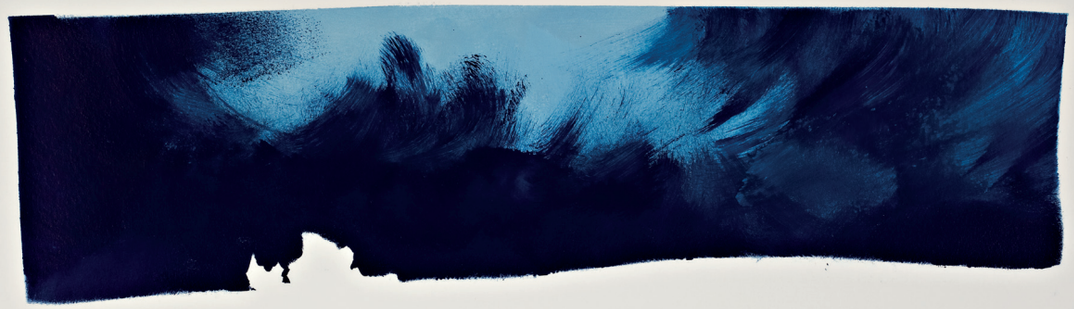
Ariadna S. Szczybelski Ciordia



Advancement of benthic indicators and biomarker-based tools for biomonitoring and risk assessment in the Barents Sea region

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But that in the simple appreciation of a world not our own to define, that poised arctic landscape,
we might find some solace by discovering the ki-lin hidden within ourselves,
like a shaft of light.
- Barry Lopez -

CONTENTS

1 General introduction

Chapter 1

The Arctic Ocean is subject to a substantial climate change [1], causing Arctic marine ecosystems to exhibit particularly fast changes in the coming decades [2,3]. Sea ice loss and an extended open water period within Arctic marine areas are expected to contribute to increased human activities such as oil and gas (O&G) production, commercial fisheries and tourism development [4,5]. This raises a global concern about Arctic marine oil pollution [6,7] and its long-term impacts [8], based on experiences and environmental impacts from previous oil spills in the Arctic or elsewhere [9,10]. Awareness is also reinforced by the vast amounts of bunker fuel on board ships currently transiting International Maritime Organization Arctic waters being operated on heavy fuel oil [11], salient difficulties during oil spill response such as remoteness, seasonal darkness, stronger winds and currents, more fragmented sea ice and a lack of effective oil spill response methods for icy conditions [12,13]. Consequently, sustained and adequate long-term monitoring of the impacts of oil spills on Arctic ecosystems and development of plans for mitigation before and after Arctic oil spills should be implemented [14-16].

Current environmental risk assessment procedures contain uncertainties on the potential long-term effects of oil spills and operational discharges of produced water in the Arctic [17]. Environmental risk assessment combines exposure assessment and chemical-specific hazard data. Oil spills may pose an acute risk to the environment, with ecological consequences being highly dependent on the type of oil, size of spill, environmental characteristics of the habitat, selection and effectiveness of emergency response systems [16]. Risk assessment of O&G and shipping activities also needs to include long-term effects of continuous exposure to drilling waste and produced water [18].

Environmental impact assessment procedures should delineate oil pollution sources, transport mechanisms, routes and duration of exposure to Arctic species or habitats prior to the implementation of forecasting methods to predict O&G environmental impacts. Arctic characteristics affecting the environmental fate and effects of oil related chemicals may result in different exposure scenarios between Arctic and temperate species. Therefore, environmental monitoring tools should rely on the identification of factors that are critical for the exposure to oil related chemicals and development of sublethal effects in Arctic biota. Such factors may be used to define acceptable environmental assessment criteria and monitoring methods for the early detection of O&G environmental impacts in the Arctic.

For any species or group of species to be an effective indicator of O&G related chemicals, the abovementioned factors should meet the following criteria:

1. Its response is sensitive to changes in the chemical concentration;
2. Its response is specific and causally related to the chemical exposure;
3. Its response is adequate given the anticipated concentration of the chemical [19];
4. The species' uptake/elimination of the chemical can be quantified;
5. The toxic effects of the chemical can be measured/interpreted.

For the specific purpose of the thesis, biological indicators (bioindicators) are defined as biological species which may be affected by the exposure to a given O&G target chemical at different levels of biological organization and under realistic O&G exposure scenarios. In addition, bioindicators may present different functional traits and habitat choices so that their combined use may indicate different levels of stress for different ranges of environmental conditions and/or exposure to an O&G chemical group. Since adaptations of Arctic marine individuals to their environment underlie their sensitivity and vulnerability to O&G chemicals, the genetic diversity and phenotypic plasticity of species traits influencing the combined effect of natural stressors (e.g., ocean acidification) and O&G waste exposure should be reflected by regional impact monitoring. This ensures that bioindicators of potential pressures arising from O&G activities are identified, and that our understanding of how certain ecological attributes increase resilience of Arctic marine ecosystems is improved and used to develop oil spill restoration plans.

Chemical characterization and environmental fate of offshore oil and gas waste in the Arctic with special emphasis on the Barents Sea region

Waste materials produced by offshore O&G activities that may be discharged into the ocean are water-based drilling muds (WBM) and cuttings, synthetic-based mud (SBM) cuttings, treated produced water (PW), treated sanitary and domestic waste, deck drainage, once-through fire water and non-contact cooling water (Figure 1). WBM are formulated mixtures of clays, natural and synthetic organic polymers, mineral weighting agents, and other additives, all dissolved or suspended in freshwater, saltwater, or in brine (Table 1). The composition of WBM may vary during drilling of a single well because different additives may be required to drill different well sections through varying geological formations [20]. WBM cuttings that are discharged to the ocean tend to accumulate at seafloor level down-stream from the discharge source at distances of about 100 m to > 1 km. SBM cuttings do not

disperse on contact with sea water and settle rapidly when discharged, mainly exposing the (local) benthic ecosystem [21].

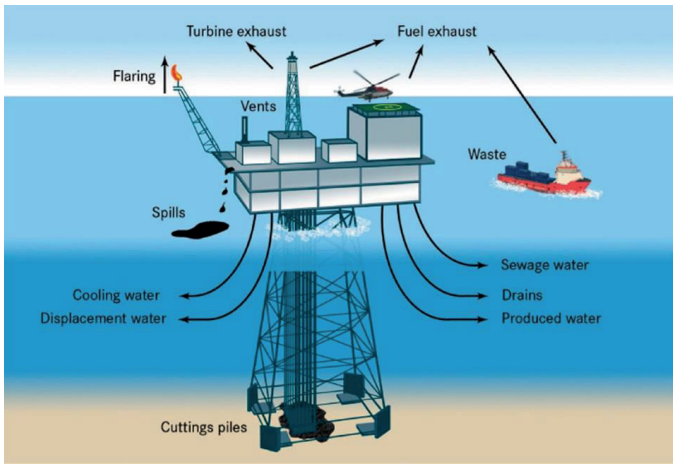

Figure 1. Offshore O&G waste discharges and air emissions depiction.

Sewage water includes industrial household and sanitary sewage in compliance with MARPOL 73/78 Annex V. Drains include ballast water, storm/rain water and other liquid waste in compliance with MARPOL 73/78 Annex I, regulation 39. Produced water (PW), cutting piles (WBM attached to WBM cuttings and SBM cuttings, if allowed) and displacement water discharges are in compliance with OSPAR Decision 2000/3. Emissions to air shall be in compliance with the provisions of MARPOL 73/78 Annex VI. Shipping discharges to sea shall be in compliance with the provisions of MARPOL 73/78 Annex I-V. Source: http://www.ospar.org/work-areas/oic/chemicals

Table 1. Functional categories of additives used in WBM to improve drilling performance and examples of chemical products as provided by the International Association of Oil and Gas Producers [20].

Functional Category of Additives	Examples
Weighting materials	barite, calcium carbonate, ilmenite or hematite
Thinners	lignite, lignosulfonates, polymers
Filtrate reducers	clay, lignite, polymers, starch
Lost circulation	inert soluble solids (e.g., calcium carbonate, ground nut shells, graphite, mica and cellulose fibres)
Shale control	soluble salts (e.g., KCl), amines, glycols
Bactericides	glutaraldehyde, triazine disinfectants
Pipe-freeing agents	water-based lubricants, enzymes, surfactants
Corrosion inhibitors	amines, phosphates
Viscosifiers	clay, organic polymers
Flocculants	inorganic salts, acrylamide polymers
pH control	inorganic acids and bases (caustic soda)
Lubricants	water-based lubricants, glycols and beads
Emulsifiers, surfactants	detergents, soaps, organic fatty acids
Defoamers	alcohols, silicones, aluminium stearate, alkyl phosphates
Calcium reducers	sodium carbonate, bicarbonate, polyphosphates
Temperature stability	acrylic or sulfonated polymers, lignite, lignosulfonate

PW is a complex mixture of dissolved and particulate organic and inorganic chemicals (Tables 2 and 3). Naturally occurring compounds that are dissolved or dispersed include inorganic salts, metals, radioisotopes, and a wide variety of organic chemicals, mainly polycyclic aromatic hydrocarbons (PAHs). Although these compounds may occur naturally, local concentrations after a spill may exceed natural concentrations by several orders of magnitude, creating a non-natural situation. Much of the petroleum hydrocarbons discharged to the ocean in properly treated PW are dissolved low-molecular-weight (LMW) PAHs and smaller amounts of saturated hydrocarbons. Because PW treatment is not always optimally effective, treated PW usually still contains some dispersed oil which includes high-molecular-weight (HMW), less soluble saturated and aromatic hydrocarbons. If PW is discharged to shallow waters, some metals and HMW aromatic and saturated hydrocarbons may accumulate in sediments near the PW source, with an increasing trend observed for compounds with a relatively high octanol-water partitioning coefficient (K_{ow}). In well-mixed waters, high concentrations of saturated hydrocarbons and PAHs in sediments sometimes can be observed as far as hundreds of meters from the PW source [14,22,23].

Table 2. Concentration of naturally occurring organic chemicals in produced water worldwide as provided by Neff [24], and heavy metals and radioisotopes in produced water from the Norwegian Continental Shelf as provided by Neff [24] and the Norwegian Radiation Protection Authority [25].

Natural chemical	Treatment conc. (mg/L)
Total organic carbon	≤0.1 ─ >11,000
Total organic acids	≤0.001 ─ >10,000
Total saturated hydrocarbons	17 ─ 30
Total benzene, toluene, ethylbenzene and xylenes (BTEX)	0.068 ─ 578
Total polycyclic aromatic hydrocarbons (PAH)	0.04 ─ 3.0
Total steranes/triterpanes	0.14 ─ 0.175
Ketones	1.0 ─ 2.0
Total phenols (primarily C_0-C_5- phenols)	0.4 ─ 23
Metals (mainly Barium)	107,000 ─ 228,000 (µg/L)
Radioisotopes (mainly ^{226}Ra and ^{228}Ra)	n.d. ─ 567 (pCi/L)

n.d.: not detected.

Table 3. Summary of additives in offshore produced water provided by Zheng et al. [26].

Function	Offshore chemical class	Treatment conc. (ppm)
Corrosion inhibitor (acid)	Amine imidazolines Amine salts Quaternary ammonium salts Nitrogen heterocyclics	25 ─ 100
Corrosion inhibitor (oxygen)	Ammonium bisulfite	5 ─ 15
Bactericide	Quaternary amine salt Amine acetate Gluteraldehyde	10 ─ 200
Hydrate inhibitor	Methanol	n.a.
	Ethylene glycol	n.a.
Dehydration	Triethylene glycol	n.a.
Scale inhibitor	Phosphate esters Phosphonates	3 ─ 10
Emulsion breaker	Oxyalkylated resins Polyglycol esters Sulfonates	10 ─ 200
Solid removal	Polyamine Quaternary polyamine	< 3

n.a.: not available.

Among all pollutants released during O&G drilling and production, alkylphenols (APs) and PAHs are seen as potential causes of long-term biological effects [23,27-29] although there is high variability in, for instance, PW composition between different fields and over drilling time (Table 2). Prolonged O&G waste discharges could have a cumulative effect on chronically exposed Arctic biota and their impacts could be particularly relevant for ecosystem components vulnerable to oil spills, such as benthic systems [30,31], since they mainly accumulate hydrocarbons from sediments to which persistent fractions of oil remain adsorbed. Despite their relevance, long-term AP and PAH effects on benthic populations or communities at concentrations found in PW effluents are basically unknown [32] and there are scarce means to evaluate their long-term impacts on the marine environment [18,33].

Besides, the effects of long-term exposure of Arctic benthos to oil or O&G waste components could be enhanced by increased primary productivity in seasonal ice regions where PAHs may be absorbed by phytoplankton, which in turn serves as major food source for benthic species [34-37]. Furthermore, exposure to oil or O&G waste may be aggravated by limited protein function (e.g., enzymatic detoxification) of benthos due to a relatively low tolerance of (sub-)Arctic temperatures (i.e., > 5 ºC) in polar ectotherms [38,39]. Chronic impacts of O&G waste discharge on Arctic benthic systems could eventually result in a reduction of the productivity of the Arctic marine ecosystem since benthic systems are mainly responsible for

carbon and nutrient recycling [30] and they form an important food source for many top predators.

Because of the important role of benthic communities within the Arctic marine ecosystem and their susceptibility to O&G pollution, the scientific goals of the current thesis focused on the Barents Sea region which is characterized by the highest benthic species diversity among the different Arctic seas [30]. Major gaps on the physical and biological conditions of the Barents Sea seabed currently prevent the integration of baseline knowledge about the benthic ecosystem in O&G monitoring programmes and environmental impact assessment [40-42].

In the following sections, I will justify and underpin the selection of PAHs and model Arctic benthic invertebrate species from the Svalbard inshore region as indicators for chemical and biological pressures arising from O&G activities in the Barents Sea benthic system.

Polycyclic aromatic hydrocarbons as oil and gas target chemicals

PAHs are one of the main components of crude oil and can be classified into (1) LMW PAHs which are highly toxic to aquatic organisms and highly volatile typically remaining in the environment for hours to days following a spill, and (2) HMW PAHs which are less volatile and degradable, and are associated with chronic effects in biota due to their persistence in the environment [43]. PAHs have both natural and anthropogenic sources which overlap, particularly for combustion, and processes such as diagenesis (i.e., alteration of sediments into sedimentary rock) can modify sediment PAH distributions over time. In the Arctic Ocean, relatively high atmospheric concentrations and increasing deposition of PAHs with increasing latitude have been attributed to combustion of biomass and/or coal [44]. Therefore, the environmental fate and effects of anthropogenic PAHs must be evaluated against a site-specific background of natural PAHs and a broad range of hydrocarbon indicators needs to be considered in order to define sources, transport and sedimentary persistence of PAHs [45].

Once released into the ocean, PAHs are subject to partitioning processes including adsorption, where the hydrocarbon attaches to the surface of a solid or other interface, and absorption, where the chemical partitions into an (a)biotic compartment [46]. Understanding the distribution or partitioning of PAHs between the dissolved phase, aquatic particles and sediment is crucial for the determination of fate and bioavailability of PAHs to marine biota, as this will strongly affect the environmental fate of PAHs and mechanisms and magnitude of exposure of aquatic organisms to PAHs [47,48]. Similarly, PAH accumulation in organisms

will be affected by PAH desorption rates from particles in biota digestive tissues and metabolic rates that degrade or eliminate PAHs.

Environmental fate of PAHs: sorption to sediment and bioavailability

The fate and persistence of petroleum in marine systems are controlled by physical, chemical and biological properties and processes that subsequently modify petroleum impact. All these properties and processes are driving the weathering of oil, which however also depends on factors such as temperature, light conditions and water mixing by, for instance, wind. A key factor of oil weathering is based on the viscosity of the oil mixture, which largely affects its tendency to spread out on surfaces [49]. Since cold temperatures can increase the viscosity of oil, the resulting spreading of oil under Arctic conditions may be lower than expected and so are the evaporation of volatile components from oil [49,50] and from the water-accommodated fraction of some fuel oils [51]. This may delay oil weathering and biodegradation [52] and increase PAH sorption affinity [46], particularly near river runoff sources where alluviums of finely dispersed materials would sequestrate the suspended fractions of oil.

Desorption rate constants of PAHs from sediments contaminated with petroleum are inversely correlated with the logarithm of K_{ow} of the PAH compound and directly correlated with its aqueous solubility [53,54]. PAHs associated with dissolved organic matter (colloidal; DOC) behave like dissolved PAHs, whereas PAHs associated with suspended organic matter will usually settle. They readily desorb from DOC, striving at an equilibrium between water, DOC, suspended and settled particles, defined by organic carbon normalised partition coefficients (K_{oc}). However, when PAHs remain adsorbed to sediment for long periods, their desorption rate will decrease since prolonged contact time of PAHs with organic materials may lead to increased K_{oc} values over time [55]. This ‘ageing’ phenomenon effectively decreases the bioavailability of adsorbed PAHs [48].

Assessment based on bioavailability is considered a useful tool for the risk evaluation of contaminated sites. PAH bioavailability can be estimated from PAH bioaccumulation which results from PAH uptake by an organism and subsequent chemical excretion after digestion and metabolism. Uptake of hydrocarbons in target tissues (bioavailability) occurs from sediments, suspended particulate matter, the water column, or through the diet, depending on the trophic level and ecological lifestyle of the organism [56]. In general, bioaccumulation is measured in order to (1) determine contaminant-specific bioavailability (for non-metabolised

contaminants), (2) identify possible causative agent(s) of toxicity, (3) relate body residues to predator-prey accumulation values (biomagnification factors) and (4) assess/predict effects of chronic, low-level exposures [57].

Under environmentally realistic PAH exposure scenarios, dealing with the fourth objective may seem compelling. Since PAHs exert toxic effects following metabolism, PAH body residues in organisms with low metabolic rate such as cold stenothermal bivalves will mainly reflect PAH bioavailability [58]. For organisms with a higher metabolic rate, measurement of parent PAH compounds is difficult due to low concentrations, hence PAH metabolic products need to be analysed to determine a relationship between bioaccumulation and toxicity. However, toxicity from bioaccumulation will also depend on the prior history of organism exposure and the differences in organism sensitivity. Thus, both bioaccumulation of PAHs (and their derivatives) and toxic effects should be simultaneously reported in benthic organisms exposed to PAHs in order to identify any likely tolerance mechanisms or sensitivity under low PAH exposure level [59].

Arctic benthic invertebrates as oil and gas bioindicators

In coastal environments, where oil is most likely to strand and accumulate, local biota will be subject to long-term impacts at source point or in surrounding areas after oil remobilization. In these coastal areas, or hydrodynamic systems, a causal link between an increase of PAHs in suspended solids and higher PAH bioavailability is suggested due to frequent sediment resuspension and ageing [60,61]. This process may be enhanced by the physical disturbance of sediment and increased riverine input of terrestrial PAH sources within inshore distance to land (< 200 m). Freshwater drainage with terrigenous particles may, in addition, create steep gradients of sedimentation and salinity [62] which generally underlie a shift from filter- to (sub-)surface-deposit feeders within the benthic community. Allowing for the coexistence of different functional groups along such small-scale gradients, a comprehensive understanding of the influence of species traits on PAH uptake and selection of bioindicators may be promoted.

Many polar benthic organisms typically display *K* strategies [63], such as reduced development and lower resource thresholds. In low water energy zones, absence or temporary drifting of larval stages and selective feeding mode would cause benthic invertebrates to be constantly exposed to local PAH sources and their possible PAH uptake routes could be more easily defined. Additionally, among true residents, ectotherms may show a high vulnerability

to increasing temperatures with greater fluctuations in their abundances on an inter-annual scale, depending on their recent recruitment [64]. Such ectotherms may be far more vulnerable to cumulative, but relatively minor impacts causing local extinctions such as the combined effect of rising temperatures, food shortage and oil spill incidents.

Validation of long-term PAH exposure indicators in Arctic benthic invertebrates

In the Arctic, development of offshore O&G has undergone extensive technological and engineering advances that have enabled the industrial sector to operate safe and efficiently from a human and economic perspective [65]. One important aspect derived from such advances is the prioritization of early detection of environmental effects of anthropogenic pollution sources, notably effects derived from release of compounds included in the List of Substances/Preparations Used and Discharged Offshore that are Considered to Pose Little or No Risk to the Environment (PLONOR) [66]. The PLONOR list encompasses compounds for which an array of ecotoxicological properties has been evaluated according to the Harmonized Offshore Chemical Notification Format (HOCNF) [67] and set against a suite of validity criteria in order to ensure environmental risks are minimized during operational discharges. In the Lofoten-Barents Sea region, the development of O&G resources is permitted under a strict regulatory policy of zero discharge of oil-based mud (OBM) and SBM cuttings [68,69], whereas most of the additives used in WBM are either PLONOR substances or if non-PLONOR their use is restricted to emergency situations.

For the scientific community, there is one key question about current ecotoxicological data validation in the Arctic, which is whether the actual parameters used in HOCNF classification criteria need to be adjusted for this region. This question is motivated by the fact that low temperatures and regional physiological adaptations in Arctic species may give a difference in sensitivity to oil components of Arctic ecosystems when compared to non-Arctic systems. It has been shown that in general, differences in sensitivity to oil or PW components between Arctic and non-Arctic test species are small [70-72], the former ones including mainly boreal species with either a planktonic or nektonic adult stage. However, factors such as O&G waste discharge conditions, ecological seasonal variations or a delayed manifestation of toxicity in polar environments as recently suggested [73], could play an important role in establishing differences between the susceptibilities of both regional groups in the long term.

Parameters such as bioaccumulation and biotransformation are currently included in national or regional biomonitoring programmes of the marine environment [74], and they have been

compared across a wide range of temperate aquatic systems and benthic species, enabling us to make a read across between Arctic and temperate species. In this thesis, both parameters are used as part of an evaluating approach for the identification of long-term PAH exposure indicators in Arctic benthic species. In addition, the thesis evaluates the sensitivity of behavioural indicators in benthic model species to reflect on their potential use for the prediction of sublethal effects of oil from the perspective of Arctic benthic biota.

PAH bioaccumulation from sediment: biota-sediment accumulation factors (BSAFs) for Arctic benthos

Bioconcentration of PAHs from water is directly proportional to their respective K_{ow} value, whereas bioaccumulation from sediments and food involves an intermediate step in which PAHs desorb from the solid matrix and partition into the lipid tissues of the organism via the aqueous gut content. Bioaccumulation models generally assume that (1) both uptake and elimination routes are regulated by passive partitioning and will finally result in steady-state conditions and that (2) there is no biological metabolism once the PAH is absorbed. Bioaccumulation from ingestion has been neglected by traditional models such as the Equilibrium Partitioning Theory (EPT) [75]. The EPT method assumes that organic chemicals such as PAHs partition between the sediment organic carbon and biota lipid phase and that there is an equilibrium. However, the use of EPT methodology and the validity of its extrapolation to sediment-dwelling organisms have been questioned because of the large variability in tissue PAH concentrations across species and environmental conditions [56,76,77]. Hence, bioaccumulation processes are not solely explained by passive partitioning.

In both filter- and deposit-feeding organisms, PAHs are mainly accumulated via food intake as these chemicals are generally adsorbed on dissolved and particulate organic matter (OM). Because of the high affinity of PAHs for (sediment) condensed carbon (e.g., black carbon; BC) [78], there is an inverse relationship between the concentration of BC in sediments and the bioavailability of PAHs in sediments to marine organisms [79]. Additionally, the relative contribution of water, food and sediment PAH sources to the total body burden greatly varies from one species to another [56,76,77,80]. Within the EPT context, the estimation of a PAH compound concentration in a certain species may be obtained by multiplying its sediment concentration by an empirically derived biota-sediment accumulation factor (BSAF) for that same PAH, species and sediment quality/composition. In practical terms, the observed

variability in PAH concentrations requires a broad interpretation since the bioavailability of PAHs will be modified by the species behaviour and the composition of their sorbent.

The BSAF has been used as a metric to evaluate the bioaccumulation of contaminants from sediment and has been compared across a wide range of aquatic ecosystems [56,80-83]. The use of the BSAF concept assumes that the concentration of chemicals in organisms is a linear, non-threshold function of the concentration in sediment. However, assumptions of steady-state conditions and absence of metabolism may not always be suitable, particularly if food sources are predominantly waterborne or if PAHs are effectively metabolised after summer periods of intense foraging. In the Arctic, BSAFs for organic pollutants have been barely reported for benthic biota under field or laboratory conditions [84,85], and have never been reported for PAHs in benthic biota.

A PAH biotransformation approach: biomarkers of PAH exposure and pyrene metabolites in Arctic benthos

Biomarkers of chemical exposure may be useful as estimators of chemical stress to monitor trends in ecological impacts of O&G activities [86,87]. For instance, aromatic xenobiotics may enhance the production of intracellular reactive oxygen species (ROS) through induction of the cytochrome P450 system (CYP). CYP is a multigene family of haem-containing enzymes, which catalyse a variety of oxidative reactions, including hydroxylation, epoxidation, dealkylation, deamination, sulfoxidation, and desulfuration (i.e., Phase I). During these reactions, the lipophilicity of the xenobiotic is lowered by the addition of a polar functional moiety, which often makes the metabolite more hydrophilic and, in most cases, ready for further conjugation and/or excretion. Metabolites produced in Phase I reactions can be eliminated or covalently conjugated to various endogenous compounds (e.g., reduced glutathione, glucuronic acid, sulfate) to further decrease their lipophilic properties. Such reactions, commonly known as Phase II, are catalysed either by glutathione transferases (GSTs), uridine-diphosphate-glucuronosyl-transferases (UDPGTs), or sulfotransferases (STs), usually yielding water-soluble products that can be readily excreted. Suites of biomarkers have been successfully applied at low levels of biological organization in combination with chemical body residues in fish and invertebrates [74,88,89]. However, the use of biomarkers also has been criticized as a result of the lack of a clear ecological relevance [86] and of difficulty in data interpretation due to the influence of confounding factors [90] or lack of baseline values [91].

The major trait that could enhance oxidative stress sensitivity in polar species is the elevated unsaturation level in membrane lipids [92]. A higher percentage of the polyunsatured fatty acid (PUFA) represents an important mechanism by which polar ectotherms maintain biological membrane structural and functional properties against low temperature. Since PUFAs are highly susceptible to ROS attack, and high unsaturation levels enhance the velocity and propagation of lipid radical chain reactions, the difference in fatty acid composition increases the vulnerability of polar organisms to oxidative stress. PAH metabolites may, in addition, exert pro-oxidant effects through the redox cycle, a well-known source of chemically-mediated ROS generation [93], while metabolites containing an epoxide may be mutagenic and/or carcinogenic or even inhibit some CYP components [94]. From a practical point of view, the analysis of PAH metabolites in polar benthos may facilitate the definition of acceptable oxidative stress thresholds since metabolic responses may not rely on a large variety of homeostatic controls and feedback mechanisms as in the case of changes in enzymatic induction (i.e., gene expression and protein production), which ensures their meaningful interpretation [95].

Behavioural response approach: avoidance of Distillate Marine grade A (DMA) oil-spiked sediment

Active spatial avoidance ('escape') of contaminated sediment relates to the ability of an organism to detect toxicants via olfaction or taste and to move to a lesser contaminated area [96]. Such behaviour can be up to 1000 times more sensitive than conventional lethal endpoints (e.g., LC50), and it may also potentially reflect changes at a population level [97]. Among benthic invertebrates, amphipods have been extensively used in avoidance assays [98-101] since they are equipped with a sensory system and they form an important food source for benthic fishes and other organisms. To date only few studies have addressed avoidance behaviour of oil-contaminated sediment by benthic amphipods, all of which were restricted to temperate marine or estuarine species such as *Corophium volutator* or *Melita plumulosa*.

In the Arctic, exposure to the water-soluble fraction (WSF) of oil revealed little mortality in sea-ice amphipods (i.e., *Gammarus wilkitzkii*), although biomarker results indicated sublethal effects after exposure to WSF [102]. However, oil toxicity in pelagic amphipods may be quickly dampened due to considerable evaporation and dissolution of oil in the water column. This may not be the case in the water-sediment interface where microbial degradation of oil under Arctic conditions [103] may result in persistent and localised sediment oil sources.

These may trigger an escape response in benthic amphipods, while their response to lethal concentrations may be slower than avoidance as previously observed in amphipods exposed to the water-accommodated fraction of oil [104,105].

Since heavy fuel oils are being gradually replaced with marine distillate fuel oils (e.g., DMA) where increased marine traffic is expected [106] and the organic fraction of distillate fuels might be able to persist sufficiently long to adsorb to sediment and cause toxicity on a similar scale than heavier fuels [51,107], escape by Arctic benthic amphipods from oil-spiked sediment could become a sensitive tool for the early detection of oil sublethal effects in benthic communities [108,109]. So far, studies have been performed on the toxicity of diesel in sediment to polar benthic species such as the ophiuroid *Ophiura crassa* [110] and the amphipod *Gammarus setosus* [111], but their ability to avoid oil-contaminated sediment has never been addressed under field or laboratory conditions. Integrating escape responses in oil risk assessment for the Arctic may not only give an indication on benthic amphipods' spatial distribution, which is inversely related to sediment toxicity, but also on oil related adverse effects from chronic exposure.

Aim and scope of the thesis

As described in the foregoing sections there are four main knowledge gaps presently hampering our ability to monitor long-term impacts of O&G activities on Arctic benthic ecosystems:

- BSAFs for organic pollutants have been barely reported for Arctic benthic biota, neither evaluated as possible indicators of sediment PAH bioavailability.
- Biomarkers of exposure to organic pollution and their responsiveness to sediment pollution sources have not been evaluated in Arctic benthos chronically exposed to PAHs.
- Biotransformation metabolites of PAHs and their persistence along sediment exposure have not been evaluated in Arctic benthos chronically exposed to PAHs.
- Avoidance of DMA-spiked sediment and its potential as an oil impact assessment tool have never been studied from the perspective of Arctic benthic biota.

Aim of thesis

The aim of this thesis is to develop candidate methods for the early identification of Arctic bioindicators of O&G and shipping related chemical stress in Arctic coastal benthic systems. The bioindicator potential of some Arctic benthic invertebrate species is evaluated by

specifically measuring the species traits influence on their PAH uptake and PAH biotransformation induction during PAH exposure. Species with either sessile or with reduced mobility, different feeding mode, food selectivity and low dispersal capacity are used to define a sensitivity range to PAHs and other stressors within our chosen taxa. From that perspective, two of the most representative Barents Sea and Chukchi Sea bivalve species, with differing feeding and reproduction traits, the filter feeder *Astarte borealis* (Schumacher, 1817) and the filter/deposit feeder *Macoma calcarea* (Gmelin, 1791) were selected as target species in combination with a predator polychaete, *Nephtys ciliata* (O.F. Müller, 1776) [112-115].

Research questions:

Q1: Can PAH body residues or BSAFs be used as bioavailability indicators in Arctic coastal benthic systems and which target species is most suitable to detect trends?

Q2: Can species traits explain differences in PAH bioaccumulation between Arctic benthic invertebrates?

Q3: Does bioaccumulation of PAHs differ between Arctic and temperate benthic invertebrate species with different traits, and can modelling assist in reading across between species?

Q4: What is the feasibility of using biomarkers of exposure to monitor PAH pollution in the Arctic? Is the identification of biotransformation metabolites a better alternative?

Q5: Can behavioural tests like avoidance behaviour become a suitable monitoring tool for oil impact assessment in the Arctic?

Q6: What are prospects and advantages of using Arctic benthic bioindicator species for monitoring long-term impacts of O&G and other maritime activities in the Arctic?

The above research questions are answered through a combination of field and laboratory research, and modelling.

To address **Q1**, PAHs, PCBs and HCB body residues were measured in the field in *A. borealis*, *M. calcarea* and *N. ciliata*. Differences in body residues among two locations in Kongsfjorden ecosystem (Svalbard, Norway), chemicals and species were assessed and BSAF values were obtained. Whether the BSAF metric is useful as an indicator of PAH availability in Arctic benthic invertebrates under field conditions is investigated in **Chapter 2**.

To address **Q2** and **Q3**, PAH body residues were measured and BSAF values were obtained for the aforementioned Arctic invertebrates and two Atlantic counterpart species (i.e., *Limecola balthica* [Linnaeus, 1758] and *Alitta virens* [Sars, 1835]) under laboratory conditions. PAH bioaccumulation and bioavailability were compared between both regional groups and bioaccumulation was modelled in order to make BSAF reading across between groups possible. Results from laboratory experiments and modelling exercises are discussed in **Chapter 3** and set against field bioaccumulation conclusions in **Chapter 2**.

To address **Q4**, the responsiveness of some biochemical biomarkers of exposure, namely acyl-CoA oxidase (AOX) and glutathione S-transferase (GST), or acetylcholinesterase (AChE) inhibition in the Arctic bivalve *A. borealis* exposed to a PAH-contaminated sediment in laboratory conditions were studied. Additionally, two biotransformation metabolites of the model PAH pyrene (e.g., 1-hydroxypyrene [OHPyr] and pyrene-1-glucuronide [GluPyr]) were measured in Arctic (*M. calcarea*, *N. ciliata*) and temperate (*L. balthica*, *A. virens*) bivalves and polychaetes exposed to the same sediment and differing (Arctic *vs* temperate) laboratory conditions. Both groups' PAH biotransformation capacity and estimated chronic toxicity derived from PAH metabolites bioaccumulation are discussed in **Chapter 4**.

To address **Q5**, short-term avoidance by the temperate freshwater amphipod *Gammarus pulex* (Linnaeus, 1758) and temperate marine benthic amphipod *Gammarus locusta* (Linnaeus, 1758) to DMA-spiked sediment was studied and set against results from the available literature in **Chapter 5**. How and to what extent avoidance may affect the long-term performance of the amphipods and how the response of the Arctic benthic amphipods may differ from their temperate counterparts are discussed in **Chapter 6**, based on available literature on oil acute toxicity in Arctic sea-ice and benthic amphipods.

Lastly, motivated by **Q6**, a synthesis is provided in **Chapter 6** which discusses how physiological and ecological traits may render Arctic coastal benthic species particularly vulnerable to chronic exposure to O&G related chemicals and how we can identify those at an early stage during O&G production as part of existing biomonitoring programmes. Incorporating sublethal effects and long-term impacts of O&G waste on Arctic benthic systems into biomonitoring and risk assessment procedures will help to determine the odds of oil spill and O&G impacts on Arctic marine ecosystems and thus, improve environmental assessment and restoration planning.

2 Bioaccumulation of polycyclic aromatic hydrocarbons, polychlorinated biphenyls and hexachlorobenzene by three Arctic benthic species from Kongsfjorden (Svalbard, Norway)

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Chapter 2

Abstract

The predicted expansion of oil and gas (O&G) activities in the Arctic urges for a better understanding of impacts of these activities in this region. Here we investigated the influence of location, feeding strategy and animal size on the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) by three Arctic benthic species in Kongsfjorden (Svalbard, Norway). No toxicity was expected based on biota PAH critical body residues. Biota PCB levels were mainly below limit of detection, whereas samples were moderately polluted by HCB. PAH concentrations in biota and biota-sediment accumulation factors (BSAFs) were generally higher in Blomstrandhalvøya than in Ny-Ålesund, which was explained by a higher abundance of black carbon in Ny-Ålesund harbour. BSAFs differed significantly among species and stations. We conclude that contaminant body residues are a less variable and more straightforward monitoring parameter than sediment concentrations or BSAFs in Arctic benthos.

1. Introduction

The Arctic region is undergoing an unprecedented change, with global rising temperatures causing an annual summer retreat of sea ice and changes to for instance, seasonal weather patterns and even ecosystems [116,117]. The retreat of sea ice will allow expansion of oil and gas (O&G) activities in the next decades, posing possible impacts on the Arctic ecosystem [14]. Besides O&G activities, changes in climatic parameters as such may affect contaminant transport to and cycling in the Arctic. Furthermore, primary productivity and food web energetics may be affected and thus the trophic transfer of contaminants. Besides allowing possible expansion of O&G activities, retreat of ice also opens alternative commercial shipping routes, with associated anthropogenic activities and risk of maritime accidents.

It has been claimed that baseline information on effects of O&G activities on the Arctic ecosystem or ecosystem components is still inadequate or unavailable [72]. Further research on linking effects in organisms to exposure to O&G related contaminants is therefore essential. In this context, identifying chemical (e.g., petroleum marker compounds like petrogenic PAHs), biological indicators for cumulative effects of O&G activities that can be applied across the Arctic is highly relevant. Petroleum hydrocarbons are generally considered to be one of the main pollutants related to O&G activities. Although anthropogenic inputs of PAHs are only a small proportion of total hydrocarbon burden in the Arctic environment [118], they can create substantial local pollution [119]. Several studies suggest that atmospheric transport of polycyclic aromatic hydrocarbons (PAHs) has only a minor influence on sedimentary PAH distributions in the Arctic Ocean [45,120]. However, petrogenic and pyrogenic PAHs have been identified as an emerging concern in the Arctic [121] and there is little information on PAH bioaccumulation in Arctic benthic species [122]. Since the hydrophobicity of PAHs may promote their adsorption to settling organic matter (OM), marine sediments and benthic systems may act first as a sink and ultimately as a secondary source of PAHs.

Besides PAHs, legacy persistent organic pollutants (POPs) like polychlorinated biphenyls (PCBs), are still ubiquitous in Arctic regions. Local sources are mainly found in the form of secondary sources after, for instance, breakdown and decommission of equipment and demolishing of buildings, although long range atmospheric transport also greatly contributes to the general supply. Contaminants deposited in polar regions typically become mobile during the summer months as glaciers, sea ice and snow melting may introduce POPs into the

marine system [123,124]. Input of these contaminants may directly impact the pelagic system when taken up by algae and zooplankton [125,126], and indirectly the benthic communities due to a strong pelagic-benthic coupling in the Arctic [127]. PCBs are persistent and hydrophobic organochlorine contaminants with a wide range of biological effects in chronic exposed biota. This makes them a suitable chemical group for environmental monitoring in general.

Hexachlorobenzene (HCB) is another chemical that is known for its persistence and ability to being transported to the cold polar regions [128]. Potential sources of HCB to the marine ecosystem may comprise by-products and waste such as solid and liquid residues, solid waste, oils and wastewater.

Coal, crude oil and atmospheric dust have been identified as possible PAH sources in sediment samples of Svalbard [129]. PAH patterns in sediment samples showed predominance of petrogenic PAHs (20 – 60% ΣPAH) and Svalbard coal was identified in all sediment sampling stations [129]. Kongsfjorden is a relatively pristine fjord, as direct anthropogenic impact on the marine system at local scales is generally much lower in Svalbard than along the Norwegian coast and in European waters [130]. Strict environmental policy has resulted in a limited contamination of the Kongsfjorden environment [131], although accidental spills or chronic impacts from local anthropogenic activities still may occur.

Arctic benthic species can be applied as relevant and sensitive bioindicators for the impact assessment of O&G activities in the Arctic [122,132]. Markert et al. [133] defined a bioindicator as an "organism (or a part of an organism or a community of organisms) that contains information on the quality of the environment (or a part of the environment)". Arctic benthic systems are characterised by a high trophic diversity, relatively long life-span, and sedentary lifestyles of the species [134]. This makes them well suited for studying spatial but also temporal variability of O&G related pollution. Omnivore strategies are common among many benthic species from Arctic shallow benthic communities such as scavenging amphipods [135] making them resilient to changes in seasonal conditions which results in little change in food web structure through the year [136]. Benthic invertebrates are an important food source for higher trophic levels in the Arctic such as benthic fishes, bottom-feeding seals (e.g., bearded seals, walruses) and birds (such as the eider duck) [137,138]. The accumulation and effects of contaminants in benthic species depends on both biological traits

such as diet, habitat preference and longevity [56], and sediment characteristics such as grain size and organic carbon content [80]. This results in differences in bioaccumulation potential among benthic invertebrate species, which may be further enhanced by differences in their biotransformation capacity of contaminants [139]. Some information is available on contaminant levels in Arctic benthic species [140,141], however effects of contaminants in Arctic invertebrates are still poorly understood [142].

The biota-sediment accumulation factor (BSAF) has been used as a metric to assess the bioaccumulation of contaminants in sediment and has been compared across a wide range of aquatic ecosystems [80-82]. Only a few examples of BSAFs for Arctic species are available. BSAFs are reported for PCBs in (sub-)Arctic shorthorn sculpins [84] and for volatile siloxanes in Svalbard Atlantic cods and shorthorn sculpins [85]. We are aware of only one study reporting BSAFs for Arctic benthic invertebrates. PCB BSAFs have been published for *Astarte* sp. and *Nephtys incisa* from an Atlantic temperate region [143].

Aim of the present study was to provide a more systematic evaluation of bioaccumulation of PAHs, PCBs and HCB by three Arctic benthic species. This includes assessing differences between species, size classes within species, contaminant groups and sampling stations. In this study we explore for the first time to what extent the BSAF metric is useful as an indicator for PAH, PCB and HCB accumulation in Arctic benthic invertebrate species. The results will be interpreted in light of the usefulness of such metric for assessment of potential impact of future O&G activities in the Arctic.

Biota was collected in Kongsfjorden (Svalbard, Norway). Three benthic species (*Astarte borealis*, *Macoma calcarea* and *Nephtys ciliata*) were selected based on their feeding habits, sessility and relative abundance or Valuable Ecosystem Component (VEC) condition (i.e., species that are specifically abundant at ecologically relevant habitats) [144].

Results of this study show the bioaccumulation potential of PAHs, PCBs and HCB in Arctic benthic species in the field and are used to select the most relevant benthic species and tools for monitoring organic contaminants in Arctic sediment.

2. Materials and Methods

2.1. Study sites

Sediment was collected in an (sub-)Arctic fjord (Kongsfjorden Bay, Svalbard) and an offshore region (Barents Sea) (Figures 1A-1B). The Svalbard inshore and Barents Sea study areas were located between 71° and 79° latitude (Table S3) along which the waters are influenced by the West Spitsbergen Current (WSC). Barents Sea sampling stations depth ranged 110 to 320 m. For a general description of PAH levels in the W Barents Sea the reader is referred to Boitsov et al. [118].

Kongsfjorden ecosystem is a high-latitude (sub-)Arctic fjord (79°N, 12°E), but the waters are influenced by both the Atlantic water masses of the WSC as well as the Arctic-type coastal waters, and additionally glacial input of melt water [145]. Depths in the outer basin average 200 to 300 m, whereas the inner basin is considerably shallower (average depth 50 – 60 m). Muds dominate the subtidal sediments throughout the fjord. Kongsfjorden inner and outer basins differ with respect to differences in their deposition rates, which are much higher in the inner basin, and differences in bioturbatory activity by infaunal organisms distribution [131].

Kongsfjorden shallow benthic macrofauna distribution and abundance are influenced by sediment characteristics such as grain size, water currents, as well as by different carbon sources such as fresh settling OM, reworked settling OM, microphytobenthos and terrestrial OM [127,136,141,146]. Two faunal communities can be distinguished in the Kongsfjorden ecosystem according to the frequency of species occurrence, and to several indices of community fidelity of each species in each community [147]. The Glacial Bay community (inner basin) is dominated by small mobile bivalves (e.g., *Yoldiella solidula*) while the Outer Basin community is dominated by larger, often less mobile, mostly tubiculous polychaetes (e.g., *Pectinaria hyperborea*).

2.2. Collection of field samples

Sampling of sediment and biota in Kongsfjorden was performed at a locally impacted harbour station (Ny-Ålesund) [148,149] and two reference stations (Blomstrandhalvøya and Kapp Guissez) (Figure 1C). Sampling took place outside the protected areas of Kongsfjorden on board of research vessel MS Teisten (Kings Bay AS, Ny-Ålesund) between 15 and 19 July 2013 and within the Outer Basin community distribution area as defined by Włodarska-

Kowalczuk and Pearson [147]. Additionally, sediment samples were collected at five stations in the West Barents Sea on board of the R/V *Lance* (Norwegian Polar Institute, Tromsø) between 3 and 10 October 2013 (Table S3). Sediment samples consisted of soft bottom substrates and were collected using a Van Veen grab (0.1 m^2). Biota samples were collected at Ny-Ålesund and Blomstrandhalvøya using either a Van Veen grab, or a small dredge. Three Arctic marine benthic invertebrate species with different feeding behaviours were collected: *A. borealis* (Schumacher, 1817) (mollusc; suspension feeder), *M. calcarea* (Gmelin, 1791) (mollusc; deposit and suspension feeder), and *N. ciliata* (O.F. Müller, 1776) (polychaete; omnivore). Sediment samples were preserved in 250 mL-glass jars at -20 °C until analysis. The organisms were allowed to depurate for 24 h in clean sea water from the fjord before dissection. Animals were weighed, measured, dissected and pooled by size class (Table S2). Size classes were defined such that each class had sufficient numbers of individuals to allow statistical testing. The soft tissue of bivalves was removed from the shell and samples were pooled in groups of 5 to 12 individuals before freezing at -20 °C. Sediment and biota samples were all shipped frozen in dry-ice to Wageningen University (Wageningen, the Netherlands) for chemical analysis.

2.3. Chemical analysis

Chemical analysis was described before [150]. The following PAHs and POPs were analysed: Phenanthrene (PHE), Anthracene (ANT), Fluoranthene (FLT), Pyrene (PYR), Benz(a)anthracene (BaA), Chrysene (CHR), Benzo(e)pyrene (BeP), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Indeno(1,2,3-*cd*)pyrene (Ind123P), Dibenz(*a*,*h*)anthracene (dBahA), Benzo(*ghi*)perylene (BghiP), hexachlorobenzene (HCB), PCB-18, -20, -28, -29, -31, -44, -52, -101, -105, -118, -138, -153, -170, -180, -194, -204 and -209.

For details on extraction, clean-up and instrumental analysis see the Supporting Information (SI). In short: sediment and biota samples were Soxhlet-extracted with hexane/acetone. Extracts were cleaned over Al_2O_3 (PAH), or Al_2O_3/silica (HCB and PCBs) and analysed using HPLC-UV and GC-ECD, respectively. Recoveries were 53 to 77% (PAHs in biota), 66 to 79% (HCB and PCBs in biota), 57 to 67% (PAHs in sediment) and 79 to 103% (HCB and PCBs in sediment). Besides recoveries, numerous blanks were used. All data were corrected for the resulting values.

Subsamples of each sediment sample were analysed for weight percentages (wt. %) of OM content, measured as loss on ignition (550 °C, 3 h). Biota lipids were extracted with chloroform:methanol:water and quantified gravimetrically.

2.4. Data analyses

Lipid-normalised biota concentrations were calculated. BSAFs were calculated as $(C_{org}/f_{lip})/(C_{sed}/f_{SOM})$ with C_{org} being the chemical concentration in the organism in wet weight (wet wt.; µg/kg), C_{sed} the chemical concentration in sediment in dry weight (dry wt.; µg/kg), f_{lip} the fraction of lipids in the organism based on wet wt., and f_{SOM} the fraction of sediment organic matter (SOM) based on dry wt. BSAFs of a specific compound were calculated provided that there were at least three detects (i.e., >LOD) for the same compound in sediment samples. Only those compounds for which ≥ 90% BSAF values were above zero in both stations were included in the statistical analysis. Data were checked for normality with Q-Q plots and Shapiro-Wilk test and equality of variances with Levene's test. Lipid-normalised biota concentrations and BSAFs were tested for location and size effect with the Mann-Whitney *U* test and Kruskal-Wallis test, respectively for each PAH and PCB compound as well as for HCB and the sum of PAHs (Σ_{13}PAH) and 7 PCBs (Σ_7PCB). Significance level of an overall statistical comparison was set at $p \leq 0.05$, while for pairwise comparisons a Bonferroni correction was applied. All statistical calculations were performed using SPSS version 22.

PAH diagnostic ratios were applied to assess emission sources of PAHs in sediment. Sources can be pyrogenic (originated from combustion), petrogenic (originated from oil) or a combination of these. It has been shown that PAH isomers with masses 202 (e.g., FLT, PYR) and 276 (e.g., BghiP, Ind123P) have the greatest range in stability of PAHs, thus they are good indicators of petroleum *vs* combustion sources [120,151]. PAH ratios FLT/(FLT+PYR) and Ind123P/(Ind123P+BghiP) were calculated. A FLT/(FLT+PYR) ratio of < 0.4, 0.4 – 0.5 and > 0.5 points out to petrogenic sources, fuel combustion or grass/wood/coal combustion, respectively. A Ind123P/(Ind123P+BghiP) ratio of < 0.5 or > 0.5 points out to fuel combustion or grass/wood/coal combustion, respectively. However, this relationship is not universal and interpretation of this measure should only be used indicatively.

Sediment (based on dry wt.) and biota (based on lipid wt.) PAH and PCB concentrations were evaluated based on Environmental Quality Standards (EQSs) established by the Norwegian

Environment Agency [152], Environmental Assessment Criteria (EACs) established by the OSPAR Commission [153] and Critical Body Residues (CBRs) established by the USEPA [154].

Concentrations below the EACs are considered to present no significant risk to the environment, and to that extent may be considered as being related to the EQSs applied to concentrations of contaminants in water [155]. CBRs established by the USEPA are based on the target lipid model suggested by Di Toro et al. [156].

3. Results and Discussion

3.1. Concentrations of PAHs, PCBs and HCB in sediment

PAHs in sediment. For Kongsfjorden samples, concentrations of Σ_{13}PAH varied between 12 and 2315 µg/kg dry wt. (geometric mean 139 µg/kg dry wt.), whereas for Barents Sea samples, this range was between 39 and 4270 µg/kg dry wt. (geometric mean 823 µg/kg dry wt.) (Table S4). Ny-Ålesund sediments were contaminated quite uniformly with Σ_{13}PAH concentrations ranging from 1815 to 2315 µg/kg dry wt. (geometric mean 2011 µg/kg dry wt.). Towards the northern fjord shelf (Blomstrandhalvøya) and the inlet from Kongsfjorden to Krossfjorden (Kapp Guissez), PAH levels decreased. In the Barents Sea, significant differences were found for PHE and BeP among distant stations (e.g., SE Edgeøya *vs* Snøhvit, Figures 1A-1B). This may indicate differences in deposition, the presence of local sources such as natural seepages of oil or other organic fossils, or differences in SOM content. No significant differences were found among stations relatively close to each other (e.g., SE Edgeøya S2-S4) or among distant stations located within the NW Barents Sea study area (e.g., E Kong Karls Land *vs* SE Edgeøya).

