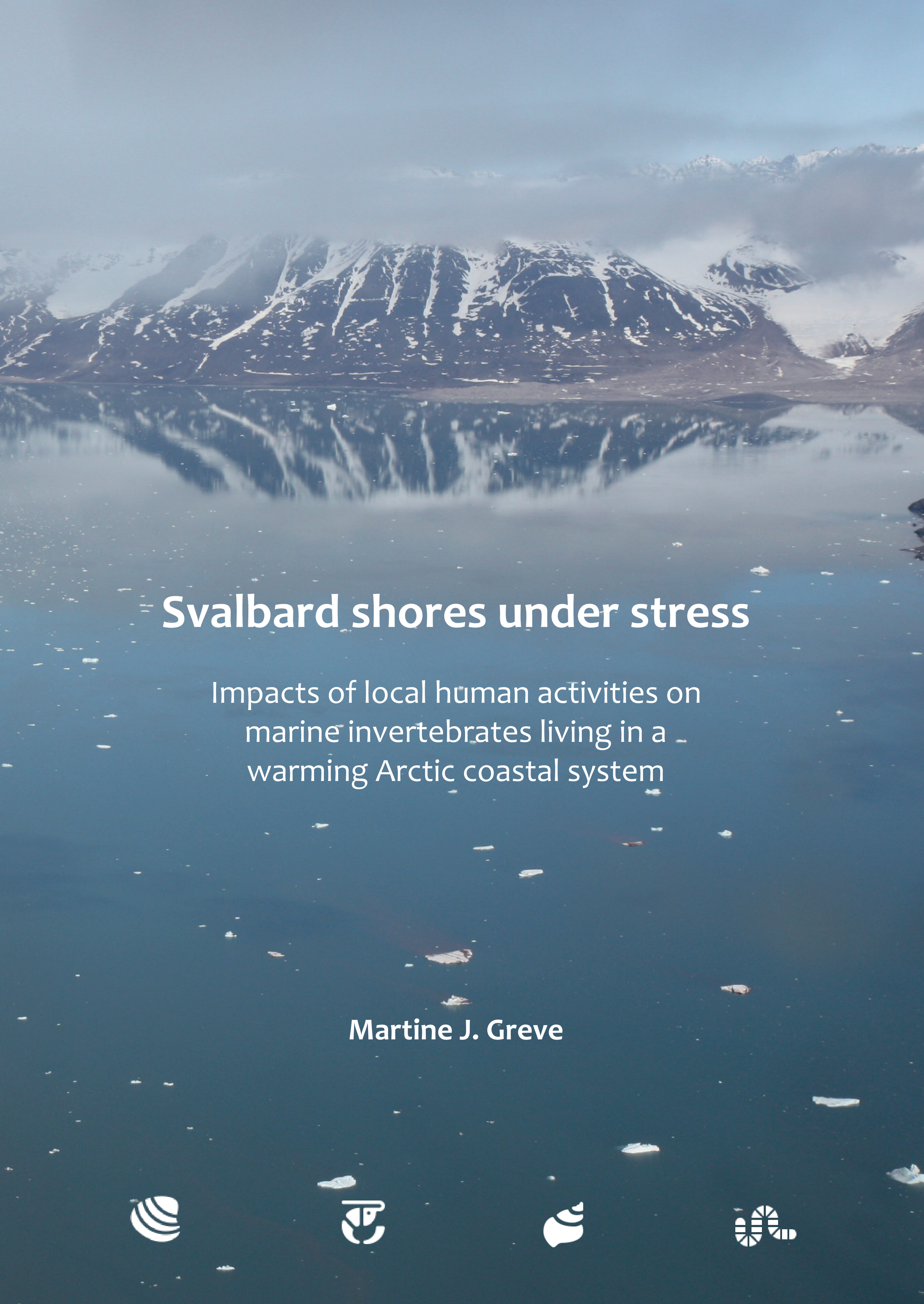

Svalbard shores under stress

Impacts of local human activities on marine invertebrates living in a warming Arctic coastal system

Martine J. Greve

Propositions

1. Contaminant-containing antifouling paints on ships are less harmful to the marine ecosystem than hull fouling.
   (this thesis)

2. Non-indigenous species will be the saviours of local Arctic ecosystems under the pressure of climate change.
   (this thesis)

3. The relevance of outliers is often overlooked in scientific research.

4. The disgust towards the consumption of putrefied meat is a matter of Westernization.

5. Arts lessons at school are a prerequisite for an innovative society.

6. Passionate laymen are more convincing in public debates than deliberate scientists.

Propositions belonging to the thesis, entitled

Svalbard shores under stress; impacts of local human activities on marine invertebrates living in a warming Arctic coastal system

Martine Johanna Greve
Wageningen, 8 June 2023

Svalbard shores under stress

Impacts of local human activities on marine invertebrates living in a warming Arctic coastal system

Martine J. Greve

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Svalbard shores under stress

Impacts of local human activities on marine invertebrates living in a warming Arctic coastal system

Martine J. Greve

Thesis
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in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
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For my parents,

Bart, Mare and Nanne

“Dreaming is a casual thing you do if you believe

And just by chance one day the lovely things will come true”

- *My Friend the Chocolate Cake, A Midlife’s Tale* -

TABLE OF CONTENT

CHAPTER 1

GENERAL INTRODUCTION

Background

The Arctic is getting hotter. Not only is the temperature rising much faster than anywhere on the globe (Rantanen et al. 2022), but ecological, economic, and geopolitical issues are also heating up among many countries that have interests in the area.

There are several definitions of 'the Arctic region' or 'the Arctic', two of which are most widely used. The first definition identifies the Arctic geographically as 'the region north of the Arctic Circle' (66°33'44" North) (fig. 1). In this region the sun does not rise during the shortest day of the year in December, and does not set during the longest day of the year in June. The second definition of the Arctic is functional: 'the region north of the 10°C isotherm in July' (fig. 1), being the area where the average air temperature is below 10°C in the warmest month (July) (AMAP 1998). By this definition the region extends further south, and is including the outflow areas of the gateways to the Arctic Ocean, on both the Atlantic and Pacific side (Fig. 1). This thesis uses the AMAP definition of the Arctic region.

The Arctic region is warming four times faster than anywhere else on the globe (Rantanen et al. 2022). As a consequence, both the extent and volume of sea ice in the Arctic Ocean is steadily decreasing (Meier et al. 2014). An earlier melt of the sea ice and later freeze of the sea water is extending the season with less or no sea-ice (Serreze & Meier 2019).

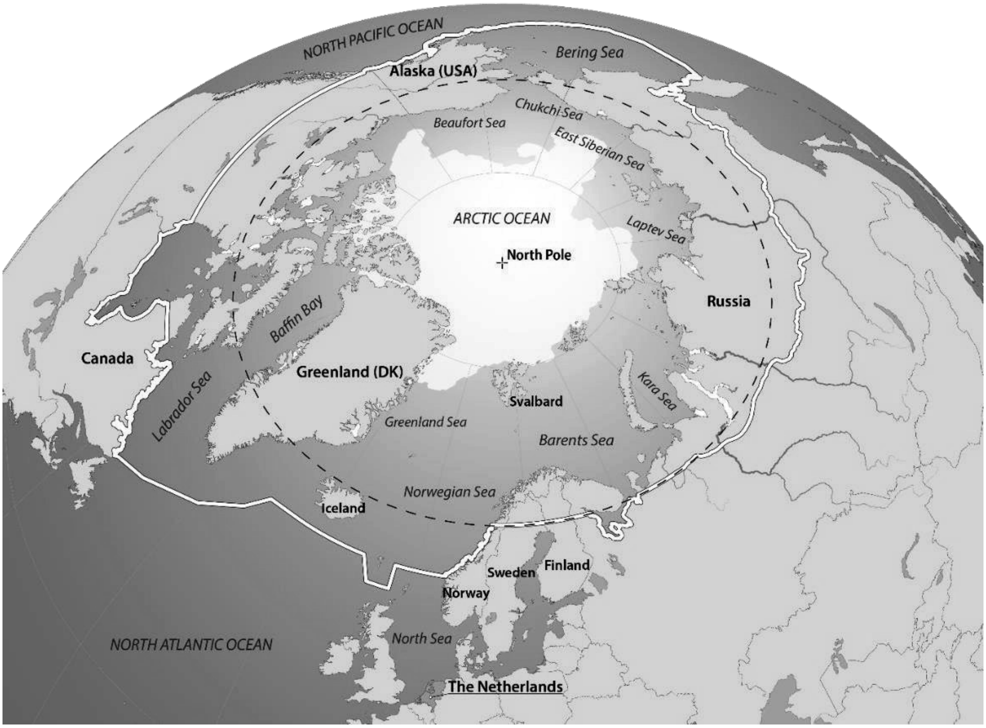


***Figure 1**. Two main definitions of the Arctic region: the area north of the Arctic Circle (dotted black line) or the area north of the 10°C July isotherm (white line based on AMAP 1998) (F. Steenhuisen).*

These changes in Arctic temperature are happening faster than forecasted in weather models (Meier et al. 2014). This is a result of processes like the increased inflow of warm and salty Atlantic Water into the Arctic Ocean and the albedo effect. The albedo effect occurs when a dark ocean surface absorbs more heat than a surface of reflecting sea-ice (Smedsrud et al. 2022, Serreze & Meier 2019, Ingvaldsen et al. 2021). Less sea ice cover therefore accelerates the heat absorption.

With the receding sea ice, the Arctic is opening up to increased human activities (Meier et al. 2014). With little or no sea ice, ships can navigate to previously inaccessible areas. Accessibility of new shipping routes increases shipping activity in the Arctic. The number of unique ships within the Arctic Polar Code Area (which closely resembles the Arctic region in the AMAP definition) increased by 25% between 2013-2019 to 1628, and the total distance sailed by ships increased with 75% to 10.7 million nautical miles in 2019 (PAME 2020). For both the increase in number and distance sailed, the large majority consisted of fishing vessels (41% in 2019), which were mainly located on the shelves of the Barents Sea and Bering Sea (PAME 2020). The number of fishing vessels grew from 535 unique ships in 2013 to 671 in 2019. Vessel transport over the Northern Sea Route, just north of the Russian coast, also increased as this is a shorter, and thereby faster and cheaper route between Asia and Europe than when travelling via the common southern route through the Suez Channel. The increasing number of tourist ships (58 ships in 2013, 73 in 2019) mostly hug the coastlines of Svalbard and Greenland. Ships that are most likely to go towards the middle of the Central Arctic Ocean are research vessels. Most shipping however is confined to the coastal shelf seas along the most common sea routes.

Shipping and other related human activities can impact the marine environment in a variety of ways. Vessels can emit contaminants and (Greenhouse) gases, be a source of household and fishery related litter, transport (non-indigenous) species on the hull of the ships or in ballast water, and generate underwater noise (Ware et al. 2014, Jalkanen et al. 2021). Unintentional oil spills may occur, for instance through bilge water emission (Jalkanen et al. 2021), as a result of rough weather or an accident. Fishing activities remove targeted and non-targeted organisms from the ecosystem and bottom trawling activities damage the sea bed (Hiddink et al. 2017). In addition to the observed higher temperatures in the Arctic, shipping imposes multiple forms of stress on Arctic nature.

In this thesis the impacts of local human activities on warming Arctic coastal systems are investigated using the archipelago of Svalbard as a case study. The marine coastal system is defined here as the area where the sea meets the land, from the intertidal part to ~50 m of depth. The system is highly influenced by inputs from fresh water (rivers, melt water), the terrestrial system and human activities that are taking place in and around the system. The shallow depths of the coastal system create a tight pelagic-benthic coupling (Søreide et al. 2020). Svalbard is experiencing the fastest warming rate on earth with 2-2.5 times the Arctic warming averages and 5-7 times the global warming averages (Isaksen et al. 2022). The archipelago is an important research hub, which means extensive knowledge is available on the coastal system (Wiencke & Hop 2016, Misund et al. 2017) and there is a substantial research infrastructure facilitating field and laboratory studies.

Characteristics of Svalbard as case study area

The archipelago of Svalbard was discovered by the Dutch explorer, Willem Barentsz, in 1596, during his quest for a northern trading sea route to East Asia. The island group consists of four larger islands (Spitsbergen, Nordaustlandet, Edgeøya and Barentsøya) and around 80 smaller islands. Svalbard is located between the latitudes of 74° to 81° (Fig. 2). The archipelago is influenced by the relatively warm West Spitsbergen Current, an extension of the Atlantic Gulf Stream that runs along the west side to the north, and a colder Arctic water mass that affects the east side of the island group (fig. 2). Therefore, most of the sea ice and land-fast ice, which is ice connected to the shores, is found on the east and northeast side of Svalbard. The formation of sea ice and land-fast ice forms in the fall and reaches its maximum extent in early spring before the melt season starts.

Svalbard has a population of around 3000 inhabitants. These are concentrated in a few settlements on the west side of Spitsbergen Island; predominantly the capital Longyearbyen, the mining town Barentsburg and the international research station Ny-Ålesund. The human population is of diverse origin, with Norwegians making up the majority. The Spitsbergen Treaty, signed in 1920, ensured that all states that signed the treaty were given equal rights to engage in commercial activities on the islands and Norway was given sovereignty (Misund et al. 2017). Svalbard does not have a historical presence of indigenous people.

The coastal marine systems of Svalbard are influenced by strong seasonality due to the full absence of light in winter and darkness in summer. Species are well adapted to the long winter and summer seasons and the periods of rapid changes in the spring and fall. As a result of this,

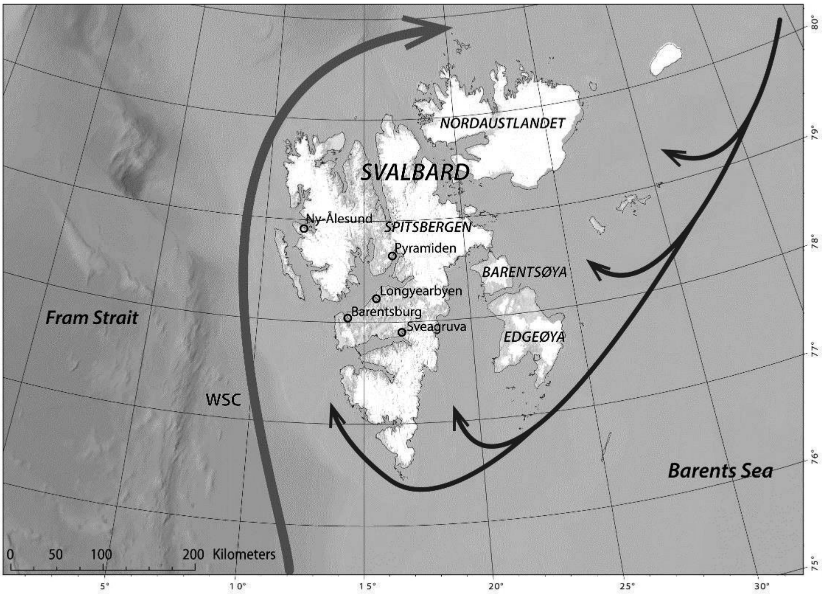


***Figure 2**. The archipelago of Svalbard and a simplified version of the predominant water masses surrounding it: the West Spitsbergen Current (WSC) containing warmer Atlantic Ocean on the west, and the Arctic current carrying colder water on the east (map: Esri, N Robinson, NCEAS, USGS, sources: Esri, USGS) (F. Steenhuisen).*

the food web relations are strongly connected (Fig. 3). Following a period of 24-hour darkness in winter, the reappearance of the sun brings back energy and warmth to the system. Even before full daylight is back, around April-May, the sea ice melts and the ice algae and phytoplankton species start to bloom. They form an important food source for pelagic zooplankton species, which mainly consists of Calanus copepods. These copepods quickly increase in number and in lipid content, providing an excellent food source for other invertebrates like amphipods, and the fish species that prey on them like polar cod. Polar cod are the preferred diet of many marine birds and seals. Benthic invertebrates living on the seafloor feed on the phytoplankton and ice algae that have sunk to the seafloor, as well as on other organic matter, such as macroalgal detritus, present in and on the sediment (Hop et al. 2002, McMahon et al. 2006, Renaud et al. 2015). The presence and abundance of benthic species in the Arctic is influenced by hydrography, depth of the sea floor, sediment properties and dynamics in food supply from the pelagic and/or sympagic (related to the sea ice) system (Søreide et al. 2013). These invertebrates form an important food source for fish, benthic feeding birds (eider duck), walruses and seals. Top predators of the Arctic marine system consist of polar bears, greenland sharks, scavenging glaucus gulls and arctic foxes.

Marine organisms in Svalbard and the wider Arctic have physiological and biological adaptations to the strong seasonality and low temperatures. They grow slower, but larger and live longer than in warmer areas (Renaud et al. 2018). Their lipid stores also differ in volume and composition compared with more temperate conditions because they much more effectively store energy when food is abundant, and provide an energy buffer for when energy is required for reproduction and food is scarce (Falk-Larsen et al. 1987). This lipid retention results in high total lipid content in Arctic invertebrates with a relatively large fraction of wax esters when compared to triacylglycerols (Sobek et al. 2010, Hünerlage et al. 2016, Ruus et al. 2021).

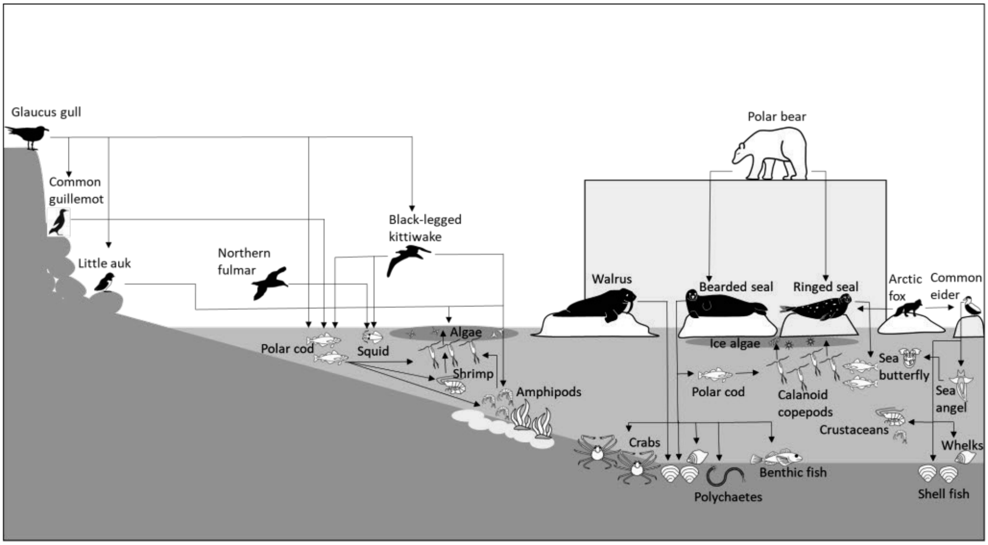


***Figure 3**. Simplified coastal food web on Svalbard (based on Gabrielsen 2009).*

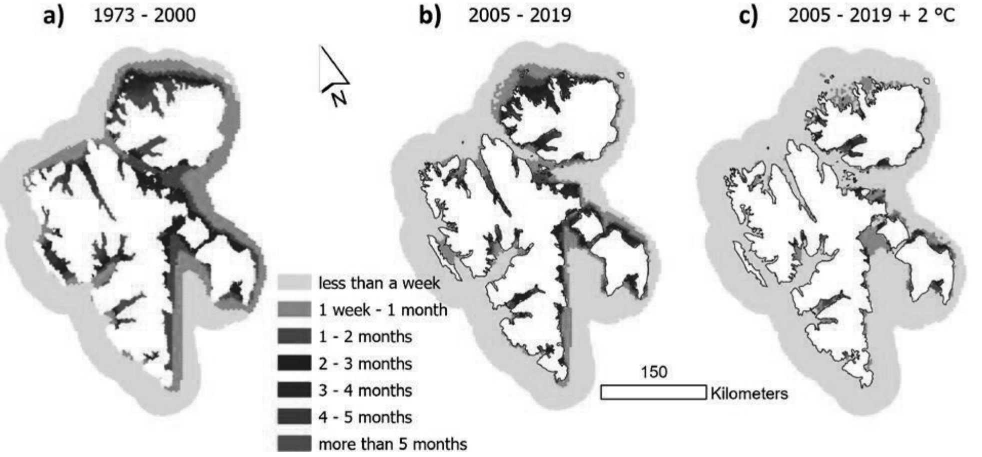


Figure 4*. The mean distribution of the land-fast ice duration in Svalbard (a) during the ice seasons of 1973—2000, (b) during the ice seasons of 2005—2019, and (c) post 2019, assuming a 2 °C increase in winter air temperature (Urbański & Litwicka 2022, © 2022 Institute of Oceanology of the Polish Academy of Sciences).*

Warming on Svalbard

The Svalbard region has experienced the highest warming rates of the entire Arctic in the last decades (Węsławski et al. 2010, Misund et al. 2016, Isaksen et al. 2022, Urbański & Litwicka 2022), and the occurrence of storms and rainfall is increasing (Nowak et al. 2021). As a result of this the extent of sea-ice and land-fast ice are declining (Fig. 4) (Urbański & Litwicka 2022, Isaksen et al. 2022). The Atlantic current is now flowing along the west coast of Svalbard to the northside and extending there to the east (Fig. 2, Polyakov et al. 2020). For instance, inflowing waters in the Kongsfjorden system in northwest Svalbard were observed to have warmed by 4–5.5 °C (Payne & Roesler, 2019). This, in combination with the presence of wind, generates enough heat north of Svalbard to prevent sea ice forming during winter time (Arntsen et al. 2019, Duarte et al. 2020, Isaksen et al. 2022). On shore, glaciers are retreating, permafrost is thawing, and unstable snow and soil packs are causing avalanches and landslides (Nowak et al. 2018, Hanssen-Bauer et al. 2019). These dangers have forced the inhabitants of Longyearbyen to relocate their houses further away from the mountains or the sea. Glacier and permafrost melt as well as increased rainfall also result in augmented run off of sediment particles, organic material, nutrients, minerals and other compounds into the marine coastal system (Nowak et al. 2021, Rudnicka-Kępa & Zaborska 2021, Hanssen-Bauer et al. 2019).

Changes in the local marine systems have been observed along the coast and in the fjords as a result of the rising temperatures and loss of sea-ice (Ingvaldsen et al. 2021, Węsławski et al. 2010). Environmental changes can lead to the opening of ecological niches that were previously exclusive to species specifically adapted to Arctic conditions. Boreal species are transported northwards with the expanding northward flowing Atlantic current (borealisation or, more specific, Atlantification). These transported species may settle and establish populations in these now more hospitable habitats (Ingvaldsen et al. 2021). Examples of this type of range extension have been found for zooplankton (Weydmann-Zwolicka et al. 2021), mussels (Leopold et al. 2019), amphipods (Grabowski et al. 2019), and fish (Misund et al. 2016). The now less frequent or absent scouring by sea-ice and fast ice in shallow areas also allows species living in the intertidal part of the coast and macrophytes, with their associated fauna, to better sustain and increase in biomass (Węsławski et al. 2010, Krause-Jensen et al. 2014, Søreide et al. 2020). Simultaneously, the number and biomass of ice-associated species is gradually declining (Węsławski et al. 2010, Ingvaldsen et al. 2021). This reduction has potential consequences for the species dependent on these organisms as a food source (McMahon et al. 2006) and the structure of the food web.

Human activities and pressures on Svalbard

Historical human activities on Svalbard include whaling and hunting in the 17th-19th centuries, and coal mining (Kotašková 2022, Hacquebord et al. 2011). Most coal mines are now abandoned, with some mining still taking place in Longyearbyen and Barentsburg. Human activities that are taking place nowadays on and around Svalbard include research, tourism and recreation, and fishing (Misund et al. 2016, Misund et al. 2017, Kotašková 2022, Hagen et al. 2012). As settlements on Svalbard are not connected by roads, travelling within the archipelago and to other areas is done mostly via ships and aircrafts. Shipping is vital for tourism and recreation, delivering supplies, research, and fishing in the area (Stocker et al. 2020, Hagen et al. 2012, Evenset & Christensen 2011). Where no piers and jetties are present, boats can anchor on the shallow coast and people go onshore using smaller (inflatable) boats. This results in a patchy presence of humans in summer time almost all around Svalbard, despite the few settlement that are located only on the west side of the archipelago (Fig. 5, Hagen et al. 2012, Evenset & Christensen 2011). The Governor of Svalbard and Association of Arctic Expedition Cruise Operators (AECO) now require tourist companies to request permission to land and to use Site Specific Guidelines for accessing these sites (AECO 2020).

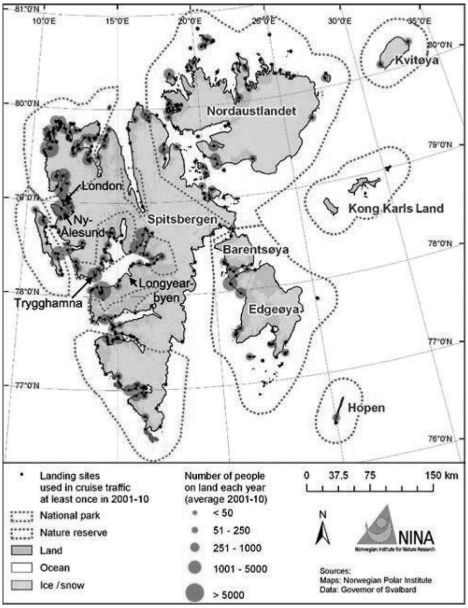


***Figure 5**. Landing sites on Svalbard used by tourist ships in the period 2001-2010, including protected areas (Hagen et al. 2012). Black dots represent the geographical distribution of landing sites used by cruise ships at least once between 2001 and 2010. Graded symbols show the average numbers of visitors during the same time period.*

Pressures from human activities in and around Svalbard can affect species, species groups and food webs along the coast. The selective extraction of fishes (Misund et al 2016), habitat destruction, the deposition of contaminating compounds (Granberg et al. 2017) and the introduction of non-indigenous species (NIS) (Ware et al. 2014, Sundet & Bakanev 2014) can eventually influence food web structure and the ecological balance. With human activities consistently increasing, a multi stressor assessment of pressures resulting from local human activities in combination with warming is needed for managing the environment of the Svalbard coast.

This thesis

In this thesis the impacts of local human activities on the Svalbard marine coastal system are presented in the context of a warming climate. An increasing number of human activities in combination with rising temperatures puts the marine coastal system under stress in multiple ways. The consequences of warming Arctic temperatures are hard to manage, so there is a need for understanding of the combined effects of pressures from local human activities. With sufficient knowledge and understanding, the critical human impacts on the Arctic might be mitigated through informed policy change and enforcement.

This study focusses on (epi)benthic species of the Svalbard coastal system, invertebrate species that live in and on the seafloor. They are key prey components in the Svalbard coastal food web, and consist also of habitat-forming species (e.g. molluscs) that can influence the diversity and functioning of the larger ecosystem (e.g. Wold et al. 2011, Leopold et al. 2019, Hjelset et al. 1999, Vollen et al. 2004, Renaud et al. 2012). Several representatives of these groups (shellfish, amphipods and worms) are often used as bioindicators for assessing the impact of warming and of pressures such as the introduction of contaminants (e.g. Drewnik et al. 2017, Szczybelski et al. 2016, Szczybelski et al. 2019). (Epi)benthic species live on a fixed location during most of their lifespan and are therefore most prone to be affected by and reflect local pressures. This makes this group very suitable for the topic of this thesis.

A human activity – pressure – ecosystem approach is applied to structure the impact assessment of local human activities on invertebrate species living in the warming Arctic coastal system of Svalbard. Similar approaches have been used in multiple studies and within international organisations as an environmental risk assessment tool (Hansen et al. 2022, ICES 2022, Borgwardt et al. 2019). It provides a clear structure of how to link human activities with their (potential) pressures and how these activities link to the different ecosystem components.

Firstly, an overview of the main human activities on and around the Svalbard coast is drafted (Fig. 6). Shipping, being the most dominant activity in the area, can be linked to the pressures of contaminants, introduction of non-indigenous species (NIS), marine litter, noise and nutrient enrichment (Fig. 6) (Jalkanen et al. 2021). As shipping is an important part of several human activities that are taking place on Svalbard (fishing, tourism, research), the environmental pressures resulting from shipping include the shipping part of all these other human activities. Besides this, fisheries may exert additional pressures such as removal of target species of the ecosystem, bycatch, physical disturbance, habitat loss and sedimentation, whereas tourism and recreation may cause disturbance (Misund et al. 2016, Hagen et al. 2012, Evenset & Christensen 2011). Research activities can lead to pressures that are similar to fisheries and tourism activities, but usually with a lower intensity.

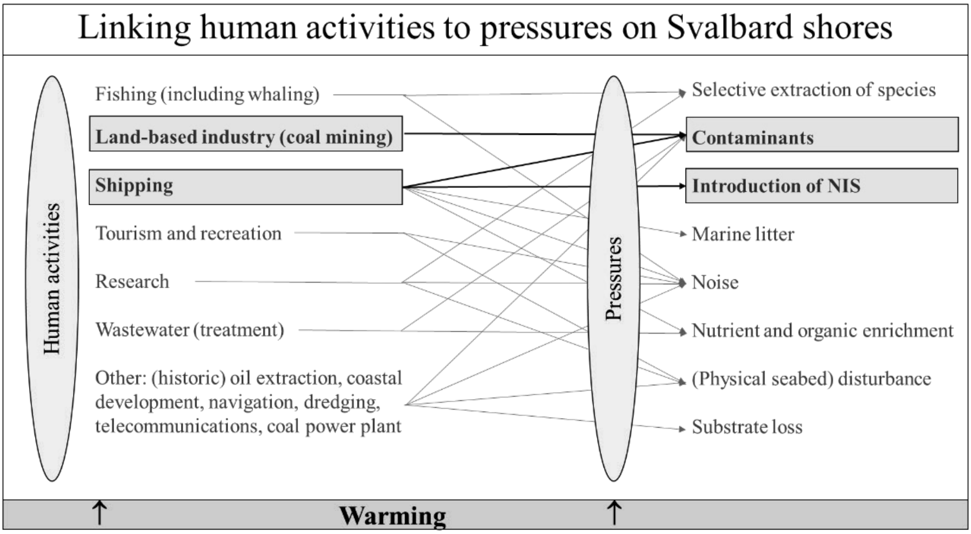


Figure 6*. Identification of relevant human activities for the Svalbard coast and the selection of human activities and pressures that are studied in this thesis (highlighted rectangles).*

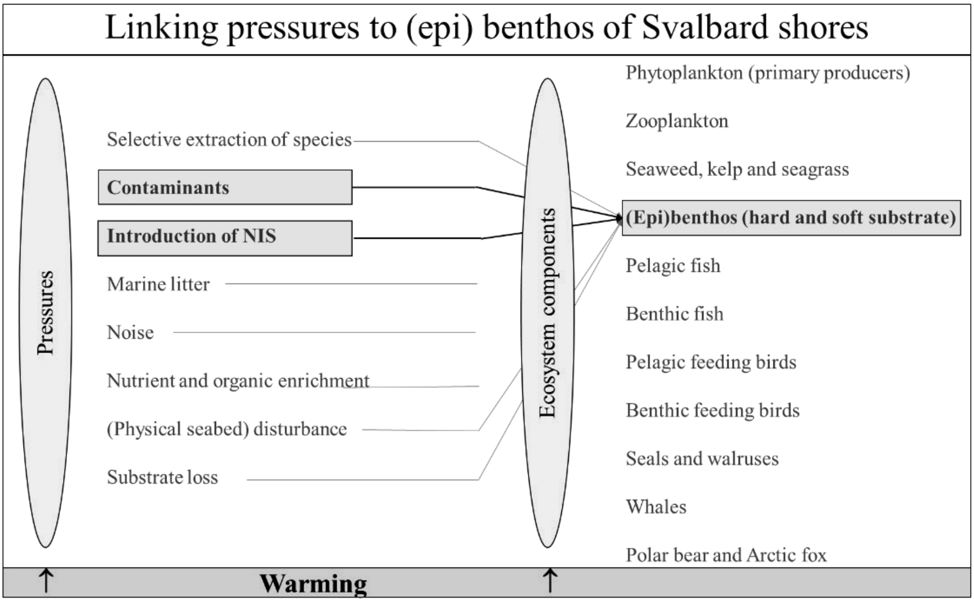


Figure 7*. Identification of the main pressures that may affect the (epi)benthic species living in and on hard and soft substrates of the Svalbard coast, and the selection of the pressures and ecosystem component that are studied in this thesis (highlighted rectangles).*

Of the pressures resulting from shipping in Svalbard waters, benthic communities were considered to have a medium vulnerability for contaminants pollution (high confidence) and for pollution from oil (high confidence) in a recent vulnerability assessment for Svalbard waters (Hansen et al. 2022) (Fig. 7). Other pressures that impact (epi)benthic species were described to be removal of target species (in this case shrimp), bycatch, physical disturbance and habitat loss (Hansen et al. 2022). These are all pressures resulting from fisheries, which seems to be taking place more in deeper waters and are therefore less likely to occur directly along the Svalbard coast. Another pressure resulting from shipping that can impact (epi)benthic invertebrates are the NIS. These, however, were not identified as potential pressure for benthic communities in the vulnerability assessment (Hansen et al. 2022). Although the reasoning behind this is not known, it may have been omitted because of a stronger focus on the deeper waters surrounding Svalbard. NIS however may very well affect (epi)benthic invertebrates as has been shown in other areas (Katsanevakis et al. 2014). Therefore, both the pressures of contaminants and NIS as a result of shipping are selected for this thesis.

Besides ships, contaminants can also reach the shore through run-off from land-based industry like coal mining, from (historic) oil extraction and from (non-treated) waste water (Granberg et al. 2017). To take into account the importance of a land-based source for the input of contaminants into the coastal environment, (historic) coal mines are also included in the assessment.

This ultimately results in the framework for this thesis (Fig. 8). All parts of the framework (human activities, pressures and ecosystem components) can be influenced by warming temperatures.

Aim of the research

The aim of the thesis is to assess the extent of current and future pressures related to local human activities on (epi)benthic marine invertebrates living in warming Arctic coastal systems, using Svalbard as a case study.

This aim is translated into the following research objectives:

1. To assess the potential impact of (current and foreseeable) contaminants originating from local human activities on (epi)benthic invertebrates living in the Svalbard coastal system

2. To assess the potential impact of (current and foreseeable) non-indigenous species related to local human activities on (epi)benthic invertebrates living in the Svalbard coastal system

Study approach

This thesis presents three studies on contaminants related to mining and shipping, and one on NIS (Fig. 9).

Both mercury (Hg) and Polycyclic Aromatic Hydrocarbons (PAHs) are naturally occurring compounds. They are concentrated in fossil fuels such as oil and coal, and can therefore be used as tracer for coal mine activities on land (AMAP/UN Environment 2019). Mercury is known

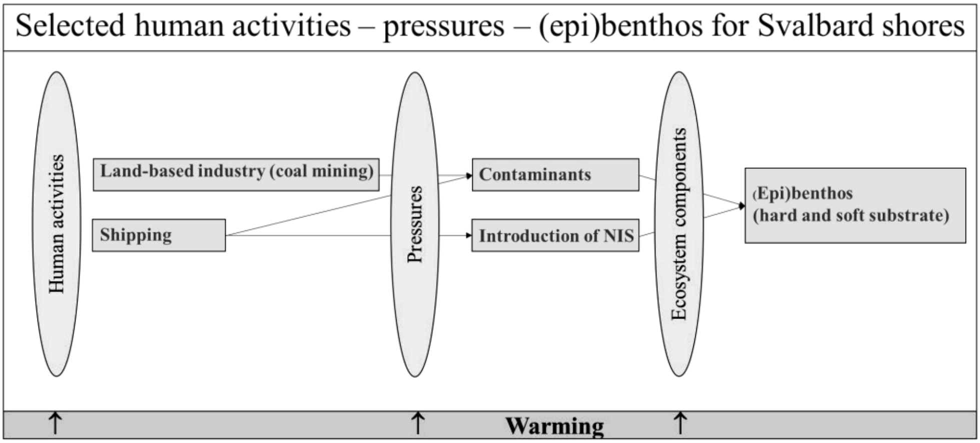


Figure 8. *The refined structure of the human activity – pressure – ecosystem component approach to be addressed in this thesis.*

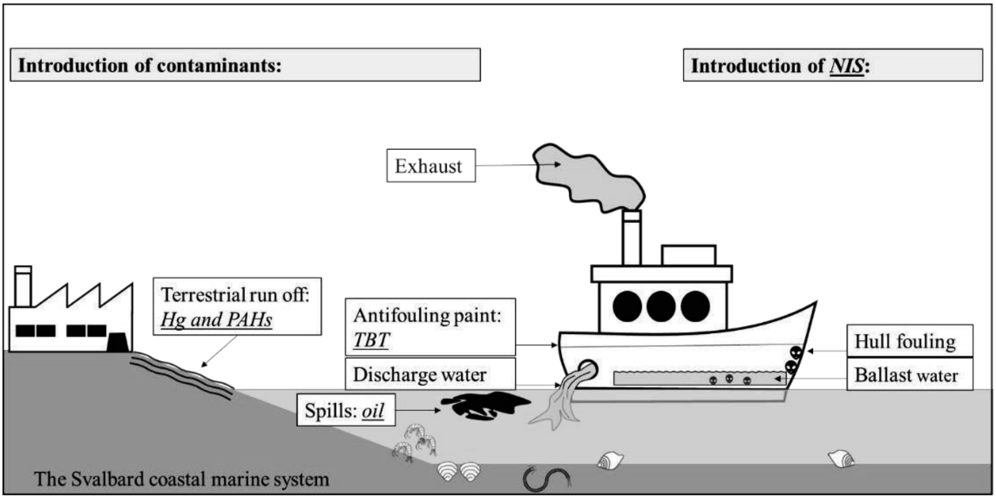


Figure 9. *The local human activities and their pressures of which the effects are addressed in this thesis: shipping and coal mining and their introductions of contaminants and NIS in the coastal system. Each underlined item in italic represents a chapter in the thesis.*

for its biomagnification potential as it cannot be degraded and the methylmercury form is especially prone to accumulate and affect species in the top of the food web (Dietz et al. 2013). Mercury has been found in high concentrations in the Arctic marine food webs, such as a few seabird species, beluga whale (Delphinapterus leucas), seals and polar bears (Ursus maritimus) (Dietz et al. 2013, Ruus et al. 2015). PAHs are less prone to bioaccumulation because several, especially vertebrate, species have the ability to biotransform PAHs (Hauck et al. 2007, De Laender et al. 2011a, Szczybelski et al. 2019). The resulting PAH metabolites, however, are also known to induce for instance cancer and oxidative stress (Van Schanke et al. 2001, Wallace et al. 2020).

Tributyltin (TBT) originates from antifouling paints that are used on ship hulls. Even though the International Maritime Organisation (IMO) has banned the use and application of TBT on ships, not all flag states have ratified this ban and there are still ships using this type of paints to prevent fouling. In addition, TBT hardly degrades, so historical pollution remains present for decades before it becomes less available by burial in deeper sediment layers. Chronic exposure to TBT has been linked to well established adverse effects that are especially found in harbours and shipping lanes (Gibbs & Bryan, 1986, Ten Hallers-Tjabbes et al. 1994, Mensink et al. 1996). The most well-known effect of TBT is the development of male characteristics in female marine snails, a hormone disruption phenomenon called ‘imposex’ (Gibbs & Bryan, 1986). Organotin compounds, such as TBT, have low biomagnification potential, especially higher up in the food web (Veltman et al. 2006), although some biomagnification has been found in (sub)Arctic cetaceans (Kucklick & Ellisor 2019).

Accidental release of oil, such as in the case of an oil spill, frequently results in a high concentration of oil in a small area, enhancing the risk of acute smothering and toxic effects. Oils consist of a combination of lighter hydrocarbons and higher-molecular weight compounds, with the exact composition depending on the origin of the oil, the way it has been processed and the weathering stage (Redman & Parkerton 2015). The acute toxicity varies, mainly depending on how much and which fraction of the oil components dissolve in the water. The general toxicity of oil consists of narcotic effects (De Laender et al. 2011b).

The (epi)benthic invertebrate groups that will be represented in the contaminant studies include worms (*Nephtys* sp. and *Polycirrus* sp), shellfish (e.g. *Astarte borealis* and *Macoma calcarea*), amphipods (*Gammarus* sp.), and whelks (*Buccinum* sp.) (Fig. 10).

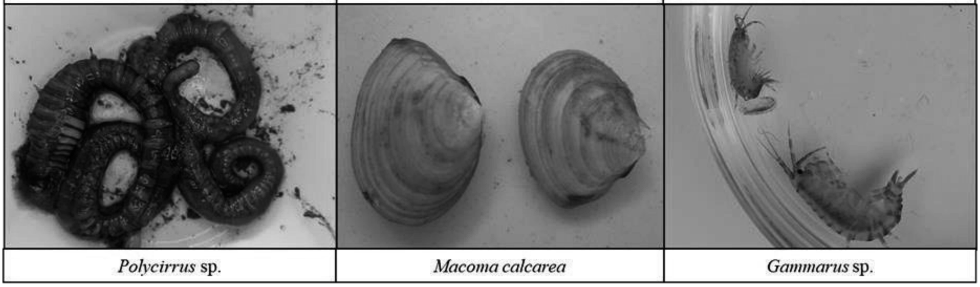
Non-indigenous species are defined in this thesis as ‘species that have been intentionally or unintentionally introduced outside of their native range as a consequence of human activity’ (Convention on Biological Diversity, in Cook et al. 2016). Secondary introduction of NIS may occur through range extension from these areas, where they have been first introduced through human activities. Species that naturally extend their living range because of climate change are not considered to be NIS.

In the NIS study, the emphasis is placed on the identification of marine non-indigenous invertebrate species in Svalbard coastal waters. The most likely route of arrival of NIS on Svalbard is through hull fouling and discharge of ballast water (Ware et al. 2014). NIS can have significant impacts on the local ecosystem, through changing community structures, altering ecosystem processes or by reef building organisms changing the physical environment; all potentially leading to impacts on the local environment and economy (Bax et al. 2003; Molnar et al. 2008), that could be considered negative, neutral and/or positive.

Nephtys sp.

Astarte borealis

Buccinum sp.



Polycirrus sp.

Macoma calcarea

Gammarus sp.

***Figure 10**. Overview of representatives of the (epi)benthic invertebrate groups of worms, shellfish, marine whelks and amphipods that are included in this thesis (Photos M. van den Heuvel-Greve & F. Steenhuisen).*

Outline of the thesis

Objective 1 on the impact of contaminants is addressed in **Chapter 2**, **3** and **4**. In **Chapter 2** the exposure radius of contaminants coming from a historic mine is studied. This provides direct information on the link between contaminant presence originating from a land-based source and levels in coastal marine invertebrates. To trace the exposure radius, two groups of contaminants were used as indicators: mercury (Hg) and Polycyclic Aromatic Hydrocarbons (PAHs). Concentrations of both contaminants were determined in sediment samples from melt water streams of the historic mine near the research station of Ny-Ålesund, and the adjacent unexposed marine system. Several shellfish species (*A. borealis*, *Serripes groenlandicus* and *M. calcarea*) and worm species (*Nephtys* sp. and *Polycirrus* sp.) were collected at the same marine location to assess the actual exposure to Hg and PAHs. To enhance the understanding of the exposure radius from the historical coal mine, several reference locations were included in the study: one located to the east of the mine further into the fjord system, one near the harbour of Ny-Ålesund (positive control) and one at the western outskirts of the Kongsfjorden. Both PAH ratios and the relationship between Hg and PAH concentration were studied to assess whether PAHs can be used to identify coal mines as source of Hg.

In **Chapter 4** information on the current pressure of contaminants in harbours and their potential impact on an invertebrate species was assessed. The concentrations of organotin compounds originating from antifouling paints on ships were analysed in sediment from the five harbours of Svalbard. Based on ratios of individual organotin compounds (Monobutyltin (MBT), Dibutyltin (DBT) and Tributyltin (TBT)) the Butyltin Degradation Index (BDI) was determined, indicating how recent the TBT input into the harbour system was. Marine whelks (*Buccinum* sp.) were collected in one of the harbours to assess effects of TBT exposure through analysing the stages of imposex. Some additional contaminant groups were assessed in sediment samples from the Kongsfjorden to further reveal the level of contamination in the coastal system.

Chapter 3 assesses the potential future risk of oil as a contaminant and its acute effects on the keystone invertebrate Arctic *Gammarus* sp. Information of acute toxicity of oil on Arctic invertebrate species is still relatively sparse compared to temperate regions, and translating temperate data to the Arctic system has been done through modelling or comparison studies with different species and/or test temperatures. In this study the influence of temperature on the acute toxicity (LC50) and Critical Body Residue (CBR) in the temperate amphipod, *Gammarus locusta*, was determined. To obtain this, temperate gammarids were exposed to the Water Accommodated Fraction (WAF) of a Distillate Marine Grade A (DMA) oil at three different temperatures (4°C, 12°C, 20°C). The Arctic *Gammarus* sp. was then exposed at 4°C under the same test conditions and the CBR measured. This enabled a direct comparison between the sensitivity of temperate and Arctic gammarids to oil, and provides further information on the influence of temperature on the characteristics of oil and on the behaviour, biology and physiology of the temperate test species.

Objective 2 on non-indigenous species (NIS) is addressed in **Chapter 5**, in which the current presence of NIS was studied in the harbour of Ny-Ålesund. A literature search was conducted to determine which NIS had been previously reported for Svalbard. The same sampling locations as in Chapter 2 were used for this study to collect sediment samples to which an innovative metabarcoding method was applied to identify species in the sediment based on their DNA. Two DNA markers were used to analyse the environmental DNA samples; the 18S marker as wide screening for eukaryotes and the COI marker to enable identification of the animal kingdom to a species level. Additionally, individual species were collected and identified for the production of new barcodes to feed the online international DNA library.

In **Chapter 6** the results of all studies are discussed in relation to the objectives in order to fulfil the main aim of the thesis. The chapter closes with viewpoints on anthropogenic stressors for management, recommendations for future research and monitoring, and perspectives for the wider Arctic,.

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CHAPTER 2

EXPOSURE RADIUS OF A LOCAL MINE IN AN ARCTIC COASTAL SYSTEM; CORRELATION BETWEEN PAHs AND MERCURY AS A MARKER FOR A LOCAL MERCURY SOURCE

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Abstract

Mercury in the Arctic originates from emissions and releases at lower latitudes and, to a lesser extent, from local and regional sources. The relationship between mercury (Hg) and polycyclic aromatic hydrocarbons (PAHs) in sediment can be applied as an indicator of the mercury source. This research examines the Hg contamination gradient from a land-based coal mine to the surrounding coastal environment to quantify the impact of local sources. Total mercury and PAH (Σ14PAH) were measured in terrestrial and marine sediments as well as in marine biota. Samples were collected at the mine and two reference sites. Mercury and Σ14PAH concentrations in samples collected at the mine site were significantly higher than those at the reference sites. This was also found in the biota samples, although less pronounced. This work addresses the complexities of interpreting data concerning very low contaminant levels in a relatively pristine environment. A clear correlation between PAH and Hg concentration in sediment was found, although a large number of samples had levels below detection limits. PAH profiles, hierarchical clustering, and molecular diagnostic ratios provided further insight into the origin of PAHs and Hg, showing that signatures in sediments from the nearest reference site were more similar to the mine, which was not the case for the other reference site. The observed exposure radius from the mine was small and diluted from land to water to marine biota. Due to low contamination levels and variable PAH profiles, marine biota was less suitable for tracing the exposure radius for this local land-based Hg source. With an expected increase in mobility and availability of contaminants in the warming Arctic, changes in input of PAHs and Hg from land-based sources to the marine system need close monitoring.

Introduction

Mercury (Hg) is a naturally occurring compound with toxic characteristics, posing risk to both humans and the environment (AMAP, 1998; AMAP/UN Environment, 2019, Gustin et al., 2016; Dietz et al., 2013). It can be found in highly variable concentrations in minerals and in fossil fuels, with coal in particular (AMAP/UN Environment, 2019). Mercury in coal originates from geogenic sources, having been assimilated from the atmosphere by the original plant species that formed the basis of coal. Mercury is emitted globally into the air and water through various natural and anthropogenic activities. For most of the Arctic with very low emissions from industrial activity (Steenhuisen & Wilson, 2019), the main source of (anthropogenic) mercury is long range atmospheric transport (AMAP, 1998; AMAP, 2011; AMAP/UN Environment, 2019). In some areas of the Arctic however industrial activities such as mining and primary metal production occur, which may introduce mercury into the environment (AMAP, 2011; AMAP/UN Environment, 2019; Steenhuisen/Wilson, 2019).

