**Dilute concentrations of maritime fuel can modify sediment reworking activity of high-latitude marine invertebrates**

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## Abstract

Multiple expressions of climate change, in particular warming induced reductions in the type, extent and thickness of sea ice, are opening access and providing new viable development opportunities in high latitude regions. Coastal margins are facing these challenges, but the vulnerability of species and ecosystems to the effects of fuel contamination associated with increased maritime traffic is largely unknown. Here, we show that low concentrations of the water-accommodated fraction of marine fuel oil, representative of a dilute fuel oil spill, can alter functionally important aspects of the behaviour of sediment-dwelling invertebrates. We find that the response to contamination is species specific, but that the range in response amongst individuals is modified by increasing fuel concentrations. Our study provides evidence that species responses to novel and/or unprecedented levels of anthropogenic activity associated with the opening up of high latitude regions can have substantive ecological effects, even when human impacts are at, or below, commonly accepted safe thresholds. These secondary responses are often overlooked in broad-scale environmental assessments and marine planning yet, critically, they may act as an early warning signal for impending and more pronounced ecological transitions.

**Keywords**: Fuel contamination, Arctic trade, Northern sea route, Arctic marine shipping, multiple pressures, bioturbation

## Introduction

The Arctic region is experiencing rapid change due to climatic forcing (Burrows et al., 2011; Meredith et al., 2022), resulting in significant transformations in offshore (Horvat et al., 2017), terrestrial (Swanson, 2021) and coastal environments (Ogorodov et al., 2020). The combination of changes in the type, extent and thickness of ice cover (Meier et al., 2014; Pizzolato et al., 2016), retreating glaciers (Vincent et al., 2001), meltwater input (Statham et al., 2008), and water mass dynamics (Fossheim et al., 2015), coupled with warming and ocean acidification (Lam et al., 2016) is modifying short- to medium-term opportunities for the exploitation of abiotic and biotic resources, causing as an uplift in transport, trade, tourism, and other ship-based activity across the Arctic region (Pizzolato et al., 2016; Eguíluz et al., 2016; Dawson et al., 2018, Bartsch et al. 2021). In addition, melting and decreasing marine and terrestrial nearshore ice and snow also disrupts traditional transportation routes and methods (e.g. sea ice pathways and sled dogs), resulting in

the need for either longer routes or the increased reliance on motorised boats to make long or otherwise inaccessible journeys (Steiro et al. 2020). Such activity brings an increasing risk of both acute and chronic marine contamination from deliberate and accidental releases of fuel oils (PAME, 2020) to a seafloor that hosts a diverse (>4600 invertebrate species, Piepenberg et al., 2011) and productive benthic ecosystem (Sørensen et al., 2015; Kędra et al., 2015). Previous infamous oil spills have led to prolonged, catastrophic impacts on marine and coastal systems (Joye, 2015). Even short-lived, smaller spills can lead to immediate toxicity responses in exposed marine biota and declines in abundance (Brussaard et al., 2016) with likely disruptions in ecosystem productivity if recurrent (Ortmann et al., 2012; Brussaard et al., 2016).

Airborne contaminant bioaccumulation in high-latitude ecosystems has long been recognized (Alexander, 1995) and, given that the physical characteristics of Arctic marine habitats render them at greater risk to petroleum contamination (Short & Murray, 2011), accidental fuel spills have received considerable scientific attention (Helle et al., 2020). Cold temperatures exert a pronounced influence on hydrocarbon behaviour in seawater, altering compound composition and concentrations by affecting fuel partitioning and diminishing evaporation and degradation rates (Payne et al., 1991; Siron et al., 1993). The presence of ice can also adjust spill dynamics by suppressing wave action, prolonging exposure periods and curbing the spread and dispersion of a spill (Fingas and Hollbone, 2003). As near-shore habitats remain covered by sea ice for much of the year, and can receive runoff from contaminated shoreline sites (Chapman and Riddle, 2003), they are particularly vulnerable to the effects of contamination. Exposure to sublethal concentrations of the water accommodated fraction of fuel can affect the behaviour (Culbertson et al., 2004; Brown et al., 2017) and metabolism (Sardi et al., 2017) of invertebrate species, the effects of which can transfer across generations (Lee et al., 2013), whilst changes in community composition has been observed following exposure to median lethal concentration (LC50) values (Payne et al., 2014).

Despite extensive ecological monitoring programs existing across intertidal, offshore, and deep-sea habitats (Blicher & Arboe, 2021; Sejr et al., 2021; Thyrring et al., 2021) the functional consequences of contaminant-driven adjustments to species behaviour and alterations in biodiversity for community resilience (Peterson et al., 2003) and ecosystem functioning (Ferrando et al., 2015) are not well constrained. Here, we establish whether exposure to the water accommodated fraction of marine fuel oil affects the behaviour of three common, but functionally contrasting, sediment-dwelling invertebrates (the bivalves *Astarte crenata* and *Macoma calcare*a and the polychaete *Cistenides hyperborea*) from the fjordic regions of Greenland. Our *a priori* expectation was that sediment particle reworking activity – an important mediator of benthic biogeochemical processes and other sediment properties – would broadly reflect interspecific differences in lifestyle traits, but that subtle aspects of reworking behaviour would be modified by the presence of marine fuel oil. Our motivation was to highlight the potential that additional pressures associated with increased human activity may have in high latitude regions that are already undergoing substantive transformation.