Sediment PAH diagnostic ratios. Based on PAH ratios, sediment PAHs seemed to originate from combustion of grass, wood, coal or petroleum (Barents Sea, Ny-Ålesund), from combustion of grass, wood and coal (Kapp Guissez), and from combustion of grass, wood and coal or from petroleum (Blomstrandhalvøya) (Table 1). In Ny-Ålesund sediments, uncombusted coal also is relevant. After all, PAHs from coal are ubiquitous in the Svalbard environment where extensive coal mining was carried out by Kings Bay in Ny-Ålesund until 1962 [157]. In a study of surface sediment collected between the Barents and Kara Sea shelves, the highest pyrogenic PAH levels (sum of 4- to 6-ring hydrocarbons, perylene

excluded) were found in sediments from Kola, the Pechenga Bays and Svalbard inshore [129]. Highest pyrogenic PAH levels in Barents Sea and Kongsfjorden were reached in sediments from station 2 (SE Edgeøya) and 8 (Kapp Guissez) (Table 1).

Table 1. Sediment parent PAH diagnostic ratios.

		Barents Sea (October 2013)					Kongsfjorden (July 2013)		
PAH ratio		S1	S2	S3	S4	S5	S6	S7	S8
FLT/(FLT+PYR)	Min	0.43 (B)	0.53 (C)	0.45 (B)	0.44 (B)	0.00 (A)	0.63 (C)	0.31 (A)	0.48 (B)
	Max	0.47 (B)	0.57 (C)	0.48 (B)	0.49 (B)	0.52 (C)	0.65 (C)	0.35 (A)	0.55 (C)
Ind123P/(Ind123P+BghiP)	Min	0.80 (C)	0.82 (C)	0.77 (C)	0.79 (C)	0.81 (C)	0.34 (B)	0.21 (B)	0.83 (C)
	Max	0.83 (C)	0.85 (C)	0.79 (C)	0.80 (C)	0.87 (C)	0.43 (B)	0.79 (C)	0.86 (C)

(A) Petrogenic sources; (B) Fuel combustion sources; (C) Grass/wood/coal combustion sources.

Sediment Quality Assessment based on PAH concentrations. Effect Range (ER) values were established as sediment quality guidelines to be used to predict adverse biological effects on marine organisms [158]. ERL (ER lower tenth percentile) values are available for 9 individual PAHs (PHE, ANT, FLT, PYR, BaA, CHR, BaP, Ind123P and BghiP) below which adverse effects on organisms are rarely observed. An ERL in sediment for the sum of PAHs (2990 µg/kg dry wt.) was calculated as the sum of the 9 PAH ERLs. The geometric means of the sum of 9 PAHs in sediment samples from our study (Barents Sea and Kongsfjorden, respectively) were 32 to 39 times lower than the 9 PAH ERL. However, in almost all Barents Sea (S2-S4) and all Ny-Ålesund (S6) samples PHE concentrations were above its individual ERL value (i.e., 240 µg/kg dry wt.). Additionally, FLT, BbF, dBahA and Ind123P concentrations in some Barents Sea samples (stations S3-S4, Figure 1A) were within the interval of acute effects for short term exposure (Class IV) established by the Norwegian Environment Agency [152]. In some cases (station S4), PYR concentrations were even within the interval of severe toxic effects (> 140 µg/kg dry wt., Class V) established by this same institution.

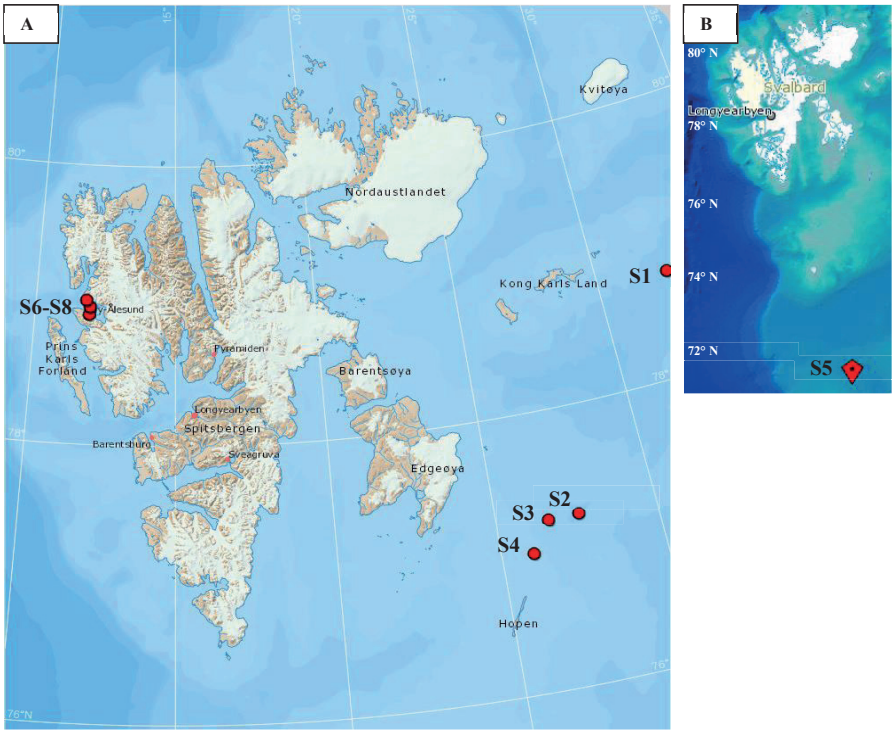

Figure 1. A: Barents Sea (S1-S4) and Kongsfjorden (S6-S8) sampling stations (Basemap © Norwegian Polar Institute). **B:** Snøhvit sampling station (S5) (MAREANO mapping © Institute of Marine Research).

PCBs in sediment. For Kongsfjorden samples, Σ_7PCB concentrations varied between 0.00002 and 0.13 µg/kg dry wt. (geometric mean 0.001 µg/kg dry wt.), whereas for Barents Sea samples this range was between below limit of detection and 0.04 µg/kg dry wt. (geometric mean below limit of detection) (Table S5). Highest concentrations of Σ_7PCB were detected in Ny-Ålesund sediments where concentrations varied between 0.002 and 0.13 µg/kg dry wt. (geometric mean 0.01 µg/kg dry wt.). Significant differences were found between Ny-Ålesund and Blomstrandhalvøya station for PCB-18, -105, -118, -138 and -155 (Mann-Whitney *U* test, $p < 0.05$). PCB concentrations in Kongsfjorden sediment were within the range of earlier reported PCB concentrations in Kongsfjorden sediment [149]. In other Svalbard fjords, Σ_7PCB sediment concentrations of 0.74 to 5.41 µg/kg dry wt. in Barentsburg (Grønfjorden) and 1.8 to 20.2 µg/kg dry wt. in Pyramiden (Billefjorden) were reported in 2008, showing 6 to 14 orders of magnitude higher concentrations than in Ny-Ålesund samples included in our study [159,160]. PCB-138 and PCB-153 made up 60% of Σ_7PCB in some Ny-

Ålesund sediment samples and 53% of Σ_7PCB in Barentsburg sediment samples in 2008 [159] which may point to different local sources [140,161,162].

Figure 1. C: Kongsfjorden sampling stations (S6-S8). From north to south: Kapp Guissez (S8), Blomstrandhalvøya (S7) and Ny-Ålesund (S6) harbour (Basemap © Norwegian Polar Institute).

HCB in sediment. HCB concentrations in Kongsfjorden samples were between below limit of detection and 0.01 µg/kg dry wt., whereas concentrations of the Barents Sea sediment samples varied between below limit of detection and 0.37 µg/kg dry wt. (Table S5). For Kongsfjorden and Barents Sea samples, significant differences were found between Ny-Ålesund and Blomstrandhalvøya station (Kruskal-Wallis test, $p = 0.037$) as well as between SE Edgeøya (S3) sediments where HCB concentrations ranged 0.17 to 0.37 µg/kg dry wt. and Snøhvit (S5) sediments where HCB concentrations were ≤ 0.00003 µg/kg dry wt. (Kruskal-Wallis test, $p = 0.037$).

3.2. Sediment organic matter content

For Kongsfjorden, SOM percentages varied between 3.7% in Kapp Guissez and 6.3% in Ny-Ålesund, and for the Barents Sea samples between 4.9% in E Kong Karls Land and 9.1% in SE Edgeøya (S2) (Table S4).

3.3. Concentrations of PAHs, PCBs and HCB in biota

3.3.1. Polycyclic aromatic hydrocarbons

PAH concentrations in biota. Among species and stations, Σ_{13}PAH concentrations in benthic invertebrates of Kongsfjorden varied between 27 and 9185 µg/kg lipid wt. (geometric mean 729 µg/kg lipid wt.) (Table S9). Lowest Σ_{13}PAH concentrations were found in *A. borealis* at the Ny-Ålesund sampling station, whereas highest Σ_{13}PAH concentrations were observed in *N. ciliata* samples at Blomstrandhalvøya (Figure 2). In general the highest concentrations in *A. borealis* and *N. ciliata* were found in Blomstrandhalvøya samples, whereas concentrations in *M. calcarea* samples did not differ much between stations. In our study, *A. borealis* specimens (Ny-Ålesund) were mainly exposed to both petroleum and coal combustion-derived PAH sources in the field based on the agreement between mean PAH ratios (FLT/[FLT+PYR] and Ind123P/[Ind123P+BghiP]) calculated for both Ny-Ålesund bivalves (0.49/0.52) and sediment (0.64/0.38) samples [163,164].

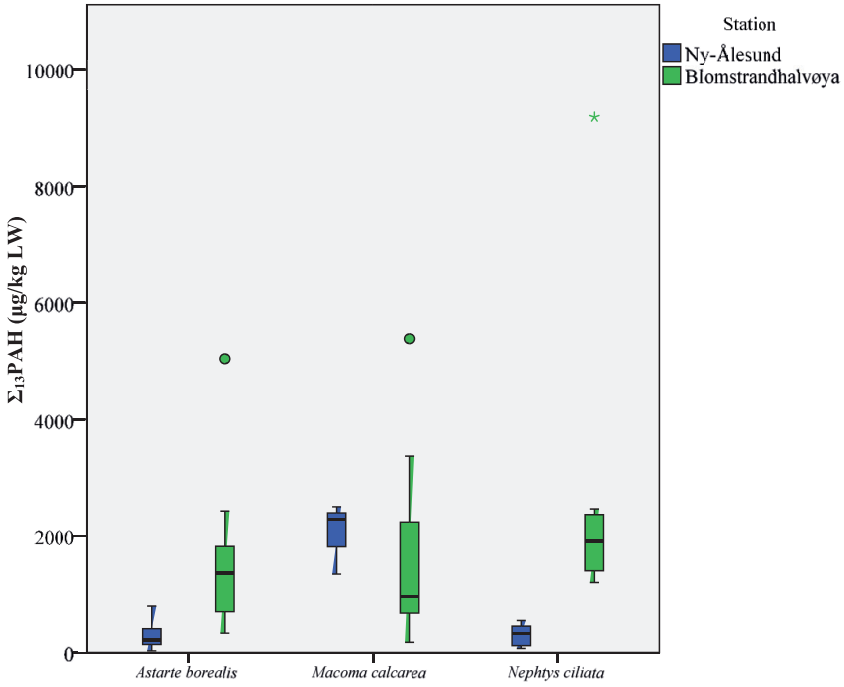

Figure 2. Σ_{13}PAH concentrations (µg/kg lipid wt.) in benthic invertebrates of Kongsfjorden.

PAH Critical Body Residues. To assess potential effects resulting from PAH bioaccumulation, the measured concentrations can be compared to USEPA CBR levels. Hansen et al. [154] calculated a Σ_{13}PAH total lipid LC50 body burden (96 h) for *N. arenaceodentata* (polychaeta) and for *L. stagnalis* (gastropoda) as 280,000 and 330,000 µg/g lipid wt. (using a Σ_{13}PAH W_m (molar weight) = 3058 µg/µmol). This is a factor of 30,500 to 65,500 times higher than the mean Σ_{13}PAH lipid-normalised concentrations found in any of our species (Blomstrandhalvøya).

Hwang et al. [165] found a CBR of Σ_{24}PAH for lysosomal destabilization (at least 50% of destabilized cells) of 2100 µg/kg dry wt. in eastern oysters (*C. virginica*). Assuming a 20% and 12% dry wt. (based on soft tissue wet wt.) in *A. borealis* [166] and in *M. calcarea* [167], respectively, the maximum Σ_{13}PAH values detected in Blomstrandhalvøya *A. borealis* and *M. calcarea* specimens are 3 to 8 times lower (258 and 620 µg/kg dry wt., respectively) than the Σ_{24}PAH CBR calculated by Hwang et al. [165].

Based on EACs for PAHs established in mussels and oysters [153], and assuming 20% dry wt. in *A. borealis*, mean concentrations of PHE, FLT and PYR in *A. borealis* Blomstrandhalvøya specimens were 22, 6 and 2 times lower than the corresponding EAC.

Liu et al. [168] observed the induction of aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin O-deethylase (EROD) enzymatic activities in *R. philippinarum* individuals with internal BaP concentrations of approximately 2.02 μg/g dry wt. in the digestive gland. Assuming 20% and 12% dry wt. (based on soft tissue wet wt.) in *A. borealis* and *M. calcarea*, respectively, and accepting that a great proportion of BaP should be accumulated in the digestive gland of bivalves [169,170], then the maximum BaP concentrations detected in *A. borealis* (Blomstrandhalvøya) and *M. calcarea* (Ny-Ålesund) would be 108 to 465 times lower (0.02 and 0.004 μg/g dry wt., respectively) than the same concentration observed by Liu et al. [168].

In summary, no potential toxicity of Σ_{13}PAH to *A. borealis*, *M. calcarea* or *N. ciliata* Blomstrandhalvøya populations was indicated according to three CBRs mentioned. Biotransformation enzymatic activity might be also negligible in the case of bivalves, according to the results obtained by Liu et al. [168] for *R. philippinarum*.

Differences in PAH concentrations among stations. Several factors were identified that can explain differences in PAH bioaccumulation between Ny-Ålesund (NY) and Blomstrandhalvøya (BL). A summary of factors in relation to ecological processes is provided as Figure 3, and in the discussion below we refer to these factors and processes as NY-*x* and BL-*y*, where *x* and *y* correspond to the numbers as indicated in Figure 3. Nearly all lipid-normalised individual PAH concentrations in the polychaete *N. ciliata* were significantly higher in Blomstrandhalvøya than in Ny-Ålesund specimens (Table S6). The lower Σ_{13}PAH content in Ny-Ålesund specimens (Table S9) can be explained by different factors. The most important factor may be differences in bioavailability related to differences in OM quality between sites. For instance, sediment in Ny-Ålesund harbour contained clearly visible black carbon (BC) particles that are known to strongly bind organic contaminants such as PAHs making them less available for uptake [78] (Figure 3, factor NY-7). BC particles (≥10 μm) predominate in surface sediments from the middle part of the Kongsfjorden compared to the outer part [171]. BC ≥10 μm are usually associated with local sources such as fossil-fuel fired power stations located in different Svalbard human settlements. Secondly, transport of coal particles from former coal mines within the Bayelva river catchment area, west of Ny-

Ålesund, might also influence the final bioavailability of sediment-based PAH sources, particularly in Ny-Ålesund *A. borealis* and *N. ciliata* specimens [148,172]. Thirdly, ongoing degradation of weathered oil in Ny-Ålesund [148,172] could have a highly significant impact on Ny-Ålesund individuals (Figure 3, factor NY-6) as this would minimize their in depth and deposit feeding in the weathered oil immediately surrounding area.

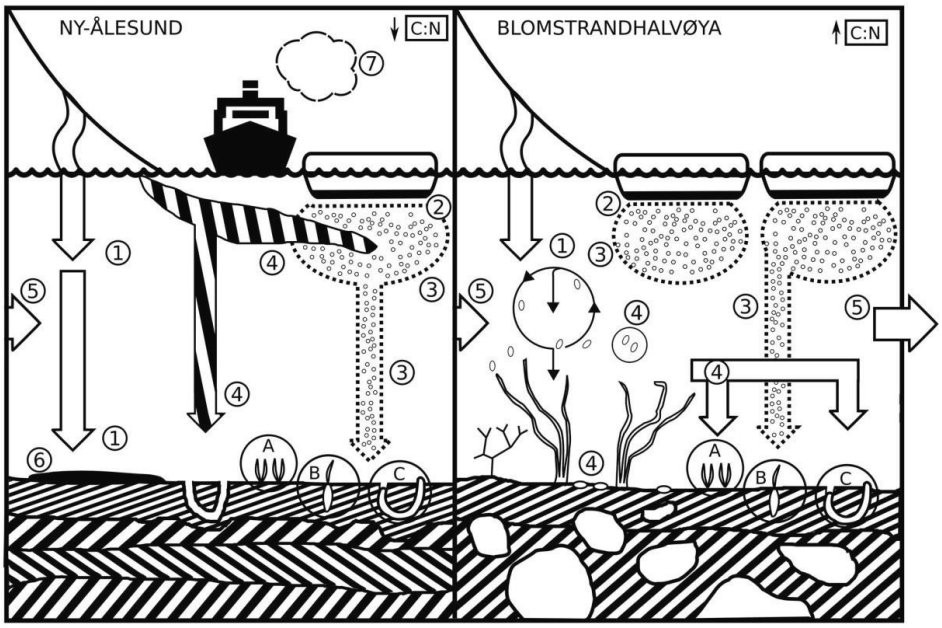

Figure 3. Possible factors contributing to observed differences between sampling stations. **Ny-Ålesund (NY):** (1) terrestrial particulate organic matter (POM), (2) ice algae, (3) fresh pelagic/settling organic matter (OM), (4) clayish river runoff, (5) advected and reworked detritus, (6) weathered oil, (7) volatile organic compounds (VOCs). **Blomstrandhalvøya (BL):** (1) terrestrial POM, (2) ice algae, (3) pelagic/settling OM, (4) brown macroalgal material, (5) advected and reworked detritus. A: *Astarte borealis*, B: *Macoma calcarea*, C: *Nephtys ciliata*.

Additionally, differences in OM quality related to differences in species and biomass composition of phytoplankton blooms and in macroalgae biomass would partly explain different benthic PAH bioaccumulation patterns between stations. A higher predominance of ice diatoms (Figure 3, factor BL-2) promoted by the resuspension of nutrients as a direct consequence of the continuous down-fjord advection of deep freshwater [145] (Figure 3, factor BL-5), as well as a higher input of macroalgal detritus [173] (Figure 3, factor BL-4),

are expected to increase the PAHs partitioning from the water phase to fresh settling OM (Figure 3, factor BL-3) as both algal materials are rich in polyunsaturated fatty acids (PUFAs) [174]. A higher relative intake of PUFA-rich material may increase the bioavailability of PAHs in Blomstrandhalvøya, particularly in the case of filter feeders (e.g., *A. borealis*). Finally, streams leaving Blomstrandhalvøya carried a much higher terrestrial settling OM (Figure 3, factor BL-1) concentration per both water volume and dry wt. of suspended matter than the Bayelva river (Ny-Ålesund) [146] (Figure 3, factor NY-1). A higher input of terrestrial OM in Blomstrandhalvøya can contribute to the mineralization of settling OM during sedimentation which eventually would increase the OC-normalised partition coefficients in settling particles [175-177].

A short response time to fresh settling OM may also increase PAH bioaccumulation in *M. calcarea* as concentrations of ANT, BaA, BaP and dBahA in *M. calcarea* Ny-Ålesund specimens were significantly higher than in Blomstrandhalvøya specimens. Bayelva river outflow usually contains a high proportion of clay materials (Figure 3, factor NY-4) that could also enhance phytoplankton mortality while zooplankton may suffer from direct mortality [131]. The immediate decrease of phytoplankton grazing by zooplankton due to the oxygen depletion of surface waters, would ultimately favour the sedimentation of more fresh phytoplankton (Figure 3, factors NY-2 and NY-3), which *M. calcarea* basically feeds on and to which PAHs may adsorb. *M. calcarea* is also one of the few benthic species in the Kongsfjorden of which a low trophic level and $\delta^{13}C$ may suggest that this species is mainly reliant on fresh phytoplankton or fresh phytodetritus during summer [136,178]. Additionally, a lower PAH excretion capacity in *M. calcarea* [179,180] compared to the other two species may contribute to the observed differences between Ny-Ålesund and Blomstrandhalvøya *M. calcarea* individuals.

In summary, lipid-normalised PAH concentrations in all species seemed to be affected by some station related variation. This may be explained by either a difference in SOM content, origin and sedimentation rate of settling OM and BC strength source between stations.

Differences in PAH concentrations among species. BaP, dBahA and Ind123P burden in *N. ciliata* was significantly higher than in both bivalve species at Blomstrandhalvøya (Table S7). This may be caused by a more opportunistic feeding pattern of *N. ciliata* when compared to the bivalve species. Regarding Ny-Ålesund samples, nearly all lipid-normalised individual PAHs concentrations were significantly higher in *M. calcarea* compared with the other two

species (Table S7). Since steady-state PAH body burdens in polychaetes can also be greatly influenced by biotransformation processes [139,181], chronic PAH exposure is suggested to increase the inducibility of PAH biotransformation enzymes (e.g., CYP) in Ny-Ålesund polychaetes, and thus the probability of finding lower PAH body burdens in Ny-Ålesund individuals [182]. Additionally, specific CYP enzymes involved in xenobiotic biotransformation often are induced by substrates upon which they act, so that the mere presence of some PAHs may trigger the expression of CYP enzymes (e.g., PYR and PYR hydroxylase) [183]. However, long-term PAH detoxification might be slower, if not negligible as explained in the PAH critical body residues section, in bivalves since the major site of uptake and accumulation in these organisms is the digestive gland [169] whereas CYP genes transcription has been described as a tissue specific process reflecting the importance of gills in PAH detoxification [184].

In general, ingestion of SOM has been described as the major uptake pathway of organic contaminants in temperate benthic species (*A. virens*, *L. balthica*) [56]. Additionally, a higher ingestion rate, more opportunistic diet and higher dermal exposure of *N. ciliata* could lead to an extended bioaccumulation of sediment-bound PAHs [185]. *A. borealis* (filter-feeder) and *M. calcarea* (deposit-feeder) feed mainly on fresh phytoplankton or fresh phytodetritus, as shown by an abundance in algal-derived fatty acids in the same species [186,187]. In a Chukchi Sea study performed by Neff et al. [188], ΣPAH (parent and alkylated compounds) concentrations were also higher in *Macoma* sp. clams and maldanid worms than in *Astarte* sp. This may be due to the fact that, as a deposit-feeder, *M. calcarea* may also actively ingest SOM.

In general, differences in species-specific PAH accumulation patterns were seen between the three species, which can be explained by differences in uptake and elimination rates between these species due to underlying mechanisms that however could not be assessed unambiguously from the data.

Differences in PAH concentrations among species size-classes. For the Kruskal-Wallis test, a Bonferroni correction was applied and all effects are reported at a 0.0167 level of significance. We conclude that there is no significant size-class effect on *A. borealis* lipid-normalised PAH concentrations (Table S10), neither on *M. calcarea* (Blomstrandhalvøya) PAH concentrations. Similarly, no significant differences were found in Σ_{13}PAH lipid-normalised concentrations between both bivalves size-classes.

3.3.2. Polychlorinated biphenyls

Concentrations of PCBs in biota were below limit of detection in ≥ 90% of the biota samples analysed for every species and from both Ny-Ålesund and Blomstrandhalvøya station. PCBs were therefore excluded from further analyses.

3.3.3. Hexachlorobenzene

Among species and stations, concentrations of HCB varied between < 0.01 and 14.16 µg/kg lipid wt. The minimum and maximum values were detected in *A. borealis* (Ny-Ålesund) and *M. calcarea* (Blomstrandhalvøya), respectively. In general the highest concentrations in biota tissues were found in Blomstrandhalvøya specimens. *M. calcarea* Blomstrandhalvøya individuals were assessed as markedly polluted (Class I to Class III for HCB, between 0.1 and 1 µg/kg wet wt.) according to the EQSs for blue mussels defined by the Norwegian Environment Agency [189]. HCB lipid-normalised concentrations were found to be significantly higher in *A. borealis* (Mann-Whitney *U* test, $p < 0.001$) and *M. calcarea* (Mann-Whitney *U* test, $p = 0.005$) Blomstrandhalvøya specimens than in their Ny-Ålesund counterparts. An interspecies comparison within Blomstrandhalvøya station samples showed significantly higher (Kruskal-Wallis test, $p = 0.027$) HCB lipid-normalised concentrations in *A. borealis* specimens. No size effect on HCB lipid-normalised concentrations was reported after applying a Bonferroni correction.

3.4. Biota-sediment accumulation factors

For organisms that are closely linked to the sediment and are exposed mainly to sediment-bound contaminants, the BSAF has been shown to be a useful metric of bioaccumulation [56,82]. Here we explore to what extent BSAF is useful to assess bioaccumulation in the Arctic species studied, hypothesizing that BSAF has the lowest value for suspension feeder *A. borealis*. Data were sufficient to calculate BSAF values only for PAHs. Mean BSAF values are presented in Tables S11, S13 and S15 for both stations.

For Blomstrandhalvøya, the 10-90th percentile range of Σ_{13}PAH BSAF values per species was 0.59 to 3.50 for *A. borealis*, 0.35 to 4.76 for *M. calcarea* and 1.88 to 5.50 for *N. ciliata*, which thus shows a somewhat higher accumulation than the value of BSAF = 1-2 as predicted by Equilibrium Partitioning Theory (EPT) for hydrophobic contaminants [190]. Similar BSAF values have been reported earlier in the literature [56,83,191-193]. One explanation is

that EPT neglects the ingestion pathway, whereas this pathway is relevant in nature. Another potential explanation is that uptake was not from the sediment compartment but merely from freshly deposited or settling suspended solids and their ambient water [136,194], having higher chemical concentrations than the sampled sediment, due to atmospheric or melt-water based sources of PAHs [121,177,195]. In Ny-Ålesund, the 10-90th percentile range of Σ_{13}PAH BSAF values per species was 0.00 to 0.02 for *A. borealis*, 0.05 to 0.07 for *M. calcarea* and 0.00 to 0.02 for *N. ciliata*. These general ranges of the Σ_{13}PAH BSAF values in Ny-Ålesund comply very well with the BSAF values found for BC impacted sites [196,197]. Sediments in Ny-Ålesund contain BC [171,198]. This is consistent with information from the mean PAH diagnostic ratios FLT/(FLT + PYR) and Ind123P/(Ind123P + BghiP), which were > 0.5 and 0.2 – 0.5, respectively (Table 1), and reflect a pyrogenic origin of these chemicals [120].

PAH BSAF values were significantly higher in Blomstrandhalvøya specimens compared to Ny-Ålesund specimens (Tables S12, S14 and S16). In the case of *A. borealis*, all considered BSAFs were significantly higher (Mann-Whitney *U* test, $p < 0.001$, Table S12) in Blomstrandhalvøya specimens. As for *M. calcarea* and *N. ciliata* Blomstrandhalvøya samples, significant differences between the stations were found in FLT, PYR, BaA, CHR and BghiP (Mann-Whitney *U* test, $p < 0.05$, Table S14) BSAFs and FLT, BaA, CHR, BbF, BghiP and Ind123P (Mann-Whitney *U* test, $p < 0.05$, Table S16) BSAFs in the first and second species, respectively.

An interspecies comparison within Blomstrandhalvøya station samples showed significantly higher BbF BSAFs in *A. borealis* (Kruskal-Wallis test, $p = 0.006$) whereas the same comparison within Ny-Ålesund showed significantly higher FLT, BaA and CHR BSAFs in *M. calcarea* (Kruskal-Wallis test, $p < 0.05$). No size class effect was observed in either of the species PAH BSAFs.

4. Implications and Conclusions

Our data analysis provides the first systematic evaluation of BSAF patterns across chemicals, locations and species in the Arctic. PAH concentrations and BSAFs were higher in Blomstrandhalvøya specimens which was explained by stronger sorption to BC and lesser uptake from ingested sediment in Ny-Ålesund which in turn implies that the use of the BSAF concept is not straightforward in these ecosystems. We conclude that PAH body residues can

be applied as a less variable and more straightforward pollution monitoring parameter than sediment concentrations or BSAFs as biota body concentrations in low trophic level species give a good estimate of the bioavailable fraction of organic contaminants present in the system. Based on this field study, the filter/deposit-feeding bivalve *M. calcarea* is proposed as a (sub-)Arctic indicator of O&G-derived environmental impacts due to its ability to accumulate PAHs in low O&G impacted areas (e.g., Ny-Ålesund) regardless of its body size class and to its selective feeding mode which decreases uncertainty on the species bioavailable PAH sources.

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Chapter 2

Supporting Information

Extraction, clean-up and instrumental analysis

Frozen sediment samples (Barents Sea) and freeze-dried sediment samples (Kongsfjorden) were thawed at room temperature. Frozen pooled biota samples were thawed at room temperature and subdivided into replicates of equal weight before drying. Sediment subsamples and biota replicates were dried and grinded with diatomaceous earth and Soxhlet-extracted for 40 min with hexane/acetone (3:1) (DionexASE 350 System, Thermo Scientific). All extracts were split in two. One part was cleaned-up over an Al_2O_3 column and used for PAH analysis, which was carried out on an HP 1100 HPLC equipped with a 4.6 mm Vydac guard and analytical reverse phase C18 column (201GD54T and 201TP54, respectively) which were kept at 22.00 °C. Detection was performed by an HP 1100 multi-wavelength fluorescence detector operating in the multi-emission wavelength mode. The mobile phase consisted of methanol/water (mixture and flow gradient). The injection volume was 20 µL.

The other part was cleaned-up using an Al_2O_3/silica column, desulfurized with Cu-powder, and used for PCB and HCB analysis. PCBs were measured by splitless injection of 1 µL of sample in an upgraded HP 5890 series II gas chromatograph equipped with an HP 7673A autosampler system, two fused silica capillary columns, CP Sil-8 CB and CP Sil-5 CB (both 50 m.; i.d. 0.15 mm; d.f. 0.20 µm), and two ^{63}Ni electron capture detectors. The injector and detector temperatures were 250 and 325 °C, respectively. Carrier gas was N_2 (1 mL/min).

Besides recoveries 3 blanks per each batch of 30 samples were used, and values were corrected for blanks.

Table S1. HCB and PCBs limit of detection (LOD, µg/L) and limit of quantification (LOQ, µg/L).

Compound	LOD	LOQ
HCB	0.08	0.27
PCB 18	0.25	0.85
PCB 20	0.13	0.43
PCB 28	0.13	0.42
PCB 29	0.47	1.58
PCB 31	0.11	0.37
PCB 44	0.34	1.14
PCB 52	0.76	2.53
PCB 101	0.12	0.40
PCB 105	0.09	0.30
PCB 118	0.09	0.32
PCB 138	0.08	0.28
PCB 153	0.13	0.42
PCB 155	0.09	0.29
PCB 170	0.08	0.28
PCB 180	0.07	0.23
PCB 194	0.08	0.27
PCB 204	0.03	0.09
PCB 209	0.10	0.32

Table S2. Biota samples size classification.

Species	Size class	Shell/body length (mm)	Blomstrandhalvøya specimens	Ny-Ålesund specimens
Astarte borealis	1	≤ 35.9	39	
	2	36.0 – 37.9	20	
	3	38.0 – 39.9	15	25
	4	≥ 40.0	12	45
Macoma calcarea	1	≤ 25.5	30	
	2	25.6 – 26.5	15	10
	3	≥ 26.6	30	
Nephtys ciliata	1	≤ 84.9	20	
	2	85.0 – 93.9	25	
	3	94.0 – 102.9		10
	4	≥ 103.0		15

Table S3. Sampling stations in Barents Sea and Kongsfjorden Bay (Svalbard, Norway).

Location	Station	Latitude	Longitude	Water depth (m)	Sediment samples (0.1 m^2)	Biota spp.
E Kong Karls Land	S1	78° 45.8'	32° 53.6'	250	4	n.a.
SE Edgeøya	S2	77° 14.0'	27° 37.0'	230	3	n.a.
SE Edgeøya	S3	77° 13.8'	26° 39.6'	130	3	n.a.
SE Edgeøya	S4	77° 00.0'	26° 00.0'	110	3	n.a.
Snøhvit	S5	71° 37.1'	21° 04.3'	320	5	n.a.
Ny-Ålesund	S6	78° 55.0'	n.a.	15-20	4	*Astarte borealis*, *Macoma calcarea*, *Nephtys ciliata*
Blomstrandhalvøya	S7	78° 59.0'	n.a.	20-25	4	*Astarte borealis*, *Macoma calcarea*, *Nephtys ciliata*
Kapp Guissez	S8	79° 02.0'	n.a.	45-50	4	*Astarte borealis*, *Nephtys ciliata*

n.a.: not available.

Table S4. PAH (µg/kg dry wt.) and OM (% dry wt.) concentrations (mean ± SD) in sediment samples collected in Kongsfjorden Bay and the Barents Sea.

	Barents Sea (October 2013)					Kongsfjorden (July 2013)		
Station	S1	S2	S3	S4	S5	S6	S7	S8
OM	4.97 ± 0.16	9.04 ± 0.10	6.97 ± 0.56	6.15 ± 0.26	8.22 ± 0.46	6.10 ± 0.11	4.25 ± 0.23	4.17 ± 0.29
PHE	86.17 ± 102.61	371.44 ± 284.13	657.79 ± 261.61	1015.60 ± 300.92	17.38 ± 17.38	707.05 ± 84.88	7.52 ± 6.54	16.86 ± 6.02
ANT	0.00 ± 0.00	4.07 ± 5.25	4.76 ± 2.37	10.08 ± 3.32	0.04 ± 0.07	52.14 ± 4.29	0.22 ± 0.25	0.00 ± 0.00
FLT	23.83 ± 25.90	143.81 ± 104.12	118.27 ± 46.70	184.89 ± 57.64	4.02 ± 3.99	197.72 ± 18.81	2.02 ± 1.46	6.88 ± 2.25
PYR	27.59 ± 28.79	113.00 ± 79.28	133.24 ± 54.59	203.51 ± 47.39	5.14 ± 4.03	113.76 ± 13.65	4.03 ± 2.65	6.63 ± 2.89
BaA	8.22 ± 7.35	33.46 ± 22.38	26.60 ± 10.39	32.53 ± 9.84	1.80 ± 0.69	45.80 ± 5.65	0.34 ± 0.07	1.07 ± 0.14
CHR	19.94 ± 20.71	106.93 ± 79.03	149.77 ± 78.80	244.57 ± 64.87	5.22 ± 2.60	63.54 ± 6.77	5.51 ± 2.45	6.25 ± 1.43
BeP	36.93 ± 52.23	217.45 ± 172.38	494.50 ± 207.24	698.89 ± 220.75	0.00 ± 0.00	566.51 ± 96.80	0.00 ± 0.00	2.33 ± 2.21
BbF	52.43 ± 49.31	208.06 ± 141.43	202.79 ± 78.64	248.90 ± 74.94	24.39 ± 16.73	34.16 ± 5.24	3.43 ± 0.60	8.95 ± 1.43
BkF	13.28 ± 11.66	57.62 ± 39.02	45.50 ± 17.86	57.07 ± 17.05	6.08 ± 4.67	27.19 ± 2.26	0.41 ± 0.36	2.32 ± 0.36
BaP	0.00 ± 0.00	71.41 ± 63.65	41.51 ± 40.88	84.99 ± 52.65	0.00 ± 0.00	67.82 ± 16.41	0.00 ± 0.00	1.84 ± 3.68
BghiP	9.26 ± 6.66	26.81 ± 16.97	24.01 ± 9.18	27.23 ± 8.54	4.22 ± 2.41	15.75 ± 1.55	1.11 ± 0.42	1.07 ± 0.18
dBahA	102.77 ± 87.86	226.68 ± 130.41	314.44 ± 144.50	437.48 ± 112.55	17.68 ± 8.56	120.17 ± 20.19	2.05 ± 1.79	9.12 ± 1.80
Ind123P	43.13 ± 34.78	138.96 ± 91.91	87.96 ± 32.30	106.86 ± 30.94	25.80 ± 19.64	9.92 ± 2.08	1.45 ± 1.01	5.68 ± 0.51
Σ_{13}PAH	423.54 ± 427.85	1719.69 ± 1221.84	2301.14 ± 963.26	3352.61 ± 991.97	111.75 ± 76.70	2021.53 ± 260.85	28.09 ± 14.24	68.99 ± 16.48

Table S5. HCB (μg/kg dry wt.), CB (μg/kg dry wt.) and OM (% dry wt.) concentrations (mean ± SD) in sediment samples collected in Kongsfjorden Bay and the Barents Sea. LOD (μg/L) values are provided on Table S1.

	Barents Sea (October 2013)					Kongsfjorden (July 2013)		
Station	S1	S2	S3	S4	S5	S6	S7	S8
OM	4.97 ± 0.16	9.04 ± 0.10	6.97 ± 0.56	6.15 ± 0.26	8.22 ± 0.46	6.10 ± 0.11	4.25 ± 0.23	4.17 ± 0.29
HCB	<0.01	0.21 ± 0.05	0.28 ± 0.10	0.27 ± 0.02	<0.01	<0.01	<0.01	<0.01
PCB 18	0.07 ± 0.05	0.03 ± 0.00	0.06 ± 0.03	0.11 ± 0.17	<0.01	0.01 ± 0.01	<0.01	<0.01
PCB 20	<0.01	<0.01	0.01 ± 0.00	0.02 ± 0.00	<0.01	<0.01	<0.01	<0.01
PCB 28	<0.01	0.01 ± 0.00	<0.01	0.01 ± 0.00	<0.01	<0.01	<0.01	<0.01
PCB 29	0.02 ± 0.02	0.08 ± 0.02	0.01 ± 0.02	<0.01	0.08 ± 0.13	<0.01	0.01 ± 0.01	<0.01
PCB 31	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 44	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 52	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 101	<0.01	<0.01	<0.01	0.01 ± 0.01	<0.01	<0.01	<0.01	<0.01
PCB 105	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 118	<0.01	0.01 ± 0.00	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 138	<0.01	0.01 ± 0.00	<0.01	<0.01	<0.01	0.01 ± 0.02	<0.01	<0.01
PCB 153	<0.01	<0.01	<0.01	<0.01	<0.01	0.02 ± 0.03	<0.01	<0.01
PCB 155	<0.01	0.01 ± 0.00	0.11 ± 0.01	0.09 ± 0.12	<0.01	<0.01	<0.01	<0.01
PCB 170	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01 ± 0.02
PCB 180	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
PCB 194	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02 ± 0.01
PCB 204	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01 ± 0.00
PCB 209	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.02 ± 0.02
Σ_7PCB	<0.01	0.03 ± 0.01	0.01 ± 0.01	0.02 ± 0.01	<0.01	0.05 ± 0.07	0.01 ± 0.01	<0.01

Table S6. Single species lipid-normalised PAH concentrations statistical comparison (Mann-Whitney *U* test *p*-value) between stations (BL: Blomstrandhalvøya; NY: Ny-Ålesund; AB: *Astarte borealis*; MC: *Macoma calcarea*; NC: *Nephtys ciliata*). Only compounds for which statistical significance was found are included.

Station – Spp.	PHE	ANT	FLT	PYR	BaA	CHR	BeP	BbF	BkF	BaP	BghiP	dBahA	Ind123P	Σ_{13}PAH
BL - AB	0.001	0.026	0.000	0.000	0.033	0.001	0.000	0.000	0.001	0.024	0.049		0.002	0.000
NY - AB														
BL - MC														
NY - MC		0.015			0.030					0.007		0.043		
BL - NC	0.003		0.020	0.016	0.003	0.003	0.004	0.009		0.020	0.020	0.004	0.004	0.003
NY - NC														

Table S7. Single station lipid-normalised PAH concentrations statistical comparison (Kruskal-Wallis test *p*-value) between species (BL: Blomstrandhalvøya; NY: Ny-Ålesund; AB: *Astarte borealis*; MC: *Macoma calcarea*; NC: *Nephtys ciliata*). Only compounds for which statistical significance was found are included.

Station – Spp.	PHE	ANT	FLT	PYR	BaA	CHR	BeP	BbF	BaP	dBahA	Ind123P	Σ_{13}PAH
BL - AB								0.006				
BL - MC												
BL - NC									0.002	0.032	0.032	
NY - AB												
NY - MC	0.011	0.003	0.013	0.037	0.023	0.048	0.037		0.005	0.028	0.018	0.023
NY - NC												

Table S8. PAH (µg/kg wet wt.) concentrations in biota samples collected in Kongsfjorden Bay (Svalbard, Norway).

Station	Blomstrandhalvøya						Ny-Ålesund					
Spp.	*Astarte borealis*		*Macoma calcarea*		*Nephtys ciliata*		*Astarte borealis*		*Macoma calcarea*		*Nephtys ciliata*	
n	86		75		45		75		15		25	
Mean lipid wt. (% wet wt.)	1.17		2.67		1.24		3.17		3.23		3.55	
PAH	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PHE	3.84	3.95	7.36	6.65	9.61	9.19	1.06	1.23	15.42	2.53	2.31	1.65
ANT	0.62	1.15	0.26	0.69	0.64	0.75	0.02	0.07	1.98	1.66	0.24	0.45
FLT	1.40	0.93	4.29	2.70	1.76	1.85	0.78	0.60	6.56	1.90	1.09	0.11
PYR	3.97	2.37	6.71	4.65	5.76	6.49	1.95	2.16	14.23	5.21	2.33	2.39
BaA	0.29	0.29	0.65	0.46	0.63	0.48	0.22	0.16	3.00	1.22	0.18	0.09
CHR	1.45	1.14	4.96	3.65	3.38	3.64	0.91	0.87	5.81	2.79	0.88	0.66
BeP	1.58	1.13	3.46	2.51	3.12	2.33	0.74	0.51	3.28	0.98	1.25	1.30
BbF	0.95	0.63	0.65	0.36	0.95	0.85	0.66	0.26	1.49	0.85	0.37	0.40
BkF	0.46	0.35	0.61	0.47	0.48	0.59	0.41	0.33	1.47	1.09	0.27	0.38
BaP	0.44	0.95	0.17	0.19	0.58	0.58	0.16	0.10	1.42	0.33	0.08	0.06
BghiP	0.38	0.40	1.13	0.84	1.07	1.54	0.28	0.18	1.38	1.07	0.35	0.45
dBahA	0.37	0.59	0.25	0.29	0.99	1.46	0.07	0.08	1.47	0.84	0.17	0.22
Ind123P	1.48	1.59	2.85	3.30	4.40	4.64	0.69	1.04	7.45	1.63	1.22	0.99
Σ_{13}PAH	17.23	12.45	33.36	21.48	33.35	32.58	7.96	5.00	64.98	15.52	10.73	7.26

Table S9. PAH (µg/kg lipid wt.) concentrations in biota samples collected in Kongsfjorden Bay (Svalbard, Norway).

Station	Blomstrandhalvøya						Ny-Ålesund					
Spp.	*Astarte borealis*		*Macoma calcarea*		*Nephtys ciliata*		*Astarte borealis*		*Macoma calcarea*		*Nephtys ciliata*	
n	86		75		45		75		15		25	
PAH	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PHE	342.85	382.59	379.76	420.04	763.11	711.10	32.31	31.51	483.54	110.56	64.79	47.00
ANT	60.28	111.00	11.80	28.72	51.81	60.71	0.42	1.52	60.99	52.88	7.06	13.77
FLT	123.91	88.75	185.52	130.43	138.90	143.57	26.33	22.92	205.59	67.23	30.75	3.85
PYR	355.52	230.85	324.16	302.83	449.05	497.74	87.11	117.54	449.77	190.63	63.73	62.50
BaA	24.25	24.51	30.71	30.45	50.22	36.67	8.93	12.07	94.10	41.53	4.95	2.48
CHR	128.34	103.90	241.95	263.73	270.09	280.77	38.98	52.72	184.03	98.54	24.85	18.74
BeP	142.19	104.87	180.21	191.05	253.40	184.58	25.21	15.89	103.23	36.88	36.52	40.24
BbF	82.17	53.70	29.27	23.05	74.90	64.75	23.49	12.82	47.60	28.78	10.14	10.73
BkF	38.32	23.70	30.28	35.54	37.57	45.96	15.99	15.85	47.38	35.85	7.81	11.53
BaP	39.39	91.91	5.62	6.86	45.24	44.24	6.04	4.55	44.05	10.22	2.32	1.54
BghiP	29.71	27.58	46.58	31.25	84.41	118.67	10.90	9.67	44.06	34.81	10.10	13.61
dBahA	31.73	49.66	14.17	21.17	77.03	112.76	3.56	5.48	47.02	28.16	4.61	5.66
Ind123P	130.84	139.79	147.84	217.94	345.74	358.12	24.48	31.13	233.28	63.41	34.59	28.62
Σ_{13}PAH	1529.50	1184.37	1627.87	1526.95	2641.48	2497.47	303.74	244.98	2044.63	613.36	302.22	207.35

Table S10. *Astarte borealis* PAH (µg/kg lipid wt.) concentrations in samples collected in Kongsfjorden Bay (Svalbard, Norway) for different size classes.

Station	Blomstrandhalvøya								Ny-Ålesund			
Size class	1		2		3		4		3		4	
n	39		20		15		12		25		45	
PAH	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PHE	641.50	529.63	148.12	105.09	124.57	144.14	322.99	235.38	51.03	38.14	22.96	26.11
ANT	90.34	147.84	1.40	2.80	6.84	11.84	142.14	137.33	1.18	2.63	0.04	0.12
FLT	179.58	107.34	94.51	64.29	73.00	17.58	121.22	110.47	29.66	22.51	24.51	25.59
PYR	471.00	270.22	315.55	194.01	212.59	171.25	359.27	272.05	135.60	99.53	67.79	129.40
BaA	35.19	28.43	20.27	23.43	28.35	30.99	7.23	4.09	6.38	4.55	10.13	15.44
CHR	171.20	147.48	135.82	62.77	75.84	38.26	99.40	120.80	56.02	34.82	33.85	62.11
BeP	232.67	125.92	99.04	52.16	83.09	15.30	108.03	98.90	36.93	19.09	19.83	11.31
BbF	129.30	59.77	62.96	34.34	68.93	44.86	42.49	18.59	30.25	16.87	20.59	10.00
BkF	45.81	20.35	35.62	25.81	42.57	37.98	25.17	14.84	22.84	13.49	13.05	17.38
BaP	89.06	155.17	22.56	29.82	9.65	11.10	8.80	7.46	6.49	3.67	6.03	5.37
BghiP	41.83	17.82	24.87	28.97	37.80	46.74	7.86	4.07	14.15	9.11	9.10	10.58
dBahA	69.00	70.62	21.88	31.89	2.80	3.11	11.68	15.06	4.53	4.88	3.42	6.20
Ind123P	241.14	187.24	111.67	90.53	43.03	18.85	60.40	62.18	50.60	31.30	12.54	23.51
Σ_{13}PAH	2437.63	1520.01	1094.29	611.32	809.06	512.61	1316.67	1043.02	445.66	195.41	243.83	256.27

Table S11. *Astarte borealis* PAH BSAFs. BSAFs of a specific compound were calculated provided that there were at least three detects in sediment samples.

Station	**Blomstrandhalvøya**								**Ny-Ålesund**			
Size class	1		2		3		4		3		4	
n	39		20		15		12		25		45	
Mean lipid wt. (% wet wt.)	1.12		1.13		1.31		1.18		3.09		3.20	
PAH	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PHE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.00	0.00	0.00	0.00
ANT	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.00	0.00	0.00	0.00
FLT	3.61	2.16	1.90	1.29	1.47	0.35	2.44	2.22	0.01	0.01	0.01	0.01
PYR	4.74	2.72	3.18	1.95	2.14	1.73	3.62	2.74	0.07	0.05	0.04	0.07
BaA	4.17	3.37	2.40	2.78	3.36	3.67	0.86	0.49	0.01	0.01	0.01	0.02
CHR	1.26	1.09	1.00	0.46	0.56	0.28	0.73	0.89	0.05	0.03	0.03	0.06
BeP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.00	0.00	0.00	0.00
BbF	1.53	0.71	0.74	0.41	0.82	0.53	0.50	0.22	0.05	0.03	0.04	0.02
BkF	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.05	0.03	0.03	0.04
BaP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.01	0.00	0.01	0.00
BghiP	1.17	0.50	0.70	0.81	1.06	1.31	0.22	0.11	0.09	0.06	0.06	0.07
dBahA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.00	0.00	0.00	0.00
Ind123P	8.84	6.86	4.09	3.32	1.58	0.69	2.21	2.28	0.20	0.12	0.05	0.09

n.a.: not available.

Table S12. *Astarte borealis* PAH BSAFs: station effect. Only compounds for which ≥ 90% BSAF values were higher than zero in both stations were included in the statistical analysis. Maximum mean ranks for all significant results were found in Blomstrandhalvøya specimens.