Svalbard is an Arctic archipelago, situated north of Norway. On Svalbard local sources of mercury can be attributed to the coal mining industry (both active and historical), the use of coal in two power plants and – to a lesser extent – waste and waste handling from settlements and shipping. While most mines on Svalbard have ceased or suspended operations (Ny-Ålesund, Pyramiden, Grumantbyen and most recently Svea), two mines are currently still in operation in 2020 (Longyearbyen and Barentsburg). At all sites, coal, mine tailings and (historic) industrial waste can be found on land. The historic coal mine of Ny-Ålesund is located at the southern shore of Kongsfjorden in the northwest of Svalbard. The mine was relatively small compared to the other mines on Svalbard and ceased operations in 1963 (Auen, 2016, Hanoa, 2016). Nevertheless, it has been related to mercury contamination as concentrations in the proximity of the mining area were reported to be elevated in soil, plants and biota when compared to reference sites (Van den Brink et al., 2018).

To assess the origin of mercury in the environment, Polycyclic Aromatic Hydrocarbons (PAHs) have been applied in earlier studies (Liu et al., 2017), as mercury and PAHs often have common sources and pathways in the environment (Parsons et al., 2014). In the Yangtze river system, a moderate correlation was found between mercury and PAHs in sediments. This is however a complex system due to the fact that multiple sources of both PAHs and Hg are present (Liu et al. 2017). In the Kongsfjorden area sources of mercury and PAHs are limited. Mercury and PAHs in soil and sediments in the mine area can originate from (1) coal present in the mine waste (through chemical (leaching) processes), (2) airborne deposition from local sources such as (historic) coal use, waste burning, diesel generators and shipping, and (3) from atmospheric deposition from long range transport of sources in other parts of Svalbard and further away. Coal has specific PAH profiles depending on its origin, with phenanthrene as the most dominant indicator compound (Achten & Hofmann, 2009).

Contaminants in coal mining piles and tailings can be transported from the original site to the marine ecosystem by fresh water runoff and melt water streams, both dissolved and bound to suspended particles. Parameters such as the temperature regime and the presence of permafrost, can influence the chemical processes such as leaching and biodegradation of contaminants, and the subsequent run off (Colombo et al., 2018). The melt season on Svalbard starts around May/June and the ground is completely snow-free during summer (Maturilli et al., 2013). Current changes in the annual snow melt regime and permafrost leads to an earlier and longer snow free period and an increase in thickness of the active layer (Boike et al., 2018, Lameris et al., 2019).

This is likely to affect the pathways through which mercury and PAHs enter and disperse into the terrestrial and marine environment.

This research examines the exposure gradient from a land-based mercury source, the historic Ny-Ålesund coal mine, to the surrounding environment. PAH profiles were used as an indicator to assess the exposure radius of Hg in this pristine Arctic environment. Mercury and PAH concentrations were therefore measured in sediments of meltwater streams in the mine area and at two reference locations, as well as in marine sediments and marine biota near the outflow of the meltwater streams. This study helps to infer how local sources contribute to mercury and PAH pollution in Svalbard (also in the context of long-range Hg transports from remote sources), and considers the complexities of interpreting data concerning very low contaminant levels in a relatively pristine environment.

Materials and methods

Sampling locations

To estimate the magnitude and extent of local Hg sources, a variety of samples were collected in July 2017 at three sampling locations in the Kongsfjorden and Krossfjorden in northwest Svalbard (Fig. 1). The first one consisted of the historic coal mine which is located southeast of Ny-Ålesund. Two other areas served as Arctic reference sites. One is situated further to the inner fjord: the area below the Austre-Lovénbreen, approximately 6 km south-east of Ny-Ålesund and halfway towards the current (2017) glacier front of the Kronebreen to the east. Initially the area just north of Kapp Guissez in the Krossfjorden, approximately 16 km northwest of Ny-Ålesund, was selected as the second reference location. However, due to the lack of suitable sediments for sampling it was decided to shift to another location nearby. An alternative reference location was found below the Willebreen in Ebeltofthamna, located roughly at the same distance from Ny-Ålesund and with soil conditions better matching those at the other sampling sites.

At all sites, terrestrial sediment samples were taken near or in (dried up) meltwater streams. Marine sediment samples were collected near the outflow of the same streams just outside the tidal zone at a depth between ~12 - 52 meter. The marine samples in the Krossfjorden were taken just outside Ebeltofthamna which was considered too shallow to provide samples comparable to the other locations. The marine samples near the Ny-Ålesund mine were taken outside the shallow area near the coast for the same reason. All marine sampling locations were outside the Kongsfjorden inner zone, which is described as being heavily influenced by large tidal glaciers, with a high influx of fresh water and glacial sediments (Hop et al. 2002, Svendsen et al. 2002, Duarte et al. 2019).

To infer the potential impacts of shipping on PAH concentrations in sediments, additional reference samples were collected at the Antarctic Peninsula in March/April 2019, a remote location without mercury sources nearby but with similar general environmental conditions (e.g. climate) as Svalbard. The samples were collected on Danco Island and Halfmoon Island (see map 1 in Supporting Information - SI). These locations are all frequently visited by ships comparable (and partly the same) to the ones which operate in Krossfjorden/Kongsfjorden area during the boreal summer.

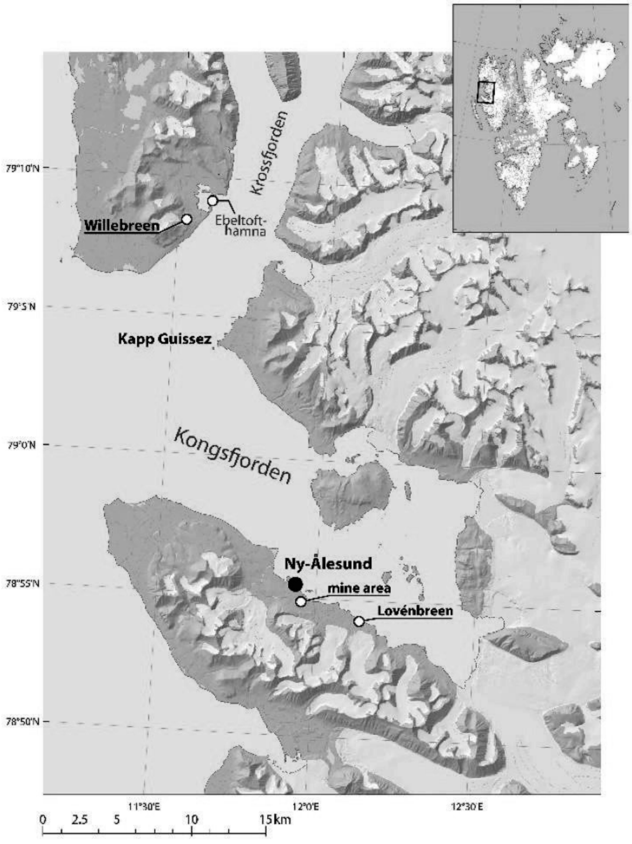

Figure 1*. Sampling locations for this study. Terrestrial and marine samples collected in July 2017 in the Kongsfjorden and Krossfjorden, Svalbard.*

Sediment collection

Sediment samples were collected from the upper 5 cm of the sediment using a plastic corer (ø4cm PVC). Two samples were stored for chemical analysis; one sample for total mercury analysis in a 135-ml plastic jar, and one sample for PAH analysis in a 200-ml glass jar. Four sediments samples were collected per melt stream along a transect starting from upstream towards the shoreline. At the mine location, additional samples were collected upstream from the mine area, where the source of contamination was presumed to be below this point. Samples were stored at -20°C within a few hours after collection until further processing. Marine sediments were collected using a Van Veen grab. Sediment samples were collected from the upper 5 cm of the sediment grab using a plastic corer and stored as specified above. In total 37 terrestrial and 24 marine sediment samples were collected (see Table 2 in the SI). A piece of coal was collected at the Ny-Ålesund coal mine location to assess the Hg and PAH concentrations.

The sample was stored in a plastic container at room temperature until further processing and homogenized prior to analysis.

Biological samples

The local Hg and PAH contamination was also assessed in organisms to determine whether exposure to contaminants of the mine can be observed at the level of marine benthic organisms. Therefore, two marine worm species (*Nephtys* sp. and *Polycirrus* sp.) and three species of marine bivalves (*Astarte borealis*, *Serripes groenlandicus* and *Macoma calcarea*) were collected from the marine sediment samples. Sediment samples were sieved on-board with ambient seawater and the specimens were collected from the sieve. The number of collected species and total volume was not sufficient to analyze both Hg and PAHs at all sites. Therefore, a selection of biota samples was made for both Hg and PAH analysis (see Tables 3 and 4 in SI). Bivalve samples were kept 24h in aerated water at 4°C to depurate their gut prior to storage (Chapman 2016). Samples were stored at -20°C after collection until further processing.

Chemical analysis

Total mercury

Total mercury was analyzed in sediment and biota using an ISO17025:2005 accredited method based on US EPA 7473 (US EPA, 2007). In short, samples were dried and decomposed at high temperature. With a supply of oxygen, the volatiles were led to a catalyst tube, where oxidation took place and halogens, nitrogen and Sulphur-oxides were removed. The residual destruction products were then led to an amalgamator, which converted mercury compounds into metallic mercury. The level of mercury was quantified using a flameless atomic absorption spectrometer. The samples were measured against a calibration curve, which was prepared from a certified standard (TraceCERT) obtained from Fluka and analyzed in the same manner. A certified reference material, Oyster Tissue NIST1566B, was measured with each set of samples with satisfactory results. The method Limit of detection (LOD) and limit of quantification (LOQ) were 0.15 and 0.3 μg/kg respectively. The LOQ could differ slightly between samples depending on sample intake with an average LOQ of 0.54 μg/kg. Samples were not corrected for blanks but blanks were required to be below LOD.

PAH

PAHs were analyzed in sediment and biota according to De Boer et al. (2001) and Van den Heuvel-Greve et al. (2016). The procedure involved PAH extraction from the sediments with soxhlet (hexane/acetone 1:1). The extract was concentrated to 10 ml, cleaned over a silica gel-aluminium oxide column and, after addition of 1 ml of acetonitril, concentrated by evaporation to 1 ml of acetonitril. The PAH levels in the acetonitril solution were analysed by HPLC equipped with fluorescence detection. The following 14 PAHs were included in the Σ14PAH: Acenaphthene, Fluorene, Anthracene, Phenanthrene, Fluoranthene, Pyrene, Benzo(a)anthracene, Chrysene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Dibenz(a,h)anthracene, Indeno(1,2,3-cd)pyrene, and Benzo(g,h,i)perylene (see Table 1 in SI for abbreviations).

The limit of quantification (LOQ) was defined as <1.5 times the method blank, or the lowest used calibration point (whichever was highest). Samples were not corrected for blanks. Certified reference material NIST2974a (freeze dried mussel tissue) and method blanks were analysed with each set of samples. All results for blanks and reference materials were within normal limits. Recoveries were between 80-120%. Method LOD was defined as 0.5×LOQ.

Dry weight

Dry weight was determined according to ISO 17025:2005 accredited SOP 2.10.3.011 "Animal tissue: Determination of the level of moisture". Samples of approximately 1 g were dried for 3 hours at 105 °C after which the dry weight was determined gravimetrically.

Organic matter

Total Organic Carbon (TOC) content was determined gravimetrically as loss on ignition after being exposed for 12h at 550°C.

Lipid weight

The lipid-level determination was modified from the Bligh & Dyer (B&D) method (De Boer, 1988). Samples were extracted three times with a mix of chloroform, methanol and demineralized water. Lipid level was determined by weighing the residue after evaporation of the solvent.

Data treatment

Hierarchical clustering was applied to explore the differences in individual PAH concentrations between the sediment samples in order to examine the dissimilarities between the samples. The dendrograms were based on the euclidean pairwise distance metric and the 'complete' linkage algorithm (Smoliński et al., 2012, Smoliński et al., 2002).

Contaminant sediment concentrations were tested using a one-way ANOVA and a Tukey multiple pairwise comparison. ANOVA assumption on homogeneity of variance and normality were met for most datasets. The data for Σ14PAH concentrations in terrestrial sediments did not fully meet the normality criterium. As the Kruskal-Wallis test revealed similar results as the Tukey multiple pairwise comparison, the Tukey test was considered valid.

To further identify PAH sources, the PAH Molecular Diagnostic Ratios (MDR) were applied (Tobizewski et al., 2012). This technique has proven its value to identify PAH sources in areas with moderate or high contamination levels. The high number of measurements close to, or below the detection limit, severely restricted the potential of MDR. Only two MDRs could be properly calculated: fluoranthene/pyrene (calculated as FLA/(FLA + PYR)) and ∑LMW/∑HMW, where ∑LMW is the sum of two and three-ring PAHs and ∑HMW is the sum of four and five-ring PAHs (Zhang et al., 2008). To visualize the relative uncertainty of the calculated PAH ratios, the markers in the ratio plots were scaled based on the number of measurements below DL, ranging from 0 (all 4 measurements <dl) to 4 (all 4 measurements >dl). The relative uncertainty per axis was indicated by horizontal and vertical markers which are scaled 0-2.

Biota–sediment accumulation factors (BSAF) for individual PAHs were calculated for *M. calcarea* as $BSAF = (C_{org}/f_{LIP})/(C_{sed}/f_{TOC})$, with C_{org} being the chemical concentration in the organism in wet weight (ww;µg/kg), C_{sed} the chemical concentration in sediment in dry weight (dw;µg/kg), f_{LIP} the fraction of lipids (%) in the organism based on wet weight (ww), f_{TOC} the fraction of TOC (%) in sediment based on dry weight (dw) (Ankley et al., 1992). For sediment the average value per location (Ny-Ålesund mine, Lovénbreen and Krossfjorden) was used for both individual PAH compounds and TOC %. The BSAF for Hg was calculated based on dry weight concentrations in both organism and sediment (C_{org}/C_{sed}).

Results and discussion

Sediment Hg and PAH concentrations

Terrestrial sediments

The highest mercury concentrations were found in sediments from meltwater streams running through the Ny-Ålesund mine area (Fig. 2 and 3). Samples contained on average 58 ±52 µg/kg dw Hg (range 7-216 µg/kg dw) and were significantly higher ($p<0.05$) than the concentration found at the Lovénbreen and Krossfjorden reference sites (<5 µg/kg dw) (Fig. 3). The Hg concentrations in the meltwater stream sediments in the mine correspond well with concentrations in soil samples (40-900 µg/kg dw) from the center of the mine waste area (Van den Brink et al., 2018). This implies that the central area of the mine contains even higher Hg levels than the sediments collected in the meltwater streams running through the mine.

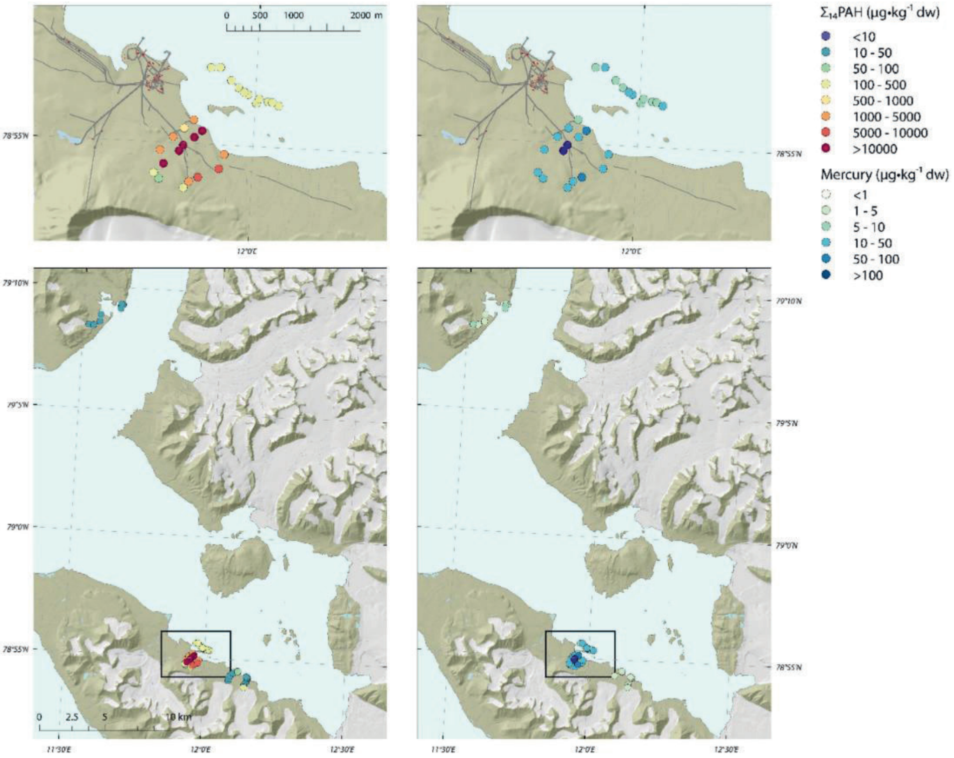

***Figure 2**. Σ14PAH (left) and Mercury (Hg) (right) concentrations in terrestrial and marine sediments of Kongsfjorden and Krossfjorden, sampled in July 2017. PAH concentrations below detection limit were included in this graph as 0.5*dl.*

Mercury concentrations in three samples collected at the 'clean, unaffected' upstream area above the mine contained 17-26 μg/kg dw Hg, a factor ten lower than the highest concentrations in the mine area and a factor two lower than the average concentration in the mine. The mercury observed in these samples most likely originated mainly from long-range atmospheric transport, and local air emissions (i.e. local use of coal and emissions from shipping) and other deposition processes. Hg deposition by long-range transport in the study area was estimated to be 4-8 μg/m2/y (modelled for year 2015, AMAP/UN Environment, 2019).

The mercury concentrations in the Antarctic samples (range 12-73 μg/kg dw, average 35 μg/kg dw) were higher than the reference locations on Svalbard and within the range of the concentrations found in the mine (Fig. 3). This is probably due to local geology, although local use of fossil fuels by research stations cannot be excluded at this point.

The Hg levels in terrestrial sediments found at the reference sites of this study were 2.6 ±1.8 (0.07- 5) μg/kg dw, based on the samples taken at Krossfjorden (n=4) and Lovénbreen (n=8). These are a result of the (highly) variable Hg presence in the local geology and soil composition as well as from Hg deposition from long range atmospheric transport. Several studies present average (background) Hg concentrations in soil on Svalbard. Halbach et al. (2017) report an average of 111 ± 36 Hg μg/kg in top soil and 25 ± 13 μg/kg in mineral soil. Gopikrishna et al. (2020) report a lower concentration of 40 ± 20 Hg μg/kg for topsoil. Both these studies report levels matching the concentrations that were found at the Ny-Ålesund mine site in our study, whereas the Hg concentrations in the reference areas were considerably lower. There are several reasons by which these differences can be explained. Both Halbach et al. (2017) and Gopikrishna et al. (2020) analysed samples collected in the vicinity of Ny-Ålesund, whereas Halbach et al. (2017) also measured in and near Longyearbyen (Halbach, 2016). These samples were collected at locations that were more exposed to Hg emissions than samples collected at more remote locations. Also, organic matter content in soil has been shown to strongly influence mercury concentrations with increasing organic matter content enhancing the ability of soil to retain Hg after deposition (Halbach et al., 2017). Average background mercury concentration in soil on Svalbard based on these samples may therefore be overestimated. Hg background concentration in soil of other remote (Arctic) locations were more in line with what we found in the Lovénbreen samples. Jiang et al. (2011) measured a range of 0.7-6.6 μg/kg dw in soil samples collected at a location just NW of Ny-Ålesund. A study in Greenland (Riget et al., 2000) reported Hg levels of <10 Hg μg/kg dw in soil, being below or just above the detection limit. We therefore believe that the Hg levels measured at the Lovénbreen and Krossfjorden provide a good indication of the local background level to assess the exposure radius of the mine.

Total PAH concentrations in stream sediments showed a similar picture as Hg. The highest PAH concentrations were found in and around the mine site (Fig. 2 and 3) with an average concentration of 2222 ± 1321 μg/kg Σ14PAH, normalized on 5% TOC, and a range of 803-6244 μg/kg ΣPAH 5% TOC, for all mine samples except the three upstream samples. In several samples we observed a high density of coal particles and consequently a high TOC (up to 51%). These samples showed relatively high PAH levels, in some cases higher than the PAH levels found in the coal sample. This may be explained by the fact that a lump of coal on average may have a lower PAH concentration than coal-dust (particles) (NGI 2003 and NGI, 2020). Σ14PAH concentrations were significantly higher than all other sites ($p<0.05$). The average total PAH concentration found in the three upstream samples above the mine was lower with 143.4 ± 25 μg/kg Σ14PAH5% TOC (range 116-177 μg/kg 5% TOC). At the

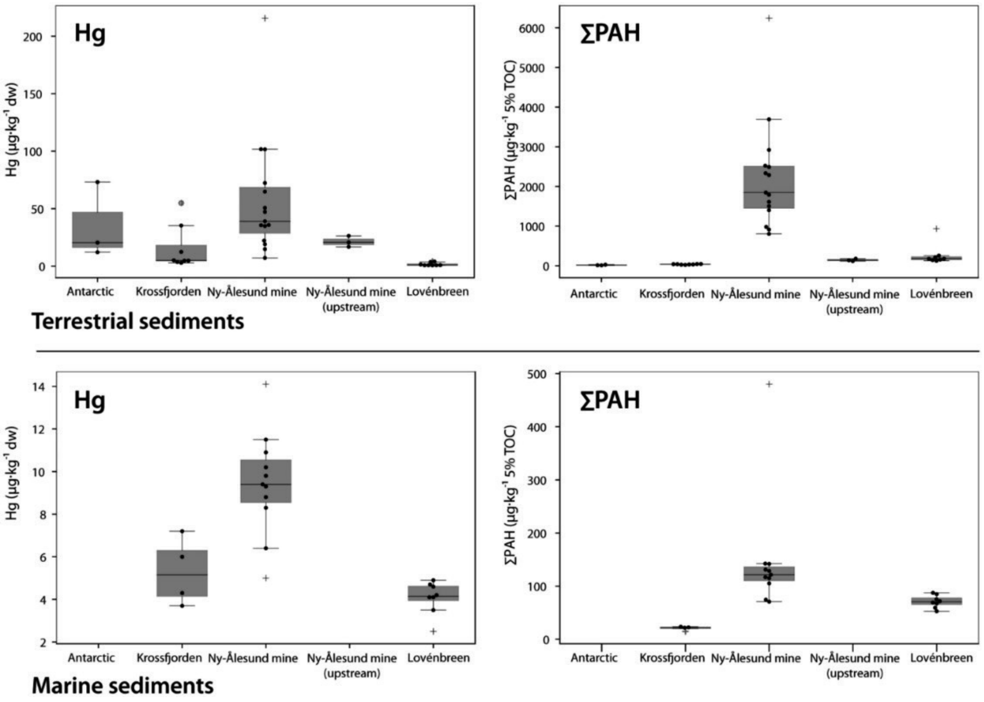

Figure 3*. Boxplot of PAHs and Hg concentrations in terrestrial (upper graphs) and marine (lower graphs) sediment samples collected in 2017 in the Kongsfjorden and Krossfjorden, and in 2019 at the Antarctic Peninsula. Concentrations are in µg/kg 5% TOC for ΣPAH and µg/kg dw for Hg. Outliers are marked with +. Individual measurements are shown as dots. PAH concentrations below detection limit were included in this graph as 0.5*dl.*

Lovénbreen reference site, total PAH levels were lower than those near the mine (average 271 ± 253 µg/kg Σ14PAH 5% TOC, range 123-933 µg/kg Σ14PAH 5% TOC), though slightly higher than those of the upstream mine samples. The Krossfjorden reference site showed the lowest total PAH levels of the Arctic samples (31-43 µg/kg Σ14PAH 5% TOC). The samples collected at the Antarctic Peninsula showed the lowest PAH concentrations of all, with 9-25 µg/kg Σ14PAH 5% TOC (fig. 3).

No exceedance of guideline limits was found for mercury (1000 µg/kg dw), whereas in 12 out of 18 samples collected at the mine site, Σ14PAH exceeded the threshold of 2000 µg/kg dw for Σ16PAH in soil (NGI, 2020).

Marine sediments

Mercury concentrations in the marine sediment samples collected near the mine area had an average Hg concentration of 9 µg/kg dw ± 0.002 (range 5-14 µg/kg dw) (Fig. 2 and 3). Both reference locations had significantly ($p<0.05$) lower concentrations: Lovénbreen 4 µg/kg dw ± 0.001 and Krossfjorden 5 µg/kg dw ± 2.

The Σ14PAH concentration in the marine sediments near the mine showed a similar picture, with an average concentration of 148 ± 107 µg/kg 5% TOC (range 71-480 µg/kg Σ14PAH 5%

TOC) (Fig. 2 and 3). The marine sediment samples taken near the Lovénbreen were lower, and contained an average concentration of 71 µg/kg ± 11 µg/kg Σ14PAH 5% TOC (range 53-87 µg/kg Σ14PAH 5% TOC). Marine sediment samples collected at the Krossfjorden marine site had Σ14PAH concentrations between 15 and 23 µg/kg 5% TOC. All locations differed significantly in Σ14PAH concentrations ($p<0.05$).

Even though marine sediment samples near the run-off from the mine showed higher Hg and PAH concentrations than the marine samples taken at the two reference sites, the contaminant signal was weaker than that in the terrestrial sediments. This can be explained by the fact that terrestrial stream sediments will be contaminated through leaching processes directly from the mine as well as atmospheric deposition. The marine system however will be influenced by dilution processes, tidal currents in the fjord and sediment load coming from the Kongsfjorden and Kongsbreen glacier system (Beldowski et al., 2015).

Mercury concentrations in the marine samples near the Ny-Ålesund mine (5-14 µg/kg dw) were slightly lower than reported in earlier studies in the Kongsfjorden (8–80 µg/kg dw; Beldowski et al., 2015; Grotti et al., 2013; Lu et al., 2012) and in the Grønfjorden (Barentsburg) (7-42 µg/kg dw, with levels increasing toward the inner parts of Grønfjorden; Lebedeva et al., 2017). The mine in Barentsburg is still operational and has had an overall higher coal production than the Ny-Ålesund mine. On top of that, the inner fjord of Grønfjorden may be influenced by deposition of Hg from the coal-burning power plants of nearby Barentsburg and Longyearbyen. The contribution from long-range atmospheric transport to Hg concentration found in this fjord is expected to be similar to that found in Kongsfjorden and Krossfjorden.

On a wider scale, mercury concentrations in the marine sediments were found to be comparable to other Arctic areas, such as the Chukchi Sea (5-55 µg/kg dw total Hg; Fox et al., 2014), Bering Sea (deep water: <2000m; 20-36 µg/kg dw total Hg; Iricanin and Trefry, 1990), and Beaufort Sea (3-97 µg/kg dw total Hg; Trefry et al., 2003), and lower than reported for the north-eastern Bering Sea (<1-130 µg/kg dw total Hg; Nelson et al, 1975) and Arctic Ocean Basin (34-116 µg/kg dw total Hg; Gobeil et al., 1999).

Hg concentrations in sediment were well below lowest reported No Observed Effect Concentrations (NOEC; 2-551 mg/kg dw) and Lowest Observed Effect Concentrations (LOEC; 7-972 mg/kg dw) for Hg in marine and estuarine sediments (Conder et al., 2015). PAH levels in marine sediment from the Kongsfjorden from this study (36-182 µg/kg Σ14PAH dw) were on the lower side compared to other studies in this fjord (12-2315 µg/kg Σ13PAH dw, Szczybelski et al., 2016; 1-2550 µg/kg Σ16PAH dw, Van den Heuvel-Greve et al., 2016; 52-1482 µg/kg Σ12PAH dw, Pouch et al., 2017). Higher PAH concentrations were observed in samples collected near the settlement of Ny-Ålesund, which is influenced by a variety of PAH sources such as shipping, run-off from waste handling and treatment, as well as (historic) coal processing. Σ14PAH concentrations in our study were well below the sediment guideline values (class 2) of 2000 µg/kg Σ16PAH (Bakke et al., 2010).

The coal sample from the mine area contained 133 µg/kg dw Hg and 554 µg/kg 5%TOC Σ14PAH.

PAH profiles

Average PAH profiles in terrestrial samples of Ny-Ålesund mine and the Lovénbreen, as well as the marine sediment near the mine were rather similar to the coal sample collected at the Ny-Ålesund mine (Fig. 4). All samples from these locations contained a clear phenanthrene peak, which has been described before as the most dominant PAH in coal, depending on the origin (Ribeiro et al., 2012). Phenanthrene dominance in Kongsfjorden marine sediment has also been observed in an earlier study, with an increasing occurrence from outer fjord towards the Kronebreen (Pouch et al., 2017). Pouch et al. (2017) however did not sample close to Ny-Ålesund and although providing an overall picture for the Kongsfjorden, they did not register the local elevated concentrations related to the mine. The Krossfjorden sediments (both terrestrial and marine) showed a low peak for phenanthrene while other compounds remained mostly below detection level. Very low PAH concentrations were observed in the terrestrial sediments from the Antarctic Peninsula.

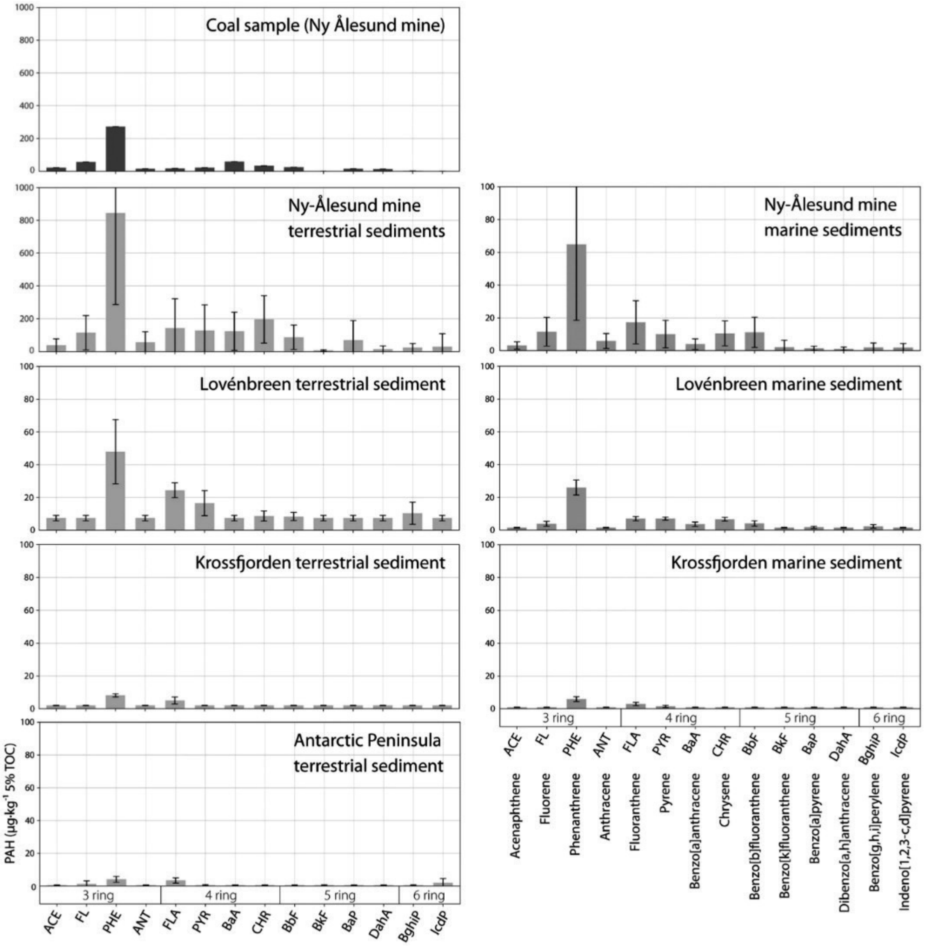

***Figure 4**. Average PAH profiles in sediment samples per location (µg/kg 5%TOC), collected in the Kongsfjorden and Krossfjorden in July 2017 and the Antarctic Peninsula in March/April 2019. Note the different y-axis scale for the Ny-Ålesund coal and terrestrial sediments. (Black – upper graph on left = coal collected at Ny-Ålesund mine, graphs on left = terrestrial melt water stream sediment, graphs on right = marine sediment). A deviating sample (terrestrial sediment from Lovénbreen) was excluded from this graph because of an extreme phenanthrene peak (414 µg/kg 5%TOC).*

Origin of PAHs in terrestrial and marine sediments

Terrestrial sediments

Hierarchical clustering of TOC normalized PAH concentrations in the terrestrial sediment samples revealed four main clusters (Fig. 5). All except one sample from the mine area were in cluster A1 and A2. Cluster A2 also included one sample from the Lovénbreen area. The Lovénbreen and Krossfjorden samples formed cluster C. Two of the samples taken at the Antarctic Peninsula formed cluster B. The two top level clusters (A and [B,C]) separated the samples which were contaminated by both direct leaching from the mine waste as well as deposition from air (A1 and A2) from the samples which were contaminated by deposition from air only (B and C). Exceptions were three samples taken just upstream from the mine area which we considered to be only contaminated by deposition from air and not by direct contamination. The single sample from the mine area in cluster C was considered an outlier.

In the Molecular Diagnostic Ratio, the Krossfjorden and Antarctica terrestrial samples separated from the Ny-Ålesund mine and the Lovénbreen samples at a fluoranthene/pyrene ratio of ~0.7 (Fig. 6). This was a higher value than that reported by De La Roche-Torre et al. (2009) as a basis for distinguishing petrogenic origin (<0.4) from pyrogenic fossil fuel origin (0.4-0.5), and >0.5 for coal combustion (Tobizewski et al., 2012). The ∑LMW/∑HMW ratio categorized most mine area samples (cluster A) as petrogenic and most other samples as pyrogenic. This is consistent with our assumption that the PAHs (from anthropogenic sources) found in the reference area mostly originated from deposition by air. An important factor to consider is that our samples were collected in an area with generally very low contamination levels, in contrast to many samples sets on which the ratios reported by Tobizewski (2012) are based. It is reasonable to assume that the low PAH values found in the current study as well as environmental degradation processes were of influence to the interpretation of the observed ratios.

Marine sediments

The marine sediment samples separated into three clusters based on their normalized PAH profiles (Fig. 5). Cluster (D), which contained all samples taken near the mine area, and cluster E, which contained the Lovénbreen samples, were both on the second level branch. All the Krossfjorden samples formed cluster F. No marine samples were collected in Antarctica. The dissimilarity between the Krossfjorden samples and those collected in the Kongsfjorden (Lovénbreen) were likely due to a different origin of the PAHs in the Krossfjorden as these samples were less likely to be affected by the mine than the Lovénbreen samples based on proximity.

Given the fact that the marine samples near the mine and those near the Lovénbreen showed a higher similarity than the terrestrial samples did from these locations, the marine sediments were thought to be more influenced by the marine environment rather than by input from the terrestrial environment alone. PAHs have a tendency to bind to organic carbon and particulates and will flow with the suspended matter in the meltwater streams into the fjord. The influence of the terrestrial run-off will therefore be highest in the shallow parts of the fjord, whereas the fjord rapidly gains depth near the coast of the mine and Lovénbreen (Husum et al., 2019). The marine samples were collected on the slope of the fjord edge, at an average depth of -26m (mine) and -29m (Lovénbreen). At these sites the influence of ice-rafted detritus, material transported and deposited from icebergs or sea ice, was also observed to be relatively high compared with

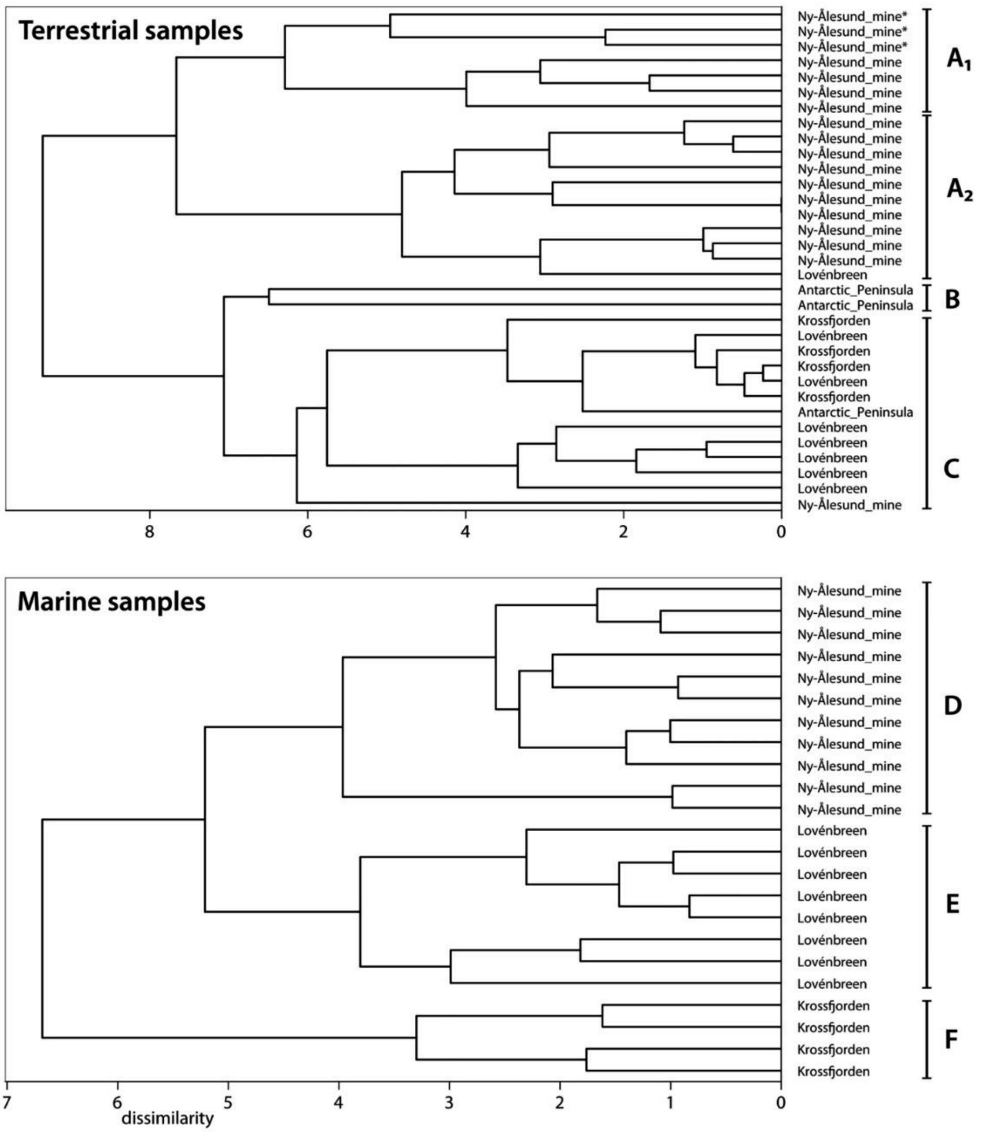

Figure 5*. Dendrograms based on the PAH profiles of all samples (normalised on* μ*g/kg 5% TOC). top: The terrestrial sediment samples that were collected in the Kongsfjorden (2017), Krossfjorden (2017) and Antarctica (2019). bottom: The marine sediment samples that were collected in the Kongsfjorden (2017) and Krossfjorden (2017). Three upstream samples from the mine area are labelled with *.*

the Krossfjorden (Husum et al., 2019). This further supports why a weakened signal of the mine was found in the marine samples compared with the terrestrial samples.

The PAH Molecular Diagnostic Ratios for the marine samples showed a much lower variation in both the fluoranthene/pyrene and the ∑LMW/∑HMW ratios (Fig. 6). This may suggest that the origin of the PAHs found in these samples is less variable than is the case with the terrestrial samples. The ∑LMW/∑HMW ratio clearly separated the mine samples (as petrogenic) from the other two locations (pyrogenic), although at a somewhat higher ratio (1.1 instead of 1.0) than reported in other studies (Zhang et al., 2008, Tobizewski et al., 2012). The mine samples showed higher variability in the ∑LMW/∑HMW ratio than the Krossfjorden and Lovénbreen samples which could indicate a more diverse PAH origin.

The Fluoranthene/Pyrene ratio showed a relatively small variation per sample location. We did not find any samples with a ratio <0.4 which would indicate a petrogenic origin of the PAHs. The ratio 0.4-0.5 (indicating fossil fuel combustion) mainly applied to the Lovénbreen samples. Most other samples were above 0.6, which indicated coal combustion (De La Roche-Torre et al., 2009, Davis et al., 2019). The source classification based on the Fluoranthene/Pyrene ration was not supporting the results from the ∑LMW/∑HMW ratio. Other PAH ratios could not be applied to differentiate between the possible petrogenic and combustion sources due to the low PAH values found in these samples. A more conclusive answer based on PAH diagnostic ratios could therefore not be provided.

Mercury - PAH relation

A clear relationship was observed between Hg and PAH concentrations in the terrestrial sediment samples as well as in the marine sediment samples (Fig. 7). For both the terrestrial and marine samples we expected the Hg concentration to be elevated compared to background levels in samples for which the PAH profiles could be related to the mine. This was the case for the terrestrial samples collected at the mine site as a result of direct contamination. The

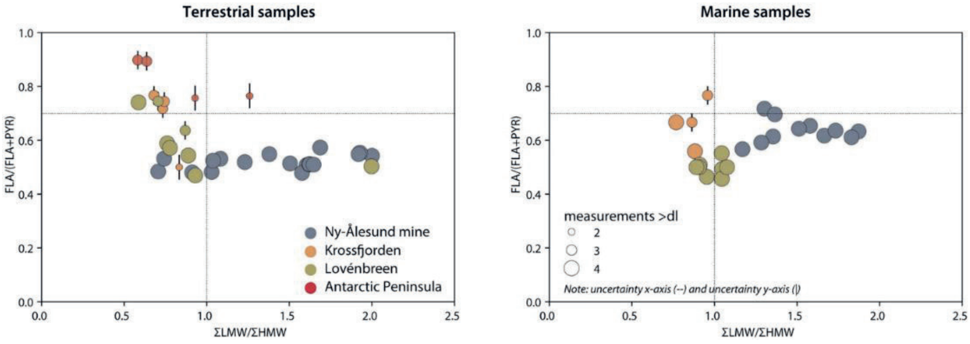

Figure 6. Bivariate plots of ∑LMW/∑HMW versus Fluoranthene/Pyrene for terrestrial and marine sediments. The size of the marker represents the number of concentrations above detection limits that are applied in the ratio calculation (see legend and Materials and Methods for more details).

correlation between PAH and Hg in the terrestrial samples collected at Lovénbreen showed a lower Hg background level in the terrestrial sediments compared with the PAH concentrations. The Hg concentrations in the terrestrial Krossfjorden samples were higher than the PAH concentrations, even though the PAH profiles and diagnostic ratios were less comparable than the mining area.

The positive correlation between the PAH and Hg levels in the Lovénbreen samples indicate that the Hg levels at this location were elevated compared with the background level. Although a clear PAH/Hg correlation was observed in the marine sediment samples, both PAHs and Hg showed much lower concentrations in these samples (Fig. 7).

Marine biota

Mercury concentrations in marine biota sampled in the Kongsfjorden and Krossfjorden varied between <10-120 µg/kg dw (see Table 4 in SI). Biota collected at Ny-Ålesund harbour and Ny-Ålesund mine contained 3.2-7.2 times higher Hg concentrations than biota collected at the Lovénbreen, and 9.6 times higher concentrations than those at the Krossfjorden (Fig. 8). Hg concentrations in biota in the harbour were in the same range as those near the mine (factor 0.8-1.0). One exception was *S. groenlandicus* for which Hg concentrations were in the same range for all locations in the Kongsfjorden (no data for Krossfjorden).

Marine biota from this study (<10-120 µg /kg dw) contained similar Hg levels compared with corresponding species sampled in the Chukchi Sea (30-150 µg/kg dw; Fox et al, 2014; Fox et al, 2017), and in the Grønfjorden, Svalbard (70-170 µg/kg dw; Lebedeva et al, 2018; note: dw concentrations were based here on average dw% of the involved species as no dw was reported in the referred study). Hg concentrations in marine benthic invertebrates in the Arctic are at the lower end of what has been reported for benthic invertebrates from lower latitudes (Fox et al, 2017).

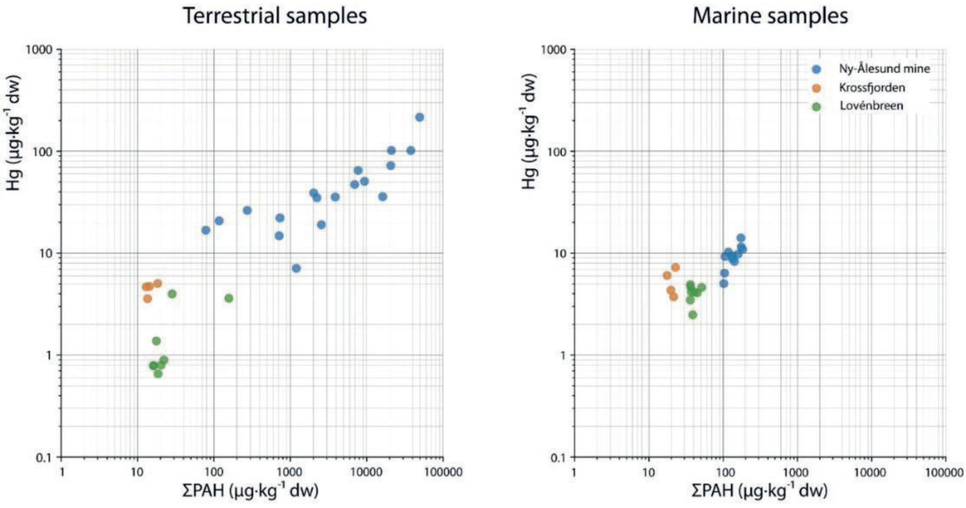

Figure 7*. Scatter plots of Hg concentration (µg/kg dw) versus PAH (µg/kg dw) concentrations in the terrestrial samples (left) and in marine samples (right), collected in Kongsfjorden and Krossfjorden in 2017. The correlation factor (R2) for the terrestrial and marine samples was 0.89 and 0.91 respectively. PAH concentrations below detection limit were included as 0.5*dl.*

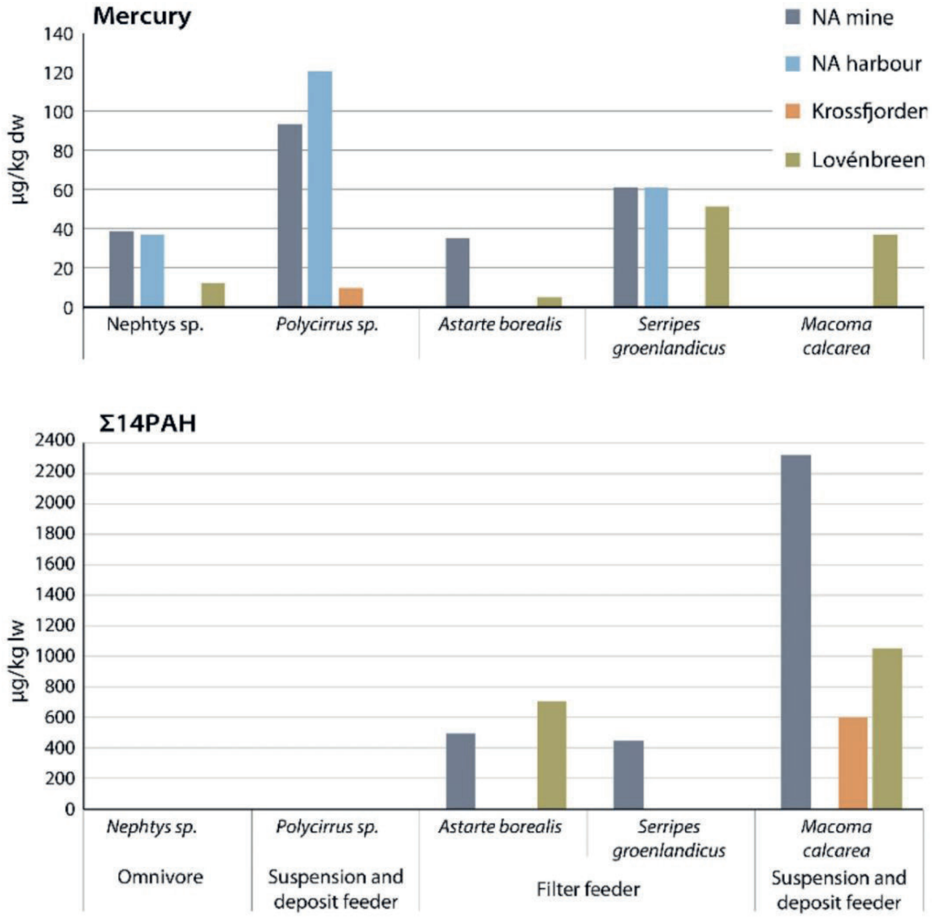

***Figure 8**. Hg (μg/kg dw) and ΣPAH (μg/kg lw) concentrations in two marine worm species (Nephtys sp. and Polycirrus sp.) and three marine shellfish species (Astarte borealis, Serripes groenlandicus and Macoma calcarea), collected in Kongsfjorden and Krossfjorden in July 2017.*

The European Commission set an Environmental Quality Standard (EQS) of 20 ng/g (μg/kg) Hg wet weight for biota to protect top predators from secondary poisoning (EC, 2008). Marine biota samples from this study contained Hg concentrations of <1.5-28 μg/kg Hg ww, with one sample (*Polycirrus* sp. in NA harbour) being slightly higher than the EQS. Our data are based on TotalHg, whereas methylmercury is most often reported for values in biota. Methylmercury has been reported to be 30-57% of TotalHg in shellfish (Pieters & Geuke, 1994).

