Effects of contaminant mixtures on marine zooplankton diversity and function

Christina Jönander

2023

UNIVERSITY OF GOTHENBURG

Department of Biological and Environmental Sciences

Faculty of Science

Effects of contaminant mixtures on marine zooplankton diversity and function

christina.jonander@bioenv.gu.se
christina.jonander@gmail.com

ISBN (Print) 978-91-8069-281-6
ISBN (Pdf) 978-91-8069-282-3
Electronic version: http://hdl.handle.net/2077/75977

Printed by: Stema Specialtryck AB, Borås, Sweden, 2023

Cover illustration: Christina Jönander

Abstract

Chemicals have important roles in our society and can be used as ingredients in personal care products, pesticides, pharmaceuticals, as well as be components of fuels used in cars or ships. More than 350 000 chemicals and mixtures have been registered for use, which only covers a part of all the chemicals that we may come into contact with. The frequent use of chemicals can result in both intentional and unintentional release of many substances to the environment, where many eventually end up in the sea. These contaminant mixtures have the potential to adversely affect marine organisms, particularly as mixtures of chemicals are known to cause larger effects than when applied individually. Some of the organisms that first encounter contaminants in the water are zooplankton. This diverse community consists of organisms that span many phyla, and that have many important functions in the pelagic food web. Some of these include grazing on microalgae that can cause harmful blooms, and constituting an important food source for larger organisms such as fish.

In this thesis, I aim to investigate the impacts of both unintentional mixtures (generated from a single source) and coincidental mixtures (originating from several sources) on the biodiversity and function of two trophic levels of marine zooplankton, and to find out which chemicals in the respective mixtures that are the main contributors to their toxicities.

The first two papers focus on the effects from unintentional mixtures originating from shipping activities, and the second two focus on effects from coincidental mixtures found in marine surface water near urban areas with industry. All studies involve effects of contaminant mixtures on natural marine zooplankton communities used in laboratory experiments.

The results in this thesis show evidence of clear mixture toxicity of all tested mixtures, in line with what has been observed elsewhere. The findings include effects on both alpha and beta diversity in zooplankton, and on mesozooplankton ability to feed and reproduce, at concentrations of contaminants that already exist or are likely to exist in the marine environment. The findings demonstrate that the estimated toxicity is generally lower using a component-based approach, where toxicity is modelled using the individual toxicities of the substances, than when using a whole mixture approach, where zooplankton are exposed to an entire mixture. The results demonstrate that there are generally few substances in each mixture that are driving the toxicity, although the number of these toxicity-drivers vary between different mixtures.

The findings of this thesis contribute to a broader perspective of how contaminant mixtures affect marine zooplankton in their environment, by including endpoints such as species diversity, and ability to feed and reproduce, which are normally not included in chemical risk assessment. Furthermore, the findings suggest that there are cause for concern regarding the impact of chemicals present in coastal environments near industry, as well as from wastewater discharged from ships with exhaust gas cleaning systems (closed-loop scrubbers), as they have the potential to harm zooplankton in coastal waters.

Keywords: zooplankton, copepods, ciliates, dinoflagellates, mixture toxicity, chemical mixtures, marine contaminants, biodiversity, reproduction, ULSFO, closed-loop scrubbers, exhaust gas cleaning systems

Sammanfattning

Kemikalier har en viktig roll i vårt samhälle och kan användas som ingredienser i till exempel hushållsprodukter, bekämpningsmedel och läkemedel, men också förekomma naturligt i bränslen som används i bilar eller fartyg. Fler än 350 000 kemikalier och blandningar har idag registrerats för användning, vilket bara omfattar en del av alla kemikalier som vi kan komma i kontakt med. De många användningsområdena för kemikalier i vår vardag betyder också att vissa kemikalier släpps ut i miljön, både avsiktligt och oavsiktligt, där de så småningom hamnar i havet där de kan benämnas som miljögifter. De här miljögiftsblandningarna kan påverka många marina organismer negativt, framför allt eftersom kemikalieblandningar orsakar större effekter än vad de enskilda kemikalierna gör, vilket brukar benämnas som ”cocktaileffekt” eller ”blandningseffekt”. Några av de organismerna som först stöter på miljögifterna i vattnet är djurplankton, vilka är (ofta mycket små) djur som driver runt med havsströmmarna. De har många viktiga funktioner i havet, bland annat genom att livnära sig på mikroskopiska alger som orsakar algblomningar, men också genom att själva vara en viktig födokälla för större djur såsom fisk.

I den här avhandlingen undersöker jag effekterna av miljögiftsblandningar genererade från en enda källa och miljögiftsblandningar som kommer från flera källor. Jag studerar hur de påverkar den biologiska mångfalden och funktionen av marina djurplanktonsamhällen, alltså grupper av flera arter, och även vilka kemikalier i de respektive blandningarna som mest bidrar till blandningarnas giftighet.

De två första studierna behandlar effekter av miljögiftsblandningar kopplade till sjöfart, och de andra två fokuserar på effekter av miljögiftsblandningar som uppmätts i marint ytvatten nära tätorter med industri. Alla studier involverar effekter av miljögiftsblandningar på naturliga marina djurplanktonsamhällen från kontrollerade laboratorieexperiment.

Resultaten i avhandlingen visar på tydliga blandningseffekter för alla blandningar, i linje med vad som har observerats i andra studier. Resultaten visar att miljögiftsblandningarna orsakar effekter på djurplanktonens biologiska mångfald, och på deras förmåga att äta och föröka sig, vid miljögiftskoncentrationer som redan finns i, eller kan komma att hamna i, den marina miljön. Studierna visar även att den beräknade giftigheten generellt är lägre när blandningens giftighet modelleras med hjälp av ämnenas individuella giftigheter, än när man exponerar djurplanktonen för hela blandningen. Resultaten visar även att det i allmänhet är ett fåtal miljögifter i varje blandning som orsakar giftigheten, även om antalet av dessa skiljer sig åt mellan olika blandningar.

Resultaten i denna avhandling bidrar med ett bredare perspektiv på hur miljögiftsblandningar påverkar marina djurplankton i deras naturliga miljö, genom att inkludera effekter på deras biologiska mångfald och förmåga att äta och föröka sig, vilket normalt inte studeras inom kemisk riskbedömning. Dessutom tyder resultaten på att det finns anledning till oro angående effekterna av miljögifter i kustnära miljöer nära industrier, samt från tvättvatten som släpps ut från fartyg med avgasreningssystem (så kallade skrubbrar), eftersom de blandningarna har potential att skada djurplankton i kustvatten.

List of papers

I. **Jönander C.**, Dahllöf I., 2020. Short and long-term effects of low-sulphur fuels on marine zooplankton communities. *Aquatic Toxicology*, 227, 105592. https://doi.org/10.1016/j.aquatox.2020.105592

Authorship contribution from Christina Jönander (according to CRediT): Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing - Original Draft

II. **Jönander C.**, Egardt J., Tiselius P., Hassellöv I.-M., Rasmussen M., Dahllöf I. Exposure to wastewater from ships with closed-loop scrubbers alters biodiversity, reproduction, and grazing in marine zooplankton, independent of season. *Submitted for publication*

Authorship contribution from Christina Jönander (according to CRediT): Conceptualization, Data Curation, Formal analysis, (Part of) Funding acquisition, Investigation, Methodology, Project administration, Visualization, Writing - Original Draft

III. **Jönander C.**, Backhaus T., Dahllöf I. (2022). Single substance and mixture toxicity of dibutyl-phthalate and sodium dodecyl sulphate to marine zooplankton. *Ecotoxicology and Environmental Safety*, 234, 113406. https://doi.org/10.1016/j.ecoenv.2022.113406

Authorship contribution from Christina Jönander (according to CRediT): Data Curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing - Original Draft

IV. **Jönander C.**, Egardt J., Töpel M., Spilsbury F., Carmona E., Inostroza P.A., Brack W., Dahllöf I. Exposure to marine contaminant mixtures with different toxicity-drivers reduce microzooplankton diversity *Manuscript*

Authorship contribution from Christina Jönander (according to CRediT): Conceptualization, Data Curation, Formal analysis, Investigation, Methodology, Project administration, Visualization, Writing - Original Draft

Publications not included in the thesis

Hassellöv I-M., Dahllöf I., **Jönander C.**, Uvegård J. (2021) En förorenande följetong. *Havsutsikt*, 1. https://www.havet.nu/havsutsikt/artikel/fororenande-foljetong

Almroth B. C., Cartine J., **Jönander C.**, Karlsson M., Langlois, J., Lindström, M., Lundin J., Melander N., Pesqueda A., Rahmqvist I., Renaux J., Roos J., Spilsbury F., Svalin J., Vestlund H., Zhao L., Asker N., Ašmonaitė G., Birgersson L., Sturve J. (2021). Assessing the effects of textile leachates in fish using multiple testing methods: From gene expression to behavior. *Ecotoxicology and Environmental Safety*, 207, 111523. https://doi.org/10.1016/j.ecoenv.2020.111523

Table of Contents

Abbreviations 1
1. Introduction 2
1.1 Marine zooplankton 2
1.1.1 Types of zooplankton 2
1.1.2 Microzooplankton and mesozooplankton 2
1.2 Contaminants in the marine environment 6
1.2.1 Contaminants from shipping 6
1.2.2 Contaminants in coastal surface water 8
1.3 Mixture toxicity of chemicals 9
1.4 Hazard data for zooplankton 11
1.4.1 Marine zooplankton in ecotoxicological assays 12
1.4.2 Modelling hazard data 14
2. Aims of this thesis 14
3. Materials and methods 15
3.1 Study designs and general methodology 15
3.1.1 Paper I 16
3.1.2 Paper II 17
3.1.3 Paper III 17
3.1.4 Paper IV 18
3.2 Chemical mixtures used in the experiments 18
3.3 Biodiversity assessments 19
3.3.1 Zooplankton identification methodology 19
3.3.2 Diversity types and how they were analysed 20
3.4 Functional endpoints 21
3.5 Survival 23
3.6 Ranking of toxicity-drivers 23
4. Main findings 24
4.1 Paper I 24
4.2 Paper II 25
4.3 Paper III 27

4.4 Paper IV 29
5. Discussion 31
5.1 Taxonomic identification for biodiversity assessments 31
5.2 Effects of data availability and type on mixture toxicity modelling 32
5.3 Whole mixture approach or component-based approach 33
5.4 Consequences of the IMO sulphur regulations 34
5.5 Consequences of complex mixtures in coastal environments 35
6. Conclusions 36
6.1 How does contaminant mixtures affect marine zooplankton? 36
6.2 Which individual chemicals are responsible for the toxicity? 37
7. Outlook 37
7.1 Ecotoxicological studies with natural zooplankton communities 38
7.2 Future studies of chemical mixtures in the marine environment 39
8. Acknowledgements 40
9. References 42

Abbreviations

BPR	Biocidal products regulation
ECHA	European chemicals agency
EFSA	European food safety authority
IMO	The international maritime organization
MEC	Measured environmental concentration
MGO	Marine gas oil
PAH	Polycyclic aromatic hydrocarbon
PEC	Predicted environmental concentration
PNEC	Predicted no-effect concentration
RCR	Risk characterisation ratio
SECA	Sulphur emission control area
TU	Toxic unit
ULSFO	Ultra low sulphur fuel oil
VLSFO	Very low sulphur fuel oil
WAF	Water accommodated fraction
WFD	Water framework directive

1. Introduction

1.1 Marine zooplankton

1.1.1 Types of zooplankton

Once you consider that the oceans cover more than 70% of the earth's surface, it might not be surprising to learn that certain tiny animals that live in the water column, zooplankton, are some of most abundant organisms on earth. The word zooplankton originates from the Greek word *zoo*, meaning animal, and *planktós*, meaning drifter, which together give a good definition of a what a zooplankton is; an animal that drifts around in the water.

Zooplankton are commonly classified in two ways, either based on how much of their lifecycle they are planktonic or how large they are. Holoplankton are planktonic their entire life cycles and include organisms such as copepods, pteropods, and krill, while meroplankton are planktonic only part of their life cycle (Figure 1), and include larval stages of many benthic, stationary organisms that can disperse their planktonic larvae many kilometres (Shanks, 2009). The size classes most commonly used for plankton are picoplankton (<2 µm), nanoplankton (2–20 µm), microplankton (20–200 µm), mesoplankton (0.2–20 mm), macroplankton (20–200 mm) and megaplankton (>200 mm) (Sieburth *et al.*, 1978) (Figure 1). Zooplankton are usually within the size classes microplankton to megaplankton, and this thesis focuses on the specific groups microzooplankton and mesozooplankton.

1.1.2 Microzooplankton and mesozooplankton

The microzooplankton community consists of mainly heterotrophic and mixotrophic organisms in the size range of 20-200 µm. This size class includes many unicellular organisms such as dinoflagellates, foraminiferans and ciliates, but also some smaller metazoans such as meroplankton or naupliar stages of copepods (Sieburth *et al.*, 1978). The groups which have been the focus of this thesis are the ciliates and dinoflagellates. It has recently been estimated that there are around 4500 free-living described species of ciliates and 2000 described species of dinoflagellates (Foissner and Hawksworth, 2009), although much fewer species occur at the Swedish west coast (Figure 2).

Free-living dinoflagellates have several different modes of feeding, some are autotrophs, some are heterotrophic, and some are mixotrophic (Schnepf and Elbrächter, 1992). Mixotrophic dinoflagellates can either contain permanent plastids attained through one or many endosymbiotic events (Keeling, 2010; Dorrell and Howe, 2015) or acquire them from their prey for temporary photosynthesis (Takishita *et al.*, 2002). Some dinoflagellates can produce toxins and cause harmful blooms, which can have a great impact on aquaculture and

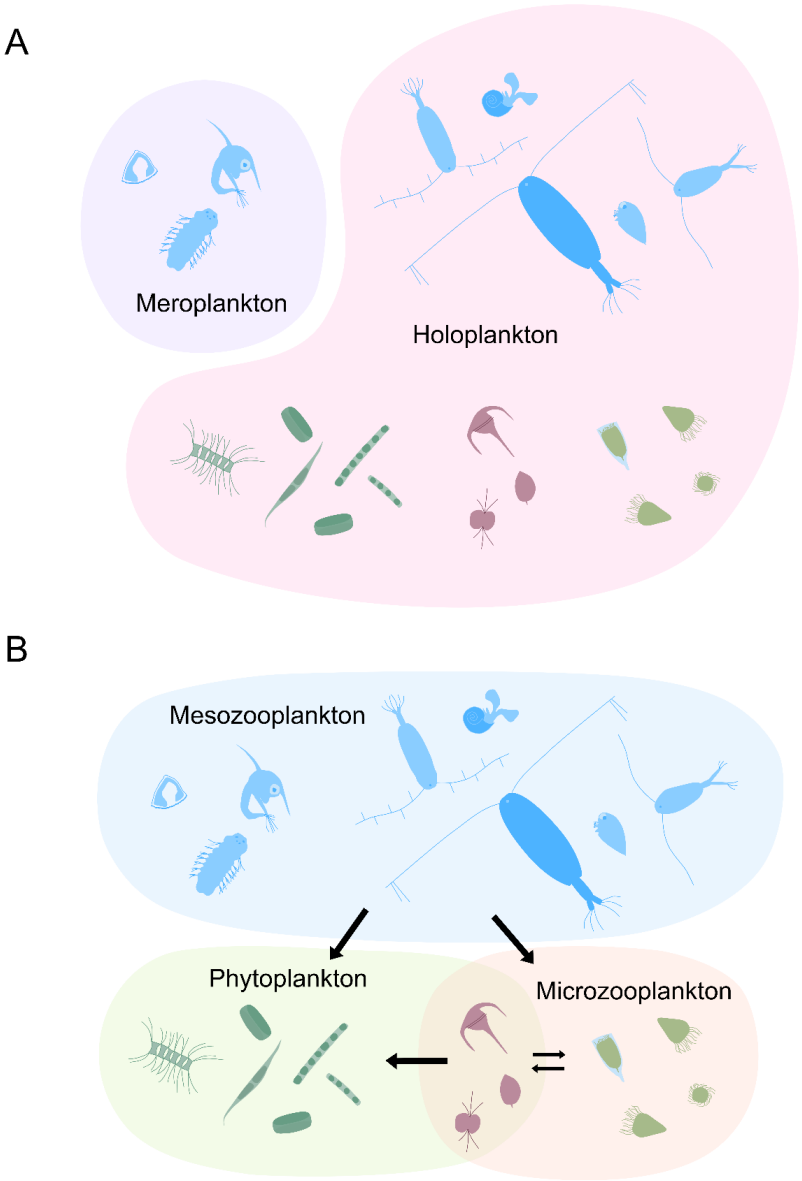

Figure 1. Simplified groupings of plankton based on A) time of lifecycle spent planktonic, and B) size and photosynthetic activity. The arrows in B) represent the main patterns of which groups that feed on other groups.

intoxicate humans (Díaz *et al.*, 2019; Mafra Jr *et al.*, 2019). Ciliates, like dinoflagellates, have different feeding modes and can be both heterotrophic or mixotrophic (Stoecker *et al.*, 1988; Stoecker *et al.*, 1989), and can also cause dense blooms but without toxins (Herfort *et al.*, 2011; Dierssen *et al.*, 2015). As primary consumers in the pelagic foodweb, microzooplankton link together primary producers and secondary consumers, and are often considered the main consumers of primary production in the ocean (Calbet and Landry, 2004). They are also an important food source for mesozooplankton such as copepods (Calbet and Saiz, 2005).

The mesozooplankton community consists of various meroplankton such as larvae of benthic echinoderms or crustaceans, but also fish larvae. The community is often dominated by a group of holoplanktonic crustaceans called copepods, which is the mesozooplankton group that has been the focus in this thesis. A copepod life cycle consists of six naupliar stages followed by another six copepodite stages of which the final stage is the adult one (Allan, 1976), and the adult females either disperse their eggs freely (broadcast spawners), or carry egg sacs from which the nauplii hatch directly (sac spawners) (Kiørboe and Sabatini, 1994). There are currently more than 14 000 accepted freshwater and marine species of copepods globally (Walter & Boxshall, 2023), although only a few species occur frequently on the Swedish west coast (Figure 2).