			Test statistics[a]				
			FLT	BaA	CHR	BbF	BghiP
Mann-Whitney *U*			0.000	0.000	2.000	0.000	8.000
Wilcoxon *W*			120.000	120.000	122.000	120.000	128.000
Z			-4.667	-4.666	-4.585	-4.666	-4.335
Asymp. sig. (2-tailed)			0.000	0.000	0.000	0.000	0.000
Exact sig. [2*(1-tailed sig.)]			0.000[b]	0.000[b]	0.000[b]	0.000[b]	0.000[b]
Monte Carlo sig. (2-tailed)	Sig.		0.000[c]	0.000[c]	0.000[c]	0.000[c]	0.000[c]
	99% confidence interval	Lower bound	0.000	0.000	0.000	0.000	0.000
		Upper bound	0.000	0.000	0.000	0.000	0.000
Monte Carlo sig. (1-tailed)	Sig.		0.000[c]	0.000[c]	0.000[c]	0.000[c]	0.000[c]
	99% confidence interval	Lower bound	0.000	0.000	0.000	0.000	0.000
		Upper bound	0.000	0.000	0.000	0.000	0.000

[a] Grouping variable: Station
[b] Not corrected for ties.
[c] Based on 10000 sampled tables with starting seed 2000000.

Table S13. *Macoma calcarea* PAH BSAFs. BSAFs of a specific compound were calculated provided that there were at least three detects in sediment samples.

Station	Blomstrandhalvøya						Ny-Ålesund	
Size class	1		2		3		2	
n	30		15		30		10	
Mean lipid wt. (% wet wt.)	2.84		2.72		2.57		3.09	
PAH	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PHE	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.05	0.00
ANT	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.07	0.09
FLT	2.07	1.72	5.60	2.57	3.63	2.75	0.07	0.02
PYR	2.73	3.17	2.35	0.83	3.99	3.86	0.29	0.08
BaA	2.40	3.21	3.48	0.76	4.34	4.77	0.15	0.06
CHR	1.03	1.12	1.43	0.67	2.34	2.62	0.22	0.09
BeP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.01	0.00
BbF	0.22	0.22	0.28	0.16	0.44	0.33	0.11	0.01
BkF	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.15	0.00
BaP	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.04	0.01
BghiP	0.96	0.81	0.79	0.35	1.74	0.96	0.37	0.17
dBahA	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	0.03	0.00
Ind123P	5.34	6.09	1.27	1.72	7.53	10.42	0.99	0.28

n.a.: not available.

Table S14. *Macoma calcarea* PAH BSAFs: station effect. Only compounds for which ≥ 90% BSAF values were higher than zero in both stations were included in the statistical analysis. Maximum mean ranks for all significant results were found in Blomstrandhalvøya specimens.

Test statistics[a]								
			FLT	PYR	BaA	CHR	BbF	BghiP	Ind123P
Mann-Whitney *U*			0.000	1.000	1.000	3.000	5.000	3.000	10.000
Wilcoxon *W*			6.000	7.000	7.000	9.000	11.000	9.000	16.000
Z			-2.598	-2.454	-2.454	-2.165	-1.876	-2.165	-1.155
Asymp. sig. (2-tailed)			0.009	0.014	0.014	0.030	0.061	0.030	0.248
Exact sig. [2*(1-tailed sig.)]			0.004[b]	0.009[b]	0.009[b]	0.031[b]	0.070[b]	0.031[b]	0.295[b]
Monte Carlo sig. (2-tailed)	Sig.		0.005[c]	0.010[c]	0.010[c]	0.032[c]	0.072[c]	0.032[c]	0.298[c]
	99% confidence interval	Lower bound	0.003	0.007	0.007	0.027	0.066	0.027	0.287
		Upper bound	0.007	0.012	0.012	0.036	0.079	0.036	0.310
Monte Carlo sig. (1-tailed)	Sig.		0.003[c]	0.006[c]	0.006[c]	0.017[c]	0.037[c]	0.017[c]	0.147[c]
	99% confidence interval	Lower bound	0.001	0.004	0.004	0.014	0.032	0.014	0.138
		Upper bound	0.004	0.007	0.007	0.020	0.042	0.020	0.156

[a] Grouping variable: Station
[b] Not corrected for ties.
[c] Based on 10000 sampled tables with starting seed 2000000.

Table S15. *Nephtys ciliata* PAH BSAFs. BSAFs of a specific compound were calculated provided that there were at least three detects in sediment samples.

Station	**Blomstrandhalvøya**				**Ny-Ålesund**			
Size class	1		2		3		4	
n	20		25		10		15	
Mean lipid wt. (% wet wt.)	1.24		1.24		3.60		3.51	
PAH	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PHE	n.a.	n.a.	n.a.	n.a.	0.00	0.01	0.01	0.00
ANT	n.a.	n.a.	n.a.	n.a.	0.00	0.00	0.01	0.02
FLT	1.86	1.35	3.54	3.70	0.01	0.00	0.01	0.00
PYR	2.57	1.95	6.08	6.37	0.04	0.06	0.03	0.02
BaA	4.61	0.95	7.02	5.82	0.00	0.01	0.01	0.00
CHR	1.43	0.63	2.43	2.78	0.02	0.01	0.03	0.02
BeP	n.a.	n.a.	n.a.	n.a.	0.00	0.00	0.01	0.01
BbF	0.95	0.54	0.84	0.97	0.02	0.02	0.02	0.02
BkF	n.a.	n.a.	n.a.	n.a.	0.01	0.01	0.02	0.03
BaP	n.a.	n.a.	n.a.	n.a.	0.00	0.00	0.00	0.00
BghiP	2.34	1.58	3.70	5.91	0.04	0.04	0.08	0.11
dBahA	n.a.	n.a.	n.a.	n.a.	0.00	0.00	0.00	0.00
Ind123P	7.61	4.18	11.37	13.45	0.12	0.10	0.14	0.14

n.a.: not available.

Table S16. *Nephtys ciliata* PAH BSAFs: station effect. Only compounds for which ≥ 90% BSAF values were higher than zero in both stations were included in the statistical analysis. Maximum mean ranks for all significant results were found in Blomstrandhalvøya specimens.

			Test statistics[a]					
			FLT	BaA	CHR	BbF	BghiP	Ind123P
Mann-Whitney *U*			5.000	0.000	0.000	0.000	0.000	0.000
Wilcoxon *W*			20.000	15.000	15.000	15.000	15.000	15.000
Z			-2.333	-3.000	-3.000	-3.000	-3.000	-3.000
Asymp. sig. (2-tailed)			0.020	0.003	0.003	0.003	0.003	0.003
Exact sig. [2*(1-tailed sig.)]			0.019[b]	0.001[b]	0.001[b]	0.001[b]	0.001[b]	0.001[b]
Monte Carlo sig. (2-tailed)	Sig.		0.020[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]
	99% confidence interval	Lower bound	0.016	0.000	0.000	0.000	0.000	0.000
		Upper bound	0.023	0.002	0.002	0.002	0.002	0.002
Monte Carlo sig. (1-tailed)	Sig.		0.010[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]	0.001[c]
	99% confidence interval	Lower bound	0.008	0.000	0.000	0.000	0.000	0.000
		Upper bound	0.013	0.001	0.001	0.001	0.001	0.001

[a] Grouping variable: Station
[b] Not corrected for ties.
[c] Based on 10000 sampled tables with starting seed 2000000.

3 Bioaccumulation of polycyclic aromatic hydrocarbons by Arctic and temperate benthic species

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Chapter 3

Abstract

Increasing oil and gas activities may substantially increase chemical stress to benthic ecosystems in the Arctic. Polycyclic aromatic hydrocarbons (PAHs) can be used as indicator compounds for this increase. For temperate benthos, more information is available on the potential accumulation of PAHs, which may be useful to address Arctic species. Here we compare for the first time, the bioaccumulation of PAHs by Arctic benthic invertebrate species, with that of temperate invertebrate species. PAH biota-sediment accumulation factors (BSAFs) ranged 0.01 to 2.48 and were generally higher in the temperate bivalve (*Limecola balthica*) than in the Arctic bivalve (*Macoma calcarea*, ranging 0.00 – 0.21). Arctic polychaetes (*Nephtys ciliata*) showed higher BSAFs (0.00 – 1.79) than temperate polychaetes (*Alitta virens*, 0.00 – 0.34). Differences in bioaccumulation were found among Arctic and temperate species, reflecting species-specific feeding modes and traits. Bioaccumulation modelling revealed that steady state was not likely to be reached in the 28-d tests for all chemicals and organisms. Due to low numbers of individuals, most species-specific parameters carried too much uncertainty to reveal differences between Arctic and temperate species. No preference for ingestion of sediment organic matter (OM) over that of suspended OM was detected, indicating a high PAH affinity for OM. The results from the present study did not detect crucial differences in bioaccumulation between temperate and Arctic species. This means that there is no indication that data from temperate species cannot be used as a proxy for Arctic species in risk assessment.

1. Introduction

The Arctic has a high sensitivity to oil spill impacts and has limited capacity for natural recovery due to a very pronounced seasonality, mainly dictated by the reduction in sea ice. Sensitivity may be increased by expanding shipping and oil and gas (O&G) activities and the lack of appropriate oil spill response methods for this area [199]. Therefore, biological targets (i.e., bioindicators) for priority monitoring during all phases of O&G activities should be used, to assess, minimize and mitigate adverse effects [199]. Polycyclic aromatic hydrocarbons (PAHs) constitute a large group of hydrophobic contaminants and have been the focus of previous environmental assessments because of their potential toxicity and bioaccumulation [23,200]. Such chemical properties may be used to select appropriate bioindicators of acute and chronic effects of O&G production.

Besides knowledge about chemical concentrations in the abiotic environment, bioindicators are considered particularly useful to monitor trends in O&G related pollution, as they integrate chemical and non-chemical stress and account for *in situ* ecological conditions [74,201]. Arctic benthic systems are characterised by a high trophic diversity, relatively long life-span and sedentary lifestyles of species [134], which makes them suitable for monitoring purposes, particularly in areas of O&G production. A particularly useful exposure metric to define the bioindicator potential of O&G related bioaccumulative chemicals in benthic invertebrates is the biota-sediment accumulation factor (BSAF) of PAHs, which can adequately mirror the pollution state of the local Arctic ecosystem near O&G activities [202]. Compared to bioaccumulation (i.e., net result of influx (uptake) and efflux of contaminants) by temperate species, little is known on bioaccumulation of PAHs from sediment by Arctic benthic species. Cross-chemical extrapolation techniques such as read-across may help to predict PAH bioaccumulation by Arctic species in the absence of experimental data [203]. If bioaccumulation would be comparable between Arctic and temperate species with similar traits, risk assessment for the Arctic might be simplified by using temperate species data as a surrogate for Arctic species. Therefore, it is useful to explore (a) the potential for reading across Arctic and temperate species and (b) the potential for reading across species with different feeding traits and from the same region, with respect to bioaccumulation. Similarity between Arctic and temperate species however may not be self-evident. For instance, exposure times to contaminants may be longer in Arctic systems due to, for instance, Arctic species having a longer life-span or biological reaction times being generally slower in polar

biota than in temperate biota [125]. Clearance of PAHs and their metabolites may also be slower in Arctic invertebrates, since chemical elimination rates are temperature-dependent and affected by seasonal variability in lipid content [14]. Finally, seasonality in the environmental conditions in the Arctic may have a major impact on the physiology of local species [36].

The BSAF has traditionally been used as a metric to assess the bioaccumulation of contaminants from sediment and has been compared across a wide range of aquatic ecosystems [80-82]. If Equilibrium Partitioning Theory (EPT) applies, BSAF values can be expected to range between 1 and 2 [204]. This is based on the assumption that chemicals partition between biota lipids and sediment organic carbon. However, EPT does not consider the possibility of feeding as a route of uptake leading to higher than equilibrium steady-state concentrations. In order to accommodate such situations where EPT does not apply, a kinetic BSAF model was used [56,83]. Potentially, the BSAF may be a useful metric to compare effects of species traits on bioaccumulation, because it can correct for differences in chemical concentration, sediment organic matter (OM) as well as organism lipid content between the sites or species that are compared. For benthic invertebrates, of which the geographic distribution can span to (sub-)Arctic areas, we are aware of only two studies reporting BSAFs [143,**Chapter 2**].

The objective of the present study is to compare bioaccumulation between four Arctic and two temperate species, with different species traits, using 28-d whole-sediment tests [56,83]. An additional objective was to assess whether the results can be generalized by using a time-dependent BSAF model, previously used for describing bioaccumulation in temperate marine and freshwater benthic invertebrates [56,83]. For the first time, we explore to what extent BSAF is useful as a metric for bioaccumulation in Arctic benthic invertebrate species and for conducting a comparison between Arctic and temperate species for bioaccumulation.

2. Materials and Methods

Based on their feeding habits, sessility and relative abundance [144], four Arctic benthic species were selected; three bivalve species: *Astarte borealis* (Schumacher, 1817), *Macoma calcarea* (Gmelin, 1791), *Nuculana pernula* (O.F. Müller, 1771) and a polychaete species: *Nephtys ciliata* (O.F. Müller, 1776). These species are primarily abundant in Arctic climate zones, although they are not restricted to this area (Table S1). Additionally, two temperate benthic species (a bivalve: *Limecola balthica* [Linnaeus, 1758] and a polychaete: *Alitta virens*

[Sars, 1835], formerly known as *Macoma balthica* and *Nereis virens*) were selected based on their comparable feeding habits, sessility, availability of chronic test protocols and thus the potential for a comparison with the Arctic species. We limited the present study to two temperate species because we prioritized comparing bioaccumulation among Arctic and temperate deposit feeding bivalves and polychaetes. Due to scarcity of *M. calcarea* individuals at the sampling area, a second Arctic deposit feeder (*N. pernula*) was included. Since *M. calcarea* may feed both on suspended and sediment OM, an obligate suspension feeder (*A. borealis*) was also included to estimate the contribution of suspended OM to the bioaccumulation of PAHs by *M. calcarea*.

2.1. Test organisms

We performed a 28-d sediment bioaccumulation experiment, with Arctic species and conditions at Kings Bay AS (Ny-Ålesund, Svalbard) between September and October 2014, here onwards named as "AE" (Arctic Experiment); and one with temperate species and conditions at Wageningen Marine Research (Yerseke, the Netherlands) between July and August 2014, here onwards named as "TE" (Temperate Experiment). Sediments in the Arctic (AE) and temperate (TE) experiments were the same for the same phylum. Emphasis was on simulating environmentally realistic PAH exposure levels, which implied use of representative species of the macrobenthic community of each climate region and field-contaminated sediments. This also implied that sometimes numbers of individuals were too low to allow for testing all sediment treatments. This is a limitation inherent to Arctic marine ecological research.

Permission for sampling of Arctic and temperate species was issued by The Governor of Svalbard and the Province of Zeeland, respectively. Sampling of Arctic species was performed along a transect from Tønsneset (79°0'22"N, 11°57'10"E) to Blomstrandhalvøya (78°59'14"N, 11°57'28"E), Kongsfjorden Bay (Svalbard, Norway), whereas *L. balthica* was collected at low tide at the Oesterdam (The Netherlands, 51°26'24"N, 4°13'16"E). *A. virens* was obtained from a professional bait farm, Topsy baits (Wilhelminadorp, the Netherlands). Arctic and temperate species were acclimatized under test conditions. Arctic species were kept in filtered natural sea water (20 µm) during 2 to 6 d without food supply, whereas in the TE experiment animals were kept in filtered natural sea water (0.2 µm) during 5 d and were fed once at the start of the acclimatization. For further details of the acclimatization of test species and sediment collection, see the Supporting Information (SI).

2.2. Sediment treatments preparation

Since both *A. borealis* and *M. calcarea* usually reside in sandy sediment and *N. ciliata* is generally found in sandy mud [40], two different batches of sediment were prepared after sediment collection to ensure an optimal habitat for the test species. Each contained different ratios of muddy and coarse sediment, respectively defined as < 0.5 and 0.5 – 1 mm grain size. Bivalves sediment (BS) contained two-thirds of coarse and one-third of muddy sediment, whereas polychaetes sediment (PS) contained two-thirds of muddy and one-third of coarse sediment, based on volume. Sediment was collected in the Oosterschelde estuary (The Netherlands, 51°36'13.5"N, 3°47'49.3"E) on 24 to 25 April 2014 and 2 May 2014 and used to create the sediment treatments for both the AE and TE experiments. This was done to ensure homogeneity of exposure of treatments among experiments. These sediment mixtures contain background PAH levels and therefore are referred to as the 'low' (L) treatment hereafter (BSL, PSL). PAH concentrations differed between BSL and PSL treatments (Tables S2 and S3) due to differences in their mud content and other variables creating sediment heterogeneity, and were one order of magnitude higher than in sediment previously collected at the Arctic species collection site (Blomstrandhalvøya) (**Chapter 2**).

From each of these two initial batches with low chemical concentration, another two sediment treatments were prepared with a higher chemical concentration, referred to as the 'medium' (M) and 'high' (H) concentration. The 'medium' treatment contained 5% (vol.) of harbour sediment (Rotterdam, the Netherlands), while the 'high' treatment contained 10% (vol.) of harbour sediment. These percentages were used (a) on a precautionary basis since neither the harbour sediment chemical concentrations nor the effect threshold for each of the species were known and (b) to be able to link non-lethal effects of chemical concentration to endpoints studied (**Chapter 4**). These preparations thus resulted in six sediments: three for bivalves (BSL, BSM, BSH) and three for polychaetes (PSL, PSM, PSH) (Figure S1). All sediment treatments were thoroughly mixed with a turbine mixer for approximately 15 min before storage/transport from the Netherlands to Svalbard and again right before use in the exposure experiments. Of all sediment treatments, chemical concentrations and other characteristics (i.e., dry wt. and OM content) were assessed (Tables S2 and S3). Due to logistics, storage time (3 – 7 °C) for sediment treatments was 9 weeks longer in the AE than in the TE experiment. Prior to start of exposure, sediment treatments were allowed to stay in contact with filtered sea water in a 1:6 sediment-to-water volume ratio without aeration for 3 d and

with aeration for the following 4 d. Some experimental units in the AE experiment were aerated for 12 to 17 d because biota field sampling took place for a longer period than initially expected (the first 3 weeks since the aeration started).

2.3. Experimental design

Experimental designs were similar for AE and TE except for ambient temperature and photoperiod. The AE experiment was a 28-d test in a temperature controlled room at 3 °C under a photoperiod of 12 h light: 12 h dark. All planned sediment treatments were run with *N. ciliata* (PSL, PSM and PSH) (n = 1 – 3; Table S4). However, due to low numbers of available organisms, only two treatments (BSL and BSH) were tested with *M. calcarea* (n = 1 – 2) and only one treatment (BSH) was tested with *A. borealis* and *N. pernula* (n = 2 – 3; Table S4).

The TE experiment was a 28-d test in a temperature controlled room at 18 °C under a photoperiod of 16 h light: 8 h dark. All sediment treatments were tested with *A. virens* (PSL, PSM and PSH) (n = 2 – 3). Due to low availability of test organisms only two treatments were tested with *L. balthica* (BSL and BSH) (n = 1 – 3).

For statistical purposes, only measured data for BSH and PSH treatments were used due to either a low level of replication of BSL and PSM treatments (Table S4) or the heterogeneity among PSL replicates in their OM content (Tables S2 and S3). Data for all treatments were used for bioaccumulation modelling.

Individuals were randomly divided in groups of 16 to 50 individuals per experimental unit (Table S4). Animals were not fed during exposure, although sediment OM content (2 – 6%) and in some cases food supply during acclimatization (i.e., temperate species) prevented body [56] and lipid weight loss (Tables S5 and S6) during the experiment.

2.4. Endpoints

Prior to the start and at end of the experiments, organisms were allowed to depurate their guts for 24 h in filtered sea water. At the end of each experiment, animals were weighed, measured for body or shell length, dissected (e.g., bivalves) and pooled by species. Samples were stored at -20 °C for later determination of wet wt. and lipid fraction, expressed as % wet wt., and chemical concentrations (µg/kg wet wt.). Experimental units were checked daily for mortality (i.e., animals at surface and immobile after poked), and dead organisms were removed daily.

At the start of the AE experiment only subsamples of *A. borealis* and *N. pernula* were archived, to assess the initial conditions ('Background'). This was however, not feasible for *M. calcarea* and *N. ciliata* due to the low number of individuals. At the end of the experiments, samples of each sediment treatment were stored at -20 °C for later determination of dry wt., OM content and chemical concentrations (µg/kg dry wt.). Sediment samples were also taken at the beginning of the AE experiment (Table S2). Biota and sediment samples from both experiments were shipped frozen in dry-ice to Wageningen University (Wageningen, the Netherlands) for chemical analysis.

2.5. Chemical analysis

Chemical analysis was conducted according to methods used by Kupryianchyk et al. [150]. The following PAHs were analysed: Phenanthrene (PHE), Anthracene (ANT), Fluoranthene (FLT), Pyrene (PYR), Benz(a)anthracene (BaA), Chrysene (CHR), Benzo(e)pyrene (BeP), Benzo(b)fluoranthene (BbF), Benzo(k)fluoranthene (BkF), Benzo(a)pyrene (BaP), Benzo(ghi)perylene (BghiP), Dibenz(a,h)anthracene (dBahA) and Indeno(1,2,3-cd)pyrene (Ind123P). PAH recoveries were 85 to 94% and 64 to 75% for biota and sediment samples, respectively. Three blanks per each batch of 30 samples were used, and values were corrected for blanks. Benzo(k)fluoranthene and benzo(a)pyrene could not be accurately determined due to overlapped retention times with an unknown compound. For further details on extraction, clean-up and instrumental analysis see the SI.

2.6. Data analyses

Lipid-normalised concentrations after 28 d were calculated in biota samples for all available sediment treatments. Biota-sediment accumulation factors were calculated as $(C_{org}/f_{lip})/(C_{sed}/f_{OC})$ with C_{org} being the chemical concentration in the organism (µg/kg wet wt.), C_{sed} the chemical concentration in sediment (µg/kg dry wt.), f_{lip} the fraction of lipids in the organism based on wet weight and f_{OC} the fraction of sediment organic carbon (OC) based on loss of ignition and an OC/OM conversion ratio of 0.4 [56,83]. Lipid-normalised biota concentrations and BSAFs of BSH exposed bivalves and PSH exposed polychaetes were checked for normality with Q-Q plots and Shapiro-Wilk test and equality of variances with Levene's test. If data were normally distributed, lipid-normalised biota concentrations and BSAFs were tested for species and climate region effect with a one-way ANOVA or an independent *t* test, respectively, for each PAH compound. If data were non-normally

distributed, these were log-transformed, and in case a normal distribution still was not reached, lipid-normalised biota concentrations and BSAFs were tested for species and climate region effect with the Kruskal-Wallis test and Mann-Whitney *U* test in combination with Kolmogorov-Smirnov *Z* test, respectively, for each PAH compound. Significance level of an overall statistical comparison was set at $p \leq 0.05$, while for pairwise comparisons among species or chemicals the Bonferroni and Holm's sequential Bonferroni corrections were applied, respectively. All statistical calculations were performed using SPSS version 22.

2.7. Bioaccumulation modelling

The usefulness of modelling bioaccumulation in invertebrate lipids was explored according to methods described in Diepens et al. [56], following previously published models (e.g., [205]). In short:

$$BSAF_t = \frac{C_{L,t=0}}{C_{OC,t=0}^{SED}} \times e^{-(k_e+k_g)t} + \frac{\frac{k_e \times K_{lip}}{K_{OC}^{SED}} + \alpha \times I \times [\beta + (1-\beta) \times \gamma]}{(k_e+k_g)} \times \left(1 - e^{-(k_e+k_g) \times t}\right) \quad (1)$$

in which $C_{L,t=0}$ (μg × kg^{-1}) is the measured concentration in the biota at time zero, normalised to lipid content, $C_{OC,t=0}^{SED}$ (μg × kg^{-1}) is the measured concentration in sediment at time zero, normalised to OC content, t (d) is time, k_e and k_g (d^{-1}) are the rate constants for overall elimination and growth dilution, K_{lip} is the ratio k_w / k_e which equates to an apparent lipid-water partition coefficient, and k_w the rate constant for dermal uptake (L × kg^{-1} × d^{-1}), α (-) is the chemical assimilation efficiency (assumed to be independent of food source) and I (≥ 0, kg$_{OC}$ × kg$_{Lipids}^{-1}$ × d^{-1}) represents the mass of ingested OC per unit of time and organism lipid weight, β ($0 < \beta < 1$) is the fraction of ingested OC originating from the sediment, whereas $1 - \beta$ is the fraction of ingested OC originating from the suspended and freshly deposited (sediment top layer) solids, and γ is a constant ratio between the sorption affinities for suspended OC and sediment OC ($K_{OC}^{SS} = \gamma K_{OC}^{SED}$). Note that Eq. (1) models an OC based BSAF, whereas Diepens et al. [56] used a similar version yet normalised on OM. The ingested OC is thus allowed to originate partly from suspended solids (SS) from the overlying water and partly from the sediment (SED). Ingestion of multiple food items by benthic invertebrates has been modelled before in a similar manner [56,80,83].

The parametrization process and calculations of the percentage of uptake through water and of the fraction of steady state reached in the bioaccumulation test have been described before

[56,83] and are provided in the SI. For further explanation of modelling and calculation of 90% Confidence Intervals (CIs) the reader is referred to the SI.

3. Results and Discussion

3.1. PAH background concentrations

In the Arctic bivalve *A. borealis* average Σ_{13}PAH concentrations when the animals were collected were 9.4 mg/kg lipid wt. This was 9 times higher than in *A. borealis* individuals collected by Szczybelski et al. (**Chapter 2**) at the same sampling area (Blomstrandhalvøya) in July 2013 (Figure S4; Table S10). Concentrations of PHE and CHR were highest (46% of the Σ_{13}PAH) in *A. borealis* collected in September, whereas individuals collected in July accumulated PHE and PYR (46% of the Σ_{13}PAH) which was similar to concentrations analysed in local sediments by Szczybelski et al. (**Chapter 2**). Changes of an order of magnitude in PAH concentrations between both years were observed for PHE, ANT, CHR and BghiP. A significant increase in CHR *A. borealis* concentration would comply with generally high 4-ring PAHs concentration in sediment surface layers from the inner areas of Kongsfjorden [206], whereas an increase in BghiP might be linked to some disturbance of deeper layers [206] and/or deposition of terrestrial petrogenic PAHs after bedrock erosion and sediment transport by Blomstrandhalvøya rivers and tributary streams during late summer [207].

In the temperate bivalve *L. balthica* average Σ_{13}PAH concentrations in individuals collected at Oesterdam (The Oosterschelde National Park, the Netherlands) were 35.5 mg/kg lipid wt. (Table S6). Concentrations of PYR and BaA were below concentrations found in *L. balthica* collected in the Westerschelde estuary (The Netherlands) [208]. In the temperate polychaete *A. virens*, Σ_{13}PAH concentrations were lower than in *L. balthica* (10.4 mg/kg lipid wt.) (Table S6). This may be expected as *A. virens* were obtained from an aquaculture farm and *L. balthica* were collected in the field.

3.2. Effects of Arctic species traits on PAH bioaccumulation

Lipid-normalised Σ_{13}PAH concentrations in Arctic invertebrates in the high treatment (BSH or PSH) were 13.1 mg/kg in *M. calcarea*, 20.2 mg/kg in *N. ciliata*, 37.7 mg/kg in *A. borealis* and 144.8 mg/kg in *N. pernula* (Table S5). The 3- to 4-ring PAHs were generally accumulated by the filter feeder *A. borealis* which agreed with a higher fraction of these compounds in

BSH sediment. The 5- to 6-ring PAHs were strongly accumulated (i.e., up to one order of magnitude higher than background concentrations) by the deposit feeder *N. pernula* which did not show this same agreement with BSH sediment composition. This disagreement might be explained by a higher PAH accumulation by *N. pernula* than the other two Arctic bivalves prior to the experiment start [209], slow PAH excretion [210] and non-equilibrium between *N. pernula* lipid tissue and sediment during the AE experiment.

The 3- to 4-ring PAHs (i.e., PHE, ANT, FLT and PYR) concentrations were on average 2.5 to 4 times higher in the BSH exposed filter feeder *A. borealis* compared to the deposit feeding bivalves (*M. calcarea* and *N. pernula*). An increase in FLT and PYR concentrations can be explained by the fact that the concentrations of these PAHs in BSH sediment were also high (Table S2). Concentrations of CHR were on average 42 times higher in BSH exposed *N. pernula* than *A. borealis* (Table S5), although CHR concentration in BSH sediment was very low (Table S2). In this case, considerably high CHR concentrations in *N. pernula* might be explained by the species ability to ingest sediment to a larger extent than the other Arctic bivalves [209] and to retain PAHs as observed by Neff et al. [210]. In the polychaete *N. ciliata*, differences in the concentration of PHE, FLT, PYR, BaA, BbF and BghiP between the low (PSL) and high (PSH) exposed individuals reflected the difference in concentrations measured in the PSL and PSH sediments (Tables S2 and S5).

No statistical differences in PAH concentrations were found between the deposit feeding bivalves *M. calcarea* and *N. pernula* in the BSH exposures. However, this conclusion should be made with caution as the number of samples available per species was low and the two species show different feeding behaviours (Table S1; [136,209,211]). Variability in metabolism of PAHs by some of our target species (*A. virens*, *L. balthica*) is well described in the literature [139], with generally higher metabolism rates in temperate polychaetes than bivalves. Although not evaluated in the present study, an increase in general metabolic activity [212,213] and a moderate production of reactive oxygen in Arctic species at the end of summer [214,215] may promote the efficiency and prevent the inhibition of PAH biotransformation, respectively. Metabolism of PAHs in Arctic species is expected to be lower than in temperate species due to low temperature and generally low food availability [73,104].

Polycyclic aromatic hydrocarbons BSAFs were generally low (i.e., BSAF <1) and higher in BSH exposed *A. borealis* than PSH exposed *N. ciliata*. This may be linked to a higher black

carbon (BC) and amorphous OM content in PSH sediment, which would decrease PAH bioavailability in polychaetes. Harbour sediments are known to contain more BC [53,216] than sediments from the collection site (The Oosterschelde National Park, the Netherlands) and the Rotterdam harbour area has been subject to considerable BC deposition [217]. The BSAFs for all PAHs ranged from < 0.0001 to 18, but they were often smaller than one which is considered to be caused by the strong sorption of the planar PAHs to BC [196,218]. The 10-90th percentiles of the BSAF for all PAHs in the high treatment (BSH or PSH) exposed biota ranged 0.02 to 1.13 for *A. borealis*, 0.01 to 0.21 for *M. calcarea*, 0.03 to 16.64 for *N. pernula* and 0.01 to 1.70 for *N. ciliata* (Figure 1; Table S11). The BSAFs for PHE, ANT, FLT and PYR were on average 2 to 5 times higher in *A. borealis* compared to the deposit feeding bivalves, whereas CHR BSAFs were up to 58 times higher in *N. pernula* than in the other two Arctic bivalves.

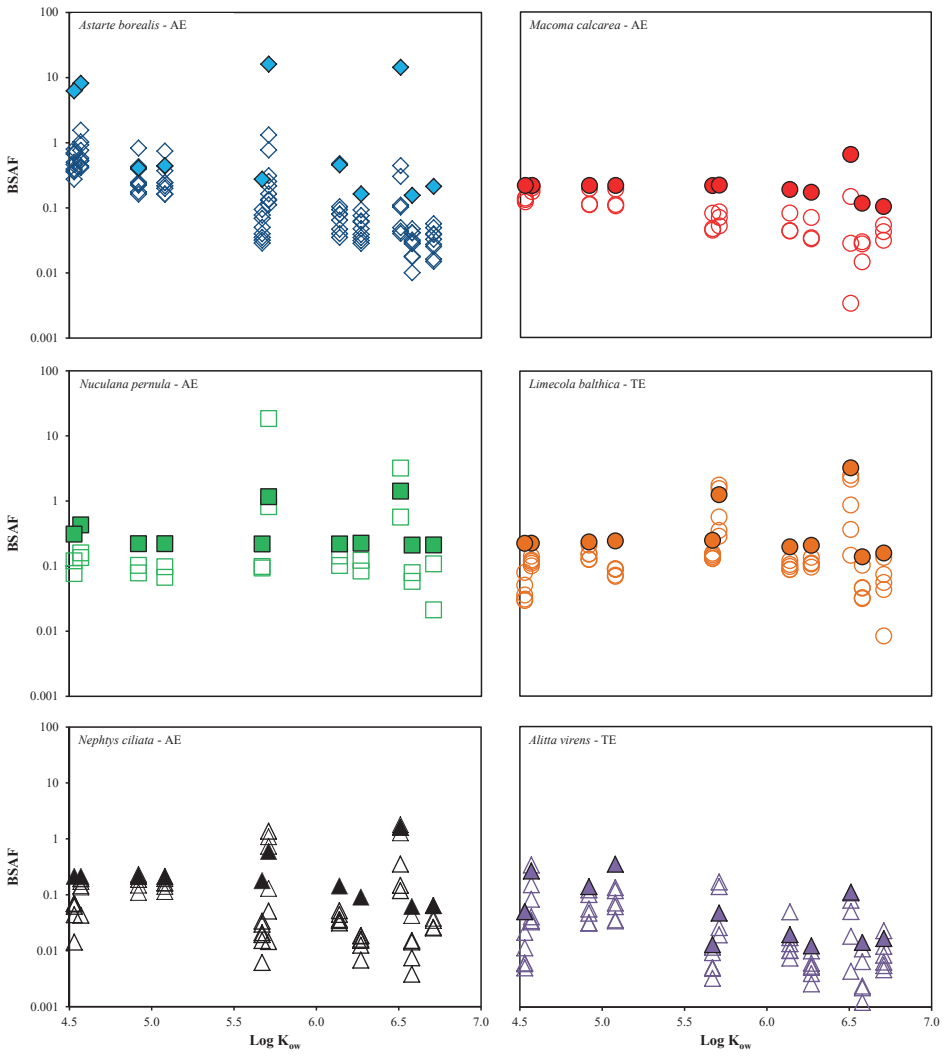

Figure 1. Modelled biota-sediment accumulation factors (BSAFs) (closed symbols) based on the minimum sum of squares and actual measured BSAFs (open symbols) for different polycyclic aromatic hydrocarbons in Arctic and temperate species exposed to the high treatment (BSH/PSH). AE: Arctic experiment; TE: temperate experiment.

Significantly higher BSAFs were found for ANT in BSH exposed *A. borealis* than in PSH exposed *N. ciliata* (Tables S11 and S15). Higher PAH BSAFs in bivalves compared to polychaetes may be explained by a higher BC content in PSH than in BSH sediment, which would decrease PAH bioavailability in the polychaete experiment, and by a higher OM content in PSH than in BSH sediment, which may result in a higher nutritional value of PSH

and reduction in polychaetes feeding. Both BSH and PSH treatments presented a similar pyrogenic PAH component, which may be indicative of BC presence in both sediment treatments [151].

3.3. Effects of temperate species traits on PAH bioaccumulation

Lipid-normalised Σ_{13}PAH concentrations in the high treatment (BSH or PSH) exposed invertebrates were 32.9 mg/kg in *L. balthica* and 9.4 mg/kg in *A. virens* (Table S6). *L. balthica* accumulated PAHs to a larger extent or excreted less than the polychaete *A. virens*, yet increasing PAH concentrations between the low and high treatment were reflected better by *A. virens* body residues. Increasing PAH concentrations in clams and sediment did not correlate between the low and high treatments. Such lack of correlation might be explained by other PAH uptake routes being dominant in *L. balthica* such as dermal absorbance or suspended solids ingestion.

Concentrations of PAHs were significantly higher in the BSH exposed deposit feeder *L. balthica* than in the PSH exposed polychaete *A. virens* (Tables S6 and S17). This may be related to several factors, like higher PAH background concentrations, higher bioavailability, lower elimination rates, longer exposure times and high food selectivity in *L. balthica* [56,179].

In *L. balthica*, PAH concentrations were 2-fold (FLT, BbF, dBahA, Ind123P), 3-fold (PYR, BeP) or 4-fold (BaA) higher in BSH than BSL individuals, whereas the concentrations were 4 to 10 times higher in BSH than in BSL sediment (Tables S3 and S6). An explanation to this disproportionality between organisms and sediment may be either a low PAH uptake from sediment compared to dermal uptake [56,219] or differences in congener-specific absorption efficiencies from sediment in *L. balthica* [220].

In *A. virens*, a 2-fold increase in FLT, PYR, BeP and dBahA body residue was observed between the low and high treatment exposed individuals which was expected from the congeners increase between PSL and PSH sediment concentrations. Fluoranthene (FLT), pyrene (PYR) and benzo(e)pyrene (BeP) were also correlated between *A. virens* body residue (wet wt. basis) and sediment concentrations after 28 d of exposure to polluted sediments from Oslo (Norway) [221].

Polycyclic aromatic hydrocarbons BSAFs were higher in BSH exposed *L. balthica* than in PSH exposed *A. virens* which is likely due to differences in biota PAH background concentrations and bioavailability between sediment treatments. The 10-90th percentiles for BSAFs for all PAHs in high treatment exposed biota ranged from 0.02 to 2.36 for *L. balthica* and 0.00 to 0.25 for *A. virens* (Figure 1; Table S12). Biota-sediment accumulation factors were on average 1.5 to 24 times higher in BSH exposed *L. balthica* than in PSH exposed *A. virens*. This may be explained by a combination of different factors such as lower PAH bioavailability in *A. virens* due to a probably higher BC and other organic material content in PSH than BSH sediment [222], lower sediment ingestion by *A. virens* compared to *L. balthica* [56] and generally higher PAH biotransformation capacity in polychaetes [181].

3.4. PAH bioaccumulation among Arctic and temperate species

Lipid-normalised PAH concentrations were generally higher in the temperate deposit feeding bivalve (*L. balthica*) than the Arctic one (*M. calcarea*), when exposed to the same sediment treatment (e.g., BSH). Only ANT concentrations were found to be 2 times higher in *M. calcarea* when compared to *L. balthica* (Figure 2; Tables S5 and S6). This may be explained by the fact that temperature affects OM-water and lipid-water partition coefficients, and thus bioaccumulation and BSAF values if other conditions are the same. Lower temperature has been found to increase PAH affinity to OM [223], and also to decrease lipid partitioning [224]. In the case of *M. calcarea*, these processes probably play a role but it was not possible to unambiguously identify the main reason for the apparent difference between the species.

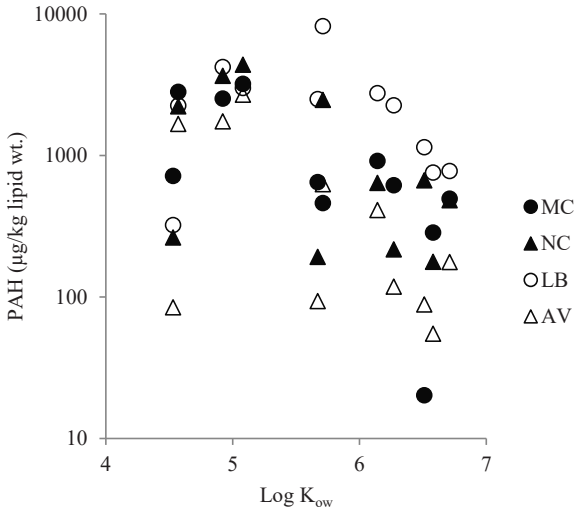

Figure 2. Geomean polycyclic aromatic hydrocarbons (PAHs) concentrations (µg/kg lipid wt.) in Arctic and temperate species exposed to the high treatment (BSH/PSH). MC: *Macoma calcarea*; NC: *Nephtys ciliata*; LB: *Limecola balthica*; AV: *Alitta virens*.

Additionally, differences between field-sampled *L. balthica* and *M. calcarea* and differences in food selectivity may have led to differences in their general performance during the TE and AE experiments. For instance, in the case of *M. calcarea*, strong temperature gradients (0 – 4 °C) described at sampling depth (20 m) within a 2 month period in late summer [145,225] could have increased energy allocation to physiological maintenance in the Arctic bivalves decreasing their energy budget [38,226], whereas the absence of phytoplankton input in the experiment can cause a rapid onset of lowered metabolic rate in *M. calcarea* adults [136,227].

The Arctic polychaete (*N. ciliata*) showed on average 2 to 3 times higher ANT, PYR, BaA and BbF concentrations than the temperate polychaete (*A. virens*) (Figure 2; Tables S5 and S6). This may be related to the fact that food conversion can be more effective in *N. ciliata* under low temperature compared to *A. virens* [228], thus lowering energy loss and improving the Arctic polychaete's fitness. Reproductive and morphological differences between the species can also affect their feeding activity rates and PAH bioaccumulation. A <12 h photophase is known to trigger onset of sexual maturity in polychaetes and to reduce their feeding rate [229,230]. It is possible that the feeding rate for the Arctic species *N. ciliata* was reduced during the period of sampling (8 – 26 September), when the natural photophase rapidly reaches <12 h [229,231]. In the case of the temperate species *A. virens*, both farm

growing and TE conditions (photophase >12 h, 18 °C) ensured gametogenic development arrest in mature females [232].

Following this last assumption, stable feeding activity would have made a PAH assimilation peak possible, which in this case was assumed to have been reached at an earlier exposure time in the temperate polychaete *A. virens* than in the Arctic polychaete *N. ciliata*. This can be due to continuous feeding by *A. virens*, which may not only increase the contact time of the species intestinal epithelium and coelomic fluids with PAHs [233], and thus increase PAH solubilisation, but may also lead to oxidative stress and ultimately to biotransformation enzymes induction [181,234]. *Nephtyidae* species such as *N. ciliata*, usually have a much smaller gut volume than other deposit feeding polychaetes and take discrete meals as part of their carnivore diet [235]. This may to some extent restrict the species PAH absorption [236], although stable pre-oogenesis conditions (12 h light: 12 h dark) in the absence of animal food (i.e., AE experimental conditions), would make it necessary for the animals to increase their deposit feeding activity over time in order to maintain PAH tolerance mechanisms. Although we have no conclusive evidence for this explanation, this could have possibly created a difference between both species so that *A. virens* energy budget at exposure start and also its PAH bioaccumulation were higher than in *N. ciliata*.

Biota-sediment accumulation factors for 2- to 3-ring PAHs were higher in BSH exposed *M. calcarea* than in *L. balthica* which, similarly to the differences between PAH lipid-normalised concentrations, could be explained by higher waterborne PAH uptake in *M. calcarea*. Biota-sediment accumulation factors for PAHs were generally higher in PSH exposed *N. ciliata* than in *A. virens*. Ranges of BSAFs for all PAHs were < 0.0001 to 18, in the order *M. calcarea* ≈ *A. virens* < *A. borealis* < *N. ciliata* < *L. balthica* < *N. pernula*. However, only species exposed to the same sediment treatment (BSH or PSH) under different climatic conditions (AE *vs* TE) and with the same feeding modes can be directly compared (i.e., *M. calcarea vs L. balthica* and *N. ciliata vs A. virens*). The BSAF 10-90th percentile ranges for all PAHs in the high treatment exposed biota were 0.01 to 0.21 for *M. calcarea*, 0.02 to 2.36 for *L. balthica*, 0.01 to 1.70 for *N. ciliata* and 0.00 to 0.25 for *A. virens*. Phenanthrene (PHE), anthracene (ANT) and fluoranthene (FLT) BSAFs were 2 to 3 times higher in the Arctic bivalve compared to the temperate one, whereas PAH BSAFs were generally 2 to 17 times higher in the Arctic polychaete compared to the temperate one (Tables S11 and S12), pointing to a generally higher PAH uptake from water in the Arctic species compared to the temperate ones.

3.5. Modelling PAH bioaccumulation by Arctic and temperate species

The modelled BSAFs matched well with the measured BSAF data. Confidence Intervals (CIs) for modelled BSAFs however were generally wide for most parameters and species, reflecting the variability in the biological data (Figure 1; Table S21). Complete data sets, with all treatments, were available for *N. ciliata* and *A. virens* and only data for the high treatment were present for all species. This also defines the cases for which parameters were estimated (Table S21).

The intercept b in the relation $LogK_{OC}^{SED} = LogK_{OW} + b$, determining the affinity of chemical partitioning to OC, was optimized to a value of 1.07 (0.81 – 1.33; 90% CI). This is higher than the well-established value of -0.21 for natural sediment [237], and can be explained from PAHs being efficiently bound to BC present in the sediment [78,197]. This is consistent with the aforementioned PAH diagnostic ratios indicating pyrogenic PAH sources, and with many measured BSAF values being smaller than one. The sorption affinity ratio γ was fitted and appeared indistinguishable from one, whereas the fractions of ingested sediment (parameter β) had 90% CIs extending beyond parameter constraints and were overlapping among species. This implies that the present experiments did not identify a difference in sorption to suspended OM and sediment OM (i.e., $K_{OC}^{SS} = K_{OC}^{SED}$), and thus that it does not matter what type of OM is ingested. Accordingly, the fraction of ingested sediment (parameter β), was set to one. This reduced the number of parameters fitted (p = 19; Table S21), which yielded narrower CIs for the remaining parameters. Hence, the present model analysis was less rigorous as those provided by Diepens et al. [56] and Sidney et al. [83] who detected significant values for β based on larger data sets.

In general, BSAFs decreased or remained at a constant value with increasing log K_{ow} (Figures 1 and S5), which agrees with earlier findings [56,83]. One explanation for this, as discussed by Diepens et al. [56], may be that steady state was only reached for PHE, ANT, FLT and PYR in *M. calcarea* and *L. balthica* during the 28-d experiment (Table S22).

However, for two chemicals (CHR and BghiP) high BSAF values were measured and predicted, which was not in line with the other values and expected trends with log K_{ow}. This can be explained by the high CHR and BghiP background concentrations in the organisms, at start of the experiment (Tables S5 and S6), and low clearance rates.

Since particle ingestion may be a dominant PAH uptake route in benthic organisms [80], ingestion rates (I) were fitted and expressed on an OM basis. In general, ingestion rates were low or even zero (Figure 3; Table S21). For Arctic species a low ingestion rate would be expected as metabolic rates are reduced and growth rates are constrained in cold-adapted stenotherms [38]. However, comparison of the temperate species *L. balthica* and *A. virens* to previously published data shows that these values are below the lower 90% CI boundary and below the range of 0.13 to 0.62 $kg_{OM} \times kg_{Lipids}^{-1} \times d^{-1}$ reported by Thomann et al. [238]. We speculate that the low apparent ingestion rates may be caused by a high nutritional value of the sediment, resulting in the dominance of dermal uptake.

For *M. calcarea* and *A. borealis*, the values for proportionality parameter 'a' in '$\log k_e = -\log K_{ow} + a$', required to calculate the elimination rate (k_e), were either overlapping or above previously published CIs for *L. balthica*, respectively (Table S21). For *N. ciliata* it was higher than previously published CIs for *A. virens* [56]. The magnitudes of 'a' for the temperate species *L. balthica* were higher than previously published CIs for PCBs [56,239]. For *A. virens*, magnitudes of 'a' were lower than previously published CIs for PCBs [56], whereas PAHs often are considered to be metabolised easier than PCBs [139,181].

The relative importance of chemical uptake pathways depends on the species, the chemical, treatment and estimated value of the ingestion parameter (Table S21). In all cases where the ingestion parameter fitted rates were zero, consequently the contribution of the OM ingestion pathway is modelled as 0%. In the high treatment (BSH or PSH), *N. pernula* and *A. virens* show 100% uptake from OM ingestion, whereas for *A. borealis*, *M. calcarea* and *N. ciliata* the model suggests 100% uptake from water (Table S23). For *L. balthica*, the chemical uptake from sediment increases with increasing log K_{ow}. These patterns and values agree with earlier published data [56,239,240]. Although lower elimination rates and ingestion rates for Arctic species compared to their temperate counterparts may be expected, this cannot be seen from the present data and parameters. Because the present bioaccumulation data set was not large enough for model validation purposes as in previous modelling studies [56,83], we ascribe this to the higher variability and uncertainty in the present bioaccumulation data.

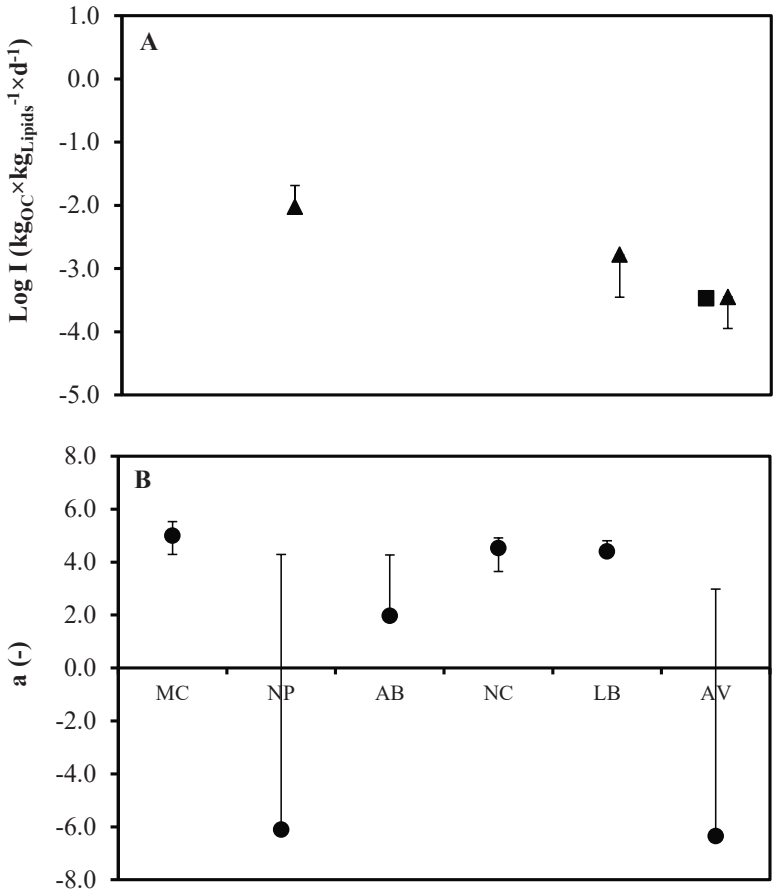

Figure 3. Optimized model parameters and 90% confidence limits (CLs) for ingestion rate (I; $kg_{OC} \times kg_{Lipids}^{-1} \times d^{-1}$) and intercept for elimination rate constant (k_e) (a; -). **A:** 'I'; **B:** 'a' for species exposed to the high treatment (BSH/PSH). 'I' was fitted separately for medium (squares) and high (triangles) treatment. Parameter values are only included if a 90% CL could be assessed in at least one direction. AB: *Astarte borealis*; MC: *Macoma calcarea*; NP: *Nuculana pernula*; NC: *Nephtys ciliata*; LB: *Limecola balthica*; AV: *Alitta virens*.