BSAF values for Hg in the analysed biota were all above 1 (1.2-12.9) pointing at accumulation from sediment. For *M. calcarea* only one Hg sample could be analysed in this study (Lovénbreen, BSAF of 9.1). The BSAF values for NA mine were 3.8-9.9, for Lovénbreen 1.2-12.6 and for Krossfjorden 1.8.

Σ14PAH concentrations in marine biota of this study varied between 46-2340 µg/kg lw, with an average concentration of 950 ± 720 µg/kg lw Σ14PAH (see Table 3 in SI). Average concentrations in marine biota were higher near the mine (1100 ± 1070 µg/kg lw Σ14PAH) than near the Lovénbreen (890 ± 250 µg/kg lw Σ14PAH) and in the Krossfjorden (610 µg/kg lw Σ14PAH) (Fig. 8). The Σ14PAH concentration in the *A. borealis* sample near the Lovénbreen was 1.4 times higher than the sample at the NA mine, whereas the concentration in *M. calcarea* was 2.2 and 3.9 times higher in the sample near the mine compared to those of the Lovénbreen and Krossfjorden. PAH concentrations were highest in *M. calcarea* (4.8-5.1 times higher than those in *A. borealis* and *S. groenlandicus* near NA mine). No PAH results could be obtained for the worms due to limited sample volumes.

Marine biota from this study contained similar PAH concentrations compared with earlier published data in marine biota of the Kongsfjorden (30-9190 µg/kg lw Σ13PAH in *A. borealis*, *M. calcarea* and *Nephtys ciliata*; Szczybelski et al., 2016). Similar to our study, lower PAH concentrations were observed in *A. borealis* compared with *M. calcarea* (Szczybelski et al., 2016). Σ16PAH concentrations in *Mytilus edulis* collected in North-Atlantic and sub-Arctic coastal environments (30-480 µg/kg dw; Jörundsdóttir et al., 2014) were also in the same range compared to our results (10-110 µg/kg dw).

The observed PAH concentrations in the marine biota of the Kongsfjorden and Krossfjorden were well below threshold PAH concentrations for effects in marine species, as reported for *Neanthes arenaceodentata* (Hansen et al., 2003), *Crassostrea virginica* (Hwang et al., 2008), and *Ruditapes philippinarum* (Liu et al., 2014).

PAH profiles in most biota samples showed a predominance of phenanthrene (Fig. 9). *M. calcarea* near the mine also contained a high chrysene concentration compared with the other PAH compounds. This was also found in the *S. groenlandicus* sample near the mine, though at a much lower concentration. Other PAH compounds showed variable concentrations in the samples.

The PAH profiles in the sediment and in *M. calcarea* of Lovénbreen and Krossfjorden were quite similar (Fig. 4 and 9). However, the phenanthrene fraction in sediment of NA mine was much higher than that in *M. calcarea*, whereas *M. calcarea* showed a higher fraction of chrysene compared with the sediment.

A high variation in BSAF values for PAH compounds in *M. calcarea* was observed for NA mine (0.1-4.5), although most BSAF values were low (<1) (Fig. 10, lowest panel). This was also found in earlier studies where BSAFs of PAHs were generally low (BSAF<1) (Szczybelski et al., 2019). BSAF values for the Lovénbreen and Krossfjorden were closer together (0.4-1.0 and 1.0-1.5). Highest BSAF values were observed for dibenzo(a,h)anthracene (3.6) and chrysene (4.5) at NA mine. Bioaccumulation of chrysene was also shown in other studies in a similar species, *Macoma balthica* (current name: *Limecola balthica*) (Pikkarainen et al., 2004). The BSAF for the Σ14PAH was 1.1 for the Krossfjorden, 0.5 for the Lovénbreen and 0.7 for NA mine.

The other two shellfish species also showed BSAF values < 1 for the Σ14PAH at NA mine and the Lovénbreen (0.1 and 0.4 respectively). The BSAF value for Σ14PAH for the Krossfjorden may be due to the fact that a lot of the concentrations were below detection limit.

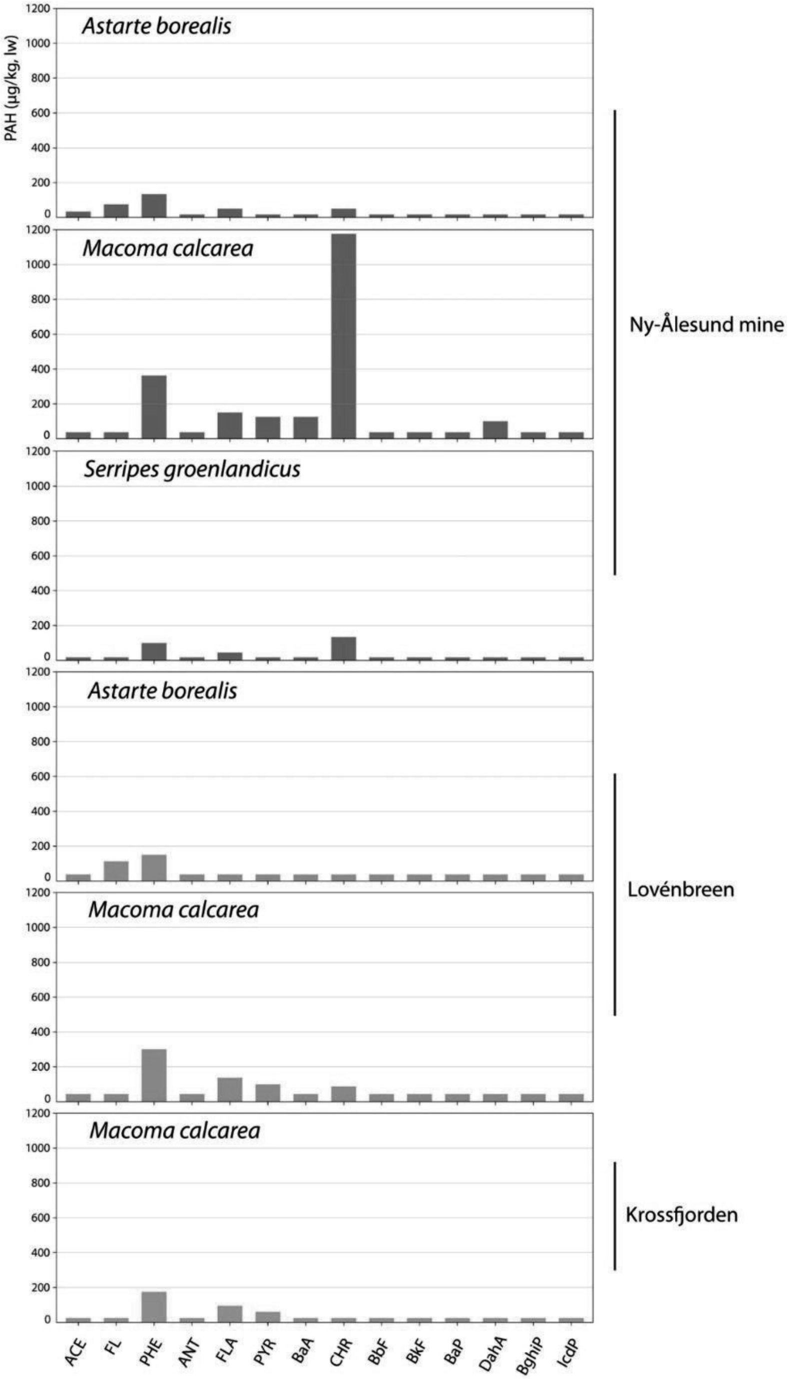

Figure 9. *PAH profiles in bivalve samples (µg/kg, based on lw), per location, of the Kongsfjorden and Krossfjorden in July 2017. Concentrations below the detection limit are include as 0.5* dl.*

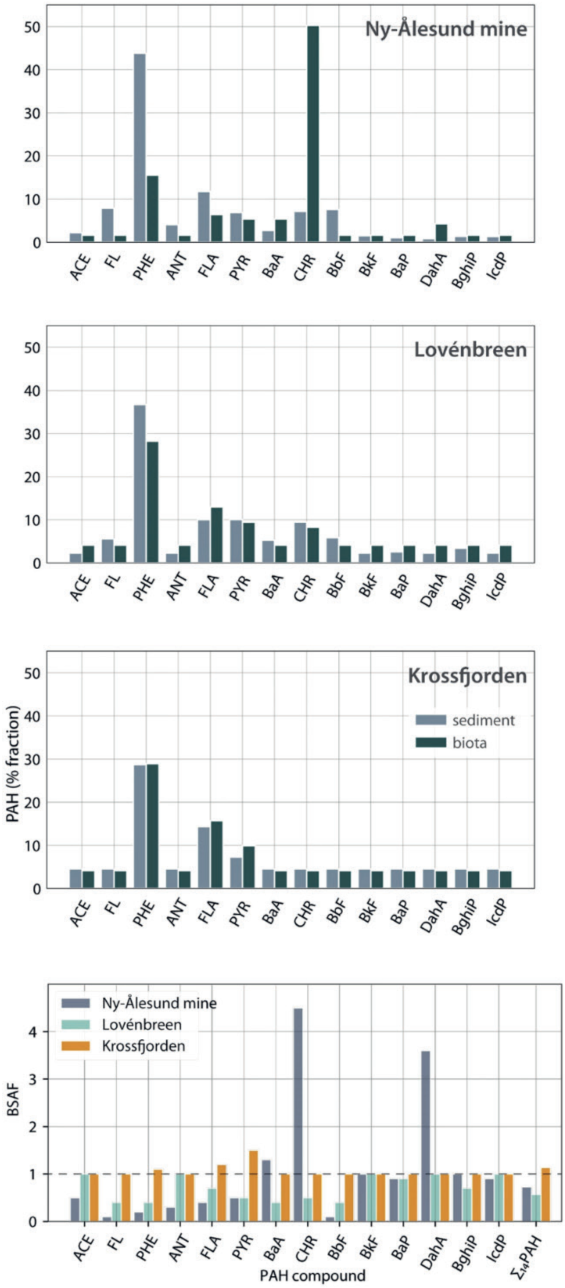

***Figure 10**. Comparison of PAH profiles (%fractions) of Macoma calcarea and marine sediments for samples from the Ny-Ålesund mine, Lovénbreen and Krossfjorden samples. Average values were used based on µg/kg lipid weight (biota) and µg/kg dw (sediments), including <dl as 0.5·dl. The bottom panel shows the biota sediment accumulation factor (BSAF) of Macoma calcarea for each PAH compound at the three locations (BSAF>1 = concentration/accumulation, BSAF<1 = metabolization/excretion).*

The fact that the PAH profiles in marine biota and sediment near NA mine were not fully similar can be explained by the chemical characteristics of the respective PAH compounds (Lofthus et al., 2018) and biological characteristics of the benthic species (Selck et al., 2012). For tracing back the source of a terrestrial coal mine in the adjacent marine system, concentrations can therefore better be assessed in sediment samples. In case the impact of these contaminants needs to be defined, concentrations in biota provide better insight into the bioaccumulation potential of these contaminants.

Conclusions

Based on the findings of this study, the exposure radius of Hg and PAH of the former coal mine in the Kongsfjorden was found to be relatively limited. The contaminant signal from the mine in both terrestrial and marine sediment samples was partly observed at the Lovénbreen reference site (~6km from the mine), while this was absent at the Krossfjorden reference site (~19km from the mine). The differences between the locations were much smaller in the marine sediments compared with the terrestrial sediments. For the biota samples the contaminant signal was even less obvious, although elevated PAH and Hg concentrations were observed in marine biota near the mine and for Hg also in the Ny-Ålesund harbour.

Clustering of PAH concentrations and PAH diagnostic ratios of the terrestrial and marine sediments showed a clear difference between the mine and the two reference sites. Also, the Lovénbreen terrestrial samples only partly clustered with the Krossfjorden samples. PAH diagnostic ratios suggested different pyrogenic sources for the Lovénbreen, compared with the Krossfjorden and Antarctic terrestrial samples.

The PAH diagnostic ratios applied in this study were based on studies conducted in areas with much higher contaminant levels. In our study the ratios based on summed PAH (∑LMW/∑HMW) and Fluoranthene/Pyrene were only applicable, hereby avoiding detection limits issues. We therefore pose that the use of PAH diagnostic ratios in low contaminated areas should be only applied in combination with other methods such as hierarchical clustering. The observed low Hg and PAH background concentrations in our terrestrial sediment samples and large differences between locations on Svalbard and more generally the Arctic region, shows the need for a more in-depth study of Hg and PAH background levels in Arctic terrestrial sediments. As the organic matter may strongly influence Hg levels in these samples, it is recommended to assess Hg and PAH concentrations in samples that are both low and high in organic content.

A clear relationship was observed between PAH and Hg concentrations in the terrestrial samples collected at the mine and at the Lovénbreen site. In the Krossfjorden samples, the different relative concentrations of Hg and PAHs indicated that the mine was not the dominant source of contamination in this area. The relatively higher concentrations of mercury at this site may reflect other sources, e.g. deposition of long-range transported mercury and local geology.

Marine biota samples showed elevated Hg and PAH concentrations near the mine outflow, and for Hg also in the harbour. PAH profiles were variable and did only slightly match the characteristic PAH profile of the mine area. Accumulation from sediment was low for PAHs but considerable for Hg. Based on these observations the marine biota was considered less suitable to trace the exposure radius from a local land-based source as the historic coal mine.

In a rapidly warming Arctic changes in precipitation, snow cover, soil temperature and permafrost will inevitably lead to an increased mobility of contaminants, as well as faster biodegradation of organic substances such as PAHs. This means that in the future increased runoff and higher availability of contaminants through leaching may enhance inputs of PAH and Hg from the terrestrial contaminated site into the marine system. Airborne deposition is not directly expected to increase, although climate-related factors can also influence atmospheric inputs from other sources.

This study shows that determining Hg and PAH concentrations in terrestrial samples and marine sediment samples is a suitable method to assess the exposure radius of a land-based coal mine and the inflow into the adjacent marine system. A follow up study is needed to more accurately determine the edge of the exposure radius of the Ny-Ålesund coal mine by conducting a more detailed sediment sampling campaign along the Kongsfjorden, both on land and in the adjacent marine system. This would also involve improved characterisation of the natural background concentrations (of Hg as well as PAH) in the region in order to define what is and what is not an impacted area. This may also include assessing whether climate change related factors are altering the edge of the exposure radius leading to for instance increased inputs from the contaminated site further into the surrounding area.

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Supporting Information (SI)

***Map 1**. Sample locations on the Antarctic Peninsula.*

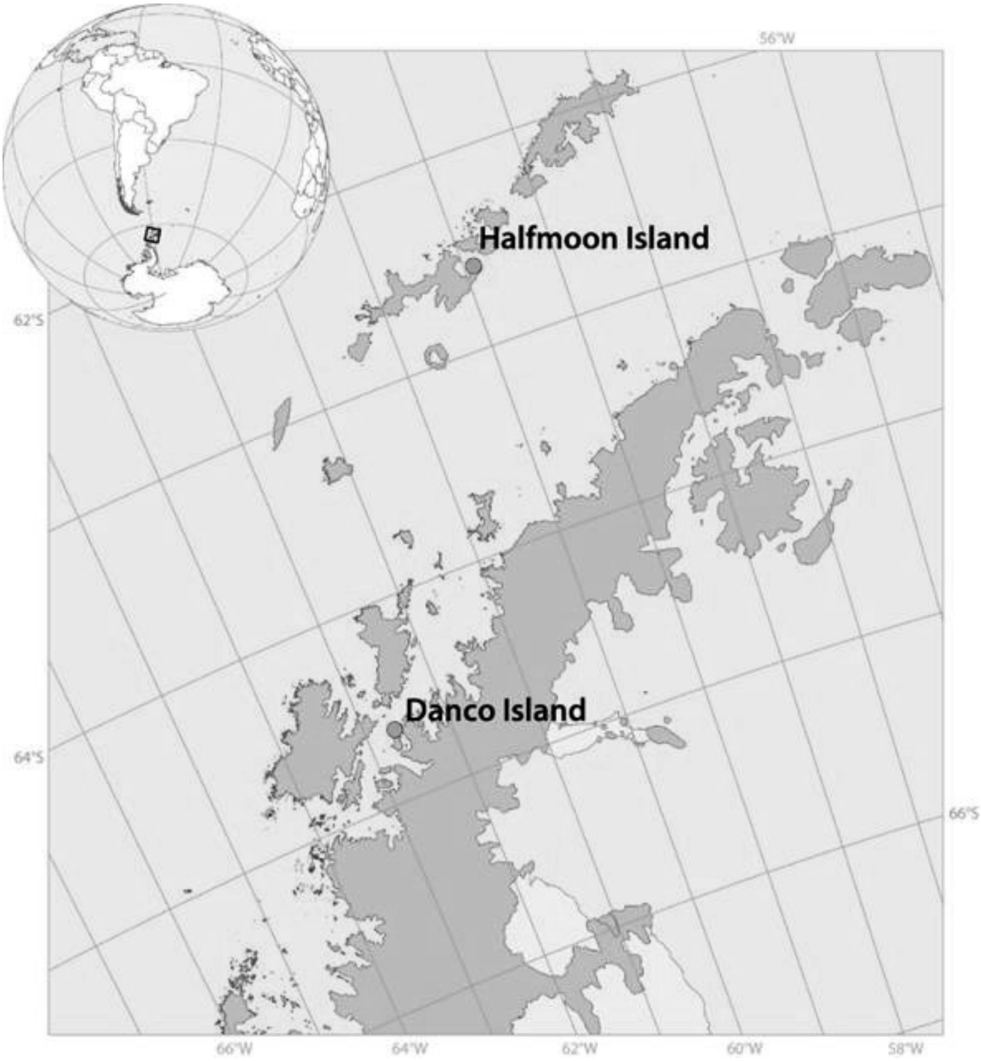

***Table 1**. PAH compounds and used abbreviations.*

PAH compound	**Abbreviation**
Naphtalene	NP
Acenaphtalene	ACE
Fluorene	FL
Phenantrene	PHE
Anthracene	ANT
Fluoranthene	FLA
Pyrene	PYR
Benzo[a]anthracene	BaA
Chrysene	CHR
Benzo[e]pyrene	BeP
Benzo[b]fluoranthene	BbF
Benzo[k]fluoranthene	BkF
Benzo[a]pyrene	BaP
Dibenzo[a,h]anthracene	DahA
Benzo[g,h,i]perylene	BghiP
Indeno[1,2,3-c,d]pyrene	IcdP

***Table 2**. Sediment samples, Hg and PAH concentrations. Values below DL are indicated with <. Marine sediment samples are marked grey.*

	Sample coordinate					mg/kg ww	µg/kg ww													
location	**latitude**	**longitude**	**depth**	**dw %**	**TOC %**	**Hg**	**ACE**	**FL**	**PHE**	**ANT**	**FLA**	**PYR**	**BaA**	**CHR**	**BbF**	**BkF**	**BaP**	**DahA**	**BghiP**	**IcdP**
Ny-Ålesund_mine	78.927	11.9684	-13.2	83.5	3.3	0.0042	2.3	6.4	37	4.5	10	5.3	1.9	5.8	5.6	<1.3	<1.3	1.9	2.3	<1.3
Ny-Ålesund_mine	78.9254	11.9829	-25.7	68.7	4	0.0057	2.1	7.4	41	3.8	18	7.1	2	7.2	5.6	<1.2	<1.2	<1.2	<1.2	<1.2
Ny-Ålesund_mine	78.9225	12.001	-16.8	72.1	5.3	0.0046	1.7	5.4	33	2.5	11	4.8	1.5	4.7	5.5	2.4	<1.3	<1.3	<1.3	<1.3
Ny-Ålesund_mine	78.9223	12.0165	-28.1	65.4	0.8	0.0067	1.5	5.9	32	3	8.9	5.6	2.1	5.1	6	2.4	<1.2	<1.2	1.6	1.5
Ny-Ålesund_mine	78.9228	12.009	-24.9	64.7	4.6	0.006	1.8	5.3	31	2.4	9.9	5.5	1.6	3.6	4.4	<1.2	<1.2	<1.2	<1.2	<1.2
Ny-Ålesund_mine	78.9227	12.012	-22.2	64.8	4.1	0.0057	<1.3	5.6	46	2.7	10	5.8	1.9	4.6	5.1	<1.3	<1.3	<1.3	1.3	<1.3
Ny-Ålesund_mine	78.9231	12.0028	-26.7	69.1	3.2	0.0065	2.1	7.7	42	3.8	10	6.2	2.6	6.2	6.6	<1.2	<1.2	<1.2	<1.3	1.5
Ny-Ålesund_mine	78.9236	11.9949	-20.4	63.4	3.9	0.0073	3.3	9.7	52	5.7	11	7	3.8	8.6	6.3	<1.3	<1.3	<1.3	<1.3	<1.3
Ny-Ålesund_mine	78.9245	11.9881	-27.1	66.5	4.5	0.0065	2.9	9	48	5.6	11	6.3	2.9	7.9	7.9	<1.2	<1.2	<1.2	1.3	<1.2
Ny-Ålesund_mine	78.9271	11.9748	-52	62.4	4.7	0.0088	2.6	9.6	44	3.1	9.7	6.7	3.8	10	12	<1.3	2.7	<1.3	<1.3	2
Ny-Ålesund_mine	78.9239	11.9922	-26	63.3	4.4	0.0069	2.2	8.6	46	4	11	8.4	3.8	11	13	<1.3	3.5	<1.3	<1.3	2
Ny-Ålesund_mine	78.9116	11.9567		66.7	7.4	0.026	14	52	750	25	110	82	42	140	84	5.9	34	<1.3	<1.3	9.6
Ny-Ålesund_mine	78.9107	11.9532		57.1	4.4	0.015	<1.2	2.7	55	<1.2	15	16	5.2	22	13	3.2	7.6	1.4	6.7	7.1
Ny-Ålesund_mine	78.9122	11.9628		78.7	13	0.051	140	580	3200	92	320	270	240	620	310	16	120	110	34	16
Ny-Ålesund_mine	78.9135	11.9771		76.3	9.1	0.036	97	400	2900	75	310	250	200	600	290	15	110	26	35	<12
Ny-Ålesund_mine	78.9154	11.9803		73.1	6.2	0.026	60	230	1500	58	170	140	110	320	160	<14	49	<14	17	<14
Ny-Ålesund_mine	78.9184	11.9641		69.2	51.1	0.05	320	770	6700	680	690	670	720	1400	880	59	580	280	520	41
Ny-Ålesund_mine	78.9184	11.9641		69.1	20.1	0.035	170	450	2900	300	340	370	340	550	380	33	270	140	200	31
Ny-Ålesund_mine	78.9175	11.959		67	21.8	0.024	290	660	4800	550	560	530	590	1000	640	48	470	350	350	29
Ny-Ålesund_mine	78.9164	11.9515		77.7	45.8	0.079	430	1100	7800	800	930	890	1500	1300	980	<15	630	<15	<15	<15
Ny-Ålesund_mine	78.9156	11.9489		43.3	22.2	0.044	430	1100	7800	800	930	890	1500	1300	980	<15	630	<15	<15	<15
Ny-Ålesund_mine	78.9118	11.9357		77.3	2.6	0.013	<1.2	<1.2	23	<1.2	4.9	5.3	2.6	10	2.6	<1.2	<1.2	<1.2	6.8	1.7
Ny-Ålesund_mine	78.9125	11.9319		72.3	3.1	0.015	<1.3	4.3	31	<1.3	6.7	7.2	2.6	15	2.1	<1.3	<1.3	<1.3	8.8	3.7
Ny-Ålesund_mine	78.9138	11.9384		64.9	25.7	0.14	790	2000	8900	1200	4200	3700	2400	3000	1300	<70	2700	<70	<71	1800
Ny-Ålesund_mine	78.9156	11.9356		78.9	4	0.015	14	37	1100	<13	170	140	130	330	50	<13	<13	<13	<13	14
Ny-Ålesund_mine	78.9175	11.9443		74.4	4.5	0.026	33	94	650	29	170	150	150	200	14	14	40	<13	70	40
Ny-Ålesund_mine	78.9175	11.9443		76.7	3.5	0.017	<14	<14	250	<14	43	39	69	75	14	<14	<14	<14	23	<14
Ny-Ålesund_mine	78.9187	11.9516		87.8	3.2	0.013	<13	33	280	<13	55	51	48	85	<13	<13	<13	<13	20	16
Ny-Ålesund_mine	78.9199	11.9581		80.2	3.2	0.0057	<15	50	510	22	54	52	80	99	41	<15	17	<15	<15	<15

Table 2. *continued.*

	Sample coordinate					mg/kg ww	µg/kg ww													
location	**latitude**	**longitude**	**depth**	**dw %**	**TOC %**	**Hg**	**ACE**	**FL**	**PHE**	**ANT**	**FLA**	**PYR**	**BaA**	**CHR**	**BbF**	**BkF**	**BaP**	**DahA**	**BghiP**	**IcdP**
Krossfjorden	79.1476	11.562		77.2	1.6	0.0036	<1.2	<1.2	2.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
Krossfjorden	79.1405	11.521		73.5	1.7	0.0037	<1.4	<1.4	2.8	<1.4	2.3	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4
Krossfjorden	79.1403	11.5383		75.4	1.2	0.0027	<1.1	<1.1	2.2	<1.1	1.4	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1
Krossfjorden	79.1428	11.5603		74.2	1.4	0.0035	<1.1	<1.1	2.4	<1.1	1.6	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1	<1.1
Krossfjorden	79.1544	11.6342	-8 - -12m	69.5	4.1	0.0042	<1.3	<1.3	3.1	<1.3	1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
Krossfjorden	79.1552	11.641	-8 - -12m	78.5	3.3	0.0034	<1.4	<1.4	4.8	<1.4	2.3	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4
Krossfjorden	79.1556	11.6355	-8 - -12m	75.1	3.7	0.0028	<1.3	<1.3	4.5	<1.3	3	1.5	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
Krossfjorden	79.1526	11.6333	-8 - -12m	69	3.5	0.005	<1.4	<1.4	4.6	<1.4	1.9	1.5	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4
Lovénbreen	78.9042	12.1562		78.3	0.5	0.0007	<1.3	<1.3	5.6	<1.3	2.5	2.1	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
Lovénbreen	78.9014	12.1514		76.2	0.5	0.0005	<1.3	<1.3	3.6	<1.3	2	1.4	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
Lovénbreen	78.8999	12.1448		75.3	0.5	0.003	<1.2	<1.2	7.6	<1.2	2.3	2.6	<1.2	1.5	1.3	<1.2	<1.2	<1.2	1.3	<1.2
Lovénbreen	78.8984	12.1442		83.2	0.7	0.003	4.1	13	58	7.8	8	7.9	3.7	9.3	8.2	<1.2	3.2	<1.2	3.8	2.4
Lovénbreen	78.9025	12.088		75.8	0.4	0.0006	<1.3	<1.3	2.6	<1.3	1.9	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3
Lovénbreen	78.9042	12.0897		75.2	0.3	0.0006	<1.3	<1.3	3.8	<1.3	2	1.5	<1.3	<1.3	<1.3	<1.3	<1.3	<1.3	1.5	<1.3
Lovénbreen	78.9059	12.0955		79.9	0.4	0.0011	<1.2	<1.2	4.2	<1.2	2.1	1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2	<1.2
Lovénbreen	78.9077	12.1047		76.4	0.5	0.0006	<1.4	<1.4	<3.9	<1.4	2	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4
Lovénbreen	78.9049	12.1572	-11.8	75.9	2	0.0031	<1.2	2.1	13	<1.2	3.3	3.4	1.9	3.1	2.1	<1.2	<1.2	<1.2	1.5	<1.2
Lovénbreen	78.9053	12.1557	-23.9	76.5	2.2	0.0019	<1.3	2.1	10	<1.3	2.6	3	1.8	2.9	1.8	<1.3	<1.3	<1.3	1.6	<1.3
Lovénbreen	78.9051	12.1541	-26	68.4	2.1	0.0032	<1.4	<1.4	9.1	<1.4	2.9	2.8	<1.4	2.3	1.5	<1.4	<1.4	<1.4	<1.4	<1.4
Lovénbreen	78.9056	12.1522	-40	67.2	2.3	0.0033	<1.2	1.5	9.1	<1.2	2.1	2.5	1.4	2.3	1.2	<1.2	<1.2	<1.2	<1.2	<1.2
Lovénbreen	78.909	12.1197	-30.4	69.5	1.8	0.0029	<1.3	<1.3	9.7	<1.3	2.6	2.6	1.6	2.5	1.6	<1.3	<1.3	<1.3	<1.3	<1.3
Lovénbreen	78.9088	12.1217	-30.3	69.1	1.8	0.0024	<1.4	1.6	9	<1.4	3.2	2.6	<1.4	2.2	<1.4	<1.4	<1.4	<1.4	<1.4	<1.4
Lovénbreen	78.909	12.1216	-34.4	71.5	2.1	0.0033	<1.2	2.1	13	<1.2	3.4	3.4	2.3	3.6	3	<1.2	1.4	<1.2	1.5	<1.2
Lovénbreen	78.9091	12.1205	-36.2	70	1.9	0.0029	<1.2	2	11	<1.2	2.7	2.7	1.7	2.7	1.6	<1.2	<1.2	<1.2	<1.2	<1.2
Ny-Ålesund_mine	78.9154	11.9803		98	84.6	0.13	360	950	4600	260	300	370	1000	560	420	<26	260	240	29	<26
Coal sample																				
Antarctic Peninsula	-64.4354	-62.3603		84.8	3.8	0.062	<0.8	<0.8	<5.5	<0.8	<2.8	<0.9	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8	<0.8
Antarctic Peninsula	-64.4351	-62.3603		91.2	1.9	0.011	<0.7	1.6	<5.1	<0.7	<2.6	<0.8	<0.7	<0.7	<0.7	<0.8	<0.7	<0.7	<0.8	<0.8
Antarctic Peninsula	-62.3544	-59.5411		73.2	3.5	0.015	<0.5	<0.5	<3.4	<0.5	2.1	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5

Table 3. *PAH concentrations in biota samples (µg/kg ww) collected in the Kongsfjorden and Krossfjorden in July 2017. Dw = dry weight. Concentrations in shell fish species were based on tissue without the shell.*

Species	Tissue- type	Location	n	Length (mm)	Weight (g)	dw %	Lipid (BD)%
Astarte borealis	Whole organism	Ny-Ålesund mine	49	24.1 ±8.7	4.5 ±5.1	15.9	1.2
Serripes groenlandicus	Whole organism	Ny-Ålesund mine	4	40.4 ±16.2	23.2 ±20.3	15.1	0.9
Macoma calcarea	Whole organism	Ny-Ålesund mine	17	24.1 ±6.3	2.7 ± 2.0	13.8	0.8
Macoma calcarea	Whole organism	Krossfjord	30	18.1 ± 5.2	1.0 ± 0.8	19.6	2
Macoma calcarea	Whole organism	Lovénbreen	32	21.0 ± 5.9	1.4 ± 1.3	12.5	0.8
Astarte borealis	Whole organism	Lovénbreen	19	25.8 ± 10.7	6.0 ± 6.5	15	0.8

Table 3. *PAH concentrations in biota samples (µg/kg ww) collected in the Kongsfjorden and Krossfjorden in July 2017. Dw = dry weight. Concentrations in shell fish species were based on tissue without the shell. Continued.*

Species	Location	ACE	FL	PHE	ANT	FLA	PYR	BaA	CHR	BbF	BkF	BaP	DahA	BghiP	IcdP
Astarte borealis	Ny-Ålesund mine	0.4	0.9	1.6	<0.4	0.6	<0.4	<0.4	0.6	<0.4	<0.4	<0.4	<0.4	<0.4	<0.4
Serripes groenlandicus	Ny-Ålesund mine	<0.3	<0.3	0.9	<0.3	0.4	<0.3	<0.3	1.2	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
Macoma calcarea	Ny-Ålesund mine	<0.6	<0.6	2.9	<0.6	1.2	1	1	9.4	<0.6	<0.6	<0.6	0.8	<0.6	<0.6
Macoma calcarea	Krossfjord	<1.0	<1.0	3.5	<1.0	1.9	1.2	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
Macoma calcarea	Lovénbreen	<0.7	<0.7	2.4	<0.7	1.1	0.8	<0.7	0.7	<0.7	<0.7	<0.7	<0.7	<0.7	<0.7
Astarte borealis	Lovénbreen	<0.6	0.9	1.2	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6	<0.6

Table 4. *Total mercury concentrations in biota samples (mg/kg ww) collected in the Kongsfjorden and Krossfjorden in July 2017. Dw = dry weight. Concentrations in shell fish species were based on tissue without the shell. NA = Ny-Ålesund.*

Sample code	Species	Type	Spec.	Location	Date	n	Length (mm)	Weight (g)	Dw%	Hg (ww)
I	*Nephtys* sp.	Whole	Tissue	NA mine	19/07/'17	1	-	3.6	19.9	0.008
X	*Nephtys* sp.	Whole	Tissue	Lovénbreen	26/07/'17	10	-	0.5 ± 0.3	19.9	0.002
XVI	*Nephtys* sp.	Whole	Tissue	NA harbour	26/07/'17	1	-	2.6	27.2	0.01
II	Tube worm	Whole	Tissue	NA mine	19/07/'17	3	-	0.9	18.2	0.017
IX	Tube worm	Whole	Tissue	Krossfjord	14/07/'17	2	-	2.5 ± 2.8	20.6	0.002
XV	Tube worm	Whole	Tissue	NA harbour	26/07/'17	4	-	2.9 ± 1.4	23.2	0.028
III	*Astarte borealis*	Whole	Tissue	NA mine	19/07/'17	48	24.9 ± 9.5	4.9 ± 5.7	16.4	0.006
XI	*Astarte borealis*	Whole	Tissue	Lovénbreen	26/07/'17	18	25.3 ± 11.2	6.2 ± 7.7	15.3	<0.002
V	*Serripes groenlandicus*	Whole	Tissue	NA mine	19/07/'17	3	49.9 ± 8.2	33.3 ± 18.6	16.3	0.01
XIII	*Serripes groenlandicus*	Whole	Tissue	Lovénbreen	26/07/'17	2	45.5 ± 14.8	25.9 ± 23.0	14.8	0.008
XIV	*Serripes groenlandicus*	Whole	Tissue	NA harbour	26/07/'17	1	65.1	72.5	16.3	0.01
XII	*Macoma calcarea*	Whole	Tissue	Lovénbreen	26/07/'17	30	21.0 ± 6.1	1.4 ± 1.2	14.1	0.005

CHAPTER 3

TEMPERATE VERSUS ARCTIC: UNRAVELLING THE EFFECTS OF TEMPERATURE ON OIL TOXICITY IN GAMMARIDS

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Abstract

With sea ice receding in the Arctic, shipping activities are increasing, leading to higher risks of accidents and oil spills. However, empirical toxicity measurements in Arctic species are rare, complicating oil spill risk assessments. In the present study, we tested if acute oil toxicity metrics acquired under temperate conditions are relevant for Arctic systems. The effect of temperature (4°C, 12°C and 20°C) on the LC50 and Critical Body Residue (CBR) of the temperate invertebrate *Gammarus locusta* exposed to Water Accommodated Fractions of a fuel oil were determined. Both toxicity metrics decreased with increasing temperature. Additionally, we compared the responses (LC50 and CBR) of the temperate *G. locusta* to that of Arctic *Gammarus* species at 4°C. The LC50 for the Arctic *Gammarus* sp. was a factor of three higher than for the temperate *G. locusta* at 4°C, but its CBR was similar, although both the exposure time and concentration were extended to reach lethality. Likely, this was caused by the larger size, and higher weight and total lipid content in Arctic gammarids compared to the temperate gammarids. This study supports the use of temperate acute oil toxicity data as basis for assessing risks for the Arctic region, provided that the effects of temperature on oil fate and functional traits (e.g. body size and lipid content) of test species are taken into account. As such, using the CBR as toxicity metric is beneficial, because although being temperature-dependent, it is independent of functional traits. To the best of our knowledge, the present study is the first to report CBRs for Total Petroleum Hydrocarbon concentrations. The present study findings are important, because empirical oil toxicity data for the Arctic are limited, while the risks of oil spills in the Arctic are increasing.

Introduction

The Arctic has been warming up rapidly in the past decades, at a four times faster rate than the globe as a whole (Rantanen et al. 2022). With the sea ice receding, Arctic regions are opening up for shipping activities, such as transport, fisheries and tourism. For instance, a 1.3 times overall increase in shipping intensity by fishing and tourist vessels was observed in the Arctic region of Svalbard during 2012-2019, coupled with a stretched operational shipping season and an expansion of navigational areas (Stocker et al. 2020). Enhanced shipping activities increase the risk of shipping accidents, especially since Arctic regions remain challenging areas with low temperatures, the likely presence of sea ice, bad visibility due to storms, fog and winter darkness, and often still unexplored shores. Shipping accidents may result in oil spills of which the consequences are expected to be worse than in temperate areas, because of slower oil spill responses, due to the remoteness of the region and reduced radio coverage (Stocker et al. 2020). Also, not all oil spill mitigation measures are suitable or feasible under Arctic conditions (Wilkinson et al. 2017), which further increases the likelihood of a prolonged presence of oil in the environment. Therefore, it is crucial to assess the effects of oil spills in Arctic marine ecosystems.

The composition and mass of the dissolved oil fraction depend on the solubility of the oil constituents in the oil, the degree of weathering, as well as the environmental conditions (Brown et al. 2016). Temperature greatly determines the solubility of petroleum substances; at lower water temperatures, oil becomes more viscous and low-molecular-weight (LMW) oil constituents will evaporate less (Brown, et al. 2016). Hence, at low temperatures, relatively more LMW hydrocarbons than higher-molecular-weight (HMW hydrocarbons will dissolve. Aquatic organisms are mainly exposed to the dissolved fraction of oil, and in particular the LMW constituents are easily taken up and consequently cause toxicological effects in aquatic animals (Redman et al. 2017a). Exposure to oil has been shown to lead to several types of acute and chronic effects in marine organisms, including narcosis and developmental effects (e.g. Gissi et al. 2021; Vrabie et al. 2009; De Laender et al. 2011). Narcosis is directly related to the accumulated oil mass and can be predicted based on the oil concentrations and profiles measured in water and oil, using the PETROTOX model (Redman et al. 2017b).

Oil toxicity data for the Arctic and other cold environment species are limited, creating a challenge in assessing the risks of oil spills for the polar regions (Chapman & Riddle 2005, De Hoop et al. 2011, Olsen et al. 2011, Olsen et al. 2013). Toxicity data for Water Accommodated Fractions (WAFs) of (crude) oil are reported for Arctic copepods, mysids and fish only (Riebel & Percy 1990, Gardiner et al. 2013, Bender et al. 2021, Hansen et al. 2011). Some additional data exist for arthropods, molluscs, and other fish, but these are from the 1970s-1990s and access to these data is limited (De Hoop et al. 2011 and references therein).

In order to fill this gap in oil toxicity data, the accuracy of extrapolating toxicity data from temperate to Arctic conditions has been explored in modelling studies These studies suggest that temperate data can be used as a surrogate or a first indication for Arctic toxicity data, as the variation in sensitivity between temperate and polar organisms to oil was within a factor of three, which was considered negligible (De Hoop et al. 2011, Olsen et al. 2013, Bejarano et al. 2017). Still, because of the limited data availability, additional research was recommended in order to further support this extrapolation approach, also taking into account the specific Arctic species characteristics (Camus 2014).

In the present study, the sensitivity of temperate and Arctic gammarid species to oil was further investigated. Firstly, the effect of temperature (4°C, 12°C, 20°C) on WAF composition and the acute toxicity of oil, in terms of LC50 and the Critical Body Residue (CBR), was studied for the temperate gammarid, *Gammarus locusta*. The CBR provides the internal exposure concentration at which organisms are lethally affected and was included here as it is believed to be a more robust toxicity metric, independent of external conditions (Van der Heijden et al. 2015). Secondly, the LC50 and CBR experiments were repeated at 4°C with an Arctic gammarid, *Gammarus* sp.. This allowed a direct comparison of the sensitivity of Arctic gammarids at their natural ambient temperature to the sensitivity of temperate gammarids.

Materials & Methods

Test species

For the temperate experiments, the amphipod *Gammarus locusta* was used. This species lives on sediment and macrophytes, occurs from the coasts of Iceland and Norway to the south of Portugal (Costa et al. 2004), and is frequently used in eco(toxico)logical research (e.g. Szczybelski et al. 2018). Organisms were collected in the intertidal area at two sites in the Oosterschelde, the Netherlands (Goese Sas (51°32'43.36"N, 3°55'28.79"E) and Kattendijke (51°31'45.71"N, 3°57'57.07"E)), between March and June 2016. Individuals with a length of 6-15 mm (from head to end of tail) were used in the experiments.

For the Arctic experiments, *Gammarus* sp. were collected in June 2016 and July 2017 in the intertidal area of Ny-Ålesund harbour, Kongsfjorden, northwest Svalbard (78°55'39.5"N, 11°56'17.5"E). A few individuals were examined and identified as *Gammarus setosus*, an arctoboreal gammarid with a circumpolar distribution (Opalinski & Węsławski 1989). This species has a life span of over three years and is believed to mature in its third year (Węsławski & Legezyńska 2002). However, a mixture of *G. setosus* and *Gammarus oceanicus* has previously been observed in the Kongsfjorden and the presence of *G. oceanicus* is increasing (Węsławski et al. 2018). Therefore, it cannot be excluded that *G. oceanicus* individuals were also present in the batch of test organisms. *G. oceanicus* is a boreal gammarid species with a North Atlantic distribution (Opalinski & Węsławski 1989). To acknowledge this uncertainty in test species, the test species name for the toxicity tests was set to *Gammarus* sp.. As the test organisms were collected in the harbour, with the potential of site pollution, an additional blank was collected outside the harbour for chemical analysis. All test organisms were 16-20 mm in length.

To obtain further information on the functional traits of the test species (size, weight and lipid%), additional batches of temperate and Arctic gammarids were collected from the field. After collection, gammarids were rinsed with demineralised water, blotted dry, weighed, pooled per size class (1-7 mm, 8-15 mm, 16-20 mm, 21-25 mm, >26 mm), and stored at -20° C until further processing. Frozen pooled gammarid samples were freeze-dried and homogenised using a mortar and pestle. Total lipids in these samples were extracted with dichloromethane-methanol-water (8:4:3), following the protocol by Folch et al. (1957), with some modifications.

Test oil and WAF production

The oil used for the experiments was Distillate Marine Grade A (DMA; Shell marine gas oil), a low-sulphuric (<1.5%) diesel petroleum, containing a blend of refined (distilled) oils. It has been previously used in other toxicity studies (Jonker et al. 2006, Vrabie et al. 2009, Szczybelski et al. 2018). Water Accommodated Fractions (WAFs) of DMA oil were prepared in 5-L glass Duran® bottles with a bottom tap. Each bottle was filled with 5200 mL of Artificial Sea Water (ASW) and a glass stir bar was added. ASW was used to eliminate potentially interfering components that may be present in natural seawater and to allow stable test conditions for all treatments. To obtain the ASW, Pro Reef Salt Mix (Colombo®) was dissolved in Milli-Q water to a salinity of 34-36‰. A predetermined volume of DMA oil was carefully pipetted to the glass wall of the Duran® bottle, just above the water surface (see Table S1 in the Supporting Information (SI) for applied loadings). Then, the bottle was closed and gently stirred on a magnetic stirrer, creating a small vortex of 2 cm. Stirring was done in the dark for 48 h, which was considered sufficient for obtaining an equilibrium WAF of a light fuel oil like DMA at all test temperatures (Mitusova et al. 2005, Faksness et al. 2008). Each WAF was produced in a temperature-controlled room, at the test temperature that was maintained during the LC50 and CBR experiments.

LC50 experiments

After collection, gammarids were acclimatized for two days without feeding in an aquarium with aerated filtered seawater prior to the start of the experiments. The temperate organisms were exposed to three temperatures: 4, 12 and 20°C. Experiments were run at temperatures that were similar to the ambient water temperature at the collection site at the time of each experiment. In the Oosterschelde, the water temperature ranged from 6°C in March (4°C experiment) to 17°C in June (20°C experiment). The Arctic specimens were tested at 4°C, with a recorded water temperature of 6.9°C in Ny-Ålesund harbour on 20 July 2017.

Gammarids were exposed to a gradient of WAFs derived from DMA oil in 1-L glass bottles. The bottles were gently filled with 900 mL of the respective WAF at the desired temperature and at low light intensity to minimize any photodegradation of oil constituents. Eight pre-selected gammarid specimens were sieved from 50-mL beakers filled with ASW and added to the 1-L test bottles. The bottles were immediately closed and sealed with aluminium-lined caps to minimize any evaporation of chemicals. The test bottles were randomly divided over the climate rooms. Survival was scored every 24 hours during five days (120 hours), starting at t=0. Additionally, activity of the animals was qualitatively monitored every 24 hours. For each temperature, five to seven WAF concentrations were tested at the same time, including a blank. Each concentration was simultaneously tested in five (temperate species) or three replicates (Arctic species) at the same time. The experiments were run twice for each temperature. Experiments were performed in the climate rooms of Wageningen Marine Research in Yerseke, the Netherlands (temperate conditions), and KingsBay Marine Laboratory in Ny-Ålesund, Svalbard (Arctic conditions).

Dissolved oxygen concentrations (mg/L) in the WAF solutions were measured in all replicates before and after (120 h) the experiments, using a Hach© HQ-40d multi meter with a LDO probe, to ascertain that reduced oxygen levels were not the cause of gammarid mortality.

Critical Body Residue (CBR) experiments

Based on the LC50 results, three oil loadings were selected for the CBR experiments: a control loading (C0), a medium WAF loading, which had been demonstrated to lead to mortality within 48 hours (C-medium), and a high WAF loading (C-high) (Table S1). For all temperate CBR experiments, the C-high loading was 0.3 mL/L of DMA, whereas the C-medium loadings differed per temperature: 0.1 mL/L (4°C), 0.03 mL/L (12°C), and 0.0065 mL/L (20°C). For the

Arctic CBR experiment, the oil loadings for the C-medium and C-high were 1 mL/L and 2 mL/L, respectively. These higher WAF loadings were required to reach lethal body burdens within the test duration.

Test bottles were filled with WAF and eight gammarids, as described above for the LC50 experiments. Directly after the start of the experiments, the gammarids were observed every 15-30 minutes (day and night), for 48 hours in total. The CBR experiment with the Arctic gammarids was prolonged to 72 hours, to be able to reach lethal body burdens. Upon death, the concerning individual was immediately removed from the test bottle, rinsed with demineralised water and blotted dry with a clean tissue. The time of death, weight (mg) and length (mm) were recorded before storage in a glass vial at -20°C. All dead individuals removed from the experimental systems were pooled per replicate, frozen (-20°C), and ultimately pooled per oil concentration per test for chemical analysis. Each concentration was tested in five (temperate species) or three replicates (Arctic species), with eight individuals per replicate.

Chemical analysis

After WAF production (at t=0), 500 mL of the remaining 700 mL of WAF in each of the 5-L Duran® bottles was gently poured into an amber-coloured 500-mL bottle containing 50 mL of *n*-hexane (Biosolve B.V., Netherlands). The storage bottles were closed with aluminium-lined lids and stored at a dark place at or below room temperature until chemical analysis.