Many copepod species can alternate between feeding on microzooplankton and phytoplankton (Jonsson and Tiselius, 1990; Calbet *et al.*, 2007), but it has also been shown that their ability or preference to feed on the ciliates is species dependent. The combination of copepod and ciliate species in a community can change the impact of copepod grazing on ciliates, and ultimately impact the microalgae abundance (Gismervik, 2006). Grazing experiments involving copepods, microzooplankton and phytoplankton has shown that microzooplankton typically exert a stronger predation pressure on microalgae than copepods (Maar *et al.*, 2004; Löder *et al.*, 2011).

Marine zooplankton have a central role in the marine food web, and thus changes in community composition and loss of species have the potential to impact both higher and lower trophic levels. Change in calanoid copepod size has been found to reduce recruitment of cod in the North Sea, which likely was driven by compositional changes in the mesozooplankton community. Furthermore, the gradual substitution of the copepod species *Calanus finmarchicus* to *Calanus helgolandicus* (that occurs in higher abundance later during the year) reduced the available food for cod larvae during a period of reduced recruitment (Beaugrand *et al.*, 2003). It has also been found that an increase in the copepod *Acartia spp.* in the Baltic Sea contributed to an increase in sprat recruitment (Möllmann *et al.*, 2008). Given that both top-down and bottom-up effects in the marine food web can be initiated by changes in abundance of individual zooplankton species, it is important to study the changes in zooplankton communities, whether it may be due to climate change related stressors or chemical ones.

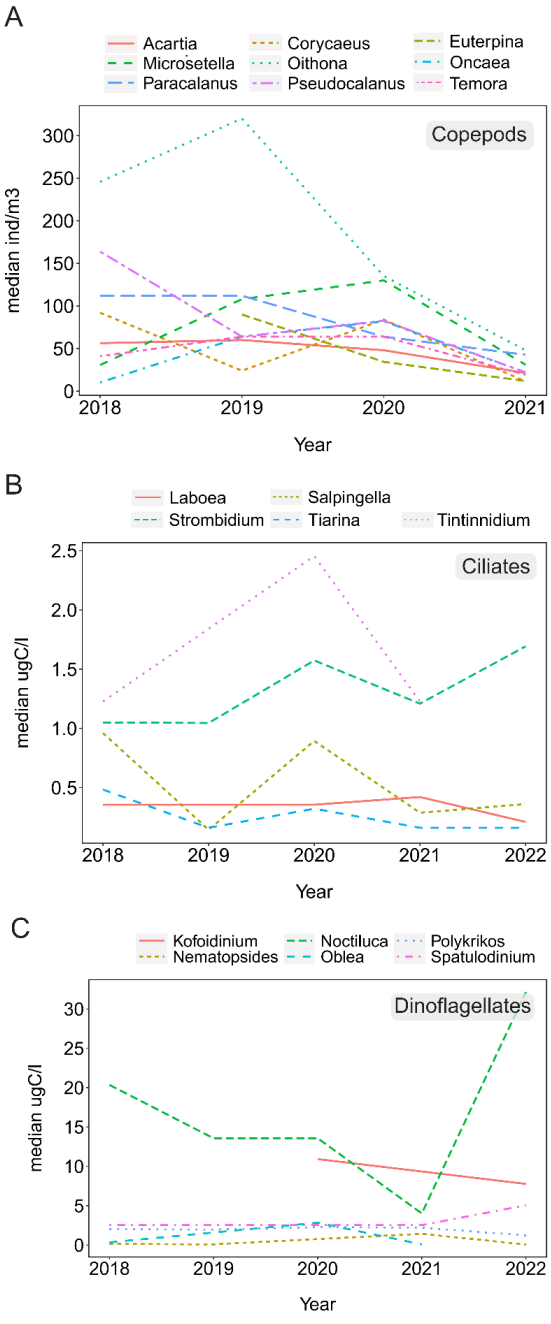

Figure 2. Selection of common plankton taxa on the Swedish west coast during the years 2018-2022. A) copepods, B) ciliates, and C) dinoflagellates. Data retrieved from SMHI Sharkweb (2023-04-04).

1.2 Contaminants in the marine environment

Chemicals provide many important functions in our society but can also end up in the environment where they have the potential to cause harm to organisms and ecosystems. Chemical production has increased over time and it has recently been estimated that more than 350 000 individual chemicals and mixtures have been registered for use and production (Wang *et al.*, 2020). Furthermore, it has been found that the planetary boundary for novel entities, including intentionally and unintentionally manufactured chemicals, has been exceeded as there is an insufficient capacity to monitor and assess the amount released into the environment (Persson *et al.*, 2022).

Chemicals can enter the marine environment through various pathways both from land and sea (Jartun and Pettersen, 2010; Tornero and Hanke, 2016; Ojemaye and Petrik, 2019), and can originate from both diffuse and point sources such as land run-off (Solaun *et al.*, 2020), industry (El Zrelli *et al.*, 2015), sewage treatment plants (STPs) (Gustavsson *et al.*, 2017a), shipping and recreational boating (Tournadre, 2014; Egardt *et al.*, 2018). In this thesis, two different sets of contaminants are specifically looked into; those originating from shipping, and complex mixtures found in surface water near areas with a lot of industries and sewage treatment plants.

1.2.1 Contaminants from shipping

Shipping is a source of many contaminants that can enter the marine environment, either as individual ones, such as biocides in antifouling paints (Dafforn *et al.*, 2011), or via different waste waters that can contain a range of different substances, such as bilge water (Magnusson *et al.*, 2018). The specific contaminant sources that have been studied in this thesis are low sulphur fuel oils, and wastewater generated from exhaust gas cleaning systems, also known as scrubbers.

Low sulphur fuels and scrubber water are very different products, as one is meant to be used in engines and should not enter the marine environment, whereas the other one is a waste product that is intentionally discharged to the sea. However, both products are in different ways related to the recent reductions of the allowed sulphur content of ship fuels implemented by the International Maritime Organization (IMO) (IMO, 2008).

Combustion of ship fuels generate exhaust that contains several hazardous substances such as SO_x and NO_x gases, particles, and hydrocarbons, and has been linked to both ocean acidification (Hassellov *et al.*, 2013) as well as human premature deaths (Winebrake *et al.*, 2009). To mitigate these effects, IMO has reduced the allowed sulphur content in ship fuels several times. In 2015, the sulphur content of fuels was reduced to 0.1% w/w in sulphur emission control areas (SECAs) such as the Baltic Sea and the North Sea areas, and in 2020 the limit was reduced globally to 0.5% w/w (IMO, 2008). Ships could comply with these new regulations by either switching to a low sulphur fuel with the appropriate sulphur content for the respective regions, or they could install a scrubber.

Some of the low sulphur fuel options on the market are non-petroleum products like hydrogen or ammonia, but there are also petroleum-based ones such as distillate marine fuels (DM grade), residual marine fuels (RM grade), or different types of hybrid fuels named after the level of sulphur content (Vedachalam *et al.*, 2022). Distillate fuels are the lighter products generated in the distillation of oil, whereas residual fuels are the heavier ones, and hybrid fuels are blends made to reach a specific sulphur level (Kass *et al.*, 2019). Hybrid fuels are grouped into either ultra-low sulphur fuel oil (ULSFO) that contains ≤0.1% S w/w, or very-low sulphur fuel oil (VLSFO) that contains ≤0.5% S w/w. When characterising hybrid fuels, it has been found that they can vary greatly in physical properties between different batches and that they have high pour points (temperature thresholds below which the oil will no longer flow) and wax content that make them particularly difficult to clean up in the event of a spill (Kass *et al.*, 2019; Hellstrøm, 2017). This means that after a spill, the fuels may stay in the water for a longer time and continue to leach contaminants and oil droplets into the water.

Large spills of fuel oils have been scarce in Swedish waters during recent years. In 2020, a total of 251 shipping accidents were registered in the Baltic Sea region, of which two events resulted in pollution and 22 lacked information about consequences in terms of pollution (HELCOM, 2021). However, on a global scale, seven oil spills over seven tonnes each were registered in 2022, four of which included fuel oil (ITOPF, 2023). Reports of specifically hybrid fuel spills have been rare, but during 2020, a large cargo vessel running on VLSFO stranded outside Mauritius and released nearly 1000 t of fuel (Hebbar and Dharmasiri, 2022). More recently, in April 2022, there was a smaller spill of VLSFO on the Swedish west coast that occurred during bunkering, and the Swedish coast guard removed 1000 L of oil or oil and water mixture in the area (Swedish coast guard, 2022).

Instead of running on a low sulphur fuel, ships can comply with the IMO sulphur restrictions by installing a scrubber unit that removes sulphur and other hazardous substances from the exhaust, which means that the ship can continue to run on a fuel with high sulphur content (Ushakov *et al.*, 2020). When a scrubber is used, the exhaust is sprayed with water that depending on the scrubber type can be recirculated to some extent, or that is discharged directly to the surrounding water. Open-loop scrubbers use the naturally alkaline seawater as input that is discharged back to the ocean at volumes of 24 000-33 000 m^3d^{-1} (based on a medium sized ship, 15 MW) (Hermansson *et al.*, 2021), whereas a closed-loop scrubber uses freshwater with an added base (often NaOH), and recirculate the water. However, closed-loop scrubbers still generate a bleed-off in the range of 126-150 m^3d^{-1} (based on a medium sized ship, 15 MW) (Hermansson *et al.*, 2021) that generally contain much higher concentrations of metals and organic pollutants than the wastewater generated from the open-loop scrubber (Thor et al., 2021).

Before the 2015 implementation of the sulphur cap in SECAs, few ships were equipped with scrubbers, but there has since been a rapid increase in installations. By the end of 2022, around 4800 ships had installed scrubbers, 19% of which were hybrid and closed-loop, and 81% of which were open-loop (DNV-GL, 2023). Given this rapid expansion in the use of scrubbers, there has also been an increase in the discharge of contaminants to the marine environment.

1.2.2 Contaminants in coastal surface water

Surface water in many marine coastal areas has been found to contain complex mixtures of chemicals that sometimes exceed their individual environmental thresholds (Loos *et al.*, 2013; Gustavsson *et al.*, 2017a; Vanryckeghem *et al.*, 2019; Solaun *et al.*, 2020). These chemicals can include a range of different classes such as pharmaceuticals, pesticides, and chemicals used in industry or in personal care products. In this thesis, I have put particular focus on the coastal waters near the municipality of Stenungsund on the Swedish west coast, where there are many potential sources of marine contaminants. Some of these include the many industries in the area, including companies that produce chemicals and plastics, as well as two sewage treatment plants, agricultural land, shipping, and recreational boating (Figure 3). The most recent status classification of the three coastal water bodies in this area shows that none of them achieved good chemical or ecological status according to the Water Framework Directive (WFD) (VISS, 2023a, VISS 2023b, VISS, 2023c).

Although chemicals typically occur in mixtures, contaminants in coastal marine environments are usually monitored on an individual basis. Only 45 priority substances are monitored in coastal waters under the WFD (Directive 2013/39/EU) with respective individual environmental quality standards (EQS), i.e., thresholds that should not be exceeded if good status is to be achieved. Mixtures of chemicals have not been considered to a large extent in different guidance and regularity documents, but this has started to change in the last decade. For mixtures with known or partly known relatively constant composition, such as pesticide and biocide preparations, the European commission states that EQSs can be derived based on mixtures rather than for individual substances (European-Commission, 2011). Furthermore, implementation of a mixture assessment factor in chemical risk assessment, that would account for increased toxicity of mixtures compared to individual substances, has recently been suggested by the European Commisson (European Commission, 2020).

Two parts of legislation that consider mixtures in their guidance documents on risk assessment are the European chemicals agency's (ECHA) guidance on the Biocidal Products Regulation (BPR) and the European food safety authority's (EFSA) guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. The guidance document on the BPR states that all ingredients in a biocidal product should be considered relevant to include in a mixture risk assessment of the product, but that further steps can be taken to narrow these down based on expected contribution to the additive mixture effect, or based on which part of the mixture that is expected to end up in the environment (ECHA, 2017). As for EFSA, the guidelines are somewhat vague. They state that within ecological risk assessment, mixture risk assessment could be appropriate for some regulated products that are, will be, or have recently been removed from the market (EFSA-Scientific-Committee *et al.*, 2019). ECHA and EFSA both propose the use of mixture models in mixture risk assessment that will be further introduced in the next section.

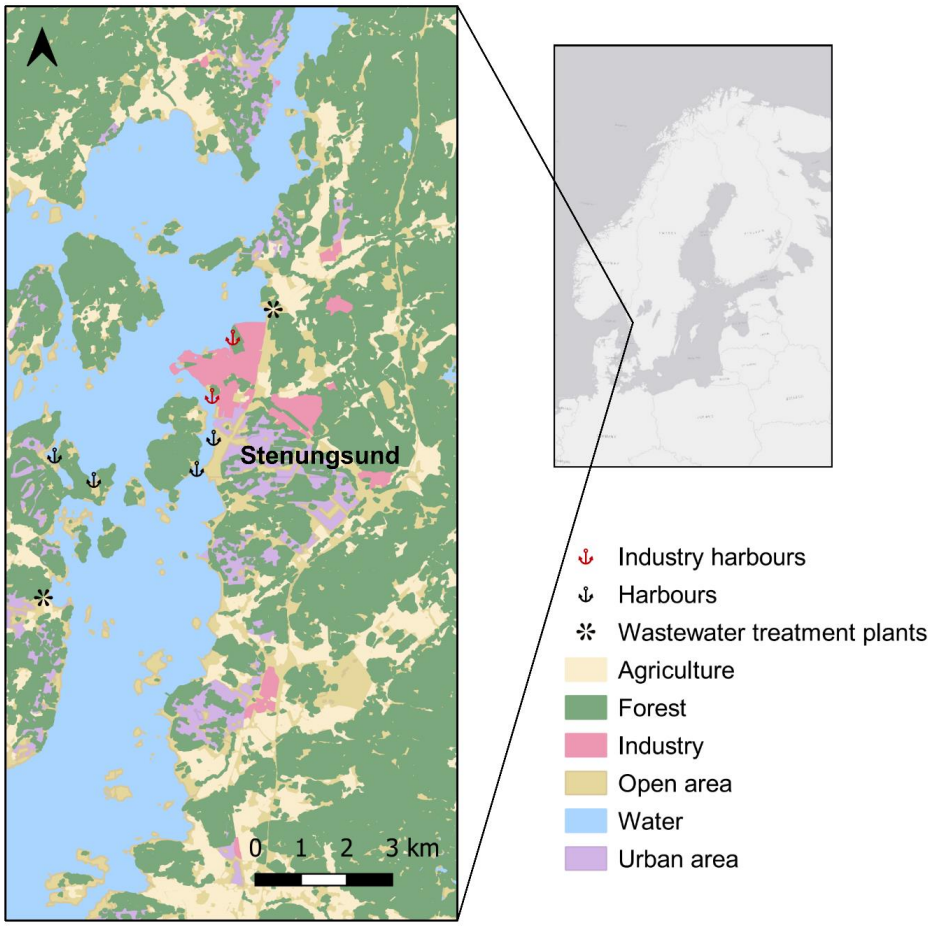

Figure 3. Possible sources of contaminants in marine surface water near Stenungsund. Map created using GSD-Topographic Map © Lantmäteriet.

1.3 Mixture toxicity of chemicals

By now we know that mixtures of chemicals generally cause effects that are larger than those from its individual components (Kortenkamp *et al.*, 2009), and that mixtures can cause toxicity even when individual substances are non-toxic at their respective concentrations (Silva *et al.*, 2002; Altenburger and Greco, 2009). Chemical mixtures can be classified in three ways according to the European commission (2012), and can either be intentional, unintentional, or coincidental mixtures (nomenclature from EFSA, 2019). Intentional mixtures are products that contain mixtures of chemicals and that are marketed as such, for example paints, while unintentional mixtures originate from a single source, and are discharged to the environment during the production, transport, use or disposal. Coincidental mixtures originate from several

sources and are discharged through multiple pathways, and sometimes lack information about the specific chemicals in the mixture. This thesis covers the effect of unintentional mixtures as well as coincidental mixtures.

Toxicity of a mixture can be evaluated in two ways, either based on the whole mixture (whole mixture approach) or based on its components (component-based approach). In a mixture risk assessment, the whole mixture approach treats the whole mixture as a single entity and uses exposure and effect data for the specific mixture or a model mixture (EFSA-Scientific-Committee *et al.*, 2019). The component-based approach, on the other hand, uses the exposure and effect the of the individual components in the mixture to model the total mixture toxicity, usually using either of two common models: independent action (also known as response addition) or concentration addition (also known as dose addition).