4. Conclusions and Implications

We showed significant differences in PAH bioaccumulation among temperate species with different feeding traits, and only for ANT among Arctic species. Differences between Arctic and temperate species with similar feeding traits, were generally not significant.

Bioaccumulation of PAHs from sediment was generally higher in the Artic polychaete *N. ciliata* than in the temperate *A. virens*. On the contrary, the temperate deposit feeding bivalve *L. balthica* accumulated PAHs to a greater extent from sediment than its Arctic counterpart *M. calcarea*. Consequently, bioaccumulation metrics experimentally determined in temperate species might become too conservative in the risk assessment for similar Arctic species and in other cases too moderate.

The time-dependent BSAF model shows that model parameters did not significantly differ among species and climate regions. This is supported by the generally non-significant differences in PAH bioaccumulation between Arctic and temperate species. This implies that although modelling has been shown to be a valid tool in earlier work [56,83], insufficient data for an appropriate parametrization limited the insight provided by modelling in the present study. Field validation of bioaccumulation models, of which data assumptions are built on temperate species data, will however require Arctic standard single-species tests to be able to further characterize sediment bioaccumulation mechanisms, such as contaminant uptake and elimination routes, under local conditions.

The present study used the same sediments and conditions mimicking Arctic and temperate environmental conditions and used Arctic and temperate species with pairwise matching functional groups. Differences in bioaccumulation in a direct comparison of the two climate regions characteristics were observed, which however were not statistically significant due to considerable variability in the biological control data, and similarity in exposure routes. This means that a cautionary note should be sounded on the use of temperate benthic species as a surrogate for Arctic benthic species in bioaccumulation assessment.

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Chapter 3

Supporting Information

Field sampling

Sampling of Arctic species. Sampling in Kongsfjorden was mainly performed near Blomstrandhalvøya and to a lesser extent at Gluudneset and Tyskerhytta, Kongsfjorden Bay (Svalbard, Norway). Sampling took place outside the protected areas of Kongsfjorden on board of research vessel MS Teisten (Kings Bay AS, Ny-Ålesund) between 8 and 26 September 2014 and within the Outer Basin community distribution area as defined by Włodarska-Kowalczuk and Pearson [147]. Biota samples were collected using a Van Veen grab (0.1 m^2). Four Arctic marine benthic invertebrate species with different feeding behaviors were collected: *Astarte borealis* (mollusc; suspension feeder [Schumacher, 1817]), *Macoma calcarea* (mollusc; surface deposit and suspension feeder [Gmelin, 1791]), *Nuculana pernula* (mollusc; sub-surface deposit feeder [O.F. Müller, 1771]) and *Nephtys ciliata* (polychaete; predator [O.F. Müller, 1776]) (Table S1).

Sampling of temperate species. *Limecola balthica* (mollusc; surface deposit and suspension feeder [Linnaeus, 1758]) was collected at low tide at the Oesterdam, Zeeland, the Netherlands, between 16 and 17 July 2014. *Alitta virens* (polychaete; predator and deposit feeder [Sars, 1835]) were obtained from a professional bait farm, Topsy baits, Wilhelminadorp, the Netherlands (Table S1).

Acclimatization

Arctic species were kept in glass aquaria with aerated, filtered (20 μm) and UV-treated sea water from the fjord (3 °C) for 2 to 6 d, before start of the experiment. In the case of *Nephtys ciliata*, a thin layer of sieved (1 mm Ø) sediment from the original sampling area was also included. Animals were not fed during acclimatization.

Temperate species were kept in glass aquaria with aerated filtered (0.2 μm) sea water for 5 d and were fed once with spiked ground fish food (TetraMin) suspended in deionised water at the start of the acclimatization. In order to have sufficient food supply, 10 mg of dry food for *A. virens* and 3 mg (shell length ≥ 15 mm) or 1.5 mg (shell length < 15 mm) for *L. balthica* was added per individual.

Water quality variables such as pH, dissolved oxygen, salinity and temperature were measured every two days during exposure time (Tables S7 and S8).

Sediment collection

A moderately contaminated sub-toxic marine sediment was prepared by mixing a non-contaminated 'clean' sediment with a naturally contaminated harbour sediment. Batches of muddy sediment (132 L) and coarse sediment (176 L) were collected near Wissenkerke (The Oosterchelde Natural Park, the Netherlands) at low tide between 24 and 25 April 2014 and 2 May 2014. Coarse sediment was sieved in the field with a 1-mm sieve whereas muddy sediment was sieved at laboratory inside a 1-mm sieve with some filtered sea water (0.2 μm). Once sieved both samples were stored at 3 °C.

A batch of harbour sediment (30 L) was collected near Rotterdam Nesserdijk by Nautisch Service Centrum (Rotterdam, the Netherlands) in mid-April 2014 and sieved with a 1-mm sieve on 1 May 2014. A subsample of the sieved wet harbour sediment (800 mL) was sent to Wageningen Marine Research laboratories (IJmuiden, the Netherlands) for further chemical analyses.

Test species

Arctic experiment. *A. borealis*, *M. calcarea*, *N. pernula* and *N. ciliata* were obtained as described in the field sampling section. *A. borealis* randomly selected healthy individuals with an average and standard deviation (SD) shell length of 31.30 (4.63) mm and wet wt. (flesh) of 7.47 (4.16) g were used. *N. pernula* randomly selected healthy individuals with an average (SD) shell length of 14.60 (3.66) mm and accumulated (n = 16 – 17) wet wt. (flesh) of 7.38 (1.65) g were used. As for *M. calcarea* and *N. ciliata*, we did not measure any morphological parameters due to the scarcity of individuals. Animals were not fed during the acclimatization, neither during the exposure experiments.

Temperate experiment. *L. balthica* and *A. virens* were obtained as described in the field sampling section. *L. balthica* randomly selected healthy individuals with an average (SD) shell length of 13.49 (2.46) mm and wet wt. (shell + flesh) of 0.47 (0.31) g were used. *A. virens* randomly selected healthy individuals with an average (SD) length of 10.83 (1.70) cm and wet wt. of 2.75 (0.98) g were used. Animals were fed once at the beginning of the acclimatization.

Chemical analysis

Subsamples of each sediment sample were analyzed for weight percentages of organic matter (OM) content, measured as loss on ignition (550 °C, 3 h). Biota lipids were extracted with chloroform:methanol:water and quantified gravimetrically.

Frozen sediment samples were thawed at room temperature. Frozen pooled biota samples were thawed at room temperature and subdivided into replicates of equal weight before drying. Sediment subsamples and biota replicates were dried and grinded with diatomaceous earth and Soxhlet-extracted for 40 min with hexane:acetone (3:1, v/v) (DionexASE 350 System, Thermo Scientific). Extracts were cleaned-up over an Al_2O_3 column and used for polycyclic aromatic hydrocarbons (PAHs) analysis, which was carried out on an HP 1100 HPLC equipped with a 4.6 mm Vydac guard and analytical reverse phase C18 column (201GD54T and 201TP54, respectively) which were kept at 22.00 °C. Detection was performed by an HP 1100 multi-wavelength fluorescence detector operating in the multi-emission wavelength mode. The mobile phase consisted of methanol/water (mixture and flow gradient). The injection volume was 20 µL.

Bioaccumulation modelling

Bioaccumulation modelling was used to link observed bioaccumulation with species-specific traits. Bioaccumulation in invertebrate lipids was modelled according to Diepens et al. [56] and is briefly described here:

$$\mathrm{BSAF_t} = \frac{C_{L,t=0}}{C_{OC,t=0}^{SED}} \times e^{-(k_e+k_g)\times t} + \frac{\frac{k_e \times K_{lip}}{K_{OC}^{SED}} + \alpha \times I \times [\beta + (1-\beta)\times \gamma]}{(k_e+k_g)} \times \left(1 - e^{-(k_e+k_g)\times t}\right) \quad \text{(S1)}$$

in which $C_{L,t=0}$ (µg × kg^{-1}) is the measured concentration in the biota at time zero, normalised to lipid content, $C_{OC,t=0}^{SED}$ (µg × kg^{-1}) is the measured concentration in sediment at time zero, normalised to OC content, t (d) is time, k_e and k_g (d^{-1}) are the rate constants for overall elimination and growth dilution, K_{lip} is the ratio k_w/k_e which equates to an apparent lipid-water partition coefficient, and k_w the rate constant for dermal uptake (L × kg^{-1} × d^{-1}), α (-) is the chemical assimilation efficiency (assumed to be independent of food source) and I (≥ 0, kg_{OC} × kg_{LIP} × d^{-1}) represents the mass of OC ingested per unit of time and organism lipid weight, β ($0 < \beta < 1$) is the fraction of ingested OC originating from the sediment whereas $1 - \beta$ is the fraction of ingested OC originating from the suspended and freshly deposited (sediment top

layer) solids and γ is a constant ratio between the sorption affinities for suspended OC and sediment OC ($K_{OC}^{SS} = \gamma K_{OC}^{SED}$). The ingested OC thus is assumed to originate partly from suspended solids (SS) from the overlying water and partly from the sediment (SED). Ingestion of multiple food items by benthic invertebrates has been modelled in a similar manner [56,80,83]. A detailed explanation of Eq. S1 is provided below.

The percentage of uptake through water is calculated based on Eq. S1 as:

$$\%WaterUptake = \frac{k_e}{k_e + \alpha I[\beta + (1-\beta)\gamma]K_{OC}^{SED}/K_{lip}} \quad \text{(S2)}$$

The fraction of steady state reached (F_{SS}, $0 < F_{SS} < 1$) in the bioaccumulation test (t = 28 d) was calculated as:

$$F_{SS} = 1 - e^{-(k_e+k_g)t} \quad \text{(S3)}$$

Model parametrization

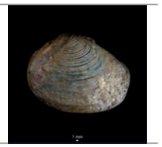
Eq. S1 was implemented in Microsoft Excel and the model was fitted to the log-transformed experimental BSAF data using the Excel Solver tool with scaling of parameters and a relative least-squares criterion. The first term in Eq. S1 ($C_{L,t=0}$) is omitted when concentrations in organism lipids at time zero are below limit of detection. The sediment OC-water partition coefficient K_{OC}^{SED} was assumed proportional to $LogK_{OW}$: $LogK_{OC}^{SED} = LogK_{OW} + b$ [241,242]. The parameter k_g could be obtained from either soft tissue weight differences between t = 28 d and t = 0 d for *A. borealis* (0.032), *N. pernula* (0.001), *L. balthica* (-0.001) and *A. virens* (-0.019), or estimated from previously calculated yearly growth rates (mm/year) [56,243] for *M. calcarea* (0.002) and *N. ciliata* (-0.001). The parameters k_e, I, β and γ were estimated using a two-stage iterative approach [83]. First, the chemical sorption parameters b and γ were set at default literature values [175,241,242], and the species-specific parameters a, I (constrained: $I \geq 0$) and β (constrained: $0 < \beta < 1$) were optimized for each species separately by minimizing their individual sum of squares. Subsequently, the parameters b and γ were optimized by minimizing the total sum of squares, after which the parameters a, I and β were fitted again for each of the species. This procedure was repeated until all minimum sums of squares had been reached.

Confidence intervals (90% CIs) were calculated according to Draper and Smith [244]:

$$SS_{90} = SS_{min}\left(1 + \frac{p}{(n-p)}F(p, n-p, 90\%)\right) \quad \text{(S4)}$$

in which SS_{90} is the sum of squares at the 90% confidence contour, SS_{min} is the minimum sum of squares, n is the number of BSAF measurements (n = 479), p is the number of estimated parameters (p = 19) and F (p, n-p, 90%) is the F-distribution according to Fisher. Confidence intervals were estimated using n, p and F either for the whole data set for the general parameters or the species-specific data set. Negative confidence limits for I were set to zero. Since the present experiments did not identify a difference in sorption to suspended OC and sediment OC (i.e., $K_{OC}^{SS} = K_{OC}^{SED}$), the fraction of ingested sediment (β), was set at one. An overview of all model parameters is provided as Table S9.

Table S1. Overview of target species and their ecological traits.

Experiment	Species	Geographical distribution	Feeding mode	Picture
Arctic	*Astarte borealis* (Schumacher, 1817)	Chukchi Sea [a] Barents Sea [b] Baltic Sea [c,d] Temperature: -2 − 16 °C	Suspension feeder [e]	
	Macoma calcarea (Gmelin, 1791)	Bering Sea [f] Chukchi Sea [a] Barents Sea [b] Kara Sea [g] Baltic Sea [d] Temperature: -2 − 12 °C	Surface deposit and suspension feeder [e]	
	Nuculana pernula (O.F. Müller, 1771)	Bering Sea [f] Barents Sea [b] Temperature: -2 − 9 °C	Sub-surface deposit feeder [h]	
	Nephtys ciliata (O.F. Müller, 1776)	Bering Sea [i] Barents Sea [b] Baltic Sea [c,d,j] Temperature: -1 − 14 °C	Predator [e]	
Temperate	*Limecola balthica* (Linnaeus, 1758)	Extensive distribution in the northern hemisphere [k] Temperature: 0 − 25 °C	Surface deposit and suspension feeder [k,l]	
	Alitta virens (Sars, 1835)	Cosmpolitan, often found in areas of chronic oil pollution [m] Temperature: +3 − 12 °C	Predator and deposit feeder [n,o]	

[a] [114], [b] [245], [c] [166], [d] [246], [e] [136], [f] [247], [g] [248], [h] [249], [i] [250], [j] [251], [k] [240], [l] [252], [m] [253], [n] [254], [o] [255]

Table S2. Polycyclic aromatic hydrocarbons concentrations (mean ± SD; µg/kg dry wt.) in sediment treatments at the start of the Arctic experiment (AE). Concentrations at AE end (t = 28 d) were assumed to be close to the experiment start values.

Treatment	BSL	BSH	PSL	PSM	PSH
OM (% dry wt.)	2.05 ± n.a.	2.52 ± 0.03	6.02 ± n.a.	3.89 ± n.a.	3.76 ± n.a.
PHE	48.11 ± 8.46	164.69 ± 48.81	155.03 ± 26.35	159.71 ± 15.59	250.26 ± 29.11
ANT	8.52 ± 1.25	61.62 ± 14.90	34.09 ± 3.75	49.27 ± 9.77	81.30 ± 8.37
FLT	58.83 ± 11.28	306.13 ± 128.02	249.20 ± 35.11	288.08 ± 58.13	406.93 ± 52.78
PYR	42.94 ± 5.80	240.91 ± 109.12	180.19 ± 24.54	238.76 ± 75.43	313.86 ± 38.50
BaA	15.45 ± 3.14	148.82 ± 70.13	83.17 ± 20.31	121.87 ± 54.17	156.96 ± 18.12
CHR	n.d.	92.95 ± 59.11	19.64 ± 11.43	80.57 ± 41.76	170.90 ± 110.17
BeP	25.42 ± 7.53	209.65 ± 88.39	128.04 ± 19.40	216.94 ± 117.22	251.10 ± 31.37
BbF	30.44 ± 6.45	174.13 ± 69.34	145.95 ± 16.18	166.15 ± 32.69	234.81 ± 30.82
BghiP	0.80 ± 0.65	11.45 ± 7.23	8.17 ± 2.22	13.46 ± 5.70	18.84 ± 6.94
dBahA	32.09 ± 12.89	146.19 ± 58.01	129.82 ± 17.92	140.35 ± 27.26	203.35 ± 25.71
Ind123P	24.12 ± 3.57	152.92 ± 60.80	150.68 ± 20.01	154.70 ± 32.43	218.81 ± 39.02
Σ_{13}PAH	286.72 ± 58.50	1709.46 ± 709.16	1283.99 ± 184.63	1629.86 ± 470.15	2307.13 ± 162.56

BS: bivalves sediment; PS: polychaetes sediment; L: low treatment; M: medium treatment; H: high treatment; n.d.: not detected; n.a.: not available.

Table S3. Polycyclic aromatic hydrocarbons concentrations (mean ± SD; µg/kg dry wt.) in sediment treatments at the end of the temperate experiment (t = 28 d).

Treatment	BSL	BSH	PSL	PSM	PSH
OM (% dry wt.)	2.18 ± 0.06	2.73 ± 0.76	3.52 ± 0.04	4.01 ± 0.04	4.00 ± 0.04
PHE	53.71 ± 4.21	229.66 ± 42.90	159.69 ± 82.88	278.72 ± 98.45	381.60 ± 258.88
ANT	14.43 ± 2.23	98.66 ± 48.61	39.03 ± 21.19	80.80 ± 35.04	128.92 ± 85.59
FLT	80.04 ± 9.79	460.54 ± 196.09	316.06 ± 156.73	430.07 ± 125.39	667.36 ± 280.22
PYR	60.09 ± 7.37	349.31 ± 149.85	254.04 ± 146.96	321.96 ± 101.17	509.52 ± 215.43
BaA	21.79 ± 3.26	227.37 ± 130.50	140.35 ± 122.63	158.02 ± 32.66	256.14 ± 70.35
CHR	n.d.	158.98 ± 125.12	220.13 ± 340.36	113.51 ± 38.92	207.44 ± 127.47
BeP	34.62 ± 6.50	352.06 ± 195.45	205.57 ± 141.23	257.50 ± 74.64	479.18 ± 250.18
BbF	42.21 ± 5.79	255.53 ± 116.72	168.62 ± 85.51	243.55 ± 63.64	387.37 ± 169.03
BghiP	4.26 ± 3.91	19.16 ± 10.47	19.88 ± 23.15	16.31 ± 4.89	42.54 ± 25.68
dBahA	49.43 ± 11.12	212.64 ± 82.18	127.75 ± 53.39	232.47 ± 82.85	352.82 ± 194.89
Ind123P	39.37 ± 9.81	205.33 ± 88.24	136.06 ± 67.36	204.31 ± 54.36	374.39 ± 202.59
Σ_{13}PAH	400.01 ± 52.03	2569.23 ± 1182.50	1787.18 ± 1103.86	2337.23 ± 698.72	3787.26 ± 1880.29

BS: bivalves sediment; PS: polychaetes sediment; L: low treatment; M: medium treatment; H: high treatment. n.d.: not detected.

Table S4. Overview of the number of experimental units (*n*) per species and sediment treatment.

Experiment	Species	Ind./EU[b]	Treatment (*n*)[a]					
			Background	BSL	BSH	PSL	PSM	PSH
Arctic	*Astarte borealis*	25	3	n.t.	3			
	Macoma calcarea	40-50	n.t.	1	2			
	Nuculana pernula	40-50	1	n.t.	2			
	Nephtys ciliata	20	n.t.			1	1	3
Temperate	*Limecola balthica*	50	3	1	3			
	Alitta virens	16	3			2	2	3

[a] Sediment treatments with different levels of PAH contamination are referred as BSL: Low Bivalve Sediment; BSH: High Bivalve Sediment; PSL: Low Polychaete Sediment; PSM: Medium Polychaete Sediment; PSH: High Polychaete Sediment. 'Background' refers to pooled field samples in which individuals were allowed to depurate for 24 h in filtered sea water before storage.

[b] 'Experimental unit' (EU) refers to a sediment treatment replicate or aquarium in which a specific number of individuals of a single species are exposed during 28 d to the corresponding sediment treatment, allowed to depurate for 24 h in filtered sea water and pooled for analytical treatment.

n.t.: not tested.

Table S5. Polycyclic aromatic hydrocarbons (mean ± SD; µg/kg lipid wt.) in test species from the Arctic experiment.

Spp.	*Macoma calcarea*		*Nephtys ciliata*			*Astarte borealis*		*Nuculana pernula*	
Treatment	BSL	BSH	PSL	PSM	PSH	Background	BSH	Background	BSH
n	1	2	1	1	3	3	3	1	2
Lipid wt. (% wet wt.)	0.82	0.84	0.39	0.41	0.45	0.42	0.46	1.71	1.70
PHE	3566 ± 393	2821 ± 287	1402 ± 681	729	2462 ± 941	9697 ± 1482	11746 ± 4998	2457	2792 ± 324
ANT	890 ± 89	718 ± 48	336 ± 143	149	297 ± 120	2384 ± 442	2977 ± 957	359	686 ± 216
FLT	1612 ± 178	3329 ± 1174	2353 ± 788	1771	4468 ± 969	1376 ± 280	8504 ± 4206	374	3192 ± 837
PYR	1314 ± 87	2623 ± 930	1713 ± 530	1384	3758 ± 920	1119 ± 120	6926 ± 3575	346	2828 ± 531
BaA	n.d.	673 ± 240	113 ± 70	77	218 ± 104	n.d.	n.d.	129	1847 ± 70
CHR	7434 ± 6614	470 ± 113	1327 ± 1709	0.15	6425 ± 6647	6164 ± 1276	2926 ± 2658	9860	122550 ± 158586*
BeP	316 ± 0	957 ± 374	427 ± 178	298	650 ± 129	884 ± 169	2066 ± 2300	183	3222 ± 761
BbF	175 ± 30	658 ± 305	117 ± 78	100	228 ± 69	223 ± 56	845 ± 269	218	2200 ± 586
BghiP	1162 ± 745	50 ± 65	344 ± 454	n.d.	1106 ± 955	1409 ± 2154	n.d.	1192	2953 ± 2925
dBahA	275 ± 2	506 ± 133	166 ± 76	40	n.d.	n.d.	479 ± 233	296	1173 ± 1112
Ind123P	69 ± 40	298 ± 102	34 ± 37	n.d.	n.d.	205 ± 144	421 ± 187	113	1307 ± 283
Σ_{13}PAH	16933 ± 6714	13104 ± 3552	8333 ± 267	4550	20242 ± 9762	34245 ± 20336	37668 ± 17371	15526	144750 ± 156791

BS: bivalves sediment; PS: polychaetes sediment; L: low treatment; M: medium treatment; H: high treatment. * Individual data are 10413 and 234688 µg/kg lipid wt.
n.d.: at least one sample shows a concentration below the limit of detection.

Table S6. Polycyclic aromatic hydrocarbons (mean ± SD; µg/kg lipid wt.) in test species from the temperate experiment.

Spp.	***Limecola balthica***			***Alitta virens***			
Treatment	Background	BSL	BSH	Background	PSL	PSM	PSH
n	3	1	3	3	2	2	3
Lipid wt. (% wet wt.)	0.85	0.84	0.78	1.30	0.97	1.71	1.39
PHE	2499 ± 571	2849	2257 ± 165	2497 ± 798	2267 ± 1376	1324 ± 496	2278 ± 2000
ANT	225 ± 60	262	334 ± 102	136 ± 64	108 ± 70	100 ± 27	113 ± 96
FLT	1431 ± 394	2853	3061 ± 685	5421 ± 1780	1596 ± 598	1824 ± 664	3151 ± 1903
PYR	1484 ± 325	3513	4340 ± 1153	1611 ± 530	893 ± 379	1157 ± 419	2034 ± 1254
BaA	680 ± 177	1101	2547 ± 484	n.d.	83 ± 62	134 ± 19	104 ± 51
CHR	863 ± 98	964	11585 ± 9242	268 ± 278	n.d.	887 ± 1061	n.d.
BeP	937 ± 247	1618	2833 ± 702	125 ± 50	249 ± 220	279 ± 102	475 ± 286
BbF	938 ± 259	1442	2295 ± 432	51 ± 26	100 ± 88	151 ± 26	125 ± 45
BghiP	2216 ± 4746	47	1754 ± 1454	76 ± 66	n.d.	134 ± 134	n.d.
dBahA	717 ± 491	702	1083 ± 818	83 ± 29	115 ± 79	137 ± 36	239 ± 238
Ind123P	452 ± 206	448	839 ± 473	n.d.	68 ± 79	96 ± 56	75 ± 62
Σ_{13}PAH	35512 ± 56664	15799	32928 ± 13246	10360 ± 3099	8005 ± 5818	6223 ± 2229	9371 ± 5439

BS: bivalves sediment; PS: polychaetes sediment; L: low treatment; M: medium treatment; H: high treatment. n.d.: at least one sample shows a concentration below the limit of detection.

Table S7. Mean water quality parameters in the Arctic experiment H treatment aquaria.

	Astarte borealis - BSH					*Macoma calcarea* - BSH					*Nuculana pernula* – BSH				
Day	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)
1	8.1	82.7	9.0	34.7	2.7	7.9	81.0	8.8	35.6	2.9	7.9	80.5	8.8	35.9	3.1
2	8.1	81.3	8.8	34.7	3.0	7.9	82.5	8.9	35.7	2.9	7.9	82.0	8.8	36.0	3.1
3	8.0	82.3	8.9	34.8	2.7	8.0	84.0	9.0	35.8	2.9	8.0	84.5	9.0	36.2	3.1
4	7.9	83.1	9.0	34.9	2.7	7.9	85.0	9.2	35.8	2.9	7.9	82.0	8.8	36.3	3.1
5	7.9	83.5	9.0	34.9	2.6	7.9	85.5	9.3	35.9	2.9	7.9	82.0	8.9	36.5	3.1
6	7.8	83.7	9.1	35.0	2.6	7.8	81.5	8.7	35.7	2.9	7.8	83.0	8.9	36.1	3.1
7	7.9	85.0	9.2	35.1	2.6	7.9	83.5	9.1	36.0	2.9	7.9	81.5	8.8	36.5	3.0
8	7.8	84.4	9.1	35.2	2.7	8.0	86.0	9.4	35.8	2.8	8.0	88.5	9.7	36.0	3.0
9	7.8	83.8	9.0	35.2	2.7	n.a.	82.0	9.1	36.0	2.9	n.a.	83.5	9.1	36.3	3.0

BSH: High Bivalves Sediment containing 10% (vol.) of harbour sediment; D.O.: dissolved oxygen; n.a.: not available.

	Nephtys ciliata - PSH				
Day	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)
1	8.0	81.2	8.7	35.1	2.9
2	8.0	81.5	8.8	35.2	2.8
3	8.0	82.5	8.9	35.2	2.8
4	8.0	82.8	8.9	35.3	2.8
5	7.9	82.3	8.9	35.5	2.8
6	7.9	82.7	8.9	35.5	2.8
7	8.0	81.7	8.8	35.7	2.9
8	8.0	84.8	9.2	35.8	2.8
9	8.0	85.3	9.0	35.8	2.8

PSH: High Polychaetes Sediment containing 10% (vol.) of harbour sediment; D.O.: dissolved oxygen.

Table S8. Mean water quality parameters in the temperate experiment H treatment aquaria.

	Limecola balthica - BSH					*Alitta virens* - PSH				
Day	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)
1	8.2	83.8	6.6	32.8	17.8	8.1	79.4	6.2	33.8	18.1
2	n.a.	80.3	6.3	n.a.	17.8	n.a.	77.9	6.1	n.a.	18.1
3	n.a.	79.3	6.2	n.a.	17.7	n.a.	75.3	5.9	n.a.	17.9
4	8.2	80.8	6.2	33.3	18.0	8.1	76.7	5.9	34.4	18.2
5	8.2	82.0	6.4	33.5	18.1	8.2	78.9	6.1	34.0	18.2
6	8.2	82.2	6.4	33.6	17.9	8.2	80.7	6.2	34.5	18.0
7	8.3	84.2	6.5	34.1	17.8	8.2	80.9	6.2	35.2	18.0
8	8.2	84.2	6.5	34.5	17.8	8.2	81.6	6.3	35.1	18.0
9	8.3	86.0	6.6	34.7	18.1	8.2	79.7	6.0	35.5	18.3
10	8.3	85.2	6.6	35.0	17.9	8.2	82.1	6.3	34.8	18.0
11	8.3	85.3	6.6	35.4	17.9	8.2	82.4	6.3	35.2	18.0
12	8.3	84.5	6.5	35.7	17.9	8.2	81.4	6.2	35.7	17.9

BSH: High Bivalves Sediment containing 10% (vol.) of harbour sediment; PSH: High Polychaetes Sediment containing 10% (vol.) of harbour sediment; D.O.: dissolved oxygen; n.a.: not available.

Table S9. Parameters for the biota-sediment accumulation factor model.

Parameter	Symbol	Unit
Affinity of chemical partitioning to peat in the relation $\boldsymbol{LogK_{OC}^{SED} = LogK_{OW} + b}$	b	-
Biota-sediment accumulation factor	$BSAF_t$	-
Chemical assimilation efficiency	α	-
Concentration in invertebrate lipids	C_L	µg × kg^{-1} lipids
Concentration in water	C_W	µg × L^{-1}
Concentration in sediment	C_{OC}^{SED}	µg × kg^{-1} OC
Concentration in suspended solids	C_{OC}^{SS}	µg × kg^{-1} OC
Constant ratio between sorption affinities for suspended OC and sediment OC in the relation $\boldsymbol{K_{OC}^{SS} = \gamma K_{OC}^{SED}}$	γ	-
Dermal uptake rate constant	k_w	L × kg^{-1} × d^{-1}
Elimination rate constant	k_e	d^{-1}
Food ingestion rate	I	kg$_{OC}$ × kg$_{Lipids}^{-1}$ × d^{-1}
Fraction of ingested OC originating from the sediment	β ($0<\beta<1$)	-
Growth rate constant	k_g	d^{-1}
Lipid water partition coefficient ($\boldsymbol{k_w/k_e}$)	K_{lip}	L × kg^{-1}
Sediment water partition coefficient	K_{OC}^{SED}	L × kg^{-1}
Species-specific elimination parameter in the relation $\boldsymbol{LogK_e = -LogK_{ow} + a}$	a	-
Suspended solids water partition coefficient	K_{OC}^{SS}	L × kg^{-1}
Time	t	d

Table S10. *Astarte borealis* background polycyclic aromatic hydrocarbons (PAHs) concentrations (mean ± SD; µg/kg lipid wt.).

PAH	2013[a]	2014
PHE	193.52 ± 169.41	2708.86 ± 341.85
ANT	45.25 ± 93.27	664.10 ± 91.68
FLT	96.07 ± 67.43	385.65 ± 80.61
PYR	297.78 ± 198.32	313.13 ± 30.09
BaA	18.78 ± 21.82	n.a.
CHR	106.90 ± 74.79	4614.76 ± 5290.49
BeP	96.95 ± 56.92	245.85 ± 32.57
BbF	58.61 ± 32.37	62.18 ± 14.44
BkF	34.57 ± 25.36	n.a.
BaP	14.56 ± 19.59	n.a.
BghiP	23.65 ± 30.32	393.05 ± 616.28
dBahA	13.09 ± 21.49	n.a.
Ind123P	75.70 ± 68.40	56.89 ± 39.89
$\sum_{13}$PAH	1075.43 ± 684.05	9444.47 ± 6537.91

[a] Individuals collected in July 2013 by Szczybelski et al. (**Chapter 2**) belong to size classes 2 to 4 as described by the authors (36 to ≥ 40 mm shell length), whereas individuals from 2014 collected by the present study averaged 31.3 ± 4.63 mm (shell length). Both groups were collected in different sampling years near Blomstrandhalvøya, Kongsfjorden Bay (Svalbard, Norway).
n.a.: not available.

Table S11. High treatment (BSH) polycyclic aromatic hydrocarbons biota-sediment accumulation factors (BSAFs) percentile ranges for Arctic (*Astarte borealis*, *Macoma calcarea* and *Nuculana pernula*) and temperate (*Limecola balthica*) bivalves.

Spp.	*Astarte borealis*		*Macoma calcarea*		*Limecola balthica*		*Nuculana pernula*	
BSAF percentile	10th	90th	10th	90th	10th	90th	10th	90th
PHE	0.43	1.13	0.18	0.21	0.10	0.13	0.14	0.16
ANT	0.34	0.72	0.12	0.14	0.03	0.07	0.08	0.12
FLT	0.16	0.50	0.11	0.17	0.07	0.09	0.07	0.09
PYR	0.17	0.51	0.11	0.18	0.13	0.19	0.08	0.10
BaA	0.03	0.08	0.04	0.07	0.13	0.15	0.09	0.10
CHR	0.13	0.88	0.06	0.08	0.31	1.67	2.57	16.64
BeP	0.04	0.18	0.04	0.07	0.09	0.11	0.11	0.14
BbF	0.03	0.08	0.03	0.06	0.10	0.13	0.09	0.12
BghiP	0.04	0.36	0.01	0.12	0.23	2.36	0.83	2.94
dBahA	0.02	0.05	0.03	0.05	0.02	0.11	0.03	0.10
Ind123P	0.02	0.04	0.02	0.03	0.03	0.08	0.06	0.08

Table S12. High treatment (PSH) polycyclic aromatic hydrocarbons biota-sediment accumulation factors (BSAFs) percentile ranges for Arctic (*Nephtys ciliata*) and temperate (*Alitta virens*) polychaetes.

Spp.	*Nephtys ciliata*		*Alitta virens*	
BSAF percentile	10th	90th	10th	90th
PHE	0.09	0.19	0.03	0.25
ANT	0.03	0.07	0.01	0.04
FLT	0.13	0.20	0.04	0.13
PYR	0.13	0.22	0.03	0.11
BaA	0.01	0.03	0.00	0.01
CHR	0.03	1.23	0.02	0.16
BeP	0.03	0.05	0.01	0.03
BbF	0.01	0.02	0.00	0.01
BghiP	0.13	1.70	0.01	0.10
dBahA	0.03	0.05	0.01	0.02
Ind123P	0.01	0.03	0.00	0.01

Table S13. Statistical test for differences in lipid-normalised polycyclic aromatic hydrocarbons concentrations between Arctic species exposed to the high treatment (Kruskal Wallis test *p*-value).

Spp.	PHE	ANT	FLT	PYR	CHR	BeP	BbF
Macoma calcarea							
Nephtys ciliata							
Astarte borealis	0.110	0.046	0.088	0.080			
Nuculana pernula					0.091	0.075	0.054

Table S14. Statistical test for differences in lipid-normalised polycyclic aromatic hydrocarbons concentrations between Arctic bivalve species exposed to the high treatment (Kruskal Wallis test *p*-value).

Spp.	PHE	ANT	FLT	PYR	CHR	Ind123P
Macoma calcarea						
Astarte borealis	0.095	0.105	0.095	0.105		
Nuculana pernula					0.069	0.069

Table S15. Statistical test for differences in biota-sediment accumulation factors of polycyclic aromatic hydrocarbons between Arctic species exposed to the high treatment (Kruskal Wallis test *p*-value).

Spp.	PHE	ANT	FLT	PYR	CHR	BbF
Macoma calcarea						
Nephtys ciliata						
Astarte borealis	0.068	0.037	0.103	0.103		
Nuculana pernula					0.103	0.054

Table S16. Statistical test for differences in biota-sediment accumulation factors of polycyclic aromatic hydrocarbons between Arctic bivalve species exposed to the high treatment (Kruskal Wallis test *p*-value).

Spp.	PHE	ANT	FLT	PYR	CHR	Ind123P
Macoma calcarea						
Astarte borealis	0.069	0.069	0.107	0.107		
Nuculana pernula					0.069	0.107

Table S17. Statistical test for differences in lipid-normalised polycyclic aromatic hydrocarbons (PAHs) concentrations between temperate species exposed to the high treatment after log transformation (independent *t* test).

					Levene's test for equality of variances		*T* test for equality of means						
Group statistics					F	Sig.	*t*	df	Sig. (2-tailed)	Mean difference	SE difference	95% confidence interval of the difference	
PAH		N	Mean	SE mean								Lower	Upper
ANT	AV	3	1.99	0.18	3.91	0.12	-2.88	4	0.045	-0.55	0.19	-1.07	-0.02
	LB	3	2.54	0.07									
FLT	AV	3	3.49	0.07	0.04	0.86	0.35	4	0.748	0.03	0.10	-0.24	0.31
	LB	3	3.45	0.07									
PYR	AV	3	3.30	0.07	0.00	1.00	-3.00	4	0.040	-0.31	0.10	-0.59	-0.02
	LB	3	3.60	0.07									
BaA	AV	3	2.01	0.07	0.03	0.86	-14.43	4	0.000[a]	-1.37	0.09	-1.63	-1.11
	LB	3	3.38	0.06									
BeP	AV	3	2.67	0.06	0.57	0.49	-7.23	4	0.002	-0.74	0.10	-1.03	-0.46
	LB	3	3.41	0.08									
BbF	AV	3	2.09	0.03	3.43	0.14	-18.27	4	0.000[a]	-1.24	0.07	-1.42	-1.05
	LB	3	3.33	0.06									
dBahA	AV	3	2.30	0.18	0.88	0.40	-3.36	4	0.030	-0.69	0.20	-1.25	-0.12
	LB	3	2.99	0.09									
Ind123P	AV	3	1.81	0.18	1.47	0.29	-4.90	4	0.008	-1.05	0.21	-1.64	-0.46
	LB	3	2.86	0.11									

[a] Significant *p*-value after Holm's sequential Bonferroni correction based on the number of possible paired comparisons among chemicals is applied ($p \leq 0.0014$).
AV: *Alitta virens*; LB: *Limecola balthica*.

Table S18. Statistical test for differences in biota-sediment accumulation factors of polycyclic aromatic hydrocarbons between temperate species exposed to the high treatment (Mann Whitney *U* test *p*-value).

Spp.	ANT	PYR	BaA	BeP	BbF	dBahA	Ind123P
Limecola balthica	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Alitta virens							

Table S19. Statistical test for differences in lipid-normalised polycyclic aromatic hydrocarbons concentrations between Arctic and temperate species (Mann Whitney *U* test *p*-value) based on input data from Tables S5 and S6.

Spp.	PHE	ANT	BaA	CHR	BeP	BbF	BghiP	dBahA	Ind123P
Macoma calcarea	0.083	0.083							
Limecola balthica			0.083	0.083	0.083	0.083	0.083	0.083	0.083
Nephtys ciliata	0.827	0.127	0.127	n.t.	0.127	0.050	n.t.	n.t.	n.t.
Alitta virens									

n.t.: not tested.

Table S20. Statistical test for differences in biota-sediment accumulation factors of polycyclic aromatic hydrocarbons between Arctic and temperate species exposed to the high treatment (Mann Whitney *U* test *p*-value) based on input data from Tables S11 and S12.

Spp.	PHE	ANT	FLT	PYR	BaA	CHR	BeP	BbF	BghiP	dBahA	Ind123P
Macoma calcarea	0.083	0.083	0.083								
Limecola balthica				1.000	0.083	0.083	0.083	0.083	0.083	0.248	0.083
Nephtys ciliata	0.827	0.127	0.050	0.050	0.050	n.t.	0.050	0.050	n.t.	n.t.	n.t.
Alitta virens											

n.t.: not tested.

Table S21. Parameters and their 90% confidence intervals (CIs) (Eq. S4) obtained from fitting with data for *Astarte borealis*, *Macoma calcarea*, *Nuculana pernula*, *Limecola balthica*, *Nephtys ciliata* and *Alitta virens* using the full model.

Parameters	CI	*Astarte borealis*	*Macoma calcarea*	*Nuculana pernula*	*Limecola balthica*	*Nephtys ciliata*	*Alitta virens*	*Limecola balthica*[a]	*Alitta virens*[a]
a (-)	L90	*	4.29	*	3.15	3.64	*	2.8	
		1.97	5.00	-6.11	4.40	4.53	-6.35	3.9	2.7
	H90	4.27	5.53	4.29	4.81	4.91	2.98	4.3	3.4
I ($kg_{OC} x kg^{-1} x d^{-1}$) - L	L90	n.a.	#*	n.a.	#*	#*	#*	0.07-0.16[b]	0.11-0.20[b]
		n.a.	0.00	n.a.	0.00	0.00	0.00	0.12-0.24[b]	0.14-0.25[b]
	H90	n.a.	0.02	n.a.	0.06	0.01	0.00	0.16-0.32[b]	0.17-0.31[b]
I ($kg_{OC} x kg^{-1} x d^{-1}$) - M	L90	n.a.	n.a.	n.a.	n.a.	#*	#*		
		n.a.	n.a.	n.a.	n.a.	0.00	0.00		
	H90	n.a.	n.a.	n.a.	n.a.	0.01	0.00		
I ($kg_{OC} x kg^{-1} x d^{-1}$) - H	L90	#*	#*	0.00	#*	#*	#*		
		0.00	0.00	0.01	0.00	0.00	0.00		
	H90	0.00	0.01	0.02	0.01	0.00	0.00		
b (-)	L90			0.41					
				0.67					
	H90			0.93					
N of experimental data points	Per species	96	52	22	65	95	149		
Parameters (*p*)		3	4	3	4	5	5		
F-ratio value		2.14	2.07	2.40	2.04	1.91	1.89		
SS_{min}		59.13	11.35	3.72	11.43	34.57	40.45		
N of experimental data points	Total			479					
Parameters (*p*)				19					
F-ratio value				1.45					
SS_{min}				303.16					

Table S21. Continued.

[a] Diepens et al. [56], [b] Ranges for all treatments as described in Diepens et al. [56]; # = confidence limit not within two orders of magnitude above or below estimated value, * = parameter set to zero or one because fit was out of constrain boundary, L90 = lower boundary of the 90% CI, H90 = higher boundary of the 90% CI. Ingestion I was fitted separate for low (L), medium (M) and high (H) treatments. n.a.: not available.

Table S22. Fraction of steady state reached (F_{ss}) (Eq. S3) in 28-d bioaccumulation main tests with *Astarte borealis*, *Macoma calcarea*, *Nuculana pernula*, *Limecola balthica*, *Nephtys ciliata* and *Alitta virens* for low (L), medium (M) and high (H) treatments for the full model.

		Fraction of steady state reached										
Treatment	Species	PHE	ANT	FLT	PYR	BaA	CHR	BeP	BbF	BghiP	dBahA	Ind123P
L	*A. borealis*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	M. calcarea	1.00	1.00	1.00	1.00	1.00	1.00	0.87	0.79	0.60	0.45	0.54
	N. pernula	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	L. balthica	1.00	1.00	0.99	1.00	0.46	0.38	-0.45	-0.65	-0.94	-1.10	-1.00
	N. ciliata	1.00	1.00	1.00	1.00	0.91	0.89	0.66	0.60	0.50	0.45	0.48
	A. virens	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
M	*A. borealis*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	M. calcarea	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	N. pernula	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	L. balthica	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	N. ciliata	1.00	1.00	1.00	1.00	0.94	0.92	0.75	0.71	0.64	0.60	0.62
	A. virens	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32	0.32
H	*A. borealis*	-8.02	-7.96	-8.48	-8.39	-8.63	-8.64	-8.67	-8.67	-8.68	-8.68	-8.68
	M. calcarea	1.00	1.00	1.00	1.00	1.00	1.00	0.87	0.78	0.59	0.43	0.53
	N. pernula	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
	L. balthica	1.00	1.00	1.00	1.00	0.63	0.57	-0.01	-0.15	-0.34	-0.46	-0.39
	N. ciliata	1.00	1.00	1.00	1.00	0.90	0.88	0.62	0.55	0.44	0.38	0.42
	A. virens	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31

n.a.: not available.

Table S23. Relative importance of polycyclic aromatic hydrocarbons (PAHs) uptake by sediment ingestion in 28-d bioaccumulation main tests with *Astarte borealis*, *Macoma calcarea*, *Nuculana pernula*, *Limecola balthica*, *Nephtys ciliata* and *Alitta virens* for low (L), medium (M) and high (H) treatments for the full model.

		% PAH uptake by sediment ingestion										
Treatment	Species	PHE	ANT	FLT	PYR	BaA	CHR	BeP	BbF	BghiP	dBahA	Ind123P
L	*A. borealis*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	M. calcarea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	N. pernula	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	L. balthica	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	N. ciliata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	A. virens	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
M	*A. borealis*	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	M. calcarea	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	N. pernula	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	L. balthica	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
	N. ciliata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	A. virens	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
H	*A. borealis*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	M. calcarea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	N. pernula	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
	L. balthica	0.90	0.82	2.86	2.00	10.29	11.17	25.29	31.35	44.13	53.14	47.30
	N. ciliata	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	A. virens	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

n.a.: not available

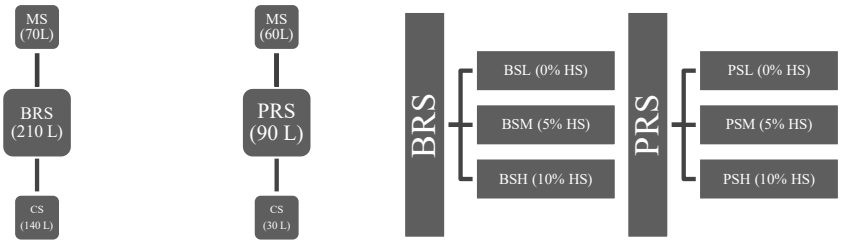


Figure S1. Preparation of Bivalves Reference Sediment (BRS) and Polychaetes Reference Sediment (PRS) from both muddy (MS) and coarse (PS) sediment.

BRS: Bivalves Reference Sediment; BSL: Low Bivalves Sediment containing background levels of contaminants; BSM: Medium Bivalves Sediment containing 5% (vol.) of harbour sediment (HS); BSH: High Bivalves Sediment containing 10% (vol.) of harbour sediment (HS); PSL: Low Polychaetes Sediment containing background levels of contaminants; PSM: Medium Polychaetes Sediment containing 5% (vol.) of harbour sediment (HS); PSH: High Polychaetes Sediment containing 10% (vol.) of harbour sediment (HS).

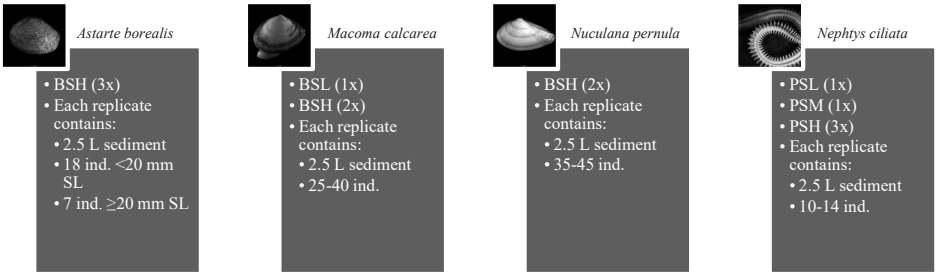


Figure S2. Arctic experiment (AE) replicates per species and treatment.

BSL: Low Bivalves Sediment containing background levels of contaminants; BSH: High Bivalves Sediment containing 10% (vol.) of harbour sediment; PSL: Low Polychaetes Sediment containing background levels of contaminants; PSM: Medium Polychaetes Sediment containing 5% (vol.) of harbour sediment; PSH: High Polychaetes Sediment containing 10% (vol.) of harbour sediment; SL: shell length; ind.: number of individuals.

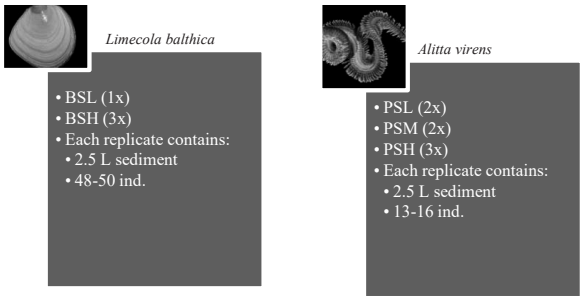


Figure S3. Temperate experiment (TE) replicates per species and treatment.

BSL: Low Bivalves Sediment containing background levels of contaminants; BSH: High Bivalves Sediment containing 10% (vol.) of harbour sediment; PSL: Low Polychaetes Sediment containing background levels of contaminants; PSM: Medium Polychaetes Sediment containing 5% (vol.) of harbour sediment; PSH: High Polychaetes Sediment containing 10% (vol.) of harbour sediment; ind.: number of individuals.

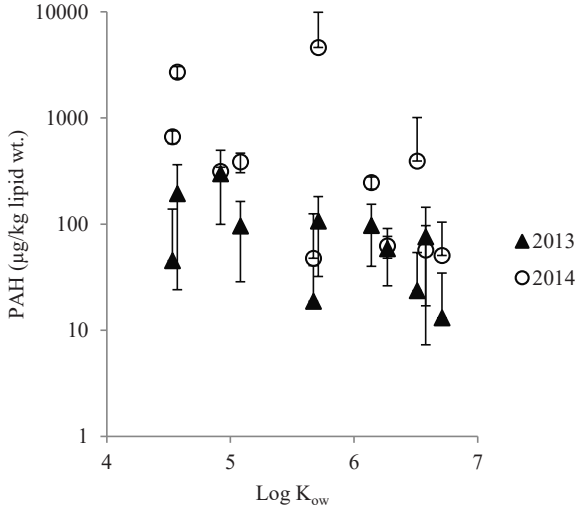


Figure S4. *Astarte borealis* background polycyclic aromatic hydrocarbons (PAHs) concentrations (mean ± SD; µg/kg lipid wt.).

Individuals collected in July 2013 by Szczybelski et al. (**Chapter 2**) belong to size classes 2 to 4 as described by the authors. Individuals were collected in different sampling years near Blomstrandhalvøya, Kongsfjorden Bay (Svalbard, Norway).