Actual, dissolved Total Petroleum Hydrocarbon (TPH) concentrations in the WAFs were determined by liquid-liquid extraction with *n*-hexane. The bottles containing WAF and *n*-hexane were shaken on a heavy-duty reciprocal shaker for 1 h, after which the hexane phase was transferred to a calibrated pointed flask with a pipette. The WAF was extracted a second time with an additional 20 mL of hexane and the extracts were pooled and concentrated to 0.5 mL with a modified Kuderna-Danish setup and a gentle flow of nitrogen gas, respectively. Then, 1.0 mL of *n*-heptane was added, after which the extract was concentrated under nitrogen to 0.4 mL and 100 µL of internal standard solution (500 mg/L of decane (C10) and 300 mg/L of tetracontane (C40) in *n*-heptane) was added.

Oil concentrations in the gammarids from the CBR experiment were determined as follows: approximately 150-300 mg of freeze-dried organism material was Soxhlet-extracted with *n*-hexane/acetone (3:1) for 16 h in pre-extracted glass fiber extraction thimbles. The extracts were concentrated as described above, to 1 mL and cleaned-up through basic/acid-treated silica gel columns with *n*-hexane (Muijs & Jonker, 2009a). The hexane eluates were concentrated, solvent-exchanged to 0.4 mL of *n*-heptane, and 100 µL of internal standard was added as described above. For both WAF and biota extraction series, four blank and four recovery determinations were included. Oil concentrations in the extracts were quantified by GC-FID, as described before (Muijs & Jonker, 2009a), using 9 calibration standards (measured in fourfold) for each analysis series. Final concentrations in water and gammarids were recovery-corrected and expressed as µg TPH (i.e., C_{10}-C_{40})/L water and mg TPH/g dry or lipid weight in organisms, respectively. Additionally, to obtain information on chemical profiles, final concentrations were expressed in terms of hydrocarbon blocks (HB; i.e., boiling point fraction distribution): C_{10}-C_{12}, C_{12}-C_{14}, C_{14}-C_{16}, C_{16}-C_{18}, C_{18}-C_{22}, C_{22}-C_{28}, and C_{28}-C_{40} µg/L or mg/kg dw.

In several cases, the chemical analysis was compromised with emulsion and precipitation formation in the stored WAF. Also, a few unexpectedly high TPH concentrations were observed, which were inconsistent with expected aqueous concentrations. This was probably due to experimental artefacts, as the t=0 WAF aliquots used for chemical analysis consisted of what was left in the 5-L glass bottle after draining the bottle for the toxicity experiments. Contamination of the WAF with the oil layer used for loading may have occurred in these cases. The results of the chemical analysis were therefore carefully evaluated and deviating data points were noted and omitted. To be able to express the LC50 results on an actual TPH concentration basis for all test concentrations, temperature-specific relationships between WAF loading and measured TPH concentration were constructed, using all available data (also including earlier pilot experimental data), though excluding the biased datapoints mentioned above. Based on these fitted temperature-specific relationships, all corresponding TPH concentrations in the WAFs could be derived for each loading (Text S1, Table S1). The relationships between nominal and measured concentrations were not linear, but followed an one-site-total-binding model, as included in Prism GraphPad, version 9.5.0 (Tables S1 and S2).

Total lipid content of the gammarid samples in the CBR experiment was quantified according to Bligh and Dyer (1959). Samples of approximately 200 mg of freeze-dried material were extracted three times with a water/methanol/chloroform mixture. The chloroform phases were pooled and evaporated to dryness, after which lipid weights were determined gravimetrically.

Data analysis

LC50 values were determined by fitting a dose-response model with variable slope (GraphPad, version 9.5.0) to the experimental data, using the actual, dissolved (fitted) TPH concentrations. The data were normalized to values between 0 and 100% (see Text S2 and Table S3) and 95%-confidence intervals were interpreted for assessing if treatments were significant. The average time-of-death in each treatment of the CBR experiments was calculated. After confirmation of the normal distribution by means of the D'Agostino & Pearson test, a one-way ANOVA was performed, followed by Tukey's Multiple Comparisons test (GraphPad, version 9.5.0) to test for statistical differences between treatments.

Results

TPH concentrations in WAF

TPH concentrations in the produced WAF series ranged from 83-3163 µg/L for all temperatures and loadings, with ASW blanks containing 44 ± 25 µg/L TPH. Differences between the fitted curves of the test temperatures were observed, mainly up to the loading of 0.3 mL/L, with lower TPH concentrations in the 20°C as compared to the 4°C systems (Fig. S1). Oil profiles of the WAFs differed per temperature (Fig. S3): at 20°C, the relative contribution of the first HB (C_{10}-C_{16}) to the overall WAF composition was lower as compared to that at 4°C.

Test organism characteristics

On average, the Arctic gammarids were larger and had a higher individual weight (factor 2-3) than the temperate gammarids (Fig. S5 and Table S4). The Arctic gammarids, in particular the smaller individuals, also had a higher lipid % than the temperate gammarids (Fig. S6). The total lipid % of the Arctic gammarids decreased with size ($p<0.05$), whereas for the temperate gammarids an opposite pattern was observed (not significant).

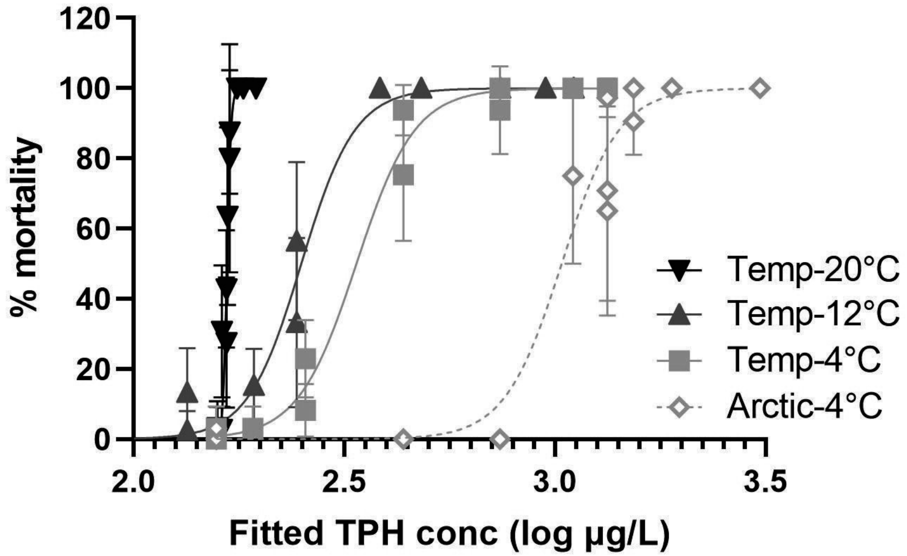

***Figure 1**. Concentration-response curves of Gammarus locusta (temperate) exposed to different concentrations of Water Accommodated Fractions at three test temperatures (4 °C - R^2=0.96, 12 °C - R^2=0.93, 20 °C - R^2=0.83), and Gammarus sp. (Arctic) tested at 4 °C (R^2= 0.91). The actual Total Petroleum Hydrocarbon (TPH) concentrations in these curves are derived from fitted temperature specific curves, based on oil loadings and measured TPH concentrations (see Table S1 and Fig. S1). Error bars represent 95% confidence intervals. An additional, detailed graph of the 20°C data is presented in Figure S2.*

LC50 experiments

The LC50 values for the temperate *G. locusta* decreased with increasing temperature upon exposure to the WAFs for five days. The LC50 at 4°C (338 µg/L TPH; 95% CI: 321-356) was two times higher than at 20°C (167 µg/L TPH; 95% CI: 166-167), with the 12°C value lying in between these two values (250 µg/L TPH; 95% CI: 240-262) (Fig. 1; Table S3). Arctic *Gammarus* sp. had a factor of three lower sensitivity to WAF (LC50 value of 1038 µg/L, 95% CI: 957-1111) compared to the temperate *G. locusta* at 4°C, and a factor of 4-6 lower than the temperate gammarids when these were tested at 12-20°C (Fig. 1; Table S3). Since the 95% confidence intervals of the LC50 values for none of the treatments overlapped, it can be concluded that temperate gammarids exposed to three different temperature resulted in significantly different LC50s, and also the LC50 values of Arctic versus temperate *Gammarus* species differed significantly (Table S3).

Critical Body Residue experiments

The CBR for the temperate *G. locusta* ranged between 6.0-11.9 mg/g TPH based on lipid weight (lw) (Fig. 2) and 0.4-0.9 mg/g TPH based on dry weight (dw) (Fig. S7). The unexposed *G. locusta* (blanks) had body burdens of 0.6-1.4 mg/g TPH lw and up to 0.1 mg/kg TPH dw (n=4),

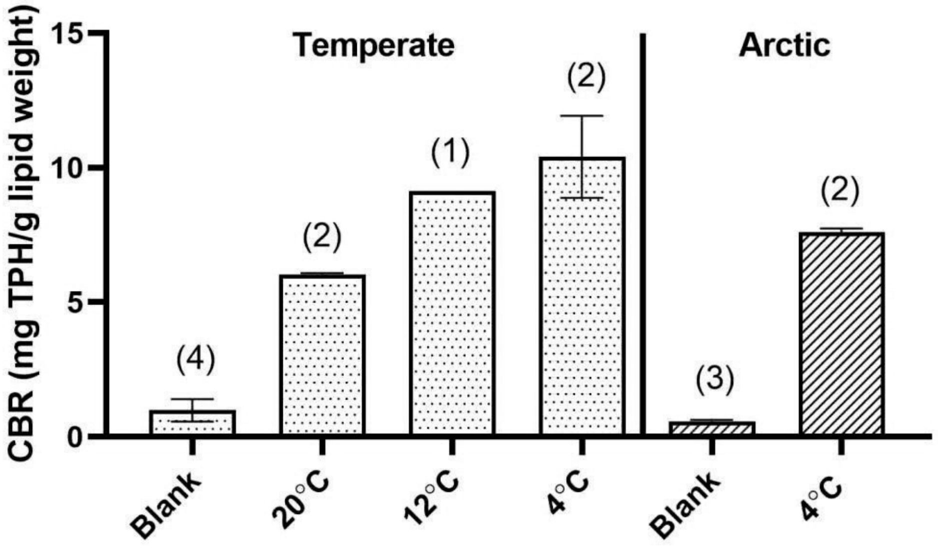

Figure 2*. Averaged Critical Body Residues (CBRs) of Total Petroleum Hydrocarbons (TPH) (mg/g lipid weight, including ranges) in the temperate G. locusta (dotted bars) after exposure to DMA gas oil at test temperatures of 4 °C (n=2), 12 °C (n=1) and 20 °C (n=2); and in Arctic Gammarus sp. (n=2; striped bars) at 4 °C. The blanks indicate body residues of individuals exposed to artificial sea water only. Numbers in brackets above the bars indicate how many times the experiments were replicated.*

irrespective of the test temperature. The CBR was the lowest at 20°C (6.0 ± 0.1 mg/g TPH lw; n=2) and the highest at 4°C (10.4 ± 2.2 mg/g TPH lw; n=2) (Fig. 2). The CBR of the 12ºC treatment was again in between these values (9.1 mg/g TPH lw; n=1). Note that the data of the second CBR exposure at 12°C were excluded, as a thin oil layer was observed on the exposure water surface in one of the replicate systems and a deviating high TPH concentration was measured in the corresponding gammarid sample.

The CBR for the Arctic *Gammarus* sp. at 4°C was 7.6 ± 0.2 mg/g TPH lw and 1.0 ± 0.1 mg/g dw (Fig. 2, Fig. S7). Background THP concentrations in the blanks (e.g. through harbour activities) were negligible for the Arctic gammarids (0.5-0.6 mg/g TPH lw and less than 0.1 mg/g TPH dw; n=3), with the additional blank collected outside the harbour being in the same range (0.6 mg/g TPH lw; n=1).

The CBR for the Arctic *Gammarus* sp. was similar to that for *G. locusta* at 4°C, with the Arctic gammarids having a slightly higher dry weight-based CBR than the temperate gammarids (factor 1.1-1.6 difference), but a lower CBR when based on lipid weight (factor 0.6-0.9) (Fig. 2, Fig. S7). The averaged total lipid % of the Arctic gammarids at 4°C (13.5%; n=3) was 1.8 times higher than that of the temperate gammarids at 4°C (7.4%, n=2). Due to the small sample sizes, accurate statistical analyses were not possible.

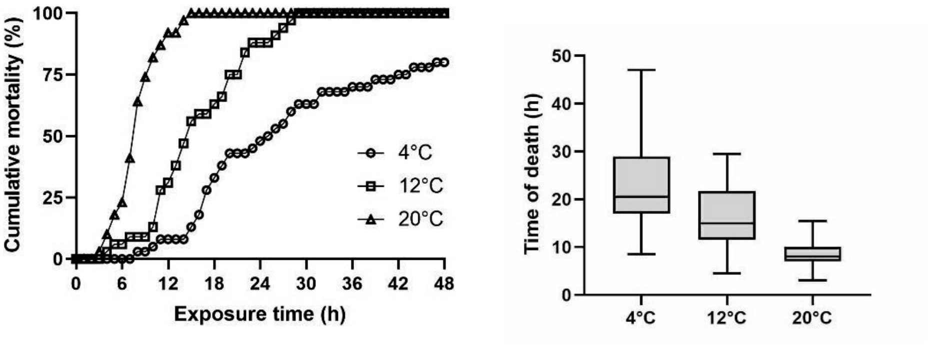

Figure 3*. Cumulative time of death for the individual gammarids in the CBR experiment with a test duration of 48 hours (left panel); and a Box and Whiskers plot, showing median, 25-75 percentile and minimum and maximum values for the time of death for the temperate G. locusta, upon exposure to a water accommodated fraction having a loading of 0.3 mL oil/L at 4 °C, 12 °C and 20 °C (right panel).*

The TPH profiles in all exposed gammarids demonstrated preferential uptake of the C_{14}-C_{16} and C_{16}-C_{18} fractions, as these were enriched by a factor of 2-2.5, as compared to the profile in the WAFs (Fig. S3, Fig. S4). TPH profiles of the exposed organisms were similar for all temperatures, although the C_{18}-C_{22} and C_{22}-C_{28} fractions were up to a factor of 2 enriched at 4°C and 12°C, as compared to the observed profiles at 20°C. TPH profiles in Arctic and temperate gammarids of the CBR experiment were equal (Fig. S4).

At the higher exposure temperatures, organisms died sooner (Fig. 3). A significant difference in the time of death was observed between temperate gammarids exposed at different temperatures to a loading of 0.3 mL/L (Fig. 3) (one-way ANOVA $p<0.0001$, $F=45.25$; Tukey's multiple comparison tests $p<0.0001$). The averaged time of death was 8.4 ± 2.8 hours at 20°C, 16.4 ± 6.5 hours at 12°C, and 23.8 ± 9.9 hours at 4°C, with in the latter treatment eight individuals still being alive after 48 hours of exposure. A direct comparison with the time of death in the Arctic experiment was not possible, because a different, higher oil loading was applied to produce the two WAFs of this experiment. The exposure time of the Arctic gammarids in the CBR experiment at 4°C was extended with another day, up to 72 hours in total, such that lethality was caused in all organisms and CBRs could be determined.

Discussion

This study demonstrates that temperature had an effect on the acute toxicity of the WAF of oil in temperate gammarids, expressed as LC50, CBR and time of death. Furthermore, a three-fold difference in LC50 between Arctic and temperate gammarids exposed at 4°C was observed, whereas the CBR was similar for both species.

The effect of temperature on toxicity in temperate gammarids

The observed inverse relationship between LC50 and exposure temperature is in agreement with earlier study findings. Higher temperatures (32°C versus 25°C) significantly increased the toxicity of oil to larvae of the shrimp *Palaemonetes pugio*, of the mud snail *Tritia obsoleta*, and of the fish *Cyprinodon variegatus* (DeLorenzo, et al. 2021). Likewise, LC50 values of a copepod and a rotifer were inversely related to temperature when exposed to chemicals, such as copper and DDT (Li et al. 2014). Several mechanisms may cause the observed temperature related differences in LC50 values. Firstly, temperature influences the aqueous solubility and evaporation rate of organic compounds from water, and thereby their bioavailability. Both solubility and evaporation are temperature-dependent and increase with increasing temperature (Brown et al. 2016). In the present experiments, the TPH concentrations in WAFs were lower at high temperatures and contained a lower percentage of the LMW hydrocarbons. This suggests that at higher temperatures the LMW compounds were already depleted in the oil loading floating on the water or that evaporation had taken place during WAF production.

Additionally, the temperature-dependent physiology of the test organisms may have influenced the sensitivity towards oil. A toxic effect level is reached when internal concentrations exceed the CBR. In the case of a general acutely toxic, narcotic effect, the concentration of organic contaminants within the lipid bilayer of the cell membrane of an organism exceeds a certain molar threshold concentration (Redman et al. 2022, Van Wezel & Opperhuizen 1995). Petroleum hydrocarbons generally exert such a nonpolar narcotic mode of action (De Laender et al. 2011). In addition to partitioning into membranes, hydrophobic contaminants also accumulate in the storage lipids of organisms (Van Wezel & Opperhuizen 1995), where they, however, will not exert any toxicity. Membrane and storage lipids may differ in lipid/water partition coefficients, but both are inversely related to temperature (Van Wezel & Opperhuizen 1995, Van Wezel et al. 1996,), just like bioaccumulation factors (Muijs & Jonker 2009b). Furthermore, the membrane composition may be adapted by organisms in response to a changing temperature, in order to maintain membrane fluidity (Hazel 1997). Considering these processes, the higher CBR values at lower temperature observed in the present, as well as a previous study (Van Wezel & Jonker, 1998), may be explained as follows. First of all, uptake kinetics are slower at low temperatures, due to reduced molecular diffusion and organism activity, explaining the observed longer times to death (Fig. 3). At lower temperatures partitioning of oil constituents to lipids is enhanced, thereby increasing chemical mass transport and transport times, adding to an increase in the time of death. During the longer exposure, petroleum hydrocarbons will also accumulate in storage lipids, thereby increasing internal concentrations and the final CBR. Note that part of the accumulated mass thus does not concern a ‘toxic mass’ (i.e., chemicals associated with the non-target storage lipids), but these are included in the total lipid-based CBR (Van der Heijden et al., 2015).

The reduced activity of the organisms observed at the lowest temperature (4°C) agrees with findings from other studies. For instance, an increase in test temperature from 15°C to 20°C resulted in an accelerated and condensed life cycle of *G. locusta* (Neuparth et al. 2002), whereas the copepod *Tigriopus japonicus* and the rotifer *Brachionus koreanus* even displayed a dormant behaviour when tested at a lower test temperature of 4°C (Li et al. 2014). In the present study, a higher oxygen consumption was observed at 20°C, as compared to that at the lower test temperatures (Fig. S8). The increased activity may result in faster chemical uptake, e.g. due to increased ventilation. Hence, oil fate (dissolution and evaporation), as well as the physiology and activity of the test organism are all influenced by temperature, potentially explaining the observed differences in gammarid sensitivity at the different exposure temperatures.

To the best of our knowledge, the present study is the first to report CBRs for TPH. To contextualise the obtained CBRs in terms of narcotic effects, the values were translated to lipid-based molar concentrations, by assuming the C_{14}-C_{16} fraction to be the most abundant HB in the exposed gammarids (this fraction represents 31-37% of the overall TPH). Using the molecular weight of *n*-pentadecane (C_{15}) as a representative of this HB, resulted in an estimated CBR of 28, 43 and 49 mmol/kg lipid for 20°C, 12°C and 4°C, respectively. These CBRs differ less than a factor two and are at the lower end of the 40-160 mmol/kg lipid range, as defined for polar and nonpolar narcotic compounds causing mortality in a wide variety of aquatic organisms (Van Wezel & Opperhuizen 1995, Van der Heijden et al. 2015). Although they are somewhat low, these values make sense and, considering the crudeness of the calculation, can be considered acceptable.

Sensitivity of temperate versus Arctic gammarids

The Arctic gammarids had a three times higher LC50 value than the temperate gammarids, when tested under the same conditions at 4°C. This difference is equal to the previously reported differences for marine species, as obtained by modelling (De Hoop et al. 2011, Olsen et al. 2013, Bejarano et al. 2017). However, when compared to the temperate LC50 obtained at 12 and 20°C, the Arctic LC50 (obtained at 4°C) was 4-6 times higher. A similar factor of 3-6 higher LC50 (so lower toxicity) was found for the Arctic copepod *C. glacialis* compared to the boreal *C. finmarchicus* after exposure to marine diesel oil at 2 and 10°C, respectively (Hansen et al. 2013), and for Arctic and temperate copepod and shrimp species, when exposed to produced water (i.e., the discharge from oil and gas production), at their ambient temperatures (Camus et al., 2015). However, a smaller factor of 1.3 in difference was found when *C. glacialis* and *C. finmarchicus* were exposed to a WAF of weathered crude oil (Hansen et al. 2011) and when the toxicity of multiple Arctic and temperate species were considered, after exposure to produced water (Camus et al., 2015). Overall, the observed factor of 2-3 difference in LC50s based on the compiled and modelled toxicity data for oil (De Hoop et al. 2011, Olsen et al. 2013, Bejarano et al. 2017) slightly underestimated the lower sensitivity of the Arctic gammarids to oil that was observed in the present study. The CBR of the tested Arctic gammarids, however, was similar to that of the temperate gammarids, although a longer exposure time and a higher exposure concentration was required to reach the CBR, even at the same temperature of 4°C. Such a need for modifications of test methods has also been reported in previous studies with test species from the polar regions (Chapman & Riddle 2005, Olsen, et al. 2011, Hansen et al., 2011).

The differences in sensitivity and the required test modifications are probably related to the morphological and physiological adaptations of Arctic crustaceans to their cold environment, such as larger body size, higher lipid content, adapted lipid composition and differences in activity. These factors affect the accumulation rate leading to the acute toxicological responses of the animals to organic contaminants, such as oil (Camus 2014). The Arctic gammarids in the present study had a factor of 2-3 higher body weight than their temperate equivalents. A larger body size implies a longer time needed until enough oil has partitioned to the cell membranes to reach the CBR and produce narcotic effects (Fig. S9). Still, in a recent modelling study, only a limited influence of weight on the final hydrocarbon concentrations in organisms was found, although it should be noted that this conclusion was drawn on the basis of pooled data from different literature sources (Redman et al. 2022).

Besides size and weight, the total lipid content is generally higher in Arctic than in temperate species. In our study, the total lipid % of the Arctic *Gammarus* sp. was almost two times higher than in temperate gammarids. The total lipid % of the Arctic *Gammarus* sp. in the present study

(1.6-3.6 % lipid based on wet weight (ww)) was in the same range as the 0.9-2.6 % lipid based on ww that was recently reported for the Arctic *G. setosus* on Svalbard (Skogsberg et al. 2022). In other studies, Arctic copepods and the arctoboreal *Thysanoessa inermis* krill species also had a 2-5 times higher lipid % than their equivalent temperate and subtropical counterparts (Kattner & Hagen 2009, Huenerlage et al. 2016). Moreover, the lipid composition of Arctic and temperate invertebrate species may differ. Arctic and arctoboreal crustaceans have been observed to contain a higher percentage of wax esters and triacylglycerol (i.e., nonpolar storage lipids) compared to their counterparts from lower latitudes. In the arctoboreal euphausiid *T. inermis*, approximately 65% of its total lipid pool consisted of wax esters and triacylglycerol, which was substantially more than in the boreal-subtropic *Nematoscelis megalops* (~15% wax esters and triacylglycerol; Huenerlage et al. 2016). As a result, Arctic species contain a lower relative (though not necessarily absolute) phospholipid% (Huenerlage et al. 2016). Both wax esters and triacylglycerol are used for energy storage (Falk-Petersen et al. 2000, Huenerlage et al. 2016). The higher total lipid content, the higher storage-to-membrane lipid ratio, and the difference in membrane composition in Arctic crustaceans, compared to their temperate counterparts, will have consequences for the CBR and the time needed for uptake and distribution of narcotic compounds within the organisms up to the CBR level (Fig. S9; Van der Heijden et al. 2015; Hazel 1997). Further studies into the role of lipid composition are recommended to advance the understanding of compound fate and thus toxicity in temperate and Arctic species (Van Der Heijden & Jonker 2011; Van der Heijden et al. 2015). Another factor to take into account in future studies is the influence of seasonality on the total lipid content of organisms, e.g. during their reproductive cycle (Skogsberg et al. 2022). For instance, *G. setosus* showed the highest total lipid content between June and August (>2% based on ww) and lower lipid percentages in early spring (<2%) (Skogsberg et al. 2022). Although this may not have large consequences for their overall sensitivity, it will assist in further finetuning the knowledge on the sensitivity of Arctic species.

The observed LC50 value of 1.0 mg TPH/L in the Arctic amphipods in this study is similar to, though somewhat lower than, earlier published LC50 values for oil in Arctic marine species; i.e., 2.3-9.6 mg TPH/L for the copepod *Calanus glacialis* (Hansen et al. 2013), 1.6-4.0 mg TPH/L for *C. glacialis*, juvenile Arctic cod, *Boreogadus saida*, and larval sculpin, *Myoxocephalus* sp. (Gardiner et al. 2013), and 4.6 ± 2.9 mg TPH/L for arthropods (Camus 2014). The present value being somewhat lower may be the result of the selected oil type for the tests, as DMA marine gas oil has been found to be more toxic compared to other gas and crude oils (Foekema et al. 1997). Testing oil is challenging and the results will depend on the test species, the type of oil, its degree of weathering, the WAF production procedure, and the exposure conditions and duration (Faksness et al. 2008). As such, one should be careful when directly comparing oil toxicity data from different studies.

Concluding remarks

The observed differences in sensitivity between temperate and Arctic gammarids were within a factor of 3-6, when expressed in terms of LC50. This is similar to the factor of five, which was considered acceptable for risk assessment purposes in modelling studies (Hendriks et al., 2001). The differences in sensitivity in terms of the CBR, however, were less than a factor two. Therefore, this study further supports the use of temperate acute oil toxicity data as a proxy for the sensitivity of species living in the Arctic region. This finding is highly relevant, because the risks of oil spills in the Arctic are increasing, while at the same time empirical oil toxicity data for the Arctic are limited. However, when applying temperate toxicity data to the Arctic, the effects of temperature on the availability of petroleum hydrocarbons, as well as organism traits should be considered.

This study only concerned acute, narcotic effects, and it should be noted that oil can also cause adverse effects by direct smothering of organisms, by inducing specific toxicity, or by causing sublethal effects upon chronic exposure to low concentrations (Stark et al. 2017, Szczybelski et al. 2018). Obviously, all such potential effects should be included when assessing or modelling the potential risks of oil in Arctic environments.

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Supporting Information (SI)

Text

Text S1 - Correlation fits between oil loadings and TPH concentrations in the produced WAFs

All relevant data on DMA loadings and TPH concentrations from this project, thus including pilot experiments, were included in constructing the correlation fits. The correlation between the oil loadings and the TPH concentration of the WAFs was not linear. Although not specifically developed to described oil concentrations in water, the standard equation for 'one site total binding' included in Graphad Prism 9.5.0 described the non-linear relationship most accurately (R2 values between 0.92-0.95). We used this equation to calculate the exposure concentration during the LC50 experiments. See equation and parameters below.

Equation 'one site total binding' included in Graphad Prism 9.5.0:

$$Y=Bmax * X/(Kd+X)+NS * X+Background$$

Y is in our application the concentration (μg/L) of TPH in the WAF
X is in our application the concentration (ml/L) of DMA oil in the WAF
Bmax is in our application the maximum concentration of THC in the WAF [originally the maximum specific binding]
Kd is the equilibrium dissociation constant, in the same units as X.
NS is the slope of nonspecific binding in Y units divided by X units.
Background value in our application indicates the LoQ of the THC analyses.

Text S2 - LC50 calculation

The LC50 values from the experiments were determined by using the equation for a dose response curve with variable slope, as included in Prism Graphpad 9.5.0. The survival data were normalized to values between 0 and 100%.

Equation Dose response (log(agonist) vs. normalized response) included in Graphad Prism 9.5.0:

$$Y=100/(1+10^{(LogEC50-\log X)})$$

Y= percentage observed mortality
X= exposure concentration

Tables

Table S1. *Applied DMA loadings in the Water Accommodated Fractions (WAFs) and corresponding Total Petroleum Hydrocarbon concentrations (µg/L, based on fitted curves) for each of the LC50 and CBR experiments with temperate and Arctic gammarids. For fitted curves, see Fig.S1.*

	Temperate									**Arctic**		
	4°C	**4°C**	**4°C**	**12°C**	**12°C**	**12°C**	**20°C**	**20°C**	**20°C**	**4°C**	**4°C**	**4°C**
DMA (ml/L)	**LC50**	**CBR**	**LC50**	**LC50**	**CBR**	**LC50**	**LC50**	**CBR**	**LC50**	**LC50**	**CBR**	**LC50**
0	157	157	157	66	66	66	158	158	158	157	157	157
0.001	192			134		134	162		162			
0.002						193	166		166			
0.0025									167			
0.003	257		257	245		245	169		169			
0.00475									176			
0.0065						386	182	182	182			
0.01	438		438	484		484	195			438		
0.02							230					
0.03	742		742	743	743	743				742		
0.1	1105	1105	1105	952						1105		
0.3	1332	1332		1109	1109			830		1332		1332
0.6												
1											1537	1537
2											1724	
3												1897
10												3068

Table S2. *Fitted curves for DMA loadings in the Water Accommodated Fractions (WAFs) and measured Total Petroleum Hydrocarbon (TPH) concentrations in these WAFs, constructed per test temperature.*

	4°C	**12°C**	**20°C**	**All temperatures**
One site -- Total				
Best-fit values				
Bmax	1257	952.2	1500	1258
Kd	0.03506	0.01309	0.4132	0.09146
NS	165.9	435.8	135.3	161.0
Background	156.8	65.57	158.1	171.0
95% CI				
Bmax	934.3 to 1640	707.5 to 1260	947.5 to 3292	986.0 to 1582
Kd	0.006478 to 0.1362	0.003631 to 0.06043	0.1050 to 2.188	0.02644 to 0.2268
NS	93.18 to 232.9	265.5 to 577.9	-4.917 to 203.2	110.9 to 209.0
Background	-66.54 to 347.9	-112.9 to 225.7	48.77 to 264.6	65.95 to 266.1
Goodness of Fit				
Degrees of Freedom	19	13	26	66
R squared	0.9230	0.9509	0.9465	0.9016
Sum of Squares	1026831	301048	1067958	3951296
Sy.x	232.5	152.2	202.7	244.7

Table S3. *Calculated LC50 values for the temperate Gammarus locusta exposed to WAF (DMA) at three test temperatures (4 °C, 12 °C and 20 °C), and Gammarus sp. (Arctic) tested at 4 °C. TPH concentrations are derived from fitted temperature-specific concentration curves. As mortality was used as endpoint in the experiments, all EC50s in the table are in fact LC50s.*

	Arc-4C	**Temp-4C**	**Temp-12C**	**Temp-20C**
Best-fit values				
LogEC50*	3.016	2.529	2.398	2.221
HillSlope	6.378	6.247	6.800	90.12
EC50*	1038	338.0	250.0	166.5
95% CI				
LogEC50*	2.981 to 3.046	2.507 to 2.551	2.380 to 2.418	2.220 to 2.223
HillSlope	4.741 to 8.839	5.182 to 7.625	4.857 to 11.60	59.64 to 159.6
EC50	956.9 to 1111	321.0 to 355.8	240.0 to 261.7	165.8 to 167.0
Goodness of Fit				
Degrees of Freedom	61	51	63	58
R squared	0.9094	0.9593	0.9296	0.8278
Sum of Squares	11598	4256	8763	17136
Sy.x	13.79	9.136	11.79	17.19

Table S4. *Individual wet weights, dry weights and lipid % of gammarids of the populations that were used in the toxicity experiments, based on samples with multiple individuals.*

Location	**Species**	**Size range (mm)**	**# of samples**	**# of individuals per sample**	**Mean (stdev) individual wet weight (mg)**	**Mean (stdev) individual dry weight (mg)**	**Mean (stdev) lipid % (of dw)**
Temperate	*G. locusta*	1-7	3	204 - 1000	21 (± 9.9)	5 (± 2.0)	4.3 (± 2.2)
		8-15	3	52 - 86	51 (± 5.5)	13 (± 1.6)	4.0 (± 1.8)
		16-20	3	1-4	128 (± 19.8)	28 (± 3.9)	6.0 (± 3.3)
Arctic	*Gammarus* sp.	<16*	3	200 - 237	49 (± 17.7)	12 (± 4.4)	11.9 (± 2.0)
		16-20	2	120 -158	158 (± 57.4)	37 (± 15.9)	9.7 (± 0.8)
		21-25	2	30-107	228 (± 15.9)	56 (± 15.1)	7.0 (± 0.8)
		>26	2	12 - 20	402 (± 43.1)	99 (± 11.1)	7.0 (± 0.2)

*this smallest size range was collected near Longyearbyen, Isfjorden.

Figures

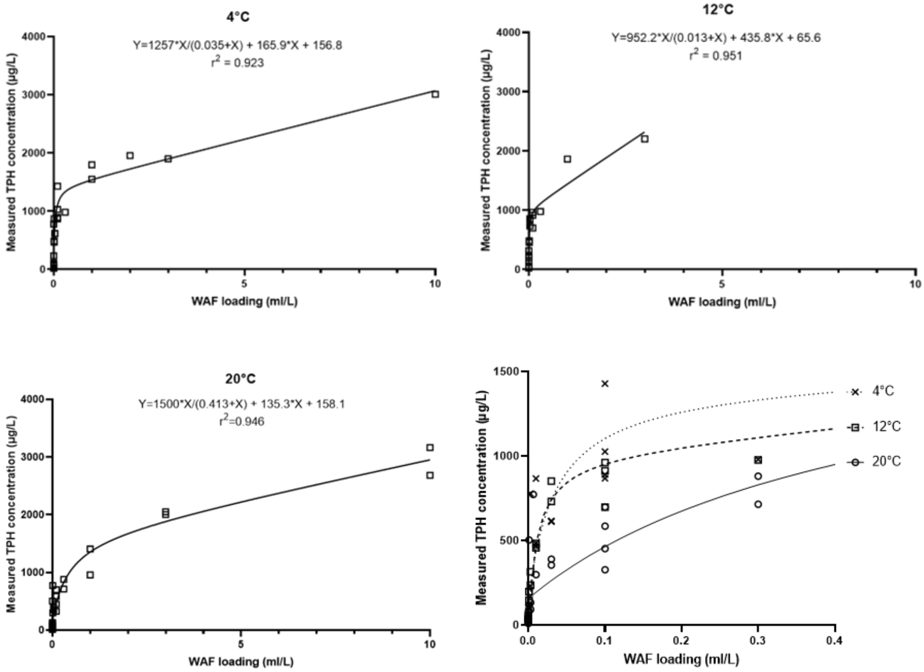

Figure S1. Fitted curves of measured TPH concentrations (µg/L) versus WAF loadings (mL/L) for each of the test temperatures (4 °C, 12 °C and 20 °C) for the LC50 and CBR experiments with Gammarus sp.; and a comparison of all the fitted curves for the different test temperatures (up to a WAF loading of 0.4 ml/L for better comparison of the lower loadings).

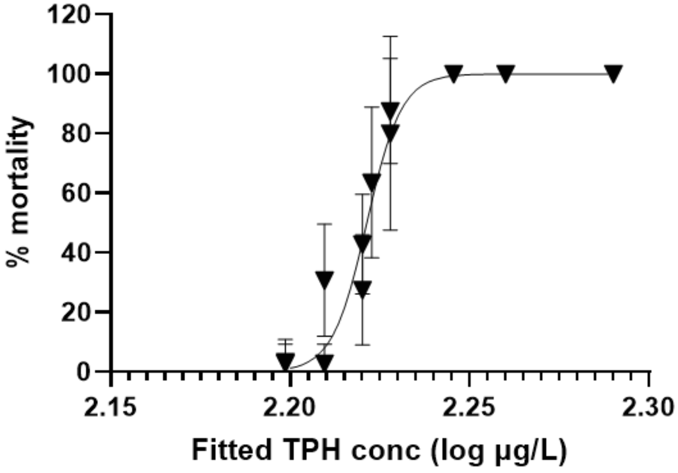

Figure S2. Concentration-response curve of Gammarus locusta (temperate) exposed to different concentrations of Water Accommodated Fractions at 20°C (R^2=0.83). The graph is also shown in Figure 1 of the manuscript, but is plotted here at a different x-axis scale.

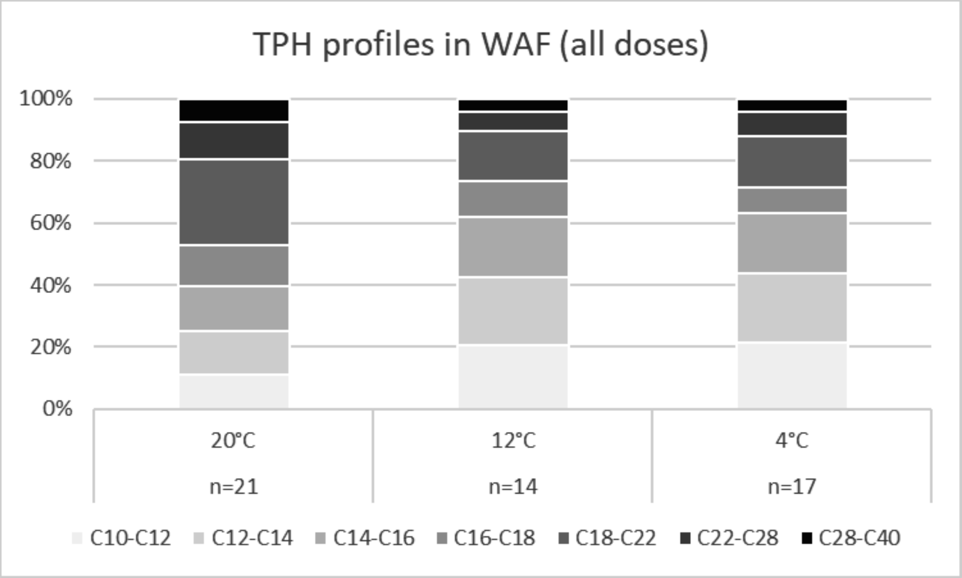

Figure S3. *Hydrocarbon block distribution profiles (relative contributions of different boiling point fractions) per temperature for all DMA loadings in the WAF, as produced in artificial sea water after 48 hours of gentle stirring in the dark at test temperatures of 20 °C, 12 °C and 4 °C.*

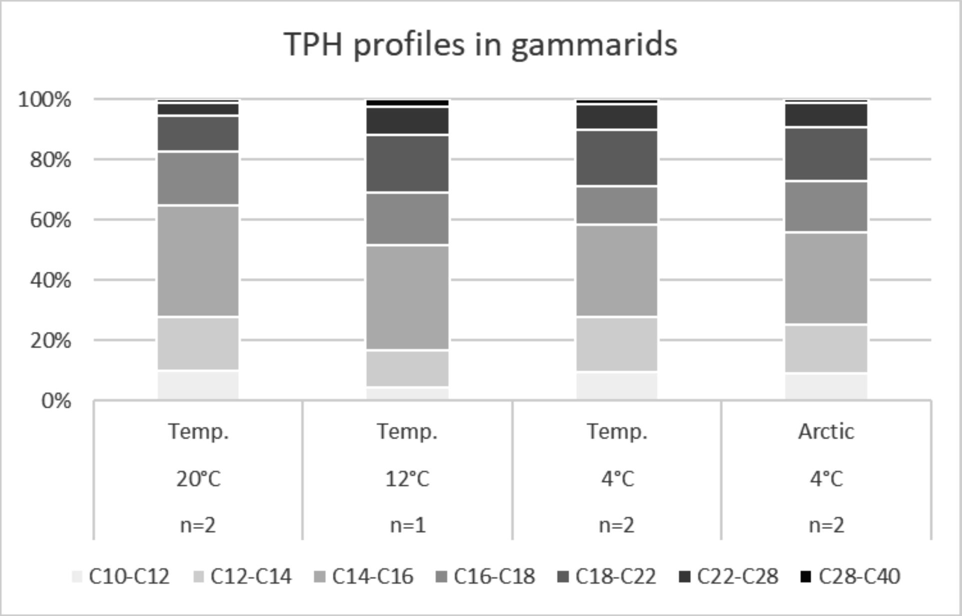

Figure S4. *Hydrocarbon block distribution profiles (relative contributions of different boiling point fractions) in extracts from the temperate G. locusta, exposed to oil (DMA) in the CBR experiment at 20 °C, 12 °C and 4 °C, and the Arctic Gammarus sp. tested at 4 °C.*

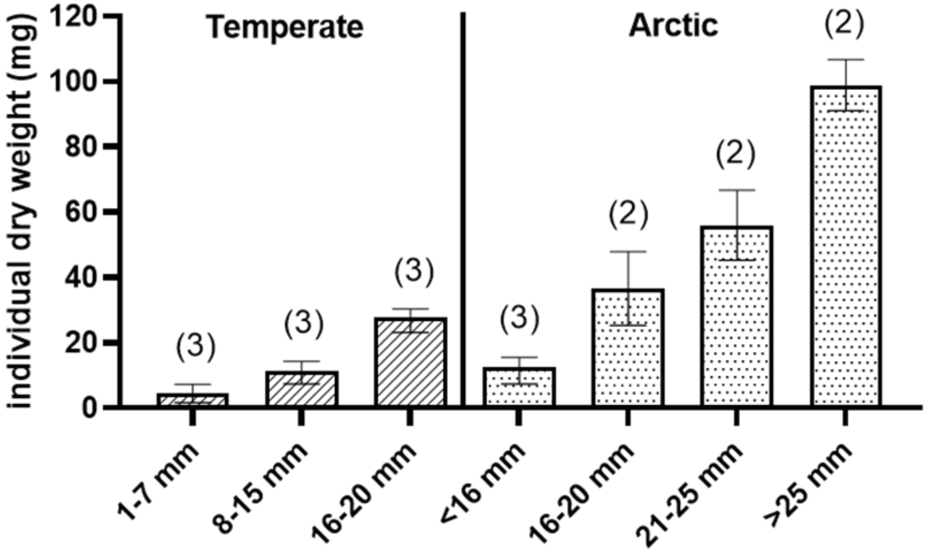

Figure S5*. Individual body weight (mg dw) versus length class for the temperate gammarids (G. locusta; bars with diagonal lines) and Arctic gammarids (Gammarus sp.; dotted bars). Error bars represent standard deviations and the numbers in brackets above bars indicate the number of samples (see Table S4).*

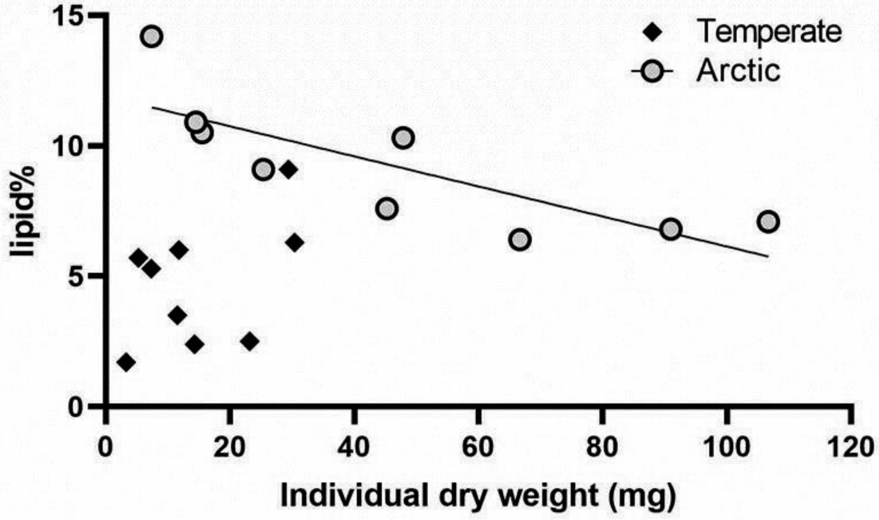

Figure S6*. Total lipid concentration (% lipid of total dry weight) in temperate (G. locusta; diamonds) and Arctic (Gammarus sp.; circles) gammarids of the populations that were used in the toxicity experiments (see Table S4). The Arctic trendline is significant (p= 0.0092); for the temperate data points a significant trendline could not be drawn.*

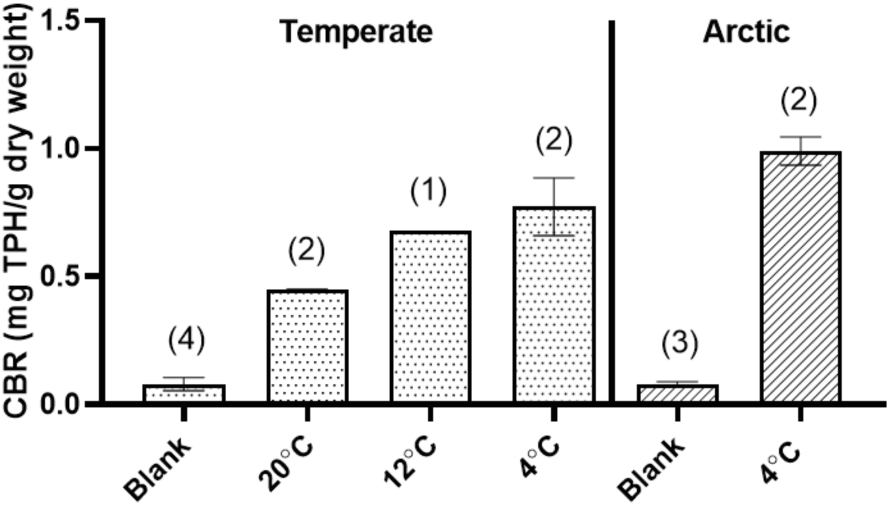

Figure S7. *Critical Body Residue (CBR) of TPH (Total Petroleum Hydrocarbons) (mg/kg dry weight) in the temperate G. locusta (dotted bars) after a 48-hr exposure to oil (DMA) at test temperatures of 4 °C (n=2), 12 °C (n=1) and 20 °C (n=2), compared with the CBR in the Arctic Gammarus sp. (n=2) (bars with diagonal lines) at 4 °C. Error bars represent the range and the numbers in brackets indicate how many times the experiment were reproduced.*

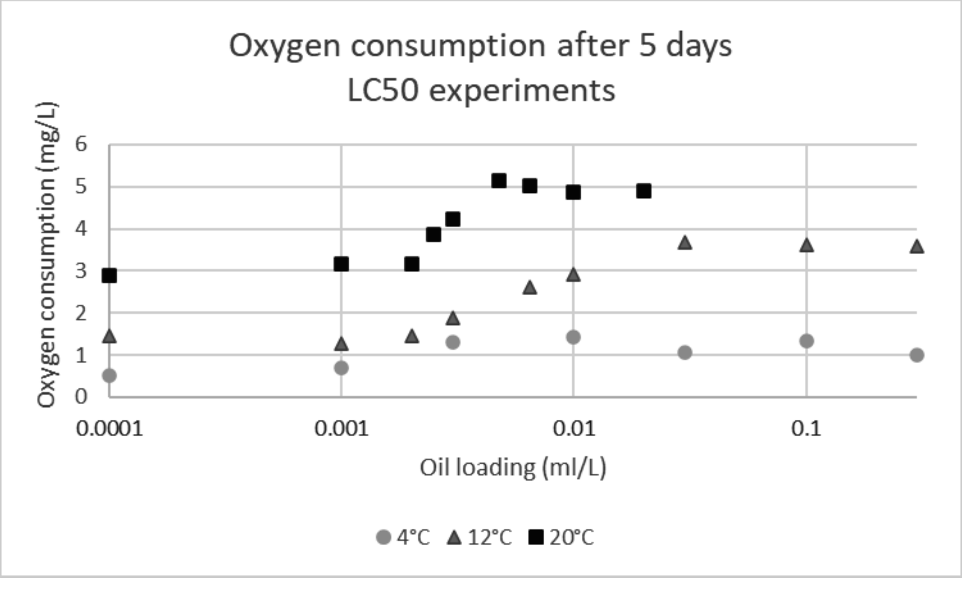

Figure S8. *Oxygen consumption (mg/L) at the end of the LC50 experiments with the temperate G. locusta exposed to DMA WAF for five days, when tested at 4 °C, 12 °C and 20 °C. The loading of 0.0001 mL/L represents the blanks (0 mL/L). Note the x-axis is on a log scale.*

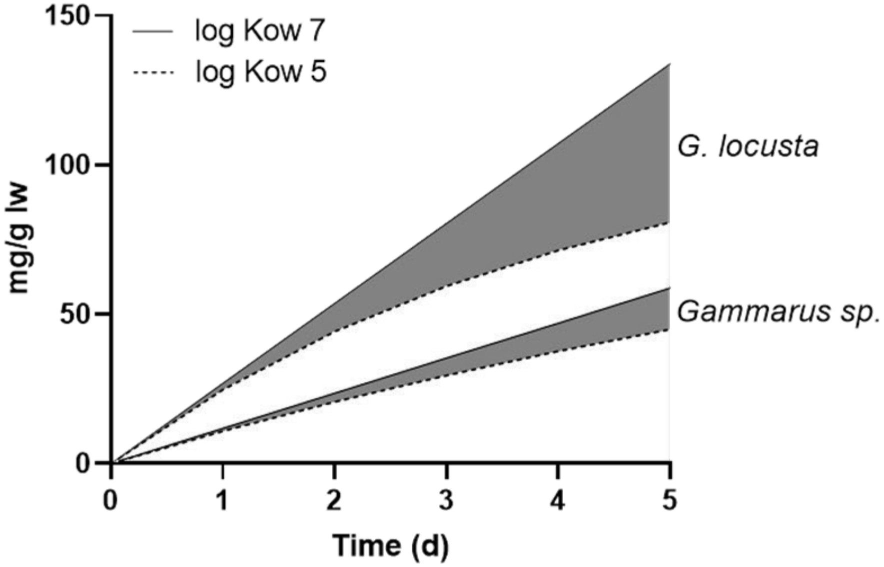

Figure S9. *Modelled Critical Body Residues (mg/g lipid weight) for hydrocarbons with a logK$_{ow}$ of 5 and 7 (as TPH representatives) in the temperate G. locusta (upper lines) and Arctic Gammarus sp. (lower lines,) taking into account the body weight and lipid content. Calculations were done for an oil loading of 1 mL/L, using the OMEGA model (Hendriks et al. 2001).*

CHAPTER 4

LOW ORGANOTIN CONTAMINATION OF HARBOUR SEDIMENT IN SVALBARD

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Abstract

Arctic sea routes are opening up for maritime transport due to sea ice retreat leading to increasing human activities in the Arctic and concomitant pressures on the environment. Organotin compounds are used in antifouling paints of large seagoing vessels and are known to leach into the marine environment and accumulate in sediments and biota. As organotin levels in Svalbard sediments have not been documented in peer-reviewed literature before, this study describes the levels in sediment of harbours around Svalbard (Ny-Ålesund, Longyearbyen, Svea, Pyramiden and Barentsburg). Organotin levels in sediments of Svalbard harbours were low (below the detection limit up to 14 ng Sn/g dw sum-butyltin) compared to other Arctic regions with a longer history of shipping. Levels were below known no effect levels and in accordance, no imposex was found in marine whelks from Ny-Ålesund harbour. Of all other analysed compounds in sediments of Kongsfjorden (polycyclic aromatic hydrocarbon (PAHs), polychlorinated biphenyls, polybrominated diphenylethers and perfluorinated compounds) PAH levels were highest and in one sample above action levels. It is advised to continue monitoring contaminant levels, for which the current results form a good basis. If contaminant levels rise, mitigation measures can be taken in time.