Independent action works under the assumption that the individual chemicals have different modes of actions and therefore causes effects that are independent from the other components of the mixture (Bliss, 1939; Greco *et al.*, 1995). The model predicts a scaled effect (0-1) from a mixture (E_{mix}) by multiplying the effects caused by the *n* individual chemicals (Ec_i) at their respective concentrations c_i in the mixture, and can be defined as:

$$E_{mix} = \prod_{i=1}^{n} E(c_i)$$

Concentration addition is traditionally used for mixtures of chemicals that share a mode of action and works under the assumption that chemicals with equal potency contributes equally to the toxicity of the mixture if applied at the same concentrations (Löewe and Muischnek, 1926; Cedergreen *et al.*, 2008). The concentration addition concept is built on toxic units (TUs), where the TU of an individual substance (TU_{ind}) is defined as the ratio between its concentration (c) and effect concentration (EC_x):

$$TU_{ind} = \frac{c}{EC_x}$$

TUs can also be used to estimate the toxicity of an entire mixture, where a cumulative TU (TU_{mix}) is calculated by adding the concentration (c_i) and effect concentration (EC_x) ratio (i.e., TU) for each substance (i) in the mixture:

$$TU_{mix} = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

Although concentration addition assumes a similar mode of action of the chemicals in the mixture, it has been shown to predict toxicity for mixtures of chemicals with different modes of action within a factor of 2 (Belden *et al.*, 2007). Concentration addition has generally been shown more conservative than independent action (Kortenkamp *et al.*, 2009; Kienzler *et al.*, 2016) and is recommended for deriving EQSs for chemical mixtures in coastal environments (European commission, 2011), as well as for mixture risk assessment of chemicals in the food chain (EFSA-Scientific-Committee *et al.*, 2019), and biocidal products (ECHA, 2017). The principles of concentration addition can also be used to calculate toxicity-driving substances in a mixture by ranking the individual TUs in a mixture. There are typically a few substances that

are responsible for the toxicity of a mixture with smaller contributions of the majority (Gustavsson *et al.*, 2017b; Syberg *et al.*, 2017).

The alternative to using a component-based approach is the whole mixture approach, which implies testing the toxicity of the entire mixture. This type of experimental approach has some advantages, such as not having to know the entire content of the mixture, as well as to integrate all possible interactions between the substances (Bopp *et al.*, 2019). It is however not possible to test all possible combinations of chemicals that may end up in the environment. Nonetheless, whole mixture approaches and effect-based methods have been recommended and are increasingly being used with mixtures of unknown content (Brack *et al.*, 2017; Bopp *et al.*, 2019).

Mixtures of chemicals can cause additive, antagonistic, or synergistic effects. Additive toxicity means that there is no interaction between the substances in the mixture that cause them to inhibit or enhance the effects of each other. Antagonism and synergism means that the effects respectively deviate downwards or upwards from mixture toxicity predictions (Nørgaard and Cedergreen, 2010). Chemical mixtures generally cause additive toxicity, whereas synergistic toxicity has been shown more rare, and often occur at high concentrations and for specific classes of chemicals such as combinations of triazine, azole and pyrethroid pesticides (Cedergreen, 2014; Martin *et al.*, 2021). ECHA has defined synergism as an interaction between two or more mixture components that causes toxicity that deviates by a factor of 5 from the toxicity predicted by concentration addition (ECHA, 2022).

1.4 Hazard data for zooplankton

Chemical risk assessment involves exposure assessment, that estimates the concentration of a chemical or mixture that organisms will be exposed to, and the hazard assessment, where the toxicological effect on organisms is estimated. During exposure assessment, a predicted environmental concentration (PEC) or a measured environmental concentration (MEC) is determined for a substance. In the hazard assessment, the concentration of a chemical that has an effect on one or several organisms is estimated, and this is in turn divided by an assessment factor to extrapolate to natural conditions. The assessment factor becomes larger when the uncertainty is higher, for example when few species have been tested. The risk characterization ratio (RCR) is calculated by dividing the PEC (or MEC) by the PNEC (ECHA, 2016), and a value ≥ 1 indicates risk:

$$RCR = \frac{PEC\ (or\ MEC)}{PNEC}$$

The first tier of the hazard assessment for aquatic organisms involves testing the short-term toxicity of a substance to single species of organisms from three trophic levels: primary producers, primary consumers, and secondary consumers (ECHA, 2008). These trophic levels are usually represented by algae, invertebrates (usually genus *Daphnia*) and fish, hence, the hazard for zooplankton (both micro and meso) is usually represented by freshwater daphnids. There is considerably more hazard data produced for freshwater organisms than for marine

ones (de LG Solbé *et al.*, 1993; Yanagihara *et al.*, 2022), hence, toxicity to marine organisms is mostly extrapolated from tests with freshwater species.

1.4.1 Marine zooplankton in ecotoxicological assays

Even if sensitivity among marine and freshwater species can be comparable, there is a larger species diversity among marine organisms than freshwater ones, and several taxonomic groups such as echinoderms, cephalopods and ctenophores only exist in marine environments. Because of this larger abundance of taxa in the marine environment that cannot be represented by a freshwater equivalent, and hence the broader possible distribution of sensitivities, an additional assessment factor of 10 is normally applied in hazard assessment to account for the uncertainty (ECHA, 2008; ECHA, 2017).

Some of the few marine zooplankton taxa that are routinely used in ecotoxicological tests are the brine shrimp *Artemia* (Nunes *et al.*, 2006), the copepod *Acartia* (Gorbi *et al.*, 2012), and the rotifer *Brachionus* (Li *et al.*, 2020). Although the use of these organisms in ecotoxicological tests can be representative for the entire community of zooplankton, there are many possible effects and interactions that are not considered when including a single test species. Different taxa, even within the same trophic level, can have a range of sensitivities to the same substance or chemical mixture. Calanoid and cyclopoid copepods have had different sensitivities to insecticides (Willis & Ling, 2003), but similar sensitivity to the water accommodated fraction (WAF) of diesel (Payne, King, Zamora, & Virtue, 2014). Dinoflagellates have also been found more tolerant than ciliates to crude oil and dispersants (Almeda *et al.*, 2018). Furthermore, size, feeding preference, and life stage are also factors that have played a role in mesozooplankton sensitivity to chemicals. Smaller zooplankton have been shown more sensitive to oil and dispersant than larger ones (Almeda *et al.*, 2014a; Almeda *et al.*, 2014b), and predatory species have been shown to accumulate more hydrophobic contaminants than herbivores as a result of biomagnification (Borgå *et al.*, 2002; Hallanger *et al.*, 2011). Like for many other organisms, sensitivity to contaminants have been higher among copepod juveniles than the adults (Heuschele et al., 2022; Medina et al., 2002; Saiz et al., 2009),

When a community is exposed to a contaminant or a mixture, the most sensitive species and genotypes can be replaced by more tolerant ones, which is known as toxicant-induced succession (Blanck, 2002) (Figure 4). The new community will be more tolerant to the specific type of contaminant(s), known as pollution induced community tolerance (PICT) (Blanck *et al.*, 1988), but can also become more sensitive to other types of stressors (Bach and Dahllof, 2012). In studies involving effects of contaminants on marine zooplankton, exposure has altered the diversity in the community (Hjorth *et al.*, 2008; Payne *et al.*, 2014), and as diversity can be closely linked with function (Hooper *et al.*, 2005; Downing *et al.*, 2014), such changes can have severe consequences for a community. Worm and Duffy (2003) describes three fundamental properties of an ecosystem: quantity (biomass and productivity), quality (biodiversity) and stability (resistance to, or resilience after disturbance). By including the aspects of quantity, as well as quality, this thesis aims to broaden the traditional scope of ecotoxicity studies, where effects on quantity on a single species level is most common.

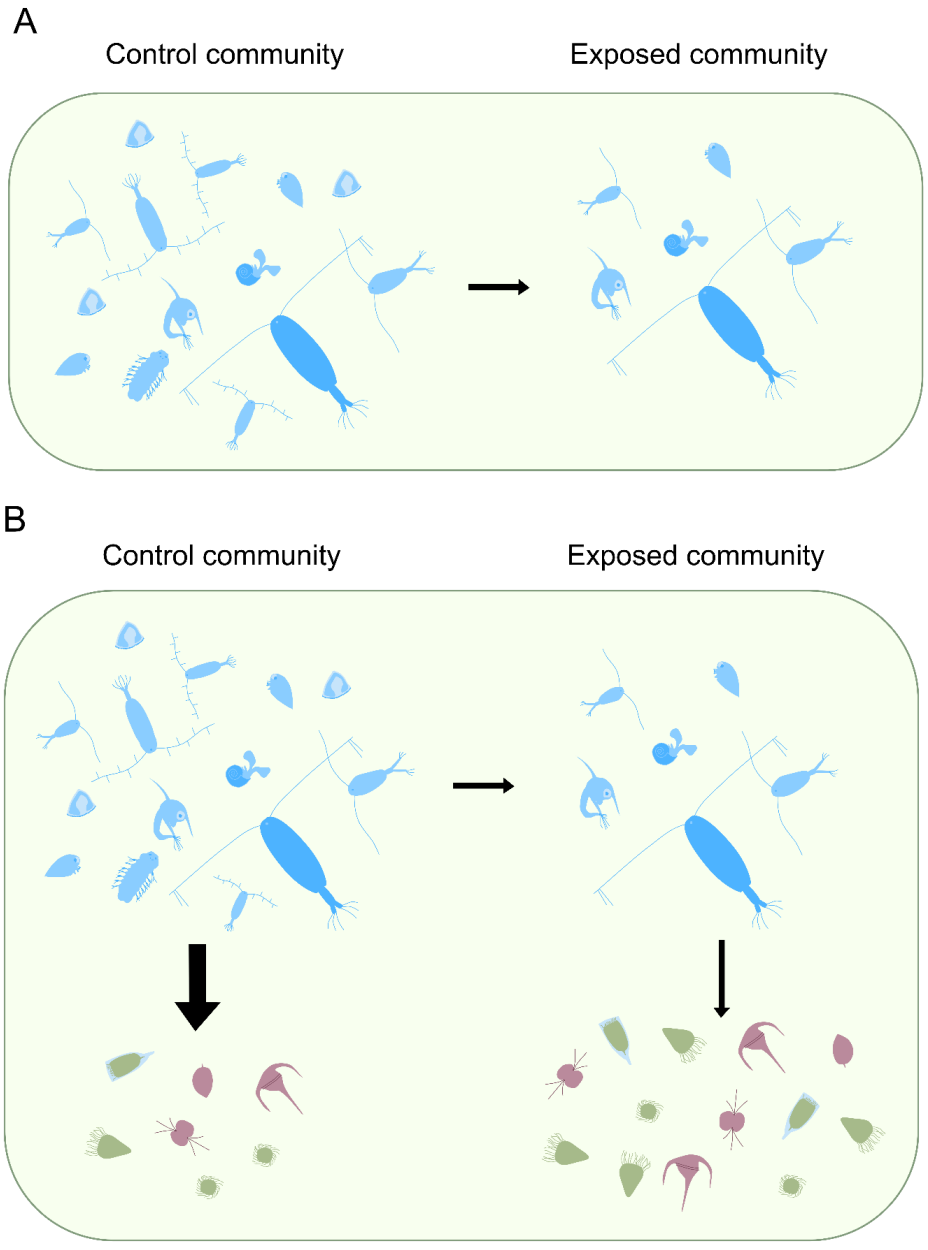

Figure 4. Example of how contaminant exposure can affect a zooplankton community according to the first part of toxicant induced succession, where the most sensitive species and genotypes are lost. A) Effects of contaminant exposure on one trophic level of zooplankton where abundance and diversity is reduced, and B) effects on two trophic levels where reduced abundance and diversity in mesozooplankton causes indirect growth in microzooplankton

1.4.2 Modelling hazard data

One of many challenges in chemical risk assessment of either individual chemicals or mixtures is the lack of hazard data (Backhaus and Karlsson, 2014; Bopp *et al.*, 2018), and one option to overcome this issue is to model the toxicity for individual chemicals. Toxicity of a chemical can be modelled using quantitative structure activity relationships (QSARs), which like the name suggests, can model the toxicity of a substance based on molecular structure or other properties of a compound. In this thesis, two types of QSARs have been used; the ECOlogical Structure-Activity Relationship Model (ECOSAR) developed by the US Environmental Protection Agency (US EPA) (**paper IV**), and a QSAR for polycyclic aromatic hydrocarbons (PAHs) developed by Ha *et al.* (2019) (**paper I**).

ECOSAR contains several QSARs for different chemical classes, which have been developed by grouping organic chemicals with similar structure and correlating them to physico-chemical properties (US EPA, 2022). ECOSAR has been shown to predict the acute toxicity within a factor of 10 for between 63-85% of chemicals, depending on which organism group the toxicity was predicted for (Reuschenbach *et al.*, 2008; Melnikov *et al.*, 2016; Zhou *et al.*, 2021).

The QSAR developed by Ha *et al.* (2019) is based strictly on the octanol/water partition coefficient (K_{ow}), which defines how much of a substance that that would end up in either the octanol or water phase and therefore describes its hydrophobicity. The log K_{ow} has been found to correlate with the toxicity of chemicals and can increase their potential to accumulate in animal tissue (Bradbury *et al.*, 2003; Bekele *et al.*, 2018). It has therefore become common to use in QSARs a basis for toxicity predictions (Netzeva *et al.*, 2008), and it has been shown that these QSARs have better accuracy when they are based on chemical mode of action (Lambert *et al.*, 2022).

2. Aims of this thesis

The overarching aims of this thesis were to find out how unintentional and coincidental contaminant mixtures that exist or may end up in the marine costal environment affect biodiversity and function of marine zooplankton, and to find out which individual chemicals in these mixtures that are most responsible for the effects. The two first papers investigate the toxicity of shipping-related contaminants, and the second two studies cover the effects of complex mixtures in coastal marine surface water near urban areas with industry. The specific aims of the four research papers included in this thesis are as follows:

Paper I: The aims of this study were to find out how toxic the water accommodated fraction (WAF) of an ultra-low sulphur fuel oil (ULSFO) was in relation to that from a marine gas oil (MGO), which individual organic chemicals in the WAFs that were driving the respective toxicities, and to test the long-term effects on mesozooplankton diversity and reproduction from exposure to the ULSFO WAF.

Paper II: The aims of this study were to test the toxicity of closed-loop scrubber water to marine mesozooplankton, the indirect effects on microzooplankton, and to find out which the toxicity-driving substances in the closed-loop scrubber water were. Specifically, we wanted to find out if the mesozooplankton diversity, abundance and reproduction were affected by the closed-loop scrubber water, if this affected their ability to predate on microzooplankton, and if effects were different depending on seasonal species composition.

Paper III: The aims of this study were to find out which individual substances in a marine contaminant mixture that were driving the toxicity, and to test their individual and combined toxicity to mesozooplankton. Specifically, we wanted to find out how these substances and their mixture affected diversity, survival, reproduction, and indirect effect in algae growth, and to find out how well the mixture toxicity was predicted using the independent action model.

Paper IV: The aims of this study were to find out how microzooplankton diversity was affected by three marine chemical mixtures, and to find out which substances in the mixtures that were driving the toxicity. Another aim was to evaluate ECOSAR performance by comparing QSAR generated EC50s for daphnids to observed zooplankton EC50s from various databases for individual chemicals in the mixtures. The comparison was made to evaluate whether supplementing the datasets of observed EC50s with QSAR data was appropriate or not when calculating the cumulative toxic units for the mixtures.

3. Materials and methods

3.1 Study designs and general methodology

All four studies included in this thesis are based on laboratory experiments where we exposed field-sampled natural communities of either microzooplankton or mesozooplankton to contaminant mixtures. All zooplankton communities were sampled near to the monitoring station Släggö (N 58° 15.5', E 11° 26.0') close to Kristineberg Center (formerly Kristineberg marine research station) where the experiments were performed (Figure 5). The respective study designs and measured endpoints are presented for each study in the next sections.

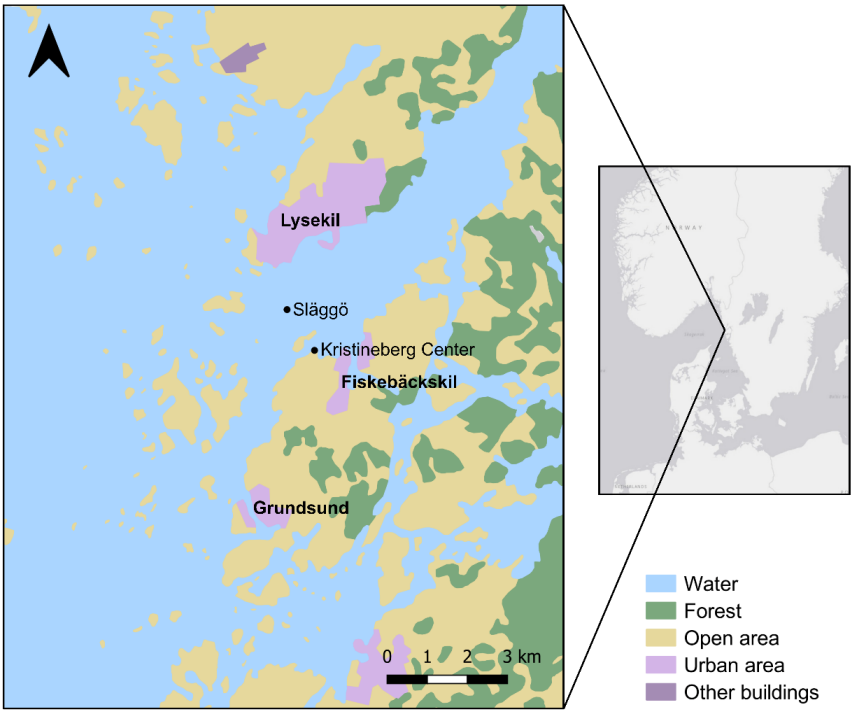

Figure 5. Map of the sampling station Släggö near Kristineberg Center. Map created using GSD-General Map © Lantmäteriet.

3.1.1 Paper I

We performed two experiments in this study. Initially, we performed a short-term study comparing the toxicities of the water accommodated fractions (WAFs) of an ultra-low sulphur fuel oil (ULSFO) and a DMA grade marine gas oil (MGO). We then performed a long-term study investigating the toxicity of the ULSFO WAF alone. In the short-term study, natural mesozooplankton communities were exposed to three concentrations of WAFs of the two respective fuel types with a negative control ($n=3$) for 48 h. During and after the exposure we collected copepod eggs to estimate how the exposure affected community copepod egg production and hatching. Using the principles of concentration addition, we modelled the cumulative toxicity and the toxicity-driving substance in the undiluted WAFs of each fuel type based on their content of hydrocarbons and on QSAR modelled ecotoxicity data for mesozooplankton based on their respective log K_{ow} using the model from Ha *et al.* (2019).