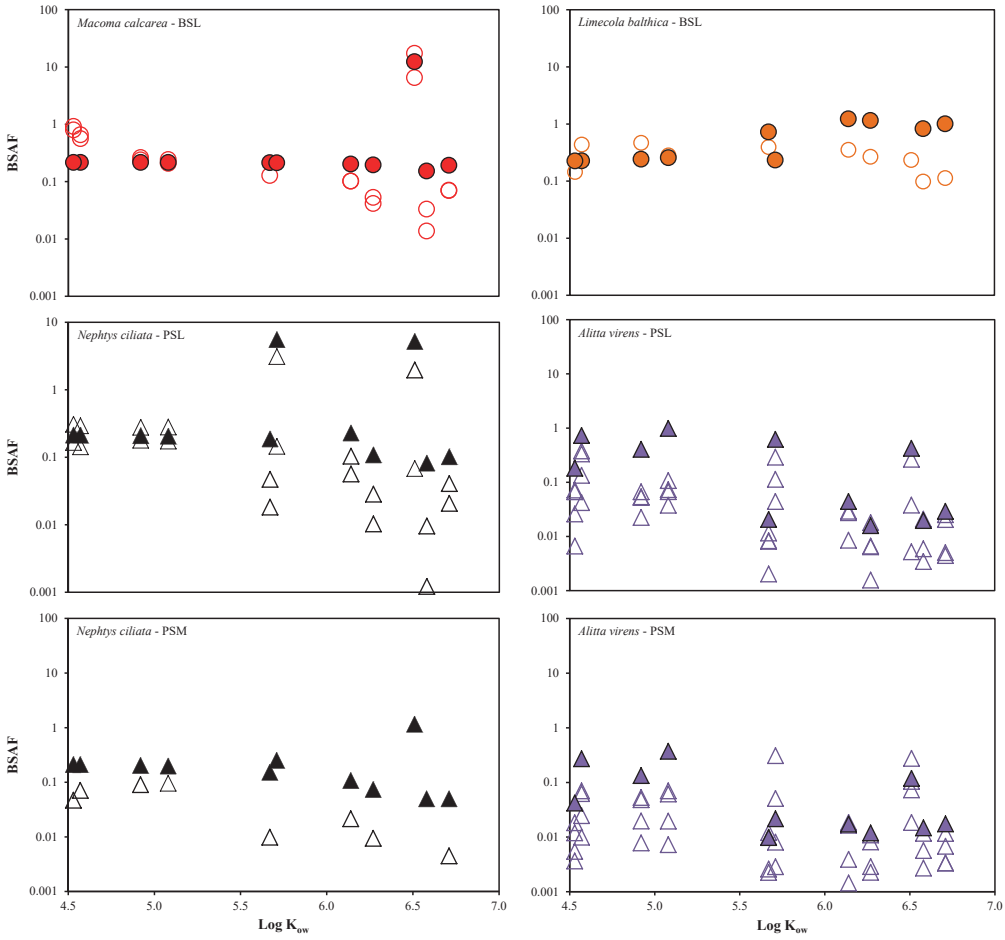


Figure S5. Modelled polycyclic aromatic hydrocarbons biota-sediment accumulation factors (BSAFs) (closed symbols) and actual measured BSAFs (open symbols) for Arctic (*Macoma calcarea* and *Nephtys ciliata*) and temperate (*Limecola balthica* and *Alitta virens*) species exposed to the low (BSL/PSL) and medium (PSM) treatments.

4 Biomarker responses and biotransformation capacity in Arctic and temperate benthic species exposed to polycyclic aromatic hydrocarbons

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Science of the Total Environment, submitted

Abstract

Monitoring parameters for the assessment of oil and gas related contaminants and their biological effects need validation before application in the Arctic. For such monitoring purposes, we evaluated the potential use of three biomarkers (acetylcholinesterase, acyl-CoA oxidase and glutathione S-transferase) for application to an Arctic bivalve (*Astarte borealis*) and determined the body residue of pyrene and two pyrene metabolites (1-hydroxypyrene and pyrene-1-glucuronide) in Arctic benthic species (bivalve: *Macoma calcarea*; polychaete: *Nephtys ciliata*) and temperate benthic species (bivalve: *Limecola balthica*; polychaete: *Alitta virens*) in order to establish the potential of polycyclic aromatic hydrocarbons (PAHs) metabolite profiles as biomarkers of exposure in such species. Experimental PAH exposure levels were probably too low (0.2 – 1.7 mg/kg dry wt. in sediment) to induce or inhibit biomarker responses in *A. borealis*. Concentrations of pyrene and pyrene metabolites varied between species, although no consistent patterns could be established among taxonomic groups and locations. Metabolites made up to 79% of the total pyrene concentrations indicating that basal metabolic activity is affecting pyrene kinetics even at low concentrations in all species. This indicates that Arctic and temperate species could show similar metabolism patterns of PAHs, although more insight into the effects of confounding factors is needed.

1. Introduction

Current baseline information on potential effects of oil and gas (O&G) activities on the Arctic ecosystem or ecosystem components is still inadequate or unavailable [72], particularly on the cumulative effects of O&G related toxic compounds. More information is needed because the retreat of sea ice in the Arctic will cause an increase in O&G related activities [14]. Hence, further studies on assessing the vulnerability of Arctic biota to O&G related chemicals are essential, in order to inform policy and to ensure that O&G risk assessment procedures are targeted to the Arctic environment.

Polycyclic aromatic hydrocarbons (PAHs) are the main organic pollutants related to O&G activities, which have been identified as an emerging concern in the Arctic [121]. Once released into the marine environment, partitioning of PAHs to settling particulate organic matter generally results in contamination of sediments and chronic exposure of benthic organisms. Accumulation of PAHs by benthic organisms depends on several factors such as the species' feeding behavior, physiological responses to PAH exposure like detoxification and excretion of PAHs [256], as well as sediment characteristics [80]. Biological responses (biomarkers) to exposure to PAHs may be used as indicators of exposure to or sublethal ecological effects of O&G related activities, and the use of such biomarkers may provide an early warning for hazard [257] and ecological risk assessment [86,258].

Studies have documented effects of oil at the cellular, individual and community level in Arctic benthic communities [104,122,259,260], but they provide little information on the specific chemicals in the complex oil matrix causing the effects. Although PAHs are the major group of organic contaminants in oil, there is a large knowledge gap on their specific role in affecting the responsiveness of biochemical biomarkers in Arctic benthos chronically exposed to O&G derived chemicals [261]. Furthermore, almost no information on PAH metabolites in benthic invertebrates is available that may be used to assess oil-derived hazards and risks for Arctic benthic organisms [213]. In order to address these knowledge gaps the aims of the present study were to (1) identify the responsiveness of biochemical biomarkers of exposure and effects in Arctic benthic species, and to (2) assess the use of profiles of metabolites of a model PAH (i.e., pyrene) for monitoring of exposure and effects in Arctic benthic invertebrates. This was performed experimentally, using representative species of the Arctic macrobenthic community (the suspension feeder *Astarte borealis* [Schumacher, 1817], the suspension/deposit feeder *Macoma calcarea* [Gmelin, 1791] and the predator/deposit feeder

Nephtys ciliata [O.F. Müller, 1776]), which are compared to temperate counterpart species (the suspension/deposit feeder *Limecola balthica* [Linnaeus, 1758] and the omnivore/deposit feeder *Alitta virens* [Sars, 1835], formerly known as *Macoma balthica* and *Nereis virens*, respectively).

Selected biomarkers of exposure were the peroxisomal *β*-oxidation enzyme acyl-CoA oxidase (AOX, E.C.1.3.3.6) and Phase II biotransformation enzyme glutathione S-transferase (GST, E.C.2.5.1.18). The biomarker of neurotoxicity consisted of acetylcholinesterase (AChE, E.C.3.1.1.7). These were determined in *A. borealis*, upon exposure to PAH-contaminated sediment under laboratory conditions. In a second experiment, selected pyrene metabolites (1-hydroxypyrene [OHPyr, Phase I metabolite, Figure 1] and pyrene-1-glucuronide [GluPyr, Phase II metabolite, Figure 1]) were analyzed in Arctic (*M. calcarea*, *N. ciliata*) and temperate (*L. balthica*, *A. virens*) bivalves and polychaetes exposed to PAH-contaminated sediment under laboratory conditions. Due to limited availability of test animals it was not feasible to analyze biochemical biomarkers and metabolite profiles in the same experiments. In the first experiment, an Arctic species was selected for which ample information was available on biomarker responses in similar temperate species. As for metabolite profiles, much less information is available, so we conducted a broader experimental approach, comparing species with different feeding modes and geographical origin.

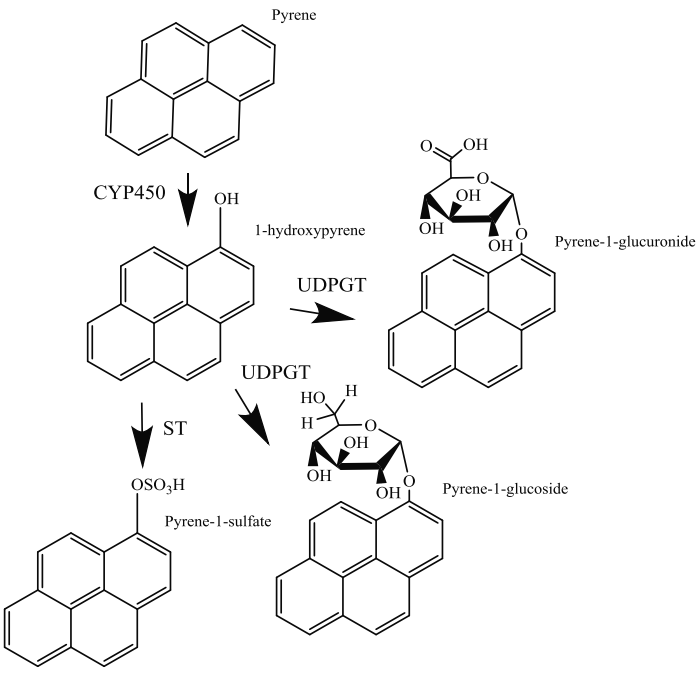

Figure 1. Biotransformation pathway for pyrene in *Alitta virens* (adapted from Jørgensen et al. [183]). CYP450: cytochrome P450 enzymes; ST: sulfotransferase enzymes; UDPGT: glucuronosyltransferase enzymes. CYP450 forms Phase I metabolites, whereas ST and UDPGT form Phase II metabolites.

2. Materials and Methods

2.1. Test organisms

Based on their feeding habits, sessility and relative abundance in Kongsfjorden Bay (Svalbard, Norway), three Arctic benthic species were selected: the suspension feeder *A. borealis*, the suspension/deposit feeder *M. calcarea*, and the carnivore/omnivore *N. ciliata*. Additionally, two counterpart temperate species (the suspension/deposit feeder *L. balthica* and the deposit feeder/omnivore *A. virens*) were selected based on their comparable habitat and feeding traits with Arctic species. Permission for sampling of Arctic and temperate (*L. balthica*) species was issued by The Governor of Svalbard and the Province of Zeeland, respectively. *A. virens* was obtained from a professional bait farm, Topsy baits

(Wilhelminadorp, the Netherlands). For details of the sampling and acclimatization of test species and sediment collection, the reader is referred to the Supporting Information (SI).

2.2. Sediment treatments preparation

Reference sediment was collected in the Oosterschelde estuary (The Netherlands, 51°36'13"N, 3°47'49"E) on 24 to 25 April 2014 and 2 May 2014, and naturally contaminated sediment was collected near Nesserdijk (Rotterdam, the Netherlands, 51°54'14"N, 4°31'17"E) by Nautisch Service Centrum in April 2014. Two different batches of reference sediment were prepared to ensure an optimal habitat for the test species. Since the bivalve species (*A. borealis*, *M. calcarea* and *L. balthica*) usually reside in sandy sediment, their sediment batch consisted of two thirds of coarse and one third of muddy sediment, based on volume. *N. ciliata* and *A. virens* are generally found in sandy mud, so a sediment batch consisting of two thirds of muddy and one third of coarse sediment was prepared for these species.

The reference sediments are referred to as the 'low' (L) treatment: BSL (Bivalve Sediment Low for bivalves) and PSL (Polychaete Sediment Low for polychaetes). Two additional sediment treatments were prepared with a higher amount of harbour sediment increasing the chemical concentrations, referred to as 'medium' (BSM and PSM with 5% harbour sediment) and 'high' (BSH and PSH with 10% harbour sediment) treatments. These preparations thus resulted in six sediments: three for bivalves (BSL, BSM, BSH) and three for polychaetes (PSL, PSM, PSH) (Tables S5 and S6).

All sediment treatments were thoroughly mixed before storage and transport from the Netherlands to Svalbard, before use in the exposure experiments. Due to logistics, storage time (at 3 – 7 °C) for sediment treatments was 8 and 17 weeks in the temperate and Arctic experiment, respectively. Prior to the start of exposure, sediment was allowed to settle in contact with filtered sea water in a 1:6 sediment-to-water volume ratio without aeration during 3 d and with aeration during the following 4 d. In the Arctic experiment some aquaria were aerated for 7 to 14 d because biota field sampling took longer than initially expected.

Σ_{13}PAH concentrations in the high (H) treatment were selected to be above or within average sediment Σ_{16}PAH concentration detected in Arctic oil-impacted areas like Ny-Ålesund harbour (Svalbard, Norway) [149], and Gulf of Alaska (USA) after 4 and 13 years of the *Exxon Valdez* oil spill [262] (Table 1).

Table 1. Total polycyclic aromatic hydrocarbons (PAHs) concentrations (min-max; µg/kg dry wt.) in sediment and biota (soft tissue) available in the literature.

			Total PAH	
Present study[a]		Analytes (No. of compounds)	Sediment	Biota
Treatment	BSL	3- to 6-ring PAHs (13)	219 – 322	n.a.
	BSM		n.a.	n.a.
	BSH		839 – 2780	446 – 1878
	PSL		1130 – 1489	249 – 264
	PSH		2125 – 2437	147 – 1183
Payne et al. [262][b]				
1993	Gold Creek	2- to 6-ring PAHs and selected alkylated homologues (44)	~50	~600
	Alyeska Marine Terminal		20 – 300	300 – 400
	Disk Island		1830[c]	~200
Payne et al. [262][b]				
1993-2002	Gold Creek	2- to 6-ring PAHs and selected alkylated homologues (44)	40 – 125	100 – 800
Van den Heuvel-Greve et al. [149]				
2012-2013	Kongsfjorden	3- to 6-ring PAHs (16)	1 – 26	n.a.
	Ny-Ålesund		476 – 2550	n.a.

[a] Sediment and *A. borealis* samples from the Arctic experiment.
[b] Sediment and *Mytilus trossulus* samples.
[c] Sediment samples from Boehm et al. [263].
BSL: Low Bivalve Sediment; BSH: High Bivalve Sediment; PSL: Low Polychaete Sediment; PSM: Medium Polychaete Sediment; PSH: High Polychaete Sediment; n.a.: not available.

2.3. Experimental design

Two 28-d exposure experiments were conducted: a first with Arctic species and conditions at Kings Bay AS (Ny-Ålesund, Svalbard) (September to October 2014); and another with temperate species and conditions at Wageningen Marine Research (Yerseke, the Netherlands) (July to August 2014). The Arctic experiment was performed in a temperature controlled room (3±1 ºC) with a photoperiod of 12 h light: 12 h dark, whereas the temperate experiment was performed in a temperature controlled room (18±1 ºC) with a photoperiod of 18 h light: 6 h dark.

Exposure treatments were achieved in quadruplicate or more for *A. borealis* (BSL, BSM and BSH), whereas for the rest of the test species, these were achieved in duplicate or more due to low availability of test organisms (Table S1). For *A. borealis*, each aquarium contained 25 individuals of which 18 individuals were classified as 'small' (< 2 cm) and 7 individuals were classified as 'large' (2 – 4 cm), based on shell length. For *M. calcarea*, *L. balthica*, *N. ciliata* and *A. virens*, each aquarium contained 40 to 50, 50, 20 and 16 individuals, respectively (Table S1). Individuals were not fed during exposure.

No mortality was observed for any species in any treatment. At the end of the experiments, organisms were allowed to depurate their guts for 24 h in filtered sea water. Animals were weighed, measured for body or shell length, dissected (e.g., bivalves), snap-frozen and stored at -80 °C for the determination of enzymatic activities in *A. borealis* samples or at -20 °C for the analysis of pyrene and pyrene metabolites in Arctic (*M. calcarea*, *N. ciliata*) and temperate (*L. balthica*, *A. virens*) species.

A. borealis samples were shipped in a dry-shipper in liquid nitrogen (Arctic Express 20, Thermo Fisher Scientific) to Plentzia Marine Station (University of the Basque Country) at Plentzia (Biscay, Spain), while the remaining samples were shipped in dry-ice to the Department of Analytical Chemistry (University of the Basque Country) at Leioa (Biscay, Spain).

2.4. Chemicals

2′,7′-dichlorofluorescin diacetate (DCF), N,N-dimethylformamide (DMF), palmitoyl coenzyme A lithium salt, peroxidase from horseradish, sodium azide, Triton™ X-100 and ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-N_2) (Sigma-Aldrich) were used for the determination of AOX activity. Butylated hydroxytoluene (BHT), acetylthiocholine iodide (ATC), 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) (Sigma-Aldrich) and sodium bicarbonate (VWR) were used for the determination of AChE activity. 1-chloro-2,4-dinitrobenzene (CDNB) and L-glutathione reduced (GSH) (Sigma-Aldrich) were used for the determination of GST activity. Quick Start™ Bradford Kit 4 (Bio-Rad) was used for protein determination.

Pyrene (98%), 1-hydroxypyrene (98%) (Sigma-Aldrich), pyrenyl-1-O-glucuronide (98.2%) (Isostandards Material), acetone, methanol, acetonitrile (HPLC grade) (Macrom Fine Chemicals), 0.45 µm polyamide filters (Macherey-Nagel) and Oasis HLB (200 mg) cartridges (Waters) were used for the determination of pyrene metabolites.

2.5. Enzymatic activities

Pooled digestive glands of either 9 ‘small’ or 3 ‘large’ *A. borealis* individuals were homogenized in 1:5 volumes of 100 mM potassium phosphate buffer (pH 7.4) in a tissue homogenizer (Precellys®24, Bertin Technologies) at 6,000 *rpm* × 30 s (5 °C). Homogenates for AOX determination were obtained after a centrifugation of the homogenate at 500 *g* x 15

min (4 °C) (Allegra® 25R Benchtop Centrifuge, Beckman Coulter). 100 µL of supernatant was used for AOX determination whereas the remaining volume was centrifuged at 13,280 *g* × 20 min (4 °C) (Microfuge® 22R Microcentrifuge, Beckman Coulter) to obtain the post-mitochondrial fraction (S12) in the supernatant for further biochemical determinations of AChE and GST activities. Samples were stored at -80 ºC if not directly used. All assays were carried out in quadruplicate per sample at 22 °C (AChE, GST) or in duplicate at 25 °C (AOX).

AOX activity was analyzed in *A. borealis* 1:4 500 *g* homogenates following Small et al. [264]. AChE activity was analyzed in *A. borealis* S12 homogenates following Guilhermino et al. [265]. GST activity was analyzed in S12 homogenates following Habig et al. [266] with some modifications (i.e., reaction medium is 100 mM potassium phosphate buffer pH 7.4). AChE and GST activity rates were recorded in 96-well Sterilin™ Clear Microtiter™ plates (Thermo Fisher Scientific) at 412 and 340 nm every 60 s during 5 and 3 min, respectively (PowerWave HT Microplate Spectrophotometer, BioTek). Total cytosolic protein was measured in the same homogenate fraction where enzymatic activities were analyzed following Lowry et al. [267].

2.6. Pyrene metabolites

Samples were frozen and freeze-dried prior to the extraction step. Each sample was weighed and extracted in 10 mL of acetone in a 40 mL polypropylene vessel. Focused ultrasound solid-liquid extraction (FUSLE) was performed in the pulsed mode for 2 min, with a pulsed time 'on' of 0.8 s and pulsed time 'off' of 0.2 s, at 20% of irradiation power [268]. Extractions were performed at 0 °C in an ice-water bath [269]. After the extraction step, the supernatant was filtered through 0.45 µm poliamide filters and evaporated to 500 µL under a nitrogen stream at 35 °C using a Turbovap LV evaporator (Zymark).

200-mg Oasis HLB cartridges were conditioned with 10 mL of Milli-Q:acetonitrile (1:9, v/v). Then, the sample was loaded and 1 mL of Milli-Q water was added and cartridges were dried for an hour under vacuum. Then, the analytes were eluted using 10 mL of acetonitrile and collected in a test tube. The eluate was evaporated until dryness under a gentle stream of nitrogen at 35 °C and reconstituted in 150 µL of methanol. Finally, the analyses of pyrene metabolites were performed by high-performance liquid chromatograph with fluorescence detector (HPLC-FLD). For further details on the extraction, clean-up and instrumental analysis see the SI.

2.7. Data analyses

Data were checked for normality with Q-Q plots and the Shapiro-Wilk test and for equality of variances with Levene's test. Differences among treatment groups were assessed either by one-way ANOVA and Tukey HSD as a post-hoc test in case of normally distributed data, or by the Kruskal-Wallis non-parametric rank test for each of the biomarkers in *A. borealis*. For each taxonomic group (i.e., bivalves and polychaetes), differences in the content of GluPyr among L and H treatment groups and among climatic groups were analyzed by either ANCOVA, or multiple linear regression, respectively. For the ANCOVA test the content of pyrene (Pyr) was used as the covariate and for multiple linear regression the treatment group and source region of the species were used as predictors. A simple linear regression between Pyr and GluPyr concentration was also calculated for each of the species. The simple linear regression was used to analyze any likely difference in GluPyr/Pyr ratios among species. Significance level of an overall statistical comparison was set at $p \leq 0.05$, while for pairwise comparisons a Bonferroni correction was applied. All statistical calculations were performed using SPSS version 22.

3. Results and Discussion

3.1. Enzymatic activities

Exposure to the selected Σ_{13}PAH concentration range (i.e., 287 – 1710 µg/kg dry wt. sediment; Table S5) had no significant effect on any biomarker response in *A. borealis* digestive gland (Tables 2, S11-S13). Nevertheless, AChE activity in BSH exposed *A. borealis* was relatively low and within the range as detected in mussels from either historically polluted areas [270] or transplanted to harbour areas [271], indicating a potential neurotoxic response. Examples of classical AChE inhibitors are organophosphate and carbamate pesticides, although PAHs may also show AChE inhibition capacity [270,272]. However, low AChE activity could also be due to either a low AChE substrate specificity in digestive gland with respect to gills as observed in scallops [273] or a stressed physiological status in *A. borealis* as observed in caged mussels [274].

The absence of AChE inhibition in *A. borealis* is also in line with the lack of GST and AOX induction (Table 2). GST activity in *A. borealis* was below baseline levels as detected in digestive gland of scallops (*Chlamys islandica*) [215,275] and slightly lower than those in

mussels (*Mytilus galloprovincialis*) experimentally exposed to similar PAH concentrations (Table 3; [276]). GST activity is mainly correlated to the concentration of 5- to 6-ring PAHs in mussels [277]. Yet, higher concentrations of high-molecular-weight PAHs in BSH exposed *A. borealis* compared to sediment PAH exposed *M. galloprovincialis* yielded lower GST activity values in *A. borealis* than in mussels [276]. Furthermore, a negative correlation was observed between GST activity rates in *M. galloprovincialis* and sediment PAH exposure (Table 3; [276]). This indicates that even if lower-molecular-weight PAHs such as phenanthrene, anthracene, fluoranthene and pyrene were mainly accumulated by *A. borealis* during BSH exposure (**Chapter 3**), pointing to an absence of GST induction, likely confounding factors such as the nutritional and reproductive state of *A. borealis* could have masked the bivalves' response to organic pollution [278].

Table 2. Acyl-CoA oxidase, acetylcholinesterase and glutathione S-transferase activities (mean ± SD) in *Astarte borealis* digestive gland.

Treatment	EA (*n*)	AOX	AChE	GST
BSL	4	2.1 ± 0.4	27.9 ± 8.4	49.8 ± 13.4
BSM	4	2.0 ± 0.4	35.5 ± 4.2	47.8 ± 14.2
BSH	6	2.3 ± 0.6	30.7 ± 14.4	46.7 ± 11.2

EA: experimental aquarium; AOX: Acyl-CoA oxidase (mU AOX/mg prot.); AChE: Acetylcholinesterase (nmol/min/mg prot.); GST: Glutathione S-transferase (nmol/min/mg prot.). Additional abbreviations are defined in Table 1.

AOX activity in all *A. borealis* pooled samples was above levels as detected in digestive gland of mussels from or transplanted to harbour areas [279] and at O&G produced water discharge points [280]. This indicates that AOX levels in the present study were high, even in the controls (i.e., BSL exposed *A. borealis*). Relatively high AOX activity in *A. borealis* may be related to non-toxic factors like low food availability or changing metabolic status under post bloom conditions [134]. Although AOX induction has been described as a rapid and reversible response to PAH and PCB exposure [279,281], this process can also be affected by seasonal changes [281]. AOX activity in mussels (*M. galloprovincialis*) is usually induced during late winter and spring while AOX levels are negatively correlated with lipid content in digestive tubules of mussels during phytoplankton blooms [282]. AOX may be further induced in *A. borealis* due to the increased synthesis of prostaglandins during the species spawning in early autumn [282,283]. Hence, nutritional and reproductive status impacts on peroxisome proliferation should be considered as likely confounding factors when interpreting AOX as a biomarker for exposure to environmental contaminants [281].

Table 3. Total polycyclic aromatic hydrocarbon (PAH) concentrations in whole body soft tissue and glutathione S-transferase activity in digestive gland of exposed bivalve species.

Source	Species (type of exposure)	Total PAH		GST
		Analytes (No. of compounds)	Whole body soft tissue (weight units)	
Present study	*Astarte borealis* [a] (sediment)	3- to 6-ring PAHs (13)	170 (wet wt.) 34830 (lipid wt.)	34 – 62
Baussant et al. [275]	*Chlamys islandica* (dispersed oil)	2- to 6-ring PAHs (16)	0 (wet wt.) 6 (wet wt.) 8 (wet wt.)	475 355 425
	Mytilus edulis (dispersed oil)		0 (wet wt.) 3 (wet wt.) 10 (wet wt.)	71 62 85
Nahrgang et al. [215]	*Chlamys islandica* [b] (bakground)	2- to 6-ring PAHs (16)	< 5 – 5.7 (wet wt.)	500 – 800
	Mytilus edulis [b] (background)		5.7 – 6.3 (wet wt.)	0 – 100
Giuliani et al. [276]	*Mytilus galloprovincialis* (sediment)	2- to 6-ring PAHs (15)	140 (dry wt.) 300 (dry wt.) 600 (dry wt.)	120 110 90

[a] *A. borealis* exposed to BSH treatment.
[b] Biota samples collected from the field in September 2010.
Whole body soft tissue (µg/kg); GST: Glutathione S-transferase (nmol/min/mg prot.).

In summary, we observed a general lack of dose related responses of the selected biomarkers in digestive gland of *A. borealis*. This may indicate that exposure levels were too low to induce such changes. However, within treatment variation in catalytic activity was rather large, which prevented a sensitive analysis of dose response relationships, but for which knowledge of possible confounding factors was lacking. Therefore, in order to interpret variation in biomarker responses in Arctic marine invertebrates, relevant confounding parameters should be identified such as time scales for enzymatic induction, conditions of the assays, and maturation and nutritional status for field individuals.

3.2. Pyrene metabolites

Biotransformation and excretion of hydrophobic organic pollutants is mostly mediated by CYP enzymes, although biotransformation capacity may be low in marine invertebrates [284]. Only a few studies showed the existence of the aryl hydrocarbon receptor (AHR) transcription factor in marine invertebrates and suggested that the expression of Phase I enzymes through AHR in response to hydrocarbon exposure might be tissue-specific in bivalves [285,286]. Additionally, relatively large differences in dominating Phase II biotransformation pathways between invertebrates could yield different PAH metabolite patterns. In the present study, GluPyr was selected as the major Phase II metabolite because glucuronosyltransferases

(UDPGTs) have a higher activity rate than sulfotransferases (STs), and also glucuronidation is the main conjugation pathway in *A. virens* (Figure 1). In the following subsections, pyrene metabolites results are discussed based on the possibilities and limitations for an interspecies comparison inherent in our selection of metabolites.

3.2.1. Pyrene hydroxylation

OHPyr concentrations were often below the limit of detection among all analyzed individuals (Tables 4, S9 and S10). This agrees with typically low OHPyr/Pyr ratios found in other freshwater [287] and marine worms [183,233], as well as in marine clams [288] due to generally high Phase II biotransformation levels and/or low induction capacity of Phase I biotransformation pathways in polychaetes and bivalves. Based on the species-specific sediment threshold for *A. virens* (i.e., 10 µg pyrene/g dry wt. [183]), an absence of pyrene hydroxylase induction and therefore low OHPyr concentrations, would be expected in *A. virens* exposed to PSH (i.e., 0.5 µg pyrene/g dry wt.). This is also expected in *L. balthica* since its PAH biotransformation capacity is generally lower than *A. virens* [139].

3.2.2. 1-hydroxypyrene glucuronidation

A significant effect of the high treatment (PSH) compared to the low sediment treatment (PSL) was observed on the concentration of GluPyr in polychaetes ($p = 0.005$, Table S14). This was not detected between BSH and BSL exposed bivalves (Table S15). Similarly, a multiple linear regression on the concentration of GluPyr did generate a significant model for polychaetes when both treatment and climatic groups were included as predictors ($R^2 = 0.466$, $p < 0.001$, Table S16). This was not the case for bivalves (Table S17).

3.2.3. Biotransformation of pyrene among climatic groups

For polychaetes and bivalves different GluPyr/Pyr ratios were observed when comparing Arctic and temperate species (Figures 2 and 3). GluPyr concentrations in the Arctic *N. ciliata* were on average twice as high as in the temperate *A. virens* in both L and H treatments. This agrees with higher pyrene concentrations in the former species (Figure 2) and a positive correlation between pyrene and GluPyr concentrations in *N. ciliata* ($R^2 = 0.252$, $p = 0.012$), but not in *A. virens* (Tables S18 and S19). In H exposed *A. virens*, low GluPyr concentrations may be due to too low pyrene concentrations in *A. virens* to lead to significant induction of Phase I (e.g., pyrene hydroxylase) and thus, consecutive Phase II (UDPGT) biotransformation

[233]. However, GluPyr/Pyr ratios in H exposed *A. virens* were on average 3.5 times higher than in *N. ciliata*. This shows that biotransformation rates were limited among polychaete species according to the low level of exposure (Table 4), although a higher induction of CYP450 enzymes may be expected in *A. virens* than *N. ciliata* possibly due to a stronger bioactivity of PAHs and additional inducer chemicals, such as PBDEs (Table S4; [289]), in the broad omnivore *A. virens* than in *N. ciliata* [136,235].

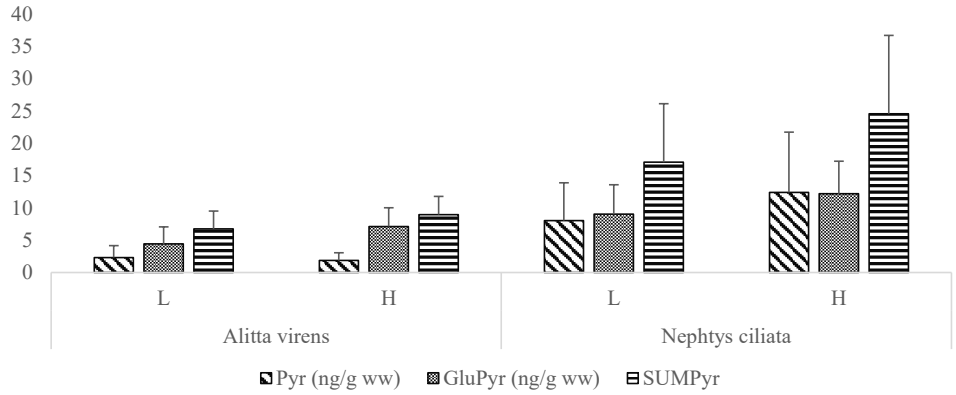

Figure 2. Concentrations of pyrene (Pyr; ng/g wet wt.), pyrene-1-glucuronide (GluPyr; ng/g wet wt.) and the sum of both compounds (SUMPyr) in whole body tissue of temperate (*Alitta virens*) and Arctic (*Nephtys ciliata*) polychaete species exposed to the low (L) and high (H) sediment treatments.

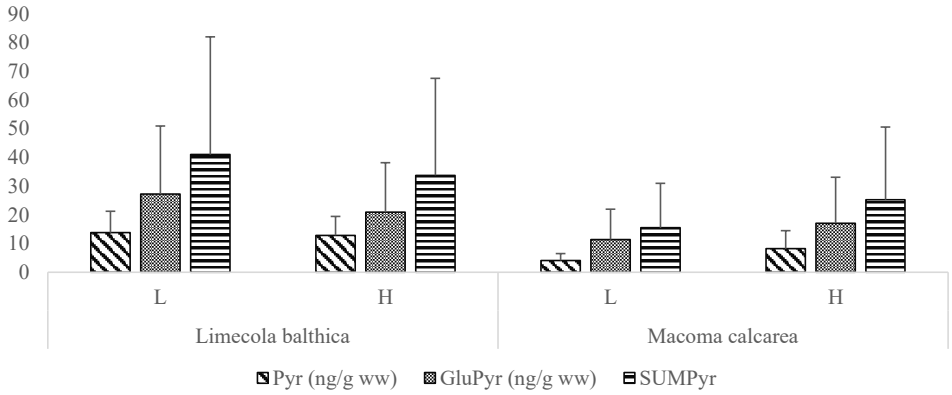

Figure 3. Concentrations of pyrene (Pyr; ng/g wet wt.), pyrene-1-glucuronide (GluPyr; ng/g wet wt.) and the sum of both compounds (SUMPyr) in soft body tissue of temperate (*Limecola balthica*) and Arctic (*Macoma calcarea*) bivalve species exposed to the low (L) and high (H) sediment treatments.

Table 4. Pyrene, 1-hydroxypyrene and pyrene-1-glucuronide concentrations (geomean [min-max]; ng/g wet wt.) in Arctic (*Macoma calcarea*, *Nephtys ciliata*) and temperate species (*Limecola balthica*, *Alitta virens*).

Species	Treatment	Pyr	OHPyr	GluPyr
Limecola balthica	BSL	11.6 (<LOQ – 36.4)	0.4 (<LOD – 2.98)	17.4 (<LOD – 74.0)
	BSH	11.8 (<LOQ – 26.7)	0.4 (<LOD – 2.22)	12.1 (<LOQ – 50.7)
Macoma calcarea	BSL	3.6 (0.7 – 12.3)	0.5 (<LOD – 1.5)	7.8 (<LOD – 34.7)
	BSH	5.8 (1.1 – 21.3)	0.6 (<LOD – 3.1)	7.1 (<LOQ – 41.6)
Alitta virens	PSL	1.7 (0.5 – 5.4)	0.1 (<LOD – 1.5)	3.9 (2.2 – 10.0)
	PSH	1.5 (0.5 – 4.3)	<LOD	6.7 (4.5 – 13.7)
Nephtys ciliata	PSL	5.9 (2.0 – 15.5)	0.2 (<LOD – 0.9)	8.2 (4.1 – 20.9)
	PSH	9.2 (3.6 – 28.5)	0.3 (<LOD – 3.7)	11.4 (6.4 – 22.2)

Pyr: Pyrene; OHPyr: 1-hydroxypyrene; GluPyr: pyrene-1-glucuronide; LOD: limit of detection; LOQ: limit of quantification. Additional abbreviations are defined in Table 1.

Mean GluPyr concentrations in the temperate *L. balthica* were higher than in the Arctic *M. calcarea*. This complies with higher pyrene concentrations in *L. balthica* than in *M. calcarea* (Figure 3). In H exposed bivalves, pyrene concentrations were within the same range as observed concentrations of another 4-ring PAH, chrysene, in scallops (*Chlamys farreri*) after exposure to 0.1 µg/L (i.e., 35 – 45 ng chrysene/g dry wt. [290]). Within such range, concentrations of chrysene in scallops did not reveal a significant correlation with 7-ethoxyresorufin O-deethylase activity, which is the catalytic measurement of CYP450

induction. Absence of CYP induction would explain similar biotransformation rates between *L. balthica* and *M. calcarea* (Figure 3), but it would not account for a seemingly decreasing trend in GluPyr concentration with PAH exposure (Table 4). This stresses the importance of considering different Phase II biotransformation pathways for the characterization and comparison of pyrene metabolite patterns among bivalves [288,291].

In summary, pyrene metabolite patterns appeared to be similar among all target species after exposure to PAHs in sediment at the different sediment treatment levels. However, GluPyr yielded different concentration profiles between Arctic (*N. ciliata*) and temperate polychaetes (*A. virens*) exposed to the high treatment, possibly related to a higher induction of CYP450 enzymes in *A. virens*. Besides, the suitability of this metabolite as biomarker of exposure could be confirmed for *N. ciliata* according to its positive correlation with pyrene exposure. In bivalves, both temperate (*L. balthica*) and Arctic species (*M. calcarea*) showed similar GluPyr concentration profiles, although lower GluPyr concentrations with PAH exposure pointed towards the activation of alternative Phase II biotransformation pathways in *L. balthica*.

4. Conclusions and Implications

No effects of environmentally relevant PAH concentrations in sediment were found on biomarkers AChE, GST and AOX activities in digestive gland of *A. borealis*. Overall exposure levels were probably too low to induce detectable biomarker responses in *A. borealis* digestive gland, while relatively low AChE levels and high baseline AOX levels potentially indicated a stressed physiological status of *A. borealis*. A further identification of crucial confounding parameters in both enzymatic induction and inhibition, as well as optimizing the biomarker assays for Arctic species is therefore needed for an appropriate evaluation.

In Arctic and temperate benthic invertebrates similarly exposed to PAHs, the concentrations of Phase I and Phase II biotransformation metabolites of pyrene did not increase with pyrene exposure. Biotransformation rates appeared to be limited by low exposure to sediment-bound chemicals among bivalve and polychaete species, although glucuronide conjugates represented up to 74% and 79% of the total pyrene concentrations in Arctic bivalves and temperate polychaetes, respectively. This shows that in order to characterize and quantify

PAH exposure among invertebrate species, the metabolic products of Phase I and Phase II biotransformation should be equally considered.

Acknowledgements

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Chapter 4

Supporting Information

Field sampling

Sampling of Arctic species. Sampling in Kongsfjorden Bay (Svalbard, Norway) was mainly performed along a transect from Tønsneset (79°0'22"N, 11°57'10"E) to Blomstrandhalvøya (78°59'14"N, 11°57'28"E), and to a lesser extent at Gluudneset and Tyskerhytta, on board of research vessel MS Teisten (Kings Bay AS, Ny-Ålesund) on 8 to 26 September 2014. Biota samples were collected using a Van Veen grab (0.1 m^2). Three Arctic marine benthic invertebrate species with different feeding behaviours were collected: *Astarte borealis* (mollusc; suspension feeder [Schumacher, 1817]), *Macoma calcarea* (mollusc; surface deposit and suspension feeder [Gmelin, 1791]) and *Nephtys ciliata* (polychaete; predator and deposit feeder [O.F. Müller, 1776]).

Sampling of temperate species. *Limecola balthica* (mollusc; surface deposit and suspension feeder [Linnaeus, 1758]) were collected at low tide at the Oesterdam (The Netherlands, 51°26'24"N, 4°13'16"E) on 16 to 17 July 2014. *Alitta virens* (polychaete; predator and deposit feeder [Sars, 1835]) were obtained from a professional bait farm, Topsy baits (Wilhelminadorp, the Netherlands).

Acclimatization

Arctic species were kept in glass aquaria with aerated, filtered (20 µm) and UV-treated sea water from the fjord (3 °C) for 2 to 6 d, before start of the experiment. In the case of *N. ciliata*, a thin layer of sieved (1 mm Ø) sediment from the original sampling area was also included. Animals were not fed during acclimatization.

Temperate species were kept in glass aquaria with aerated, filtered (0.2 µm) sea water for 5 d and were fed once with spiked ground fish food (TetraMin) suspended in deionised water at the start of the acclimatization. In order to have sufficient food supply, 10 mg of dry food for *A. virens* and 3 mg (shell length ≥ 15 mm) or 1.5 mg (shell length < 15 mm) for *L. balthica* was added per individual.

Water quality variables such as pH, dissolved oxygen, salinity and temperature were measured every two days during exposure time (Tables S2 and S3).

Sediment collection

A moderately contaminated sub-toxic marine sediment was prepared by mixing a non-contaminated 'clean' sediment with a naturally contaminated harbour sediment. Batches of muddy sediment (132 L) and coarse sediment (176 L) were collected in the Oosterchelde estuary (The Netherlands, 51°36'13"N, 3°47'49"E) at low tide on 24 to 25 April 2014 and 2 May 2014. Coarse sediment (0.5 – 1 mm grain size) was sieved in the field with a 1-mm sieve whereas muddy sediment (< 0.5 mm grain size) was sieved at laboratory inside a 1-mm sieve with some filtered sea water (0.2 µm). Once sieved both samples were stored at 3 °C.

A batch of harbour sediment (30 L) was collected near Nesserdijk (Rotterdam, the Netherlands, 51°54'14"N, 4°31'17"E) by Nautisch Service Centrum in April 2014 and sieved with a 1-mm sieve on 1 May 2014. A subsample of the sieved wet harbour sediment (800 mL) was sent to Wageningen Marine Research laboratories (IJmuiden, the Netherlands) for further chemical analyses (Table S4).

Test species

Arctic experiment. *A. borealis*, *M. calcarea* and *N. ciliata* were obtained as described in the field sampling section. *A. borealis* randomly selected healthy individuals with an average and standard deviation (SD) shell length of 31.30 (4.63) mm and wet wt. (flesh) of 7.47 (4.16) g were used. As for *M. calcarea* and *N. ciliata*, we did not measure any morphological parameters due to the scarcity of individuals. Animals were not fed during the acclimatization, neither during the exposure experiments.

Temperate experiment. *L. balthica* and *A. virens* were obtained as described in the field sampling section. *L. balthica* randomly selected healthy individuals with an average (SD) shell length of 13.49 (2.46) mm and wet wt. (shell + flesh) of 0.47 (0.31) g were used. *A. virens* randomly selected healthy individuals with an average (SD) length of 10.83 (1.70) cm and wet wt. of 2.75 (0.98) g were used. Animals were fed once at the beginning of the acclimatization.

Pyrene metabolites analysis

Chemicals. Pyrene (98%) and 1-hydroxypyrene (OHPyr) (98%) were purchased from Sigma Aldrich. Pyrenyl-1-O-glucuronide (GluPyr) (98.2%) was supplied by Isostandards Material.

The dilutions at lower concentrations were prepared daily, depending on the experiment. All chemical standards were stored at 4 °C and stock solutions at -20 °C. Acetone, methanol and acetonitrile (all HPLC grade) were purchased from Macrom Fine Chemicals. For filtration 0.45 µm polyamide filters (Macherey Nagel) were used. Oasis HLB (200 mg) cartridges were purchased from Waters in order to perform clean-up step.

High-performance liquid chromatograph with a fluorescence detector (HPLC-FLD) analysis. A high-performance liquid chromatograph (Agilent Technologies, Series 1100) with a fluorescence detector (FLD, Agilent Technologies, Series 1100) was used for all the quantitative measurements. The chromatographic method was modified from Beach et al. [292]. A Supelcolsil LC-PAH HPLC column (10 cm × 4.6 mm, 3 µm) was used for the separation. Mobile phases were prepared: of A: only Milli-Q water, B: methanol:acetonitrile:Milli-Q water (38:57:5, v/v/v), both with 10 mM ammonium acetate. The solvent gradient was 15 min, starting with 20% B with a linear gradual increase to 100% B from 0 to 2.5 min with a 4 min hold at 100% B and continued with a linear decrease to 80% B from 6.5 to 8.5 min with a 6.5 min hold at 80% B. An injection volume of 5 µL and a flow rate of 800 µL/min were used throughout. Fluorescence detection was performed at an excitation/emission wavelength pair of 235/388 nm for pyrene, OHPyr and GluPyr.

Limits of detection (LODs) were 0.2, 0.35 and 0.20 ng/mL for pyrene, OHPyr and GluPyr, respectively. LODs were expressed in biomass units taking 0.4, 0.8, 0.05 and 1.7 g as the average wet wt. per sample of *M. calcarea*, *N. ciliata*, *L. balthica*, *A. virens*, respectively. Therefore, pyrene, OHPyr and GluPyr LODs were recalculated as 0.5, 0.88 and 0.5 ng/g for *M. calcarea*; 0.25, 0.44 and 0.25 ng/g for *N. ciliata*; 4, 7 and 4 ng/g for *L. balthica*; and 0.12, 0.21 and 0.12 ng/g for *A. virens*. Samples with pyrene or GluPyr below the limit of quantification (LOQ) were assigned a “LOQ/2” value.

Table S1. Overview of the number of experimental aquaria per species and sediment treatment.

			Treatment (n)[a]				
Experiment	Species	Ind./aq.[b]	BSL	BSM	BSH	PSL	PSH
Arctic	*Macoma calcarea*	40-50	2	n.a.	2		
	Astarte borealis	25	4	4	6		
	Nephtys ciliata	20				2	3
Temperate	*Limecola balthica*	50	2	n.a.	3		
	Alitta virens	16				3	4

[a] n: number of replicates per sediment treatment.
[b] Ind./aq.: number of organisms per aquarium.
BSL: Low Bivalve Sediment; BSM: Medium Bivalve Sediment; BSH: High Bivalve Sediment; PSL: Low Polychaete Sediment; PSH: High Polychaete Sediment; n.a.: not available

Table S2. Mean water quality parameters in the Arctic experiment H treatment aquaria.

	Astarte borealis - BSH					*Macoma calcarea* - BSH				
Day	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)
1	8.1	82.7	9.0	34.7	2.7	7.9	81.0	8.8	35.6	2.9
2	8.1	81.3	8.8	34.7	3.0	7.9	82.5	8.9	35.7	2.9
3	8.0	82.3	8.9	34.8	2.7	8.0	84.0	9.0	35.8	2.9
4	7.9	83.1	9.0	34.9	2.7	7.9	85.0	9.2	35.8	2.9
5	7.9	83.5	9.0	34.9	2.6	7.9	85.5	9.3	35.9	2.9
6	7.8	83.7	9.1	35.0	2.6	7.8	81.5	8.7	35.7	2.9
7	7.9	85.0	9.2	35.1	2.6	7.9	83.5	9.1	36.0	2.9
8	7.8	84.4	9.1	35.2	2.7	8.0	86.0	9.4	35.8	2.8
9	7.8	83.8	9.0	35.2	2.7	n.a.	82.0	9.1	36.0	2.9

	Nephtys ciliata - PSH				
Day	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)
1	8.0	81.2	8.7	35.1	2.9
2	8.0	81.5	8.8	35.2	2.8
3	8.0	82.5	8.9	35.2	2.8
4	8.0	82.8	8.9	35.3	2.8
5	7.9	82.3	8.9	35.5	2.8
6	7.9	82.7	8.9	35.5	2.8
7	8.0	81.7	8.8	35.7	2.9
8	8.0	84.8	9.2	35.8	2.8
9	8.0	85.3	9.0	35.8	2.8

D.O.: dissolved oxygen; n.a.: not available. Additional abbreviations are defined in Table S1.

Table S3. Mean water quality parameters in the temperate experiment H treatment aquaria.

Limecola balthica - BSH

Day	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)
1	8.2	83.8	6.6	32.8	17.8
2	n.a.	80.3	6.3	n.a.	17.8
3	n.a.	79.3	6.2	n.a.	17.7
4	8.2	80.8	6.2	33.3	18.0
5	8.2	82.0	6.4	33.5	18.1
6	8.2	82.2	6.4	33.6	17.9
7	8.3	84.2	6.5	34.1	17.8
8	8.2	84.2	6.5	34.5	17.8
9	8.3	86.0	6.6	34.7	18.1
10	8.3	85.2	6.6	35.0	17.9
11	8.3	85.3	6.6	35.4	17.9
12	8.3	84.5	6.5	35.7	17.9

Alitta virens - PSH

Day	pH	D.O. (%)	D.O. (mg/L)	Salinity (g/L)	T (°C)
1	8.1	79.4	6.2	33.8	18.1
2	n.a.	77.9	6.1	n.a.	18.1
3	n.a.	75.3	5.9	n.a.	17.9
4	8.1	76.7	5.9	34.4	18.2
5	8.2	78.9	6.1	34.0	18.2
6	8.2	80.7	6.2	34.5	18.0
7	8.2	80.9	6.2	35.2	18.0
8	8.2	81.6	6.3	35.1	18.0
9	8.2	79.7	6.0	35.5	18.3
10	8.2	82.1	6.3	34.8	18.0
11	8.2	82.4	6.3	35.2	18.0
12	8.2	81.4	6.2	35.7	17.9

D.O.: dissolved oxygen; n.a.: not available. Additional abbreviations are defined in Table S1.

Table S4. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls and polybrominated diphenyl ethers concentrations in Rotterdam harbour sediment (Nesserdijk, Rotterdam).

Compound	Mean (μg/kg dry wt.)
PHE	1378
ANT	459
FLT	2551
PYR	1811
BaA	1174
CHR	1352
BeP	510
BbF	1403
BkF	536
BaP	1148
BghiP	612
dBahA	135
Ind123P	1148
CB-28	< 0.20
CB-52	0.64
CB-101	0.97
CB-118	0.89
CB-138	0.97
CB-153	0.64
CB-180	< 0.50
BDE-28	0.23
BDE-47	2.04
BDE-99	6.12
BDE-100	0.51
BDE-153	10.71
BDE-154+BDE-155	0.26

PHE: phenanthrene; ANT: anthracene; FLT: fluoranthene; PYR: pyrene; BaA: benz(a)anthracene; CHR: chrysene; BeP: benzo(e)pyrene; BbF: benzo(b)fluoranthene; BkF: benzo(k)fluoranthene; BaP: benzo(a)pyrene; BghiP: benzo(ghi)perylene; dBahA: dibenz(a,h)anthracene; Ind123P: indeno(1,2,3-cd)pyrene.

Table S5. Polycyclic aromatic hydrocarbons concentrations in sediment treatments at the start of the Arctic experiment. Source: **Chapter 3**.