Introduction

Arctic sea routes are opening up for maritime transport due to sea ice retreat. This leads to increasing human activities in the Arctic and concomitant pressures on the environment (Stępień et al. 2014). Local sources of contaminants are closely linked to activities in the area such as shipping, mining and harbour activities and may cause local hotspots of contamination. Contaminants are also transported to the Arctic region from diffuse sources far away by evaporation and air currents, or via rivers and ocean currents (AMAP 1998, 2010), causing widespread contamination of pristine areas, and in particular the marine environment (MacDonald et al. 2000; Letcher et al. 2010; Andersen et al. 2015).

Svalbard is an archipelago in the Barents Sea, bordering the Greenland Sea in the west and the Arctic Ocean in the north, about midway between continental Norway and the North Pole. Svalbard has a few human settlements consisting of the main settlement of Longyearbyen, the Russian mining community of Barentsburg, the historical mining settlement of Pyramiden, the research station of Ny-Ålesund, and the Norwegian mining community of Svea. The main industries on Svalbard are coal mining, tourism, education and research.

Local sources of contamination in Svalbard are a consequence of local settlements, shipping and (historical) coal mining (Kozak et al. 2013). Persistent contaminants such as polycyclic aromatic hydrocarbon (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) and perfluorinated compounds (PFCs) are associated with local settlements and coal mining on Svalbard (Hop et al. 2001; Evenset et al. 2006a, 2009). Contaminants related to shipping include air pollution (such as PAHs, fine particles, S and NOx), waste (solid and liquid) and organotin compounds (OTCs), such as tributyltin (TBT; Van Aardenne et al. 2013; Eckhardt et al. 2013; Matthiessen 2013). Organotin compounds are biocides that are added to paint used on ship hulls to prevent hull fouling and thereby decrease drag and fuel consumption (Lindholdt et al. 2015). These anti-fouling compounds leach into the marine environment and have caused shipping-related accumulation of TBT in sediment resulting in the chronic exposure of aquatic organisms (Matthiessen 2013). A well-known effect of TBT exposure is imposex in gastropod molluscs such as marine whelks (Mensink et al. 2002). Imposex is the formation of male characteristics in female snails. It was estimated that about 150 species showed signs of imposex worldwide (Matthiessen et al. 1999). Since the 1960s, TBT has been applied as anti-fouling biocide in paint, until in 2003, the International Maritime Organization (IMO) banned new application of TBT coatings on ships and since 2008 no TBT is allowed to be present on ships (IMO 2001). Not all countries have ratified the treaty yet. In 2012, some 61 contracting states representing 80 % of the worlds tonnage had ratified the treaty (IMO 2012). New input of TBT from TBT-coated ships cannot be excluded in the Arctic in addition to persistent historical TBT pollution. As TBT slowly degrades over time into dibutyltin (DBT) and finally into monobutyltin (MBT), the relative presence of these three compounds can indicate how recent the TBT input was. For this purpose, the Butyltin Degradation Index (BDI) was developed (Diez et al. 2002).

In Ny-Ålesund harbour, imposex has been reported once for the marine snail *Buccinum undatum* in the mid-1990s (Brick and Bolte 1994). There is, however, no peer-reviewed information available on past or current organotin levels in sediments or biota of Svalbard harbours, nor has imposex been studied further. Organotin contamination and imposex have been studied and identified in other Arctic harbours in Greenland (Jacobsen and Asmund 2000; Strand and Asmund 2003; Strand et al. 2006), Iceland (Skarphédinsdóttir et al. 1996; Svavarsson 2000) and Alaska (Tallmon 2012).

This study focuses on providing insight into organotin contamination of sediments of Svalbard harbours and the occurrence of imposex in marine whelks (*Buccinum* sp.) of Ny-Ålesund harbour, Kongsfjorden. In addition, levels of other contaminants (PAHs, PCBs, PBDEs and PFCs) were analysed in the sediment of several locations within Kongsfjorden to get a further understanding of local inputs of contaminants in this Svalbard fjord. This will provide a basis to assess pressures and impacts of current and future activities in Svalbard in order to develop relevant and effective mitigation measures in the future.

Materials and methods

Sediment sampling

Sediment in and near harbours of Svalbard was collected in 2009, 2012 and 2013 using a Van Veen grab (Table 1; Fig. 1). In Kongsfjorden (in 2012 and 2013), sediment was sampled at three sites (Ny-Ålesund harbour, Ny-Ålesund Thiisbukta and Kongsfjorden reference). Sampling location Ny-Ålesund Thiisbukta is located in the intertidal part of a bay adjacent to the west of Ny-Ålesund harbour and close to an abandoned waste dump on land. In Kongsfjorden, the upper 5 cm sediment layer from five grabs was mixed and homogenized on board and two subsamples of 200 ml each of this homogenate were stored in pre-rinsed 250-ml glass jars with aluminium foil between sample and lid. Sediment at Ny-Ålesund Thiisbukta was collected directly from shore during low tide. Five sediment surface samples were mixed and homogenized and two subsamples of 200 ml each from this location were stored. In Longyearbyen and Svea (in 2013), sediment from four grabs was mixed and homogenized on board and two subsamples were stored according to the same procedures used in Kongsfjorden. In Pyramiden and Barentsburg (2009) the sampling procedure was slightly different; the upper 0–1 cm from one grab at each sampling station was taken out using a small Plexiglas core and stored directly in pre-cleaned glass jars or polypropylene containers. All sediment samples were frozen to −20 °C immediately after sampling until further analysis.

Sampling of organisms

Nineteen individuals of *Buccinum* sp. were sampled using baited traps in Ny-Ålesund harbour (near the old coal pier) in July 2013. Snails were kept alive and cool prior to imposex staging. Snails were examined for imposex occurrence using standard procedures (OSPAR Commission 2009).

Chemical analysis

Organotin analysis in sediments consisted of MBT, DBT, TBT, monophenyltin (MPhT), diphenyltin (DPhT) and triphenyltin (TPhT), using an RvA accredited method (Verslycke et al. 2005). In short, tripropyltinchloride was added to the samples as internal standard after which the organotin compounds were extracted by adding methanol, acetic acid and hexane. Compounds were subsequently derivatized using sodium tetraethylborate (4 % in water) after which sodium hydroxide was added to the samples. The hexane layer containing the derivatized compounds was then cleaned by eluting over an aluminium oxide column with pentane. Extracts were concentrated to one ml using a turbovap and subsequently analysed by GC–MS.

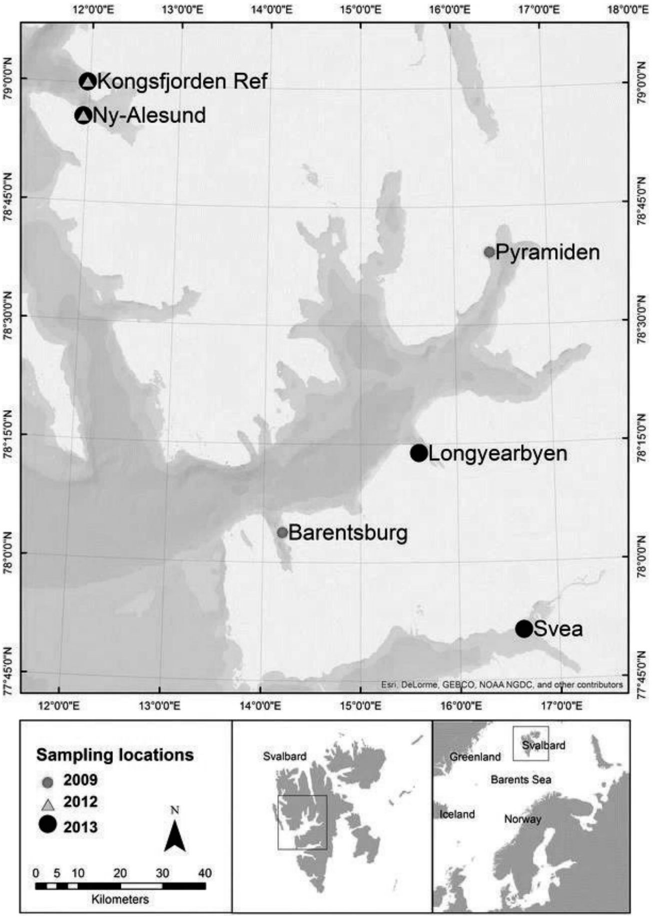

***Figure 1**. Sediment sampling locations in harbours around Svalbard (2009–2013).*

PFCs were analysed according to the procedure by Kwadijk et al. (2010). In short, samples were extracted using acetonitrile and cleaned up using hexane and ENVI-Carb. Extracts were analysed using LC–ESI–MS.

PBDEs and PCBs were extracted using the accelerated solvent extraction technique with in-cell clean-up using florisil. Extraction took place over three cycles using a static time of 10 min with a mixture of pentane: dichloromethane (85:15) at 40 °C. Extracts were concentrated to 1 ml.

For PCBs, 1 μl of sample was injected on a Shimadzu GCMS2010 (GC) coupled to a GCMS-QP2010 Ultra (MS) detector (Shimadzu, 's Hertogenbosch, the Netherlands). Column used was a 30 m × 0.25 mm i.d. HT8 with a film thickness of 0.25 μm. Detection was performed using electron impact (EI) in single ion monitoring (SIM) mode. Injection port and source temperatures were 250 and 200 °C, respectively. Oven temperature program started at 90 °C, hold for 3 min, increased by 20 °C/min to 170 °C followed by an increase by 2.5 °C/min to 292 °C. At the end of the program, a column was heated to 320 °C for 10 min. The following

***Table 1**. Sediment sampling locations in harbours around Svalbard (2009–2013).*

Year	Date	Sampling site	Specifics	Fjord	Depth (m)	N coordinate	E coordinate	# Samples OTC analysis	# Samples additional
2009	24-06-2009	Pyramiden	Harbour	Billefjorden	14	78,392	16,231	1	
2009	24-06-2009	Pyramiden	Harbour	Billefjorden	18	78,389	16,234	1	
2009	24-06-2009	Pyramiden	Harbour	Billefjorden	42	78,386	16,246	1	
2009	24-06-2009	Pyramiden	Harbour	Billefjorden	45	78,390	16,252	1	
2009	24-06-2009	Pyramiden	Harbour	Billefjorden	165	783,585	163,080	1	
2009	22-06-2009	Barentsburg	Harbour	Grønfjorden	70	780,380	141,200	1	
2009	22-06-2009	Barentsburg	Harbour	Grønfjorden	40	780,530	141,010	1	
2009	22-06-2009	Barentsburg	Harbour	Grønfjorden	112	780,270	141,165	1	
2009	22-06-2009	Barentsburg	Harbour	Grønfjorden	95	780,570	140,840	1	
2013	12/10/2013	Longyearbyen	Harbour	Adventfjorden	45	781,390	153,630	1	
2013	12/10/2013	Longyearbyen	Harbour	Adventfjorden	45	781,390	153,630	1	
2013	12/10/2013	Longyearbyen	Harbour	Adventfjorden	23	781,374	153,650	1	
2013	12/10/2013	Longyearbyen	Harbour	Adventfjorden	23	781,374	153,650	1	
2013	10/9/2013	Svea	Harbour	Van Mijenfjorden	40	–	–	2	
2013	10/9/2013	Svea	Harbour	Van Mijenfjorden	25	775,137	163,864	2	
2013	10/9/2013	Svea	Harbour	Van Mijenfjorden	18	775,140	163,853	2	
2012	13-06-2012	Ny-Ålesund	Harbour	Kongsfjorden	20	7,855,816	1,156,544	3	1
2012	13-06-2012	Ny-Ålesund	Coal pier	Kongsfjorden	22	7,855,859	1,155,409	3	1
2012	15-06-2012	Reference	Tonsneset	Kongsfjorden	17	7,900,145	1,158,348	2	1
2012	15-06-2012	Reference	Tonsneset	Kongsfjorden	22	7,900,301	1,156,758	2	1
2013	27-06-2013	Ny-Ålesund	Harbour	Kongsfjorden	21	7,855,816	1,156,544	1	1
2013	27-06-2013	Ny-Ålesund	Coal pier	Kongsfjorden	19	7,855,859	1,155,409	1	1
2013	27-06-2013	Ny-Ålesund	West of coal pier	Kongsfjorden	19	7,855,920	1,153,940	1	1
2013	29-06-2013	Ny-Ålesund	Thiisbukta	Kongsfjorden	0	7,855,572	1,154,420	2	1
2013	28-06-2013	Reference	Blomstrand grotten	Kongsfjorden	19	7,859,406	1,158,764	1	1
2013	28-06-2013	Reference	Blomstrand glacier	Kongsfjorden	19	7,900,006	1,158,674	1	1

quantifier and qualifier ions were monitored, respectively, 256 and 258 for PCB 28, 292 and 290 for PCB 52, 326 and 324 for PCB 101, PCB 112 and PCB 118, 360 and 362 for PCB 138 and PCB 153, 394 and 396 for PCB 180. Recovery was typically between 80 and 110 % for all compounds. Calibration curves consisted of nine points within a range of 1–650 ng/ml. R 2 ≥ 0.999 was achieved for each calibration curve for all compounds.

For PBDEs, 1 µl of sample was injected on an Agilent 6890 GC–MS (Agilent, Amstelveen, the Netherlands) using a 50 m × 0.25 mm i.d. CPsil8 column with a film thickness of 0.25 µm. Injection port and source temperatures were 275 and 200 °C, respectively. Oven temperature program started at 90 °C, hold for 3 min, increased by 30 °C/min to 210 °C followed by an increase by 5 °C/min to 290 °C. At the end of the program, a column was heated to 325 °C for 25 min. M/z 79 and 81 were used as quantifier and qualifier ions, respectively. Recovery was typically between 80 and 110 % for all compounds. Calibration curves consisted of nine points within a range of 0.1–500 ng/ml. R 2 ≥ 0.999 was achieved for each calibration curve for all compounds.

PAHs were analysed according to De Boer et al. (2001). In short, PAHs were extracted from the sediments with soxhlet (hexane/acetone 1:1). The extract is concentrated to 10 ml, cleaned over a silica gel–aluminium oxide column and, after addition of 1 ml of acetonitril, concentrated by evaporation to 1 ml of acetonitril. The PAH levels in the acetonitril solution were then analysed by HPLC equipped with fluorescence detection. acenaphthylene was not measured since it was not part of the standard method used for the measurements of PAH in the samples. Naphthalene was excluded from analyses due to fluctuating high background levels in the blanks which would lead to questionable results.

Limit of detection (d limit) was defined as <5 times the method blank or the lowest used calibration point, whichever is highest. Reference materials and method blanks were analysed with each set of samples. All results for blanks and reference materials were within normal limits.

Data treatment

In cases where the TBT concentration was above d limit in a sediment sample, the BDI was calculated as following (Diez et al. 2002):

$$BDI = ([MBT] + [DBT])/[TBT]$$

In case MBT and DBT concentrations were below d limit, the values for these compounds to be included in the calculation were calculated as 0.5 * d limit.

The PAH concentrations were summed as the 16 US EPA PAHs (sum-16-PAH) (naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Fl), phenanthrene (Phe), anthracene (An), fluoranthene (Flu), pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), indeno[1,2,3 cd]pyrene (Inp), dibenz[a,h]anthracene (DBA) and benzo[ghi]perylene (BgP). Naphthalene and acenaphthylene were, however, not included in the analysis. The PCB concentrations were presented as sum-7-PCB (PCB-28, PCB-52, PCB-101, PCB-118, PCB-138, PCB-153 and PCB-180).

Results

Organotin concentrations

Organotin concentrations in sediments of Svalbard harbours were low (<d limit—14 ng Sn/g dw; Table 2). MBT and DBT were only detected in Kongsfjorden sediment. TBT was present in 20–67 % of the samples, except for samples from Longyearbyen harbour where no TBT was found above d limit. The highest number of samples with TBT was found in Svea harbour (67 % above d limit), whereas highest sum-butyltin concentrations were found in Ny-Ålesund harbour (<d limit—14 ng Sn/g dw). TPhT was only detected in a single sediment sample of Pyramiden harbour (1.4 ng/g dw), whereas MPhT and DPhT were not detected in any of the sediment samples.

A BDI could be calculated for 13 of the sediment samples with a TBT concentration above d limit (Table 3). The BDI for these samples ranged between 0.4 and 8.3. The BDI was <1 in samples of Pyramiden, Barentsburg, Svea and one sample of Ny-Ålesund. The BDI was >1 for most samples of Ny-Ålesund.

Imposex scores

No imposex was found in females of *Buccinum* sp. of Ny-Ålesund harbour (Table 4). Of the investigated 19 individuals, three were males and 16 were females.

***Table 2**. Summarized organotin data in the sediment of Svalbard harbours (2009–2013), dl = d limit.*

			% > dl	% > dl	% > dl	ng/g dw	ng/g dw
Sampling site	**Fjord**	**n**	**MBT**	**DBT**	**TBT**	**Range sum-butyltin (Sn)**	**Range sum-butyltin (Kation)**
Ny-Ålesund - reference	Kongsfjorden	6	0	33	33	<dl-0.5	<dl-1.2
Ny-Ålesund	Kongsfjorden	9	44	33	44	<dl-14	<dl-24.5
Ny-Ålesund - Thiisbukta	Kongsfjorden	2	0	0	50	<dl-2.1	<dl-5.3
Pyramiden	Billefjorden	5	0	0	20	<dl-1.8	<dl-4.4
Barentsburg	Grønfjorden	4	0	0	25	<dl-1.6	<dl-4.1
Longyearbyen	Adventfjorden	4	0	0	0	<dl	<dl
Svea	Van Mijenfjorden	6	0	0	67	<dl-2.1	<dl-4.9

***Table 3**. BDI scores for the sediment of Svalbard harbours (for all samples with [TBT] > d limit) (2009–2013). In case MBT and/or DBT were <d limit, the value was calculated as 0.5*d limit.*

			n > d limit	Range	Median
Sampling site	**Fjord**	**n**	**TBT**	**BDI**	**BDI**
Ny-Ålesund - reference	Kongsfjorden	6	2	1.0-1.3	1
Ny-Ålesund	Kongsfjorden	9	4	0.8-8.3	4
Ny-Ålesund - Thiisbukta	Kongsfjorden	2	1	1.1	1.1
Pyramiden	Billefjorden	5	1	0.4	0.4
Barentsburg	Grønfjorden	4	1	0.4	0.4
Longyearbyen	Adventfjorden	4	0	-	-
Svea	Van Mijenfjorden	6	4	0.5-0.7	0.7

***Table 4**. Imposex scores of Buccinum sp. collected at Ny-Ålesund harbour, Kongsfjorden, in 2013.*

Nr	**Length (mm)**	**Sex**	**Imposex stage**	**Penis length (mm)**
1	29.1	M	-	15
2	23.8	F	0	
3	23.5	M	-	21
4	21.3	F	0	
5	19.8	F	0	
6	30.0	F	0	
7	27.0	F	0	
8	28.1	F	0	
9	28.4	F	0	
10	31.9	F	0	
11	33.1	F	0	
12	29.0	F	0	
13	27.4	F	0	
14	27.5	F	0	
15	25.3	F	0	
16	25.6	F	0	
17	25.6	M	-	18
18	21.7	F	0	
19	23.6	F	0	

Table 5. *PAH concentrations in the sediment of Kongsfjorden, Svalbard (2012–2013), all summed concentrations were above d limit.*

		µg/kg dw	µg/kg dw	µg/kg dw	µg/kg dw
		Geometric mean	Average	SD	Range
Location	**n**	**Sum-16-PAH USEPA**	**Sum-16-PAH USEPA**	**Sum-16-PAH USEPA**	**Sum-16-PAH USEPA**
Ny-Ålesund - reference	4	7	13	12	1-26
Ny-Ålesund harbour	5	1399	1630	859	476-2550
Ny-Ålesund - Thiisbukta	1	16787	16787	0	16787

Table 6. *PCB concentrations in the sediment of Kongsfjorden, Svalbard (2012–2013), dl = d limit.*

			µg/kg dw	µg/kg dw	µg/kg dw
		% > dl	Average	SD	Range
Location	**n**	**Sum-7-PCB**	**Sum-7-PCB**	**Sum-7-PCB**	**Sum-7-PCB**
Ny-Ålesund - reference	4	25	0.14	0.28	<dl-0.57
Ny-Ålesund harbour	5	40	0.54	0.76	<dl-1.61
Ny-Ålesund - Thiisbukta	1	100	0.33	-	0.33

Other contaminants

The sediment sample collected at Thiisbukta contained the highest sum-16-PAH concentration (16,787 µg/g dw), about ten times higher than concentrations in Ny-Ålesund harbour and more than a factor 1000 higher than at the Kongsfjorden reference sites (Table 5). PAH concentrations varied within Ny-Ålesund harbour with sum-16-PAH concentrations being almost two times higher in the sediments around the coal pier of the harbour than in sediments at the commercial pier and further westwards of the coal pier.

PCB concentrations in sediment were low in Kongsfjorden (<d.l.—1.61 ng/g dw sum-7-PCB; Table 6). Highest concentrations were found in Ny-Ålesund harbour (two out of five samples), but there were also concentrations above detection levels at Thiisbukta (one out of one sample) and the reference site (one out of four samples) in Kongsfjorden. All PBDE concentrations in sediment of Kongsfjorden were below d limits. PFC concentrations in sediment of Kongsfjorden were all below d limits, except at one sample at Thiisbukta that contained levels of two PFCs just above detection level (0.2 ng/g perfluorodecane sulphonate (PFDS) dw and 0.2 ng/g perfluorooctane sulphonate (PFOS) dw).

Discussion

Organotin concentrations

Organotin concentrations in sediment of Svalbard harbours were low (<d.l.—14 ng Sn/g dw/24.5 ng kation/g dw for sum-butyltin; 64 % of all sediment samples had all organotin compounds below d limit). TBT levels above d limit varied from 0.3 to 2.9 ng Sn/g dw. Most of the Ny-Ålesund harbour samples (with TBT levels above d limit) had a BDI of >1 suggesting no recent inputs of butyltins in this harbour (Diez et al. 2002), except for one sample with a BDI of 0.8. The latter, however, consisted of a low TBT concentration of 0.3 ng Sn/g dw, and both MBT and DBT concentrations were below d limit. These low concentrations may have resulted in a deviating BDI value, since the other BDI values in Ny-Ålesund harbour were calculated on OTC concentrations mainly above d limit. The few samples of Pyramiden, Barentsburg and Svea with TBT levels above d limits all had a BDI < 1, showing that there may still be some fresh input of TBT in the area. Pyramiden and Barentsburg only had one sediment sample above d limit with a BDI of <1. Svea had four out of six sediment samples above d limit with all showing a BDI of <1. Shipping traffic to Pyramiden is relatively low with only tourist vessels frequenting the area during summer whereas shipping traffic to Barentsburg and Svea is considerably higher with an active coal mine industry at these places. OTC levels in the sediment of another study in Isfjorden (Pyramiden, Longyearbyen, Barentsburg and Colesbukta) confirm low to non-detectable levels of OTC in Svalbard sediments (Evenset et al. 2006a). In this study, two out of 18 sediment samples in 2005 had TBT concentrations above d limit and contained 1.8 ng Sn/g dw (Longyearbyen) and 3.6 ng Sn/g dw (Barentsburg) (Evenset et al. 2006a). Low levels of TBT were explained by seasonally restricted shipping activities around Svalbard due to heavy ice conditions in winter time and a high sedimentation rate, for example in Van Meijenfjord (Svea), covering and diluting sediment concentrations (Cochrane et al. 2001).

Information on OTC concentrations in sediment of Arctic harbours is very limited. In Nuuk harbour, Greenland, sediment concentrations were reported up to 171 ng Sn/g dw for TBT and 180 ng Sn/g dw for sum-butyltin in 1997 (Jacobsen and Asmund 2000). OTCs were not detected in sediment samples of the coastal area of Thule Air Base and Qaanaaq, West Greenland, in 2002 (Strand et al. 2006). This suggests that OTC levels in Arctic sediment are low compared to more populated regions around the world, where the lowest OTC concentrations were reported in the range of up to 27–124 ng Sn/g dw (along the coasts of Italy in 1999–2000, of Japan in 2003, and of Vietnam in 2003; Antizar-Ladislao 2008). Highest TBT concentrations were reported in sediment of an American harbour in 2001–2003 and a Japanese harbour in 2005 (up to 14,000 ng Sn/g dw; Antizar-Ladislao 2008). Even though decreases of OTC concentrations have been reported in water and biota, sediment does not tend to show clear (>20 %) decreasing trends globally since TBT may not be easily broken down in (especially anaerobic) sediment (Matthiessen 2013; Kim et al. 2014).

More information is available on OTC levels in Arctic benthic invertebrates. TBT concentration varied between 0.5 and 4 ng Sn/g ww in *Nucella lapillus* and 5–65 ng Sn/g ww in *Mytilus edulis* near Reykjavík harbour in 1993–1994, whereas DBT concentrations fluctuated around 5 ng Sn/g ww in *N. lapillus* and between 1 and 6 ng Sn/g ww in *M. edulis* (Skarphédinsdóttir et al. 1996). Concentrations of TBT and degradation products in *M. edulis* of Nuuk harbour in Greenland were close to 1 ng Sn/g ww in 1997 (Jacobsen and Asmund 2000). At one station in West Greenland, low OTC levels were detected in biota in 2002, in whelks (3.1 ng Sn/g dw sum-butyltin) and clams (11 ng Sn/g dw sum-butyltin; Strand et al. 2006). In other south-western harbours of West Greenland, organotin concentrations were above detection level in biological samples (bivalves) in 66 % of the samples in 1999–2000, with concentrations up to 283 ng/g ww sum-butyltin (Strand and Asmund 2003). In Alaska, evidence of TBT contamination in mussels collected in 2007–2009 was shown in four out of 10 harbours, with a range of 29–54 ng TBT/g ww in mussels at the contaminated harbours (Tallmon 2012). No OTC levels were measured in biota of Svalbard harbours.

All TBT concentrations in the sediment of Svalbard harbours were below the lowest action level of 3 ng Sn/g dw for dredged materials of OSPAR contracting parties (OSPAR Commission 2004; Schipper et al. 2010) and below no observed effect concentrations (NOEC) of 80 ng Sn/g dw (Stronkhorst and Van Hattum 2003).

A field study in Greenland harbours showed that imposex in gastropods may be a more sensitive biomarker for the presence of TBT in the marine environment than the analysis of TBT in sediment or biota samples (Strand et al. 2006). In the gastropod samples collected in Ny-Ålesund harbour in 2013, none of the 16 female specimen showed any stage of imposex. Imposex occurrence has been found in Ny-Ålesund, Kongsfjorden, in marine snail samples of 1992 (Brick and Bolte 1994). At that time, five out of eight female specimen showed signs of imposex. The BDI in Kongsfjorden suggested that no new sources of TBT were presented in Ny-Ålesund harbour. This may be a further sign that OTC concentrations in Ny-Ålesund harbour have decreased over the past two decades and are currently below effect levels in the harbour. However, other Arctic harbours in Alaska and Greenland did show imposex occurrence (Strand et al. 2006; Tallmon 2012). Imposex development occurred in *Buccinum* sp. in all five sampled harbours in west Greenland in 1999–2000 (Strand and Asmund 2003) and in *Buccinum finmarkianum* at seven of eight marine stations off Thule Air Base in Northwest Greenland in 2002 (Strand et al. 2006). *Nucella lima* samples from harbour sites in Alaska in 2007–2009 exhibited imposex, with 36–87.5 % females affected per site (Tallmon 2012). Studies on imposex in the dogwhelks (*N. lapillus*) in Icelandic waters in 1998 show that levels of imposex have decreased considerably since the early 1990s after banning the use of TBT-containing paints on small vessels (Svavarsson 2000).

Other contaminants

PAHs concentrations were highest of all compounds analysed in Kongsfjorden sediment (1–16,787 ng/g dw), and below lowest European action levels of 2000 ng/g dw for sum-16-PAH for dredged sediment, except for the sediment sample from Thiisbukta (16,787 ng/g dw; OSPAR Commission 2004). The sediment sample of Thiisbukta fell into category 2 (poor/bad) for sum-16-PAH of 2000–20,000 ng/g dw in Norway and were above action level 1 for sum-6-PAH of 1000 ng/g dw in Germany (OSPAR Commission 2004). The sediment sample of Thiisbukta bay consisted of fine sediment with clear black carbon particles which may explain the elevated PAH levels in this sample. In 1997 PAH levels in Kongsfjorden sediment were 927 ng/g dw (Olsson et al. 1998). There are records of several old oil spills in Ny-Ålesund due to leakage from oil tanks or damaged pipes (Skei 1994) with major spills in 1986 and 1990 (Olsson et al. 1998). Additionally, local coal mining related activities may have caused a further input of PAHs into the harbour.

Low levels of PCBs were detected in Kongsfjorden sediments (d.l.—1.6 ng/g sum-7-PCB dw), both in the harbour area and near the dumpsite. Levels are below lowest European action levels of 20 ng/g dw for sum-7-PCB as set for dredged sediment (OSPAR Commission 2004). Low levels of PCBs in Kongsfjorden sediments have been described before in 1991 (0.32 ng/g sum-7-PCB dw; Skei 1994), 1997 (0.07 ng/g sum-7-PCB dw; Olsson et al. 1998) and 2007 (0.17–0.25 ng/g sum-7-PCB dw; Tessmann 2008). Sum-7-PCB concentrations in sediment of other Svalbard fjords were 0.02–3.50 ng/g (Barentsburg), 0.85–4.30 ng/g (Longyearbyen), 0.03–0.82 ng/g (Pyramiden), and 1.84 ng/g (Isfjorden) in September 1998 (Cochrane et al. 2001), 0.70–6.72 ng/g (Barentsburg), 0.16–0.70 ng/g (Longyearbyen) and 1.91–12.4 ng/g (Pyramiden) in 2005 (Evenset et al. 2006a), and 0.10–0.38 ng/g (Longyearbyen), 0.74–5.41 ng/g (Barentsburg) and 1.8–20.2 ng/g (Pyramiden) in 2009 (Evenset et al. 2009). Flux calculations indicated that local sources are important sources outside these settlements (Governor of Svalbard 2008). Elevated levels outside Pyramiden and Barentsburg were probably due to a flooding event in the summer of 2005. High levels of PCB have been measured in soil from these Russian settlements (Jartun et al. 2009), and flood water probably transported contaminated soil to the marine environment.

Other analysed contaminant groups showed levels below d limit in Kongsfjorden sediment, except for the sediment sample at Thiisbukta that showed low levels of PFCs consisting of PFDS and PFOS concentration just above detection level (0.2 ng/g dw). To our knowledge, PFC levels in Kongsfjorden sediment have not been described in peer-reviewed journals before. PFOS concentrations in sediment of Isfjorden varied between 0.10 and 0.54 ng/g dw, whereas sum-PBDE concentrations were between 0.05 and 1.10 ng/g dw in 2005 (Evenset et al. 2006b).

The fact that, of all Kongsfjorden sediment samples, the sampling location at Thiisbukta often showed levels of contaminants above d limit does not come as a surprise. This site is located directly below an old terrestrial garbage dump and may receive contaminants through run-off from the dumpsite area. In a study from 2000/2001 concentrations of PAHs and PCBs in macrobenthos were highest close to the settlement in Kongsfjorden compared to samples taken further out in the fjord, suggesting a local input of these compounds (Hop et al. 2001). Hop et al. (2001) showed that PAH levels in macrobenthos of the main fjords of Svalbard were quite variable both between and within fjords with highest levels in Isfjorden, followed by Grønfjorden, Billefjorden and Adventfjorden, and lowest levels in Kongsfjorden (Hop et al. 2001). Based on the Norwegian guide for classification of fjords, Kongsfjorden macrobenthos was judged insignificantly polluted for PAH (Hop et al. 2001).

Sediment is highly heterogeneous in terms of composition (sediment fractions, organic carbon) and contaminant load as is also shown by this study. Often, a considerable number of sediment samples are needed from each location to obtain an acceptable standard error. In order to get a better insight into contaminant load in sediment and temporal trends of contaminants, a reduction in variance may be achieved by sampling sediments using sediment traps in low turbidity areas. This way mainly freshly transported sediment is sampled giving a more representative picture of the contents and contaminant load of freshly formed sediment layers. However, due to glacial run-off Svalbard fjords such as Kongsfjorden, Isfjorden (including Adventfjorden, Grønfjorden and Billefjord) and VanMijenfjorden receive large amounts of sediment every year (Evenset et al. 2006a, b; Governor of Svalbard 2008). This sediment has a low organic content and some of it will accumulate close to the settlements and thus 'dilute' any contaminants that are emitted from the settlements. Therefore, sampling locations should also be carefully selected with knowledge on the sedimentation rates and sources around a settlement.

Future developments

TBT-containing paint has been shown to be highly effective as antifouling paint preventing organisms attaching to ship hulls, thereby preventing the introduction of non-indigenous species and reducing drag on the ship. For the Antarctic region, the best option to prevent the introduction of non-indigenous species is under discussion including the question of whether local sources of TBT contamination may be preferred over the introduction of non-indigenous species with possible catastrophic and non-reversible effects (Lewis et al. 2004). With the predicted increased shipping in the Arctic, the prevention of introduction of invasive species is also a high priority. However, in the meantime, alternative methods to stop fouling have been developed or are under development (for example Müller et al. 2012; Pérez et al. 2016).

Levels of organotin in sediment of Svalbard harbours are currently low and below NOECs based on the results of this study. However, organotin levels below detection level are no guarantee that effects do not occur as TBT may also cause effects below detection level (Strand et al. 2006). A further study on imposex occurrence in all Svalbard harbours may give a more thorough insight into current effects of OTCs in Svalbard.

In the future, with sea ice receding and shipping opportunities in the area rising, more ships may frequent Svalbard shipping lanes and harbours (via the Northern Sea Route and Transpolar Sea Route). As not all countries have ratified the ban on TBT-containing paints for vessels, chances that TBT-containing vessels frequent the area increase. This may result in an elevation of TBT concentrations in areas along shipping lanes and harbours (Ten Hallers-Tjabbes et al. 2003; Schipper et al. 2010). Regular monitoring of sediment organotin concentrations and/or imposex occurrence in gastropods will provide information on organotin trends in the area. If organotin concentrations increase, mitigation is then possible by actively limiting the access of TBT-containing vessels in the Arctic region.

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CHAPTER 5

EARLY DETECTION OF MARINE NON-INDIGENOUS SPECIES ON SVALBARD BY DNA METABARCODING OF SEDIMENT

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Abstract

Non-indigenous species (NIS) in the Arctic have an increased likelihood of arrival from ship traffic in the region, while the survival potential of the species becomes more likely in a warming environment. Monitoring is essential to detect the rate and magnitude of the establishment of NIS. In this study, a list of 123 potential marine NIS for Svalbard was drafted and the presence of marine NIS in soft sediment of the Kongsfjorden in Svalbard was assessed using molecular metabarcoding techniques. For 37 species, including eight potential Arctic NIS, we generated new 18S and/or COI barcode sequences in order to improve the available online reference databases. In total, 299 species were identified in the sediment samples, including seven potential NIS. Three of these potential NIS have not been reported before in Svalbard: the harpacticoid copepod *Euterpina acutifrons*, and the ascidians *Botrylloides violaceus* and *Molgula manhattensis*. Another novel observation for Svalbard was the polychaete *Chone mollis*. Additional studies are needed to assess whether the NIS have been established on Svalbard and what their potential impact on the local system may be. Metabarcoding proved to be an effective monitoring tool to detect the presence of new species in Svalbard marine waters. We advise its use to set up a baseline record for the presence of NIS at points of entry, especially harbours. This approach is also valuable for biodiversity monitoring, in particular the detection of small organisms and life stages that are hard to identify using current visual techniques.

Introduction

Non-indigenous species (NIS) have the potential to transform local ecosystems by changing the community structure, altering fundamental ecosystem processes or changing the physical environment, with potentially large environmental and economic consequences (Molnar et al. 2008; Bax et al. 2003). Early detection is essential for NIS management (Trebitz et al. 2017; Simberloff et al. 2005), especially when vectors of introduction can be rapidly identified and mitigated.

With the Arctic experiencing its highest rate of global warming over the past decades, NIS are more able than ever to arrive and establish in regions in which they previously could not survive and reproduce (Walther et al. 2009). The risk of introducing marine NIS is particularly high in environments where shipping traffic and the discharge of ballast water present efficient vectors for their spread (Brown et al. 2016). A closer monitoring of possible vectors for the introduction of NIS in the Arctic is recommended to be prepared for development of potential pests (Goldsmit et al. 2014). Chan et al. (2018) reported 54 introductions involving 34 NIS in the Arctic region, 26 of which have led to established populations in Arctic waters. As the Arctic includes a variety of habitats and covers an extensive area, species endemic to one part of the Arctic may be non-indigenous to others. The introduction of NIS in the Arctic, therefore, can result not only from transport to the Arctic, but also from within the Arctic region.

A comprehensive baseline of the current biodiversity in an area is crucial to enable the assessment of changes taking place in the biodiversity as well as the identification of potential anthropogenic routes of introduction of NIS. Both are needed for the development of a strategy for the protection of native species from human-facilitated invasions of NIS (Goldsmit et al. 2014). Research on NIS is ongoing in several areas of the Arctic (CAFF 2013; Reimer et al. 2017). The Circumpolar Biodiversity Monitoring Program (CBMP) of the Arctic Council working group on Conservation of Arctic Flora and Fauna (CAFF) has developed a program to establish a biodiversity baseline and monitor change in species composition of coastal and open-sea habitats of the Arctic. The efforts of the CBMP, which include ongoing scientific projects and studies of time series across the Arctic, are an important step toward resolving which species are native, thereby permitting the identification of new species.

Svalbard is an island archipelago located north of Norway (Fig. 1). Recent warming of the waters west and north of Svalbard is resulting in more suitable habitat conditions for the settlement of non-native species (Førland et al. 2011; Węsławski et al. 2018; Leopold et al. 2019). New marine species may arrive in Svalbard either by natural dispersion, by human assistance (ship's hull fouling, ballast water) or a combination of both. Mussels of the genus *Mytilus* reappeared on Svalbard between 2000 and 2004 after 1000 years of absence (Berge et al. 2005; Leopold et al. 2019). This was suggested to be either due to a fluctuating slow natural range extension (Węsławski et al. 2011), a single exceptional warm inflow of Atlantic water carrying larvae north (Berge et al. 2005), and/or adult specimens transported to Svalbard by rafted debris or ship traffic (Węsławski and Kotwicki 2018b, Leopold et al. 2019). Other examples of temperate and boreal species being transported northwards with the extension of the North Atlantic Current are the euphausiid *Meganyctiphanes norvegica* (Buchholtz et al. 2010) and the starfish *Asterias rubens* (Deja et al. 2016). The Norwegian Biodiversity Information Centre has identified 31 species to be 'doorstep species' for Svalbard. These are species not yet established in the area but expected to settle within the next 50 years through various vectors of introduction (https://www.artsdatabanken.no). Of the 24 marine species on this list, 23 were identified as likely to soon become established in Svalbard. The list covered NIS that are likely to expand to

Svalbard based on their current distribution and did not include NIS that need transport over long distances.

Information on the actual presence of marine NIS in the Svalbard environment is currently sparse and anecdotal. In the bordering Barents Sea, seven NIS were reported as established in the area: the arthropods *Caprella mutica*, *Eurytemora americana*, *Chionoecetes opilio*, *Eriocheir sinensis* and *Paralithodes camtschaticus*, the bivalve *Mytilus galloprovincialis*, and the platyhelminth *Gyrodactylus salaris* (Chan et al. 2018). The Alaskan king crab (P. *camtschaticus*) has an established population in the Barents Sea. It was introduced deliberately for fisheries purposes in the 1960s (Ruiz and Hewitt 2009; Jørgensen et al. 2005). The snow crab (*C. opilio*) was first observed in Svalbard waters in 2017 and was probably unintendedly introduced into the Barents Sea via ballast water before spreading throughout the region (Chan et al. 2018; Berge et al. 2017). Pacific pink salmon (*Oncorhynchus gorbusa*) escaped from Murmansk cage cultures and was found on Svalbard in the 1960s; it may now be spawning in the region (Witkowski and Glowacki 2010). Some studies have reported the presence of marine NIS on Svalbard as biofouling on ship hulls (e.g. cargo and tourist vessels), ballast water (of coal bulk carriers), and on floating debris (Ware et al. 2016; Węsławski and Kotwicki 2018). Multiple non-indigenous zooplankton species (23) were identified from ship ballast water discharged near Svalbard ports (Ware et al. 2016), though none of these have as of yet been reported as being established in the Svalbard environment.

The main reason for the paucity of information on NIS in Svalbard marine waters is a lack of effective and rapid techniques for monitoring marine biodiversity along the extensive coast. Traditional, morphological identification methods are labour intensive and require specialist knowledge, and species detection is low when populations occur at low densities. Moreover, visual identification techniques often do not allow identification of juvenile life stages of benthic or pelagic animals because species characteristics have not yet been fully developed. Identifying these juvenile stages, however, is critically important for monitoring the establishment and spread of NIS populations (Brown et al. 2016). Many sessile marine species can only disperse as juvenile stages in the water column before settlement and therefore signify successful recruitment.

Molecular technology can offer an efficient approach to the identification of NIS (Chan et al. 2018). In particular, metabarcoding of DNA extracted from environmental samples (environmental DNA or eDNA) enables the simultaneous detection of multiple species (Van der Loos and Nijland, 2020; Jeunen et al. 2018). This method allows the identification of DNA contained in live-collected organisms, but also in fragments that animals release into their environment (Jeunen et al. 2018). It is useful in monitoring the presence of species in water, soil or air samples (Leray et al. 2013), and especially in cold water regions where DNA degradation is slower due to low tempparetures (Lacoursière-Roussel et al. 2018). DNA metabarcoding may therefore provide a valuable alternative method for the identification of NIS living in the direct vicinity of the sampled site compared with traditional methods (Holman et al. 2019). Yet, despite of the increasing number of studies using eDNA metabarcoding for the marine realm, the field is still in development and optimisation of protocols for e.g. sampling, DNA conservation and extraction, barcode selection as well as data analysis is ongoing (Ruppert et al. 2019). Important challenges in eDNA metabarcoding include the spatial relevance of eDNA-based findings (DNA potentially being redistributed by e.g. water currents), the development of markers that allow a better coverage of all key taxonomic groups within the wide marine diversity, and the availability of sufficient and unambiguous DNA sequences in reference databases (Van der Loos and Nijland, 2020; Zaiko et al. 2018; Lacoursière-Roussel et al. 2018). Reference databases are currently incomplete, sometimes lacking common taxa, including those already identified as potential or likely invaders. Further development of barcoding reference databases is therefore essential to support future monitoring and detection of NIS using molecular methods (Bylemans et al. 2019).

In this study, we developed a list of potential marine NIS for Svalbard waters, based on the published literature and records of NIS introductions elsewhere in the Arctic. The currently available DNA library was expanded by adding DNA barcodes for two marker regions (18S and COI) of 37 native and potential NIS for the Arctic. Furthermore, the eDNA method was applied for the first time in marine soft sediments of northwest Svalbard to assess the presence of native and potential marine NIS. This study provides insight into the effectiveness of this technique for monitoring NIS in the Arctic.

Material & Methods

Preparing an overview of marine NIS for Svalbard

An initial list of 103 marine species recorded as potential NIS for the Arctic was derived from the list assembled on EMODnet's SeaBasin Checkpoint – Arctic page on alien species (http://www.emodnet-arctic.eu/alien-species, access date 9 December 2020). This list includes a wide variety of groups ranging from phytoplankton to fish and plants. It was subsequently expanded with the marine NIS reported for Svalbard by Ware et al. (2016) and the Norwegian Biodiversity Information Centre (2018), as well as the marine NIS listed for the Arctic (mainly Barents Sea) by Chan et al. (2018).

Selection of barcode regions

The selection of barcode markers was based on an extensive screening for three complementary criteria: 1) the potential discrimination among taxa occurring in the North Sea and Arctic marine environment, 2) the availability of primers and protocols in peer-reviewed literature, and 3) the availability of reference data in online databases. Based on the first two criteria, 18S and COI were selected, being the most commonly used fragments and the only two barcodes for which sufficient reference data are available (Günter et al. 2018; Stefanni et al. 2018). The 18S functioned as a backbone, discriminating between key taxonomic groups among eukaryotes, and providing a general wide screening of species. Despite of its often lower taxonomic resolution, generic 18S markers have proven useful for genus and even species-level identifications in some groups including copepods (Wu et al. 2015). To obtain more information on the metazoa (animal kingdom) COI was used, enabling identification to species level because of the much higher resolution. The combination of 18S and COI therefore explored the added value of 18S and CO1 for NIS detection.