In the long-term study, natural mesozooplankton communities (F0 generation) were exposed to four concentrations of ULSFO WAFs or a negative control ($n=5$) for 8 days. We measured algae density to find out if any toxicity to the zooplankton indirectly affected algae growth, and we estimated direct effects on copepod egg production and hatching rate during the exposure.

During the last days of the exposure, we collected copepod eggs to start the F1 generation, which was cultured for another 22 days. We collected samples for species identification and diversity estimations from the F0 generation before and after the exposure, and from the F1 generation at day 22, to estimate how the exposure affected copepod diversity in the community during two generations.

3.1.2 Paper II

We repeated an experiment during three different seasons, where we evaluated the direct effects of closed-loop scrubber water on mesozooplankton communities, and indirect effects on microzooplankton communities. Mesozooplankton were exposed to two concentrations of scrubber water or a negative control for 72 h ($n=5$) and we estimated copepod community egg production and hatching rate at the end of the exposure. The mesozooplankton were then incubated with microzooplankton communities for another 24 h to estimate how the exposed mesozooplankton were able to predate on and affect the microzooplankton abundance and diversity. We also compared mesozooplankton diversity before and after the exposure.

Using the principles of concentration addition, we estimated the cumulative toxicity as well as the toxicity-driving substances in the scrubber water, based on the metal and hydrocarbon content of the scrubber water and observed ecotoxicity data for mesozooplankton acquired from US EPA ECOTOX Knowledgebase.

3.1.3 Paper III

We modelled the cumulative toxicity and toxicity-driving substances in surface water near Stenungsund using the principles of concentration addition, based on data of detected chemicals in surface water near Stenungsund from Gustavsson *et al.* (2017a) and observed ecotoxicity data for mesozooplankton acquired from US EPA ECOTOX Knowledgebase.

After identifying di-butyl phthalate and sodium dodecyl sulphate as the toxicity-driving substances, we performed an experiment where a mesozooplankton community was exposed to individual and mixture treatments or a negative control ($n=6$) of these chemicals for 48 h. The treatments consisted of two lower and two higher concentrations of both the individual substances and their binary mixtures. Copepod eggs were collected during and after the exposure to estimate the effects on copepod community egg production and hatching rate, and samples before and after the exposure were collected to assess effects on diversity. The mixture toxicity of the two chemicals was predicted based on the effects of the individual substances using the independent action model.

3.1.4 Paper IV

We used mixture extracts of polar, organic chemicals generated from six marine surface water samples collected near Stenungsund, Sweden, and selected mixtures from three sites to use in experiments with microzooplankton based on their difference in chemical profiles. Natural microzooplankton communities were exposed to the three mixtures (at three concentrations each) or a negative control ($n=5$) for five days, and samples were collected before and after the exposure to characterise the effects on diversity ($n=5$) and on ciliate and dinoflagellate abundances ($n=3$).

Based on the principles of concentration addition, we estimated cumulative toxicity and toxicity-driving chemicals in each of the three mixtures using observed ecotoxicity data for micro and mesozooplankton acquired from US EPA ECOTOX Knowledgebase, EFSA and REACH, supplemented with QSAR generated toxicity data from ECOSAR for the substances that lacked observed data. We compared the QSAR data (LC50s for daphnids) with the observed ecotoxicity data for each chemical detected in any of the six sampling sites to evaluate performance and whether supplementing the observed data with modelled data was appropriate.

3.2 Chemical mixtures used in the experiments

The fuels used in **paper I** were donated from Preemraff Lysekil. The WAFs were prepared in the lab by mixing the respective fuels with filtered seawater in closed containers and separating the saturated water from the fuel. The WAFs were finalized on the same days as the start of the respective experiments.

The closed-loop scrubber water used in **paper II** was donated from a ship equipped with a scrubber that operates in Swedish waters. The same batch of scrubber water was used in all three experiments and stored in a closed container in the dark at approximately 5°C in between the experiments.

The chemical mixture data on which we modelled the toxicity-drivers in **paper III** was generated from a marine surface water sample collected near Stenungsund in 2012 (Figure 6). Individual and mixture stocks of the toxicity-driving chemicals, sodium dodecyl sulphate and di-butyl phthalate, were prepared from chemicals obtained from Sigma-Aldrich.

The chemical mixtures used in **paper IV** were obtained from extracts collected from six sites near Stenungsund in 2020 (Figure 6). The extracts were generated from 100 L surface water samples collected with an onsite large volume solid phase extraction device (Schulze *et al.*, 2017), and the chemicals were extracted into methanol and stored at -20 ºC until they were used in the experiments.

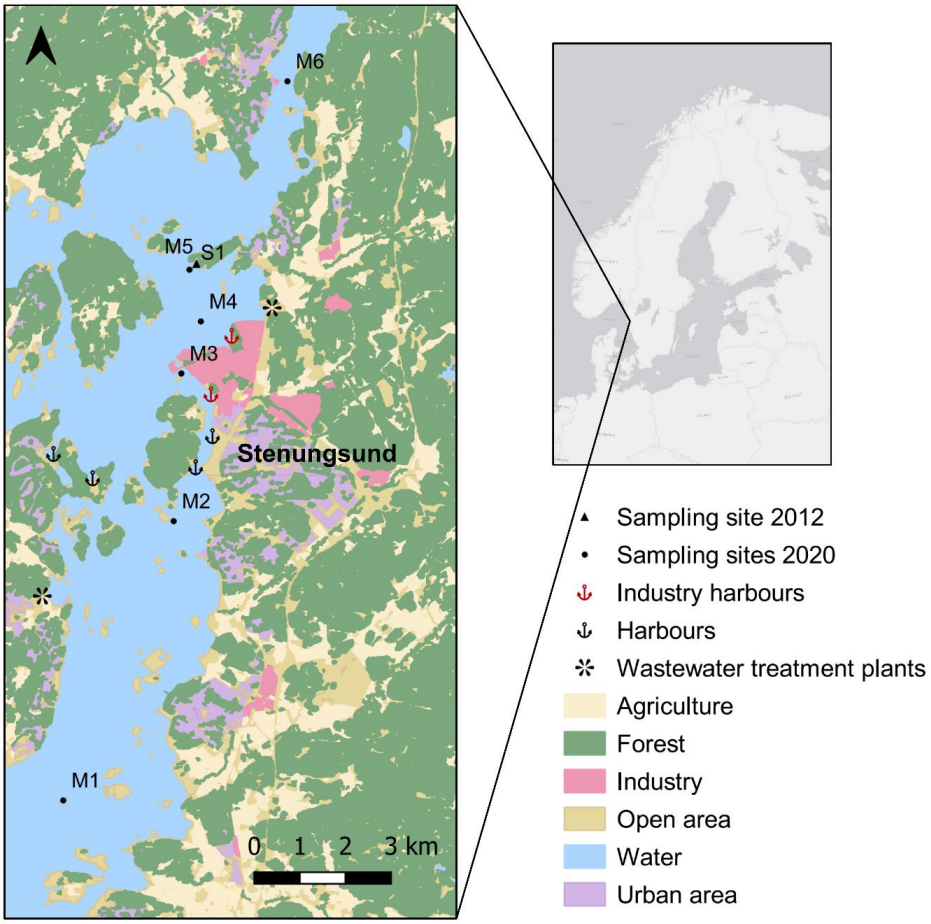

Figure 6. Sampling sites of chemical mixtures in marine surface water near Stenungsund in 2012 and 2020. Coordinates and data for sampling site S1 retrieved from (Gustavsson *et al.*, 2017a). Map created using GSD-Topographic Map © Lantmäteriet.

3.3 Biodiversity assessments

3.3.1 Zooplankton identification methodology

Effects on zooplankton diversity was analysed in each of the four studies included in this thesis using three types of methodology: taxonomic identification through microscopy (**paper I, II**), image analysis (**paper III**) and metabarcoding (**paper IV**). The taxonomic identification through microscopy (**paper I, II**) was done to the lowest level possible, usually to species level

for copepods and to order for other groups, and were based on various sources of literature such as Enckell (1980), Gissel Nielsen & Hansen (1999), and Larink & Westheide (2011).

The taxonomic identification through image analysis (**paper III**) was performed using the software ZooImage (version 1.2-1) (Grosjean and Denis, 2007). Samples of mesozooplankton were digitally scanned, and the individuals were classified to either genus (copepods) or order (other taxa) using a classifier based on a random forest algorithm (Breiman, 2001). The classifier was built from a training set where digital images of manually identified organisms were sorted in a hierarchical taxonomic structure.

The taxonomic identification through metabarcoding (**paper IV**) was based on water samples collected from each experimental bottle. RNA was extracted from the filtered water samples, and reverse transcribed into cDNA. During a first PCR, the 18S V4 region was amplified using a primer set adapted for eukaryotic microbes (Stoeck *et al.*, 2010). The following steps of the library preparation such as clean up steps and indexing, as well as the sequencing on the Illumina MiSeq system, were performed at SciLifeLab National Genomics Infrastructure (Solna, Sweden). The analysis of the metabarcoding sequences was performed using the nf-core/ampliseq pipeline (Straub and Peltzer, 2019), where the sequences were quality controlled, leftover primers were trimmed, and amplicon sequence variants (ASVs) were inferred from the sequences. The ASVs were taxonomically classified using the PR^2 (Protist Ribosomal Reference) database, which is a database with focus on protists and 18S sequences (Guillou *et al.*, 2012). Non-target ASVs and ASVs that were taxonomically classified with low confidence (<0.8) were removed before the diversity analyses.

3.3.2 Diversity types and how they were analysed

The types of diversity that have been used to determine effects of chemical exposure on zooplankton species in this thesis are alpha and beta diversity. Alpha diversity is a descriptor of within treatment diversity (Figure 7a), and there are many indices that can be used to calculate this diversity type. In **paper IV**, we used species richness that simply describes the number of observed species in a sample, and the Shannon index that also considers the relative quantity of each species in a sample.

Beta diversity can be used as a descriptor of change in composition between samples (Figure 7a) and was used to evaluate pairwise differences in control and treatment diversity in **papers I, II, III** and **IV**. We used the Bray-Curtis dissimilarity index (Bray and Curtis, 1957) as the specific diversity metric to compare differences between treatments in all four studies, which was visualised using non-metric multidimensional scaling (nMDS). nMDS is a method of visualisation that iteratively tries to find the most representative visualisation of the differences in diversity between sites, while preserving their rank-order (Clarke *et al.*, 2014).

We tested for diversity differences between treatments using either ANOSIM (**paper I, paper II, paper III**) or PERMANOVA together with PERMDISP (**paper IV**). The ANOSIM uses rank similarities between treatments from the dissimilarity matrix to test whether there are any significant differences between treatments (Clarke *et al.*, 2014), whereas the PERMANOVA

compares within and between group sums of squares similar to an ANOVA but in a multivariate space (Anderson, 2008). Both tests generate a test statistic and test the significance of these by performing permutation tests where the treatment labels are shuffled many times.

The PERMDISP tests if there is a difference in average distance to the group centroid of the respective groups, i.e., the dispersion. This type of test is a good complement to a PERMANOVA or an ANOSIM, which both are sensitive to differences in dispersion among treatments, and could generate a significant result for a specific treatment based on either location, dispersion of both of them (Anderson, 2008) (Figure 7b). By combining a PERMANOVA and a PERMDISP, you can disentangle the location and dispersion effects, although if both indicate significant differences between treatments, they should be complemented with a visualisation of treatment differences such as an ordination plot.

3.4 Functional endpoints

We estimated effects on community functioning in three of the papers in this thesis by measuring effects on community feeding (**paper I, III**) and copepod reproduction (**paper I, II, III**). The effects on feeding was assessed by measuring density of the algae culture added as a food source, and in both experiments a mixture of *Rhodomonas salina* and *Thalassiosira weissflogii* was added at the start of the exposures. Chlorophyll a was measured in the control and in all treatments as a proxy for algae density in both studies, using either a Turner Trilogy Laboratory Fluorometer fitted with an in-situ Chlorophyll In-Vivo Module (**paper I**) or a 10-AU Turner fluorometer (**paper III**).

We measured the effects on copepod reproduction by quantifying the number of eggs produced as well as the % of these eggs that hatched (**paper I, II, III**). The eggs were filtered out from each experimental replicate and left to hatch in filtered seawater without contaminants, and the eggs and hatched nauplii were later fixed with acidified Lugol's solution and counted using a stereo microscope. The egg production was quantified by adding the number of unhatched eggs and nauplii, and the % of hatched eggs were quantified by dividing the counted nauplii by the total egg production.

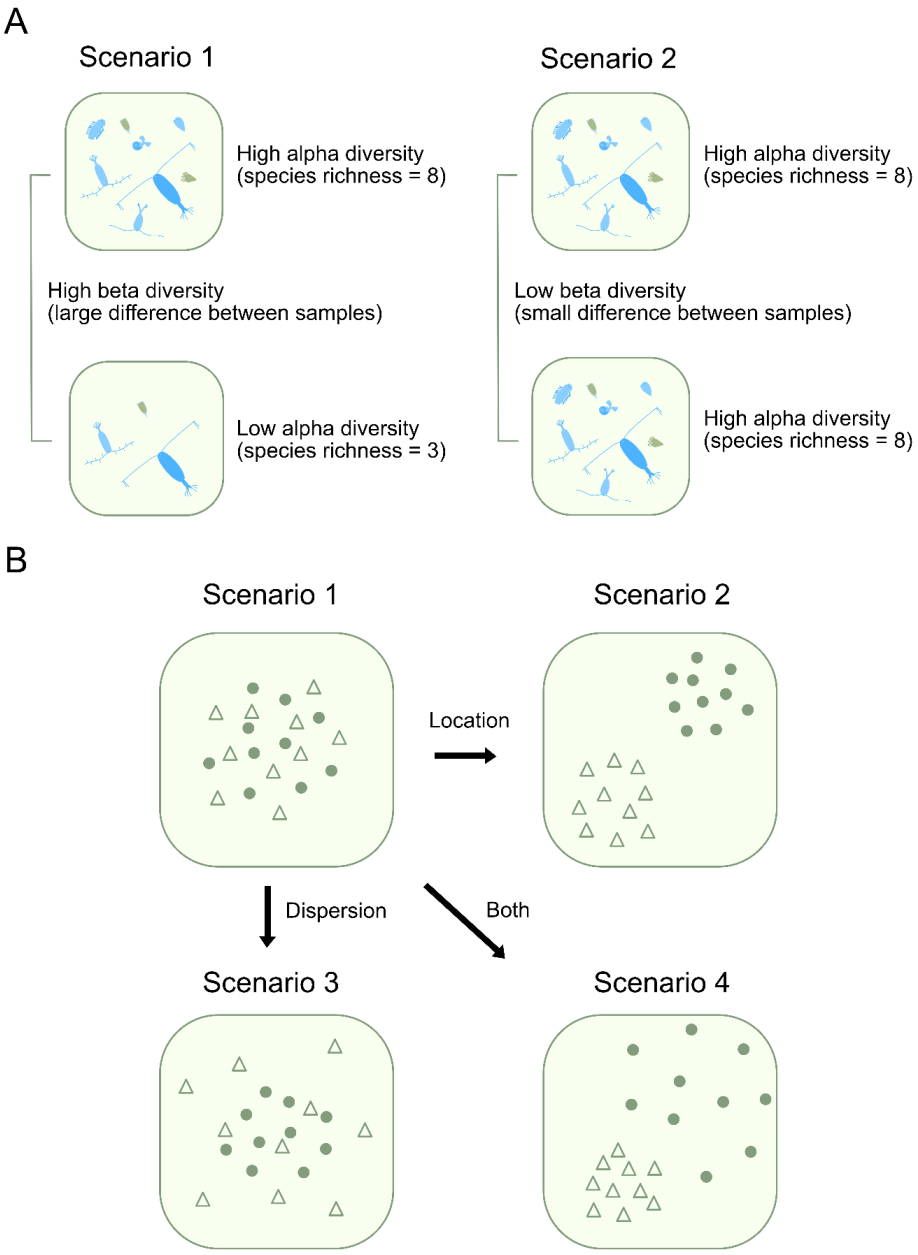

Figure 7. Examples of how species diversity can differ within and between samples. A) Scenario 1 shows two samples that each have low and high alpha diversity, and thus a large beta diversity. Scenario 2 shows two samples that each have high alpha diversity and contain the sample species in each sample, which gives a small beta diversity. B) Different patterns of diversity that can cause differences in beta diversity between treatments. Two groups of samples are shown in two dimensions that 1) do not differ in location or dispersion 2) differ only by location, 3) differ only in dispersion, and 4) differ in both location and dispersion. Figure 7B is modified from Anderson (2008).

3.5 Survival

Copepod survival was estimated in three studies (**paper I, II, III**) by fixing zooplankton samples after exposure in the control and treatments, and prior to exposure from separately prepared bottles. The fixation was either performed by preserving samples in ethanol (**paper I**), in a 5:95 glycerol:ethanol mix (**paper II**), or by staining with neutral red and freezing samples on a filter (**paper II, III**). Neutral red stains viable cells and a bright red concentration of stain in the prosome tissues indicates a live individual (Elliott and Tang, 2009).

Copepod survival was estimated either by comparing number of individuals in control and treatments at the end of the exposure (**paper I, III**), or by comparing number of individuals in control and treatments to the quantity in the samples collected prior to exposure (**paper II**). Copepod survival in the controls was always estimated by comparing the number of live individuals to the live quantity in the samples collected prior to exposure (**paper I, II, III**).

Microzooplankton survival was estimated by quantifying the number of individuals prior to and at the end of each experiment (**paper II, IV**). Water samples were collected from each experimental replicate, stained using acidified Lugol's solution, and counted after settling in Utermöhl chambers (Utermöhl, 1958) using an inverted microscope.