## Materials and methods

### Sediment and invertebrate collection

Sediment (mean ± s.e., n = 4: $D_{50}$ = 150.25 ± 23.16 µm ($D_{10}$-$D_{90}$, 21.8 ± 2.91 – 418.5 ± 16.56 µm); organic matter content = 0.30 ± 0.07 %) and individuals of the bivalves *Astarte crenata* and *Macoma calcarea*, and the polychaete *Cistenides hyperborea,* were collected from the inner Kobbefjord (64°08.364 N, 51°23.621' W; 12m water depth) using a van Veen grab (0.1 $m^2$) deployed from the r.v *Age V Jensen II*. Sediment was sieved (1 mm mesh) in a seawater bath to remove any macrofauna and allowed to settle to retain the fine fraction (< 63 µm). Sediment particle size frequency distributions (see electronic supplementary material, figures S1) were determined optically using a Malvern Mastersizer 3000 LASER diffraction sizer at the School of Geography and Environmental Science, University of Southampton. Briefly, samples were broken down from aggregates using a surface active cleaning agent (Decon™) and a rubber pestle and suspended in distilled water during analysis. Between samples, the instrument was cleaned using pre-programmed standard settings of 3 rinses using tap then distilled water. These data were used to resolve mean particle size, sorting, skewness and kurtosis (Folk, 1974) using GRADISTAT v9.1 (Blott & Pye, 2001; see electronic supplementary material table S1). Loss on ignition was used to estimate sediment organic matter content (%; Lamb, 2005). Here, the weight of clean and labelled crucibles was recorded using a Mettler Toledo Analytical Balance (± 0.0001g) prior to the weighing of each wet sediment sample. The crucibles were overnight dried at 105˚C using a Gallenkamp Hotbox Oven (Size one), then heated to 550 ˚C for 2h and to 950 ˚C in a Carbolite Muffle Furnace.

**Experimental design**

Transparent square glass aquaria (internal dimensions, LWH; 11.0 × 11.0 × 23.5 cm) were each filled with ~11 cm (1.331 L) sieved sediment and overlain with ~9 cm (1.08 L) of seawater (salinity, 33 @ 12 m depth ; Sørensen et al., 2015) and maintained in a temperature-controlled water bath (5 ± 1° C, following Solan et al. 2020, see electronic supplementary figure S2) in the dark. We set the abundance and biomass (mean ± s.e.) of species to levels indicative of those found in inner Kobbefjord : *Astarte crenata,* 3 ind. and 11.61 ± 0.31 g aquarium$^{-1}$ (= 248 ind./959 g $m^{-2}$); *Macoma calcarea*, 4 ind. and 7.51 ± 0.48 g aquarium$^{-1}$ (= 331 ind./621 g $m^{-2}$); and *Cistenides hyperborea,* 6 ind. and 3.09 ± 0.14 g aquarium$^{-1}$ (= 496 ind./255 g $m^{-2}$); see electronic supplementary figure S3). Each aquarium was continually aerated and maintained for seven days.

**Introduction of marine fuel**

Marine fuel oil was sourced directly from a local marine supplier in Nuuk, Greenland. A low energy Water Accommodated Fraction (LE-WAF) was prepared following established guidelines from the Chemical Response to Oil Spills: Ecological Research Forum (CROSERF), using a modified method from that previously described, in closed vessels (Faksness and Altin, 2017). Specifically, an oil-to water ratio of 1:40 was used, with reduced contact time of 2 h (due to laboratory and experimental constraints). At this contact ratio, the system is assumed to be 'saturated' and therefore represents a conservative estimate of concentrations introduced by vessel traffic (Faksness and Altin, 2017). Using LE-WAF is preferential to introducing fuel oil directly as it avoids the generation of oil droplets, which may lead to dosing variability between treatments and enhanced exposure through organism adherence (e.g. Hansen et al., 2021). Following preparation, the Water Accommodated Fraction of the oil was removed and used to dose treatments. Each species was maintained in one of three marine fuel oil concentrations (n = 4 replicates species$^{-1}$

fuel concentration$^{-1}$): 0% (control), 0.1% and 0.5% marine fuel oil (Water Accommodated Fraction, Table 1). These concentrations are at the lower end of dosing rates observed to generate low, developmental, effects in marine organisms (e.g. Pereira et al., 2018). A water sample was taken from the Water Accommodated Fraction immediately before dosing to determine WAF composition and hydrocarbon concentration. The sample was sealed using parafilm, stored in darkness during transport and analysed for total petroleum hydrocarbons (aliphatic and aromatic fractions) via gas chromatography of solvent extracts (FID) and total and individual PAHs via gas chromatography linked mass spectrometry (GC-MS) at an accredited commercial laboratory.

**Quantification of bioturbation behaviour**

Faunal reworking of sediment particles (bioturbation) was estimated using non-invasive sediment profile imaging (f-SPI) (Solan et al., 2004) of the redistribution of a fluorescent dyed particle tracer, imaged under ultraviolet (UV) light (50g aquarium$^{-1}$, green colour; $D_{50}$ = 301 µm ($D_{10}$-$D_{90}$, 206-438 µm); Glass Pebbles Ltd, UK) after 7 days. Images (94 µm pixel$^{-1}$ resolution) were taken of all four aquarium sides using a digital camera (CANON 400D) in a UV illuminated dark box and stitched together in Adobe Photoshop CS6 (version 13.0 x64). The vertical distribution of luminophores were determined from the stitched images (see electronic supplementary material, figures S4 and S5) using a custom-made, semi-automated macro that runs within ImageJ (v. 1.47), a Java-based public domain program (Schneider et al., 2012). From these data, the mean ($^{f\text{-}SPI}L_{mean}$, time dependent indication of mixing), median ($^{f\text{-}SPI}L_{med}$, typical short-term depth of mixing), and maximum (f-$^{f\text{-}SPI}L_{max}$, maximum extent of mixing over the long-term) mixed depth of particle redistribution were calculated. In addition, the maximum vertical deviation of the sediment-water interface (surface boundary roughness, SBR) provided an indication of surficial activity (Hale et al. 2014).