Treatment	BSL		BSH		PSL		PSM		PSH	
Compound (µg/kg dry wt.)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
OM (% dry wt.)	2.05	n.a.	2.52	0.03	6.02	n.a.	3.89	n.a.	3.76	n.a.
PHE	48.11	8.46	164.69	48.81	155.03	26.35	159.71	15.59	250.26	29.11
ANT	8.52	1.25	61.62	14.90	34.09	3.75	49.27	9.77	81.30	8.37
FLT	58.83	11.28	306.13	128.02	249.20	35.11	288.08	58.13	406.93	52.78
PYR	42.94	5.80	240.91	109.12	180.19	24.54	238.76	75.43	313.86	38.50
BaA	15.45	3.14	148.82	70.13	83.17	20.31	121.87	54.17	156.96	18.12
CHR	n.d.	n.d.	92.95	59.11	19.64	11.43	80.57	41.76	170.90	110.17
BeP	25.42	7.53	209.65	88.39	128.04	19.40	216.94	117.22	251.10	31.37
BbF	30.44	6.45	174.13	69.34	145.95	16.18	166.15	32.69	234.81	30.82
BghiP	0.80	0.65	11.45	7.23	8.17	2.22	13.46	5.70	18.84	6.94
dBahA	32.09	12.89	146.19	58.01	129.82	17.92	140.35	27.26	203.35	25.71
Ind123P	24.12	3.57	152.92	60.80	150.68	20.01	154.70	32.43	218.81	39.02
Σ_{13}PAH	286.72	58.50	1709.46	709.16	1283.99	184.63	1629.86	470.15	2307.13	162.56

OM: organic matter; n.a.: not available; n.d.: not detected. Additional abbreviations are defined in Tables S1 and S4.

Table S6. Polycyclic aromatic hydrocarbons concentrations in sediment treatments at the end of the temperate experiment (t = 28 d). Source: **Chapter 3**.

Treatment	BSL		BSH		PSL		PSM		PSH	
Compound (µg/kg dry wt.)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
OM (% dry wt.)	2.18	0.06	2.73	0.76	3.52	0.04	4.01	0.04	4.00	0.04
PHE	53.71	4.21	229.66	42.90	159.69	82.88	278.72	98.45	381.60	258.88
ANT	14.43	2.23	98.66	48.61	39.03	21.19	80.80	35.04	128.92	85.59
FLT	80.04	9.79	460.54	196.09	316.06	156.73	430.07	125.39	667.36	280.22
PYR	60.09	7.37	349.31	149.85	254.04	146.96	321.96	101.17	509.52	215.43
BaA	21.79	3.26	227.37	130.50	140.35	122.63	158.02	32.66	256.14	70.35
CHR	n.d.	n.d.	158.98	125.12	220.13	340.36	113.51	38.92	207.44	127.47
BeP	34.62	6.50	352.06	195.45	205.57	141.23	257.50	74.64	479.18	250.18
BbF	42.21	5.79	255.53	116.72	168.62	85.51	243.55	63.64	387.37	169.03
BghiP	4.26	3.91	19.16	10.47	19.88	23.15	16.31	4.89	42.54	25.68
dBahA	49.43	11.12	212.64	82.18	127.75	53.39	232.47	82.85	352.82	194.89
Ind123P	39.37	9.81	205.33	88.24	136.06	67.36	204.31	54.36	374.39	202.59
Σ_{13}PAH	400.01	52.03	2569.23	1182.50	1787.18	1103.86	2337.23	698.72	3787.26	1880.29

OM: organic matter; n.d.: not detected. Additional abbreviations are defined in Tables S1 and S4

Table S7. Polycyclic aromatic hydrocarbons concentrations (mean ± SD; µg/kg lipid wt.) in biota samples collected from the Arctic experiment. Source: **Chapter 3**.

Species	*Macoma calcarea*		*Nephtys ciliata*			*Astarte borealis*	
Treatment	BSL	BSH	PSL	PSM	PSH	Background	BSH
PHE	3566 ± 393	2821 ± 287	1402 ± 681	729	2462 ± 941	9697 ± 1482	11746 ± 4998
ANT	890 ± 89	718 ± 48	336 ± 143	149	297 ± 120	2384 ± 442	2977 ± 957
FLT	1612 ± 178	3329 ± 1174	2353 ± 788	1771	4468 ± 969	1376 ± 280	8504 ± 4206
PYR	1314 ± 87	2623 ± 930	1713 ± 530	1384	3758 ± 920	1119 ± 120	6926 ± 3575
BaA	n.d.	673 ± 240	113 ± 70	77	218 ± 104	n.d.	n.d.
CHR	7434 ± 6614	470 ± 113	1327 ± 1709	0.15	6425 ± 6647	6164 ± 1276	2926 ± 2658
BeP	316 ± 0	957 ± 374	427 ± 178	298	650 ± 129	884 ± 169	2066 ± 2300
BbF	175 ± 30	658 ± 305	117 ± 78	100	228 ± 69	223 ± 56	845 ± 269
BghiP	1162 ± 745	50 ± 65	344 ± 454	n.d.	1106 ± 955	1409 ± 2154	n.d.
dBahA	275 ± 2	506 ± 133	166 ± 76	40	n.d.	n.d.	479 ± 233
Ind123P	69 ± 40	298 ± 102	34 ± 37	n.d.	n.d.	205 ± 144	421 ± 187
Σ_{13}PAH	16933 ± 6714	13104 ± 3552	8333 ± 267	4550	20242 ± 9762	34245 ± 20336	37668 ± 17371

n.d.: not detected. Additional abbreviations are defined in Tables S1 and S4.

Table S8. Polycyclic aromatic hydrocarbons concentrations (mean ± SD; µg/kg lipid wt.) in biota samples collected from the temperate experiment. Source: **Chapter 3**.

Species	*Limecola balthica*			*Alitta virens*			
Treatment	Background	BSL	BSH	Background	PSL	PSM	PSH
PHE	2499 ± 571	2849	2257 ± 165	2497 ± 798	2267 ± 1376	1324 ± 496	2278 ± 2000
ANT	225 ± 60	262	334 ± 102	136 ± 64	108 ± 70	100 ± 27	113 ± 96
FLT	1431 ± 394	2853	3061 ± 685	5421 ± 1780	1596 ± 598	1824 ± 664	3151 ± 1903
PYR	1484 ± 325	3513	4340 ± 1153	1611 ± 530	893 ± 379	1157 ± 419	2034 ± 1254
BaA	680 ± 177	1101	2547 ± 484	n.d.	83 ± 62	134 ± 19	104 ± 51
CHR	863 ± 98	964	11585 ± 9242	268 ± 278	n.d.	887 ± 1061	n.d.
BeP	937 ± 247	1618	2833 ± 702	125 ± 50	249 ± 220	279 ± 102	475 ± 286
BbF	938 ± 259	1442	2295 ± 432	51 ± 26	100 ± 88	151 ± 26	125 ± 45
BghiP	2216 ± 4746	47	1754 ± 1454	76 ± 66	n.d.	134 ± 134	n.d.
dBahA	717 ± 491	702	1083 ± 818	83 ± 29	115 ± 79	137 ± 36	239 ± 238
Ind123P	452 ± 206	448	839 ± 473	n.d.	68 ± 79	96 ± 56	75 ± 62
Σ_{13}PAH	35512 ± 56664	15799	32928 ± 13246	10360 ± 3099	8005 ± 5818	6223 ± 2229	9371 ± 5439

n.d.: not detected. Additional abbreviations are defined in Tables S1 and S4

Table S9. Pyrene (Pyr), 1-hydroxypyrene (OHPyr) and pyrene-1-glucuronide (GluPyr) concentrations in polychaete samples (ng/g wet wt.).

Species	Treatment	Aquarium	Pyr	OHPyr	GluPyr
A. virens	L	PSL-1	1.5	1.5	10
A. virens	L	PSL-1	4.7	<LOD	2.4
A. virens	L	PSL-1	5.2	<LOD	2.9
A. virens	L	PSL-1	0.5	<LOD	2.2
A. virens	L	PSL-2	5.4	<LOD	3.5
A. virens	L	PSL-2	1	<LOD	2.7
A. virens	L	PSL-2	3.4	<LOD	3.2
A. virens	L	PSL-2	1.3	<LOD	9.2
A. virens	L	PSL-3	1.5	<LOD	3.2
A. virens	L	PSL-3	0.6	<LOD	3.6
A. virens	L	PSL-3	1.8	<LOD	6.1
A. virens	L	PSL-3	1	<LOD	4.3
A. virens	H	PSH-1	1.4	<LOD	8.1
A. virens	H	PSH-1	0.9	<LOD	7.6
A. virens	H	PSH-1	2	<LOD	5.7
A. virens	H	PSH-2	1.6	<LOD	13.7
A. virens	H	PSH-2	0.5	<LOD	11.8
A. virens	H	PSH-3	3.6	<LOD	5.1
A. virens	H	PSH-3	1.4	<LOD	4.6
A. virens	H	PSH-3	1.1	<LOD	7
A. virens	H	PSH-3	1.7	<LOD	4.8
A. virens	H	PSH-4	4.3	<LOD	6.2
A. virens	H	PSH-4	3.1	<LOD	6.4
A. virens	H	PSH-4	0.6	<LOD	4.5
N. ciliata	L	PSL-1	3	<LOD	4.1
N. ciliata	L	PSL-1	2.8	0.6	10
N. ciliata	L	PSL-1	11.8	<LOD	6.9
N. ciliata	L	PSL-1	15.5	<LOD	7.5
N. ciliata	L	PSL-1	2.6	<LOQ	9.1
N. ciliata	L	PSL-1	14.3	<LOD	8.7
N. ciliata	L	PSL-2	2.7	0.5	8.3
N. ciliata	L	PSL-2	2	0.8	5.6
N. ciliata	L	PSL-2	15.3	<LOD	13.8
N. ciliata	L	PSL-2	2.7	<LOQ	4.5
N. ciliata	L	PSL-2	10.1	<LOD	8.9
N. ciliata	L	PSL-2	13.8	<LOD	20.9
N. ciliata	H	PSH-2	28.5	<LOD	22.2
N. ciliata	H	PSH-2	19.1	<LOD	15.7
N. ciliata	H	PSH-2	5.2	2.5	8.2
N. ciliata	H	PSH-2	27	<LOD	10.9
N. ciliata	H	PSH-2	4.2	1.5	7.8
N. ciliata	H	PSH-2	4.3	<LOD	10
N. ciliata	H	PSH-5	3.6	3.7	6.4
N. ciliata	H	PSH-5	4.6	2.6	20.6
N. ciliata	H	PSH-5	15.8	<LOD	10.7
N. ciliata	H	PSH-6	15.8	<LOD	9.3
N. ciliata	H	PSH-6	3.8	<LOD	14.5
N. ciliata	H	PSH-6	16.9	<LOD	10

L: low treatment; H: high treatment; LOD: limit of detection; LOQ: limit of quantification.
LOQs: 0.39 ng/mL (Pyr), 0.9 ng/mL (OHPyr) and 0.38 ng/mL (GluPyr).

Table S10. Pyrene (Pyr), 1-hydroxypyrene (OHPyr) and pyrene-1-glucuronide (GluPyr) concentrations in bivalve samples (ng/g wet wt.).

Species	Treatment	Aquarium	Pyr	OHPyr	GluPyr
L. balthica	L	BSL-1	17.90	2.18	47.51
L. balthica	L	BSL-1	16.50	2.04	40.94
L. balthica	L	BSL-1	10.50	1.87	48.87
L. balthica	L	BSL-1	11.60	<LOD	5.40
L. balthica	L	BSL-1	22.70	<LOD	5.90
L. balthica	L	BSL-1	19.69	2.20	56.60
L. balthica	L	BSL-1	17.90	<LOD	7.40
L. balthica	L	BSL-1	13.70	<LOD	6.70
L. balthica	L	BSL-1	22.30	<LOD	12.50
L. balthica	L	BSL-1	11.30	<LOD	6.00
L. balthica	L	BSL-1	36.38	2.98	73.97
L. balthica	L	BSL-1	10.00	1.19	56.36
L. balthica	L	BSL-2	1.30	<LOD	1.30
L. balthica	L	BSL-2	2.40	<LOD	<LOQ
L. balthica	L	BSL-2	14.12	1.83	50.06
L. balthica	L	BSL-2	9.23	1.40	46.43
L. balthica	L	BSL-2	9.09	1.56	34.64
L. balthica	L	BSL-2	13.50	<LOD	11.70
L. balthica	L	BSL-2	6.45	1.06	45.73
L. balthica	L	BSL-2	11.00	<LOD	6.50
L. balthica	L	BSL-2	13.60	<LOD	4.80
L. balthica	L	BSL-2	13.20	<LOD	15.10
L. balthica	L	BSL-2	24.07	2.66	67.66
L. balthica	L	BSL-2	7.40	<LOD	22.80
L. balthica	L	BSL-2	8.90	<LOD	5.50
L. balthica	H	BSH-1	8.04	0.88	16.43
L. balthica	H	BSH-1	14.49	1.26	44.81
L. balthica	H	BSH-1	11.40	1.27	37.22
L. balthica	H	BSH-1	18.00	<LOD	1.80
L. balthica	H	BSH-1	13.00	<LOD	1.40
L. balthica	H	BSH-1	11.90	<LOD	5.00
L. balthica	H	BSH-1	13.10	<LOD	2.20
L. balthica	H	BSH-2	3.30	<LOD	2.10
L. balthica	H	BSH-2	6.34	0.96	26.35
L. balthica	H	BSH-2	6.89	2.03	24.31
L. balthica	H	BSH-2	16.72	2.22	48.66
L. balthica	H	BSH-2	2.90	<LOD	3.90
L. balthica	H	BSH-2	10.88	1.47	43.56
L. balthica	H	BSH-2	19.60	<LOD	5.00
L. balthica	H	BSH-2	14.31	1.66	36.31
L. balthica	H	BSH-2	<LOQ	<LOD	1.50
L. balthica	H	BSH-2	12.10	<LOD	4.50
L. balthica	H	BSH-3	16.25	0.99	50.65
L. balthica	H	BSH-3	12.55	1.75	47.08
L. balthica	H	BSH-3	6.03	1.01	22.13
L. balthica	H	BSH-3	11.85	1.30	18.75
L. balthica	H	BSH-3	22.60	<LOD	11.10
L. balthica	H	BSH-3	21.50	<LOD	22.70
L. balthica	H	BSH-3	21.00	<LOD	14.20
L. balthica	H	BSH-3	26.70	<LOD	31.30
M. calcarea	L	BSL-1	2.06	0.68	4.24
M. calcarea	L	BSL-1	0.70	1.10	0.70
M. calcarea	L	BSL-1	2.49	0.54	11.09
M. calcarea	L	BSL-1	5.11	0.88	23.48
M. calcarea	L	BSL-1	0.70	0.90	0.30
M. calcarea	L	BSL-1	2.16	0.69	8.37

Table S10. Continued.

Species	Treatment	Aquarium	Pyr	OHPyr	GluPyr
M. calcarea	L	BSL-1	3.95	0.74	18.65
M. calcarea	L	BSL-1	4.10	<LOD	<LOD
M. calcarea	L	BSL-1	2.80	1.10	<LOQ
M. calcarea	L	BSL-1	5.50	<LOD	5.30
M. calcarea	L	BSL-1	6.52	0.79	24.78
M. calcarea	L	BSL-1	3.50	<LOD	<LOQ
M. calcarea	L	BSL-1	5.41	0.89	<LOQ
M. calcarea	L	BSL-1	5.20	1.50	<LOD
M. calcarea	L	BSL-1	4.76	0.55	15.50
M. calcarea	L	BSL-1	2.92	0.33	9.40
M. calcarea	L	BSL-1	12.25	0.93	25.12
M. calcarea	L	BSL-1	5.54	1.00	21.15
M. calcarea	L	BSL-1	3.99	0.70	26.40
M. calcarea	L	BSL-1	3.10	<LOD	3.20
M. calcarea	L	BSL-1	5.30	0.95	34.72
M. calcarea	L	BSL-1	4.70	<LOD	2.90
M. calcarea	L	BSL-1	5.64	0.79	17.74
M. calcarea	L	BSL-2	2.34	0.51	6.80
M. calcarea	L	BSL-2	3.30	<LOD	1.70
M. calcarea	H	BSH-6	2.30	<LOD	<LOD
M. calcarea	H	BSH-6	1.20	0.60	<LOQ
M. calcarea	H	BSH-6	5.90	<LOD	0.50
M. calcarea	H	BSH-6	11.40	<LOD	1.70
M. calcarea	H	BSH-6	1.20	<LOD	0.30
M. calcarea	H	BSH-6	1.10	1.10	0.80
M. calcarea	H	BSH-6	4.30	<LOD	0.40
M. calcarea	H	BSH-6	16.67	1.17	33.63
M. calcarea	H	BSH-6	8.10	<LOQ	2.00
M. calcarea	H	BSH-6	2.90	<LOD	3.00
M. calcarea	H	BSH-6	6.30	<LOD	3.90
M. calcarea	H	BSH-6	8.30	<LOQ	0.80
M. calcarea	H	BSH-6	13.07	1.23	15.24
M. calcarea	H	BSH-6	21.30	<LOQ	2.50
M. calcarea	H	BSH-6	17.34	1.30	24.78
M. calcarea	H	BSH-6	17.39	1.66	40.16
M. calcarea	H	BSH-6	21.18	1.50	30.23
M. calcarea	H	BSH-6	3.54	1.28	32.48
M. calcarea	H	BSH-6	6.11	0.89	34.12
M. calcarea	H	BSH-6	5.25	0.86	38.24
M. calcarea	H	BSH-7	6.04	1.67	41.63
M. calcarea	H	BSH-7	5.72	1.37	15.23
M. calcarea	H	BSH-7	3.66	1.25	23.47
M. calcarea	H	BSH-7	3.78	1.63	29.22
M. calcarea	H	BSH-7	4.83	1.84	36.21

L: low treatment; H: high treatment; LOD: limit of detection; LOQ: limit of quantification.
LOQs: 0.39 ng/mL (Pyr), 0.9 ng/mL (OHPyr) and 0.38 ng/mL (GluPyr).

Table S11. Mean acetylcholinesterase activity statistical comparison between high (BSH), medium (BSM) and low (BSL) treatment exposed *Astarte borealis* individuals.

Descriptives								
Mean AChE								
	N	Mean	SD	SE	95% confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
BSL	7	0.0284	0.00824	0.00311	0.0207	0.0360	0.01	0.04
BSM	8	0.0355	0.01005	0.00355	0.0271	0.0439	0.02	0.05
BSH	8	0.0353	0.01614	0.00571	0.0218	0.0488	0.00	0.05
Total	23	0.0333	0.01202	0.00251	0.0281	0.0385	0.00	0.05

Test of homogeneity of variances			
Mean AChE			
Levene statistic	df1	df2	Sig.
1.163	2	20	0.333

ANOVA							
Mean AChE							
			Sum of squares	df	Mean square	F	Sig.
Between groups	(Combined)		0.000	2	0.000	0.827	0.452
	Linear term	Unweighted	0.000	1	0.000	1.234	0.280
		Weighted	0.000	1	0.000	1.176	0.291
		Deviation	0.000	1	0.000	0.478	0.497
	Quadratic term	Unweighted	0.000	1	0.000	0.478	0.497
		Weighted	0.000	1	0.000	0.478	0.497
Within groups			0.003	20	0.000		
Total			0.003	22			

Multiple comparisons

Mean AChE

(I) Treatment			Mean difference (I-J)	SE	Sig.	95% confidence interval	
						Lower bound	Upper bound
Tukey HSD	BSL	BSM	-0.00715	0.00627	0.501	-0.0230	0.0087
		BSH	-0.00697	0.00627	0.519	-0.0228	0.0089
	BSM	BSL	0.00715	0.00627	0.501	-0.0087	0.0230
		BSH	0.00019	0.00606	0.999	-0.0151	0.0155
	BSH	BSL	0.00697	0.00627	0.519	-0.0089	0.0228
		BSM	-0.00019	0.00606	0.999	-0.0155	0.0151
Games-Howell	BSL	BSM	-0.00715	0.00472	0.317	-0.0196	0.0053
		BSH	-0.00697	0.00650	0.550	-0.0246	0.0107
	BSM	BSL	0.00715	0.00472	0.317	-0.0053	0.0196
		BSH	0.00019	0.00672	1.000	-0.0178	0.0182
	BSH	BSL	0.00697	0.00650	0.550	-0.0107	0.0246
		BSM	-0.00019	0.00672	1.000	-0.0182	0.0178
Dunnett t (>control)[a]	BSM	BSL	0.00715	0.00627	0.215	-0.0055	
	BSH	BSL	0.00697	0.00627	0.223	-0.0057	

[a] Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table S12. Mean glutathione S-transferase activity statistical comparison between high (BSH), medium (BSM) and low (BSL) treatment exposed *Astarte borealis* individuals.

Descriptives								
Mean GST								
	N	Mean	SD	SE	95% confidence interval for mean		Minimum	Maximum
					Lower bound	Upper bound		
BSL	8	49.7688	26.64573	9.42069	27.4924	72.0452	18.26	95.48
BSM	8	47.8317	20.47659	7.23957	30.7128	64.9505	25.99	84.19
BSH	11	46.5008	15.16479	4.57236	36.3130	56.6887	27.73	79.98
Total	27	47.8634	19.85929	3.82192	40.0074	55.7195	18.26	95.48

Test of homogeneity of variances			
Mean GST			
Levene statistic	df1	df2	Sig.
1.909	2	24	0.170

ANOVA							
Mean GST							
			Sum of squares	df	Mean square	F	Sig.
Between groups	(Combined)		49.475	2	24.737	0.058	0.944
	Linear term	Unweighted	49.463	1	49.463	0.116	0.736
		Weighted	48.961	1	48.961	0.115	0.737
		Deviation	0.513	1	0.513	0.001	0.973
	Quadratic term	Unweighted	0.513	1	0.513	0.001	0.973
		Weighted	0.513	1	0.513	0.001	0.973
Within groups			10204.707	24	425.196		
Total			10254.181	26			

Multiple comparisons							
Mean GST							
(I) Treatment			Mean difference (I-J)	SE	Sig.	95% confidence interval	
						Lower bound	Upper bound
Tukey HSD	BSL	BSM	1.93708	10.31014	0.981	-23.8103	27.6845
		BSH	3.26796	9.58143	0.938	-20.6596	27.1955
	BSM	BSL	-1.93708	10.31014	0.981	-27.6845	23.8103
		BSH	1.33088	9.58143	0.989	-22.5967	25.2584
	BSH	BSL	-3.26796	9.58143	0.938	-27.1955	20.6596
		BSM	-1.33088	9.58143	0.989	-25.2584	22.5967
Dunnett t (>control)[a]	BSM	BSL	-1.93708	10.31014	0.730	-22.5711	
	BSH	BSL	-3.26796	9.58143	0.782	-22.4436	

[a] Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table S13. Mean acyl-CoA oxidase activity statistical comparison between high (BSH), medium (BSM) and low (BSL) treatment exposed *Astarte borealis* individuals.

Test statistics[a,b]			
			Mean AOX
Chi-Square			0.050
Df			2
Asymp. sig.			0.976
Monte Carlo sig.	Sig.		0.977[c]
	99% confidence interval	Lower bound	0.973
		Upper bound	0.981

[a] Kruskal Wallis test

[b] Grouping variable: Treatment

[c] Based on 10000 sampled tables with starting seed 2000000.

Table S14. ANCOVA analysis of pyrene-1-glucuronide (GluPyr) concentration for polychaete species (*Alitta virens* and *Nephtys ciliata*). L: low treatment (PSL); H: high treatment (PSH).

Levene's test of equality of error variances[a]			
Dependent variable: Log_GluPyr			
F	df1	df2	Sig.
1.594	1	46	0.213
Tests the null hypothesis that the error variance of the dependent variable is equal across groups.			

[a] Design: Intercept + Log_Pyr + Treatment

Tests of between-subjects effects					
Dependent variable: Log_GluPyr					
Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	1.030[a]	2	0.515	12.657	0.000
Intercept	10.735	1	10.735	263.876	0.000
Log_Pyr	0.607	1	0.607	14.933	0.000
Treatment	0.350	1	0.350	8.603	0.005
Error	1.831	45	0.041		
Total	37.255	48			
Corrected total	2.860	47			

[a] R squared = 0.360 (adjusted R squared = 0.332)

Parameter estimates						
Dependent variable: Log_GluPyr						
Parameter	B	SE	t	Sig.	95% confidence interval	
					Lower bound	Upper bound
Intercept	0.804	0.054	14.804	0.000	0.694	0.913
Log_Pyr	0.239	0.062	3.864	0.000	0.114	0.363
[Treatment=L]	-0.171	0.058	-2.933	0.005	-0.289	-0.054
[Treatment=H]	0[a]					

[a] This parameter is set to zero because it is redundant.

Estimates				
Dependent variable: Log_GluPyr				
Treatment	Mean	SE	95% confidence interval	
			Lower bound	Upper bound
L	0.761[a]	0.041	0.678	0.844
H	0.932[a]	0.041	0.849	1.015

[a] Covariates appearing in the model are evaluated at the following values: Log_Pyr = 0.5385.

Pairwise comparisons						
Dependent variable: Log_GluPyr						
(I) Treatment		Mean difference (I-J)	SE	Sig.[b]	95% confidence interval for difference[b]	
					Lower bound	Upper bound
L	H	-0.171*	0.058	0.005	-0.289	-0.054
H	L	0.171*	0.058	0.005	0.054	0.289
Based on estimated marginal means						

* The mean difference is significant at the 0.05 level.

[b] Adjustment for multiple comparisons: Bonferroni.

Table S15. ANCOVA analysis of pyrene-1-glucuronide (GluPyr) concentration for bivalve species (*Limecola balthica* and *Macoma calcarea*). L: low treatment (BSL); H: high treatment (BSH).

Levene's test of equality of error variances[a]			
Dependent variable: Log_GluPyr			
F	df1	df2	Sig.
0.108	1	95	0.743
Tests the null hypothesis that the error variance of the dependent variable is equal across groups.			

[a] Design: Intercept + Log_Pyr + Treatment

Tests of between-subjects effects					
Dependent variable: Log_GluPyr					
Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	18.669[a]	2	9.334	10.950	0.000
Intercept	0.012	1	0.012	0.014	0.906
Log_Pyr	18.393	1	18.393	21.576	0.000
Treatment	0.063	1	0.063	0.074	0.786
Error	80.130	94	0.852		
Total	166.421	97			
Corrected total	98.799	96			

[a] R squared = 0.189 (adjusted R squared = 0.172)

Parameter estimates						
Dependent variable: Log_GluPyr						
Parameter	B	SE	t	Sig.	95% confidence interval	
					Lower bound	Upper bound
Intercept	0.049	0.224	0.219	0.827	-0.395	0.493
Log_Pyr	0.964	0.208	4.645	0.000	0.552	1.377
[Treatment=L]	-0.051	0.188	-0.272	0.786	-0.424	0.322
[Treatment=H]	0[a]					

[a] This parameter is set to zero because it is redundant.

Estimates				
Dependent variable: Log_GluPyr				
Treatment	Mean	SE	95% confidence interval	
			Lower bound	Upper bound
L	0.809[a]	0.133	0.544	1.074
H	0.860[a]	0.132	0.598	1.122

[a] Covariates appearing in the model are evaluated at the following values: Log_Pyr = 0.8412.

Pairwise comparisons						
Dependent variable: Log_GluPyr						
(I) Treatment		Mean difference (I-J)	SE	Sig.[a]	95% confidence interval for difference[a]	
					Lower bound	Upper bound
L	H	-0.051	0.188	0.786	-0.424	0.322
H	L	0.051	0.188	0.786	-0.322	0.424
Based on estimated marginal means						

[a] Adjustment for multiple comparisons: Bonferroni.

Table S16. Multiple linear regression of pyrene-1-glucuronide (GluPyr) concentration for polychaete species (*Alitta virens* and *Nephtys ciliata*).

Model summary[b]

Model	R	R square	Adjusted R square	SE of the estimate	Change statistics					Durbin-Watson
					R square change	F change	df1	df2	Sig. F change	
1	0.682[a]	0.466	0.442	0.18429	0.466	19.610	2	45	0.000	1.853

[a] Predictors: (Constant), Treatment, Source

[b] Dependent variable: Log_GluPyr

ANOVA[a]

Model		Sum of squares	df	Mean square	F	Sig.
1	Regression	1.332	2	0.666	19.610	0.000[b]
	Residual	1.528	45	0.034		
	Total	2.860	47			

[a] Dependent variable: Log_GluPyr

[b] Predictors: (Constant), Treatment, Source

Coefficients[a]

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.	95% confidence interval for B		Correlations			Collinearity statistics	
		B	SE	Beta			Lower bound	Upper bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	0.152	0.116		1.312	0.196	-0.081	0.386					
	Source	0.275	0.053	0.564	5.175	0.000	0.168	0.382	0.564	0.611	0.564	1.000	1.000
	Treatment	0.188	0.053	0.384	3.526	0.001	0.080	0.295	0.384	0.465	0.384	1.000	1.000

[a] Dependent variable: Log_GluPyr

Table S17. Multiple linear regression of pyrene-1-glucuronide (GluPyr) concentration for bivalve species (*Limecola balthica* and *Macoma calcarea*).

Model summary[b]

Model	R	R square	Adjusted R square	SE of the estimate	Change statistics					Durbin-Watson
					R square change	F change	df1	df2	Sig. F change	
1	0.272[a]	0.074	0.054	0.98647	0.074	3.764	2	94	0.027	1.591

[a] Predictors: (Constant), Treatment, Source
[b] Dependent variable: Log_GluPyr

ANOVA[a]

Model		Sum of squares	df	Mean square	F	Sig.
1	Regression	7.326	2	3.663	3.764	0.027[b]
	Residual	91.473	94	0.973		
	Total	98.799	96			

[a] Dependent variable: Log_GluPyr
[b] Predictors: (Constant), Treatment, Source

Coefficients[a]

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.	95% confidence interval for B		Correlations			Collinearity statistics	
		B	SE	Beta			Lower bound	Upper bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	1.467	0.433		3.386	0.001	0.607	2.327					
	Source	-0.539	0.200	-0.267	-2.692	0.008	-0.937	-0.142	-0.267	-0.268	-0.267	1.000	1.000
	Treatment	0.112	0.200	0.056	0.561	0.576	-0.285	0.510	0.053	0.058	0.056	1.000	1.000

[a] Dependent variable: Log_GluPyr

Table S18. Linear regression of pyrene (Pyr) and pyrene-1-glucuronide (GluPyr) concentration for the polychaete species *Alitta virens*.

Model summary[b]

Model	R	R square	Adjusted R square	SE of the estimate	Change statistics					Durbin-Watson
					R square change	F change	df1	df2	Sig. F change	
1	0.187[a]	0.035	-0.009	0.21989	0.035	0.796	1	22	0.382	1.407

[a] Predictors: (Constant), Log_Pyr
[b] Dependent variable: Log_GluPyr

ANOVA[a]

Model		Sum of squares	df	Mean square	F	Sig.
1	Regression	0.038	1	0.038	0.796	0.382[b]
	Residual	1.064	22	0.048		
	Total	1.102	23			

[a] Dependent variable: Log_GluPyr
[b] Predictors: (Constant), Log_Pyr

Coefficients[a]

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.	95% confidence interval for B		Correlations			Collinearity statistics	
		B	SE	Beta			Lower bound	Upper bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	0.736	0.054		13.602	0.000	0.624	0.848					
	Log_Pyr	-0.128	0.144	-0.187	-0.892	0.382	-0.427	0.170	-0.187	-0.187	-0.187	1.000	1.000

[a] Dependent variable: Log_GluPyr

Table S19. Linear regression of pyrene (Pyr) and pyrene-1-glucuronide (GluPyr) concentration for the polychaete species *Nephtys ciliata*.

Model summary[b]

Model	R	R square	Adjusted R square	SE of the estimate	Change statistics					Durbin-Watson
					R square change	F change	df1	df2	Sig. F change	
1	0.502[a]	0.252	0.218	0.16988	0.252	7.400	1	22	0.012	2.215

[a] Predictors: (Constant), Log_Pyr
[b] Dependent variable: Log_GluPyr

ANOVA[a]

Model		Sum of squares	df	Mean square	F	Sig.
1	Regression	0.214	1	0.214	7.400	0.012[b]
	Residual	0.635	22	0.029		
	Total	0.848	23			

[a] Dependent variable: Log_GluPyr
[b] Predictors: (Constant), Log_Pyr

Coefficients[a]

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.	95% confidence interval for B		Correlations			Collinearity statistics	
		B	SE	Beta			Lower Bound	Upper Bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	0.760	0.089		8.506	0.000	0.575	0.945					
	Log_Pyr	0.258	0.095	0.502	2.720	0.012	0.061	0.455	0.502	0.502	0.502	1.000	1.000

[a] Dependent variable: Log_GluPyr

Table S20. Linear regression of pyrene (Pyr) and pyrene-1-glucuronide (GluPyr) concentration for the bivalve species *Limecola balthica*.

Model summary[b]

Model	R	R square	Adjusted R square	SE of the estimate	Change statistics					Durbin-Watson
					R square change	F change	df1	df2	Sig. F change	
1	0.423[a]	0.179	0.162	0.62617	0.179	10.471	1	48	0.002	1.789

[a] Predictors: (Constant), Log_Pyr
[b] Dependent variable: Log_GluPyr

ANOVA[a]

Model		Sum of squares	df	Mean square	F	Sig.
1	Regression	4.106	1	4.106	10.471	0.002[b]
	Residual	18.820	48	0.392		
	Total	22.926	49			

[a] Dependent variable: Log_GluPyr
[b] Predictors: (Constant), Log_Pyr

Coefficients[a]

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.	95% confidence interval for B		Correlations			Collinearity statistics	
		B	SE	Beta			Lower bound	Upper bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	0.484	0.209		2.320	0.025	0.065	0.904					
	Log_Pyr	0.605	0.187	0.423	3.236	0.002	0.229	0.981	0.423	0.423	0.423	1.000	1.000

[a] Dependent variable: Log_GluPyr

Table S21. Linear regression of pyrene (Pyr) and pyrene-1-glucuronide (GluPyr) concentration for the bivalve species *Macoma calcarea*.

Model summary[b]

Model	R	R square	Adjusted R square	SE of the estimate	Change statistics					Durbin-Watson
					R square change	F change	df1	df2	Sig. F change	
1	0.398[a]	0.159	0.140	1.13464	0.159	8.482	1	45	0.006	1.444

[a] Predictors: (Constant), Log_Pyr
[b] Dependent variable: Log_GluPyr

ANOVA[a]

Model		Sum of squares	df	Mean square	F	Sig.
1	Regression	10.919	1	10.919	8.482	0.006[b]
	Residual	57.934	45	1.287		
	Total	68.853	46			

[a] Dependent variable: Log_GluPyr
[b] Predictors: (Constant), Log_Pyr

Coefficients[a]

Model		Unstandardized coefficients		Standardized coefficients	t	Sig.	95% confidence interval for B		Correlations			Collinearity statistics	
		B	SE	Beta			Lower bound	Upper bound	Zero-order	Partial	Part	Tolerance	VIF
1	(Constant)	-0.359	0.356		-1.010	0.318	-1.075	0.357					
	Log_Pyr	1.387	0.476	0.398	2.912	0.006	0.428	2.346	0.398	0.398	0.398	1.000	1.000

[a] Dependent variable: Log_GluPyr

5 Avoidance tests as a tool to detect sublethal effects of oil-impacted sediments

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Based on:

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Chapter 5

Abstract

Currently, risk assessment for oil contamination does not consider behavioral responses of benthos to oil toxicity. Avoidance of oil-contaminated sediment by benthic amphipods, however, may be a highly sensitive endpoint for sublethal effects of commonly used distillate fuels. In the present study, the avoidance behavior of temperate freshwater (*Gammarus pulex*) and marine (*Gammarus locusta*) amphipods is tested, allowing them to choose between a reference sediment and a Distillate Marine grade A (DMA) oil-spiked sediment. Avoidance of DMA-spiked sediment at 1000 mg/kg (dry weight) was significant within total exposure time (96 h) in *G. pulex* and within the first 72 h in *G. locusta* in one of two tests. Absence of DMA avoidance at lower concentrations (≤ 250 mg/kg dry weight) indicates that test species can only detect DMA above these concentrations. However, sensitivity to oil may vary according to the phenology and physiological conditions of the populations involved, such as the species temperature tolerance and reproductive stage. The results suggest that avoidance tests may be used as an alternative to traditional chronic toxicity tests provided that a causal link between avoidance and long-term effects can be established.

1. Introduction

Increasing global offshore oil and gas (O&G) production, oil transportation and shipping entail higher risks of accidental oil spills which are known to cause severe environmental impacts. The assessment of potential environmental consequences should be based on the relationship between the extent of oil exposure and the sensitivity of marine species and their habitats. Following a spill, the composition of oil and its toxicity will mostly vary according to the timing and magnitude of oil spreading and evaporation on sea surface [293]. In this process, the more water soluble and bioavailable fraction of oil volatilizes or degrades in the first several days, while the more hydrophobic oil components will be deposited to the seafloor where degradation is much slower [294]. Exceptions may apply to polar regions where the presence of ice can greatly reduce oil spreading and low temperatures may lower oil hydrocarbons partitioning and depletion rates [51]. Thus, the identification of biological features affecting oil exposure in local benthic organisms may help to better characterize the sensitivity of environmental receptors such as shoreline habitats [295,296].

Monitoring of environmental stress resulting from O&G activities can be based on assays that use behavioral endpoints, like movement ability [105,297]. Animal behavior integrates biochemical and physiological processes in response to chemical stressors. As such it is expected to be more sensitive than a lethal response [298], provided that an organism is able to sense a toxicant via olfaction or taste [299], and it may potentially reflect changes at a population level [97]. In a natural environment, mobile species may be able to move away from oil-contaminated sites before the oil can exert its toxic effects. In some cases, such responses may be absent. For example, in case of chronic toxicity [300] or when the individuals' energy allocation to locomotion, and thus avoidance, is attenuated [301-303].

Previous research addressed avoidance behavior in laboratory assays, studying spatial avoidance of either water or sediment containing different chemicals (summarised by Araújo et al. [96]). Amphipods have been used in avoidance assays since they are equipped with a sensory system [98-101]. Studies on the avoidance behavior of oil-contaminated sediment by benthic organisms are limited and restricted to the marine environment [99,304-306]. In these studies, generally a bi-compartmented system container was used to test the ability of amphipods to discern between a clean and a contaminated sediment. However, a preference for the clean sediment or avoidance of the contaminated sediment is not always evident, because for instance exposure to sublethal concentrations of oil may lead to contrasting

sensitivities among different groups of amphipods or 'attractive' odours may be masked by oil [108,109]. The effect of time is generally not considered or limited to a single time point per exposure day, which eliminates the possibility of identifying likely experimental biases such as a (pre-)adaptation to the chemical cue [100].

Spatial avoidance can be divided into active and passive avoidance [96], each occurring under the changing physical state of chemicals over exposure time. Active avoidance or 'escape' response relates to the ability of an organism to detect a chemical and to move to a lesser contaminated area, whereas passive avoidance or 'drift' response will occur when water flow is responsible for the organism displacement. In a bi-compartmented static system, amphipods will display active spatial avoidance provided that no other physical disturbances are present (e.g., sand, seaweed). The effect of time on the amphipods distribution may help to predict possible interactions between avoidance and their physiological susceptibility to oil [307] as well as their long-term implications [308].

The objective of the present study is to evaluate the potential of sediment avoidance tests using benthic amphipods as a monitoring tool for oil spill impact assessment. Prior to testing, oil was mixed with the sediment. We tested the avoidance behavior of a freshwater amphipod *Gammarus pulex* and a marine benthic amphipod *Gammarus locusta* as model invertebrate species, to sediment spiked with a commonly used distillate fuel oil (i.e., Distillate Marine grade A [DMA]) during a 96-h test period. DMA oil is widely used as fuel in midsized to larger ships such as large inland vessels, large cutters and coasters [309], and is often used in oil toxicity studies [43,46]. These species were deployed as a first step to develop ecologically relevant tests for oil impact assessment.

2. Materials and Methods

2.1. Test organisms

Freshwater experiment (FW). Following De Lange et al. [100], *G. pulex* (Linnaeus, 1758) adult individuals (9.7±1.8 mm) were collected from a non-polluted freshwater stream near Heelsum (The Netherlands, 51°58'40.3"N, 5°45'27.8"E) on 13 March 2014. Animals were transported to the laboratory in plastic buckets with water from the collection stream and acclimatized in a water bath during 10 d (15±1 °C). Since animals were collected in winter and seasonal effects may influence tolerance to toxicants exposure [310], the temperature and

duration of acclimatization were chosen according to the temperature range used by similar avoidance studies [99,100] and studies on effects of temperature on *G. pulex* physiology [311,312]. During acclimatization, oxygenated copper-free tap water was used and animals were fed unconditioned poplar leaves ad libitum. Prior to their use, poplar leaves were leached in tap water to remove active leachable substances, and dried.

Water quality variables such as temperature, dissolved oxygen, electric conductivity and pH were measured regularly during acclimatization (Table S1).

Marine water experiments (MW1 and MW2). *G. locusta* (Linnaeus, 1758) individuals were collected over 4 d in the Oosterschelde estuary (The Netherlands, 51°32'41.3"N, 3°55'30.0"E) on 24 to 25 June 2016 and 25 to 26 July 2016, hereafter MW1 and MW2 experiments. Since *G. locusta* life-span and maturity age are known to be greatly reduced by water temperature [313], individuals of the species were collected at two different times during summer in order to assess the consistency of experimental results. Animals were transported to the laboratory in 15-L jerry cans with water from the collection site and acclimatized during 5 d (15±1 °C). Sea water temperature from the collection site was 17±1 °C in June and 21±1 ºC in July. During acclimatization, animals were kept in 18-L glass aquaria each of them holding approximately 200 to 500 individuals in 15 L of oxygenated artificial sea water (ASW) (40 g/L, Pro Reef Salt, Colombo B.V.). A few stones from the collection site were also placed inside each aquarium for comfort of the test animals and animals were fed fish food tabs (Tetra Wafermix) ad libitum.

2.2. Sediment spiking

For the freshwater experiment, sediment was collected from a pond at Wageningen University campus. Sediment was sieved with a 2-mm sieve (83% dry wt., 0.88% organic matter) and spiked with DMA to obtain four different nominal concentrations: 0 (Control), 50 (Low), 250 (Medium) and 1000 (High) mg DMA/kg sediment (dry wt.). DMA oil was supplied by Gulf Oil (Nigtevecht, the Netherlands) and was the same as used in our earlier studies, where also chemical characteristics are provided [43,46]. Nominal DMA concentrations were chosen to match field-relevant concentrations [314,315] and to match the range of oil concentrations for which PAH sediment-water partition coefficients (K_d) are generally independent of oil concentration (i.e., 100 – 1000 mg/kg dry wt. [46]). This was observed after a 2 year weathering process of oil-spiked sediment, although in some cases (i.e., 3-ring PAHs) K_d

values were increased after weathering [46]. A relative stability of 2- to 3-ring PAHs in weathered surface oil [316] and in slick oil spiked to sediment [317], as well as their sublethal effects on benthic biota exposed at 1.7 mg/kg (dry wt.) ($\sum_{50}$PAH = 2.6 mg/kg dry wt.) or more, have been reported in literature [317,318]. Therefore, concentrations of 3-ring PAHs (e.g., phenanthrene, anthracene) in the high DMA treatment were considered sublethal (~1 mg/kg dry wt.) assuming a fair similarity in weathering conditions between Jonker et al. [43] and the present study.

For each sediment treatment containing DMA-spiked sediment (50 – 1000 mg/kg dry wt.), the required volume of DMA was divided into three batches to minimize the adsorption of DMA onto the barrel walls and its evaporation during stirring. Sediment was continuously stirred while each of the DMA batches was added drop by drop from a glass pipette. Once each oil batch was added, sediment stirring continued for about 5 min and allowed to rest for another 5 min before addition of following batch under the same procedure. After spiking, each sediment treatment was mixed on a roller bank during approximately 22 h and stored (7±1 °C) for 4 weeks before use. For the control treatment, sediment was stirred and mixed in the same way without the addition of oil, and stored.

For the marine *G. locusta* experiments, sediment was collected at the animals collection site during low tide. Sediment was sieved (86% dry wt., 0.82% organic carbon) and spiked with DMA following the same procedure as in the freshwater experiment. After spiking, each sediment treatment was mixed on a roller bank during approximately 24 h and stored (4±1 °C) for 2 to 6 weeks before use. The control sediment treatment was prepared as mentioned above.

2.3. Experimental design

The avoidance experiments were performed in glass aquaria (20 × 20 cm). A glass barrier was placed in the middle of each aquarium dividing the bottom into two equal parts. One side was filled with a 2-cm layer of control sediment and the other side with a 2-cm layer of control or spiked sediment. In each experiment, 800 mL of either copper-free tap water or ASW was gently added to each aquarium in order to avoid sediment disturbance, after which the systems were allowed to stabilise for 24 h. The freshwater *G. pulex* experiment was performed at Wageningen University laboratories, whereas the marine experiments with *G. locusta* were performed at the marine research laboratories of Wageningen Marine Research (Yerseke, the Netherlands). In the freshwater experiment, the aquaria were randomly distributed within a

water bath (15±1 °C), whereas for marine water experiments the aquaria were randomly placed on a table located inside a climate room (15±1 °C). Once the systems were stabilised, another 800 mL of either copper-free tap water or ASW was carefully added, after which the systems were allowed to stabilise for 3 d. Five replicates (aquaria; $n = 5$) were used for each of the four sediment treatments (control, low, medium and high DMA concentration) during each experiment.

Prior to the addition of the animals, the water layer was partially renewed. A volume of 800 mL of the system water was pumped out of each aquarium and renewed with 800 mL of clean copper-free tap water or ASW, respectively. This was done to minimize the concentration of water soluble DMA toxicants in the water column with respect to the concentration in the spiked sediment layer at start. The glass barriers were carefully removed and 20 individuals were added in the middle of each aquarium to ensure that the starting position did not influence the experiment outcome [100]. Animals were kept under a 16 h light: 8 h dark red light cycle since *Gammarus* spp. usually become photonegative when they reach an adult size [319]. However, dim light was needed for maintenance and to facilitate the visual recording of the animal distribution.

2.4. Endpoints

During the removal of barriers, some mixing of control and spiked sediment could not be prevented at surface level in the middle area. Therefore, the middle area was excluded from the statistical analyses. Each system sediment surface was divided into three areas: control (37.5% of the surface), middle (25%) and spiked (37.5%). The number of individuals staying for at least 5 to 6 consecutive seconds within the control or spiked area during an observation bout was recorded as '*control*' or '*spiked*', respectively. Recordings took place during a 96-h period allowing an estimation of the effect of DMA concentration (treatment) on *Gammarus* spp. avoidance of the DMA-spiked area over time (day). Only the results of the '*control*' scores of each replicate were used to test whether their relative distribution at the control section was higher than 37.5%, in which case the null hypothesis was rejected and avoidance of the spiked area was considered occurred. Water quality variables were measured at the beginning of each recording day in three experimental control replicate aquaria without animals during the first marine experiment (Table S2) and in each experimental replicate at the end (Table S3) of both marine experiments.

2.5. Data analyses

Logistic regression within the framework of Generalized Linear Mixed Models (GLMM) was used to analyse the relationship between the number of individuals in the control section treated as repeated measures for each aquarium (experimental unit) and the effect of treatment, day and their respective interaction as effect builders. GLMM statistical tests provide a more flexible approach than other non-parametric tests for analysing non-normal data when random effects are present. Only observations obtained from the second to the fourth experimental day were used since they include an equal number of recordings per day and handling of the animals could also have induced stress on the first day. Observations were collected 3 times per day for each aquarium and treatment in the freshwater experiment, that is 180 observations in total; and 7 times per day for each aquarium and treatment in the marine experiments, making a total of 420 observations for each experiment. Observations were assumed to be Poisson distributed following the repeated recording of a single event (i.e., '*control*' score takes integer values between 0 and 20) and a pairwise contrast among treatments and recording days was chosen in order to calculate the estimated means. The significance level of an overall statistical comparison was set at $p \leq 0.05$, while for pairwise comparisons the least significant difference correction was applied. All statistical calculations were performed using SPSS version 22.

3. Results and Discussion

3.1. Freshwater *Gammarus pulex* avoidance

G. pulex avoidance was significantly and consistently higher in the high DMA treatment than in the control and low treatment (day 2 to 4) (Figure 1; Tables S4-S6). *G. pulex* avoidance behavior in the medium DMA treatment was significantly different from that in the control, low and high treatment on day 2, after which an apparent attenuation of avoidance was observed (Figure 1). No mortality was observed within the total test duration (4 d) throughout the range of nominal DMA concentrations used (0 – 1000 mg/kg dry wt.).