3.6 Ranking of toxicity-drivers

The ranking of the toxicity-driving chemicals in the mixtures were always performed by estimating the individual toxic units of each substance in the mixture, and then calculating the relative contribution of these to the summarised toxic unit of the mixture (**paper I, II, III, IV**). Concentrations of the individual substances in the mixtures were either collected from literature (**paper III**) or from analytical measurements of substances in the mixtures (**paper I, II, IV**). The water accommodated fractions of the fuels in **paper I** were analysed for aliphatic and aromatic hydrocarbons, methylpyrenes/methylfluoranthenes, methylchrysenes/methylbenz(a)anthracenes, and the standard set of 16 polycyclic aromatic hydrocarbons (PAHs). The scrubber water **in paper II** was analysed for the same set of compounds as the fuels but with the addition of metals. The marine surface water mixtures in **paper IV** was analysed for 750 organic, polar compounds including pharmaceuticals, pesticides and chemicals used in industry.

The hazard data for the individual substances in the mixtures were either observed ecotoxicity data collected from publicly available databases (**paper II, III**), QSAR modelled ecotoxicity data (**paper I**), or a mix of the two (**paper IV**).

4. Main findings

4.1 Paper I

Our findings indicate that the water accommodated fraction (WAF) of the marine gas oil (MGO) was less toxic than that from the ultra-low sulphur fuel oil (ULSFO), based on copepod community egg production. Egg production was significantly reduced by exposure to 25% ULSFO WAF, but no effects were detected from the MGO WAF. The component-based toxicity estimation of each fuel WAF found the same pattern as the experiment, where the ULSFO had a higher cumulative toxic unit (TU) than the MGO (Figure 8). The ranking of the individual TUs revealed that the toxicity of the ULSFO was driven mainly by the two polycyclic aromatic hydrocarbons (PAHs) naphthalene and phenantrene (Figure 8d), whereas the MGO toxicity was driven by several aromatic hydrocarbons (Figure 8c).

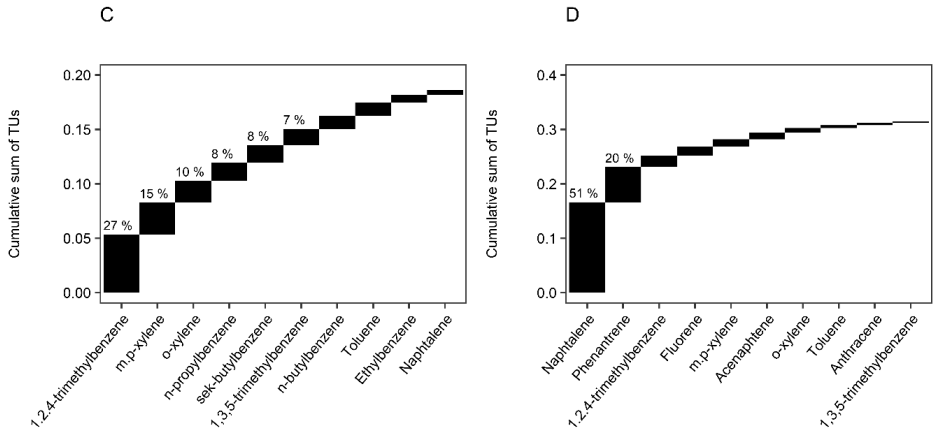

Figure 8. The individual toxic units (TUs) for all detected compounds in 100 % WAF of the C) DMA grade MGO, and D) ULSFO (RMD80). The predictions are based on QSAR modelling of toxicity based on log K_{ow} (Ha et al., 2019). Percentages on top of bars represent contribution to the overall toxicity of each fuel. Figure modified from Jönander et al. (2020).

The effects of exposure to the ULSFO included initially reduced algae concentrations on average, connected to increased feeding at lower exposure levels (0.5-1% WAF), but this did not result in an increased copepod egg production. The egg production was unaffected up to 2% WAF and decreased significantly at 3%. We found significant effects on mesozooplankton species diversity in the exposed generation (F0) starting at 0.5% WAF, but no effects on the second generation (F1) (Figure 9). The copepod *Acartia clausi* appeared to be one of the more sensitive species in the community, whereas *Centropages spp.* and *Temora longicornis* were more tolerant.

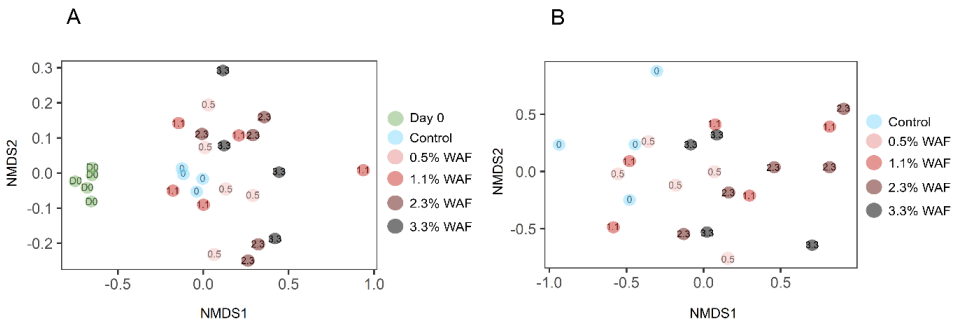

Figure 9. nMDS visualising copepod community composition based on Bray-Curtis dissimilarities of square root transformed data in the exposed parent generation and the unexposed second generation. A) Effects on the parent generation (F0) (stress = 0.10), and B) Effects on the second generation (F1) (stress = 0.17). Figure modified from Jönander and Dahllöf (2020).

4.2 Paper II

We found that mesozooplankton reproduction, survival, and biodiversity were significantly affected at <1.5% of closed-loop scrubber water, and that these effects indirectly increased the microzooplankton abundance. Generally, all the effects were observed independently of seasonal species composition of the communities. One of the most consistent effects were those on copepod reproduction, where 3% closed-loop scrubber water reduced the total community egg production to 35-42% of the control (Figure 10a), and the hatching success to 3-27% (Figure 10b).

Modelling of the cumulative toxicity of the undiluted closed-loop scrubber water based on detected hydrocarbons and metals, as well as observed ecotoxicity data for the individual substances, revealed that the closed-loop scrubber water had a cumulative toxic unit of 17 (Figure 11). This meant that the undiluted closed-loop scrubber water was 17 times more toxic than what affects 50% of a zooplankton population. The toxicity was driven by the substances V (57%), Cu (26%), benzo[*ghi*]perylene (11%) and Ni (5%), which together were responsible for >99% of the total toxicity.

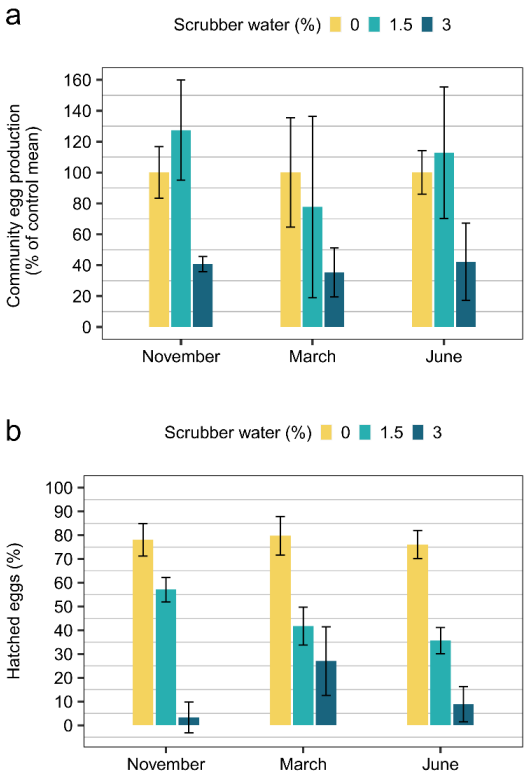

Figure 10. Effects of closed-loop scrubber water on copepod reproduction during all three seasons. a) Copepod community egg production shown as % of control mean (mean ± 95% confidence interval, *n*=5), and b) hatching success displayed as % hatched eggs of the total amount of eggs produced during the exposure (mean ± 95% confidence interval, *n*=5). The mean copepod community egg production was 452, 103, and 734 respectively for the months November, March, and June. Figure from Jönander et al. (*Manuscript*).

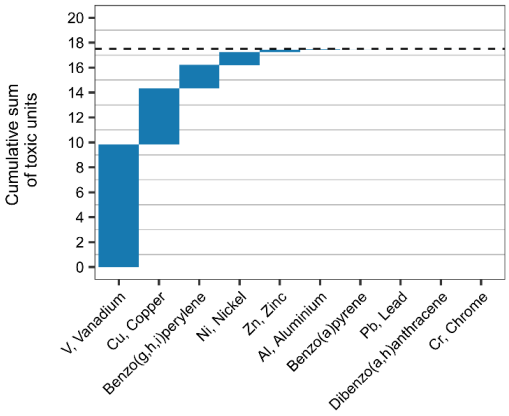

Figure 11. The cumulative toxic unit of the undiluted closed-loop scrubber water based on ranked individual toxic units of the detected chemicals. The dashed line marks the total cumulative toxic unit. Figure from Jönander et al. (*Manuscript*).

4.3 Paper III

We found that DBP significantly affected community feeding through indirect increase in algae growth (Figure 12c), as well as reduced the copepod ability to reproduce at 4 µmol/l (Figure 12d), which corresponds to 2000x the measured environmental concentration (MEC). As for SDS, we found that the significantly reduced feeding shown through indirect algae growth, as well as reduced reproduction, were caused by exposure to 12 µmol/l (Figure 12), which corresponds to 40x the MEC. At this concentration, SDS also significantly affected the community diversity, which was changed in composition compared to the control community (Figure 13). The reduced survival of the copepod genus *Acartia* was the main cause of changes in the community diversity.

We found that the high concentration mixture of DBP and SDS (4 µmol/l + 12 µmol/l) significantly affected all tested endpoints (Figure 12, Figure 13). The toxicity was higher than what had been predicted by the independent action model by 21% on average (Figure 12). Finally, we found that the SDS (at 12 µmol/l) and a mixture of DBP and SDS (at 4 µmol/l + 12 µmol/l) affected mesozooplankton diversity in unique ways (Figure 13).

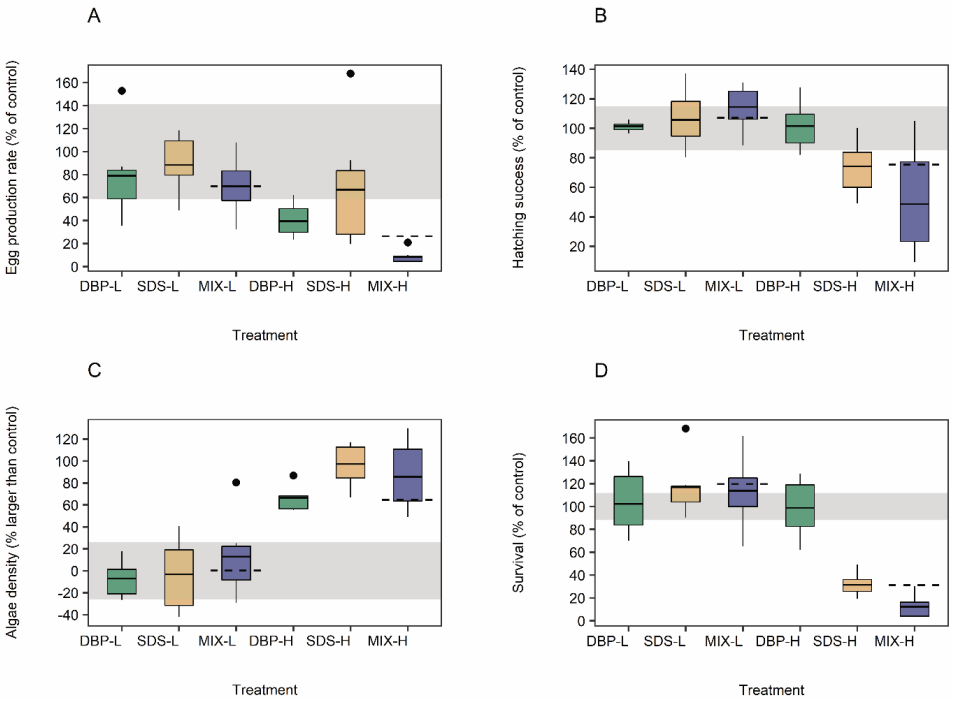

Figure 12. Observed and modelled toxicity of individual compounds and the mixture. A) Egg production per broadcast spawning copepod between 24 and 48 h, B) hatching success of eggs produced between 24 and 48 h, C) increase in algae concentration at 48 h relative to the control, and D) copepod survival after 48 h (% of surviving individuals). Horizontal lines in the box-and-whisker plot indicate arithmetic means, and dots represent outliers in each treatment. Independent Action model predictions for the mixtures are represented by dashed lines. 95% CI of the controls are represented by the horizontal grey area. Figure from Jönander *et al*. (2022).

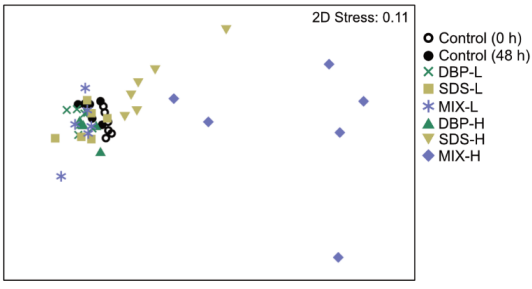

Figure 13. Non-metric Multidimensional Scaling (NMDS) of the zooplankton species diversity in the 0 h and 48 h control communities, DBP, SDS and Mixture exposed communities. Visualizations are based on Bray-Curtis index using species occurrence data (standardised by total abundance and square root transformed). Figure from Jönander *et al*. (2022).

4.4 Paper IV

We found that all three mixtures significantly reduced diversity within each sample at x10 the measured environmental concentrations (MECs) (Figure 14a) but changed the species composition significantly compared to the controls already at x1 MEC (Figure 14b). At 10x MEC, the samples were dominated by the dinoflagellate *Dinophysis acuminata* and had fewer ciliates and other dinoflagellates compared to lower concentration treatments and controls (based on sequences reads). These patterns were supported by microscopy counts of the samples, where both dinoflagellate and ciliate abundances were significantly reduced at 1x-10x MEC depending on the specific site.

The cumulative toxicity estimations of each mixture showed that they should be similar in toxicity to zooplankton, which was in line with the observed toxicity from our experiments. However, the component-based modelled toxicity was much lower than the observed toxicity from the whole mixture. The cumulative toxicity estimations were built on observed ecotoxicity data for different zooplankton species retrieved from different databases, supplemented with QSAR modelled ecotoxicity to daphnids when observed data was missing.

Comparisons of these two data types revealed that the QSAR data represents the approximate average of the observed zooplankton data, and that there were no systematic difference in sensitivity to the chemicals detected in the marine surface water among different types of zooplankton taxa (Figure 15). However, there was a large spread in sensitivity to certain chemicals among all zooplankton taxa, where the largest difference in EC50 was by a factor of 100 000. Evaluation of the QSAR performance revealed ECOSAR could predict the toxicity of 65% of the detected chemicals within a factor of 10.

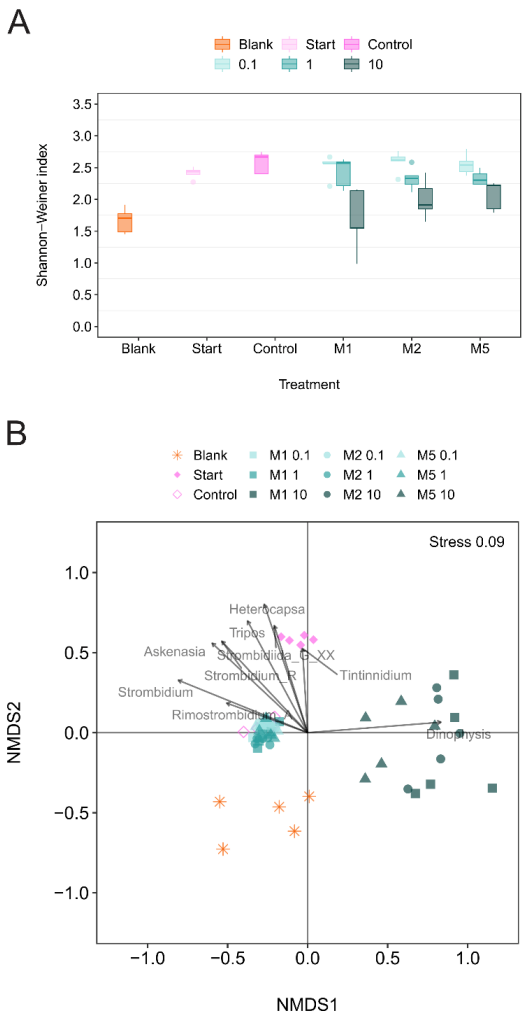

Figure 14. Alpha and beta diversity of microzooplankton (and other microplankton groups) exposed to extracts of concentration 0.1x, 1x and 10x measured environmental concentrations from sites M1, M2 and M5 (n=5). The diversity metrics is based on number of 18S sequence reads. A) Alpha diversity in the form of Shannon index in each sample. The boxes display median and the 25th and 75th percentiles. The whiskers extend from the box to the highest and smallest datapoints (at most 1.5 * the interquartile range from the box), and any point outside this range is plotted individually as an outlier. B) Beta diversity in the form of Bray-Curtis index on standardised and square root transformed data, visualised in an NMDS. The direction of the vector arrows display the direction of increased proportions of the respective ciliate and dinoflagellate taxa that most explain the variance of NMDS1 and NMDS2. Figure from Jönander et al. (*Manuscript*).