**Statistical analysis**

Analysis of Variance (ANOVA) models were developed for each dependant variable (SBR, $^{f\text{-}SPI}L_{mean}$, $^{f\text{-}SPI}L_{median}$, $^{f\text{-}SPI}L_{max}$) to determine the effects of the marine fuel oil concentration (3 levels: 0 %, 0.1 %, 0.5 %, Water Accommodated Fraction). As our focus was to determine species specific responses to marine fuel oil at indicative natural densities, we used an independent model for each species to avoid the confounding effects caused by differences in species abundance and biomass. Model assumptions were visually assessed using standardised residuals vs fitted values plots, Q-Q plots and Cook's distance (Zuur et al., 2010). Where there was a violation of homogeneity of variance, we used a *varIdent* variance-covariance structure and generalised least-squares (GLS) estimation (Pinheiro & Bates, 2000, West et al., 2014) to allow residual spread to vary amongst groups. We determined the optimal fixed-effects structure using backward selection informed by Akaike Information Criteria (AIC) and inspection of model residual patterns. For the GLS analysis, we determined the optimal variance-covariance structure using restricted maximum-likelihood (REML) estimation by comparing the initial ANOVA model without variance structure to equivalent GLS models incorporating specific variance terms. These models were compared against the initial ANOVA model using AIC informed by visualisation of model residuals. We determined the optimal fixed structure of the most suitable model by applying backward selection using the likelihood ratio test with maximum-likelihood (ML) estimation (West et al., 2014, Zuur et al., 2010). Details of

initial and minimal adequate models (Models S1 to S12), as well as model coefficient tables, are provided in electronic supplementary material.

## Results

The activities of all three species used in our study resulted in the vertical mixing of luminophore tracers, with subtle differences in the form of the profile (electronic supplementary material, figure S5) reflecting differences in species behaviour. Surface boundary roughness ranged from 0.48 cm (0 % marine fuel oil) to 1.21 cm (0 % marine fuel oil) in *Astarte crenata*, from 0.48 cm (0 % marine fuel oil) to 1.24 cm (0.5 % marine fuel oil) in *Macoma calcarea*, and from 0.39 cm (0.1 % marine fuel oil) to 1.03 cm (0 % marine fuel oil) in *Cistenides hyperborea.* Similarly, the range of the mean ($^{f\text{-}SPI}L_{mean}$: *A. crenata*, from 0.32 cm (0 % marine fuel oil) to 0.47 cm (0.1 % marine fuel oil); *M. calcarea*, from 0.35 cm (0.1 % marine fuel oil) to 0.68 cm (0 % marine fuel oil); and *C. hyperborea*, from 0.35 cm (0.1 % marine fuel oil) to 0.51 cm (0.5 % marine fuel oil)), median ($^{f\text{-}SPI}L_{med}$: *A. crenata*, from 0.31 cm (0 % marine fuel oil) to 0.40 cm (0.1 % marine fuel oil); *M. calcarea*, from 0.28 cm (0.5 % marine fuel oil) to 0.44 cm (0.5 % marine fuel oil); and *C. hyperborea*, from 0.33 cm (0.1 % marine fuel oil) to 0.43 cm (0.5 % marine fuel oil)) and maximum ($^{f\text{-}SPI}L_{max}$: *A. crenata*, from 0.50 cm (0 % marine fuel oil) to 1.33 cm (0.5 % marine fuel oil); *M. calcarea*, from 0.66 cm (0.1 % marine fuel oil) to 3.90 cm (0 % marine fuel oil); and *C. hyperborea*, from 0.57 cm (0.5 % marine fuel oil) to 1.13 cm (0.1 % marine fuel oil)) depths of mixing varied across marine fuel oil concentrations.

Analysis of total biomass across marine fuel oil concentration treatments confirmed no differences in biomass for each species (*A. crenata*: $F_{2,9} = 0.271$, $p = 0.765$; *M. calcarea*, $F_{2,9} = 0.850$, $p = 0.463$; *C. hyperborea*, $F_{2,9} = 0.860$, $p = 0.455$), negating the need to include biomass as a random effect in our statistical models.