Specimen collection for generation of reference barcodes

The initial list of 103 NIS for the Arctic based on the EMODnet's SeaBasin Checkpoint was used to identify key taxa with barcodes missing in online databases. Online barcoding libraries (e.g. Genbank, BOLD) were consulted for existing DNA barcoding markers (COI and 18S) for these Arctic NIS. From this screening, a total of 23 species were found to have full barcode coverage of the entire marker regions, 48 species had partial coverage (sequences covering e.g. only the V4 or V9 region of 18S) and 32 species had no coverage at all for COI and 18S

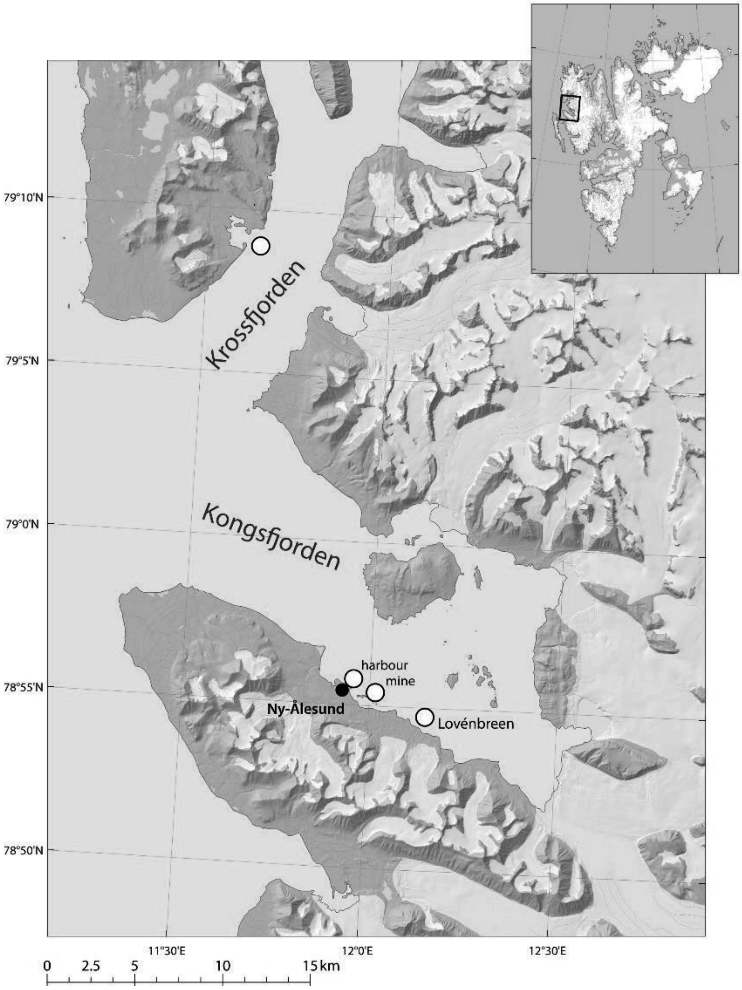

Figure 1*. Sediment sampling sites (open circles) in Kongsfjorden and Krossfjorden, Svalbard, in July 2017.*

markers (Online Resource 1). To generate both 18S and COI barcodes for the database for selected species with no (three species) or partial (nine species) coverage, specimens were collected from locations where the species were well established.

European specimens were collected by hand in various locations in the Oosterschelde in the Netherlands in August-September 2017 (Online Resource 1). Additionally, several Arctic specimens were collected from Kongsfjorden, northwest Svalbard, in July 2017, and barcoded to serve as positive controls of native species in the eDNA samples (Fig. 1). Each collected specimen was morphologically identified to species level, stored in 97% ethanol in a plastic tube, and transported to the Ecological Genetics Laboratory of Wageningen Environmental Research for DNA barcoding.

Generation of reference barcodes

DNA extraction from tissues (for Barcoding purposes) was performed using the DNeasy Blood & Tissue Kit of Qiagen according to the kit protocol.

Primers and PCR protocols were identical both for the reference barcoding and eDNA metabarcoding samples. For both markers (18S and CO1) we selected primer pairs that are most commonly used in the recent literature on eDNA metabarcoding of the marine realm. For 18S, primers were based on Stoeck et al. (2010), amplifying a ~270bp fragment of the V4 region of the eukaryote SSU rRNA gene. We adopted their forward primer TAReuk454FWD1 while using an optimized version of their reverse primer TAReukREV3_(TAReukREV3_v1; 5'-ACTKTCGYTCWTGAYYRA-3'). Although the V9 region of 18S may have a higher taxonomic resolution and may therefore detect a higher diversity, a lack of reference data compared to V4 so far limits its potential for detailed taxonomic annotation of this diversity (see e.g. Choi & Park 2020). For COI, primers were based on Leray et al. (2013), amplifying a ~bp fragment. Here, we adopted their reverse primer jgHCO2198, while using an optimized version of their forward primer mlCOIintF (mlCOIintF_v2; 5'-GGIACIGGITGRACWGTNTAYCCNCC-3'). Optimization consisted in both cases of creating a more degenerate version of the primer to increase amplification success for specific taxonomic groups (particulary Annelida, Arthropoda and Mollusca). In a direct comparison using the same PCR and bioinformatic protocols these versions detected a clearly higher taxon diversity than the original primers for the same samples (Glorius, Laros & De Groot, unpublished data). Forward and reverse primers were augmented with CS1 and CS2 tag sequences (Fluidigm, South San Francisco, CA, USA) to be used as sequence primers in Sanger sequencing (for reference barcodes, see below) and to allow the indexing PCR for multiplexed analysis via high-throughput sequencing (see below). PCR reactions were performed in a 25µl reaction volume, consisting of 1U Platinum Taq (Fisher Scientific), 1x PCR buffer, 2.5 mM MgCl2, 5%(m/m) Trehalose, 200ng/µl BSA, 200µM dNTP and 250µM of each primer. The cycling program was as follows: 2 minutes at 94°C followed by 15 cycles of 30 seconds at 94°C, 3 minutes at 56°C reduced by 1°C each cycle and 1 minute at 72°C, followed by 20 additional cycles of 30 seconds at 94°C, 3 minutes at 42°C and 1 minute at 72°C and ended by a 10 minutes hold at 72°C.

PCR products were then sent to and external company (Macrogen Europe B.V., Amsterdam, the Netherlands) for Sanger sequencing with both the forward and reverse primer per sample, and final consensus sequences per sample were determined by hand using Seqman (DNAStar).

Environmental sample collection

Samples for eDNA analysis were collected in four locations in Kongsfjorden, northwest Svalbard, in July 2017 (Fig. 1). A total of 30 marine sediment samples were collected using a Van Veen grab on-board the r/v Teisten: 26 in the Kongsfjorden (8 near Ny-Ålesund mine, 10 in Ny-Ålesund harbour, 8 near Lovénbreen) and 4 in the entrance of the Krossfjorden (see Online Resource 2). Plastic samples tubes (50 ml) were filled with approximately 40 g of sediment (wet weight) that was randomly collected from the upper 1 cm layer of the grab surface. The sample was kept cool until return to the Kings Bay Marine Laboratory at the end of each sampling day, where it was stored frozen (-20°C). Samples were transported in frozen condition to the laboratory in Wageningen for DNA metabarcoding.

eDNA metabarcoding

DNA extraction from sediments was performed using the Powermax soil DNA isolation kit (Mobio) with the following adaptation from the kit protocol: dispersing of the sediment samples by vortexing with beads was replaced by a dispersing using an Ultra Turrax (T25) instrument equipped with an in house constructed titanium dispersing shaft, while keeping the samples cooled on ice. This results in a better homogenization of the entire sample volume (see e.g. Aylagas et al. 2016). A subsample of 8 grams of this homogenized volume was then included in the Powermax extraction.

PCR reactions were conducted following the above mentioned protocols for reference barcodes. The produced amplicons were then sent to Genome Quebec (Canada) for high-throughput sequencing. Here, a second PCR reaction was conducted to add sample-specific index barcodes and Illumina adaptor sequences. Indexed amplicons were then normalized and pooled per marker, before being sequenced in a 250bp paired-end run on a Illumina Miseq flow cell platform.

Raw sequence data were processed in the R programming environment (R Core Team, 2017) making use of functions available in the DADA2 packages (Callahan et al., 2016). Primer sequences were removed from the raw sequence reads and the read quality was inspected by plotting the quality scores per base position for each sample. The following filtering steps were carried out; sequences with undetermined nucleotides, exceeding the expected number of errors of two and reads contaminated with the Phix genome were removed. All reads were trimmed at the point where read quality dropped below a score of two. The minimum sizes for the forward and reverse read lengths were set by inspecting the quality plots and varied between 202 and 230 depending on gene and forward/reverse read. The DADA 2 error model parameter learning algorithm was carried out on the sequences passing the filtering steps, followed by a dereplication step and inferring the sequence variance using standard settings of the DADA2 functions 'derepFastq' and 'dada'. Forward and reverse reads were merged, and a table containing the number of reads per unique sequence variant per sample was constructed. In a final step, chimeras were identified and removed.

Automated taxonomic annotation of all obtained unique sequence variants was performed using a home-made script based on the BLAST® program, using the NCBI nucleotide database as a reference. To avoid false positive detections of NIS, we used a conservative approach for this automated annotation, assigning a taxonomy only to reads for which the top hits in BLAST had a sequence coverage of >90% and a sequence similarity >97% (following e.g. Holman et al. 2019). Reads were assigned to genus level in case all hits with a similarity >97% belonged to the same genus. Likewise, reads were identified to species level in case the top hits had a similarity >99% and all hits with a similarity >99% belonged to the same species. Sequences that could not be identified at least to genus level were recorded as unidentified, as such results do rarely allow a reliable detection of NIS.

For all reads identified as potential NIS, we assessed the validity of this result via manual BLAST searches, analysing the diversity observed among the top 50 hits (based on similarity). For some reads assigned to genus level only, this allowed us to assess whether it more likely concerned a native species or a species reported as NIS for the Svalbard region, thereby discarding some reads as belonging to potential NIS.

Results

List of potential marine NIS for Svalbard

Based on all available information, a list of potential NIS was drafted for Svalbard marine waters consisting of 123 species. The list predominantly consists of arthropods (55%), along with chordates (14%), ochrophytes (7%), rhodophyte (5%), molluscs and nematodes (both 4%) species (Online Resource 3).

New reference barcodes

In total 37 species were barcoded; 31 of these were developed for the 18S marker, and 28 species for the COI marker (Online Resource 1). This included eight species identified as potential Arctic NIS and 13 Arctic species that acted as positive DNA references for the identification of species in the sediment samples. These barcodes were submitted to the NCBI database (https://www.ncbi.nlm.nih.gov/nucleotide). Successful barcodes could not be produced for the chordate *Botryllus schlosseri*, the chlorophyte *Codium fragile*, the ochrophyte *Fucus serratus* and the rhodophyte *Caulacanthus okamurae/ustulatus* (all collected from the Oosterschelde), as well as an unknown tube worm and *Mya* sp. from Kongsfjorden, Svalbard.

Metabarcoding of sediment samples

A total of 299 species were identified from the molecular analysis of the sediment samples, of which 243 were identified using the 18S marker and 64 using the COI marker. The following eight species were identified using both markers: the annelids *Lysippe labiata*, *Scoloplos* sp. and *Terebellides stroemii*; the ochrophytes *Desmarestia* sp. and *Thalassiosira* sp.; the chlorophyte *Micromonas* sp.; the mollusc *Musculus niger*; and the myzozoan *Polarella glacialis*. More species were detected in the sediment samples using the 18S marker (range of total number of species per location was 121-141) compared to the COI marker (range of total number of species per location was 28-33) (Table A-B in Online Resource 4). The phyla with the greatest number of species in the DNA reads obtained from the Kongsfjorden sediment samples using the 18S marker were nematods (18-30 species), followed by ochrophytes (17-20 species), chlorophytes (8-18 species), arthropods (8-13 species), and platyhelminthes (3-10 species) (Fig. 2A, Table A in Online Resource 4). The group ‘other’ comprises other, less common, taxa, including a wide diversity unicellular protists (see Online Resource 4). From the COI results, mainly ochrophytes (8-14 species) and annelids (7-13 species) were identified, followed by molluscs (2-4 species) (Fig. 2B, Table B in Online Resource 4). The COI results also included the identification of the terrestrial chironomid fly *Micropsectra radialis*, which was found despite it not being a marine species.

Of the 243 species identified using the 18S marker, 79 (33%) were identified to species level, and the remaining 164 to genus level. Of the 64 species identified using the COI marker 39 (61%) were identified to species level, and the remaining 25 to genus level.

Five of the newly barcoded Arctic species were positively identified in the sediment samples collected in Kongsfjorden, Svalbard. This included one species using the 18S marker (the chordate *Pelonaia corrugata* - 5 samples) (Table A in Online Resource 4) and four species based on the COI marker (the molluscs *Macoma calcarea* - 11 samples, *Astarte borealis* - 2 samples, *Mytilus* sp. - 1 sample, and the rhodophyte *Polysiphonia arctica* - 7 samples) (Table B in Online Resource 4).

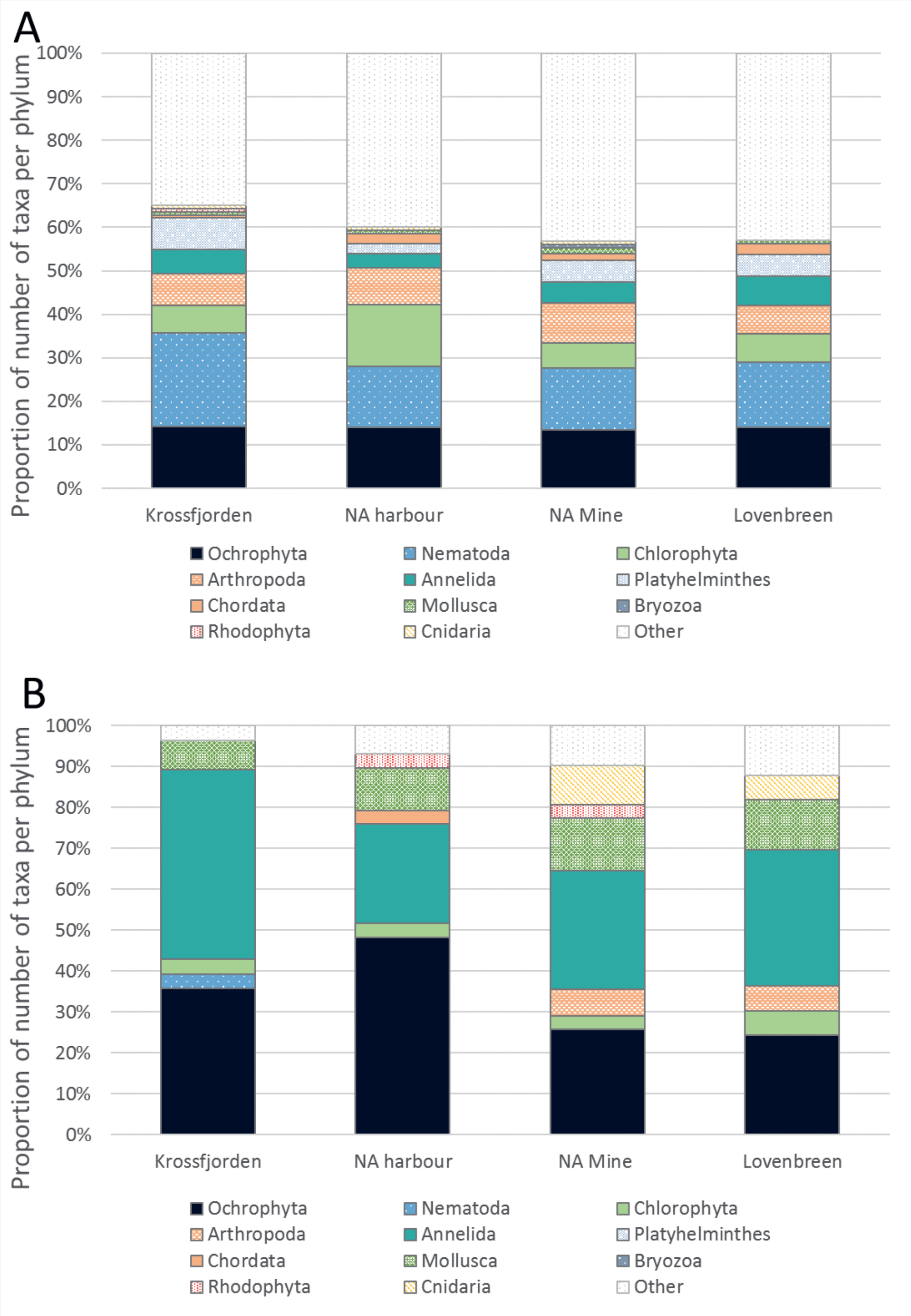

Figure 2*. Number of taxa (grouped per phylum), identified via metabarcoding based on 18S (A) or COI (B), in sediment samples collected in Kongsfjorden, Svalbard, in July 2017.*

Identification of NIS

Based on our drafted list of potential NIS for Svalbard marine waters, seven potential NIS species were identified in the sediment samples collected in the Kongsfjorden; three using the 18S marker, and four using the COI marker (Table 1). The potential NIS consisted of three arthropods (copepods and a cirriped), one annelid (polychaete), two chordates (ascidians) and one mollusc (bivalve). Four were identified to species level: the copepods *Euterpina acutifrons* and *Oithona similis*, and the ascidians *Botrylloides violaceus* and *Molgula manhattensis*. Three were identified to a genus level: the polychaete *Scolelepis* sp., the cirriped *Balanus* sp. and the bivalve *Mytilus* sp.. Four were observed in a single sample: in the harbour (*Mytilus* sp., *B. violaceus*), near the mine (*Balanus* sp.), and near the Lovénbreen (*M. manhattensis*), whereas *Scolelepis* sp. and *Euterpina acutifrons* were found in 4 and 11 samples respectively.

Discussion

Detection of NIS

eDNA metabarcoding shows great potential as an effective monitoring tool to assess the presence of NIS in environmental samples. This pilot study using sediment samples from Kongsfjorden, Svalbard, revealed seven species that are listed as potential Arctic NIS based on their DNA (Table 1). One of these is however the copepod *O. similis*, which is, although considered an NIS for Canadian ports (Brown et al. 2016), ubiquitous and widespread throughout the Arctic Basin and occurring naturally in Svalbard (Gulliksen et al. 1999; Ormanczyk et al. 2017). It should therefore be removed from the draft list of potential NIS for Svalbard.

E. acutifrons is a harpacticoid copepod that was detected in 11 sediment samples in Kongsfjorden (based on the 18S marker). This may suggest that the species has established in the region, although its presence has not been reported in the Svalbard region before. It was identified earlier as a NIS in Canadian East Arctic-West Greenland and Hudson Bay, with vessels mentioned as a possible vector and with an unknown source region (Brown et al. 2016; Chan et al. 2018). No information is available on its potential impact on the ecology around Svalbard. In temperate and tropical areas *E. acutifrons* is a dominant member of some planktonic communities (Yahia et al. 2004; Sautour & Castel 1993; Vidjak et al. 2006). The dominance of the species is reliant on high concentrations of suspended particulate matter (Sautour & Castel 1993) and although *E. acutifrons* is reported to overwinter in the sediment at low temperatures (<9°C) breeding is limited in temperatures below 16°C (D'Apolito & Stancyk 1979). Under current climate conditions the Arctic waters do not seem to meet the environmental parameters necessary for the species to become dominant.

The colonial chain tunicate *B. violaceus* and solitary tunicate *M. manhattensis* (sea grape) were both identified in one sediment sample. Based on these data it cannot be concluded whether they were settled, or whether the positive identifications were based on alive or dead cells. Both tunicates have been invasive in other regions and can cause significant negative impacts (Carver et al. 2006; Fofonoff et al. 2018). They can smother other species, and compete with filter feeders for food, potentially leading to serious changes in the local biodiversity (Carver et al. 2006; Carman et al. 2010). Economic problems of these species have been reported for aquaculture,

Table 1. *Number of (potential) Arctic non-indigenous species (NIS) observed in sediment samples of Kongsfjorden, Svalbard, in July 2017 (based on species listed in the draft Svalbard marine NIS, see Online Resource 3).*

Phylum	**Potential Arctic NIS in sediment samples**	**# samples 18S**	**# samples COI**	**Species name on Arctic NIS list(s)**	**Mentioned as (potential) Arctic NIS by**	**Previously detected on Svalbard**
Annelida	*Scolelepis* sp.		4	*Scolelepis* sp.	EMODnet Arctic / Ware et al., 2016	Reported in Svalbard region: *S. burkovskii or S. squamata* (Sikorski & Pavlova, 2015) In ballast water: *Scolelepis* sp. (NIS) (Ware et al., 2016)
	Chone mollis		1	Not listed	-	Reported in Svalbard region: *Chone duneri*, *Chone infundibuliformis*, *Chone paucibranchiata* (Gulliksen et al., 1999; Palerud et al., 2004) *Chone mollis* unknown
Arthropoda	*Euterpina acutifrons*	11		*Euterpina acutifrons*	EMODnet Arctic / Chan et al., 2018 / Brown et al., 2016	Unknown
	Balanus sp.		1	*Balanus trigonus*	EMODnet Arctic	Reported in Svalbard region: *Balanus balanus*, *Balanus crenatus* (Palerud et al., 2004) In ballast water: *Balanus balanus* (native) (Ware et al., 2016)
	Oithona similus	1		*Oithona similus*	EMODnet Arctic	Reported as common in Svalbard region (Palerud et al., 2004). **Not an NIS**
Chordata	*Botrylloides violaceus*		1	*Botrylloides violaceus*	EMODnet Arctic	Unknown
	Molgula manhattensis	1		*Molgula manhattensis*	Chan et al., 2018	Reported in Svalbard region: *Molgula citrina*, *Molgula complanata*, *Molgula griffithsii*, *Molgula herdmani*, *Molgula retortiformis*, *Molgula romeri*, *Molgula siphonalis* (Palerud et al., 2004) *Molgula manhattensis* unknown
Mollusca	*Mytilus* sp.		1	*Mytilus galloprovincialis*	EMODnet Arctic / Chan et al., 2018	Reported in Svalbard region: *M. edulis*, *M. galloprovincialis* or hybrids of *M. edulis/M. galloprovincialis* and *M. edulis/M. trossulus* (Berge et al., 2005; Mathiesen et al., 2016; Leopold et al., 2019)

fishing and shipping by clogging equipment and increasing maintenance costs (Carver et al. 2006; Fofonoff et al. 2018). Potential vectors of introduction on Svalbard are shipping and floating debris (Lejeusne et al. 2011; Carver et al. 2006). Under current climate conditions, *B. violaceus* can survive year-round in large areas of the Bering Sea shelf, but with limited opportunities for reproduction (Reimer et al. 2017). The temperature range for survival of this species lies between -0.6°C and 29°C with a salinity range of 20-38 ‰, whereas for sexual reproduction this range was between 15°C and 25°C with a salinity of 26-38 ‰ (Reimer et al. 2017). Colony growth, however, can also occur by asexual budding and fragmentation which may enhance vegetative growth where established. Temperature survival of *M. manhattensis* is reported between -1.5°C and 32.1°C with a mean salinity of 30.6‰ ± 5.6 (Lins et al. 2018). Both tunicates recently had first observations in southwestern Iceland in 2018, which is considered a first station for expansion of NIS towards very cold areas (Ramos-Esplá et al. 2020). The observed effects of both species in other invasion areas indicates the urgency to assess the route of introduction and establishment potential on Svalbard.

Another novel observation for Svalbard was that of the sabellid polychaete, *Chone mollis*, in one sediment sample of the Krossfjorden (based on the COI marker, Table 1). This species was not included in the list of potential NIS for Svalbard. It is known to occur in muddy sands of estuaries of the Pacific in the northern hemisphere (Bonar 1972), but has not been previously described for Svalbard. Related *Chone* spp. are however common to the Svalbard coast, such as *Chone duneri*, *Chone infundibuliformis* and *Chone paucibranchiata* (Gulliksen et al. 1999; Palerud et al. 2004). Further monitoring is needed to confirm whether *Ch. mollis* has established in the Svalbard region.

One sample in the harbour contained a positive identification for the mussel *Mytilus* sp.. Nowadays, several *Mytilus* spp. can be found on Svalbard: *Mytilus edulis*, *Mytilus galloprovincialis* or hybrids of *M. edulis*/*M. galloprovincialis* and *M. edulis*/*Mytilus trossulus*, although *M. edulis* is so far the most abundant species (Mathiesen et al. 2017; Węsławski and Kotwicki 2018). A recent study indicated that mussels (*M. edulis*) found on floating debris around Svalbard were hybrids of boreal and warm temperate taxa, some with genetic structure resembling populations in the UK, Iceland, and western mainland Europe (Wenne et al. 2020). This shows the complex interpretation of origin that may also account for other NIS.

Of the list of 123 potential NIS for Svalbard, 12 species have already been observed in earlier studies in this region (Online Resource 3): the annelid *Spiophanes kroeyeri* (Gulliksen et al. 1999; Palerud et al. 2004); the arthropods *Crassicorophium bonellii*, *Lithodes maja* and *Paralithodes camtschaticus* (Gulliksen et al. 1999; Palerud et al. 2004); the bryozoan *Schizoporella unicornis* (Gulliksen et al. 1999; Palerud et al. 2004); the chordates *Botryllus schlosseri* and *Ciona intestinalis* (Gulliksen et al. 1999; Palerud et al. 2004); the echinoderm *Echinus esculentus* (Gulliksen et al. 1999; Palerud et al. 2004); the mollusc *Mya arenaria* (Gulliksen et al. 1999; Palerud et al. 2004); the ochrophyte *Fucus serratus* (Gulliksen et al. 1999), and the rhodophyte *Dumontia contorta* (Gulliksen et al. 1999). Whether all these species were correctly labeled as potential NIS to Svalbard needs further study. The annelid *Heteromastus filiformis*, also listed as potential NIS for Svalbard and observed in earlier studies, is not considered an NIS to Svalbard. Another 26 species of the list of potential NIS for Svalbard have been identified in ballast water samples collected from ships arriving to Svalbard (Ware et al. 2016) (Online Resource 3).

It is important to realise that not all newly arriving marine species become invasive or form a nuisance to the receiving habitat. A newly arrived NIS may settle, grow and reproduce, while slowly obtaining an ecological niche in the new habitat. With the warming waters in the Arctic region, the coastal waters around Svalbard are expected to become more hospitable to more southern species in the near future (Ware et al. 2016), while indigenous species may be less adapted to the new conditions. Although only a few of the many introduced species may become pests, the impact of these may be large (Mack et al. 2000). Therefore, it is better to prevent anthropogenic introductions of new species, as measures for prevention are more realistic than measures for removal of already established NIS.

Using eDNA metabarcoding as monitoring tool

Some of the potential NIS found in the sediment samples could only be identified at a higher taxonomic level than specified in the list of potential Arctic NIS (Table 1). Based on genus level, no confirmation on the presence of the actual Arctic NIS can be made as many of the taxa identified to genus level may be species that are native to Svalbard, such as e.g. *Balanus balanus*, *Mytilus edulis*, *Scolelepis burkovskii*, and *Scolelepis squamata* (Gulliksen et al. 1999; Varpe 2012, Sikorski and Pavlova 2015). Incomplete identification of species in the sediment samples was generally due to the absence in the references library of either the native or the invasive species , and/or the 18S and COI marker regions showing insufficient polymorphisms between species within a genus to properly distinguish them.

This further stresses the need for the publication of species-specific barcodes for commonly used DNA markers (such as 18S and COI) in international reference databases, such as the online BOLD and NCBI databases. This will ultimately lead to a more detailed identification so that NIS sharing a genus with native species can be successfully distinguished. In this study, we provided new barcodes for 31 marine species, of which eight are listed as potential Arctic NIS. Unfortunately, barcodes for 14 of the 123 species from the list of potential marine NIS for Svalbard, are still missing for both the 18S and COI marker (Online Resource 5). An additional 33 have missing barcodes for either the 18S or the COI marker. A follow-up project to update the international barcode databases for these species is therefore essential to enable a more complete identification of marine NIS on Svalbard.

This study was a proof-of-principle for using sediments for the identification of NIS. These data can however also be used for assessing species diversity. Already in the relatively small sample volume (40g ww of the top 1 cm), 299 species were revealed. For a proper baseline description of biodiversity, of course, an optimised number of samples is needed per sampling site. In our study we found that for the COI marker up to seven to eight sediment samples were needed to obtain 95% of the total observed species at NA harbour, NA mine and the Lovénbreen (Fig. A in Online Resource 6). For the 18S marker, up to ten samples were needed to obtain 95% of the observed species at the sampling sites. As the species-accumulation curves from all sampling sites for this marker had not reached asymptotes yet, this indicates the ideal number of samples will be more than ten (Fig. B in Online Resource 6). Care should be taken to use these minimum sample numbers as a fixed number of samples for all sites. Not all species in the environment are currently covered by the molecular markers and site heterogeneity also influences the required number of samples. For marine water samples it was suggested to collect at least 15 samples for a comprehensive estimate of biodiversity at a site (Lacoursière-Roussel et al., 2018).

A combination of different sample types will further complement the information on the presence of marine species (including NIS) in a particular area, such as water samples for mobile species, hard substrate scrapes or plastic debris. For example, we identified 26 species on a piece of floating soft plastic in Ny-Ålesund harbour that were not found in the harbour sediment (see Online Resource 7). Floating debris can act as rafts onto which species can attach and travel significant distances on ocean currents (Carlton et al. 2017). Encrusting species such as the bryozoans *Electra* spp. and *Eucratea loricata*, the cirripeds *Semibalanus balanoides* and *Lepas anatifera*, and the bivalve *Mytilus* sp. were previously observed on large plastics items such as boxes, barrels and liquid containers that were washed upon beaches of Prins Karl Forlandet, Svalbard (Węsławski and Kotwicki 2018), with *L. anatifera* being not native to Svalbard. The considerable amount of floating plastic in the oceans increases the risk of introducing hitchhiking NIS. Coastal monitoring efforts should therefore also include floating plastic as a potential vector of NIS introduction.

At present the identification of species in eDNA may not provide direct information on whether the species is alive or not. A positive identification however shows that DNA fragments were present at the monitoring site. For screening purposes of NIS therefore, this information forms an important first step for selecting sites for further analysis and applying a more thorough study using visual inspection.

Future perspectives

Metabarcoding is a suitable technique for rapid detection of the presence of species in coastal ecosystems. It enables analysing samples en masse without the expertise-intensive taxonomic identification of individual specimens. The 18S and COI markers provided complementary information, in total identifying 299 species in the sediment samples, with only eight species overlapping for both markers. Many of the identified species came from groups of microorganisms and from taxa that are rarely studied and sampled in this region, such as flatworms, fungi, slime molds, and parasites from a variety of phyla, especially using the 18S marker as general wide screening of species (Tables A and B in Online Resource 4). This shows that the technique provides a great potential for describing otherwise understudied local biodiversity. This also accounts for early life stages and organisms that are hard to detect by visual taxonomic identification techniques, and can assist by rapidly enhancing information on the occurrence of such species.

Already the limited sampling performed in this study indicated the presence of marine NIS on Svalbard and showed that the eDNA metabarcoding method is well-suited for the detection and monitoring of the presence of NIS. The novel observations of *E. acutifrons*, *B. violaceus*, *M. manhattensis* and *Ch. mollis* in our pilot study warrant further detailed studies to assess whether these species established in Svalbard waters and to unravel their vector of introduction and point of arrival, as well as their potential impact on the system. Passenger and cargo shipping is likely the primary vector of marine NIS to Svalbard, whereby they can facilitate NIS introduction through hull fouling, ballast waters and discards of food products (primarily from cruise ships). In addition, floating debris can serve as both long and short distance vectors, whereas natural spreading can occur from NIS introduced to mainland Norway in recent years. We argue that the likely points of arrival via most of these vectors are in areas such as harbours (Longyearbyen, Pyramiden, Barentsburg, Svea and Ny-Ålesund), exposed shorelines and places where drifting marine litter is piling up on beaches. In fact, our focus on the Kongsfjorden system (Ny-Ålesund) likely gives a very conservative picture of the presence of marine NIS on Svalbard as the

Isfjorden (Longyearbyen and Barentsburg) experiences much higher ship traffic and the exposed west coasts of Spitsbergen north of Van Mijenfjord are hotspots of drifting debris.

Monitoring the introduction vectors and presence of NIS in Svalbard waters can lead to effective mitigation measures to stop the further introduction and anthropogenic spreading of NIS. We suggest to focus on strategic points of entry, such as those mentioned above, during the busy traffic months in summer. Frequent surveillance of ship hull fouling, and ballast water (see Ware et al. 2016), combined with targeted sampling of harbour and coastal habitats and education of local residents to identify likely NIS, are also advised to increase effectiveness in monitoring. A similar monitoring strategy is advisable to other harbours in the Arctic region as information on marine NIS is urgently lacking for this region. Finally, ecological studies of the potential impacts of these organisms are needed to assess whether the detrimental effects on the ecosystem, as observed elsewhere, may also account for Svalbard.

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Supporting information of chapter 5

Online Resource 1

***Table**. Details of type, location and samples of species collected for the production of COI and 18S barcodes, scores of successfully produced barcodes, and identification of these species in sediment samples of Kongsfjorden, Svalbard, in July 2017. Applied references: EMODnet Arctic - aliens webpage; Norwegian Biodiversity Information Centre (NBIC), 2018; Chan et al., 2018.*

Area	Location	Site	Collection date	Phylum	Species	18S	CO1	Comment
Arctic	Svalbard	Kongsfjord	July 2017	Arthropoda	*Monoculodes borealis*	OK	**Failed**	
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Nuculana pernula*	OK	OK	
Arctic	Svalbard	Kongsfjord	July 2017	Annelida	*Nephtys sp.*	OK	OK	
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Macoma calcarea*	OK	OK	C01 marker: positive identification (11 of 31 samples)
Arctic	Svalbard	Kongsfjord	July 2017	Annelida	*Euchone analis*	OK	OK	
Arctic	Svalbard	Kongsfjord	July 2017	Echinodermata	*Pelonaia corrugata*	OK	**Failed**	18S marker: positive identification (5 of 31 samples)
Arctic	Svalbard	Kongsfjord	July 2017	Annelida	*Pectinaria hyperborea*	OK	OK	
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Euspira sp.*	OK	OK	
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Astarte borealis moerchi*	OK	OK	C01 marker: positive identification (2 of 31 samples)
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Buccinum sp.*	OK	OK	
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Mytilus sp.*	OK	OK	C01 marker: positive identification (1 of 31 samples). Not identified to species level
Arctic	Svalbard	Kongsfjord	July 2017	Arthropoda	*Gammarus setosus*	OK	OK	
Arctic	Svalbard	Kongsfjord	July 2017	Rhodophyta	*Polysiphonia arctica*	OK	OK	C01 marker: positive identification (7 of 31 samples)
Arctic	Svalbard	Kongsfjord	July 2017	Annelida	*Unknown*	**Failed**	**Failed**	
Arctic	Svalbard	Kongsfjord	July 2017	Mollusca	*Mya sp.*	**Failed**	**Failed**	
Europe	Netherlands	Oosterschelde	September 2017	Annelida	*Scoloplos armiger*	OK	OK	
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Palaemon longirostris*	OK	OK	
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Hippolyte varians*	OK	OK	
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Gammarus locusta*	OK	OK	
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Ammothea hilgendorfi*	OK	OK	
Europe	Netherlands	Oosterschelde	September 2017	Mollusca	*Cerastoderma edule*	OK	OK	Potential NIS: EMODnet
Europe	Netherlands	Oosterschelde	September 2017	Arthropoda	*Hemigrapsus takanoi*	OK	OK	Potential NIS: EMODnet/NBIC
Europe	Netherlands	Oosterschelde	September 2017	Cnidaria	*Actinia equina*	OK	OK	
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Caprella mutica*	OK	OK	Potential NIS: EMODnet/NBIC/Chan et al., 2018
Europe	Netherlands	North Sea	September 2017	Arthropoda	*Cancer pagarus*	OK	OK	Potential NIS: EMODnet/NBIC
Europe	Netherlands	Oosterschelde	August 2017	Chordata	*Ciona intestinalis*	OK	OK	Potential NIS: EMODnet/Chan et al., 2018
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Austrominius modestus*	OK	OK	Potential NIS: EMODnet
Europe	Netherlands	Oosterschelde	August 2017	Mollusca	*Macoma balthica*	OK	OK	
Europe	Netherlands	Oosterschelde	August 2017	Arthropoda	*Gammarus obtusatus*	OK	**Failed**	
Europe	Netherlands	Oosterschelde	August 2017	Annelida	*Nereis diversicolor*	OK	OK	
Europe	Netherlands	Oosterschelde	August 2017	Annelida	*Arenicola marina*	OK	OK	
Europe	Netherlands	Oosterschelde	August 2017	Tracheophyta	*Cotula coronopifolia*	OK	OK	Potential NIS: EMODnet.
Europe	Netherlands	Oosterschelde	August 2017	Ochrophyta	*Sargassum muticum*	OK	OK	Potential NIS: EMODnet
Europe	Netherlands	Oosterschelde	August 2017	Chordata	*Botryllus schlosseri*	**Failed**	**Failed**	Potential NIS: EMODnet. DNA interference
Europe	Netherlands	Oosterschelde	August 2017	Chlorophyta	*Codium fragile*	**Failed**	**Failed**	Potential NIS: EMODnet. Lipid interference
Europe	Netherlands	Oosterschelde	August 2017	Ochrophyta	*Fucus serratus*	**Failed**	**Failed**	Potential NIS: EMODnet. Lipid interference
Europe	Netherlands	Oosterschelde	August 2017	Rhodophyta	*Caulacanthus okamurae/ustulatus*	**Failed**	**Failed**	Potential NIS: EMODnet. Lipid interference

Online Resource 2

***Table**. Details of the marine sediment samples that were collected for metabarcoding purposes to assess the presence of marine NIS on Svalbard, July 2017. Lat DD = Latitude in decimal degrees N, Lon DD = longitude in decimal degrees E.*

Sample	Location	Date	Lat DD (N)	Lon DD (E)	Sample depth (m)	Sediment characteristics
Sediment	Kongsfjorden, Ny-Ålesund mine	19/07/'17	78.9253667	11.98285	26	Pebbles + fine mud
Sediment	Kongsfjorden, Ny-Ålesund mine	19/07/'17	78.9224833	12.001	17	
Sediment	Kongsfjorden, Ny-Ålesund mine	19/07/'17	78.9222833	12.0165	28	fine mud
Sediment	Kongsfjorden, Ny-Ålesund mine	19/07/'17	78.92275	12.0090333	25	fine mud + pebbles
Sediment	Kongsfjorden, Ny-Ålesund mine	19/07/'17	78.9226833	12.012	22	
Sediment	Kongsfjorden, Ny-Ålesund mine	19/07/'17	78.55385	12.00168	27	mud
Sediment	Kongsfjorden, Ny-Ålesund mine	19/07/'17	78.55415	11.59692	20	mud
Sediment	Kongsfjorden, Ny-Ålesund mine	19/07/'17	78.55467	11.59284	27	mud
Sediment	Krossfjorden entrance	24/07/'17	79.1544167	11.63415	8-12	
Sediment	Krossfjorden entrance	24/07/'17	79.1552167	11.6410167	8-12	
Sediment	Krossfjorden entrance	24/07/'17	79.1555667	11.6355167	8-12	
Sediment	Krossfjorden entrance	24/07/'17	79.1526	11.6332667	8-12	
Sediment	Kongsfjorden, Austre Lovénbreen	26/07/'17	78.90485	12.1571667	12	fine mud
Sediment	Kongsfjorden, Austre Lovénbreen	26/07/'17	78.90525	12.1557333	24	fine mud
Sediment	Kongsfjorden, Austre Lovénbreen	26/07/'17	78.9050667	12.15405	26	fine mud
Sediment	Kongsfjorden, Austre Lovénbreen	26/07/'17	78.9056167	12.15215	40	fine mud
Sediment	Kongsfjorden, Vestre Lovénbreen	26/07/'17	78.90895	12.1197333	30	fine sand + mud
Sediment	Kongsfjorden, Vestre Lovénbreen	26/07/'17	78.9087833	12.1217333	30	1cm oxic, black anoxic below
Sediment	Kongsfjorden, Vestre Lovénbreen	26/07/'17	78.9089667	12.1215833	34	fine mud + clay
Sediment	Kongsfjorden, Vestre Lovénbreen	26/07/'17	78.9091167	12.1205333	36	
Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment
Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment
Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment
Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment
Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment

Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment
Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment
Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment
Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment
Sediment	Kongsfjorden, Ny-Ålesund harbour	26/07/'17	78.9287333	11.9381	10-30	fine org sediment

Online Resource 3

Table. *Total list of potential marine NIS for Svalbard. The list is based on EMODnet Arctic – aliens webpage (1), NIS in Barents Sea region in Chan et al., 2018 (2), the Norwegian Biodiversity Information Centre (2018) (3), and zooplankton identified in ballast water in vessels on Svalbard (Ware et al., 2016) (4).*

Phylum	**Latin name**	**Arctic NIS list**	**Records of earlier observations on Svalbard**	**Observed in this study**	**Comments**
Annelida	*Heteromastus filiformis*	1	Gulliksen et al., 1999; Palerud et al., 2004; Somerfield et al., 2006; Kędra et al., 2010		Not an NIS for Svalbard
Annelida	*Scolelepis sp.*	1, 4	Ballast water (Ware et al., 2016)	at genus level	
Annelida	*Spiophanes kroeyeri*	1, 4	Gulliksen et al., 1999; Palerud et al., 2004; Ballast water (Ware et al., 2016)		
Arthropoda	*Acantholeberis curvirostris*	1			
Arthropoda	*Acartia clausii*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Acartia tonsa*	1, 3, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Acartiella sinensis*	1			
Arthropoda	*Ampelisca abdita*	1			
Arthropoda	*Amphibalanus amphitrite*	1			
Arthropoda	*Amphibalanus eburneus*	1			
Arthropoda	*Amphibalanus improvisus*	1, 3, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Amphibalanus reticulatus*	1			
Arthropoda	*Anomalocera patersoni*	1, 4	Ballast water (Ware et al., 2016)		

Arthropoda	*Austrominius modestus*	1, 3, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Balanus trigonus*	1		at genus level	
Arthropoda	*Calanus helgolandicus*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Cancer irroratus*	1, 2			
Arthropoda	*Cancer pagurus*	1, 3, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Caprella mutica*	1, 2, 3			
Arthropoda	*Carcinus maenas*	1, 3, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Centropages hamatus*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Centropages typicus*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Chionoecetes opilio*	1, 2, 3			
Arthropoda	*Clausocalanus furcatus*	1, 2			
Arthropoda	*Conchoderma virgatum*	1			
Arthropoda	*Crangon crangon*	1, 2, 3, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Crassicorophium bonellii*	1	Palerud et al., 2004		
Arthropoda	*Cyclops kolensis kolensis*	1			
Arthropoda	*Eriocheir sinensis*	2			
Arthropoda	*Eurydice pulchra*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Eurytemora affinis*	1, 2, 3, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Eurytemora americana*	2			
Arthropoda	*Euterpina acutifrons*	1, 2		X	
Arthropoda	*Evadne nordmanni*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Gammarus cf. tigrinus*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Gammarus cf. zaddachi*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Gammarus daiberi*	1			
Arthropoda	*Grandidierella japonica*	1			

Arthropoda	*Hemigrapsus takanoi*	1, 3, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Heterolaophonte ströemi*	1	Bjørnøya (Palerud et al., 2004)		
Arthropoda	*Homarus americanus*	2			
Arthropoda	*Homarus gammarus*	3			
Arthropoda	*Hyperacanthomysis longirostris*	1			
Arthropoda	*Idotea linearis*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Ischyrocerus commensalis*	3			
Arthropoda	*Isias clavipes*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Jassa marmorata*	1			
Arthropoda	*Limnoithona tetraspina*	1			
Arthropoda	*Lithodes maja*	3	Gulliksen et al., 1999; Palerud et al., 2004		
Arthropoda	*Megabalanus cf. spinosus*	1			
Arthropoda	*Megabalanus cf. tintinnabulum*	1			
Arthropoda	*Megabalanus coccopoma*	1			
Arthropoda	*Mesopodopsis slabberi*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Metridia lucens*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Monocorophium acherusicum*	1			
Arthropoda	*Nitocra lacustris*	1			
Arthropoda	*Oithona davisae*	1			
Arthropoda	*Oithona similis*	1	Gulliksen et al., 1999; Palerud et al., 2004	X	Not an NIS for Svalbard
Arthropoda	*Paralithodes camtschaticus*	1, 2, 3	Palerud et al., 2004		
Arthropoda	*Parapontella brevicornis*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Paronychocamptus huntsmani*	1			
Arthropoda	*Podon leuckartii*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Pseudocalanus*	1			
Arthropoda	*Pseudodiaptimus forbesi*	1			
Arthropoda	*Pseudodiaptimus marinus*	1			
Arthropoda	*Schizopera clandestina*	1			

Arthropoda	*Sinocalanus doerrii*	1			
Arthropoda	*Sinocorophium heteroceratum*	1			
Arthropoda	*Temora longicornis*	1, 4	Ballast water (Ware et al., 2016)		
Arthropoda	*Temora turbinata*	1, 2			
Arthropoda	*Tortanus dextrilobatus*	1			
Bryozoa	*Schizoporella japonica*	3			
Bryozoa	*Schizoporella unicornis*	1	Gulliksen et al., 1999; Palerud et al., 2004		
Chlorophyta	*Codium fragile*	1, 2			
Chordata	*Acanthogobius flavimanus*	1			
Chordata	*Albatrossia pectoralis*	1			
Chordata	*Ascidiella aspersa*	3			
Chordata	*Ascidiella scabra*	3			
Chordata	*Botrylloides violaceus*	1		X	
Chordata	*Botryllus schlosseri*	1	Gulliksen et al., 1999; Palerud et al., 2004		
Chordata	*Ciona intestinalis*	1, 2	Gulliksen et al., 1999; Palerud et al., 2004		
Chordata	*Clavelina lepadiformis*	3			
Chordata	*Corella parallelogramma*	3			
Chordata	*Diplosoma listerianum*	3			
Chordata	*Esox lucius*	1			
Chordata	*Molgula manhattensis*	2		X	
Chordata	*Oncorhynchus gorbuscha*	2, 3			
Chordata	*Oncorhynchus kisutch*	2			
Chordata	*Oncorhynchus mykiss*	1, 2			
Chordata	*Platichtys flesus*	1, 2			
Chordata	*Salvelinus fontinalis*	1			
Cnidaria	*Alcyonium digitatum*	3			
Cnidaria	*Garveia franciscana*	1			
Echinodermata	*Echinus esculentus*	3	Gulliksen et al., 1999; Palerud et al., 2004		
Echinodermata	*Gracilechinus acutus*	3			
Foraminifera	*Trochammina hadai*	1			
Mollusca	*Cerastoderma edule*	1			
Mollusca	*Magallana/Crassostrea gigas*	2			

Mollusca	*Mya arenaria*	1	Gulliksen et al., 1999; Palerud et al., 2004		
Mollusca	*Mytilus galloprovincialis*	1, 2	Berge et al., 2005; Mathiesen et al., 2016, Leopold et al., 2019	at genus level	
Mollusca	*Patella vulgata*	3			
Mollusca	*Ruditapes philippinarum*	2			
Myzozoa	*Karenia mikimotoi*	2			
Nematoda	*Ascolaimus sp.*	1			
Nematoda	*Axonolaimidae*	1			
Nematoda	*Chromadorina erythrophthalma*	1			
Nematoda	*Daptonema tenuispiculum*	1			
Nematoda	*Geomonhystera sp.*	1			
Ochrophyta	*Fucus cottonii*	1			
Ochrophyta	*Fucus serratus*	1, 2	Gulliksen et al., 1999		
Ochrophyta	*Heterosigma akashiwo*	1, 2			
Ochrophyta	*Mediopyxis helysia*	1, 2			
Ochrophyta	*Microspongium globosum*	1			
Ochrophyta	*Neodenticula seminae*	1, 2			
Ochrophyta	*Sargassum muticum*	1, 2			
Ochrophyta	*Stephanopyxis turris*	1, 2			
Platyhelminthes	*Gyrodactylus salaris*	2			
Porifera	*Cliona thoosina*	1			
Rhodophyta	*Bonnemaisonia hamifera*	1, 2			
Rhodophyta	*Caulacanthus ustulatus*	1			
Rhodophyta	*Ceramium sinicola*	1			
Rhodophyta	*Chroodactylon ornatum*	1			
Rhodophyta	*Dasysiphonia japonica*	2			
Rhodophyta	*Dumontia contorta*	1, 2	Gulliksen et al., 1999		
Tracheophyta	*Cotula coronopifolia*	1			

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Online Resource 4

***Table A**. Species identified in the sediment samples collected in Krossfjorden and Kongsfjorden (Ny-Ålesund (NA) harbour, Ny-Ålesund (NA) mine and Lovénbreen) in July 2017, based on the 18S marker. The numbers represent in how many samples the species were identified.*

18S		**Location**				**Total**
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
Acanthocephala	*Echinorhynchus* sp.				1	**1**
Amoebozoa	*Paraflabellula hoguae*		1			**1**
Annelida	*Amphitrite ornata*			1		**1**
	Apistobranchus sp.	1	3	2	3	**9**
	Capitella sp.	2	4	2	4	**12**
	Dipolydora bidentata				1	**1**
	Eteone longa	1			2	**3**
	Lamproderma sp.		1			**1**
	Lysippe labiata				2	**2**
	Maldane sarsi	1				**1**
	Parougia sp.	1		2	1	**4**
	Pygospio sp.	2				**2**
	Scalibregma inflatum	1				**1**
	Scoloplos sp.	1		3	2	**6**
	Terebellides stroemii		1	3		**4**
	Tharyx sp.			2	1	**3**
Arthropoda	*Acarothrix* sp.	2	9	2		**13**
	Acartia sp. (*bifilosa*)	1				**1**
	Ameira sp.		2			**2**
	Aurila disparata	2	1	6		**9**