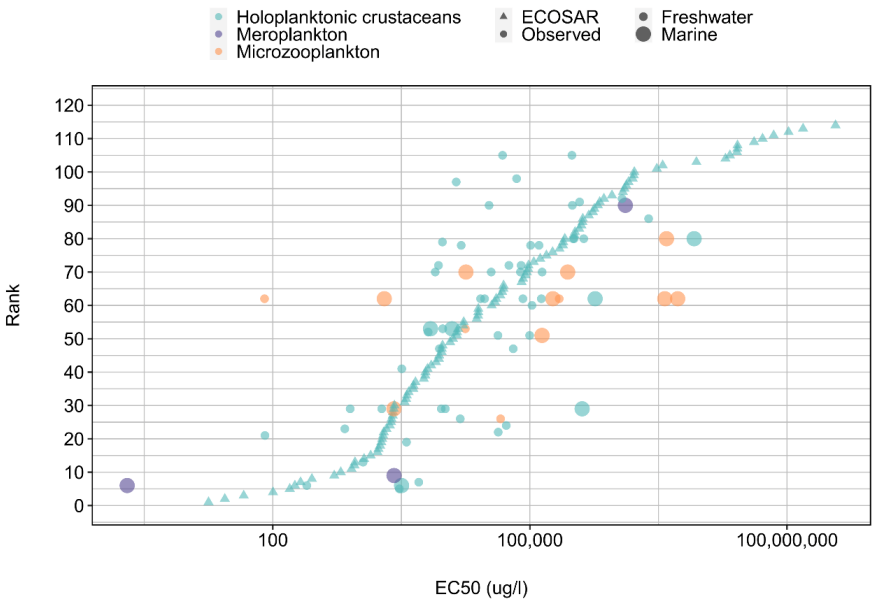

Figure 15. Observed and modelled zooplankton EC50s of each detected substance in sites M1-M6. The graph includes geometric means of QSAR (ECOSAR) acute toxicity data (LC50s) for daphnids (blue triangles), and arithmetic means of observed toxicity data (EC50s, IC50s, and LC50s) for holoplanktonic crustaceans (blue circles), meroplankton (purple circles), and microzooplankton (orange circles). Data for freshwater taxa are displayed in small triangles and circles, and marine taxa are displayed in large ones. The substances on the y axis are ranked based on high to low QSAR (ECOSAR) acute toxicity data (LC50s) for daphnids. Figure from Jönander et al. (*Manuscript*).

5. Discussion

5.1 Taxonomic identification for biodiversity assessments

We used different methods for the taxonomic identification of zooplankton in the four studies included in this thesis, all with different advantages and disadvantages. Some advantages of using image analysis for identification (as done in **paper III**), is that it can decrease the time spent on analysing samples, as well as reduce the cost of data processing (Bell and Hopcroft, 2008; Uusitalo *et al.*, 2016). It can also help reduce misclassification due to human error (Culverhouse *et al.*, 2003). However, some disadvantages are that it takes time to set up the workflow, create the training set and classifier, and that there can be a high error rate between morphologically similar taxa (Uusitalo *et al.*, 2016). We decided to only classify by copepod genera and by order for other zooplankton groups, and we still had a 27% error rate in the classification, which is similar to what has been achieved with image analysis softwares based

on similar image features (Benfield *et al.*, 2007). However, these error rates can also be comparable to manual identification through microscopy, depending on the specific analysed taxa (Culverhouse *et al.*, 2003).

An advantage of using manual classification of the zooplankton through microscopy (as done in **papers I** and **II**) are that we can gain more detailed information about the individuals, such as sex and life stage, which can be difficult to classify through image analysis as the differences can be very subtle. Such characteristics can also be useful for interpreting results, as for example copepods can have different feeding preferences and abilities depending on them (Swadling and Marcus, 1994; Saage *et al.*, 2009; Meunier *et al.*, 2016).

The use of metabarcoding for diversity assessment (as used in **paper IV**) also has both advantages and disadvantages compared to microscopy and image analysis. Metabarcoding has been able to detect more species in environmental samples compared to manual identification, particularly for meroplankton and early life stages of copepods (Lindeque *et al.*, 2013; Mohrbeck *et al.*, 2015; Serrana *et al.*, 2019). However, metabarcoding, like image analysis, cannot distinguish between developmental stages since the barcode sequence for each species is the same across egg, larval and adult stages.

Another disadvantage of using metabarcoding instead of manual identification or image analysis are the problems regarding abundance and biomass estimations, which can be explained by different numbers of gene copies (Yarimizu *et al.*, 2021; Martin *et al.*, 2022), species-specific primer affinities, and GC content (Elbrecht and Leese, 2015; Nichols *et al.*, 2018). Abundance of marine plankton, at both species and higher taxonomic level, has not been found to correlate well with read counts (Mohrbeck *et al.*, 2015; Martin *et al.*, 2022), but the use of correction factors have been found to improve this considerably (Martin *et al.*, 2022). In **paper IV**, we try to reduce some of these biases by both basing our sequence reads on reversely transcribed RNA, which has been found to reduce the gene copy bias (Not *et al.*, 2009), as well as by comparing our metabarcoding results to counts based on manual microscopy identification.

5.2 Effects of data availability and type on mixture toxicity modelling

The lack of hazard data, i.e. ecotoxicological effect data, has been identified as one of the limiting factors in risk assessment of chemical mixtures (Evans *et al.*, 2015; Bopp *et al.*, 2018), and influenced all the mixture toxicity estimations in this thesis. In **paper I**, we modelled hazard data using a QSAR for polycyclic aromatic hydrocarbons (PAHs) (Ha *et al.*, 2019), however, we still lacked data for the grouped aliphatic and aromatic substances that could also have contributed to the toxicity of the fuels. As for the closed-loop scrubber water in **paper II**, we based the cumulative toxicity estimations on available observed hazard data which was missing for 24% of the detected substances, whereas 12% were missing for the mixture in **paper III**. In **paper IV**, we lacked data for 27% of the substances in the mixtures, and we filled the data gaps by supplementing the observed hazard data with QSAR data generated with ECOSAR (US EPA, 2022). This meant that no data was missing for the detected substances in

the mixtures used in this study, however, something that all QSARs have in common is that the predictions of toxicity rarely are very precise (Golbamaki *et al.*, 2014). Hence, the outcome of a mixture risk assessment could be severely affected if the QSAR prediction underestimates or overestimates the toxicity of one or a few chemicals in the mixture. In **paper IV**, we suspected that the risk-driving role of ursolic acid was due to such an overestimation of toxicity caused by poor performance of ECOSAR, as there was little evidence of toxicity of this compound to other invertebrates than zooplankton (da Silva Ferreira *et al.*, 2010; Kalani *et al.*, 2014).

The comparisons of the observed and modelled hazard data in **paper IV** did show that the QSAR LC50s for daphnids roughly corresponded to the average of the observed EC50s for a range of marine and freshwater microzooplankton and mesozooplankton taxa. Hence, for this organism group and data type, supplementing missing observed data with modelled data was appropriate but only if the mean was used. The range of sensitivities among zooplankton taxa to some individual chemicals spanned several orders of magnitude and meant that the toxicity of only 65% of the observed data points could be predicted within a factor of 10 by ECOSAR. This is in line with other applications of ECOSAR where toxicity of 63-85% of the chemicals have been predicted within a factor of 10 (Reuschenbach *et al.*, 2008; Melnikov *et al.*, 2016; Zhou *et al.*, 2021).

The availability of hazard data could also affect the outcome of estimating the toxicity-driving substances in each mixture. This became particularly clear in **paper IV**, where most toxic units of the toxicity-driving substances were derived from QSAR generated LC50s. If data for these substances had been missing, we would have completely changed the interpretation of which chemicals in the mixtures that were most responsible for the toxicity.

5.3 Whole mixture approach or component-based approach

In **papers I**, **II**, and **IV**, we used whole mixture approaches, i.e., we exposed zooplankton to the mixture in its entirety. In **paper III**, on the other hand, we exposed zooplankton to only two toxicity-driving substances from a more complex mixture, both individually and together in a binary mixture. In all four papers we performed component-based cumulative toxic unit summations to estimate the toxicity of the mixtures and compare it to the outcome of the whole mixture exposures.

An advantage of using the whole mixture approach is that the effects can be assessed without knowing the exact content of the mixture (Brack *et al.*, 2017; Bopp *et al.*, 2019). It is impossible to identify all the components of a mixture, and in this thesis, we have usually targeted a subset of chemicals with similar properties such as hydrocarbons (**paper I**), hydrocarbons and metals (**paper II**), or polar organic chemicals (**paper IV**), which means that we lack information about the entire mixture content. This becomes evident when we compare our observed effects from the whole mixture exposure to the component-based cumulative toxic unit summations, where we usually observe effects at levels where the toxic unit summations indicate that there should be none. This is likely a result of both missing hazard data (see previous section), and from not

capturing all the mixture components in the chemical analysis on which we base the cumulative toxic units. Furthermore, many individual chemicals may fall under their respective detection limits and could therefore be excluded from a component-based cumulative toxicity assessment even though they were analysed for. Inclusion or exclusion of such non-detects could have a great impact on the interpretation of a risk assessment and could change the outcome of no risk versus risk (Gustavsson *et al.*, 2017b), and it has been shown that the detected substances in a mixture can explain as little as <0.1% of the entire toxicity of a mixture (Escher *et al.*, 2013).

An advantage of using the component-based approach is that the mixture risk assessment could be performed without having to generate new experimental data, given that there is existing hazard data available for the individual chemicals in the mixture. However, as was observed in **paper IV**, the sensitivity of different species to the same substance means that the size of the applied assessment factor needs to be carefully considered to protect the entire community of species.

5.4 Consequences of the IMO sulphur regulations

The recent reductions of allowed sulphur content in marine fuels have triggered the emergence of both new fuel types as well as technology that allows ships to continue to run on high sulphur fuels. In this thesis, the toxicity and possible impacts on marine zooplankton from two representatives of these options have been addressed; ultra-low sulphur fuel oil (ULSFO) and closed-loop scrubbers.

The findings from **paper I** revealed that that the ULSFO was more toxic to marine zooplankton than a marine gas oil (MGO), which is another petroleum-based low sulphur fuel, and that the toxicity of the ULSFO was driven by a few PAHs. After longer exposure to the ULSFO, we found that mesozooplankton species had different sensitivities which caused changes in the species diversity, and that the total copepod reproductive output was reduced in the community. This happened at a total PAH concentration of 23 µg/l (at a nominal dilution of 10% ULSFO WAF), which is much lower than what has been measured in the water after spills of diesel (222 µg/l) (Cripps and Shears, 1997) or crude oil (115 µg/l) (Reddy and Quinn, 2001). Although fuel spills are becoming less common, large spills occur every year on a global scale (see introduction section 1.2.1). Considering the difficulties involved in spill removal of hybrid fuels, such as ULSFO, we can expect that marine zooplankton will be affected adversely on at least a local scale after such a spill.

In **paper II**, we moved on to the effects on new technology on marine zooplankton, and tested the toxicity of wastewater from closed-loop scrubbers. Closed-loop scrubbers do, despite their name, generate wastewater that often is discharged to the surrounding water. In **paper II**, we found that as little as 1.5% (v/v) of this water can reduce the total reproductive output as well as survival of the mesozooplankton community, which also impacted the total ability to predate on ciliates. At the same exposure level, we also found that the sensitivity of species in the community was different, which led to changes in diversity. Closed-loop scrubber water has

previously been found to increase mortality in single species of copepods at an even lower exposure level (<0.1% v/v) (Thor *et al.*, 2021).

After analysing the content of the scrubber water, we found that the toxicity was driven by both metals and PAHs, some of which are listed as priority substances in the water framework directive (WFD) (Directive 2013/39/EU). From these results, we can conclude that mesozooplankton close to the discharge source will be adversely affected by closed-loop scrubber water.

Modelling studies have found that scrubber water can be diluted up to 2000 times depending on how the ship operates (Buhaug, 2006), which means that many of the individual substances in the scrubber water no longer exceed their environmental thresholds (Teuchies *et al.*, 2020). Furthermore, a recent study estimated the direct contribution from both open and closed-loop scrubbers in different harbour types and found that there is a low risk (0.001) of environmental impacts from the closed-loop scrubber water, at least in an OECD Baltic harbour environment (Hermansson *et al.*, 2023). However, this is based on a current scenario where most scrubbers run in open-loop mode, which could change in the near future as open-loop scrubbers have been shown to be a large contributor to contamination in certain areas (Ytreberg *et al.*, 2022; Hermansson *et al.*, 2023) and many ports and states have banned, or recommend to ban their use (Nepia, 2023, HAV, 2022). Furthermore, many of the contaminants in the closed-loop scrubber water are very persistent in the environment and have half-lives of 100-1000 days in water, and even longer once they reach the sediment (Tansel *et al.*, 2011). Hence, we can expect that marine zooplankton will be adversely affected by closed-loop scrubber discharges, at least close to the source of the discharge and in areas with heavy shipping traffic where there will be a continuous output of scrubber water.

5.5 Consequences of complex mixtures in coastal environments

Complex contaminant mixtures have been detected in many surface waters (Gustavsson *et al.*, 2017a; Vanryckeghem *et al.*, 2019), and to address their effects on marine zooplankton in these areas, we performed two studies where mesozooplankton (**paper III**) and microzooplankton (**paper IV**) were exposed to mixtures originating from these environments.

In **paper III**, we found that a phthalate and a surfactant were driving the toxicity in a mixture of contaminants from marine surface water near Stenungsund, Sweden, but we only observed effects from these at levels far above their respective measured environmental concentrations (MECs). At the concentrations where we did find effects, we found that exposure to the individual substances and the mixture had unique effects on mesozooplankton diversity, which indicates that some species in the community can tolerate exposure to a single chemical but not the mixture. At these high concentrations, we also found that independent action model underestimated the toxicity of the mixture at all tested endpoints, which implies at least additive, but possibly synergistic toxicity between the two chemicals. Binary mixtures of phthalates with other contaminants have previously been found to cause toxicity in copepods, even where the concentrations of the individual substances were non-toxic (Forget-Leray *et*

al., 2005), and we suspect that the surfactant facilitated the uptake of the phthalate through membrane disruption (Jacobi *et al.*, 1996; Syberg *et al.*, 2008).

Despite the interaction between the two contaminants tested in **paper III**, our findings suggest that there should be little risk of adverse effects on marine zooplankton from exposure to this specific mixture, based on the high effect concentrations. However, in the studies where we used whole mixture approaches (**papers I, II, IV**) we found effects even when the component-based toxicity modelling suggested there should be none. Hence, it is possible that the mesozooplankton in **paper III** had been adversely affected at the environmental mixture concentration if they had been exposed to the entire mixture.

In **paper IV**, we instead exposed a microzooplankton community to three whole mixtures collected from surface water sites. We found that environmental concentrations of all mixtures reduced the abundances of both ciliates and dinoflagellates, but that the ciliates generally were more sensitive, which reduced the community diversity. Furthermore, we found that ciliate abundance was reduced after exposure to concentrations below what had been measured in the environment, although not significantly. One dinoflagellate species, *Dinophysis acuminata*, stood out as particularly tolerant to the contaminant exposure. This is a bloom-forming species that can produce toxins which can accumulate in filter-feeders and cause diarrhetic shellfish poisoning (DSP) (Hattenrath-Lehmann *et al.*, 2013; Mafra Jr *et al.*, 2019).

Our findings suggest that the chemical mixtures of polar organic substances present in the coastal environments pose a threat to the diversity of microzooplankton. As this group of organisms have a key role in the pelagic foodweb as some of the main grazers of smaller phytoplankton (Calbet and Landry, 2004), reductions in their diversity as well as biomass could have negative implications of the status of coastal waters. Furthermore, the possible competitive advantage of the bloom-forming and toxic *D.acuminata* in contaminated water could potentially even worsen the water quality in the area.

6. Conclusions

6.1 How does contaminant mixtures affect marine zooplankton?

The first aim of this thesis was to **"*find out how unintentional and coincidental contaminant mixtures that exist or may end up in the marine costal environment affect biodiversity and function of marine zooplankton*".** From the results presented in this thesis, I found evidence of clear mixture toxicity of the contaminants, which is in line with other literature in this field. Effects on zooplankton biodiversity was detected through both alpha and beta diversity, at concentrations of contaminants that exist, and that may appear in the marine environment. Some of the most prominent effects were observed on marine microzooplankton diversity from exposure to contaminant mixtures samples in coastal surface water near urban areas. These effects occurred at the measured environmental concentrations (MECs) of only a subset of

contaminants in the area, and there were indications of effects even below these concentrations. As for the community function, reduced reproductive ability, as well as altered feeding, were also found. The most prominent effects on function were found from exposure to closed-loop scrubber water that reduced the copepod egg production and hatching success at only a few percentages. Given that this type of wastewater can be discharged from a single ship at a rate of 126-150 m^3d^{-1} (based on a medium sized ship, operating at 15 MW) (Hermansson *et al.*, 2021), this is of high concern for zooplankton (and other marine organisms) in areas near shipping traffic.

Mixture toxicity was generally underestimated using the component-based approach, compared to the whole mixture approach. In **paper I**, and **paper IV**, effects from whole mixture exposure were observed, even when the component-based toxicity modelling suggests that there should be none. In **paper II**, the component-based toxicity modelling suggests little or no effect at the respective treatment levels, but clear effects were still observed from these.

6.2 Which individual chemicals are responsible for the toxicity?

The second aim of this thesis was to **"*find out which individual chemicals in these mixtures that are most responsible for the effects*"**. Based on the component-based toxicity estimations, I found that there were generally a few chemicals that were more responsible for the toxicity in each mixture, but that the number of such toxicity-drivers varied between each type of mixture. Polycyclic aromatic hydrocarbons (PAHs) were found to be the toxicity-driving substances in marine petroleum-based fuels, specifically in the ultra-low sulphur fuel oil (ULSFO), whereas in closed-loop scrubber water, a mixture of metals and PAHs were driving the toxicity, specifically V, Cu, benzo[*ghi*]perylene, Ni, and Zn. In complex chemical mixtures in coastal surface water, the toxicity-driving substances differed both in quantity and identity depending on the sampling site.

7. Outlook

My ambition with this thesis was to contribute with knowledge about how chemical mixtures could affect marine zooplankton at the community level at realistic conditions. However, although some questions may be answered, several unanswered ones remain.