In aquaria containing *A. crenata*, surface boundary roughness (SBR) and median mixing depth ($^{f\text{-}SPI}L_{med}$) were unaffected by marine fuel oil (intercept only models: SBR, L-ratio = 1.300, d.f. = 2, $p = 0.523$; $^{f\text{-}SPI}L_{med}$, L-ratio = 4.636, d.f. = 2, $p = 0.096$), whilst mean mixing depth became notably shallower ($^{f\text{-}SPI}L_{mean}$, L-ratio = 9.430, d.f. = 2, $p < 0.01$) and maximum mixing depth, albeit marginally, showed evidence of a deepening ($^{f\text{-}SPI}L_{max}$, L-ratio = 5.770, d.f. = 2, $p = 0.056$; Figure 1). Closer examination of model coefficients revealed a difference in $^{f\text{-}SPI}L_{mean}$ between marine fuel oil concentrations of 0 % and 0.1 % (coefficient ± s.e.: 0.068 ± 0.025, t = 2.670, $p < 0.05$) and 0 % and 0.5 % (coefficient ± s.e.: 0.055 ± 0.012, t = 4.396, $p < 0.01$) but not between 0.1 % and 0.5 % (coefficient ± s.e.: -0.013 ± 0.024, t = 0.548, $p = 0.597$). Specifically, $^{f\text{-}SPI}L_{mean}$ increased from (mean ± s.e.) 0.35 ± 0.01 cm in the absence of marine fuel oil to 0.42 ± 0.02 cm and 0.40 ± 0.01 cm at concentrations of 0.1 % and 0.5%, respectively. The maximum mixing depth (mean ± s.e.) increased from 0.58 ± 0.04 cm in the absence of marine fuel oil to 0.81 ± 0.16 cm under 0.1 % and 0.93 ± 0.16 cm under 0.5 % of marine fuel oil. Model coefficients for $^{f\text{-}SPI}L_{max}$ demonstrated no difference between marine fuel oil concentrations of 0 % and 0.1 % (coefficient ± s.e.: 0.221 ± 0.162, t = 1.368, $p = 0.205$) or between 0.1 % and 0.5 % (coefficient ± s.e.: 0.127 ± 0.218, t = 0.583, $p = 0.574$), but there was some weak evidence for a deepening of $^{f\text{-}SPI}L_{max}$ between 0 % and 0.5 % (coefficient ± s.e.: 0.349 ± 0.159, t = 2.195, $p = 0.056$).

For aquaria containing *C. hyperborea,* surface boundary roughness, median mixing depth and maximum mixing depth were independent of marine fuel oil (intercept only models: SBR, L-ratio = 0.222, d.f. = 2, p = 0.895; $^{f\text{-}SPI}L_{median}$, L-ratio = 4.039, d.f. = 2, p = 0.133; $^{f\text{-}SPI}L_{max}$, L-ratio = 0.540, d.f. = 2, p = 0.763). However, there was some weak evidence that the mean mixing depth extends deeper in the presence marine fuel oil ($^{f\text{-}SPI}L_{mean}$, L-ratio = 5.433, d.f. = 2, p = 0.067; Figure 2). This observation is driven by a deepening of $^{f\text{-}SPI}L_{mean}$ between 0.1 % and 0.5 % of marine fuel oil (coefficient ± s.e., 0.067 ± 0.029, t = 2.364, p < 0.05), but we found no difference in $^{f\text{-}SPI}L_{mean}$ between 0 % and 0.1 % (coefficient ± s.e., -0.026 ± 0.017, t = 1.524, p = 0.162) or 0 % and 0.5 % (coefficient ± s.e., 0.040 ± 0.025, t = 1.599, p = 0.144) of marine fuel oil. Specifically, $^{f\text{-}SPI}L_{mean}$ was 0.41 ± 0.01 cm in the absence of marine fuel oil, 0.38 ± 0.02 cm under 0.1 % and 0.45 ± 0.02 cm under 0.5 % marine fuel oil.

In contrast to *A. crenata* and *C. hyperborea*, we found no evidence that the bioturbation behaviour of *M. calcarea* is affected by the applied concentrations of marine fuel oil (intercept only models: SBR, L-ratio = 1.022, d.f. = 2, p = 0.600; $^{f\text{-}SPI}L_{mean}$, L-ratio = 1.082, d.f. = 2, p = 0.582; $^{f\text{-}SPI}L_{median}$, L-ratio = 0.142, d.f. = 2, p = 0.931; $^{f\text{-}SPI}L_{max}$, L-ratio = 0.093, d.f. = 2, p = 0.955; Figure 3). However, absolute values of surface boundary roughness and median mixing depth (mean ± s.e.) did show a directional trend, increasing from 0.77 ± 0.13 cm and 0.35 ± 0.01 cm in the absence of marine fuel oil to 0.97 ± 0.20 cm and 0.36 ± 0.05 cm under 0.5 % marine fuel oil, respectively.

## Discussion

Our findings demonstrate that the accumulation of the Water Accommodated Fraction of marine fuel oil can lead to shifts in functionally important aspects of sediment-dwelling invertebrate behaviour, although these effects are species-specific and are not necessarily linear as contamination concentrations increase. Such changes in behaviour - here burying deeper, indicative of classic avoidance behaviour (Maire et al. 2010) - are known to influence the fate of sediment-associated pollutants (Tian et al. 2019, Tong et al. 2019) and can be sufficient to change the functional role of the species (Wohlgemuth et al. 2017). Burying deeper to avoid contamination in the surficial layers can enhance particle mixing, but can have negative consequences for the growth and survival of individuals over an extended period of time (de Goeij & Luttikhuizen 1998). Conversely, altered surficial reworking activity, as observed here, can either confine sediment-associated contaminants to the sediment surface or stimulate both downward and outward contaminant transfer (Gilbert et al. 1994), whilst the presence of the contaminant can directly disrupt important microbial mediated pathways (e.g. biogeochemical cycling, Gilbert et al. 1997).