18S		**Location**				**Total**
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Bradya sp.			1		**1**
	Bythoceratina sp.			2		**2**
	Calanus sp. (*finmarchicus*)	1	1	5	6	**13**
	Cobanocythere japonica	1				**1**
	Euterpina acutifrons	3	5	2	1	**11**
	Howeina sp.		1	6	1	**8**
	Itunella sp.			1		**1**
	Leptocythere sp.	1		2		**3**
	Mesocletodes sp.		1		1	**2**
	Metarhombognathus sp.		2			**2**
	Oithona similis		1			**1**
	Paramphiascella sp.				1	**1**
	Sentiropsis sp.				1	**1**
	Spinileberis quadriaculeata	1		1		**2**
	Stenhelia sp.			1		**1**
	Thompsonula sp.	2	2	1	1	**6**
	Trachyleberis sp.	3	1	7	2	**13**
Ascomycota	*Saccharomyces cerevisiae*		1			**1**
Bigyra	*Aplanochytrium* sp.	4	2	4	5	**15**
	Oblongichytrium sp.	1			1	**2**
Bryozoa	*Alcyonidium mamillatum*			1		**1**
Cephalorhyncha	*Pycnophyes* sp.			3	2	**5**
Cercozoa	*Allas* sp.	2	2	8	7	**19**
	Bodomorpha sp.		2		2	**4**

18S		Location				Total
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Cryothecomonas aestivalis	2	4	8	7	**21**
	Cryothecomonas sp.	4	10	8	7	**29**
	Paulinella sp.	1		6	6	**13**
	Phagomyxa sp.			1	2	**3**
	Protaspis grandis	2	6	6	2	**16**
	Protaspis sp.	4	9	8	8	**29**
	Pseudopirsonia mucosa	1				**1**
	Pseudopirsonia sp.	2				**2**
Chlorophyta	*Acrosiphonia* sp.	4	8	1	6	**19**
	Bolbocoleon piliferum		1			**1**
	Chaetomorpha melagonium		1			**1**
	Chlamydomonas sp.	2	4			**6**
	Chlorella sp.		1			**1**
	Chromochloris zofingiensis		1			**1**
	Cladophora sp.		1			**1**
	Crustomastix sp.		1			**1**
	Dolichomastix sp.	2	10	7	8	**27**
	Mamiella sp.		1	1	1	**3**
	Mantoniella sp.	3	10	7	6	**26**
	Micromonas sp.	4	10	8	8	**30**
	Planophila sp.		1			**1**
	Pterosperma cristatum				2	**2**
	Pterosperma sp.	1	5	6	7	**19**
	Pyramimonas australis	1	1	1	1	**4**

18S		Location				Total
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Pyramimonas sp.	3	3	3		**9**
	Scotinosphaera sp.		1			**1**
	Trebouxia sp.		1			**1**
	Ulva sp.	1				**1**
Choanozoa	*Lagenoeca* sp.			1		**1**
Chordata	*Boltenia* sp.			1		**1**
	Dendrodoa grossularia		1			**1**
	Halocynthia sp.		1			**1**
	Molgula sp. (*manhettensis*)				1	**1**
	~~*Ophioblennius* sp.~~ (Apicomplexa)	2	2	1	1	**6**
	Pelonaia corrugata				5	**5**
Ciliophora	*Acineta* sp.	4	10	8	7	**29**
	Amphileptus sp.			1		**1**
	Anigsteinia sp.			1		**1**
	Aspidisca fusca	1				**1**
	Cyclotrichium cyclokaryon	3	4	7	6	**20**
	Epiclintes auricularis			1		**1**
	Lynnella semiglobulosa	1			2	**3**
	Mesanophrys carcini			1	1	**2**
	Paracineta sp.	2	5	3	1	**11**
	Parastrombidinopsis shimi	1	1	1		**3**
	Parauronema sp.			1		**1**
	Phialina sp.	1				**1**
	Platyophrya sp.		1			**1**

18S		Location				Total
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Spirotrachelostyla tani	2				**2**
	Strombidinopsis sp.		3	4	2	**9**
	Strombidium sp.	4	10	8	7	**29**
	Tintinnopsis sp.	1	2	3	4	**10**
	Urotricha sp.	4	3	8	5	**20**
Cnidaria	*Alveopora* sp.			1		**1**
	Aulactinia sp.		1			**1**
	Euphysa sp.	1				**1**
Cryptophyta	*Goniomonas* sp.			1		**1**
	Katablepharis japonica		3	6	6	**15**
	Telonema sp.			2		**2**
	Telonema subtile		1			**1**
Euglenozoa	*Cercomonas* sp.	1	2		2	**5**
Gastrotricha	*Heterolepidoderma loricatum*	1		2		**3**
Microsporidia	*Microsporidium* sp.		1			**1**
Mollusca	*Musculus niger*			1		**1**
	Arca (Navicula) sp.	3	5	4	1	**13**
Myzozoa	*Alexandrium* sp.	2		5	3	**10**
	Amoebophrya sp.	1				**1**
	Amphidinium sp.	3	1		1	**5**
	Ancora sagittata	4	9	7	7	**27**
	Ancora sp.	3	8	5	6	**22**
	Ansanella granifera	4	9	8	8	**29**
	Biecheleria sp.		9	8	7	**24**

18S		**Location**				**Total**
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Crypthecodinium sp.	1				**1**
	Cryptosporidium sp.	2	1	1	1	**5**
	Gymnodinium sp.		2	7	7	**16**
	Heterocapsa sp.	4	10	8	8	**30**
	Impagidinium sp.				1	**1**
	Islandinium minutum	2	4	7	7	**20**
	Islandinium tricingulatum			1		**1**
	Lankesteria sp.			1		**1**
	Lecudina cf. tuzetae			5		**5**
	Lecudina sp.	1	1	1		**3**
	Madanidinium loirii			1		**1**
	Nusuttodinium poecilochroum		1			**1**
	Polarella glacialis	1	1	4	3	**9**
	Prorocentrum foraminosum		2			**2**
	Prorocentrum sp.		8	4	5	**17**
	Protodinium simplex				3	**3**
	Psammosa pacifica			1		**1**
	Rhytidocystis sp.				2	**2**
	Selenidium sp.			5		**5**
	Urospora ovalis	2	4	1		**7**
	Warnowia sp.	4		5	1	**10**
Nematoda	*Adoncholaimus* sp.	3	5		1	**9**
	Alaimella sp.	1		2		**3**
	Bathyeurystomina sp.	2		5		**7**

18S		Location				Total
		Krossfjorden	NA harbour	NA Mine	Lovénbreen	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Calomicrolaimus parahonestus	4		4	3	**11**
	Calomicrolaimus sp.	1				**1**
	Calyptronema sp.	2	1	7		**10**
	Camacolaimus sp.	1		4	2	**7**
	Daptonema sp. (*normandicum* + *oxycerca*)	1			1	**2**
	Desmodora communis	1				**1**
	Desmodora sp.	1	1	7	5	**14**
	Desmolaimus sp.	2		1		**3**
	Desmoscolex sp.		1			**1**
	Diploscapter sp.		1			**1**
	Enoplus sp.	1	1			**2**
	Eumonhystera sp.				1	**1**
	Eurystomina sp.	1				**1**
	Halalaimus sp.	3		8	5	**16**
	Halomonhystera sp.	4	4	8	6	**22**
	Leptolaimus sp.	4	5	8	5	**22**
	Mesacanthion sp.	3	9	5	7	**24**
	Metenoploides sp.	4	1	7	3	**15**
	Monoposthia sp.	1		6		**7**
	Oncholaimus sp.	4	5	8	5	**22**
	Oxystomina sp.	3	2	8	5	**18**
	Paracyatholaimus sp.	1	2		1	**4**
	Pellioditis marina	2	2			**4**
	Sabatieria pulchra	1	1			**2**

18S		**Location**				**Total**
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Sabatieria sp.	4	2	8	6	**20**
	Sphaerolaimus sp.	1		2	5	**8**
	Terschellingia longicaudata	1		1		**2**
	Theristus sp.	1				**1**
	Thoracostomopsis sp.	1	2	7	1	**11**
	Viscosia sp.	3	7	1	1	**12**
Nemertea	*Carinoma* sp.		2			**2**
	Cephalothrix sp.				1	**1**
	Micrura sp.			2	1	**3**
Ochrophyta	*Ankylochrysis* sp.		2			**2**
	Apedinella radians	3	2	4	3	**12**
	Chaetoceros cinctus	1	1	6	3	**11**
	Chaetoceros mannaii		1		1	**2**
	Chaetoceros sp.	4	7	8	7	**26**
	Cylindrotheca sp.			4		**4**
	Desmarestia sp.	1	1	2		**4**
	Dinobryon sp.		1	1		**2**
	Diploneis sp.	2	1	3		**6**
	Giraudyopsis sp.	1			3	**4**
	Gyrosigma sp.			1		**1**
	Helicopedinella sp.	3	2	4	7	**16**
	Heribaudiella sp.		1			**1**
	Licmophora juergensii	1	1			**2**
	Nannochloropsis sp.		1			**1**

18S		**Location**				**Total**
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Nitzschia sp.	1		4	1	**6**
	Paraphysomonas sp.	1	4	8	6	**19**
	Pelagomonas sp.	4	8	8	7	**27**
	Pleurosigma intermedium	1		5	2	**8**
	Pleurosigma sp.	1		3	4	**8**
	Pseudopedinella elastica	4	10	8	8	**30**
	Pseudopedinella sp.	1		1	1	**3**
	Pteridomonas sp.			1		**1**
	Pylaiella littoralis	1	1			**2**
	Sellaphora sp.	1				**1**
	Skeletonema sp.	2			2	**4**
	Thalassiosira concaviuscula				1	**1**
	Thalassiosira sp.	4	3	7	7	**21**
	Thaumatomastix sp.	2		7	7	**16**
	Uroglena sp.		1			**1**
Oomycota	*Anisolpidium rosenvingei*		2			**2**
	Anisolpidium sp.	1	1		3	**5**
	Lagenidium sp.		1			**1**
	Olpidiopsis sp.			1		**1**
Platyhelminthes	*Danorhynchus* sp.	3	1	4	3	**11**
	Dolichomacrostomum sp.	1				**1**
	Gyratrix sp.	1				**1**
	Limipolycystis sp.			1		**1**
	Limipolycystis wallbergi	1		3		**4**

18S		**Location**				**Total**
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Maehrenthalia agilis	1				**1**
	Mesorhynchus terminostylis	1		1		**2**
	Microstomum sp.	1		3	5	**9**
	Odontorhynchus sp.	4	3	6	5	**18**
	Paracrorhynchus sp.	2	1		4	**7**
	Placorhynchus sp.				3	**3**
	Plicastoma cuticulata			1	2	**3**
	Proxenetes sp.	1				**1**
Rhodophyta	*Clathromorphum* sp.	2				**2**
Rotifera	*Proales reinhardti*	3	1	1		**5**
Tardigrada	*Isohypsibius* sp.				2	**2**
Xenacoelomorpha	*Archaphanostoma agile*	1				**1**
	Kuma viridis	1			1	**2**
	Nemertoderma sp.			2		**2**
#N/A	*Anystis* sp.				1	**1**
	Aplanochytrium blankum	1		1		**2**
	Babesia sp.			1		**1**
	Characium perforatum		1		1	**2**
	Creolimax fragrantissima			1		**1**
	Cryptosporidium serpentis	2	2	7	3	**14**
	Cryptosporidium testudinis	4	6	2	1	**13**
	Cyranomonas australis			3	2	**5**
	Cyranomonas sp.	3	8	7	5	**23**
	Kentrophyllum setigerum	1				**1**

18S		Location				
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	**Total**
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Labyrinthulochytrium sp.	1				**1**
	Pseudoperkinsus tapetis		2			**2**
	Ventrifissura sp.			3		**3**
	Ebria tripartita	1	3	6	5	**15**
	Heteromita globosa		1			**1**
	Heteromita sp.		2			**2**
	Pirsonia sp.	**3**	**5**	**8**	**7**	**23**

Table B*. Species identified in the sediment samples collected in Krossfjorden and Kongsfjorden (Ny-Ålesund harbour, Ny-Ålesund mine and Lovénbreen) in July 2017, based on the COI marker. The numbers represent in how many samples the species were identified.*

COI		**Location**				**Total**
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
Amoebozoa	*Copromyxa sp.*		1			**1**
	Squamamoeba sp.	1			1	**2**
Annelida	*Amphitrite cirrata*	1				**1**
	Chaetozone setosa	1		7	6	**14**
	Chaetozone sp.		1		3	**4**
	Chone mollis	1				**1**
	Galathowenia oculata				1	**1**
	Harmothoe sp.	2	1		1	**4**
	Leitoscoloplos sp.	2	1	5	1	**9**
	Lumbrineris sp.	1		1	3	**5**
	Lysippe labiata			1	2	**3**
	Melinna maculata	3		1		**4**
	Pholoe baltica	2	2	2	2	**8**
	Pholoe sp.	1				**1**
	Scolelepis sp.			3	1	**4**
	Scoloplos sp.	3	2	6	6	**17**
	Spio sp.	1	2			**3**
	Tubificoides kozloffi	1				**1**
	Lumbrineris fragilis			1	1	**2**
	Maldane sarsi	1				**1**
	Terebellides stroemii		1			**1**
Arthropoda	*Balanus sp.*			1		**1**

COI		Location				Total
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
	Micropsectra radialis				1	**1**
	Semibalanus balanoides			1	1	**2**
Cephalorhyncha	*Priapulus caudatus*			2	1	**3**
Chlorophyta	*Micromonas pusilla*	4	10	8	7	**29**
	Micromonas sp.				3	**3**
Chordata	*Botrylloides violaceus*		1			**1**
Cnidaria	*Euphysa flammea*			1		**1**
	Plotocnide borealis			2	4	**6**
	Plotocnide sp.				1	**1**
	Sarsia tubulosa			1		**1**
Mollusca	*Admete viridula*			1		**1**
	Astarte borealis				2	**2**
	Ennucula tenuis			3	2	**5**
	Hiatella sp.	1				**1**
	Macoma calcarea	3	2		6	**11**
	Margarites sp.		1			**1**
	Musculus niger			1		**1**
	Mytilus sp.		1			**1**
	Oenopota sp.				1	**1**
	Thyasira cf. gouldii			1		**1**
Myzozoa	*Polarella glacialis*			1		**1**
	Woloszynskia sp.		2	2	5	**9**
Nematoda	*Terschellingia sp.*	1				**1**
Nemertea	*Cephalothrix iwatai*				1	**1**

COI		**Location**				**Total**
		Krossfjorden	**NA harbour**	**NA Mine**	**Lovénbreen**	
Number of samples		**4**	**10**	**8**	**8**	**30**
Phylum	**Latin name**					
Ochrophyta	*Alaria esculenta*	3	2	1	1	**7**
	Chorda filum		3			**3**
	Chordaria chordaeformis		2	1		**3**
	Chordaria flagelliformis	1	3	1	2	**7**
	Desmarestia sp.	4	4	6	4	**18**
	Dictyosiphon foeniculaceus	1	1			**2**
	Dictyosiphon sp.		1		1	**2**
	Ectocarpus sp.		1			**1**
	Fucus sp. (disticus)	4	4	1	1	**10**
	Grammonema striatula	3			4	**7**
	Haplospora globosa	2	2	1		**5**
	Pylaiella washingtoniensis		1		2	**3**
	Saccharina groenlandica		1			**1**
	Saccharina sp.	4	5	5	5	**19**
	Thalassiosira punctigera	3	4	1		**8**
	Thalassiosira sp.	1				**1**
Rhodophyta	*Devaleraea ramentacea*		1			**1**
	Polysiphonia arctica			7		**7**

Online Resource 5

Table. *List of potential NIS for the Svalbard region with incomplete 18S and COI barcode markers in NCBI (EMODnet Arctic, Norwegian Biodiversity Information Centre, 2018; Chan et al., 2018).*

Phylum	**Latin name**	**Presence in NCBI**	**Arctic NIS list**
Arthropoda	*Acartiella sinensis*	Two COI sequences available, 18S absent	EMODnet Arctic
Arthropoda	*Ampelisca abdita*	Neither COI nor 18S present	EMODnet Arctic
Arthropoda	*Amphibalanus improvisus*	COI present, 18S absent	Norwegian Biodiversity Information Centre
Arthropoda	*Crassicorophium bonellii*	Two COI sequences available, 18S absent	EMODnet Arctic
Arthropoda	*Eurytemora americana*	Neither COI nor 18S present	Chan et al., 2018
Arthropoda	*Gammarus daiberi*	COI present, 18S absent	EMODnet Arctic
Arthropoda	*Gammarus zaddachi*	COI present, 18S absent	EMODnet Arctic
Arthropoda	*Heterolaophonte stroemii stroemii*	Neither COI nor 18S present	EMODnet Arctic
Arthropoda	*Hyperacanthomysis longirostris*	One COI sequence available, 18S absent	EMODnet Arctic
Arthropoda	*Ischyrocerus commensalis*	Neither COI nor 18S present	Norwegian Biodiversity Information Centre
Arthropoda	*Limnoithona tetraspina*	One 18S sequence available, COI absent	EMODnet Arctic
Arthropoda	*Lithodes maja*	COI present, 18S absent	Norwegian Biodiversity Information Centre
Arthropoda	*Megabalanus spinosus*	One 18S sequence available, COI absent	EMODnet Arctic
Arthropoda	*Parapontella brevicornis*	Neither COI nor 18S present	EMODnet Arctic
Arthropoda	*Paronychocamptus huntsmani*	Neither COI nor 18S present	EMODnet Arctic
Arthropoda	*Pseudodiaptomus forbesi*	Eight COI sequences available, 18S absent	EMODnet Arctic
Arthropoda	*Schizopera clandestina*	Neither COI nor 18S present	EMODnet Arctic
Arthropoda	*Sinocalanus doerrii*	Neither COI nor 18S present	EMODnet Arctic
Arthropoda	*Sinocorophium heteroceratum*	Neither COI nor 18S present	EMODnet Arctic
Bryozoa	*Schizoporella japonica*	Neither COI nor 18S present	Norwegian Biodiversity Information Centre
Bryozoa	*Schizoporella unicornis*	Neither COI nor 18S present	EMODnet Arctic
Chlorophyta	*Codium fragile*	Neither COI nor 18S present	EMODnet Arctic, Chan et al., 2018
Chordata	*Albatrossia pectoralis*	COI present, 18S absent	EMODnet Arctic
Chordata	*Ascidiella scabra*	COI present, 18S absent	Norwegian Biodiversity Information Centre
Chordata	*Botrylloides schlosseri*	Neither COI nor 18S present	EMODnet Arctic
Chordata	*Corella parallelogramma*	Neither COI nor 18S present	Norwegian Biodiversity Information Centre
Chordata	*Oncorhynchus gorbuscha*	COI present, 18S absent	Chan et al., 2018
Chordata	*Oncorhynchus mykiss*	COI present, 18S absent	EMODnet Arctic
Cnidaria	*Garveia franciscana*	Neither COI nor 18S present	EMODnet Arctic

Echinodermata	*Gracilechinus acutus*	COI present, 18S absent	Norwegian Biodiversity Information Centre
Foraminifera	*Trochammina hadai*	18S present, COI absent	EMODnet Arctic
Nematoda	*Axonolaimidae*	Family name, not able to search	EMODnet Arctic
Nematoda	*Chromadorina erythrophthalma*	Neither COI nor 18S present	EMODnet Arctic
Nematoda	*Daptonema tenuispiculum*	Neither COI nor 18S present	EMODnet Arctic
Nematoda	*Prochromadora orleji*	Neither COI nor 18S present	EMODnet Arctic
Ochrophyta	*Fucus cottonii*	Neither COI nor 18S present	EMODnet Arctic
Ochrophyta	*Heterosigma akashiwo*	18S present, COI absent	Chan et al., 2018
Ochrophyta	*Mediopyxis helysia*	One 18S sequence available, COI absent	EMODnet Arctic, Chan et al., 2018
Ochrophyta	*Microspongium globosum*	Neither COI nor 18S present	EMODnet Arctic
Ochrophyta	*Neodenticula seminae*	Five 18S sequences available, COI absent	EMODnet Arctic
Porifera	*Cliona thoosina*	Neither COI nor 18S present	EMODnet Arctic
Rhodophyta	*Ceramium sinicola*	Neither COI nor 18S present	EMODnet Arctic
Rhodophyta	*Chroodactylon ornatum*	One 18S sequence available, COI absent	EMODnet Arctic
Rhodophyta	*Dasysiphonia japonica*	One COI sequence available, 18S absent	Chan et al., 2018
Rhodophyta	*Dumontia contorta*	COI present, 18S absent	Chan et al., 2018
Tracheophyta	*Cotula coronopifolia*	Three 18S sequences available, COI absent	EMODnet Arctic

References

EMODnet Arctic: http://www.emodnet-arctic.eu/alien-species

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Norwegian Biodiversity Information Centre (2018). The alien species list of Norway – ecological risk assessment 2018. Retrieved in January 2019 from https://www.biodiversity.no/alien-species-2018

Online Resource 6

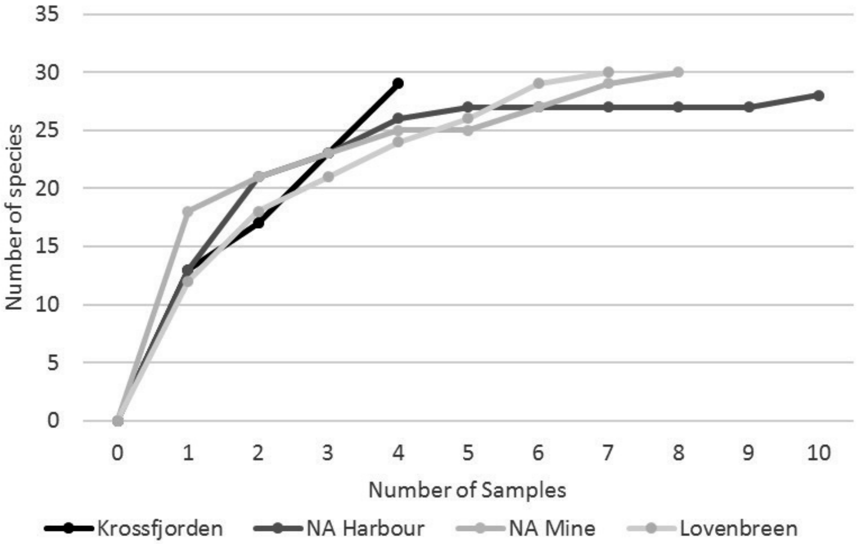

Figure A. *Cumulative number of new species collected per number of sediment samples taken based on COI marker.*

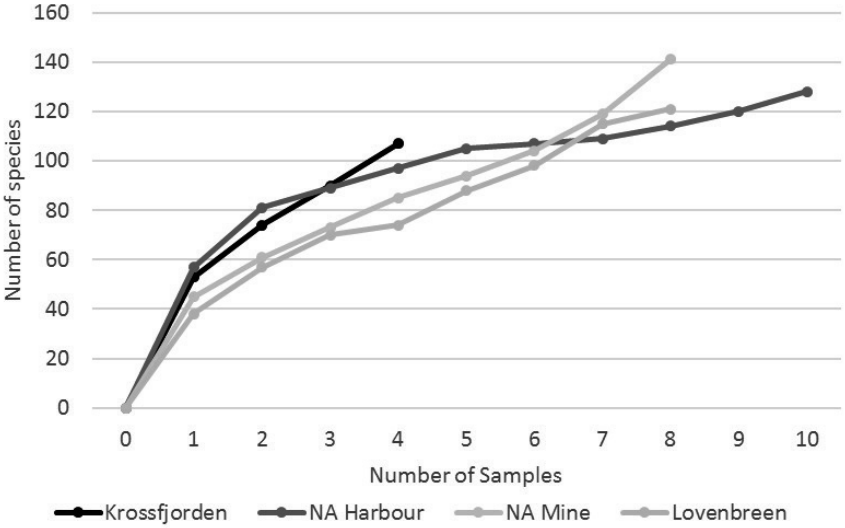

Figure B. *Cumulative number of new species collected per number of sediment samples taken based on 18S marker.*

Online Resource 7

Material and Methods

A small (<15 cm) single piece of translucent soft plastic floating in Ny-Ålesund harbour was sampled, stored frozen and analysed via DNA metabarcoding using the same approach as described for the soft sediments.

In total, 67 species were identified on the piece of floating plastic (Online Resource 5), of which 26 were not found in the sediment samples. From the plastic, 52 species were identified using the 18S marker; 24 of these were not identified in any of the sediment samples. Using the COI marker, 15 species were detected on the plastic, two of which were not found in the sediment samples.

In the one piece of floating soft plastic that was analysed in this pilot study, 26 species were found in addition to those in the sediment, implying the additional value of multiple sample types for biodiversity assessments representing different habitats.

Results

***Table A**. Species identified on a piece of floating plastic in the harbour of Ny-Ålesund, Kongsfjorden, in July 2017, based on the 18S and COI DNA markers.*

Phylum	**Latin name**	**18S**	**CO1**	**Exclusively on plastic compared with the sediment samples**
Arthropoda	*Calanus sp.*	x		
	Oithona similis	x	x	
Bacteroidetes	*Olleya sp.*		x	x
Bigyra	*Aplanochytrium sp.*	x		
	Oblongichytrium sp.	x		
	Pseudobodo sp.	x		x
Chlorophyta	*Acrosiphonia sp.*	x		
	Chlamydomonas sp.	x		
	Micromonas pusilla		x	
	Micromonas sp.	x	x	
	Pyramimonas sp.	x		
Choanozoa	*Bicosta minor*	x		x
	Calliacantha natans	x		x
	Stephanoeca apheles	x		x
Chordata	~~*Ophioblennius sp.*~~ (Apicomplexa)	x		
Ciliophora	*Acineta sp.*	x		
	Cothurnia sp.	x		x
	Cyclotrichium cyclokaryon	x		

	Dysteria lanceolata	x		x
	Ephelota mammillata	x		x
	Epicarchesium sp.	x		x
	Loxophyllum sp.	x		x
	Paracineta sp.	x		
	Pseudovorticella sinensis	x		x
	Pseudovorticella sp.	x		x
	Thigmokeronopsis stoecki	x		x
	Uronema orientalis	x		x
Cnidaria	*Aglantha digitale*		x	x
	Cyanea capillata		x	
	Cyanea sp.	x		x
Cryptophyta	*Telonema sp.*	x		
	Telonema subtile	x		
Mollusca	*Hiatella sp.*		x	
Myzozoa	*Amphidinium sp.*	x		
	Ansanella granifera	x		
	Biecheleria sp.	x		
	Gymnodinium sp.	x	x	
	Gyrodinium helveticum	x		x
	Gyrodinium heterogrammum	x		x
	Gyrodinium sp.	x		x
	Heterocapsa sp.	x		
	Karenia papilionacea	x		x
	Lepidodinium sp.	x		x
	Podolampas sp.	x		x
Nematoda	*Halomonhystera sp.*	x		
	Theristus sp.	x		
Ochrophyta	*Alaria esculenta*		x	
	Chorda filum		x	
	Fucus sp.		x	
	Grammonema striatula		x	
	Hydrurus sp.	x		x
	Licmophora juergensii	x		
	Paraphysomonas sp.	x		
	Pelagomonas sp.	x		

	Pteridomonas sp.	x		
	Pylaiella littoralis	x		
	Pylaiella washingtoniensis		x	
	Saccharina sp.		x	
	Skeletonema sp.	x		
Rhodophyta	*Devaleraea ramentacea*		x	
Rotifera	*Proales reinhardti*	x		
#N/A	*Incisomonas sp.*	x		x
	Pirum gemmata	x		x
	Amastigomonas sp.	x		x

CHAPTER 6

GENERAL DISCUSSION

Introduction

This thesis aimed to assess the extent of current and future pressures related to local human activities on (epi)benthic marine invertebrates living in warming Arctic coastal systems, using Svalbard as a case study. Two of the most important and widespread activities currently on Svalbard were selected to be ship traffic and mining. Two consequences of these activities, the pressures, were identified as potentially important for the ecosystem: contamination with toxic compounds and the introduction of non-indigenous species (NIS). These pressures were selected to further study their impacts on local benthic invertebrates, a group of organisms important both ecologically and as monitoring targets. Firstly, the pressure of the introduction of contaminants was assessed in the connection to two types of human activities: run off from historical coal mines and through shipping (via antifouling paints and potential oil spills). Secondly the pressure of the introduction of non-indigenous species in Svalbard coastal systems was investigated, also being primarily related to shipping (via ballast water and fouling).

In this chapter the results of these studies are discussed in relation to the objectives as outlined in Chapter 1, consisting of:

1. To assess the potential impact of (current and foreseeable) contaminants originating from local human activities on (epi)benthic invertebrates living in the Svalbard coastal system.

2. To assess the potential impact of (current and foreseeable) non-indigenous species related to local human activities on (epi)benthic invertebrates living in the Svalbard coastal system.

This general discussion will close with advice for management, recommendations for future research and monitoring, and perspectives for the wider Arctic.

Objective: 1. Assess the potential impact of (current and foreseeable) contaminants originating from local human activities on (epi)benthic invertebrates living in the Svalbard coastal system

In this thesis considered sources of contaminants are either land-based or ship-based.

Emission of mercury and Polycyclic Aromatic Hydrocarbons (PAHs) from land-based sources

Land-based sources of contaminants that are due to human activities can influence the coastal environment (Granberg et al. 2017, **Chapter 2**). In the Arctic, human activities often take place directly on the coastline for logistical reasons (Granberg et al. 2017), which leads to a greater chance of contaminants reaching the marine coastal system through these activities. Contaminants can either be directly emitted into the marine environment (for instance waste water) or indirectly as run-off from contaminated sites on land. How far the emissions reach into the coastal system can be determined by assessing the exposure radius of land-based sources.

In **Chapter 2** the exposure radius of contaminants from the historical coal mine of Ny-Ålesund, in northwest Svalbard, was assessed, using mercury (Hg) and Polycyclic Aromatic Hydrocarbons (PAHs) as tracers. Based on the mercury concentrations, a clustering of PAH concentrations and the PAH diagnostic ratios in sediment samples, the contamination from this land-based source could be detected up to a few kilometres away. The contaminant signatures were mainly observed on land in the sediment of meltwater streams, and less in the marine sediment collected directly below the outflow of these meltwater streams. The levels in the receiving coastal marine system were clearly influenced by the dilution from marine currents. A similar exposure radius was found in a study on Antarctica, with decreasing concentrations of Polybrominated Biphenylethers (PBDEs) found both on land, in lichens and mosses, as well as in marine amphipods and fish with increasing distance from the studied research station (Wild et al. 2015). The extent of such an exposure radius will largely depend on the the total mass of the contaminants at the source, in combination with the outflow pathways (allowing for sedimentation or not) and characteristics of the coastal system such as the degree of dilution. Besides the (historic) coal mines, mercury can enter the environment locally via emission from the two coal-power plants in Longyearbyen and Barentsburg and, to some extent, via rock types that naturally contain mercury (AMAP 2021). PAHs can enter the environment, apart from the coal-power plants, via fuel storage, garbage dumpsites and harbour activities (Granberg et al. 2017, and references therein, Ademollo et al. 2021).

The next step after quantification of the contaminant exposure radius is a risk assessment, in which the bioavailable concentration of the contaminants is compared to the safe level to decide whether the contaminants may pose an ecotoxicological risk. Mercury concentrations in marine biota were at least two orders of a magnitude below reported lethal critical body residues for mercury in invertebrates and also below those that may result in sublethal effects (AMAP 2021, Barst et al. 2022). Therefore, the observed mercury levels are not expected to pose toxicological risks in these Arctic benthic invertebrates.

One of the biota samples in the harbour of Ny-Ålesund, as described in **Chapter 2**, had a mercury concentration just above the Environmental Quality Standard (EQS) of 20 ng/g (µg/kg) Hg wet weight, as established for biota by the European Commission to protect top predators from secondary poisoning (EC, 2008). Mercury is known to bioaccumulate and biomagnify in food webs, leading to enhanced contaminant concentrations at top trophic levels (Ruus et al. 2015, Øverjordet et al. 2015). Benthic invertebrates are considered an important link in the transfer of mercury in food webs (Korejwo et al. 2022). In the Arctic, there are numerous reported health risks in marine birds and mammals in the top of the food web related to exposure to mercury (Jæger et al. 2009, Ruus et al. 2015, van den Brink et al. 2018, Chastel et al. 2022). On Svalbard, elevated mercury levels were found in seabirds, such as the black-legged kittiwake, *Rissa tridactyla*, the northern fulmar, *Fulmarus glacialis*, and the glaucous gull, *Larus hyperboreus*, at a level posing a risk for mercury-mediated health effects (AMAP 2021). More specifically, a negative relationship between mercury levels and breeding success was observed in black-legged kittiwakes breeding in the Kongsfjorden on Svalbard (Tartu et al. 2016, Goutte et al. 2015). These seabirds feed during the breeding season predominantly in the fjords, with their main diet consisting of fish as well as of invertebrate species, such as crustaceans and to a lesser extent polychaetes (Vihtakari et al. 2018). Mammalian top predators on Svalbard, such as polar bears and arctic fox did not reach levels posing adverse health risks from their mercury levels (Lippold et al. 2022, AMAP 2021).

The PAH concentrations found in marine biota, as described in **Chapter 2**, were also well below threshold PAH concentrations for effects in marine species. Therefore there is no indication for concern about adverse effects of PAHs on the local ecosystem nor for the top predators in the region.

Emission of tributyltin (TBT) and oil from shipping

Shipping is the most common human activity along the Svalbard coastline with vessels being used for cargo transport, tourism, fisheries and research. They are responsible for emission of a variety of contaminants through exhausts, ballast, black, grey and scrubber water, bilge discharges, antifouling paint components, stern tube oil leaks and the potential of spills (Jalkanen et al. 2021, Thor et al. 2021). Other contaminants related to shipping activities include heavy metals, PAHs and other hydrocarbons, and biocides from antifouling paints.

A contaminant that is clearly related with shipping is Tributyltin (TBT), an organotin compound which is the active ingredient in many antifouling paints that are applied on ship hulls to protect them from fouling. The International Maritime Organisation (IMO) has banned the use of TBT containing antifouling paints since 2008, but not all flag states have ratified this ban and therefore ships with TBT containing antifouling paints are still around. Harbours are a relevant location to assess the occurrence and impact of TBT as harbours and shipping lanes were found to be the hotspots for TBT pollution (Ten Hallers-Tjabbes et al. 1994). The results of this thesis (**Chapter 4**) show that the current TBT exposure in harbour sediment of Svalbard is low compared to more populated shipping routes and harbours around the world. Organotin levels around Svalbard were found to originate from historical TBT input based on the Butyltin Degradation Index. As this was the first study to measure organotin concentrations in harbour sediments on Svalbard, trends could not yet be established but levels are expected to go down as is the case in other parts of the world.

Chronic exposure to TBT can lead to a variety of sublethal effects, of which imposex is the most well-known. When developing marine snails are exposed to TBT male characteristics can be formed in females with concomitant hormonal changes, which can render them infertile and eventually result in an overall population decline (Bryan et al. 1987). Imposex has been observed to occur in whelks of Ny-Ålesund harbour, northwest Svalbard, in the mid-1990s (Brick & Bolte 1994). However in the current assessment of imposex in female whelks collected in this harbour, no signs of this phenomenon were found (**Chapter 4**). Organotin compounds generally do not biomagnify in top predators (Kucklick & Ellisor 2019). Limited data on organotin concentrations in the liver of ringed seals from Kongsfjorden in 2000 reported low organotin concentrations (Berge et al. 2004). Overall, current organotin exposure around Svalbard is relatively low and has probably decreased, and as imposex occurrence has not been observed anymore, there is no immediate reason for concern, neither for the benthic invertebrates nor for higher trophic levels on Svalbard.

Another contaminant directly related to shipping is oil. It can be unintentionally released via for instance bilge water or spills (Jalkanen et al. 2021) and can result in short-term acute exposures. For a proper risk assessment of oil spills in the Arctic, acute toxicity data are needed which, unfortunately, are hardly available. Therefore, in this thesis, it was studied whether temperate acute toxicity data for oil can be used as indication for the Arctic region. To do this, the effect of temperature on the toxicity of oil was studied in an experimental set-up. The results show that temperature influenced the concentration and composition of dissolved oil in sea water, as well as the acute toxicity of oil to temperate marine gammarid amphipods (**Chapter 3**). At a lower test temperature, the LC50 for oil was twofold higher (so induced lower toxicity) in the gammarids. A direct comparison of the exposure of Arctic and temperate *Gammarus* sp. to the same oil type and at similar experimental conditions (at 4°C), showed that the Arctic gammarids had a threefold higher LC50 for oil than temperate gammarids. In addition to differences in oil concentrations and composition dissolved in the water, the functional traits of the gammarids may largely explain this difference, with Arctic gammarids having a larger body size and weight, a higher total lipid content and a different lipid composition than their temperate counterparts. Also a Critical Body Residue (CBR) was determined in the exposed temperate and Arctic gammarids, which was the first time to our knowledge to be established for oil as complex mixture. Surprisingly the CBR in Arctic gammarids was in the same range of that of the temperate gammarids (**Chapter 3**). The results of the study showed that temperate oil toxicity data were roughly within half an order of a magnitude of that of Arctic species for LC50 and within a factor two for CBRs. This was considered 'close' in modelling studies (Hendriks et al., 2001) and therefore it was concluded that they can be used as proxy for the sensitivity of Arctic species to oil, which was also posed by modelling studies (De Hoop et al. 2011, Olsen et al. 2013). This risk assessment did not consider the sublethal effects or effects after chronic exposure. With invertebrate species being a key component of the Svalbard coastal food web, exposure to oil may ultimately also affect other trophic levels that depend on these species for food.

Other relevant contaminants and their sources on Svalbard

This thesis focused on three types of contaminants with a characteristic representative for each of these types: 1) contaminants having the potential to exert acute effects (oil), 2) contaminants having the potential to cause chronic or sublethal effects (TBT), and 3) contaminants that are prone to bioaccumulate or biomagnify in food webs (mercury). Especially for the last type of contaminants, several other contaminant (groups) could have been selected as an example, such as Polychlorinated Biphenyls (PCBs), PBDEs, Per- and polyfluoroalkyl substances (PFAS) and organochlorine pesticides. These have all been shown to bioaccumulate and biomagnify in Arctic marine food webs, including the Svalbard region (Haukås et al. 2007, Hallanger et al. 2011, Ali et al. 2021). Relationships between high concentrations of these contaminants and certain biological effects have also been reported on Svalbard (Routti et al. 2019, Scotter et al. 2019, Dietz et al. 2019). These include for instance changes in thyroid and immune systems in adult male walruses linked to lipophilic contaminants (including PCBs, PBDEs and some pesticides), the suggestion that the immune responses may be compromised in walruses as a result of PFAS exposure (Routti et al. 2019), and specific effects on some steroid hormones in female polar bear after exposure to PCBs and PCB metabolites (Gustavson et al. 2015).

These other contaminants can also be related to local human activities on Svalbard. Local sources of PCBs and pesticides are pointing towards historic pollution too, such as garbage dumpsites or landfills (Granberg et al. 2017). PFAS emissions can be directly linked to firefighting training stations at Longyearbyen and Ny-Ålesund airport as well as to diffuse releases from the local settlement (waste water) as the main sources, and also to landfills (Skaar et al. 2019, Ali et al. 2022).

Moreover, sewage treatment is still lacking in most settlements on Svalbard, and currently only installed at the research stations of Ny-Ålesund and Hornsund. As a result, untreated sewage is directly released into the marine environment also carrying traces of heavy metals, pharmaceuticals, personal care products and siloxanes in to the fjords (Warner et al. 2010, Gunnarsdóttir et al, 2013, Kalinowska et al. 2020). Marine exposure concentrations of these are not very well known, but currently it is to be expected that the released volumes are too low to exceed effect limits given the great dilution taking place in the sea water. Siloxanes were found to be taken up by fish in Adventfjorden and the sampled gradient pointed towards the settlement of Longyearbyen as the source, with concentrations decreasing with increasing distance from the settlement (Warner et al. 2010). No risk of accumulation of siloxanes in bearded seals was observed in the same study.

Mitigation potential of local sources of contaminants

Overall, the local contaminant sources on Svalbard that are of relevance for the coastal system can be grouped as following: contaminants resulting from: 1) waste water and effluents from settlements, 2) historic (historic coal mines, landfills, garbage dumps) and current land-based activities (coal mines that are still in operation, coal-power plants, fire-fighting training stations, harbour activities), and 3) shipping activities, in particular related risks on unintentional release of chemicals such as oil. Each group has its own mitigation potential in case of exceeding safe exposure levels or from a precautionary principle perspective.

Firstly, the discharge of waste water and effluents provide a continuous flow of water and potential contaminants into the coastal area of the settlements. Such a direct input of contaminants can be minimised by a proper treatment prior to discharge into the aquatic system (Granberg et al. 2017), resulting in a direct decrease of potential contaminants into the system.

Secondly, historic and current activities on land are hotspots for a variety of contaminants (mercury, PAHs, PCBs, PFAS, etcetera) and meltwater can carry these contaminants from land into the coastal system (Granberg et al. 2017, Chapter 2). Prevention of run-off will benefit the coastal system, especially for those contaminants that can accumulate in food webs, such as mercury, PCBs and PFAS. The current remediation of the Mine 7 site in Longyearbyen is a good example of this. Lessons from other regions can be learned, for instance how the clean-up of an old dumpsite at the Australian research station of Casey, Antarctica, was handled. Here, three research priorities were identified to structure the process: 1) understanding the contaminant mobilisation processes, 2) developing an ecological risk assessment for use in monitoring and setting priorities, and 3) the development of clean-up and remediation procedures (Snape et al. 2021). Such an approach helps to support the most suitable and cost-effective decisions for each case.

Thirdly, all relevant management bodies need to be well prepared for accidents that may happen along the coast, to be able to minimise risks and impacts of, for instance, an oil spill. With increased marine trafficking, the likelihood of such accidents are rising. In December 2018 a fishing vessel ran aground in the north of Svalbard and although a major oil spill could be prevented, it illustrates the need for good contingency planning and preparedness (Stocker et al. 2020). In this thesis, specific oil toxicity data were produced for an Arctic coastal invertebrate species, and it was shown that data from more temperate regions can also be used for such a risk management (**Chapter 3**).

Finally, invertebrate species are very suitable to provide information on the bioavailability and uptake of contaminants that are present in the environment. Contaminant concentrations in invertebrates can be used to predict the biomagnification and potential effects at the top of the food web, based on chemical characteristics and food web modelling. It also provides information on trends in space and time, and can show whether mitigation measures of, for instance, waste water treatment are effective, or when new challenges appear. Examples of such bioindicator species are shellfish, worms, amphipods or other benthic invertebrates (Szczybelski et al. 2016, Korejwo et al. 2022, Skogsberg et al. 2022, Turja et al. 2020, Duquesne et al. 2000). On the contrary, for source identification of contaminants it is more appropriate to sample the abiotic compartment (sediment), as biological and physiological characteristics of the indicator species may influence contaminant specific profiles that are needed to trace the sources of the observed contaminants (**Chapter 2**).

Diffuse and secondary contaminant sources on Svalbard

Besides local contaminant sources as described above, several other, more diffuse sources occur in the Arctic. Mitigation potential for these is non-existent, but they contribute to the contaminant levels that are found in the coastal system of Svalbard, and are therefore of importance to briefly include here.

Long range transport of contaminants is the transfer of contaminants from lower latitudes to and within the Arctic region via the atmosphere, sea and ocean currents, (sea) ice and rivers (AMAP 1998, Burkow & Kallenborn 2000). This process results in the deposition of a variety of contaminants, such as mercury, PCBs and PFAS, on Svalbard. As a result of this these widespread levels can be found, though in low concentrations throughout the abiotic compartment, such as in sea water, soil, snow and glacial ice (**Chapter 2**, Zaborska et al. 2017, Garmash et al. 2013, Ademollo et al. 2021, Hartz et al. 2023).

Secondary sources of contaminants are associated with the release of contaminants from glaciers, land and water that were earlier deposited via long range transport and/or local human activities. These can be remobilised due to environmental processes, mainly melting of glaciers, snow and permafrost, and the consequent run off of meltwater into the sea (Rudnicka-Kępa & Zaborska 2021). For example, freshwater inputs from land-terminating glaciers were suggested to be a secondary source of organochlorine contaminants to the Kongsfjorden system (Ademollo et al. 2021, McGovern et al. 2022a). Still, concentrations in the outflowing melt water streams of glaciers were lower than in the receiving marine environment where the slightly polluted terrestrial particles were shown to act as sorbents of dissolved contaminants in the coastal water column (Johansen et al. 2021). Interestingly, coastal organisms featured an overall decrease in contaminant concentrations from April, when there was no terrestrial run-off, to August, after several months of terrestrial run-off (Skogsberg et al. 2022, McGovern et al. 2022b). Ademollo et al. (2021) however found that PCB concentrations in sea water decreased with increasing distance from a glacier. A secondary source of heavy metals for the marine system is groundwater flow from contaminated soils near the coast (Rudnicka-Kępa & Zaborska 2021). Lastly, the input of contaminants, such as PCBs, PBDEs and HCB, via migratory birds, that are feeding at sea and upon return are excreting on the coast, has been described for Svalbard and the island of Bjornøya just south of Svalbard (Evenset et al. 2007, Kristiansen et al. 2019). Despite the input of contaminants via migratory birds that can lead to very localised hotspots, many of the secondary sources seem to dilute contaminant concentrations in the receiving coastal environment rather than enhancing them.

Trend information for contaminants in air is available for a variety of contaminants through the Zeppelin station in northwest Svalbard. These data show that concentrations of PAHs, PCBs, PBDEs, and several OCPs have been or are decreasing (Xie et al. 2022). Declining trends in air for mercury and PFAS concentrations are less clear (Muir et al. 2019, MacSween et al. 2022). Overall, in Arctic biota PCBs and OCPs have also declined over the past 2-3 decades (Rigét et al., 2019). PBDEs in Arctic biota peaked around 2003 and have since then decreased. PFAS concentrations in polar bear show a compound specific trend with PFOS decreasing in polar bear plasma since 2001-2003, but with shorter chained PFAS slightly increasing (Routti et al. 2017, Muir et al. 2019). Levels in arctic fox on Svalbard also appeared to increase although a change in diet selection may have played an important role in this (Routti et al. 2017). The continued input of PFAS via the North Atlantic Current is expected to continue (Routti et al. 2017).

Even though there is a well-documented input of multiple contaminants into the Svalbard coastal system through long-range transport, concentrations via this route appear to be too low to result in toxic effects at the level of invertebrates. Invertebrate species however take up contaminants originating from all sources: local sources, long range transport and secondary sources, thereby contributing to the transfer and bioaccumulation of these contaminants in the food web.

Monitoring

Based on the compiled information of this thesis, a monitoring strategy for contaminants can be constructed (Fig. 1). Monitoring of the abiotic part of the coastal system (sea water, sediment) can give a first indication of contaminant levels. Whether sea water and/or sediment is more suitable for monitoring depends on the contaminant (Table 1). When monitoring is conducted in invertebrate species, additional information on the bioavailability of the contaminant can be obtained (Fig. 1, Table 1). In case increasing or elevated levels of contaminants are detected, source identification and exposure quantification is needed. This can best be done in the abiotic matrix to pick up signatures from the source (Table 1 and **Chapter 2**). In case local source are identified, a further risk assessment can be started, including the prediction or identification of potential risks higher up in the food web. Risk identification or a precautionary principle approach can lead to the development of potential mitigation measures.

