operating numerous research vessels, and the availability of supporting services (i.e. airborne surveillance), additional time is not always possible. It is crucial any sampling limitations are overcome, as *in situ* oil-spill experiments can provide insightful results and observations into the processes that drive the fate and transport of oil in marine waters and thus guide oil-spill response management.

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