An important aspect of our findings is that not all species changed behaviour in response to the presence of marine fuel oil, perhaps reflecting differences in vulnerability or thresholds. However, whilst these results are consistent with the findings elsewhere on contamination (O'Brien & Keough, 2014; Dorgan et al., 2020), we obtained contrasting responses for two similarly sized bivalves that ordinarily adopt very similar functional roles. Though identifying the mechanistic basis for this difference is beyond the scope of the present study, having species that respond

differently to the same pressure could be viewed as being ecologically advantageous, as it increases the capacity of a system to recover following chronic disturbance events (Duffy, 2009), especially among organisms that coexist (Pagès-Escolà et al., 2018) or, as in this case, perform overlapping functional roles (Williams et al., in review). Moreover, communities with diverse response capacities have higher probabilities of including organisms that persist under specific environmental conditions and functionally compensate following species loss (Bernhardt & Leslie, 2013; Thomsen et al. 2017; Thomsen et al. 2019), minimising the impact on linked ecosystem services when environmental conditions fluctuate over time (Truchy et al., 2015). Although the effects of the conservative contamination event simulated here were relatively subtle, we did note a change in intra-specific variation of sediment reworking activity akin to other studies on responses to pressures in high-latitude marine invertebrates (Williams et al., in press). Intraspecific variation in trait expression plays a pivotal role in population maintenance (Dingemanse & Wolf, 2013; Bolnick et al., 2011), adaptation to dynamic environmental conditions (Henn et al., 2018, Sanders et al. 2024) and contributes to the stability of ecosystem functioning (Wright et al., 2016). Consequently, alterations in this variability bear significant implications for broader ecological processes, potentially influencing species interactions, community dynamics, and overall ecosystem resilience (Des Roches et al., 2018; McEntire et al., 2022).

Our study provides evidence that species responses to novel and/or unprecedented levels of anthropogenic activity associated with the opening up of high latitude regions can have substantive ecological effects, even when the source of perturbation is below acute toxicity thresholds. Investment in the Arctic is expected to be in the order of billions of dollars over the next decade (Kudryashova et al., 2019) as nations not only take advantage of economic opportunities presented by the rapid opening up of the region (O'Garra, 2017) but also prepare for the challenges associated with its governance (Ebinger & Zambetakis 2009). In Greenland, where this study is grounded, opportunistic resource exploration following the convergence of climate warming and glacial retreat has strong indigenous backing (Bendixen et al., 2022) but concerns persist regarding the sustainability of such decisions (Hanaček et al., 2022) and the possible negative feedback of expanded infrastructure on the climate (Masson-Delmotte et al., 2012) and local ecosystems (Bendixen et al., 2019), the latter of which is already affected by regional climate change (Gross, 2018). Though we find mixed results in the responses of benthic biodiversity to low marine fuel oil contamination, it is important to note that the responses observed here are at contaminant concentrations below the threshold of current environmental risk assessments (Faksness and Altin, 2017) and at the lower end of previously observed effects (Pereira et al., 2018). Hence, these observations may act as an early warning for impending and more pronounced ecological transitions. As contaminants accumulate, either directly from spillages associated with shipping activity or resuspension via dredging (Hedge et al., 2009), it follows that there will likely be more pronounced species responses, including demographic or functional transitions where lethal concentrations are reached (O'Brien and Keough, 2014), or more complex ecological outcomes when contaminants co-occur and lead to non-additive responses (Millward et al., 2004). Identifying general response patterns to sub-lethal contamination in the Arctic environment is critical for reliable assessments of ecosystem health (Eldridge et al., 2022), and

demands effective management of the expansion of human activity within the context of biodiversity and climate change within the region (Wu et al., 2017).

## Data Accessibility statement

All f-SPI images and their corresponding extracted luminophore profiles are available at http://xxxxx.xxxxx.xxxx. The derived data used for statistical analysis are provided in electronic supplementary material, Table S2.

## Competing Interests Statement

The authors declare that there are no competing interests.

## Author Contributions

**Thomas J. Williams:** Conceptualisation (equal); Data curation (equal); Data analysis (equal); Funding acquisition (supporting); Investigation (equal); Methodology (equal); Project administration (supporting); Visualization (lead); Writing – original draft (lead); Writing – review & editing (lead). **David Blockley:** Conceptualisation (supporting); Data curation (equal); Investigation (equal); Funding acquisition (supporting); Resources (lead); Data curation (equal); Writing – review and editing (supporting)., **Andrew Cundy:** Conceptualisation (equal); Data curation (equal); Investigation (equal); Methodology (equal); Resources (supporting); Validation (equal); Writing – original draft (supporting); Writing – review and editing (supporting). **Jasmin A. Godbold:** Conceptualisation (equal); Investigation (equal); Methodology (equal); Project administration (lead); Supervision (supporting); Validation (equal); Writing – original draft (supporting); Writing – review & editing (supporting). **Rebecca M. Howman:** Data curation (equal); Data analysis (supporting); Investigation (equal); Writing – review & editing (supporting); **Martin Solan:** Conceptualization (equal); Data curation (equal); Data analysis (equal); Funding acquisition (lead); Investigation (equal); Methodology (equal); Project administration (supporting); Resources (supporting); Supervision (lead); Validation (equal); Visualization (supporting); Writing – original draft (supporting); Writing – review & editing (supporting).