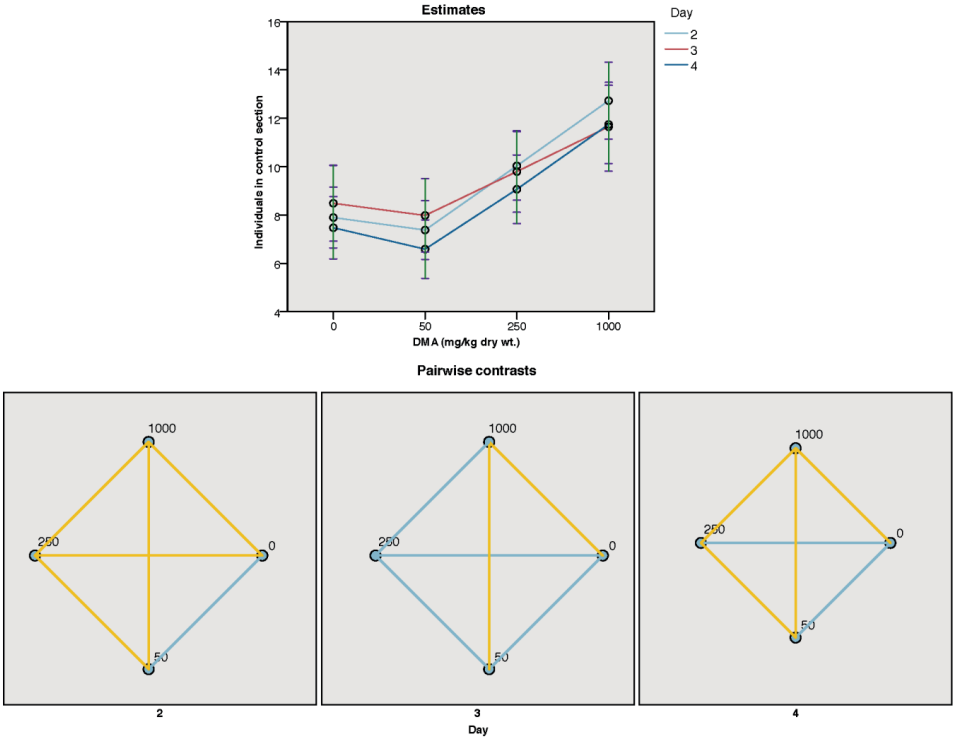

Figure 1. Generalized linear mixed models avoidance estimated means with confidence intervals and 'treatment × day' pairwise contrasts for *Gammarus pulex* exposed to Distillate Marine grade A (DMA) oil (FW experiment). Avoidance is expressed as number of individuals staying for at least 5 to 6 consecutive seconds within the control section per DMA treatment (0 – 1000 mg/kg dry wt.) and day (2 – 4). Significant contrasts ($p \leq 0.05$) are shaded gold.

A consistent avoidance response of *G. pulex* exposed to the high DMA treatment from day 2 to 4 could be explained by a combined result of exposure to a high DMA concentration and to the species upper thermal tolerance limit (i.e., 15 °C) in northern populations of *G. pulex* [311]. According to the 'oxygen- and capacity-limited thermal tolerance' model, failure of oxygen transport systems to match body oxygen demand dictates thermal tolerance [320]. An increase in the oxygen (energy) demand of *G. pulex* winter populations (~5 °C at which the organisms were sampled) at the exposure temperature (15 °C) is expected to be met by an increase in their ventilation rate [321,322]. An increase in ventilation may facilitate the detection and transmission of chemical cues [323] and possibly triggered avoidance in *G. pulex* exposed to the high DMA treatment from the first day of exposure. Another

consequence of the maintenance of *G. pulex* within its optimal thermal window can be that glycogen reserves, which is the main energy fuel for oxygen deficiency and sustained activity, are relatively stable provided that food is not limiting [324]. The mobilization of these energy stores would allow *G. pulex* to actively avoid DMA-spiked sediment and to counterbalance the stress produced by DMA exposure at the experiment start.

DMA exposure in the low and medium DMA treatments was most probably too low to elicit a continuous avoidance by *G. pulex*. The observed differences in DMA avoidance response between the low and medium treatment exposed *G. pulex* on day 2 and 4 of exposure were most likely a reflection of within treatment variability (Figure 1; Table S4). Variability in the actual exposure to DMA and avoidance response of *G. pulex* exposed to the medium DMA treatment may be underlain by intraspecific differences in *G. pulex* molecular response to starvation or reproduction [311,325] among other factors.

In summary, *G. pulex* active avoidance of DMA-spiked sediment was significant and continuous over exposure time (day 2 to 4) at a DMA concentration of 1000 mg/kg (dry wt.), and sometimes at 250 mg/kg (dry wt.). Since no mortality was observed within the total test duration (4 d) in none of the treatments, it was assumed that no deleterious effects were caused by DMA on the sensory system of individuals.

3.2. Marine *Gammarus locusta* avoidance

G. locusta avoidance during the first marine experiment was significantly higher in the high DMA treatment compared to the control, the low and medium DMA treatments from day 2 to 3 (Figure 2), whereas in the second marine experiment no effect of treatment or day on *G. locusta* avoidance was found (Figure 3). *G. locusta* avoidance was also significantly higher in individuals exposed to the high DMA treatment in the first compared to the second marine experiment from day 2 to 3 (Tables S13 and S14). No mortality was observed within the total test duration (4 d) throughout the range of nominal DMA concentrations used (0 – 1000 mg/kg dry wt.).

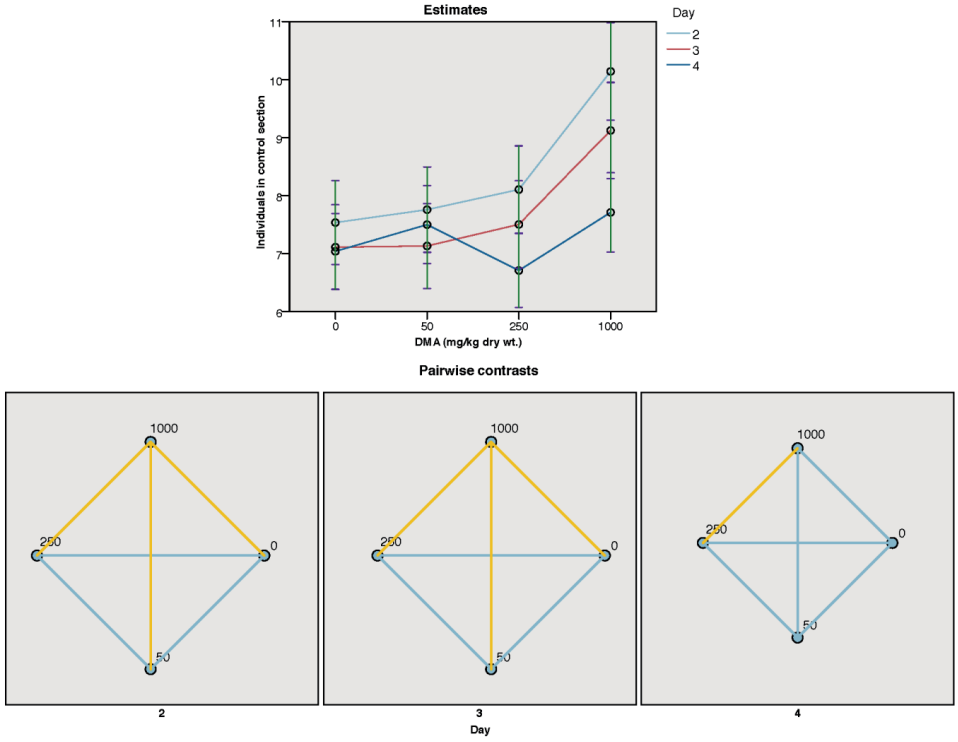

Figure 2. Generalized linear mixed models avoidance estimated means with confidence intervals and 'treatment × day' pairwise contrasts for *Gammarus locusta* exposed to Distillate Marine grade A (DMA) oil (MW1 experiment). Avoidance is expressed as number of individuals staying for at least 5 to 6 consecutive seconds within the control section per DMA treatment (0 – 1000 mg/kg dry wt.) and day (2 – 4). Significant contrasts ($p \leq 0.05$) are shaded gold.

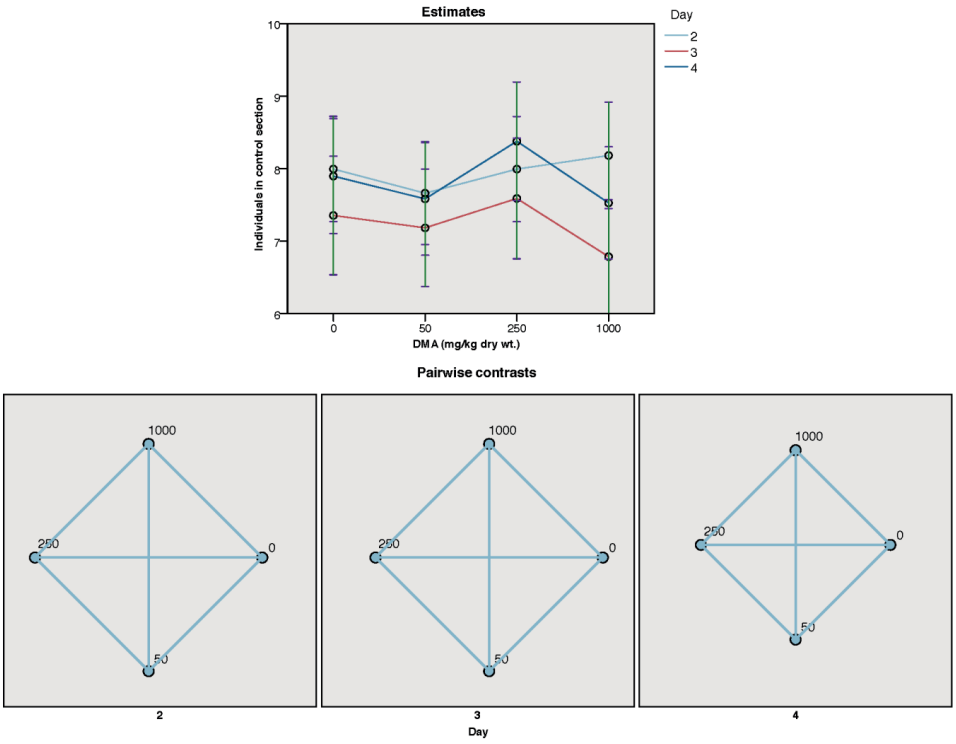

Figure 3. Generalized linear mixed models avoidance estimated means with confidence intervals and 'treatment × day' pairwise contrasts for *Gammarus locusta* exposed to Distillate Marine grade A (DMA) oil (MW2 experiment). Avoidance is expressed as number of individuals staying for at least 5 to 6 consecutive seconds within the control section per DMA treatment (0 – 1000 mg/kg dry wt.) and day (2 – 4). Significant contrasts ($p \leq 0.05$) are shaded gold.

In an experiment performed by Hellou et al. [99], the preference for reference sediment could also not be clearly elucidated in the marine amphipod *Corophium volutator* exposed to freshly diesel oil-spiked sediment (> 0.01%). As explained by the authors, a high variation in the response between exposure replicates and high mortality of *C. volutator* were caused by a narcotic effect of diesel oil. Besides avoidance behavior, re-emergence and failure to burrow did not follow a concentration-related response in *C. volutator* exposed to sediment spiked with artificially weathered crude oil at 220 to 880 mg/kg (dry wt.) [326]. In our experiments, the highest two DMA concentrations were 0.02% and 0.1%, respectively, but neither mortality nor narcotization were observed in *G. locusta*. As in *C. volutator* example [326], *G.*

locusta avoidance behavior did not correlate with DMA concentration (50 – 1000 mg/kg dry wt.) over exposure time (i.e., day 2 to 4).

Although the cause of the difference in *G. locusta* avoidance behavior between the first and second experiments is not known, this may be linked to differences in life stage-related factors such as body size [327], lipid content or maturity stage [99,313] between the two experiments. Since body size and structural (membrane) lipid content may influence the chemical exchange at body surface, changes in these variables may be reflected in differences in the apparent sensitivity to DMA and avoidance between summer cohorts. Variability in body size and structural lipid content [328] may be further increased by a reduction in the life-span of *G. locusta* above 15 °C [313], the maximum expression of which can be found among summer cohorts. A *G. locusta* generational succession was observed between June and July by Kolding and Fenchel [329] in the Limfjorden strait (Denmark) and by Costa and Costa [327] in the Sado estuary (Portugal), according to which a higher proportion of larger-sized gravid females would be expected in *G. locusta* collected in June compared to July.

Lack of avoidance by *G. locusta* exposed at 50 and 250 mg DMA/kg (dry wt.) (respectively low and medium concentration) in both marine experiments could be related to a low exposure of sediment treatments as also observed in the freshwater experiment. Both age- and sex-related differences in their oxidative stress tolerance [327,330] might account for the lack of a DMA concentration- nor time-dependent avoidance response by *G. locusta* [331].

3.3. Comparison of avoidance between *Gammarus pulex* and *Gammarus locusta*

Avoidance behavior of DMA-spiked sediment (50 – 1000 mg/kg dry wt.) could not be clearly observed in *G. locusta*, whereas in *G. pulex* avoidance was significant and continuous over exposure time (i.e., day 2 to 4) at the highest DMA concentration (Figures 4 and 5; Tables S16 and S17). Interpretation of avoidance results may be complicated by differences in behavior of DMA in freshwater and marine systems as the aqueous solubility of PAHs tends to decrease with increasing salinity [24]. Therefore, oil may have been easier detectable by *Gammarus* sp. in the freshwater experiment. PAH degradation will be generally higher in freshwater than in marine systems, although degradation will be strongly influenced by oxygen availability in the sediment and the molecular size of PAHs [43]. In the present study, oxygen concentration was not measured in the freshwater experiment, but lower oxygen levels were detected at the end of exposure in the second compared to the first marine experiment

(Table S3). This might point to a higher microbial degradation of PAHs [43] and therefore, lower DMA toxicity, in the second marine experiment than in the first marine experiment.

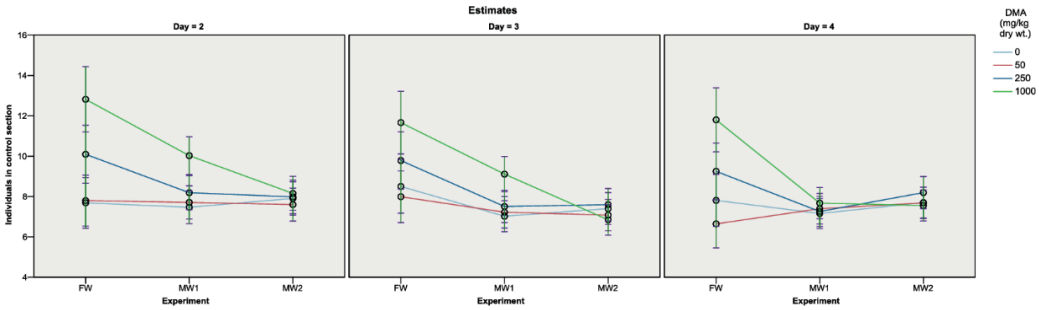

Figure 4. Generalized linear mixed models avoidance estimated means with confidence intervals for *Gammarus pulex* (FW experiment) and *Gammarus locusta* (MW1 and MW2 experiments) exposed to Distillate Marine grade (DMA) oil. Avoidance is expressed as number of individuals staying for at least 5 to 6 consecutive seconds within the control section per DMA treatment (0 – 1000 mg/kg dry wt.) and day (2 – 4).

As indicated by previous studies [99,306], lack of reproducibility of avoidance results among test organisms may be caused by differences in their growth rate and reproductive stage. Factors such as the individual size of *G. pulex* (~10 mm) and temperature at collection time (~5 °C) may have affected the experimental results. *G. pulex* has a natural life-span of 1 to 2 years and possibly experienced a low reproductive effort during the freshwater experiment [332]. A longer life-span of *G. pulex* (11 – 15 months at 15 °C [333]) compared to *G. locusta* (5 – 6 months at 15 °C [313]) and/or lower reproductive effort of female *G. pulex* during the freshwater experiment may have caused an increase in the energy budget in *G. pulex* compared to that of *G. locusta*, and thus their capacity of avoidance during DMA exposure.

In addition, it is not known whether slightly hypersaline conditions (40 – 42 mS/cm, Table S3) could have resulted in intraspecific differences in *G. locusta* growth [334] or if a likely osmotic imbalance in *G. locusta* [335] may have been enhanced by oil exposure. Osmoregulation is an energy-consuming regulatory function in *Gammarus* spp. in a way that its alteration may reduce the energy allocated to locomotion and ventilation. A combined effect of osmoregulation- and reproduction-related energy consumption may have reduced the ability of *G. locusta* to 'escape' from DMA, whereas *G. pulex* energy allocation to hyperventilation during acclimatization, may be compensated by an early DMA detection, via ventilation, and long-term stability of glycogen reserves.

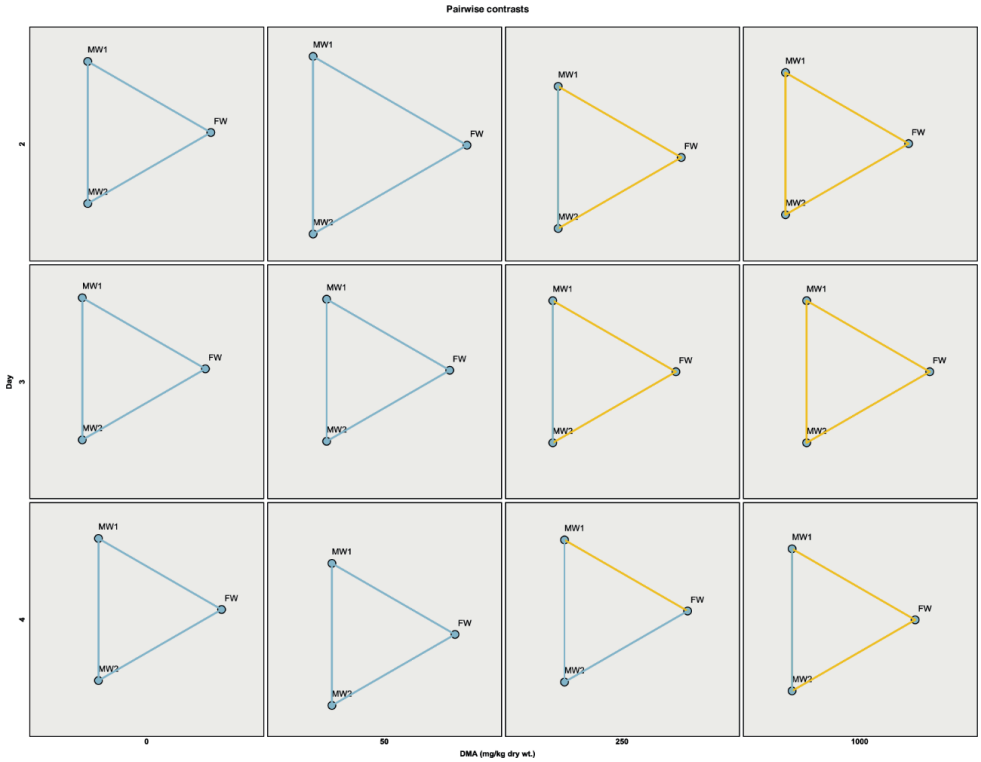

Figure 5. Generalized linear mixed models 'experiment × treatment × day' pairwise contrasts for *Gammarus pulex* (FW experiment) and *Gammarus locusta* (MW1 and MW2 experiments) exposed to Distillate Marine grade A (DMA) oil. Avoidance is expressed as number of individuals staying for at least 5 to 6 consecutive seconds within the control section per DMA treatment (0 – 1000 mg/kg dry wt.) and day (2 – 4). Significant contrasts ($p \leq 0.05$) are shaded gold.

3.4. Implications for the use of avoidance tests with *Gammarus* spp.

The present study showed that spatial avoidance may be integrated in the current risk assessment framework due to its higher sensitivity than mortality [326]. However, the interpretation of spatial avoidance results required some assumptions to be made related to inter-individual variability. Such variability may be greater in summer generations because their reproductive effort should be maximized when their life-span is shortened by high temperatures and this would restrict their capacity to cope with metabolic stress. Therefore, a supporting analysis of energy consumption in amphipods may help to establish if their energy budget is affected by the energy allocation to avoidance of oil and if such trade-off follows a seasonal pattern according to the species reproductive strategy. Avoidance results obtained

under such combined analysis could also be translated into a recognizable pattern of effects on growth and reproduction in short life-span species.

In the present study, nominal DMA concentrations were chosen according to the range of DMA oil concentrations for which a K_d decrease had been observed for most PAHs by Jonker et al. [46]. However, variability in PAH partitioning due to differences in the storage time of sediment treatments might have affected the comparability between the present avoidance tests. This stresses the importance of including a chemical characterization of sediment treatments prior to and after avoidance tests, as well as of the overlying water during exposure for each replicate [83]. The interpretation of avoidance results may also be improved after the inclusion of a statistically sufficient number of recordings between the time to achieve a uniform distribution in control treatment replicates [101] and the end of recording on the first day of exposure. Such additional recordings would serve to identify the possibility of a reversible narcotic effect, reducing the risk of bias.

The interpretation of results may be further improved using a more gradual acclimation to standard temperature and salinity conditions in field-collected organisms. Acclimation can be energy demanding in amphipods and may influence their energy allocation to avoidance. This was hypothesized for *G. pulex* as the acclimation to standard temperature regimes could have affected its energy metabolism and thus, sensitivity to temperature and other stressors [336]. Similarly, it is not known if the variation in salinity could have contributed to the observed variability in *G. locusta* experiments. In both cases, the observed variability could have been associated to different rates of energy consumption among life-span stages during acclimatization. In addition, factors such as the reproductive or moulting stage could influence the chemical exchange at body surface and sensitivity to oil of amphipods.

Consequently, sampling efforts should be addressed to specific periods at which contributing factors to the sensitivity to oil in amphipods are more uniform among gammarid populations. One needs to consider as well how seasonality may influence the energy reserves and activity of test organisms as this would facilitate the extrapolation of avoidance results between studies from different climate regions. Avoidance of oil can be a useful tool to relate energy consumption to growth reduction and delated maturation in adult amphipods, helping to predict the environmental risks derived from their behavioral reaction. A combined monitoring of spatial avoidance and metabolic responses and its integration in risk analysis

may therefore help to anticipate in which exposure scenarios gammarid populations can recover from an oil spill following habitat fragmentation [16,295].

4. Conclusions

In our experiments, it was observed that the freshwater amphipod *G. pulex* avoided DMA-spiked sediment at 1000 mg/kg (dry wt.). For the marine amphipod *G. locusta*, avoidance was either not consistent with time or below effect threshold. Oil behavior may have differed in the freshwater experiment compared to the marine experiments, despite a similar spiking protocol. Season-related differences in the physiology of amphipod species may restrict the applicability of avoidance tests to non-reproductive and/or winter individuals. Besides the inclusion of scoring on the first day of exposure, we recommend the analysis of energy reserves dynamics in amphipods in order to ascertain whether any experimental factors other than the exposure duration can significantly alter the energy allocation to locomotion. The predictive power of avoidance tests using amphipod species could be increased after the identification of biological factors that may influence sensitivity to oil exposure in sediment.

Acknowledgements

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Chapter 5

Supporting Information

Table S1. *Gammarus pulex* average water quality variables during acclimatization.

Day	Temperature (°C)	Oxygen (mg/L)	Electric conductivity (µS/cm)	pH
1	15.6 ± 0.1	9.3 ± 0.1	401.8 ± 0.5	7.3 ± 0.0
2	15.6 ± 0.0	9.2 ± 0.2	404.5 ± 1.7	7.2 ± 0.0
3	15.6 ± 0.0	9.9 ± 0.1	378.8 ± 4.3	7.5 ± 0.0
4	15.7 ± 0.1	9.5 ± 0.2	357.5 ± 5.1	7.5 ± 0.0
5	15.8 ± 0.0	9.5 ± 0.2	342.3 ± 7.3	7.6 ± 0.0
6	15.7 ± 0.1	9.4 ± 0.2	327.3 ± 9.0	7.7 ± 0.0
7	16.4 ± 0.0	10.1 ± 0.1	198.6 ± 4.2	7.9 ± 0.0
8	16.4 ± 0.0	9.8 ± 0.2	204.6 ± 7.1	7.8 ± 0.0
9	16.4 ± 0.0	9.4 ± 0.1	208.8 ± 8.2	7.8 ± 0.0

Table S2. First marine experiment (MW1) average water quality variables based on three experimental replicates without *Gammarus locusta* individuals.

Exposure day	Salinity (mS/cm)	Temperature (°C)	Oxygen (mg/L)	pH
1	40.1	16.1	7.1	7.8
2	40.7	16.8	6.5	8.0
3	41.3	16.6	7.0	8.2
4	41.5	16.3	7.4	8.2

Table S3. End of exposure (day 4) water quality variables per Distillate Marine grade A (DMA) oil sediment treatment (average ± SD) in *Gammarus pulex* (FW) and *Gammarus locusta* (MW1 and MW2) experiments.

Experiment	DMA (mg/kg dry wt.)	Salinity (mS/cm)	Temperature (°C)	Oxygen (mg/L)	pH
FW	0	-	n.a.	n.a.	n.a.
	50	-	n.a.	n.a.	n.a.
	250	-	n.a.	n.a.	n.a.
	1000	-	n.a.	n.a.	n.a.
MW1	0	42.2 ± 0.2	16.3 ± 0.1	6.3 ± 0.3	8.2 ± 0.0
	50	42.1 ± 0.1	16.4 ± 0.2	6.0 ± 0.3	8.2 ± 0.0
	250	42.2 ± 0.3	16.3 ± 0.1	6.3 ± 0.4	8.2 ± 0.0
	1000	42.2 ± 0.1	16.4 ± 0.2	6.1 ± 0.2	8.2 ± 0.0
MW2	0	41.4 ± 0.9	15.4 ± 0.1	5.2 ± 0.3	8.1 ± 0.1
	50	40.5 ± 0.9	15.4 ± 0.1	5.4 ± 0.8	8.0 ± 0.2
	250	40.7 ± 1.9	15.4 ± 0.1	5.2 ± 0.3	8.0 ± 0.0
	1000	40.2 ± 0.6	15.4 ± 0.0	5.3 ± 0.4	8.0 ± 0.0

n.a.: not available.

Table S4. Estimated means of target variable (Individuals in control section) for "Treatment × Day" effect on *Gammarus pulex* avoidance.

DMA treatment (mg/kg dry wt.)	Day	Mean	SE	95% confidence interval	
				Lower	Upper
0	2	7.896	0.636	6.639	9.152
	3	8.485	0.794	6.919	10.052
	4	7.474	0.652	6.186	8.762
50	2	7.378	0.615	6.164	8.593
	3	7.982	0.770	6.462	9.501
	4	6.588	0.613	5.379	7.797
250	2	10.034	0.717	8.618	11.450
	3	9.798	0.853	8.114	11.482
	4	9.066	0.719	7.647	10.485
1000	2	12.727	0.808	11.131	14.322
	3	11.648	0.930	9.812	13.483
	4	11.746	0.818	10.131	13.360

Table S5. Pairwise contrasts for "Treatment × Day" effect on *Gammarus pulex* avoidance.

Day	DMA treatment pairwise contrast (mg/kg dry wt.)	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
							Lower	Upper
2	0 - 50	0.517	0.885	0.584	168	0.560	-1.230	2.265
	0 - 250	-2.138	0.959	-2.230	168	0.027	-4.032	-0.245
	0 - 1000	-4.831	1.029	-4.697	168	5.461E-06	-6.862	-2.801
	50 - 0	-0.517	0.885	-0.584	168	0.560	-2.265	1.230
	50 - 250	-2.656	0.945	-2.810	168	0.006	-4.521	-0.790
	50 - 1000	-5.348	1.016	-5.266	168	4.212E-07	-7.353	-3.343
	250 - 0	2.138	0.959	2.230	168	0.027	0.245	4.032
	250 - 50	2.656	0.945	2.810	168	0.006	0.790	4.521
	250 - 1000	-2.693	1.081	-2.492	168	0.014	-4.826	-0.560
	1000 - 0	4.831	1.029	4.697	168	5.461E-06	2.801	6.862
	1000 - 50	5.348	1.016	5.266	168	4.212E-07	3.343	7.353
	1000 - 250	2.693	1.081	2.492	168	0.014	0.560	4.826
3	0 - 50	0.504	1.106	0.456	168	0.649	-1.679	2.686
	0 - 250	-1.313	1.165	-1.127	168	0.262	-3.612	0.987
	0 - 1000	-3.162	1.223	-2.587	168	0.011	-5.576	-0.749
	50 - 0	-0.504	1.106	-0.456	168	0.649	-2.686	1.679
	50 - 250	-1.816	1.149	-1.581	168	0.116	-4.084	0.452
	50 - 1000	-3.666	1.207	-3.037	168	0.003	-6.049	-1.283
	250 - 0	1.313	1.165	1.127	168	0.262	-0.987	3.612
	250 - 50	1.816	1.149	1.581	168	0.116	-0.452	4.084
	250 - 1000	-1.850	1.262	-1.466	168	0.145	-4.341	0.641
	1000 - 0	3.162	1.223	2.587	168	0.011	0.749	5.576
	1000 - 50	3.666	1.207	3.037	168	0.003	1.283	6.049
	1000 - 250	1.850	1.262	1.466	168	0.145	-0.641	4.341

Table S5. Continued.

Day	DMA treatment pairwise contrast (mg/kg dry wt.)	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
							Lower	Upper
4	0 - 50	0.886	0.895	0.991	168	0.323	-0.880	2.653
	0 - 250	-1.592	0.971	-1.640	168	0.103	-3.508	0.324
	0 - 1000	-4.271	1.046	-4.083	168	6.875E-05	-6.337	-2.206
	50 - 0	-0.886	0.895	-0.991	168	0.323	-2.653	0.880
	50 - 250	-2.478	0.944	-2.625	168	0.009	-4.342	-0.614
	50 - 1000	-5.158	1.022	-5.048	168	1.154E-06	-7.175	-3.141
	250 - 0	1.592	0.971	1.640	168	0.103	-0.324	3.508
	250 - 50	2.478	0.944	2.625	168	0.009	0.614	4.342
	250 - 1000	-2.680	1.089	-2.461	168	0.015	-4.829	-0.530
	1000 - 0	4.271	1.046	4.083	168	6.875E-05	2.206	6.337
	1000 - 50	5.158	1.022	5.048	168	1.154E-06	3.141	7.175
	1000 - 250	2.680	1.089	2.461	168	0.015	0.530	4.829

Table S6. Overall generalized linear mixed models test results for "Treatment × Day" effect on *Gammarus pulex* avoidance.

Day	F	df1	df2	Sig.
2	11.084	3	168	1.122E-06
3	3.567	3	168	0.015
4	9.433	3	168	8.541E-06

Table S7. Estimated means of target variable (Individuals in control section) for "Treatment × Day" effect on *Gammarus locusta* avoidance in the first marine experiment (MW1).

DMA treatment (mg/kg dry wt.)	Day	Mean	SE	95% confidence interval	
				Lower	Upper
0	2	7.536	0.369	6.812	8.261
	3	7.113	0.372	6.382	7.844
	4	7.041	0.332	6.389	7.692
50	2	7.759	0.374	7.024	8.495
	3	7.133	0.372	6.401	7.865
	4	7.502	0.342	6.829	8.174
250	2	8.106	0.382	7.354	8.857
	3	7.505	0.382	6.754	8.256
	4	6.711	0.324	6.075	7.347
1000	2	10.140	0.428	9.300	10.981
	3	9.123	0.421	8.295	9.951
	4	7.712	0.347	7.030	8.394

Table S8. Pairwise contrasts for "Treatment × Day" effect on *Gammarus locusta* avoidance (MW1 experiment).

Day	DMA treatment pairwise contrast (mg/kg dry wt.)	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
							Lower	Upper
2	0 - 50	-0.223	0.525	-0.424	408	0.672	-1.256	0.810
	0 - 250	-0.569	0.531	-1.072	408	0.284	-1.614	0.475
	0 - 1000	-2.604	0.565	-4.612	408	5.357E-06	-3.714	-1.494
	50 - 0	0.223	0.525	0.424	408	0.672	-0.810	1.256
	50 - 250	-0.346	0.535	-0.648	408	0.518	-1.398	0.705
	50 - 1000	-2.381	0.568	-4.190	408	3.415E-05	-3.498	-1.264
	250 - 0	0.569	0.531	1.072	408	0.284	-0.475	1.614
	250 - 50	0.346	0.535	0.648	408	0.518	-0.705	1.398
	250 - 1000	-2.035	0.574	-3.547	408	0.000	-3.163	-0.907
	1000 - 0	2.604	0.565	4.612	408	5.357E-06	1.494	3.714
	1000 - 50	2.381	0.568	4.190	408	3.415E-05	1.264	3.498
	1000 - 250	2.035	0.574	3.547	408	0.000	0.907	3.163
3	0 - 50	-0.021	0.526	-0.039	408	0.969	-1.055	1.014
	0 - 250	-0.392	0.533	-0.736	408	0.462	-1.440	0.656
	0 - 1000	-2.010	0.562	-3.578	408	0.000	-3.115	-0.906
	50 - 0	0.021	0.526	0.039	408	0.969	-1.014	1.055
	50 - 250	-0.372	0.533	-0.697	408	0.486	-1.420	0.677
	50 - 1000	-1.990	0.562	-3.539	408	0.000	-3.095	-0.885
	250 - 0	0.392	0.533	0.736	408	0.462	-0.656	1.440
	250 - 50	0.372	0.533	0.697	408	0.486	-0.677	1.420
	250 - 1000	-1.618	0.569	-2.846	408	0.005	-2.736	-0.500
	1000 - 0	2.010	0.562	3.578	408	0.000	0.906	3.115
	1000 - 50	1.990	0.562	3.539	408	0.000	0.885	3.095
	1000 - 250	1.618	0.569	2.846	408	0.005	0.500	2.736

Table S8. Continued.

Day	DMA treatment pairwise contrast (mg/kg dry wt.)	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
							Lower	Upper
4	0 - 50	-0.461	0.476	-0.967	408	0.334	-1.397	0.476
	0 - 250	0.330	0.463	0.712	408	0.477	-0.581	1.241
	0 - 1000	-0.671	0.480	-1.398	408	0.163	-1.614	0.272
	50 - 0	0.461	0.476	0.967	408	0.334	-0.476	1.397
	50 - 250	0.791	0.471	1.679	408	0.094	-0.135	1.717
	50 - 1000	-0.210	0.487	-0.431	408	0.667	-1.168	0.748
	250 - 0	-0.330	0.463	-0.712	408	0.477	-1.241	0.581
	250 - 50	-0.791	0.471	-1.679	408	0.094	-1.717	0.135
	250 - 1000	-1.001	0.474	-2.110	408	0.036	-1.934	-0.068
	1000 - 0	0.671	0.480	1.398	408	0.163	-0.272	1.614
	1000 - 50	0.210	0.487	0.431	408	0.667	-0.748	1.168
	1000 - 250	1.001	0.474	2.110	408	0.036	0.068	1.934

Table S9. Overall generalized linear mixed models test results for "Treatment × Day" effect on *Gammarus locusta* avoidance (MW1 experiment).

Day	F	df1	df2	Sig.
2	8.378	3	408	2.042E-05
3	5.463	3	408	0.001
4	1.818	3	408	0.143

Table S10. Estimated means of target variable (Individuals in control section) for "Treatment × Day" effect on *Gammarus locusta* avoidance in the second marine experiment (MW2).

DMA treatment (mg/kg dry wt.)	Day	Mean	SE	95% confidence interval	
				Lower	Upper
0	2	7.995	0.369	7.270	8.720
	3	7.355	0.416	6.537	8.174
	4	7.897	0.403	7.104	8.689
50	2	7.662	0.361	6.952	8.371
	3	7.184	0.412	6.375	7.993
	4	7.583	0.395	6.807	8.360
250	2	7.994	0.369	7.269	8.718
	3	7.589	0.423	6.757	8.420
	4	8.378	0.415	7.561	9.194
1000	2	8.182	0.373	7.448	8.915
	3	6.787	0.400	6.001	7.574
	4	7.527	0.394	6.754	8.301

Table S11. Pairwise contrasts for "Treatment × Day" effect on *Gammarus locusta* avoidance (MW2 experiment).

Day	DMA treatment pairwise contrast (mg/kg dry wt.)	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
							Lower	Upper
2	0 - 50	0.334	0.516	0.646	408	0.518	-0.681	1.348
	0 - 250	0.001	0.521	0.003	408	0.998	-1.024	1.027
	0 - 1000	-0.186	0.525	-0.356	408	0.722	-1.218	0.845
	50 - 0	-0.334	0.516	-0.646	408	0.518	-1.348	0.681
	50 - 250	-0.332	0.516	-0.644	408	0.520	-1.346	0.682
	50 - 1000	-0.520	0.519	-1.002	408	0.317	-1.540	0.500
	250 - 0	-0.001	0.521	-0.003	408	0.998	-1.027	1.024
	250 - 50	0.332	0.516	0.644	408	0.520	-0.682	1.346
	250 - 1000	-0.188	0.525	-0.358	408	0.720	-1.219	0.843
	1000 - 0	0.186	0.525	0.356	408	0.722	-0.845	1.218
	1000 - 50	0.520	0.519	1.002	408	0.317	-0.500	1.540
	1000 - 250	0.188	0.525	0.358	408	0.720	-0.843	1.219
3	0 - 50	0.171	0.585	0.293	408	0.770	-0.979	1.322
	0 - 250	-0.233	0.594	-0.393	408	0.694	-1.400	0.933
	0 - 1000	0.568	0.577	0.984	408	0.326	-0.567	1.703
	50 - 0	-0.171	0.585	-0.293	408	0.770	-1.322	0.979
	50 - 250	-0.405	0.590	-0.686	408	0.493	-1.565	0.755
	50 - 1000	0.397	0.574	0.691	408	0.490	-0.731	1.525
	250 - 0	0.233	0.594	0.393	408	0.694	-0.933	1.400
	250 - 50	0.405	0.590	0.686	408	0.493	-0.755	1.565
	250 - 1000	0.802	0.582	1.377	408	0.169	-0.343	1.946
	1000 - 0	-0.568	0.577	-0.984	408	0.326	-1.703	0.567
	1000 - 50	-0.397	0.574	-0.691	408	0.490	-1.525	0.731

Table S11. Continued.

Day	DMA treatment pairwise contrast (mg/kg dry wt.)	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
							Lower	Upper
3	1000 - 250	-0.802	0.582	-1.377	408	0.169	-1.946	0.343
4	0 - 50	0.314	0.564	0.556	408	0.579	-0.796	1.423
	0 - 250	-0.481	0.579	-0.831	408	0.407	-1.618	0.657
	0 - 1000	0.369	0.563	0.656	408	0.512	-0.738	1.477
	50 - 0	-0.314	0.564	-0.556	408	0.579	-1.423	0.796
	50 - 250	-0.795	0.573	-1.387	408	0.166	-1.921	0.332
	50 - 1000	0.056	0.558	0.100	408	0.921	-1.040	1.152
	250 - 0	0.481	0.579	0.831	408	0.407	-0.657	1.618
	250 - 50	0.795	0.573	1.387	408	0.166	-0.332	1.921
	250 - 1000	0.850	0.572	1.486	408	0.138	-0.274	1.975
	1000 - 0	-0.369	0.563	-0.656	408	0.512	-1.477	0.738
	1000 - 50	-0.056	0.558	-0.100	408	0.921	-1.152	1.040
	1000 - 250	-0.850	0.572	-1.486	408	0.138	-1.975	0.274

Table S12. Overall generalized linear mixed models test results for "Treatment × Day" effect on *Gammarus locusta* avoidance (MW2 experiment).

Day	F	df1	df2	Sig.
2	0.351	3	408	0.789
3	0.682	3	408	0.564
4	0.916	3	408	0.433

Table S13. Estimated means of target variable (Individuals in control section) for "Experiment × Treatment × Day" effect on *Gammarus locusta* avoidance (MW1 and MW2 experiments).

Experiment	DMA treatment (mg/kg dry wt.)	Day	Mean	SE	95% confidence interval	
					Lower	Upper
MW1	0	2	7.443	0.381	6.695	8.192
		3	7.027	0.394	6.253	7.801
		4	7.159	0.379	6.414	7.903
	50	2	7.682	0.388	6.921	8.443
		3	7.197	0.399	6.414	7.981
		4	7.396	0.386	6.639	8.153
	250	2	8.206	0.401	7.420	8.992
		3	7.495	0.407	6.696	8.295
		4	7.257	0.382	6.507	8.007
	1000	2	9.918	0.440	9.053	10.782
		3	9.099	0.449	8.218	9.980
		4	7.674	0.393	6.903	8.444
MW2	0	2	7.952	0.394	7.178	8.726
		3	7.422	0.405	6.626	8.218
		4	7.701	0.393	6.928	8.473
	50	2	7.644	0.387	6.885	8.403
		3	7.058	0.395	6.282	7.834
		4	7.684	0.393	6.913	8.456
	250	2	8.068	0.397	7.288	8.847
		3	7.584	0.410	6.779	8.388
		4	8.194	0.406	7.398	8.991
	1000	2	8.085	0.398	7.305	8.866
		3	6.872	0.390	6.106	7.637
		4	7.555	0.390	6.790	8.320

Table S14. Pairwise contrasts for "Experiment × Treatment × Day" effect on *Gammarus locusta* avoidance (MW1 and MW2 experiments).

DMA treatment (mg/kg dry wt.)	Day	Experiment pairwise contrast	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
								Lower	Upper
0	2	MW1 - MW2	-0.508	0.549	-0.927	816	0.354	-1.585	0.568
		MW2 - MW1	0.508	0.549	0.927	816	0.354	-0.568	1.585
	3	MW1 - MW2	-0.395	0.566	-0.698	816	0.485	-1.505	0.715
		MW2 - MW1	0.395	0.566	0.698	816	0.485	-0.715	1.505
	4	MW1 - MW2	-0.542	0.547	-0.992	816	0.322	-1.615	0.531
		MW2 - MW1	0.542	0.547	0.992	816	0.322	-0.531	1.615
50	2	MW1 - MW2	0.038	0.547	0.069	816	0.945	-1.037	1.112
		MW2 - MW1	-0.038	0.547	-0.069	816	0.945	-1.112	1.037
	3	MW1 - MW2	0.139	0.562	0.247	816	0.805	-0.964	1.242
		MW2 - MW1	-0.139	0.562	-0.247	816	0.805	-1.242	0.964
	4	MW1 - MW2	-0.288	0.551	-0.524	816	0.601	-1.369	0.792
		MW2 - MW1	0.288	0.551	0.524	816	0.601	-0.792	1.369
250	2	MW1 - MW2	0.138	0.564	0.245	816	0.806	-0.969	1.246
		MW2 - MW1	-0.138	0.564	-0.245	816	0.806	-1.246	0.969
	3	MW1 - MW2	-0.089	0.578	-0.153	816	0.878	-1.223	1.046
		MW2 - MW1	0.089	0.578	0.153	816	0.878	-1.046	1.223
	4	MW1 - MW2	-0.937	0.557	-1.681	816	0.093	-2.031	0.157
		MW2 - MW1	0.937	0.557	1.681	816	0.093	-0.157	2.031
1000	2	MW1 - MW2	1.832	0.593	3.088	816	0.002	0.668	2.997
		MW2 - MW1	-1.832	0.593	-3.088	816	0.002	-2.997	-0.668
	3	MW1 - MW2	2.227	0.595	3.745	816	0.000	1.060	3.395
		MW2 - MW1	-2.227	0.595	-3.745	816	0.000	-3.395	-1.060
	4	MW1 - MW2	0.118	0.553	0.214	816	0.831	-0.968	1.204
		MW2 - MW1	-0.118	0.553	-0.214	816	0.831	-1.204	0.968

Table S15. Overall generalized linear mixed models test results for "Experiment × Treatment × Day" effect on *Gammarus locusta* avoidance (MW1 and MW2 experiments).

DMA treatment (mg/kg dry wt.)	Day	F	df1	df2	Sig.
0	2	0.859	1	816	0.354
	3	0.488	1	816	0.485
	4	0.984	1	816	0.322
50	2	0.005	1	816	0.945
	3	0.061	1	816	0.805
	4	0.274	1	816	0.601
250	2	0.060	1	816	0.806
	3	0.023	1	816	0.878
	4	2.827	1	816	0.093
1000	2	9.538	1	816	0.002
	3	14.026	1	816	0.000
	4	0.046	1	816	0.831

Table S16. Estimated means of target variable (Individuals in control section) for "Experiment × Treatment × Day" effect on *Gammarus pulex* (FW experiment) and *Gammarus locusta* avoidance (MW1 and MW2 experiments).

Experiment	DMA treatment (mg/kg dry wt.)	Day	Mean	SE	95% confidence interval	
					Lower	Upper
FW	0	2	7.688	0.639	6.434	8.942
		3	8.498	0.674	7.174	9.821
		4	7.810	0.658	6.520	9.101
	50	2	7.800	0.644	6.537	9.063
		3	7.991	0.654	6.707	9.274
		4	6.644	0.607	5.454	7.835
	250	2	10.093	0.732	8.656	11.530
		3	9.784	0.724	8.364	11.204
		4	9.249	0.716	7.844	10.653
	1000	2	12.819	0.825	11.199	14.438
		3	11.663	0.790	10.112	13.214
		4	11.802	0.808	10.215	13.388
MW1	0	2	7.467	0.412	6.659	8.276
		3	7.024	0.392	6.255	7.793
		4	7.159	0.379	6.414	7.903
	50	2	7.709	0.418	6.888	8.530
		3	7.222	0.397	6.443	8.002
		4	7.396	0.386	6.639	8.153
	250	2	8.190	0.431	7.344	9.036
		3	7.506	0.405	6.711	8.300
		4	7.257	0.382	6.508	8.007
	1000	2	10.026	0.477	9.090	10.963
		3	9.111	0.446	8.236	9.987
		4	7.674	0.393	6.903	8.444
MW2	0	2	7.905	0.424	7.074	8.737
		3	7.402	0.402	6.612	8.191
		4	7.701	0.393	6.929	8.473
	50	2	7.601	0.415	6.785	8.416
		3	7.079	0.393	6.307	7.851
		4	7.684	0.393	6.913	8.456
	250	2	7.985	0.426	7.149	8.820
		3	7.598	0.407	6.798	8.397
		4	8.194	0.406	7.398	8.991
	1000	2	8.154	0.430	7.309	8.998
		3	6.845	0.387	6.086	7.604
		4	7.555	0.390	6.790	8.320

Table S17. Pairwise contrasts for "Experiment × Treatment × Day" effect on *Gammarus pulex* (FW experiment) and *Gammarus locusta* avoidance (MW1 and MW2 experiments).

DMA treatment (mg/kg dry wt.)	Day	Experiment pairwise contrast	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
								Lower	Upper
0	2	FW - MW1	0.221	0.760	0.290	984	0.772	-1.271	1.712
		FW - MW2	-0.217	0.767	-0.283	984	0.777	-1.722	1.287
		MW1 - FW	-0.221	0.760	-0.290	984	0.772	-1.712	1.271
		MW1 - MW2	-0.438	0.591	-0.741	984	0.459	-1.597	0.722
		MW2 - FW	0.217	0.767	0.283	984	0.777	-1.287	1.722
		MW2 - MW1	0.438	0.591	0.741	984	0.459	-0.722	1.597
	3	FW - MW1	1.473	0.780	1.889	984	0.059	-0.057	3.004
		FW - MW2	1.096	0.785	1.396	984	0.163	-0.445	2.637
		MW1 - FW	-1.473	0.780	-1.889	984	0.059	-3.004	0.057
		MW1 - MW2	-0.378	0.561	-0.672	984	0.501	-1.479	0.724
		MW2 - FW	-1.096	0.785	-1.396	984	0.163	-2.637	0.445
		MW2 - MW1	0.378	0.561	0.672	984	0.501	-0.724	1.479
	4	FW - MW1	0.652	0.759	0.859	984	0.391	-0.838	2.142
		FW - MW2	0.110	0.766	0.143	984	0.886	-1.394	1.614
		MW1 - FW	-0.652	0.759	-0.859	984	0.391	-2.142	0.838
		MW1 - MW2	-0.542	0.547	-0.992	984	0.322	-1.615	0.530
		MW2 - FW	-0.110	0.766	-0.143	984	0.886	-1.614	1.394
		MW2 - MW1	0.542	0.547	0.992	984	0.322	-0.530	1.615
50	2	FW - MW1	0.092	0.768	0.119	984	0.905	-1.415	1.598
		FW - MW2	0.200	0.766	0.260	984	0.795	-1.304	1.703
		MW1 - FW	-0.092	0.768	-0.119	984	0.905	-1.598	1.415
		MW1 - MW2	0.108	0.590	0.183	984	0.855	-1.049	1.265
		MW2 - FW	-0.200	0.766	-0.260	984	0.795	-1.703	1.304
		MW2 - MW1	-0.108	0.590	-0.183	984	0.855	-1.265	1.049

Table S17. Continued.

DMA treatment (mg/kg dry wt.)	Day	Experiment pairwise contrast	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
								Lower	Upper
50	3	FW - MW1	0.768	0.765	1.004	984	0.316	-0.733	2.270
		FW - MW2	0.911	0.763	1.194	984	0.233	-0.586	2.409
		MW1 - FW	-0.768	0.765	-1.004	984	0.316	-2.270	0.733
		MW1 - MW2	0.143	0.559	0.256	984	0.798	-0.954	1.240
		MW2 - FW	-0.911	0.763	-1.194	984	0.233	-2.409	0.586
		MW2 - MW1	-0.143	0.559	-0.256	984	0.798	-1.240	0.954
	4	FW - MW1	-0.752	0.719	-1.046	984	0.296	-2.162	0.659
		FW - MW2	-1.040	0.723	-1.439	984	0.150	-2.459	0.378
		MW1 - FW	0.752	0.719	1.046	984	0.296	-0.659	2.162
		MW1 - MW2	-0.288	0.551	-0.524	984	0.601	-1.369	0.792
		MW2 - FW	1.040	0.723	1.439	984	0.150	-0.378	2.459
		MW2 - MW1	0.288	0.551	0.524	984	0.601	-0.792	1.369
250	2	FW - MW1	1.903	0.850	2.240	984	0.025	0.235	3.570
		FW - MW2	2.108	0.847	2.489	984	0.013	0.446	3.770
		MW1 - FW	-1.903	0.850	-2.240	984	0.025	-3.570	-0.235
		MW1 - MW2	0.205	0.606	0.339	984	0.735	-0.984	1.395
		MW2 - FW	-2.108	0.847	-2.489	984	0.013	-3.770	-0.446
		MW2 - MW1	-0.205	0.606	-0.339	984	0.735	-1.395	0.984
	3	FW - MW1	2.279	0.829	2.748	984	0.006	0.651	3.906
		FW - MW2	2.187	0.831	2.633	984	0.009	0.557	3.816
		MW1 - FW	-2.279	0.829	-2.748	984	0.006	-3.906	-0.651
		MW1 - MW2	-0.092	0.574	-0.160	984	0.873	-1.219	1.035
		MW2 - FW	-2.187	0.831	-2.633	984	0.009	-3.816	-0.557
		MW2 - MW1	0.092	0.574	0.160	984	0.873	-1.035	1.219

Table S17. Continued.