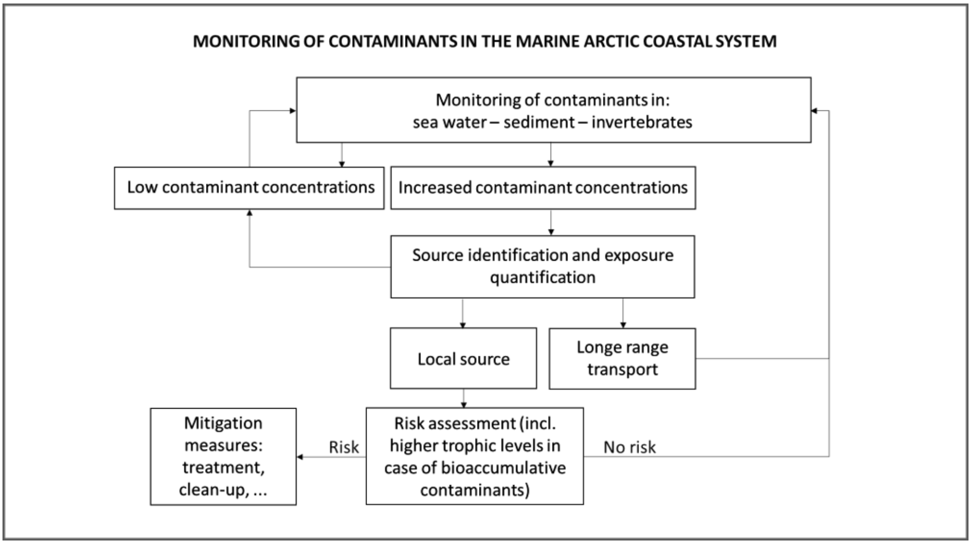

Figure 1. *Proposed monitoring strategy for contaminants in marine Arctic coastal systems.*

Table 1. *Monitoring of contaminants in the marine Arctic coastal system. Advice on the selection of sample matrices for source identification and monitoring of (non)-bioaccumulative contaminants, depending on the contaminant group. LRT = long range transport.*

Contaminant	**Source**	**Sample matrix**	**Source identification**	**Assessment of bioavailability**	**Monitoring of non-bioaccumulative contaminants**	**Monitoring of bioaccumulative contaminants**	**Reference**
Mercury	Local and LRT	Sediment	X				Chapter 2
		Invertebrates		X		X	Chapter 2
		Higher trophic levels, e.g. bird eggs				X	Chastel et al. 2022
PAHs	Local and LRT	Sediment	X				Chapter 2
		Invertebrates		X	X		Szczybelski et al. 2016
TBT	Local	Sediment	X				Chapter 4
PCBs/PBDEs /OCPs	Local and LRT	Invertebrates		X		X	Szczybelski et al. 2016
		Higher trophic levels, e.g. bird eggs				X	Helgason et al. 2011
PFAS	Local and LRT	Seawater	X				Ali et al. 202
		Invertebrates		X (short-chained)		X (short-chained)	Ali et al. 202
		Higher trophic levels, e.g. bird eggs				X (longer-chained)	Jouanneau et al. 2021
Personal Care Products	Local	Effluents and (fresh/ sea) water	X				Kallenborn et al. 2018

Conclusion on contaminants

Overall, for a sparsely populated archipelago with little industrial development, such as Svalbard, contaminant concentrations in (epi)benthic invertebrates are low (**Chapters 2 and 4**, Evenset et al. 2016, Szczybelski et al. 2016, Vieweg et al. 2012). The acute toxicity of contaminants at the level of (epi)benthic invertebrates in the marine coastal system is only expected when an accidental release of a high concentration of a contaminant occurs, such as in case of an oil spill. Chronic effects are not expected to occur in (epi)benthic invertebrates at the current contaminant levels, neither in harbours nor along the coast of Svalbard. Only one report of imposex in marine whelks on Svalbard as a result of exposure to TBT has been found, that dated back to the 1990s. Therefore, based on the present information, (epi)benthic invertebrates can be considered being not exposed to toxic levels of contaminants. However, as discussed in the case of mercury (and other biomagnifying contaminants), they are important components of the diet for higher trophic level species and therefore play a role in the transfer of mercury to their predators. Mercury in top predators is concentrated to levels that may eventually result in risks for mercury-mediated health effects.

Projections for the future

With the knowledge that contaminant concentrations in (epi)benthic invertebrates in the Svalbard coastal system currently do not raise concern for health effects, it is important to ensure that these concentrations do not increase in the future. With proper waste water and effluent treatment, clean-up of hotspots on land, and preparedness for accidents resulting in spills, contaminant concentrations can even be reduced. Coal mining and the coal-power plant on Svalbard may cease in the near future, depending on the global fossil fuel requirements, as geopolitical developments have shown how quickly the supply and demand of fossil fuels can change. Other industrial activities, however, may become possible with an increasingly milder climate and care should be taken not to introduce new contamination problems related to these.

With human activities expecting to continue to rise on and around Svalbard, the importance of controlling local contaminant sources through mitigation measures, regulations or bans is obvious. The recent tourist vessel guidelines and regulations that have been developed both by the Association of Arctic Expedition Cruise Operators (AECO, https://www.aeco.no/guidelines/) and the Governor of Svalbard (https://www.sysselmesteren.no/en/tour-operators/regulations-and-travel-restrictions/) are therefore important tools to reduce current and future potential effects of these activities.

With the continued warming of the Arctic system, the behaviour and fate of contaminants in the coastal system may change, as well as the invertebrate species living here. Specific effects of warming on contaminants consist of the (re)mobilisation of contaminants from glacial ice and permafrost, the further transport of contaminant via species that are expanding to the north, the changes in contaminant patterns and concentrations in Arctic species and their food webs, changes in available diet sources, and warming as additional stressor besides contaminants affecting the health of Arctic species (Borgå et al. 2022, McKinney et al. 2015). Replacement of Arctic species by more boreal species (Węsławski et al. 2018, Hop et al. 2019) may also have an influence on the sensitivity towards contaminants which may become more comparable to those of temperate areas, as were tested in **Chapter 3**.

Objective 2. Assess the potential impact of (current and foreseeable) non-indigenous species related to local human activities on (epi)benthic invertebrates living in the Svalbard coastal system

Non-indigenous species (NIS) have been defined as 'species that have been intentionally or unintentionally introduced outside of their native range as a consequence of human activity' (Convention on Biological Diversity, in Cook et al. 2016). Clearly what is non-indigenous also depends on the baseline considered, which will be further discussed below based on shipping-related sources of NIS.

Introduction of NIS through shipping

The introduction of marine NIS in Svalbard coastal waters is directly and predominantly linked to shipping activities through hull fouling and ballast water discharge (Ware et al. 2014). The number of scientific reports on marine NIS on Svalbard is very limited (**Chapter 5**). The only available information so far consisted of an overview of observed NIS in the entire Arctic region, in which Svalbard was grouped together with the Barents Sea (Chan et al. 2019), a description of the potential entry of marine NIS based on identification of species using DNA techniques in ballast water (based on COI, 12S, 18S markers) (Ware et al. 2016), and the particular observation of the snow crab, *Chionoecetes opilio*, in Svalbard waters (Berge et al. 2017).

A first overview of potential NIS was therefore drafted specifically for the Svalbard marine ecosystem, based on existing overviews of Arctic species and additional literature searches (**Chapter 5**). The term 'potential' is meaning that species were considered an NIS in the studied literature from other (Arctic) areas, but the status as NIS is not yet confirmed for the Svalbard region itself. Of those listed as NIS for the Arctic, some species turned out to have been already reported for Svalbard waters without being labelled as NIS. These included, amongst others, two tunicates: *Botryllus schlosseri* and *Ciona intestinalis* (Gulliksen et al. 1999, Palerud et al. 2004). Over 20 species from the newly drafted list of potential NIS for Svalbard were identified in ballast water samples of bulk carriers on Svalbard, showing the potential for the unintentional introduction of these species through ballast water discharge (Ware et al. 2016). Using DNA metabarcoding, a further identification of three potential NIS was detected, based on their DNA, in harbour sediment samples of Ny-Ålesund: *Botryllus violaceus*, *Molgula manhattensis* and *Euterpina acutifrons* (**Chapter 5**).

Impacts of NIS in general can range from ecological competition to economic consequences, even changing ecosystem services (Bax et al. 2003, Molnar et al. 2008). The establishment of NIS can have consequences at the community level of (epi)benthic invertebrates through competition for food or space depending on the competitive power of the new species under the abiotic and biotic conditions present. Introduced species may also slowly adapt to the new habitats finding their own niche which often means their arrival remains unnoticed and only results in a minor community shift, minimally influencing the food web as a total. Sometimes NIS may be introduced without their natural enemies to control their population development. If the local predators cannot take over this role and the environmental conditions are favourable enough, the NIS may quickly develop into a pest, outcompeting the native Arctic species, downgrading the local diversity and eventually affecting higher trophic levels as well, for instance because they have a lower nutritional quality than the original prey species. One example of adverse effects of such an NIS is when tunicates quickly overgrow substrates including benthic organisms, thereby obstructing other species and competing for food (Costello et al. 2021). These types of effects can eventually result in economic impacts. It is, on the other hand, also possible, that NIS take over the ecosystem functioning of indigenous species that gradually become less adapted to the changed (e.g. warmer) situation. In that case these newcomers may fulfil a crucial role in the local ecosystem in transition.

No actual impacts of NIS have been described in Svalbard coastal waters although several (probably) non-indigenous tunicate species already have been identified (**Chapter 5**). In southwest Iceland, examples from harbours show that after a first discovery of *C. intestinalis* in 2007 and *B. schlosseri* in 2011, five new non-indigenous tunicate species were recorded in 2018 (*Diplosoma listerianum*, *Ascidiella aspersa*, *B. violaceus*, *M. manhattensis* and *Ciona cf. robusta*) (Ramos-Esplá et al. 2020). This illustrates the possible speed of a new settlement and consequent spread. It depends on how adapted the tunicates are to the environmental situation of that moment how fast they can grow, smother other sessile species and compete for space and food (Lambert 2007). Current low water temperatures of the Svalbard coastal system are still expected to limit NIS growth and reproduction rates (Reimer et al. 2021, Lins et al. 2018). Another example of a recently discovered NIS is the observation of young snow crabs (*Chionoecetes opilio*) in a Svalbard fjord in 2017 (Berge et al. 2017). This crab species could develop into a pest as it is a well-adapted to cold conditions and an opportunistic feeder that may compete for food with local crab species, as has been observed in the Pechora Sea (Gebruk et al. 2021). With its large size and claws, it can easily compete for food with local crab species.

Continued warming will facilitate the successful settlement and population development of NIS after introduction via the growing number of visiting ships in a for them becoming more hospitable environment, that in turn may become less favourable for the indigenous species. A comparison of scenarios for 2016, 2050 and 2100 projected a poleward shift of several non-indigenous invertebrate species (Ware et al. 2016). The barnacle *Austrominius modestus* and crustacean *Podon leuckartii* were predicted to find suitable conditions for reproduction in Svalbard harbours under the 2050 scenario, whereas the 2100 scenario conditions would also favour the barnacle *Amphibalanus improvisus*, the copepods *Acartia tonsa* and *Eurytemora affinis*, and the shorecrab *Carcinus maenas* (Ware et al. 2016). The presence of NIS in combination with warming temperature is therefore expected to put additional pressure on the local benthic communities of the coastal Arctic ecosystems. Besides this, it is not certain whether current Arctic species will be able to find a refuge for their existence. Coastal species will retract to the colder parts of the fjords or areas further north to avoid warming (Yletyinen 2019). However, with continued warming, these refuges will further shrink, fragmenting and decreasing the habitats where these Arctic coastal species can thrive. This may result in impoverished genetic diversity and ultimately extinction of such species.

Cryptogenic species

A major challenge in the identification and mapping of the spread of marine NIS in the Arctic, is that species labelled as 'potential NIS' for the wider Arctic, may in fact be indigenous in a specific area of the Arctic. One example is the copepod *Oithona similis*, which is considered an NIS for Canadian ports (Brown et al. 2016), but naturally occurs in Svalbard waters. For several species on the potential NIS list it is unknown what their origin and/or original distribution is, which makes them 'cryptogenic'. The tunicate *Ciona intestinalis*, considered an NIS for Iceland (Ramos-Esplá et al. 2020), is registered as native to the Barents Sea in the CABI Digital library (www.cabidigitallibrary.org) and was not labelled as being an NIS when earlier observed and reported on Svalbard (Gulliksen et al. 1999, Palerud et al., 2004). Another cryptogenic tunicate, *Molgula manhattensis*, was considered 'introduced' to areas such as Norway and Iceland in the CABI Digital library, but 'cryptogenic' for the European side of the Atlantic according to another study (Haydar et al. 2011). Whether a species is indigenous or not also depends on the baseline that is considered. For example the mussel *Mytilus edulis* reappeared on Svalbard after 1,000 years of absence (Berge et al. 2005; Leopold et al. 2018, Kotwicki et al. 2021). Interestingly, genetic analyses revealed that *Mytilus* spp. samples collected on Svalbard not only consisted of the historically native *M. edulis*, but also of the non-native *M. trossulus* and *M. galloprovincialis*, as well as hybrids of all these species (Mathiesen et al. 2017, Kotwicki et al. 2021). Taxonomic and genetic knowledge therefore need to be combined to further reveal the origin and natural distribution of these marine invertebrate species.

Mitigation potential of problematic NIS

Mitigation of NIS is challenging. Preventing the introduction of new species by using a precautionary principle is by far the most important. This means proper prevention or removal of hull fouling of all vessels should be undertaken before they arrive in Svalbard harbours, as this was expected to be the current dominant vector of NIS introduction on Svalbard (Ware et al., 2014). With the wide variety of ships visiting Svalbard, ranging from cruise ships to large bulk carriers to small pleasure craft, this is not at all an easy task and may need the development of specific strategies including, for example, an offshore ship cleaning facility. With the large ecological, economic and potential health impacts of NIS worldwide, there is an urgency to place consequences of and mitigation measures for hull fouling much higher on the international policy agenda. Which antifouling paints are allowed for use on ship hulls is already well regulated via International Maritime Organisation (IMO) and (inter)national regulations, as well as the treatment and discharge of ballast water, although their efficacy and safe use may require some additional attention for the colder Arctic region too.

Secondly, there is a need for better information about the extent of arrival and establishment of NIS. A monitoring plan is needed for NIS at the most important places of entry (harbours), which should be complemented with more extensive assessments at relevant areas to follow the consequences of introduction and development of NIS in the Svalbard coast. Such activities can be included in the newly established 'Integrated Monitoring Program for Svalbard' run by the

Norwegian Environmental Department. eDNA metabarcoding has been shown to be a suitable tool to get a rapid first insight in the potential arrival of NIS (**Chapter 5**). A positive identification of an NIS by eDNA needs to lead to the next step in NIS monitoring: further research to also establish visual proof of the identified species *in situ*, and insights into the survival and reproduction potential of this NIS (Willassen et al. 2022). The main challenges of such an approach are that 1) the current barcodes are not yet fully covering all NIS of interest and research effort is needed to improve this, 2) the current barcodes cannot always identify the NIS to a species level and therefore not identify whether an NIS is actually present (expansion of the international DNA libraries is required), and 3) more information is needed on which species may actually be or become an actual NIS to the Svalbard coastal system, by further exploring the potential origin and range of cryptogenic species using population genetics methodology (**Chapter 5**). (e)DNA techniques are now rapidly evolving, further enlarging the potential of these type of identification techniques in the near future, and barcode databases currently being developed for temperate species can also support the identification of new arrivals in the Arctic.

When concern is raised for adverse ecological and/or economic effects of an already established NIS in the Svalbard coastal system, it can hardly or not be eradicated anymore (Atalah et al. 2015). With larger, slow growing species hand-picking, physical or chemical removal may be attempted, but this is very costly and success is not likely. The most promising way is the more ecological approach of biological control that can be applied in several ways (Table 2). Options are to use either a non-indigenous natural enemy of the introduced NIS (the classical approach), or facilitate potential natural indigenous enemies (the conservation or augmentative approaches). All have their own pros and cons, success is not guaranteed and especially the introduction of another NIS may result in additional challenges. It also depends on how specific the original natural enemy is for its prey, as in a worst-case scenario native species may become preferred prey. Therefore, the introduction of another NIS as mitigation action should only be considered, carefully studied and pursued if an NIS actually leads to unwanted consequences for the coastal system or the food web. It may take a few years before a new ecological balance is reached, and monitoring this process closely may yield very interesting insights for future situations and corrective actions.

Secondary pathways of NIS introduction

Besides shipping, secondary pathways of NIS introductions occur in the Arctic when a NIS arrives via a secondary spread from areas where they intentionally or unintentionally have been introduced through human activities before. Basically, this introduction mechanism is the same as for natural spread (see the explanation of borealisation below), but in this case the primary source is human-introduced. For example, NIS can be transported to Svalbard as eggs, larvae or adults via the northward extending West Spitsbergen Current or hitch-hike on floating marine debris. The latter has been demonstrated, for instance for mussels (*Mytilus* sp.), barnacles (*Semibalanus* sp.) and gooseneck barnacles (*Lepas* sp.) via rafting on floating debris (**Chapter 5**, Węsławski & Kotwicki 2018, Kotwicki et al. 2021). The diversity of marine litter washing up on Svalbard shores shows that plastic may form an important vector for the transport of invertebrate species into and within this area, especially on the west shores that are facing the Atlantic current (Kotwicki et al. 2021).

Table 2. *Types of biological control and key features for the corresponding agents (after Atalah et al. 2015).*

	Type of biological control	Principle	Key feature	Marine examples
Origin / source of agent	Classical	Introduction of a non-indigenous natural enemy that controls the NIS	-non-indigenous -high specificity -high dispersal and establishment capacity	A parasitic barnacle to control the European green shore crab (Goddard et al. 2005) (Experimental)
	Conservation	Protection and enhancement of particular natural enemies to reduce the effect of the NIS	-native -not released	Protect grouper in marine reserves to supress invasive lionfish (*Pterios volitans*) (Mumby et al. 2011) (Applied method)
	Augmentative	Additional release and facilitation of indigenous natural enemies to control the NIS	-high dispersal and native -easily available in large numbers	Periwinkles to reduce biofouling on oysters farms (Enright et al. 1984) (Experimental)
Mode of application	Inoculation (classical or augmentative)	Small release of a few natural enemies; control increases with multiplication of the released organism; proliferation of the species may be enhanced by supplementing critical habitat	-ability to multiply -low densities required	Sea slugs to control the invasive alga *Caulerpa taxifolia* (Coquillard et al. 2000) (Experimental)
	Inundation (classical or augmentative)	Release of natural enemies in large numbers of which proliferation is not expected	-control by enhanced agents (not their progeny) -densities may decrease rapidly over time -large densities or frequency of release needed	Sea urchins to control the Asian kelp *Undaria pinnatifida* (Atalah et al. 2013) (Applied method)

Borealisation

Like the secondary spread of NIS, also boreal species will travel north and influence Svalbard marine systems if the changing conditions allow their establishment. Range extension of boreal species, called 'borealisation' or 'atlantification' specifically for the Atlantic, is not considered a secondary spread of NIS, but a natural expansion of species from their native areas. This too can be through the transport of adult species via rafting on floating debris, or their pelagic larvae and eggs, transporting north with the West Spitsbergen Current (Kotwicki et al. 2021). Examples of this are numerous for Svalbard, such as the copepod, *Calanus finmarchicus*, (Kwasniewski et al. 2003), the jelly fish *Aglantha digitale* (Mańko et al. 2020), the Atlantic cod *Gadus morhua* (Renaud et al. 2012), the Atlantic mackerel *Scomber scombrus* (Berge et al. 2015), and the snake pipefish *Entelurus aequoreus* (Fleischer et al. 2007). The copepod, *C. finmarchicus*, which is extending northwards, is considerably smaller and contains approximately 10 to 25 times less energy (lipids) than the Arctic calanoids (Steen et al. 2007). With this species moving further northwards and replacing the Arctic counterpart, it may have direct consequences for the population success of their predators, such as the seabird, the little auk *Alle alle* (Steen et al. 2007). Besides this, borealisation may lead to increased competition, predation, habitat formation (e.g. mussel beds and kelp forests), and parasite infestation (Ingvaldsen et al. 2021, Renaud et al. 2012, Galaktionov, 2017, Csapó et al. 2021). Other than with introduced NIS, however, the chances are greater that also the boreal natural enemies come together with their prey, reducing the risk of pest development.

Monitoring

Based on the results of this thesis, a monitoring strategy for NIS in Arctic coastal systems can be drafted (Fig. 2). Rapid screening tools, such as metabarcoding of environmental DNA, can be used to monitor critical locations for the introduction of NIS. By using different types of environmental DNA (sediment, sea water, plankton and fouling) the chance of early detecting an NIS is greatly enhanced (**Chapter 5**). It is important to determine the minimally required number of samples per location to be considered effective for an optimal identification of species present at a location, also taking into account the effort and thus costs for monitoring (**Chapter 5**). After a positive DNA identification additional research efforts are advised at the specific location to further confirm the presence and life stages of the NIS via taxonomic identification. If indeed the NIS is established, the distribution and potential ecological consequences of the specific NIS can be further assessed and the developments studied. In case a species is considered a risk, based on literature or *in situ* observations, it may be decided to draft ecological mitigation measures to control the situation.

For such a strategy to work two points need to be noted. Firstly, it is of utter importance to have a better understanding of which species are actually NIS for the specific location in the Arctic and whether it is present without natural enemy, and secondly, the international DNA library needs to contain all relevant barcodes of these NIS so they can be identified in environmental DNA samples.

Given the rapidly changing Arctic environmental conditions through climate change, substantial changes in Arctic communities are occurring and expected to continue. As the Arctic contains specific seasonal dark-light conditions, it is likely that more temperate (boreal) species may not always find their ideal conditions to close their life cycles. Therefore, it is very important to also study the local ecological developments upon the introduction of new species in order to better understand key-mechanisms of ecological adaptation over time to abiotic and biotic changes. This is crucial for management to be prepared in time for potential pest developments or prevent hasty actions instead of allowing the natural feedback mechanisms of a system to reach a new ecological equilibrium.

Conclusion on NIS

To conclude, the number of observations of marine NIS on Svalbard coast is fragmented, limited, and most likely not complete. Observations of marine NIS are sparse and at the moment mainly consist of descriptions of ‘a potential arrival’ of a ‘potential NIS’, and not yet whether these species can settle, survive, and reproduce after being introduced to these new habitats. The limited available information indicates that the pressure of NIS is currently developing, for instance in the case of the snow crab, though actual observations are limited. Future NIS can to a certain extent be predicted based on monitoring fouling on hulls of ships, which is currently considered the most likely source of NIS for Svalbard (Ware et al. 2014). Secondary range expansion via floating debris may also play an important role for both NIS and boreal species

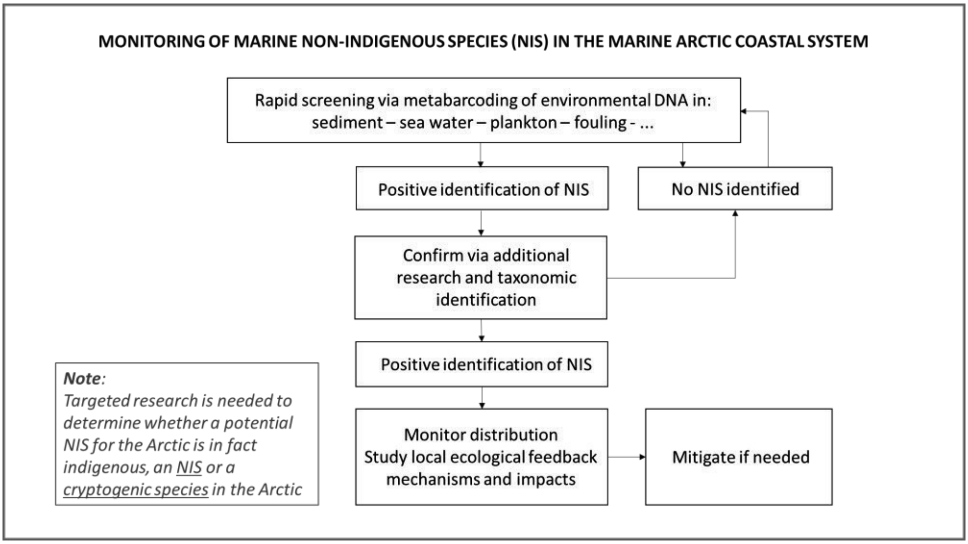

Figure 2. *Proposed monitoring strategy for non-indigenous species in marine Arctic coastal systems.*

(Kotwicki et al. 2021), and when in the future for instance (shellfish) aquaculture would be considered, such an activity should be carefully prepared and supervised, learning from earlier examples in for instance northwest Europe with the introduction of shellfish (Pezy et al. 2021).

Overall assessment of the extent of current and future pressures related to local human activities on (epi)benthic marine invertebrates living in warming Arctic coastal systems, using Svalbard as a case study

This thesis aimed to assess the extent of current and future pressures related to local human activities on (epi)benthic marine invertebrates living in warming Arctic coastal systems, using Svalbard as a case study. It is clear that warming is currently by far the most important pressure on the Svalbard coastal waters, impacting the food web at all trophic levels.

Despite several human activities, the contaminant levels in the Svalbard coastal system remained quite low, and effects on most (epi)benthic invertebrates are not likely. Invertebrate species may be considered vectors of contaminants to higher trophic levels where contaminants may ultimately lead to sub-lethal effects. Due to their important roles in food webs and sessility, invertebrates form a suitable indicator for monitoring contaminant trends in space and time.

Also the pressure of NIS does not yet seem to pose a threat to the (epi)benthic invertebrates, but detailed knowledge through observations is lacking. The future combination of an increased chance of the introduction of NIS with the warming environment make this a more likely pressure to induce changes in the Svalbard coastal system than contaminants.

Other pressures, as mentioned in the introduction, may also have consequences for the coastal systems and the (epi)benthic invertebrates that are living here. Trawling activities around Svalbard and shoreline construction will damage seafloor habitats and in addition, some fisheries are specifically targeting shrimp as main fishery product (Misund et al. 2016). This type of disturbance may disrupt ecological functioning more than the introduction of NIS, though are more likely to take place in the deeper waters of the fjords and Svalbard coast. Although very relevant for vertebrate species, pressures such as noise and ship strikes as a result of shipping activities are considered less important for the invertebrate groups.

Recommendations for management

Contaminants:

The impact of contaminants on (epi)benthic invertebrates currently appears to be low. It is important to guard this and also limit future input of contaminants into the Svalbard coastal system as much as possible, including that of new anthropogenic activities. The mitigation of local sources of contamination is much more feasible than limiting the input of contaminants via long range transport.

Therefore, the following recommendations can be given:

1. Reduce the direct input of contaminants into the coastal system, such as treatment of waste water and effluents.
2. Clean-up contaminated land-based sources (old mines, dumpsites, landfills, fire training sites) that, also under future conditions, lead to runoff of contaminants into the coastal system.
3. Arrange proper preparedness for accidents that may happen along the coast. With increased marine traffic and other activities, the likelihood of accidents, such as oil spills, increases too.
4. Establish a dedicated monitoring plan for contaminants to keep track of the most relevant contaminants in the coastal environment.

NIS:

With NIS on the verge of establishing more easily in the Svalbard coastal system, the following recommendations can be given:

1. Prevent the introduction of new species as much as possible, especially via hull fouling.
2. Establish a monitoring plan for NIS at places of entry (harbours), which will provide information on the arrival of new species, and if required, complement these with more extensive assessments to follow potential introduction and extensions of NIS along the coast.
3. Study the ecological responses to NIS introduction, to better understand coping mechanisms, adaptation processes and conditions where biological mitigation measures may be required to prevent pest outbreaks.

Recommendations for future research & monitoring

To be able to support management and mitigation decisions, the following is recommended:

Contaminants

1. Develop a strategic monitoring plan based on the local contaminant sources on Svalbard (see Fig. 1). Follow patterns of relevant contaminants, including effects of warming and/or mitigation measures, using invertebrates as indicators to obtain information on uptake of contaminants and trends in time and space. For source identification based on contaminant ratios, such as PAH ratios, it is advised to assess this using sediment samples, because processes such as bioavailability and biology of the organism will affect the investigated ratios (**Chapter 2**);
2. Further unravel the relative contribution of long-range transport of contaminants and release of historical sources from melting ice to contaminant input into the coastal system, from that of local sources. Especially more data is required for the unpopulated areas to get a better understanding of the background contaminant levels on Svalbard that are more a result of contaminant inflow via the dominant atmospheric and ocean currents.

NIS:

1. Study and monitoring the presence, settlement and distribution of marine NIS in the Svalbard coastal system, which is required to get a better estimate of the current situation (Fig. 2).
2. Obtain a further understanding if species are 'NIS' or 'cryptogenic' for the Svalbard region and what their origin and native distributions are. This involves combined research of taxonomic and genetic experts.
3. Monitor of the main sources of NIS for the Svalbard environment to better understand what is arriving via hull fouling, ballast water and floating marine debris.
4. Develop further horizon scanning studies for NIS in different Arctic regions to get a much better insight into the current distribution of marine NIS and their impacts in the Arctic, also including the understanding if species are 'NIS' or 'cryptogenic' for that specific region.
5. Research into the ecological responses and potential consequences of NIS and other new species for the local ecosystem and economy to be able to understand, predict and prepare for discussions on potential mitigation necessities.

Perspectives for the wider Arctic (coasts, seas and oceans)

The approach of this thesis will also apply for other populated coasts in the Arctic region, such as smaller settlements with a harbour and a few thousand inhabitants, similar to Svalbard, in southwest and southeast Greenland, Canada (for instance Tuktoyaktuk on the Beaufort Sea), Alaska (such as Prudhoe Bay on the Beaufort Sea, Utqiaġvik (Barrow) and Kivilina on the Chukchi Sea) and Russia (such as Dikson on the Kara Sea, and Tiksi on the Laptev Sea), but also for larger settlements around the Arctic, that may face a high contaminant input from local human activities or where the introduction of NIS due to shipping has been reported before (Fig. 3). Examples of the latter two are the former Thule bases on Greenland (Colgan 2018) and Churchill in Canada (Chan et al. 2015).

The status of these sites can be examined using a similar approach as was done in this thesis, taking into account the lessons learned and suggestions given. The first step should be conducting a pressure assessment based on the current and historic human activities in the area. This desk top study can be used to steer dedicated monitoring to assess the potential links between stressors and impacts within the ecosystem. The outcomes can be compared to the results of this thesis, which can serve as a baseline for both contaminant concentrations and the presence of NIS.

As most Arctic regions are somehow connected, knowledge of contaminant concentrations and the presence of NIS and boreal species is needed for the wider Arctic region, both along the populated parts of the coast as well as in the more pristine areas. This will enable the assessment of the current and potential future contributions of local activities to the overall pressures on the coastal system. Such insight is also needed for the Central Arctic Ocean, a much more complex system consisting of ice covered and open waters over a deep ocean with under water mountains, ridges, steep slopes and abyssal plains (ICES 2022), which is combined with unique seasonal dark-light conditions making it hard to predict ecological successes of newly introduced species. Such a vast and remote area is difficult to study. To obtain pan-Arctic data on the ocean circulation, biogeochemical cycles and marine systems in the Central Arctic Ocean, the Synoptic Arctic Survey campaign (2020-2022) was developed in which different nations coordinated their research efforts, connecting scientific cruises, exchanging sampling protocols and linking research results. A similar approach can be used for the coastal systems of the Arctic, joining forces not only in research activities but also in the applied research methodologies. This way knowledge and data can be connected and coastal systems better protected from current and future stressors.

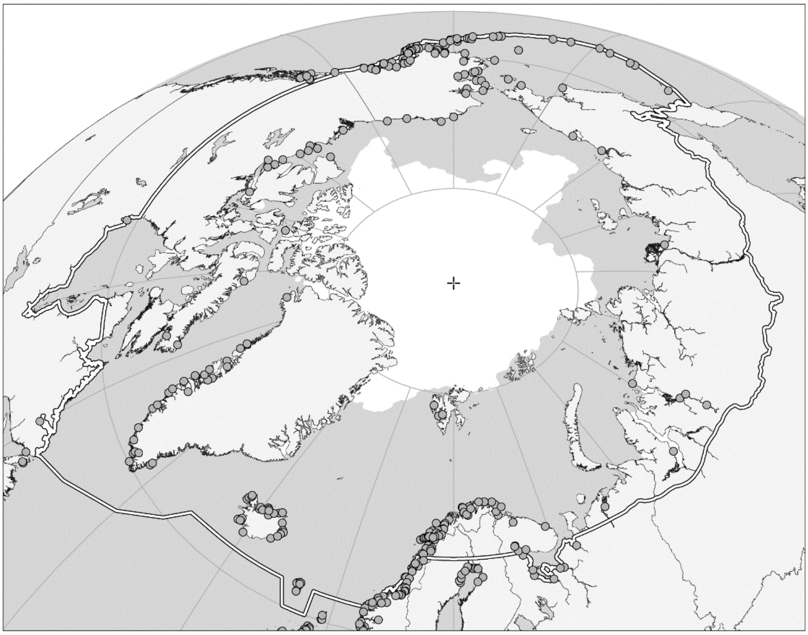

Figure 3. *Location of ports of settlements (small circles) within the Arctic region, including ports bordering this region. (Source: National Geospatial Intelligence Agency. 2019. "World Port Index." Accessed through https://msi.nga.mil/NGAPortal/ (13 April 2023) (F. Steenhuisen).*

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SUMMARY

The Arctic region is warming faster than anywhere else on the globe. This leads to rapid changes in sea-ice extent and volume and, as a consequence, the Arctic is opening up for human activities such as shipping transport, fisheries and tourism. The combination of increasing human activities and the rising temperatures places marine systems under stress. Therefore, there is a need for understanding the effects of pressures from local human activities, viewed in the context of warming. With sufficient understanding, adverse human impacts on the Arctic may be prevented or mitigated through informed management and enforcement.

In this thesis the impacts of local human activities on warming Arctic coastal systems are investigated using the archipelago of Svalbard as a case study. The study focusses on pressures on (epi)benthic species, which are invertebrate species that live in and on the seafloor. They are key prey species in the Svalbard coastal food web, also providing habitat for other species. The two selected pressures for the coastal system are the inputs of contaminants and the introduction of non-indigenous species (NIS). The contaminants studied here are introduced via run off from historical coal mines and through shipping, from antifouling paints and potential oil spills. The introduction of non-indigenous species in Svalbard coastal systems is also primarily related to shipping and ship traffic in general, via ballast water and fouling.

Chapter 1 introduces the topics covered in this thesis. It provides the relevant background information on the Arctic region, the characteristics of the Svalbard, the typical food webs of the coastal system, the biological adaptations of species to the environmental conditions, and the effects of environmental warming on these all. Furthermore, an overview of the most relevant human activities on Svalbard is given with their potential pressures.

In **Chapter 2** the spatial gradient of contaminants from a land-based coal mine to the surrounding coastal environment is examined using mercury (Hg) and Polycyclic Aromatic Hydrocarbons (PAHs) as tracer contaminants. Contaminant concentrations in sediment samples from meltwater streams on land and in the marine system near the outflow of these streams were significantly higher at the mine site than those at the reference sites. The combined presence of Hg and PAHs in sediment proved a suitable indicator to trace mercury concentrations resulting from the mine. Additionally, PAH profiles, hierarchical clustering, and molecular diagnostic ratios provided further insight into the origin of PAHs and Hg and the extent of the exposure radius from the mine site. Marine invertebrate biota were found less suitable to trace the exposure radius due to their low contamination concentrations and variable PAH profiles due to biological processes. Both Hg and PAHs were not expected to pose toxicological risks in the Arctic benthic invertebrates. Given the expected increase in the contaminant mobility and availability in the warming Arctic, it was advised to closely monitor changes in bioavailable input of mercury and PAHs from land-based sources to the marine system.

In **Chapter 3** the applicability of acute oil-toxicity data derived from temperate areas is explored for the Arctic region. Availability of Arctic oil toxicity data is limited, complicating the risk assessment for oil spills risk in this region, including the consideration of the need for mitigation. In experimental studies it was shown that temperature had an effect on both the acute oil toxicity, determined as LC50, and the Critical Body Residue in temperate gammarid amphipods. At the highest test temperature (20°C) the LC50 value of oil in temperate gammarids was a factor two lower (higher acute toxicity) than when tested at the lowest temperature (4°C), and effects occurred at an almost twofold lower Critical Body Residue. When temperate and Arctic gammarids were tested under the same environmental test conditions, the Arctic gammarids had a threefold higher LC50 than temperate gammarids (lower acute toxicity), while the Critical Body Residues for both were similar. The functional traits of Arctic gammarids in terms of body size, weight and lipid% were the most likely explanation for the observed differences. The observed difference in sensitivity to oil between temperate and Arctic gammarids were considered 'close' in modelling studies. Therefore, these results support the applicability of temperate acute oil toxicity data as basis for assessing risks for the Arctic region, provided that the effects of temperature on oil fate and eco-physiological and morphological characteristics of the tested species are taken into account.

In **Chapter 4** the exposure and chronic effects of Tributyltin (TBT) are assessed in all five harbours of Svalbard. TBT was ubiquitously used in antifouling paints of large seagoing vessels from which it leached into the marine environment and accumulated in sediments and biota. Exposure to low levels of TBT can already result in imposex in marine whelks, which is the formation of male characteristics in females whelks as a result of hormone disruption. This can eventually render the female whelks infertile and cause an overall population decline. Results showed that organotin levels in sediments of Svalbard harbours were low compared to other Arctic regions with longer shipping histories. Levels were below known effect levels and in accordance, no imposex was found in marine whelks in the harbour where the whelks were collected. Of all other compounds (PAHs, polychlorinated biphenyls (PCBs), polybrominated diphenylethers (PBDEs) and Per- and polyfluoroalkyl substances (PFAS)) analysed in sediments of one of the harbours, PAH concentrations were highest. It was advised to continue monitoring of contaminant levels to be able to act in case contaminant concentrations would be increasing.

In **Chapter 5** a first insight into the occurrence of marine non-indigenous species (NIS) for Svalbard is presented. NIS are species that have been intentionally or unintentionally introduced outside of their native range as a consequence of human activity. With increasing shipping activity the likelihood of NIS being introduced into the Arctic region increases, and the survival potential becomes more likely in a warming environment. A first list of potential marine NIS was drafted for Svalbard and subsequently, the presence of marine NIS in soft sediment of the Kongsfjorden in northwest Svalbard was assessed using innovative molecular techniques. Seven potential NIS were identified in the sediment samples based on these metabarcoding techniques, of which three were not reported before on Svalbard. Additional studies are needed to confirm these observations, assess whether the NIS have established on Svalbard and what their potential impact on the local system may be. Metabarcoding proved to be an effective monitoring tool to detect the presence of new species in Svalbard marine waters. The generation of more species-specific barcode information for international DNA libraries would be of great benefit for future identifications of all potential NIS that may or have arrived in the Svalbard region.

Chapter 6 discusses the overall outcome of the thesis, focussing on the impacts of contaminants and NIS on (epi)benthic invertebrates living in the warming coastal system of Svalbard. The results show that both contaminants and NIS currently do not raise concern for the invertebrate seafloor communities. With the invertebrate species forming an essential food source to fish, sea birds and marine mammals, they play an important role in the transfer of contaminants to higher trophic levels. Therefore it is of utmost importance to keep both contaminants and NIS at a sufficiently low level under conditions of both ocean warming and concomitant increasing human activities. It is advised to further prevent the local input of contaminants from (future) anthropogenic activities and to develop additional measures to minimise the introduction of NIS, especially through mitigation of hull fouling. Furthermore, dedicated monitoring can keep track on trends in contaminant concentrations and on NIS arrival, distribution and establishment, which is needed to be able to act in case unwanted trends appear to occur. The approach and results of this thesis are also applicable for other coastal settlements around the Arctic.

ACKNOWLEDGEMENTS

Who would have thought that… the presentation on the North Pole I gave at the age of 13 during my Dutch class at high school would lead to this? My presentation included an A0, hand-drawn map of the Arctic region (made together with my mum) and a typical Arctic ice-associated food web on the back. Although I have always been fascinated by the polar regions, the adventurous expeditions in these snow covered areas, and the Inuit culture, I never imagined my career in biology would lead me to do research in both polar regions. This was mainly thanks to one unexpected event. When searching for an fascinating second Master's thesis topic, I emailed the head of the Human Impact's programme at the Australian Antarctic Division in Tasmania, inquiring whether I could conduct a marine ecotoxicological research project down south in Australia, perhaps on an Antarctic related topic. His reply included one major question: "We need a student for an ecotoxicological research project at Casey station, Antarctica, next Antarctic fall. Are you interested?". My answer was, without any hesitation, a resounding "Yes!". I will never forget the moment, months later, that we first glimpsed the Antarctic continent (at 3 o'clock in the morning); a bit of white icecap peeking through the clouds. Here, I experienced that you can literally hear the silence, in an overwhelmingly empty landscape, consisting only of the colours white (snow), blue (ice) and grey (rocks). I fell in love.

The first spark of completing a PhD came from one of the members of the research team with whom I worked at Casey station in 1997. He asked me, years after our Antarctic expedition, why I never pursued my doctorate degree even though I was consistently developing new research projects at my institute. I had missed the 'regular PhD route', because I accepted an offer to become a policy trainee at a Ministry straight after completing my Master's degree ('doctorandus' at that time). A decision I never regret for multiple reasons, including that it broadened my understanding of how applied research can be used within a managemental or political setting. However, it did mean that going back to a PhD position after this was no longer a logical decision. Gradually a new idea started to develop: to conduct my PhD alongside my regular job. This meant additional hours after work, while using data generated during my work. The research topic was therefore extra important to maintain motivation and perseverance. To be able to combine my PhD with ongoing research activities within my job also turned out to be an important driver to be able to keep going. This is why I switched from an initial plan to focus my PhD on pollution in the Westerschelde (though currently receiving a lot of public attention again) to my present research in the rapidly changing Arctic region.

I could never have been able to complete my thesis without the support of many people. First of all, Tinka, thank you so much for your help and support; for inviting me into your Marine Animal Ecology group on a part-time secondment position; for staying up late on multiple occasions to provide me with always-to-the-point feedback and advice; and for joining me on one of my Svalbard expeditions where we sampled the Kongsfjorden, conducted night shifts for the oil experiments, and where we spotted a polar bear together from the upstairs lounge in the Ny-Ålesund restaurant but could not find a telephone to alarm the KingsBay watchmen… Also thank you, dear MAE colleagues, for welcoming me into your group, for stimulating discussions on research, methodology, data, and for having fun.

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ABOUT THE AUTHOR

Martine Greve was born on 21 July 1972 in Zwolle, the Netherlands. She obtained her Bachelor and Master's degree in Marine Biology, with a specialisation in Ecotoxicology, from the University of Groningen, the Netherlands, in 1997. During her study she spent three months at the University of Bangor, Wales, UK, to obtain a further background in, amongst others, marine pollution, and polar & tropical environments. She conducted two MSc projects focused on ecotoxicology. The first one at the Royal NIOZ on Texel, the Netherlands, resulting in the thesis "Assessment of metabolism of PBDEs in livers of marine mammals and birds". The latter at the Australian Antarctic Division in Kingston, Tasmania, Australia, including a three months research stay at Casey station, Antarctica, and a three months stay at the National Research Centre for Environmental Toxicology (NRCET) in Brisbane, Australia, resulting in the thesis "Assessment of the sensitivity of Antarctic gammarids to heavy metals and pesticides". After completing her university degree, she started a position as policy trainee at the Ministry of Housing, Spatial Planning and the Environment in the Hague in 1998, followed by a position as policy maker on biocides at the environmental department of the ministry. In 2000 she moved to California, USA, first working as a volunteer at the US Geological Survey in Menlo Park, and later as a guest scientist at the Environmental Engineering group of Stanford University on a combined USGS / Stanford research project on the bioavailability of polychlorinated biphenyls from sediment in marine hotspot locations. In 2002 she moved back to the Netherlands to work as programme leader of water quality projects at the National Institute for Coastal and Marine Management (RIKZ) at Jacobahaven and Middelburg. With the termination of the RIKZ in 2008, her position moved to Deltares, Delft. In September 2009 she started working as project leader and scientist at IMARES, nowadays Wageningen Marine Research, in Yerseke. Her projects here include the assessment of contaminants and toxins in Westerschelde and Oosterschelde biota, contaminants in harbour porpoises that beach along the Dutch coast, the effects of (metals leaching from) underwater dyke reinforcements on the marine coastal life, and the use of environmental DNA to assess the arrival and distribution of marine non-indigenous species (on marine litter). Here she initiated her Arctic research after a first scouting trip to Svalbard in 2012. Since then, she has performed almost annual research projects on Svalbard, focusing on several types of stressors for the Arctic marine coastal environment. These Arctic research activities have ultimately led to this thesis, especially after the establishment of a secondment position of one day a week at the Marine Animal Ecology group of Wageningen University in 2018. Her Arctic research later broadened to Iceland (2018) and Greenland (2019, 2022) through the Arctic Marine Litter projects of Wageningen Economic Research. Since 2020, she is one of the chairs of the ICES/PICES/PAME Working Group on Integrated Ecosystem Assessment (IEA) for the Central Arctic Ocean, and the Dutch representative in the working group marine of the International Arctic Science Committee.

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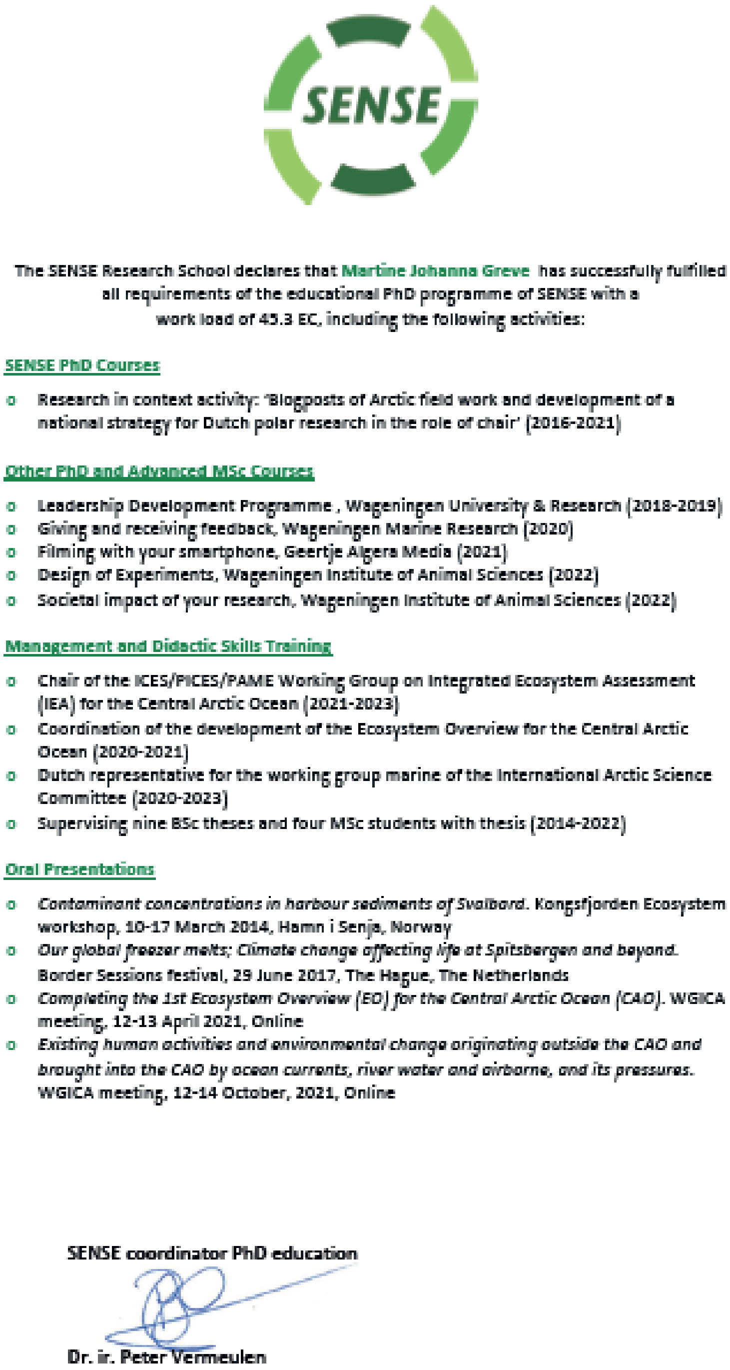

The SENSE Research School declares that **Martine Johanna Greve** has successfully fulfilled all requirements of the educational PhD programme of SENSE with a work load of 45.3 EC, including the following activities:

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Management and Didactic Skills Training

- Chair of the ICES/PICES/PAME Working Group on Integrated Ecosystem Assessment (IEA) for the Central Arctic Ocean (2021-2023)
- Coordination of the development of the Ecosystem Overview for the Central Arctic Ocean (2020-2021)
- Dutch representative for the working group marine of the International Arctic Science Committee (2020-2023)
- Supervising nine BSc theses and four MSc students with thesis (2014-2022)

Oral Presentations

- *Contaminant concentrations in harbour sediments of Svalbard*. Kongsfjorden Ecosystem workshop, 10-17 March 2014, Hamn i Senja, Norway
- *Our global freezer melts; Climate change affecting life at Spitsbergen and beyond.* Border Sessions festival, 29 June 2017, The Hague, The Netherlands
- *Completing the 1st Ecosystem Overview (EO) for the Central Arctic Ocean (CAO)*. WGICA meeting, 12-13 April 2021, Online
- *Existing human activities and environmental change originating outside the CAO and brought into the CAO by ocean currents, river water and airborne, and its pressures.* WGICA meeting, 12-14 October, 2021, Online

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