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## Tables

**Table 1 |** Summary of hydrocarbon dosing concentrations. LE-WAF = Low energy Water Accommodated Fraction. Concentrations reported as “less than” are below method detection limits. Individual PAHs (Acenaphthylene, Acenaphthene, Anthracene, Benzo(a)pyrene, Benzo(g,h,i)perylene, Benz(a)anthracene, Benzo(k)fluoranthene, Benzo(b)fluoranthene, Chrysene, Dibenzo(a,h)anthracene, Fluorene, Fluoranthene, Indeno(1,2,3-c,d)pyrene, Napthalene, Phenanthrene and Pyrene) were also analysed and were below detection limits (0.01 mg/l) in all fractions. TPH = total petroleum hydrocarbons, including split by aliphatic and aromatic fractions and carbon chain length.

| Component | Concentration in undiluted LE-WAF (mg/l) | Concentration in 0.1% dosing experiments (mg/l) | Concentration in 0.5% dosing experiments (mg/l) |
|---|---|---|---|
| Total PAHs | < 0.1 | < 0.1 | < 0.1 |
| Total TPH (C10 - C40) | 21.1 | 0.021 | 0.106 |
| TPH Aliphatic (C8-C10) | 1.43 | 0.001 | 0.007 |
| TPH Aliphatic (C10-C12) | 2.84 | 0.003 | 0.014 |
| TPH Aliphatic (C12-C16) | 0.10 | 0.0001 | 0.001 |
| TPH Aliphatic (C16-C21) | 3.01 | 0.003 | 0.015 |
| TPH Aliphatic (C21-C40) | 3.79 | 0.004 | 0.019 |
| TPH Aromatic (C8-C10) | 0.35 | 0.0004 | 0.002 |
| TPH Aromatic (C10-C12) | < 0.1 | < 0.0001 | < 0.001 |
| TPH Aromatic (C12-C16) | 5.64 | 0.006 | 0.028 |
| TPH Aromatic (C16-C21) | < 0.1 | < 0.0001 | < 0.001 |
| TPH Aromatic (C21-C40) | 5.74 | 0.006 | 0.029 |

## Figure Legends

**Figure 1 |** Summary of the bioturbation behaviour of the bivalve, *Astarte crenata*, in presence of different concentrations of the water accommodated fraction of marine fuel oil on (mean ± s.e.) (a) SBR (cm) and the depth of (b) $^{f\text{-}SPI}L_{mean}$ (cm), (c) $^{f\text{-}SPI}L_{med}$ (cm), (d) $^{f\text{-}SPI}L_{max}$ (cm). In panels (b-d), horizontal dashed lines represent the position of the sediment-water interface. Sediment profile images and associated luminophore distribution profiles are presented in electronic supplementary material, Figure S5. LE-WAF concentrations for different fuel oil hydrocarbon components are shown in Table 1.

**Figure 2 |** Summary of the bioturbation behaviour of the polychaete, *Cistenides hyperborea*, in presence of different concentrations of the water accommodated fraction of marine fuel oil on (mean ± s.e.) (a) SBR (cm) and the depth of (b) $^{f\text{-}SPI}L_{mean}$ (cm), (c) $^{f\text{-}SPI}L_{med}$ (cm), (d) $^{f\text{-}SPI}L_{max}$ (cm). In panels (b-d), horizontal dashed lines represent the position of the sediment-water interface. Sediment profile images and associated luminophore distribution profiles are presented in electronic supplementary material, Figure S5. LE-WAF concentrations for different fuel oil hydrocarbon components are shown in Table 1.

**Figure 3 |** Summary of the bioturbation behaviour of the bivalve, *Macoma calcarea*, in presence of different concentrations of the water accommodated fraction of marine fuel oil on (mean ± s.e.) (a) SBR (cm) and the depth of (b) $^{f\text{-}SPI}L_{mean}$ (cm), (c) $^{f\text{-}SPI}L_{med}$ (cm), (d) $^{f\text{-}SPI}L_{max}$ (cm). In panels (b-d), horizontal dashed lines represent the position of the sediment-water interface. Sediment profile images and associated luminophore distribution profiles are presented in electronic supplementary material, Figure S5. LE-WAF concentrations for different fuel oil hydrocarbon components are shown in Table 1.