DMA treatment (mg/kg dry wt.)	Day	Experiment pairwise contrast	Contrast estimate	SE	t	df	Adj. sig.	95% confidence interval	
								Lower	Upper
250	4	FW - MW1	1.992	0.811	2.455	984	0.014	0.400	3.584
		FW - MW2	1.054	0.823	1.282	984	0.200	-0.560	2.669
		MW1 - FW	-1.992	0.811	-2.455	984	0.014	-3.584	-0.400
		MW1 - MW2	-0.937	0.557	-1.681	984	0.093	-2.031	0.157
		MW2 - FW	-1.054	0.823	-1.282	984	0.200	-2.669	0.560
		MW2 - MW1	0.937	0.557	1.681	984	0.093	-0.157	2.031
1000	2	FW - MW1	2.792	0.953	2.930	984	0.003	0.922	4.663
		FW - MW2	4.665	0.931	5.013	984	6.356E-07	2.839	6.491
		MW1 - FW	-2.792	0.953	-2.930	984	0.003	-4.663	-0.922
		MW1 - MW2	1.872	0.643	2.914	984	0.004	0.611	3.133
		MW2 - FW	-4.665	0.931	-5.013	984	6.356E-07	-6.491	-2.839
		MW2 - MW1	-1.872	0.643	-2.914	984	0.004	-3.133	-0.611
	3	FW - MW1	2.552	0.907	2.812	984	0.005	0.771	4.333
		FW - MW2	4.818	0.880	5.477	984	5.504E-08	3.092	6.545
		MW1 - FW	-2.552	0.907	-2.812	984	0.005	-4.333	-0.771
		MW1 - MW2	2.266	0.590	3.838	984	0.000	1.107	3.425
		MW2 - FW	-4.818	0.880	-5.477	984	5.504E-08	-6.545	-3.092
		MW2 - MW1	-2.266	0.590	-3.838	984	0.000	-3.425	-1.107
	4	FW - MW1	4.128	0.899	4.593	984	4.939E-06	2.364	5.892
		FW - MW2	4.247	0.898	4.732	984	2.553E-06	2.485	6.008
		MW1 - FW	-4.128	0.899	-4.593	984	4.939E-06	-5.892	-2.364
		MW1 - MW2	0.118	0.553	0.214	984	0.831	-0.967	1.204
		MW2 - FW	-4.247	0.898	-4.732	984	2.553E-06	-6.008	-2.485
		MW2 - MW1	-0.118	0.553	-0.214	984	0.831	-1.204	0.967

Table S18. Overall generalized linear mixed models test results for "Experiment × Treatment × Day" effect on *Gammarus pulex* (FW experiment) and *Gammarus locusta* avoidance (MW1 and MW2 experiments).

DMA treatment (mg/kg dry wt.)	Day	F	df1	df2	Sig.
0	2	0.275	2	984	0.760
	3	1.784	2	984	0.168
	4	0.642	2	984	0.526
50	2	0.038	2	984	0.963
	3	0.731	2	984	0.482
	4	1.036	2	984	0.355
250	2	3.265	2	984	0.039
	3	4.124	2	984	0.016
	4	3.455	2	984	0.032
1000	2	13.590	2	984	1.507E-06
	3	17.783	2	984	2.588E-08
	4	12.033	2	984	6.873E-06

6 Synthesis

Chapter 6

Introduction

In the Arctic, combined effects of climate change and globalization will increase oil and gas (O&G) exploration prospects. This will lead to more O&G and shipping activities [337] causing higher risks of pollution (Figure 1). In Norway and Russia, oil production from existing offshore wells will likely increase in the Barents Sea and Pechora Sea, and Arctic shipping routes such as the Northern Sea Route along the Russian coast are expected to be used more frequently. Such activities will be subjected to sea ice, strong winds and currents, which may enhance the risk of accidents during O&G transport [338] (**Chapter 1**).

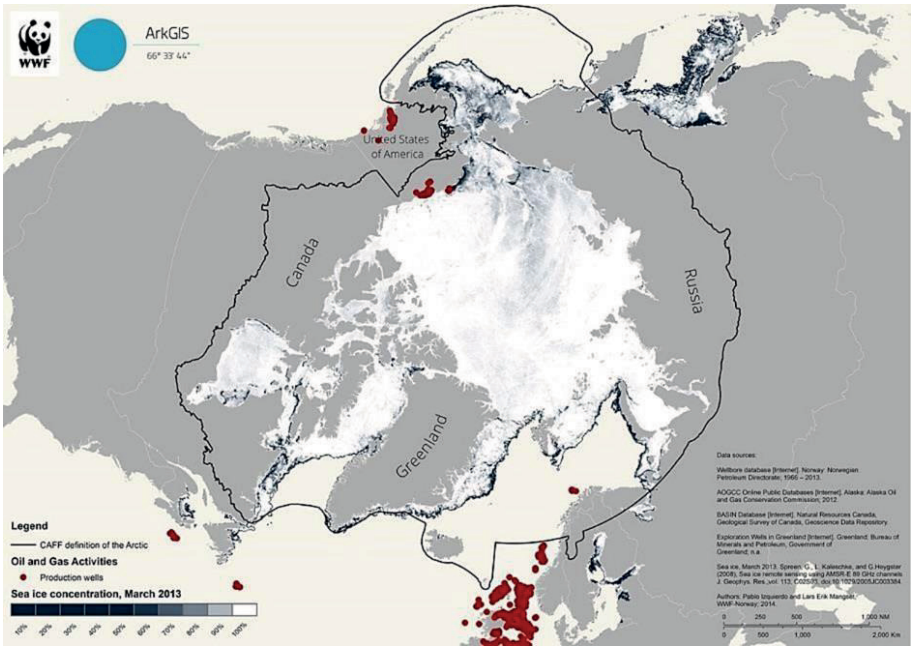

Figure 1. Arctic oil and gas production wells in 2013. Source: http://wwfarcticmaps.org/

Until now, environmental risks of O&G activities in the Arctic have mainly been evaluated using a conventional environmental risk assessment approach (Figure 2). For instance, threshold values for effects have been established based on species sensitivity distribution (SSD) curves for sub-cellular biomarker responses in water exposed species and for whole organism responses [339,340]. On the Norwegian Continental Shelf, environmental risk assessment of produced water (PW) involves the traditional steps in risk assessment (Figure 2). These are derived from the European Commission [341], and commonly using oil dispersions as proxies for PW and other crude oil discharges. Such conventional approaches

mainly apply to the water column. So far, specific approaches to assess risks for benthic systems in the Arctic have not been defined.

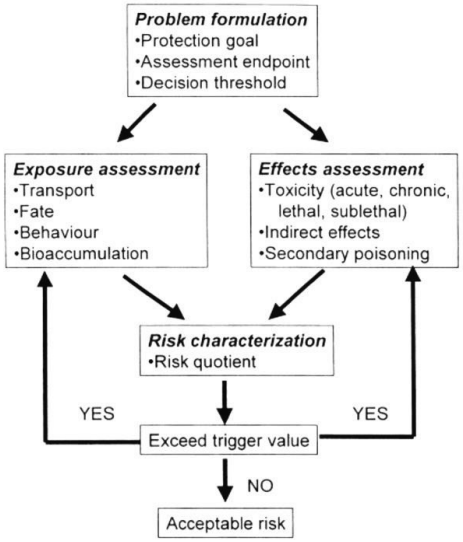

Figure 2. Environmental risk assessment process. Source: Maltby [342].

Current programmes monitoring the impacts of O&G activities on the Arctic parts of the Norwegian Continental Shelf include the water column and benthic habitats [343]. According to the Norwegian Environment Agency, both need to be surveyed every three years in both regional and field-specific stations of the same geographical region. A minimum of three wild-caught fish species and caged mussels are usually recommended for monitoring of exposure and effects in the water column [343]. As for the monitoring of benthic habitats, only the taxonomic identification and species density are required. In the Barents Sea, monitoring of benthic habitats in the Barents Sea South region is planned to start in 2019.

Polycyclic aromatic hydrocarbons (PAHs) are often used as indicators of oil related pollution [14], as petrogenic PAHs are enriched in weathered oil and reside in sediment with oil-specific chemical signatures [23,43,120]. Oil does not behave identically to PAHs in the marine environment, especially when it concerns fate processes. However, PAHs can be considered a major fraction contributing to the toxicity of oil. Therefore, in this thesis, several chapters are focused on the behaviour and effects of PAHs (**Chapters 2-4**), whereas one chapter studies a widely used gas oil; Distillate Marine grade A (DMA; **Chapter 5**).

As specified before, there is a high need for Arctic specific approaches and monitoring. To achieve this, the evaluation of environmental risks of O&G activities in the Arctic should take into account the specific physiology of Arctic species and environmental characteristics. Furthermore, the uncertainty of exposure pathways of oil related compounds should be addressed. In this thesis, four potential tools for the monitoring and risk assessment of O&G impacts on Arctic benthic species were addressed: (1) the use of biota-sediment accumulation factors (BSAFs) as indicators for sediment PAH bioavailability (**Chapters 2,3**), (2) the applicability of biomarkers of exposure (**Chapter 4**) and (3) the suitability of biotransformation metabolites in Arctic benthos chronically exposed to PAHs (**Chapter 4**), and (4) the relevance of avoidance tests as a tool for oil impact assessment (**Chapter 5**). These four methods were used to identify potential bioindicators for O&G and shipping related chemical stress in Arctic coastal benthic systems. For these tools six research questions were described (**Chapter 1**), which are discussed below.

Q1: Can PAH body residues or BSAFs be used as bioavailability indicators in Arctic coastal benthic systems and which target species is most suitable to detect trends?

In this thesis, bioaccumulation from sediment was evaluated under field (**Chapter 2**) and laboratory conditions (**Chapter 3**). The results showed that PAH partitioning deviated from what can be expected from Equilibrium Partitioning Theory (e.g., BSAF having a value of 1-2 [82]). This was explained by the presence of black carbon in the sediment, a bioaccumulation non-equilibrium, experimental variability or by any combination of these factors. This led to both lower and higher BSAF values than the range predicted by the Equilibrium Partitioning Theory.

In the field study (**Chapter 2**), significantly higher BSAFs were found in individuals from all species collected at a reference area (Blomstrandhalvøya) compared to those collected at a relatively impacted area (Ny-Ålesund). This was mostly ascribed to a higher abundance of black carbon in Ny-Ålesund sediment. For the majority of PAH compounds, body residues differed significantly between species. In Ny-Ålesund, body residues were highest in *M. calcarea* compared to body residues in *A. borealis* and *N. ciliata*.

In the laboratory study (**Chapter 3**), significantly higher BSAFs (i.e., for 3-ring PAHs) and body residues (i.e., for 3- and 4-ring PAHs) were observed in *A. borealis* than in *N. ciliata*. This was explained by a difference in feeding activity between these species and by a higher content in black carbon and amorphous organic matter in the polychaetes than in the bivalves

sediment treatment that may have resulted in a lower bioavailability and thus lower PAH uptake in *N. ciliata*.

In the literature, many examples apply body residues and/or BSAF values as indicators for bioavailability and bioaccumulation (e.g., [56,80,82]). However, deviations from BSAF values of 1-2 have also been reported frequently, sometimes limiting the applicability of BSAF as an unambiguous and reliable monitoring metric. This is also seen in the present research, where BSAFs were observed to vary widely. This means that the present work confirms the limited applicability of BSAF values as a relevant metric for biomonitoring in the Arctic as well.

As for body residues, also many literature reports exist where biomonitoring is based on concentrations in organisms over time and space [344,345]. When lipid-normalised, these concentrations are a more pure descriptor of bioavailability and bioaccumulation as they directly reflect exposure on the individual level. Furthermore, body residues can be compared directly with lipid based toxicity thresholds (e.g., the critical body burden – CBR – concept, see **Chapter 2**), which is a well-established approach in environmental risk assessment [154].

Based on the above, I conclude that PAH body residues provide a more realistic estimate of the bioavailable PAH fraction than BSAFs. This also implies that body residues are a better estimator of exposure in the context of effect- and risk assessment in Arctic benthic communities than BSAFs. Therefore, body residues are more suitable to derive relevant environmental quality standards for Arctic species. However, BSAFs are still useful indicators to help detect possible mechanisms affecting body residues, like for instance, variability in organic matter composition (e.g., black carbon), biomagnification and/or non-equilibrium as mentioned above.

Of all evaluated species I recommend *Macoma calcarea* as target species being most suitable to detect trends. This is based on its ability to accumulate PAHs in low impacted areas (e.g., Ny-Ålesund), regardless of their body size, and on their selective feeding mode, which decreases the variability of body residues measured in the species (**Chapter 2**). Recently, *M. calcarea* also was suggested as an ecological indicator in the context of developing trait-based approaches to explore changes in rapidly changing ecosystems [346]. However, in the absence of *M. calcarea* a similar method can be applied to select the next suitable indicator organism, using the criteria from **Chapter 1**. For instance, *Nuculana pernula* would be the closest species based on its traits (**Chapter 3**).

Recommendations. Because of the extensive variability in BSAFs and body residues it is recommended to minimize experimental variability. This can be achieved by enhancing sediment homogeneity, representativeness and uniformity of test animals taken from the field, quality and homogeneity of food supply, in laboratory tests with Arctic species. Standardized sediment toxicity testing [347] can be used to further develop protocols for Arctic benthic testing. A limiting practical factor is the presence of often less elaborated laboratory facilities for Arctic research that has to be dealt with.

A second recommendation is to further explore the relation between bioaccumulation (body residues) and environmental factors (e.g., temperature, particulate organic carbon, contaminant concentration) through multivariate statistical techniques [315,348-350] and based on this develop bioaccumulation models and scenarios for exposure testing.

A final recommendation is to apply the suggested biomonitoring tool, that is, use *M. calcarea* to detect trends and spatial differences in Arctic areas with O&G related pollution gradients.

Together, this will provide necessary information on spatial and temporal scales of gradients in bioaccumulation in the Arctic. When sediments need to be sampled for laboratory exposure experiments, such information on spatial heterogeneity in chemical exposure can be used to select sampling locations for these sediments.

Q2: Can species traits explain differences in PAH bioaccumulation between Arctic benthic invertebrates?

In this thesis, bioaccumulation was explored for species with different traits under both field (**Chapter 2**) and laboratory conditions (**Chapter 3**). The uncertainty in bioaccumulation metrics (BSAFs, body residues) was large, especially in the laboratory data. In the field study (**Chapter 2**), a higher bioaccumulation of PAHs was observed for *M. calcarea* in Ny-Ålesund compared to *A. borealis* and *N. ciliata*. This was explained by the high preference for freshly deposited organic matter, higher ingestion rate and lower PAH excretion capacity of *M. calcarea*. In the laboratory study (**Chapter 3**), a lower bioaccumulation of PAHs was observed for *M. calcarea* compared to *A. borealis* and *N. ciliata*. This was explained by a general decrease of PAH uptake and metabolic rate in *M. calcarea* under laboratory conditions. This decrease of uptake can be explained from the high diet selection of *M. calcarea* [211]. The decrease in metabolic rate can possibly be explained from the metabolic rate depression experienced by Arctic bivalves during starvation periods [351]. This illustrates

how laboratory settings can yield quite different results when compared to observations from field data.

According to the literature, bioaccumulation is strongly influenced by exposure routes, which in turn are determined by species traits, such as the feeding mode, ingestion – egestion rates, capability to metabolise chemicals, and diet composition [56,83,274,352,353]. Validated models are available to quantify the link between these traits and bioaccumulation [56,80,354]. For Arctic ecosystems, it has been reported that different benthic groups respond differently to the seasonality in the abundance of organic matter [127]. However, according to Włodarska-Kowalczuk et al. [355], a relatively high abundance of macroalgal detritus in the Kongsfjorden system makes benthic organisms less sensitive to the seasonality in pelagic production. Under such conditions of abundant food supply, differences in bioaccumulation will be mostly explained by other factors, such as the species' ability to metabolize and excrete PAHs.

Based on the aforementioned literature data and data from this thesis, I conclude that bioaccumulation is also dependent on species traits in Arctic species. The link between species-specific feeding mode and bioaccumulation was mechanistically explained using models (**Chapter 3**). However, the model's statistical significance remained limited as compared to earlier model evaluations, because of the considerable residual error between observations and model predictions.

Recommendations. Because of the high variability in BSAFs and body residues, it is recommended to reduce experimental variability. This will increase the rigour of tests that aim to detect differences in bioaccumulation among species with different traits, as well as the significance of models aimed at mechanistically explaining the observations.

To be able to explain differences in bioaccumulation among species from the field, the characteristics relating to quality and quantity of organic matter in all different diet components (e.g., sediment and freshly deposited organic matter) need to be determined. This includes quantifying organic matter, black carbon, oil and other possible solid components involved in binding hydrophobic chemicals.

Finally, it is recommended to assess bioaccumulation for a wider range of species in the field, together with community composition and sediment characteristics at the sites of interest. This will allow to study bioaccumulation patterns over time and space, for a much wider

range of species traits, including improved opportunities for mechanistic modelling. Furthermore, this allows for establishing causal relationships between bioaccumulation and community composition *in situ*.

Q3: Does bioaccumulation of PAHs differ between Arctic and temperate benthic invertebrate species with different traits, and can modelling assist in reading across between species?

In my research, no significant differences were observed in PAH body residues and BSAFs between Arctic and temperate benthic species under laboratory conditions (**Chapter 3**). This was mainly due to the substantial biological variability in the data.

Little is known about bioaccumulation of PAHs from sediment by Arctic benthic species, compared to bioaccumulation by temperate species. To my understanding, no studies have been published on the direct comparison of bioaccumulation by Arctic versus temperate benthic species. Two studies report PAH body residues in Arctic benthic species [121,356]. One of these studies included the modelling of bioaccumulation factors (BAFs) in blue mussels obtained from literature to infer sea water concentrations [121]. In this sense, this study (**Chapter 3**) is the first attempt to compare and to model bioaccumulation in similar Arctic and temperate benthic species.

Hypotheses were provided based on published differences in species traits with relevance for bioaccumulation. For instance, feeding rate, clearance rate and digestion rates often are lower in Arctic species [213,357,358], that may affect both bioaccumulation and risks. These differences were not observed in our experiments, due to the fact that they were probably smaller than the current variability in the data (**Chapter 3**).

For temperate species, the use of biodynamic modelling has helped to provide a mechanistic understanding of bioaccumulation processes [56,83]. The models can also be used to predict PAH bioaccumulation in the absence of experimental data [203], and to read across species, compartments (freshwater *vs* marine) and potentially climate regions. Examples of modelling of contaminants across temperate and Arctic pelagic species are provided by some studies [359,360]. These studies concluded that bioaccumulation in the Arctic may be described in the same way as bioaccumulation in temperate regions. In this thesis, however, dynamic simulations among species with similar traits and exposed to PAHs under laboratory conditions did not reveal any clear differences in the model parameters between species or

tests. This was due to the substantial variability in the biological control data and the similarity in exposure routes (**Chapter 3**).

Despite the lack of significant differences in PAH bioaccumulation between Arctic and temperate species in our study, I argue that differences between Arctic and temperate species can be detected if more rigorous testing methods are applied in these tests, and/or if other selections of species, individuals or sediments would be used in these tests.

Recommendations. As specified above, there is a need to improve the accuracy of bioaccumulation testing. This can be achieved by a series of measures, such as the use of non-exposed test animals, artificial sediment, a higher number of replicates and time points, and/or a more constant food addition.

These improvements will also increase the applicability of bioaccumulation models and lead to more certain parameter values. Additionally, several model parameters need to be measured in an experimental set up, in order to increase the reliability of bioaccumulation models for Arctic species (such as growth and feeding rates of species). To get a better understanding when comparing Arctic and temperate species, specific tests can be done where sediments either are identical for both Arctic and temperate species, or a suitable representative of the region's sediment type. The tests can be done with Arctic and temperate species at the same temperature, or at temperatures specific to Arctic and temperate conditions. This way the relative importance of both temperature and sediment properties can be identified which helps to further elucidate potential differences among species and regions.

Q4: What is the feasibility of using biomarkers of exposure to monitor PAH pollution in the Arctic? Is the identification of biotransformation metabolites a better alternative?

For an integrated monitoring and assessment programme for the Arctic, biomarkers and bioassays need to be tested and validated in Arctic organisms that are representative for the water and sediment compartment [91,361]. For the benthic compartment, benthic invertebrates may fulfil the role of bioindicators of chemical pollution, just as fish species do for the water compartment [343,362]. In this respect it is essential to causally link chemical body residues and biomarkers measured at different suborganismal levels (Figure 3), in order to increase the interpretative value of individual measurements [345]. Therefore, in my study, I analysed both PAH body residues and biomarkers of exposure/effect in target species.

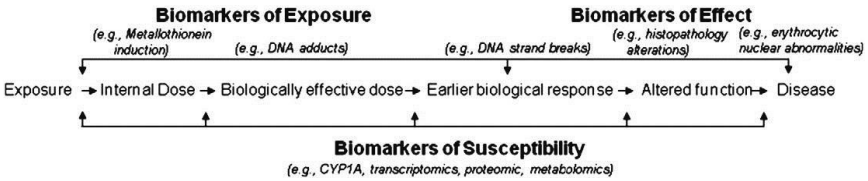

Figure 3. Continuum from chemical exposure to organism level effects. Source: Chapman et al. [363].

For an integrated exposure an effect assessment (Figure 2), two biomarkers of PAH exposure (i.e., acyl-CoA oxidase [AOX] and glutathione S-transferase [GST]) and one biomarker of neurotoxicity (i.e., acetylcholinesterase [AChE]) in *A. borealis* were addressed (**Chapter 4**). GST and AChE have been validated in temperate bivalves before. They are commonly used in the assessment of oil impact [364] and pollution monitoring [365], whereas AOX generally shows a rapid response to PAH exposure in temperate bivalves [279,281]. In the present work, interpretation of enzymatic biomarkers data in *A. borealis* was limited. This is because either baseline studies are lacking for Arctic benthic species [215], or these biomarkers were assessed in water exposed rather than sediment exposed Arctic benthic organisms [366,367].

Pyrene metabolites, and particularly 1-hydroxypyrene, have been used before as a suitable biomarker of PAH exposure in temperate species [368,369]. Our study made the first multi-species comparison of pyrene biotransformation between Arctic and temperate benthic invertebrates (**Chapter 4**). This was done to estimate differences in their biotransformation capacity as well as to validate the use of 1-hydroxypyrene and pyrene-1-glucuronide [183] as biomarkers of PAH exposure in Arctic species.

Unlike pyrene body residues, a significant increase in the concentration of pyrene-1-glucuronide was observed in polychaetes exposed to the high sediment treatment compared to the low sediment treatment (**Chapter 4**). This increase was not detected in bivalve species. In polychaetes, biotransformation rates did not vary between treatments in our study. This was illustrated by the similar relative concentrations of pyrene-1-glucuronide in polychaetes exposed to the different sediment treatments, implying biotransformation also at low concentrations. As for enzymatic biomarkers, there was no correlation between the activity of biomarkers in *A. borealis* and PAH contamination levels in sediment treatments (**Chapter 4**). This could mainly be explained by the low PAH contamination levels in *A. borealis*, although the influence of confounding factors [134,283] could not be excluded. There is a lack of

knowledge on pyrene metabolites in Arctic invertebrates and the use of biomarkers in Arctic invertebrates in general [213,370].

Based on the available literature data and data from this thesis, I conclude that the analysis of pyrene metabolites provides a feasible approach to assess PAH exposure in Arctic benthic populations. However, it would be advisable to review the sensitivity of additional biomarkers of exposure and biomarkers of effect in order to develop a further weight-of-evidence approach.

Recommendations. In order to provide a more robust and integrated assessment of PAH exposure in Arctic species, the ability of different markers of exposure needs to be evaluated to provide an early warning of risks on PAH mediated biological effects. This can be achieved by exploring the relative effect of environmental and biological factors on the activity of biomarkers with respect to PAH exposure [275]. The natural metabolic activity and seasonal dynamics of biomarkers in Arctic species are needed to be able to determine relevant biomarker responses in Arctic benthic invertebrates to PAH exposure in the field. Finally, our findings illustrate that Arctic benthic macroinvertebrates have a biotransformation capacity, which should not be overlooked when evaluating accumulation and risks of PAHs in the Arctic [93,94].

Q5: Can behavioural tests like avoidance behaviour become a suitable monitoring tool for oil impact assessment in the Arctic?

In my research, a significant avoidance of DMA oil-spiked sediment was observed at 1000 mg/kg (dry weight). Furthermore, it was shown that for the freshwater species *G. pulex* this occurred within an exposure time of 96 h, whereas for the marine species *G. locusta* in one of two tests, this occurred within an exposure time of 72 h (**Chapter 5**). Hence, this shows that the tests are able to detect behavioural effects of gammarids to DMA oil, as well as a time dependency of these effects.

Differences in time dependency of avoidance behaviour between the two species, were explained by the experimental conditions (i.e., water parameters, feeding) on the species' sensitivities to DMA oil and to their ability to maintain active over time. The sensitivity to detect DMA oil was explained by a higher ventilation rate, caused by the exposure of *G. pulex* at its upper thermal tolerance limit (i.e., 15 °C). As for *G. locusta*, the ability to detect and

avoid DMA may have been restricted by a higher salinity in the marine experiments and low feeding potential.

Within-species differences in the avoidance response of *G. locusta* among tests were considerable, thereby limiting the rigour of the test (**Chapter 5**). This variability may have been related to the variability among individuals used in the tests. This originates from differences in structural growth and reproductive status of the tested individuals, as observed more often among summer *G. locusta* generations [327,329].

In the literature, behavioural endpoints such as the avoidance response to oil of benthic organisms have been proposed before as a tool to detect sublethal effects of oil in sediment [99,304-306]. Studies on the avoidance behaviour of oil-contaminated sediment by temperate marine amphipods [298] and of PAH-spiked sediment by temperate freshwater invertebrates [100] showed that avoidance behaviour was not affected by the background exposure history of the organisms to either oil or PAHs, and that avoidance tests could be used as an alternative to chronic toxicity testing. These studies also concluded that homogeneity in exposure conditions (e.g., sediment characteristics, feeding) or in test species individuals is crucial.

Variability among individuals may be less for Arctic species than for temperate species. Variability in energy levels of organisms is likely to affect avoidance behaviour [322,371]. Energy levels are affected by environmental factors (e.g., temperature and salinity [335,372]) and biological variables (e.g., structural growth rate [313,333,372] and reproductive status [327,329,372]). Some Arctic benthic marine amphipods (e.g., *Gammarus setosus*) show less variability than *G. locusta* with regards to a longer life-span and fewer reproductive events [373]. Based on this, *G. setosus* may be a suitable candidate species for effective avoidance tests for Arctic sediments exposed to oil.

Given the above, I conclude that avoidance tests are suitable for monitoring oil impact in temperate regions, whereas their applicability in the Arctic is likely but needs more investigation.

Recommendations. The avoidance tests need to be validated with Arctic species under Arctic conditions prior to implementation.

I suggest the use of immature adult *G. setosus* collected during early spring for the avoidance tests (i.e., March-April) [373]. During this period, *G. setosus* synchronizes its activity with the

spring bloom [374,375] and water parameters are stable [62]. Immature adults will limit its energy allocation to acclimation and growth, and start building up energy reserves.

Finally, several aspects of the test conditions can be further improved. For instance, sediment oxygen demand may be used to evaluate the physiological state of individuals during tests, and the applicability of these tests at low PAH exposure level [104,259]. Similarly, the homogeneity of sediment characteristics and feeding can be considered for an optimised test.

Q6: What are prospects and advantages of using Arctic benthic bioindicator species for monitoring long-term impacts of O&G and other maritime activities in the Arctic?

Here the prospects and advantages of using Arctic benthic bioindicator species are discussed based on the criteria provided in **Chapter 1**:

1. Its response is sensitive to changes in the chemical concentration;
2. Its response is specific and causally related to the chemical exposure;
3. Its response is adequate given the anticipated concentration of the chemical;
4. The species' uptake/elimination of the chemical can be quantified;
5. The toxic effects of the chemical can be measured/interpreted.

These criteria, based on Goodsell et al. [19], do not necessarily need to apply to every single aspect measured on a bioindicator species. For instance, measuring a biomarker of exposure can be meaningful in the context of biomonitoring, even without measuring the response of a biomarker of effect (criterion 3).

As for the first two criteria, the correlation between bioaccumulation of PAHs and PAH exposure levels was tested for species with different traits under field conditions (**Chapter 2**). It was suggested that exposure was lower in the presence of black carbon, thus implying a positive correlation between exposure and uptake. However, the causality of bioaccumulation changes could also be explained by the species' feeding traits as was shown under experimental conditions (**Chapter 3**). In that respect, *M. calcarea* can be used to detect spatial and temporal gradients in bioaccumulation in the Arctic due to its selective feeding mode (**Q1**).

As for the third criterion, confounding non-target chemicals or environmental conditions have to be taken into account. The causality of the interrelationships between biomarkers at different suborganismal levels needs to be established for the bioindicator candidates (Figure 3; [376]). This is needed for the validation of the bioindicator species as these relationships will be used to select appropriate biomarkers which can provide early warning/are indicative

of PAH biological effects. In my research, a direct causality between the concentration of pyrene body residue and the rate of pyrene biotransformation was not found in *M. calcarea* (**Chapter 4**).

In addition, for the actual validation of *M. calcarea* as a biomonitoring species, future studies should address the combined effects of relevant oil chemical components and environmental factors on biomarker responses [361]. Although a long-term strategy is essential for such studies [377], the use of Arctic region specific environmental quality standards in an integrated monitoring and assessment programme is essential for the understanding of the dynamics of toxicants and their effects on Arctic marine ecosystems [345]. This implies that much work is still needed (e.g., see **Q4**) and that the present study is only a first start for what is needed to fully develop a validated system for biomonitoring in the Arctic.

The adaptation of existing methodology to Arctic species and conditions may form a suitable base for the environmental risk assessment of O&G and shipping activities in the Arctic, and the use of Arctic benthic bioindicator species is feasible and highly necessary for the prediction of long-term impacts of such activities on Arctic benthic habitats.

Conclusion

For the selection of relevant bioindicators for O&G impact assessment in the Arctic, sediment tests can be used in combination with chemical data and biomarkers measured in the organism collected *in situ* as part of an integrated weight-of-evidence approach. Bioaccumulation testing requires the implementation of more standardized methods for sediment preparation, exposure conditions and acclimatization of field organisms (**Q3**). A better understanding of the natural variability in biomarker responses in Arctic species is also needed in combination with the influence of environmental confounding factors (**Q4**).

With a few exceptions, it is not common to find all properties of a good bioindicator in one single species. Thus, different species may be used in the evaluation of different sublethal endpoints. This also accounts for the selection of biomarkers. A single biomarker response is generally not adequate, but a suite of biomarkers can be used to indicate early effects of exposure (as in this thesis) and/or effects of a toxicant. Biomarkers present early warning information that biodiversity indicators, such as biotic indexes, do not offer. Biomarkers of exposure are closely related to chemical stress, while biodiversity indicators are not specific

and may indicate all kinds of different stressors. Hence, biomarkers may enable mitigation measures before irreversible impacts occur.

In this thesis, *M. calcarea* was selected as a potential bioindicator species for O&G impact assessment in the Arctic (**Q2**). In short, *M. calcarea* was able to accumulate PAHs at a low exposure level under field conditions, although the species showed a variable level of pyrene metabolization under laboratory conditions. This was mostly related to experimental conditions, but potentially also to the likely variability in the physiological state of *M. calcarea* individuals. Its capacity for the excretion of PAH metabolites was low. Although the actual metabolization and excretion of PAHs still need to be further tested, the retention of PAH metabolites at a low exposure level in *M. calcarea* makes it worthwhile to study the implementation of several biomarkers with temporal differences in their response and differences in their effective dose.

A bioindicator like *M. calcarea* could be incorporated into a signalling system. Signalling systems can be seen as a weight-of-evidence approach, being an integrative index based on biomarkers that allow us to classify sampling sites with respect to their pollution status. For instance, the Integrated Biomarker Response (IBR) [378] is a mathematical tool based on biomarker data standardisation and their aggregation into a single value. Other approaches like the Integrated assessment of hazardous substances in the Baltic Sea (CHASE tool) combine chemical and biological measurements. Such approaches may reduce the uncertainty in interpretation of biomarker responses and can be used for regular and impact-specific biomonitoring.

A crucial part of such a signalling system is a solid knowledge on the baseline levels. Such baseline studies need to characterize natural levels in PAH body residues and biomarker responses commonly recommended for biomonitoring and to evaluate the sensitivity to oil pollution in these Arctic species. Furthermore, baseline studies help to identify the strengths and limitations for application of biomarkers to (sub-)Arctic regions as well as to determine their seasonal variability in potential bioindicator species. Seasonality may result in particular physiological characteristics of Arctic species that affect their toxic response to oil and PAHs (e.g., seasonality in lipid reserves). In addition, environmental fluctuations in the light regime and temperature may affect the bioavailability and exposure to oil-related chemicals. Baseline studies using bioindicator species will help to better characterize the general mechanisms and patterns underlying negative effects of O&G and shipping activities.

Chapter 6

The overall utility of this work consists of a first evaluation of promising candidate species for Arctic biomonitoring and research programmes, and of various alternative approaches for the early identification of negative ecological impacts of O&G and shipping in Arctic benthic bioindicators.

Particularly, this thesis proposed one potential benthic bioindicator species (*M. calcarea*) and one potential tool (avoidance tests) for the assessment of oil impacts on sediment for its further validation under Arctic conditions. It also underlined the need for standard sediment test protocols and baseline studies as an integral part of environmental risk assessment in the Arctic, and provided several recommendations based on existing guidelines and a comparative study of bioaccumulation data from field and laboratory studies with Arctic species.

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Summary

Expanding oil and gas (O&G) and shipping activities in the absence of sufficient baseline data on the sensitivity of Arctic marine biota to oil related compounds makes environmental risk assessment for the Arctic challenging. Delayed oil weathering and biodegradation, ecological seasonal variations or a delayed manifestation of toxicity in Arctic marine biota could create differences between the susceptibilities of Arctic and temperate monitoring species in the long term. Such differences may be better defined in estuarine or shallow areas, where the frequent resuspension of sediment and strong affinity of oil for sediment organic matter suggest that a large proportion of oil compounds will be transferred to the seafloor, increasing exposure of benthic organisms. Arctic benthos, and in particular sediment-dwelling organisms, can be applied as relevant and sensitive bioindicators of chemical stress and are important food sources for higher trophic levels. In addition, Arctic benthic organisms may benefit from the projected increase in phytoplankton primary production under climate change, strengthening the pelagic-benthic coupling and trophic transfer efficiency within Arctic marine ecosystems. Their intrinsic ecological value calls for the evaluation and validation of oil toxicokinetic parameters in Arctic benthos as part of current oil risk assessment procedures and monitoring programmes. Such knowledge may form an important basis for the selection of relevant Arctic benthic bioindicators of long-term environmental impacts of O&G and shipping activities.

The aim of this thesis is to develop a suite of candidate exposure methods, including bioaccumulation and biochemical markers, for the identification of bioindicators of chemical stress derived from O&G and shipping in Arctic coastal benthic systems. The second aim is to propose, based on experiments with temperate species, the avoidance of oil contaminated sediment by Arctic benthic amphipods as an oil risk assessment tool for the Arctic.

In **Chapter 2**, the applicability of biota-sediment accumulation factors (BSAFs) as chemical exposure metric was evaluated in three representative species of an Arctic coastal benthic system under field conditions. In a preliminary round, target species were selected based on their feeding mode, reduced motility and relative abundance. Two bivalves, the suspension feeder (*Astarte borealis*) and the surface deposit and suspension feeder (*Macoma calcarea*), and one polychaete, the predator and deposit feeder (*Nephtys ciliata*), were selected. These species and corresponding sediment samples were collected at a reference area (Blomstrandhalvøya) and a relatively oil impacted area (Ny-Ålesund) to study the influence of

local sediment components on the bioaccumulation of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB).

Bioaccumulation differences were detected between both areas for PAHs, with higher 10-90th $\sum_{13}$PAH BSAF percentiles (i.e., 0.35 – 5.50) in Blomstrandhalvøya than in Ny-Ålesund organisms (i.e., 0.00 – 0.07). Differences in the quality of organic matter in sediment and other food items probably affected the sediment ingestion and thus, PAH uptake by organisms from different areas. However, similar patterns across sampling areas were observed in *M. calcarea* based on $\sum_{13}$PAH body residues, with higher lipid-normalised concentrations in Ny-Ålesund specimens. This pointed at possible differences in toxicokinetic parameters such as PAH uptake and elimination, and higher preference for biogenic organic matter sources for *M. calcarea* as compared to the other species. It was concluded that PAH body residues provide a more realistic estimate of the bioaccessible PAH fraction since their variability among individuals and environmental conditions was lower than for BSAFs. The study also reflected on the necessity to include black carbon as a major influencing factor of sediment exposure to persistent organic contaminants for Arctic benthos.

In **Chapter 3**, the relative importance of feeding traits and sediment composition for the bioaccumulation of PAHs by the aforementioned species was evaluated using a dynamic modelling approach. Modelling input data were obtained from a laboratory exposure experiment performed with specimens collected at Blomstrandhalvøya. The experiment followed the design of an earlier exposure experiment developed for temperate counterpart species for comparison purposes. The latter included the surface deposit and suspension feeding bivalve (*Limecola balthica*) and the deposit feeding polychaete (*Alitta virens*) frequently used as test species in bioaccumulation studies. In both experiments, species were exposed to sediment treatments made from the combination of different ratios of a reference and a naturally contaminated sediment collected in a temperate estuary. In this laboratory study, PAH body residues were again selected as the most suitable exposure metric due to considerable BSAF variability in both the control and exposed groups.

In the Arctic experiment, differences in the bioavailability of PAHs between sediment treatments for each taxonomy group possibly explained significantly higher lipid-normalised concentrations observed for 3- and 4-ring PAHs in *A. borealis* compared to *N. ciliata*. However, PAH uptake was limited among bivalve species which was likely caused by the presence of pyrogenic PAH sources in the contaminated sediment, commonly associated with

black carbon emissions from shipping, and low nutritional value of the bivalves reference sediment. The experimental approach used did not reduce the uncertainty in the relative contribution of different PAH sources to body residues observed in **Chapter 2**. It was concluded that future studies should look at the influence of the quality and quantity of organic matter in different sediment PAH sources to help to interpret chemical exposure in Arctic species.

A comparison with the temperate experiment did not yield any significant differences in PAH body residues nor measured BSAFs between the Arctic and temperate counterpart species. Similar exposure routes and the considerable biological variability in the control groups could explain the similarity in the model output between experiments, also reflected by the absence of differences in the model parameters between test species or experiments. Because of limitations on the availability of Arctic species and the variability observed during exposure, the modelling approach did not provide any insightful information on the bioaccumulation mechanisms. Nevertheless, it is expected that such constraints may be overcome after further characterization of PAH uptake and elimination routes in Arctic species under local conditions.

In **Chapter 4**, a suite of biomarkers of PAH exposure (acyl-CoA oxidase, AOX; glutathione S-transferase, GST; pyrene metabolites) and neurotoxicity (acetylcholinesterase, AChE) were analysed in the aforementioned species in order to evaluate the species-specific sensitivity of Arctic species to PAHs in comparison to their temperate counterparts and the general applicability of standard biomarker protocols for predicting sub-lethal ecological effects in Arctic species. Due to limited sample availability and general lack of knowledge of PAH biotransformation pathways among marine invertebrates, enzymatic biomarkers (i.e., AOX, AChE, GST) were analysed in *A. borealis* samples collected from experiments included in **Chapter 3**, whereas pyrene metabolites were analysed in samples from Arctic (*M. calcarea*, *N. ciliata*) and temperate species (*L. balthica*, *A. virens*).

The results of the study showed neither induction or correlation between the activity of AOX or GST in *A. borealis* and PAH contamination levels in sediment treatments, nor evidence of PAH neurotoxicity according to AChE activity. Lack of enzymatic induction may be related to either too low PAH concentrations in sediment or to the influence of confounding factors such as low food availability or changing metabolic status of *A.borealis* during sampling. Pyrene biotransformation rates were limited among Arctic and temperate species as the ratio

of pyrene-1-glucuronide (Phase II metabolite) to pyrene content did not correlate with pyrene exposure in test organisms. It was concluded that PAH metabolites may be used to estimate PAH exposure as a flux balance between PAH parental and metabolite compounds, although Phase II biotransformation pathways need further characterization in test species.

In **Chapter 5**, the avoidance response to sediment contaminated with Distillate Marine grade A (DMA) oil of a freshwater (*Gammarus pulex*) and marine (*Gammarus locusta*) model benthic species was evaluated in a 96-h test period and used as the basis for future pilot testing in Arctic species. *G. pulex* avoided contaminated sediment at the highest concentration (1000 mg DMA/kg dry wt.), while *G. locusta* avoidance was either not consistent with exposure time or below effect threshold. Lack of consistency in *G. locusta* avoidance was possibly caused by differences in the energy allocation to growth and reproduction between *G. locusta* life-span stages, affecting their ability to escape from DMA. In contrast, a lower reproductive effort in *G. pulex* winter individuals may have increased their energy storage capacity and thus, energy allocation to avoidance. In addition, differences in DMA behaviour between freshwater and marine tests and PAH partitioning between marine tests likely affected the comparability between tests.

The results of this study highlighted the need for baseline assessments of inter-individual variability in the avoidance response of benthic amphipods as well as the chemical characterization of sediment treatments and overlying water in exposure systems. It was concluded that the combined analysis of energy consumption and spatial avoidance could help to elucidate whether the avoidance ability of amphipods may be affected by their reproductive strategy and how could avoidance be translated into a recognizable pattern of effects on life-history traits of *Gammarus* spp. Future studies should consider as well the influence of reproductive and moulting stage on the sensitivity to oil of benthic amphipods.

The final **Chapter (6)** summarizes and discusses the outcomes of Chapters 2 to 5 in order to select the most appropriate approach to risk identification of O&G and shipping activities in Arctic benthos and to identify research priorities for the validation of candidate methods. Recommendations for the selection and validation of bioindicators and biomarkers of long-term oil spill and O&G environmental effects in Arctic coastal benthic systems included (1) the implementation of more standardized methods for sediment preparation, exposure conditions and acclimatization of field organisms, (2) the collection of baseline data on PAH body residues and biomarker responses in Arctic bioindicator species, (3) the characterization

of quality and quantity of organic matter in all different diet components of exposure treatments, (4) the evaluation of the relative effect of environmental and biological factors on the activity of biomarkers, (5) the design of community assessments to explore the relationship between PAH body residues, sediment characteristics and environmental factors and (6) the inclusion of oil avoidance tests using Arctic benthic amphipods as a candidate tool for oil spill risk assessment for the Arctic. This work contributes to the development of Arctic benthic bioindicators of the cumulative effects of O&G and shipping activities through the identification of robust PAH exposure metrics in representative benthic species of the Barents Sea region.

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Y a mi pequeño pero también gran hermano, Kordian, con quien espero que podamos seguir siempre teniendo nuestras conversaciones en familia. ¡Nunca pierdas tu esencia!

How do people imagine the landscapes they find themselves in?
How does the land shape the imaginations of the people who dwell in it?
How does desire itself, the desire to comprehend, shape knowledge?
- Barry Lopez -

Curriculum vitae

Ariadna S. Szczybelski Ciordia (Zaragoza, Spain, April 2nd 1985) obtained her BSc degree in Biology from the University of Alicante, Spain. During her studies, she was acquainted with several marine biology aspects and completed an Erasmus exchange at the University of Liège (Belgium) where she became interested in the subject of marine ecotoxicology and related research performed therein. In 2010, she decided to enrol on an MSc programme in Environmental Contamination and Toxicology (University of Valencia, Spain). She focused on the use of biochemical biomarkers in freshwater and marine fish species to monitor effects of organic pollution and did an internship at the Institute of Marine Sciences (ICM-CSIC) in Barcelona, Spain. After finishing her MSc degree, she was awarded a Leonardo Da Vinci fund to complete a 6 month work-related training at the Ecotoxicology laboratory of IFREMER (Nantes, France). During her stay she was involved in the analysis of marine fish and shellfish samples from two projects funded by the French National Agency for Research (CONPHYPOP and GIMEPEC) and in the sampling of flatfish species in the Seine estuary (France) as part of the BIOCO 2012 campaign. After finishing her internship, she started a PhD in the Aquatic Ecology and Water Quality Management Group in Wageningen University (Wageningen, the Netherlands). The PhD revised the applicability of exposure assessment methods to oil related compounds in Arctic and temperate benthic macroinvertebrates for the selection of relevant Arctic benthic bioindicators to be used in current environmental risk assessment procedures in the Arctic. During her PhD she co-supervised one BSc student and three MSc students, presented at two international conferences (ARCTIC FRONTIERS and SETAC Europe) and was member of the Dutch National Committee of the Association of Polar Early Career Scientists (APECS) in 2016-2017. She is currently taking her second MSc programme in Marine Biotechnology at the University of Tromsø (Tromsø, Norway) with an eye to studying different strategies for optimizing oil bioremediation in Arctic marine sediments.

List of publications

Szczybelski A.S., M.J. van den Heuvel-Greve, A.A. Koelmans, N.W. van den Brink. Submitted. Biomarker responses and biotransformation capacity in Arctic and temperate benthic species exposed to polycyclic aromatic hydrocarbons. Science of the Total Environment.

Szczybelski A.S., N.J. Diepens, M.J. van den Heuvel-Greve, N.W. van den Brink, A.A. Koelmans. Under revision. Bioaccumulation of polycyclic aromatic hydrocarbons by Arctic and temperate benthic species. Environmental Toxicology and Chemistry.

Szczybelski, A.S., T. Kampen, J. Vromans, E.T.H.M. Peeters, M.J. van den Heuvel-Greve, N.W. van den Brink, A.A. Koelmans (2018). Avoidance tests as a tool to detect sublethal effects of oil-impacted sediments. Environmental Toxicology and Chemistry 37(6), 1757-1766.

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Akcha, F., A. Barranger, E. Bachère, C. Heude Berthelin, D. Piquemal, P. Alonso, R. Rondon Sallan, G. Dimastrogiovanni, C. Porte, D. Menard, **A. Szczybelski**, A. Benabdelmouna, M. Auffret, J. Rouxel, T. Burgeot (2016). Effects of an environmentally relevant concentration of diuron on oyster genitors during gametogenesis: responses of early molecular and cellular markers and physiological impacts. Environmental Science and Pollution Research 23(8), 8008-8020.

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The SENSE Research School declares that **Ariadna Sabina Szczybelski Ciordia** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 34.9 EC, including the following activities:

SENSE PhD Courses

- Environmental research in context (2013)
- Research in context activity: 'Preparing and organizing of Netherlands / Belgium Symposium for Association of Polar Early Career Scientists, APECS (2 November 2016)'

Selection of Other PhD and Advanced MSc Courses

- Basic statistics, Wageningen university (2013)
- Introduction to R for statistical analysis, Wageningen University (2014)
- Proficiency in English, Radboud in'to Languages (2014)
- Molecular toxicology, Postgraduate Education in Toxicology (2016)
- Writing grant proposals, Wageningen University (2016)
- Career assessment, Wageningen University (2016)
- Marine sustainability, University of Tromsø, Norway (2017)
- Toxicogenomics, Postgraduate Education in Toxicology (2017)

External training at a foreign research institute

- Enzymatic determination of a suite of exposure biomarkers, PIE Research Centre, University of the Basque Country, Spain (2015)

Management and Didactic Skills Training

- Supervising two MSc students with theses entitled 'Avoidance of DMA contaminated sediment by *Gammarus pulex*' (2014) and 'Bioaccumulation of polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) in the Arctic Ocean' (2015)
- Teaching in the BSc course 'Environmental Toxicology' (2014-2015)

Oral Presentations

- *ARCIND – Development of ARCtic biological INDicators for the impact assessment of (new) human activities in the Arctic ecosystem.* NWO Symposium 2014, 5 November 2014, The Hague, The Netherlands
- *Biological indicators for the impact assessment of (new) human activities in the Arctic.* SETAC Europe, 7 May 2015, Barcelona, Spain
- *Biological indicators for the impact assessment of (new) human activities in the Arctic.* APECS Netherlands Symposium, 6 November 2015, The Hague, The Netherlands

SENSE Coordinator PhD Education

Dr. Peter Vermeulen

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Propositions

1. Mechanistic bioaccumulation models for Arctic coastal benthic systems should account for the relative proportion of terrestrial dissolved organic matter and marine dissolved organic matter and its change over time.
   (this thesis)

2. The variation in bioaccumulation by Arctic benthic macroinvertebrates can largely be explained by site-specific differences in diet choice and food composition.
   (this thesis)

3. Community initiatives to promote physical activity and strength training among pre-frail older adults can significantly reduce health care and social assistance spending.

   J. Sacha, M. Sacha, J. Soboń, Z. Borysiuk and P. Feusette (2017). "Is It Time to Begin a Public Campaign Concerning Frailty and Pre-frailty? A Review Article." Frontiers in Physiology 8(484)

4. Geospatial data visualization should be used in decision making about climate change risks to define and communicate extreme events.

5. In the era of political micro-targeting and mass surveillance, our digital footprint can obstruct a functioning democracy.

6. Closing the gender pay gap begins by introducing a universal basic income.

Propositions belonging to the thesis, entitled

'Advancement of benthic indicators and biomarker-based tools for biomonitoring and risk assessment in the Barents Sea region'

Ariadna S. Szczybelski Ciordia
Wageningen, 5 November 2018