7.1 Ecotoxicological studies with natural zooplankton communities

Experiments with natural zooplankton communities, that consist of several species and genotypes, can be advantageous as they need less extrapolation than standardised assays with single species that usually have been cultured in the lab for many generations. Hence, working with natural communities can be an advantage when the aim is to find out how chemicals affect organisms in their natural environment. Zooplankton are a particularly important group when assessing the impacts of contaminants in the marine environment, as they are some of the first organisms that come into contact with these substances once they enter the water.

However, there are still many limitations and difficulties involved in using field-collected natural communities in laboratory experiments. Some examples of these are that communities need to be handled carefully from the field to the lab to ensure they are harmed as little as possible, and that exposure conditions should resemble those in the field to avoid unintentional stress. Furthermore, the effects of contaminants on a community level can be difficult to disentangle from interspecific interactions. Hence, comparisons of effects on the species level can be useful complements when drawing conclusions about community-level effects.

The studies in this thesis are all based on laboratory experiments with natural communities, and a way of taking these studies to the next level could involve the use of mesocosms. These types of experiments have the advantages of avoiding the net samplings that could harm the communities, and that several trophic levels with natural assemblies can be included all at once. Mesocosm studies of both freshwater and marine plankton have shown that individual contaminants can affect one trophic level more severely, which results in indirect effects up or down the food web (Hjorth *et al.*, 2008; Rumschlag *et al.*, 2020). Hence, this methodology is promising for future studies of how multiple trophic levels of zooplankton are affected by contaminant mixtures.

We examined effects on species diversity, but there are also other types of important diversity. Using trait-based approaches in studies of zooplankton have been proposed (Litchman *et al.*, 2013; Hébert *et al.*, 2016), and could provide useful knowledge about how the effects of contaminants can alter functional diversity and by extension ecosystem processes.

Once a community has been exposed to contaminants, it may have gained tolerance through the principles of pollution induced community tolerance (PICT) (described in section 1.4.1). I did not investigate such effects in the studies included in this thesis, however, they would provide good complements, and to my knowledge, no studies have been performed involving PICT on zooplankton communities. Furthermore, increased tolerance to one stressor can often be associated with increased sensitivity another one (Kashian *et al.*, 2007; Bach and Dahllof, 2012), and can for example involve increased sensitivity to global climate change after exposure to contaminants or vice versa (Hooper *et al.*, 2013). Given that these are two types of stressors that zooplankton already encounter, which may only become more severe over time, tolerance-associated costs should be further studied in this organism group.

In one of the four studies in this thesis (**paper II)** we included two trophic levels to estimate how effects of contaminant exposure on one level indirectly could affect another one. However,

the focus of that study was mainly on the mesozooplankton, and how exposure to closed-loop scrubber water affected the ability of the entire community to predate on the microzooplankton. To fully capture the effects of the contaminants on both of these trophic levels, and possibly also more, they should be exposed separately and in combination. The ciliates in the microzooplankton community have been shown sensitive to some of the metals that are found in closed-loop scrubber water, hence, it is highly relevant to test the direct toxicity of this wastewater to these organisms (Madoni and Romeo, 2006). Furthermore, as ciliates generally have been found more sensitive to contaminants than other microzooplankton (Almeda *et al.*, 2014b; Almeda *et al.*, 2018; **Paper IV**), this group should be of particular interest in future ecotoxicological studies in marine environments.

7.2 Future studies of chemical mixtures in the marine environment

As described in previous sections, the use of open-loop scrubbers are becoming increasingly criticised due to their large contribution to contaminant loads in the environment (Ytreberg *et al.*, 2022) and the toxicity of their wastewater to zooplankton (Koski *et al.*, 2017; Picone *et al.*, 2023). This means that many shipowners may turn to closed-loop scrubbers instead, which motivates further studies of the impact they could have on the marine environment. Under the current use, is has been concluded that that closed-loop scrubbers do not pose a risk to the environment in ports (Hermansson *et al.*, 2023), however, we lack information about how future increased use could impact the environment. Furthermore, every small contribution of contaminants to waterbodies, especially those that do not achieve good environmental and chemical status under the Water Framework Directive, should be considered a threat to the environment.

Based on the findings of this thesis, I can conclude that marine zooplankton already are affected by contaminant mixtures in some coastal areas. However, the marine chemical extracts that these finding were based on were collected during single occasions at each site, which means that we lack knowledge about the variability of these mixtures on a temporal scale. Levels of contaminants in marine surface waters can be affected by temporally variable factors such as precipitation and season (Smith and McLachlan, 2006; Packett *et al.*, 2009; Ademollo *et al.*, 2021). Furthermore, the sampling methodology only targeted a subset of all the contaminants that could have been present in these locations (polar, organic ones). Hence, a natural next step for these types of studies would be to use several types of solid phase extraction cartridges during samplings that each target chemicals with different properties, or to use the actual collected seawater in the experiments.

8. Acknowledgements

I would like to start by thanking my supervisors Ingela, Peter and Mats.

Ingela, I can't imagine there's a better supervisor out there! You have been an awesome support during these years. Thanks for all the smart and creative advice on my work, as well as for the numerous times you have given me a ride when I had crazy amounts of experiment material, all the laughs over coffee (or a good beer), and all the gardening tips :) It's truly the end of an era, which is exciting but also a little bit sad!

Peter, thanks for all the support during my experiments at Kristineberg! It has been very useful to have a zooplankton-guru like you as part of my project. Thanks for having the patience with the million nerdy zooplankton questions I have brought to you over the years, and for all the good times and laughs.

Mats, thanks for taking the time to teach me (at least a little) bioinformatics! I definitely had some larger ambitions at the beginning of my PhD but as everyone knows you never know where you will end up with certain projects. Anyway, I'm super grateful for all the advice and help you gave me during these last months.

I would also like to thank my examiner Malin for providing feedback on my ISP and always answering any questions I had about courses or credits quickly.

To all my co-authors, thanks for all the valuable input on the studies included in this thesis. You definitely helped me improve every study and manuscript. I would also like to thank all the funders and collaborators of the studies including FORMAS, the FRAM Centre for Future Chemical Risk Assessment and Management Strategies, Stiftelsen Birgit och Birger Wåhlströms Minnesfond för den bohuslänska havs- och insjömiljön, the Swedish Agency for Marine and Water Management, and Kustbevakningen.

There are some people that have been more involved in my projects over the years, and I would like to thank you a little extra. Jenny, I'm so grateful that you became part of our little group! Thanks for all the help with the experiments, I couldn't have done it without you! Also, thanks for all the after-work saunas and movie nights at Kristineberg and in Lysekil, for being my spider-knight in shining armour (always ready to assist when I had an unwelcome guest in my room), and for all the positive encouragement. Ida-Maja, thanks for all the guidance in the shipping-related studies. I'm so impressed with your engagement in making the industry better for the environment and all the work you do. Elisabeth, thanks for helping out in the lab and for being the GIS-support. I had a lot of fun being involved in your master project, good luck with the new job!

I have been lucky to have two working places during my PhD, Botanhuset in Gothenburg and Kristineberg marine research station (now Kristineberg Center) in Fiskebäckskil. I would like to thank my colleagues in both places for all the fun lunch and fika chats and after works during these years. You will be missed!

There are some people in Gothenburg that I would like to thank a little extra. Daniela and Frida, thanks for being the best office mates! Having two weirdos next to me to turn to for advice or just a random chat for half an hour has made the stressful times more bearable for sure. Have fun in the new house, I already have FOMO. Thanks also to doktorandrådet (Oskar, Niklas, Tobi, Maria) for all the good times and game nights! And to the ecotox gang for fun discussions and afterworks. A special thanks to Sven for helping with the randomest of things, and for taking care of literally everything at Botan.

I would also like to thank some people at Kristineberg a little extra. Special thanks to Lars, Bengt, and Rickard for dealing with all the thermoconstant rooms and lab stuff, and to the crew on Oscar von Sydow and Alice for the numerous zooplankton samplings, both in amazing and terrible weather. Thanks also to the zooplankton monitoring gang for nerdy talks about copepodite stages and random unknown larvae, and to Kerstin and Mia at IVL for encouragement and discussions about the importance of including the ecology part in ecotox.

Finally, I would like to thank my family.

Kerstin, tack för att du varit min nörd-kompis genom hela livet som på ett eller annat sätt nog bidragit till mitt naturintresse.

Emma, tack för att du förgyllde pluggtiden i Stockholm, tror inte det hade gått hälften så bra för oss om vi inte haft varandra!

Mamma och Pappa, tack för att ni inte klagade när jag valde att plugga marinbiologi av alla saker utan bara varit stolta och peppat genom åren. Det här doktorerandet var det nog inte många som såg komma, men var det några som gjorde det så var det säkert ni! Fredrika, bättre syster och supporter får man leta efter. Så tacksam för dig! <3

Sist men verkligen inte minst, Leon. Var ska man ens börja? Tack för alla timmar du lyssnat på långrandiga presentationer, för alla middagar du lagat när jag var trött efter jobbet, för att du aldrig klagat när jag vart borta på alla (ibland långa) jobbresor, och för att du peppat mig igenom de här nästan fem åren. Du är bäst, älskar dig.

9. References

Ademollo, N., F. Spataro, J. Rauseo, T. Pescatore, N. Fattorini, S. Valsecchi, S. Polesello and L. Patrolecco, 2021. Occurrence, distribution and pollution pattern of legacy and emerging organic pollutants in surface water of the Kongsfjorden (Svalbard, Norway): Environmental contamination, seasonal trend and climate change. Marine Pollution Bulletin, 163: 111900.

Allan, J.D., 1976. Life history patterns in zooplankton. The American Naturalist, 110(971): 165-180.

Almeda, R., S. Baca, C. Hyatt and E.J. Buskey, 2014a. Ingestion and sublethal effects of physically and chemically dispersed crude oil on marine planktonic copepods. Ecotoxicology, 23(6): 988-1003.

Almeda, R., C. Hyatt and E.J. Buskey, 2014b. Toxicity of dispersant corexit 9500a and crude oil to marine microzooplankton. Ecotoxicology and Environmental Safety, 106: 76-85.

Almeda, R., S. Cosgrove and E.J. Buskey, 2018. Oil spills and dispersants can cause the initiation of potentially harmful dinoflagellate blooms ("red tides"). Environmental Science and Technology, 52(10): 5718-5724.

Altenburger, R. and W.R. Greco, 2009. Extrapolation concepts for dealing with multiple contamination in environmental risk assessment. Integrated Environmental Assessment and Management, 5(1): 62-68.

Anderson, M., 2008. Permanova+ for primer: Guide to software and statistical methods. Primer-E Limited.

Bach, L. and I. Dahllöf, 2012. Local contamination in relation to population genetic diversity and resilience of an arctic marine amphipod. Aquatic Toxicology, 114-115: 58-66.

Backhaus, T. and M. Karlsson, 2014. Screening level mixture risk assessment of pharmaceuticals in stp effluents. Water research, 49: 157-165.

Beaugrand, G., K.M. Brander, J. Alistair Lindley, S. Souissi and P.C. Reid, 2003. Plankton effect on cod recruitment in the North Sea. Nature, 426(6967): 661-664.

Bekele, T.G., H. Zhao, Y. Wang, J. Jiang and F. Tan, 2018. Measurement and prediction of bioconcentration factors of organophosphate flame retardants in common carp (Cyprinus carpio). Ecotoxicology and Environmental Safety, 166: 270-276.

Belden, J.B., R.J. Gilliom and M.J. Lydy, 2007. How well can we predict the toxicity of pesticide mixtures to aquatic life? Integrated Environmental Assessment and Management, 3(3): 364-372.

Bell, J.L. and R.R. Hopcroft, 2008. Assessment of zooimage as a tool for the classification of zooplankton. Journal of Plankton Research, 30(12): 1351-1367.

Benfield, M.C., P. Grosjean, P.F. Culverhouse, X. Irigoien, M.E. Sieracki, A. Lopez-Urrutia, H.G. Dam, Q. Hu, C.S. Davis and A. Hansen, 2007. Rapid: Research on automated plankton identification. Oceanography, 20(2): 172-187.

Blanck, H., 2002. A critical review of procedures and approaches used for assessing pollution-induced community tolerance (pict) in biotic communities. Human and Ecological Risk Assessment, 8(5): 1003-1034.

Blanck, H., S.-Å. Wängberg and S. Molander, 1988. Pollution-induced community tolerance—a new ecotoxicological tool. In: Functional testing of aquatic biota for estimating hazards of chemicals. ASTM International.

Bliss, C., 1939. The toxicity of poisons applied jointly. Annals of Applied Biology, 26(3): 585-615.

Bopp, S.K., R. Barouki, W. Brack, S. Dalla Costa, J.-L.C. Dorne, P.E. Drakvik, M. Faust, T.K. Karjalainen, S. Kephalopoulos and J. van Klaveren, 2018. Current EU research activities on combined exposure to multiple chemicals. Environment International, 120: 544-562.

Bopp, S.K., A. Kienzler, A.-N. Richarz, S.C. van der Linden, A. Paini, N. Parissis and A.P. Worth, 2019. Regulatory assessment and risk management of chemical mixtures: Challenges and ways forward. Critical Reviews in Toxicology, 49(2): 174-189.

Borgå, K., G. Gabrielsen and J. Skaare, 2002. Differences in contamination load between pelagic and sympagic invertebrates in the arctic marginal ice zone: Influence of habitat, diet and geography. Marine Ecology Progress Series, 235: 157-169.

Brack, W., V. Dulio, M. Ågerstrand, I. Allan, R. Altenburger, M. Brinkmann, D. Bunke, R.M. Burgess, I. Cousins and B.I. Escher, 2017. Towards the review of the European union Water Framework Directive: Recommendations for more efficient assessment and management of chemical contamination in european surface water resources. Science of the Total Environment, 576: 720-737.

Bradbury, S.P., C.L. Russom, G.T. Ankley, T.W. Schultz and J.D. Walker, 2003. Overview of data and conceptual approaches for derivation of quantitative structure-activity relationships for ecotoxicological effects of organic chemicals. Environmental Toxicology and Chemistry: An International Journal, 22(8): 1789-1798.

Bray, J.R. and J.T. Curtis, 1957. An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs, 27(4): 326-349.

Buhaug O, H. Fløgstad, T Bakke, 2006. MARULS WP3: washwater criteria for seawater exhaust gas-SOx scrubbers. MARINTEC REPORT in: Teuchies J, Cox TJ, Van Itterbeeck K, Meysman FJ, Blust R. The impact of scrubber discharge on the water quality in estuaries and ports. Environmental Sciences Europe 2020; 32: 1-11.

Calbet, A., F. Carlotti and R. Gaudy, 2007. The feeding ecology of the copepod Centropages typicus (Kröyer). Progress in Oceanography, 72(2-3): 137-150.

Calbet, A. and M.R. Landry, 2004. Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnology and Oceanography, 49(1): 51-57.

Calbet, A. and E. Saiz, 2005. The ciliate-copepod link in marine ecosystems. Aquatic Microbial Ecology, 38(2): 157-167.

Cedergreen, N., 2014. Quantifying synergy: A systematic review of mixture toxicity studies within environmental toxicology. PloS One, 9(5): e96580.

Cedergreen, N., A.M. Christensen, A. Kamper, P. Kudsk, S.K. Mathiassen, J.C. Streibig and H. Sorensen, 2008. A review of independent action compared to concentration addition as reference models for mixtures of compounds with different molecular target sites. Environmental Toxicology and Chemistry, 27(7): 1621-1632.

Clarke, K.R., R. Gorley, P.J. Somerfield and R. Warwick, 2014. Change in marine communities: An approach to statistical analysis and interpretation.

Cripps, G.C. and J. Shears, 1997. The fate in the marine environment of a minor diesel fuel spill from an antarctic research station. Environmental Monitoring and Assessment, 46(3): 221-232.

Culverhouse, P.F., R. Williams, B. Reguera, V. Herry and S. González-Gil, 2003. Do experts make mistakes? A comparison of human and machine indentification of dinoflagellates. Marine Ecology Progress Series, 247: 17-25.

da Silva Ferreira, D., V.R. Esperandim, M.P.A. Toldo, J. Saraiva, W.R. Cunha and S. De Albuquerque, 2010. Trypanocidal activity and acute toxicity assessment of triterpene acids. Parasitology Research, 106: 985-989.