## Figures

**Figure 1:**

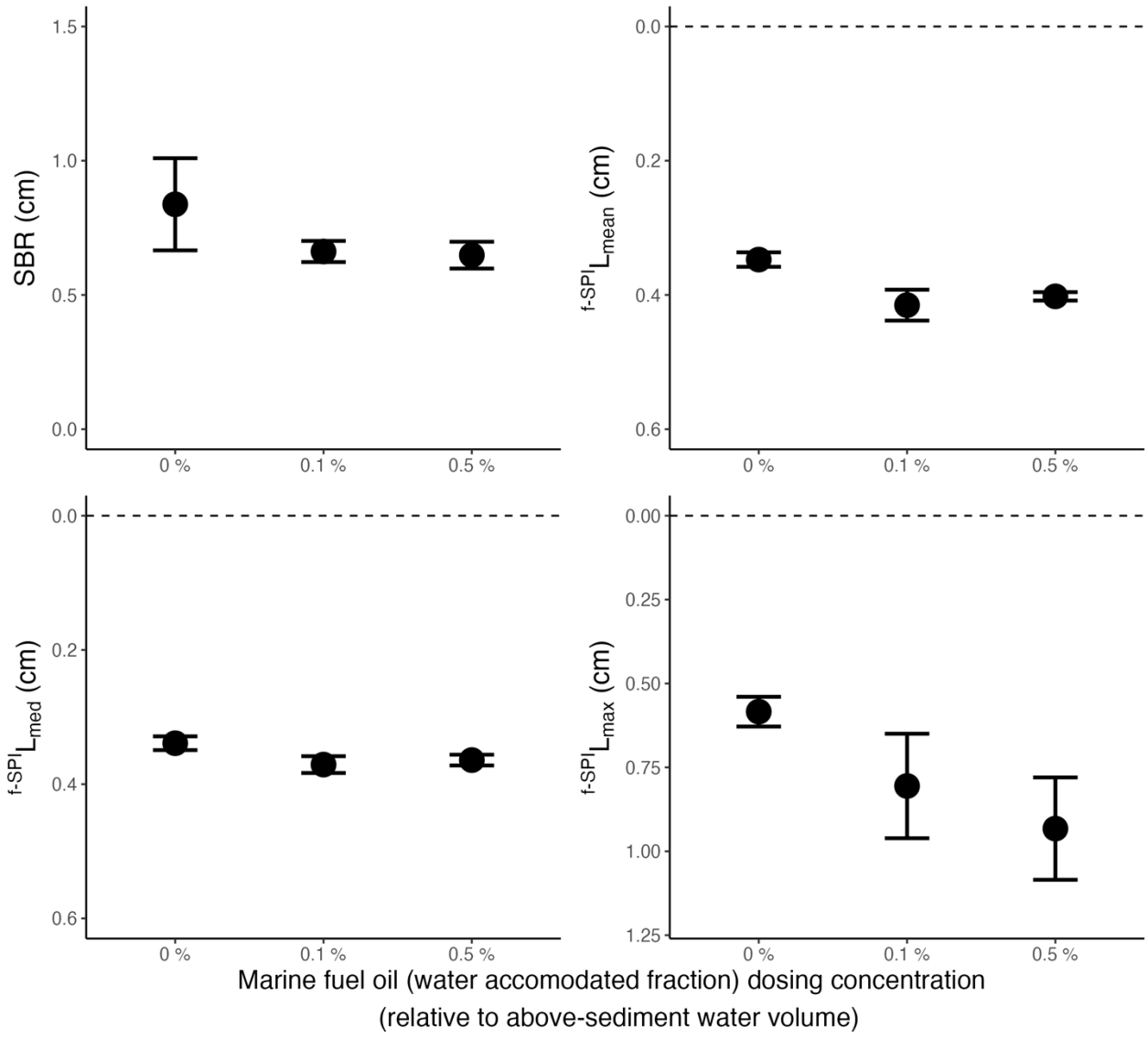


**Figure 2:**

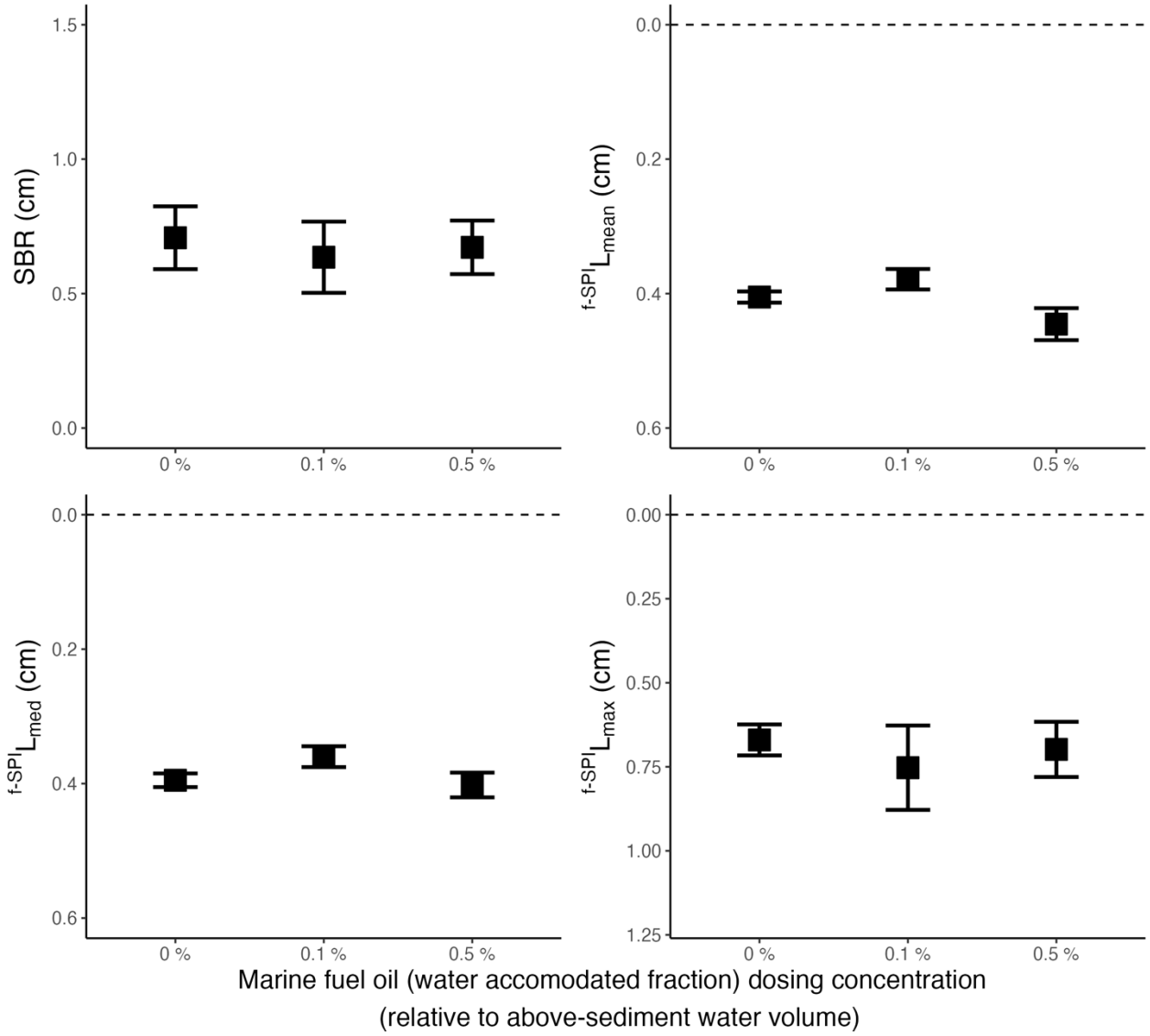
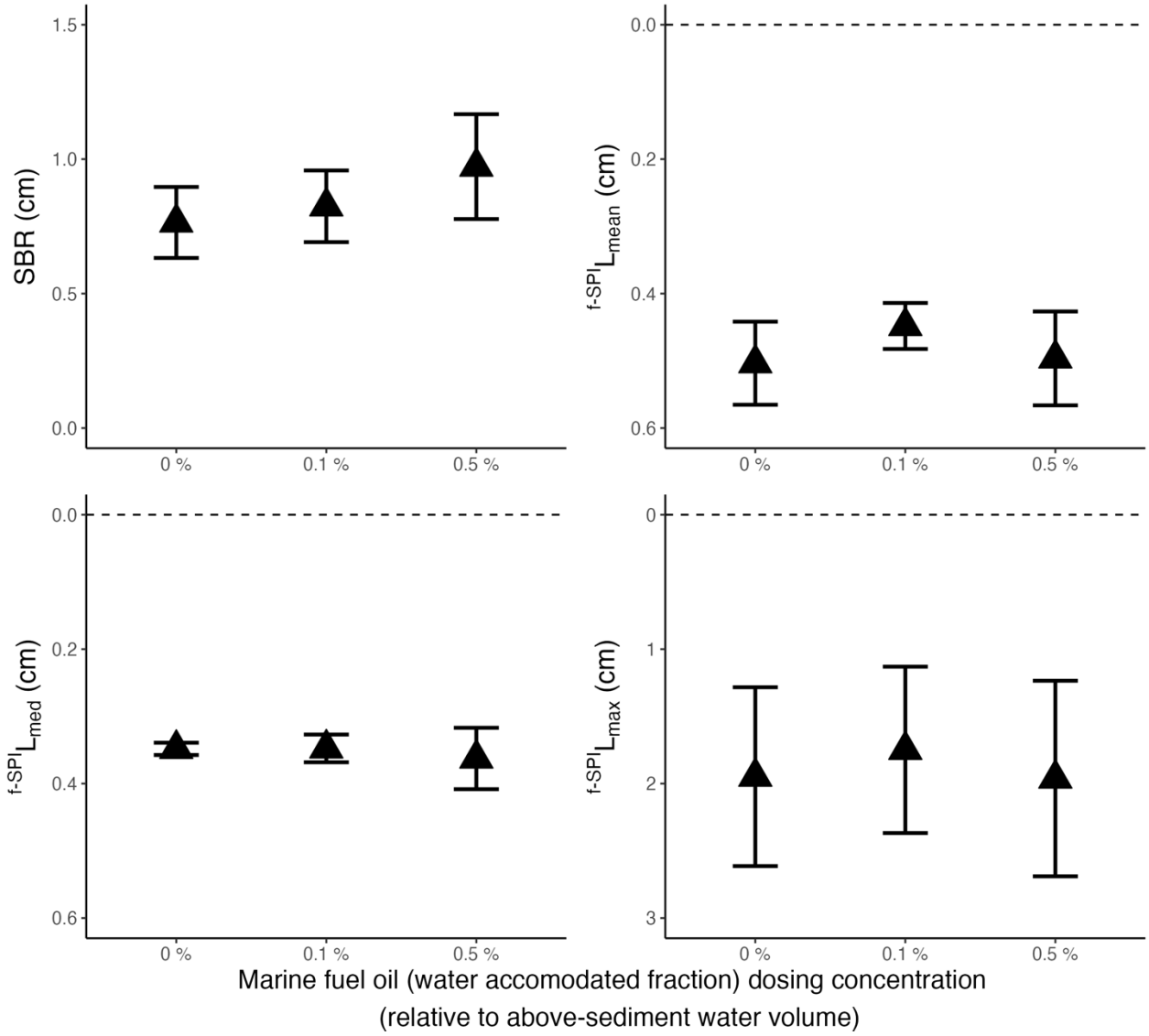

SBR (cm)
1.5
1.0
0.5
0.0
0 %
0.1 %
0.5 %
f-SPI L_mean (cm)
0.0
0.2
0.4
0.6
f-SPI L_med (cm)
f-SPI L_max (cm)
0.00
0.25
0.50
0.75
1.00
1.25
Marine fuel oil (water accomodated fraction) dosing concentration
(relative to above-sediment water volume)

***Figure 3:***


SBR (cm)
f-SPI L_mean (cm)
f-SPI L_med (cm)
f-SPI L_max (cm)
0 %
0.1 %
0.5 %
Marine fuel oil (water accomodated fraction) dosing concentration
(relative to above-sediment water volume)