Dafforn, K.A., J.A. Lewis and E.L. Johnston, 2011. Antifouling strategies: History and regulation, ecological impacts and mitigation. Marine Pollution Bulletin, 62(3): 453-465.
de LG Solbé, J., B. Buyle, W. Guhl, T. Hutchinson, R. Laenge, U. Mark, R. Munk and N. Scholz, 1993. Developing hazard identification for the aquatic environment. Science of the Total Environment, 134: 47-61.
Díaz, P.A., G. Álvarez, D. Varela, I. Pérez-Santos, M. Díaz, C. Molinet, M. Seguel, A. Aguilera-Belmonte, L. Guzmán and E. Uribe, 2019. Impacts of harmful algal blooms on the aquaculture industry: Chile as a case study. Perspectives in Phycology, 6(1-2): 39-50.
Dierssen, H., G.B. McManus, A. Chlus, D. Qiu, B.-C. Gao and S. Lin, 2015. Space station image captures a red tide ciliate bloom at high spectral and spatial resolution. Proceedings of the National Academy of Sciences, 112(48): 14783-14787.
Directive 2013/39/EU of the European parliament and of the council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. http://eurlex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013L0039&from=EN
DNV-GL. Alternative Fuels Insight: Scrubber Statistics. [Accessed on 2023-03-23] https://afi.dnv.com/Statistics?repId=2s
Dorrell, R.G. and C.J. Howe, 2015. Integration of plastids with their hosts: Lessons learned from dinoflagellates. Proceedings of the National Academy of Sciences, 112(33): 10247-10254.
Downing, A.L., B.L. Brown and M.A. Leibold, 2014. Multiple diversity–stability mechanisms enhance population and community stability in aquatic food webs. Ecology, 95(1): 173-184.
ECHA, 2008. Characterisation of dose [concentration]-response for environment (in: Guidance on information requirements and chemical safety assessment). Available at: https://echa.europa.eu/documents/10162/13632/information_requirements_r10_en.pdf/bb902be7-a503-4ab7-9036-d866b8ddce69
ECHA, 2016. Guidance on information requirements and chemical safety assessment part e: Risk characterisation. Available at: https://echa.europa.eu/documents/10162/13632/information_requirements_part_e_en.pdf/1da6cadd-895a-46f0-884b-00307c0438fd
ECHA, 2017. Guidance on the biocidal products regulation, volume iv environment - assessment and evaluation (parts b + c), version 2.0. Available at: https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation.
ECHA, 2022. Guidance on the biocidal products regulation volume iv: Environment part a: Information requirements, version 1.3, march 2022. Available at: https://echa.europa.eu/guidance-documents/guidance-on-biocides-legislation.
EFSA-Scientific-Committee, S.J. More, V. Bampidis, D. Benford, S.H. Bennekou, C. Bragard, T.I. Halldorsson, A.F. Hernández-Jerez, K. Koutsoumanis and H. Naegeli, 2019. Guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. EFSA journal, 17(3): e05634.
Egardt, J., M.M. Larsen, P. Lassen and I. Dahllöf, 2018. Release of pahs and heavy metals in coastal environments linked to leisure boats. Marine Pollution Bulletin, 127: 664-671.
El Zrelli, R., P. Courjault-Radé, L. Rabaoui, S. Castet, S. Michel and N. Bejaoui, 2015. Heavy metal contamination and ecological risk assessment in the surface sediments of the coastal area surrounding the industrial complex of gabes city, gulf of gabes, se tunisia. Marine Pollution Bulletin, 101(2): 922-929.

Elbrecht, V. and F. Leese, 2015. Can DNA-based ecosystem assessments quantify species abundance? Testing primer bias and biomass—sequence relationships with an innovative metabarcoding protocol. PloS One, 10(7): e0130324.
Elliott, D.T. and K.W. Tang, 2009. Simple staining method for differentiating live and dead marine zooplankton in field samples. Limnology and Oceanography-Methods, 7(8): 585-594.
Enckell, P., 1980. Kräftdjur. Signum i Lund, Odense
Escher, B.I., C. van Daele, M. Dutt, J.Y. Tang and R. Altenburger, 2013. Most oxidative stress response in water samples comes from unknown chemicals: The need for effect-based water quality trigger values. Environmental Science and Technology, 47(13): 7002-7011.
European Commission, 2020. COM(2020) 667 final. Communication from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions. Brussels, 14.10.2020 https://ec.europa.eu/environment/pdf/chemicals/2020/10/Strategy.pdf
European Commission, 2011. Common Implementation Strategy for the Water Framework Directive (2000/60/EC) Guidance Document No.27-Technical Guidance for Deriving Environmental Quality Standards. http://dx.doi.org/10.2779/43816
Evans, R.M., M. Scholze and A. Kortenkamp, 2015. Examining the feasibility of mixture risk assessment: A case study using a tiered approach with data of 67 pesticides from the joint fao/who meeting on pesticide residues (jmpr). Food and Chemical Toxicology, 84: 260-269.
Foissner, W. and D.L. Hawksworth, 2009. Protist diversity and geographical distribution. Springer Science & Business Media.
Forget-Leray, J., I. Landriau, C. Minier and F. Leboulenger, 2005. Impact of endocrine toxicants on survival, development, and reproduction of the estuarine copepod Eurytemora affinis (Poppe). Ecotoxicology and Environmental Safety, 60(3): 288-294.
Gismervik, I., 2006. Top-down impact by copepods on ciliate numbers and persistence depends on copepod and ciliate species composition. Journal of Plankton Research, 28(5): 499-507.
Gissel Nielsen, T and P.J. Hansen, 1999. Miljø- og Energiministeriet Danmarks Miljøundersøgelser. Dyreplankton i danske farvande. Scanprint as, Århus
Golbamaki, A., A. Cassano, A. Lombardo, Y. Moggio, M. Colafranceschi and E. Benfenati, 2014. Comparison of in silico models for prediction of Daphnia magna acute toxicity. SAR and QSAR in Environmental Research, 25(8): 673-694.
Gorbi, G., M. Invidia, F. Savorelli, O. Faraponova, E. Giacco, M. Cigar, I. Buttino, T. Leoni, E. Prato and I. Lacchetti, 2012. Standardized methods for acute and semichronic toxicity tests with the copepod Acartia tonsa. Environmental Toxicology and Chemistry, 31(9): 2023-2028.
Greco, W.R., G. Bravo and J.C. Parsons, 1995. The search for synergy: A critical review from a response surface perspective. Pharmacological Reviews, 47(2): 331-385.
Grosjean, P. and K. Denis, 2007. Zoo/PhytoImage version 1.2-0 Computer-Assisted Plankton Image Analysis user's manual. https://www.sciviews.org/software/zooimage/
Guillou, L., D. Bachar, S. Audic, D. Bass, C. Berney, L. Bittner, C. Boutte, G. Burgaud, C. de Vargas and J. Decelle, 2012. The protist ribosomal reference database (pr2): A catalog of unicellular eukaryote small sub-unit rrna sequences with curated taxonomy. Nucleic Acids Research, 41(D1): D597-D604.
Gustavsson, B.M., J. Magnér, B.C. Almroth, M.K. Eriksson, J. Sturve and T. Backhaus, 2017a. Chemical monitoring of Swedish coastal waters indicates common

exceedances of environmental thresholds, both for individual substances as well as their mixtures. Marine Pollution Bulletin, 122(1-2): 409-419.
Gustavsson, M., J. Kreuger, M. Bundschuh and T. Backhaus, 2017b. Pesticide mixtures in the swedish streams: Environmental risks, contributions of individual compounds and consequences of single-substance oriented risk mitigation. Science of the Total Environment, 598: 973-983.
Ha, H., K. Park, G. Kang and S. Lee, 2019. QSAR study using acute toxicity of Daphnia magna and Hyalella azteca through exposure to polycyclic aromatic hydrocarbons (PAHs). Ecotoxicology, 28(3): 333-342.
Hallanger, I.G., A. Ruus, D. Herzke, N.A. Warner, A. Evenset, E.S. Heimstad, G.W. Gabrielsen and K. Borgå, 2011. Influence of season, location, and feeding strategy on bioaccumulation of halogenated organic contaminants in arctic marine zooplankton. Environmental Toxicology and Chemistry, 30(1): 77-87.
Hassellöv, I.-M., D.R. Turner, A. Lauer and J.J. Corbett, 2013. Shipping contributes to ocean acidification. Geophysical Research Letters, 40(11): 2731-2736.
Hattenrath-Lehmann, T.K., M.A. Marcoval, D.L. Berry, S. Fire, Z. Wang, S.L. Morton and C.J. Gobler, 2013. The emergence of Dinophysis acuminata blooms and dsp toxins in shellfish in new york waters. Harmful Algae, 26: 33-44.
HAV, Havs och vattenmyndigheten, 2022. Uppdrag att komplettera tidigare redovisning om utsläpp av tvättvatten från skrubbrar på fartyg. [Accessed on 2023-04-13] https://www.havochvatten.se/arkiv/aktuellt/2022-06-29-forslag-om-forbud-av-tvattvatten.html
Hebbar, A.A. and I.G. Dharmasiri, 2022. Management of marine oil spills: A case study of the wakashio oil spill in mauritius using a lens-actor-focus conceptual framework. Ocean and Coastal Management, 221: 106103.
Hébert, M.-P., B.E. Beisner and R. Maranger, 2016. Linking zooplankton communities to ecosystem functioning: Toward an effect-trait framework. Journal of Plankton Research, 39(1): 3-12.
HELCOM, 2021. HELCOM report on Shipping accidents in the Baltic Sea 2020
Hellstrøm, K.C., 2017. Weathering Properties and Toxicity of Marine Fuel Oils. Summary Report. Report no. OC2017-A124. SINTEF Ocean A/S.
Herfort, L., T.D. Peterson, V. Campbell, S. Futrell and P. Zuber, 2011. Myrionecta rubra (mesodinium rubrum) bloom initiation in the columbia river estuary. Estuarine, Coastal and Shelf Science, 95(4): 440-446.
Hermansson, A.L., I.-M. Hassellöv, J.-P. Jalkanen and E. Ytreberg, 2023. Cumulative environmental risk assessment of metals and polycyclic aromatic hydrocarbons from ship activities in ports. Marine Pollution Bulletin, 189: 114805.
Hermansson, A.L., I.-M. Hassellöv, J. Moldanová and E. Ytreberg, 2021. Comparing emissions of polyaromatic hydrocarbons and metals from marine fuels and scrubbers. Transportation Research Part D: Transport and Environment, 97: 102912.
Hjorth, M., V.E. Forbes and I. Dahllöf, 2008. Plankton stress responses from pah exposure and nutrient enrichment. Marine Ecology Progress Series, 363: 121-130.
Hooper, D.U., F.S. Chapin, J.J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J.H. Lawton, D.M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setala, A.J. Symstad, J. Vandermeer and D.A. Wardle, 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs, 75(1): 3-35.
Hooper, M.J., G.T. Ankley, D.A. Cristol, L.A. Maryoung, P.D. Noyes and K.E. Pinkerton, 2013. Interactions between chemical and climate stressors: A role for mechanistic toxicology in assessing climate change risks. Environmental Toxicology and Chemistry, 32(1): 32-48.

IMO, 2008. Marpol 2008 amendments to the annex of protocol of 1997 to amend the international convention for the prevention of pollution from ships, 1973, as modified by the protocol of 1978. (Revised MARPOL Annex VI) (Resolution MEPC.176(58)).
ITOPF, 2023. Oil tanker spill statistics 2022. ITOPF, London, UK
Jacobi, H., G. Leier and I. Witte, 1996. Correlation of the lipophilicity of xenobiotics with their synergistic effects on DNA synthesis in human fibroblasts. Chemosphere, 32(7): 1251-1259.
Jartun, M. and A. Pettersen, 2010. Contaminants in urban runoff to Norwegian fjords. Journal of Soils and Sediments, 10(2): 155-161.
Jonsson, P.R. and P. Tiselius, 1990. Feeding behaviour, prey detection and capture efficiency of the copepod Acartia tonsa feeding on planktonic ciliates. Marine Ecology Progress Series: 35-44.
Jönander, C. and I. Dahllöf, 2020. Short and long-term effects of low-sulphur fuels on marine zooplankton communities. Aquatic Toxicology, 227: 105592.
Jönander, C., T. Backhaus and I. Dahllöf, 2022. Single substance and mixture toxicity of dibutyl-phthalate and sodium dodecyl sulphate to marine zooplankton. Ecotoxicology and Environmental Safety, 234, 113406.
Kalani, K., V. Kushwaha, P. Sharma, R. Verma, M. Srivastava, F. Khan, P.K. Murthy and S.K. Srivastava, 2014. In vitro, in silico and in vivo studies of ursolic acid as an anti-filarial agent. Plos One, 9(11).
Kashian, D.R., R.E. Zuellig, K.A. Mitchell and W.H. Clements, 2007. The cost of tolerance: Sensitivity of stream benthic communities to uv-b and metals. Ecological Applications, 17(2): 365-375.
Kass, M., J. Thomas, S. Sluder and T. Theiss, 2019. A guide to fuel, lubricant, and engine concerns relative to the imo 2020 fuel oil sulfur reduction mandate. Oak Ridge National Lab.(ORNL), Oak Ridge, TN (United States).
Keeling, P.J., 2010. The endosymbiotic origin, diversification and fate of plastids. Philosophical Transactions of the Royal Society B: Biological Sciences, 365(1541): 729-748.
Kienzler, A., S.K. Bopp, S. van der Linden, E. Berggren and A. Worth, 2016. Regulatory assessment of chemical mixtures: Requirements, current approaches and future perspectives. Regulatory Toxicology and Pharmacology, 80: 321-334.
Kiørboe, T. and M. Sabatini, 1994. Reproductive and life cycle strategies in egg-carrying cyclopoid and free-spawning calanoid copepods. Journal of Plankton Research, 16(10): 1353-1366.
Kortenkamp, A., T. Backhaus and M. Faust, 2009. State of the art report on mixture toxicity. Contract, 70307(2007485103): 94-103.
Koski, M., C. Stedmon and S. Trapp, 2017. Ecological effects of scrubber water discharge on coastal plankton: Potential synergistic effects of contaminants reduce survival and feeding of the copepod Acartia tonsa. Marine Environmental Research, 129: 374-385.
Lambert, F.N., D.N. Vivian, S. Raimondo, C.T. Tebes-Stevens and M.G. Barron, 2022. Relationships between aquatic toxicity, chemical hydrophobicity, and mode of action: Log Kow revisited. Archives of Environmental Contamination and Toxicology, 83(4): 326-338.
Larink, O. and W. Westheide, 2011. Coastal plankton, 2nd edition. Dr. Friedrich Pfeil, München
Li, X.-D., X.-Y. Wang, M.-E. Xu, Y. Jiang, T. Yan and X.-C. Wang, 2020. Progress on the usage of the rotifer Brachionus plicatilis in marine ecotoxicology: A review. Aquatic Toxicology, 229: 105678.

Lindeque, P.K., H.E. Parry, R.A. Harmer, P.J. Somerfield and A. Atkinson, 2013. Next generation sequencing reveals the hidden diversity of zooplankton assemblages. PloS One, 8(11): e81327.
Litchman, E., M.D. Ohman and T. Kiørboe, 2013. Trait-based approaches to zooplankton communities. Journal of Plankton Research, 35(3): 473-484.
Loos, R., S. Tavazzi, B. Paracchini, E. Canuti and C. Weissteiner, 2013. Analysis of polar organic contaminants in surface water of the northern adriatic sea by solid-phase extraction followed by ultrahigh-pressure liquid chromatography–qtrap® ms using a hybrid triple-quadrupole linear ion trap instrument. Analytical and Bioanalytical Chemistry, 405: 5875-5885.
Löder, M.G., C. Meunier, K.H. Wiltshire, M. Boersma and N. Aberle, 2011. The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at helgoland roads, north sea. Marine Biology, 158(7): 1551-1580.
Löewe, S. and H. Muischnek, 1926. Über kombinationswirkungen mitteilung: Hilfsmittel der fragestellung. Naunyn Schmiedeberg's Archives of Pharmacology, 114: 313-326.
Maar, M., T.G. Nielsen, S. Gooding, K. Tonnesson, P. Tiselius, S. Zervoudaki, E. Christou, A. Sell and K. Richardson, 2004. Trophodynamic function of copepods, appendicularians and protozooplankton in the late summer zooplankton community in the Skagerrak. Marine Biology, 144(5): 917-933.
Madoni, P. and M.G. Romeo, 2006. Acute toxicity of heavy metals towards freshwater ciliated protists. Environmental Pollution, 141(1): 1-7.
Mafra Jr, L., P. Nolli, L. Mota, C. Domit, M. Soeth, L. Luz, B. Sobrinho, J. Leal and M. Di Domenico, 2019. Multi-species okadaic acid contamination and human poisoning during a massive bloom of Dinophysis acuminata complex in southern Brazil. Harmful Algae, 89: 101662.
Magnusson, K., J.-P. Jalkanen, L. Johansson, V. Smailys, P. Telemo and H. Winnes, 2018. Risk assessment of bilge water discharges in two baltic shipping lanes. Marine Pollution Bulletin, 126: 575-584.
Martin, J.L., I. Santi, P. Pitta, U. John and N. Gypens, 2022. towards quantitative metabarcoding of eukaryotic plankton: An approach to improve 18S rRNA gene copy number bias. Metabarcoding and Metagenomics, 6: e85794.
Martin, O., M. Scholze, S. Ermler, J. McPhie, S.K. Bopp, A. Kienzler, N. Parissis and A. Kortenkamp, 2021. Ten years of research on synergisms and antagonisms in chemical mixtures: A systematic review and quantitative reappraisal of mixture studies. Environment International, 146: 106206.
Melnikov, F., J. Kostal, A. Voutchkova-Kostal, J.B. Zimmerman and P.T. Anastas, 2016. Assessment of predictive models for estimating the acute aquatic toxicity of organic chemicals. Green Chemistry, 18(16): 4432-4445.
Meunier, C.L., M. Boersma, K.H. Wiltshire and A.M. Malzahn, 2016. Zooplankton eat what they need: Copepod selective feeding and potential consequences for marine systems. Oikos, 125(1): 50-58.
Mohrbeck, I., M.J. Raupach, P. Martínez Arbizu, T. Knebelsberger and S. Laakmann, 2015. High-throughput sequencing—the key to rapid biodiversity assessment of marine metazoa? PloS One, 10(10): e0140342.
Möllmann, C., B. Müller-Karulis, G. Kornilovs and M.A. St John, 2008. Effects of climate and overfishing on zooplankton dynamics and ecosystem structure: Regime shifts, trophic cascade, and feedback loops in a simple ecosystem. ICES Journal of Marine Science, 65(3): 302-310.
Nepia, The North of England Protecting and Indemnity Association Limited (2021). No Scrubs: More Ports Declare Ban on EGCS Discharges *Update*. [Accessed on 2023-

04-13] https://www.nepia.com/industry-news/no-scrubs-more-ports-declare-banon-egcs-discharges-update/
Netzeva, T.I., M. Pavan and A.P. Worth, 2008. Review of (quantitative) structure–activity relationships for acute aquatic toxicity. QSAR & Combinatorial Science, 27(1): 77-90.
Nichols, R.V., C. Vollmers, L.A. Newsom, Y. Wang, P.D. Heintzman, M. Leighton, R.E. Green and B. Shapiro, 2018. Minimizing polymerase biases in metabarcoding. Molecular Ecology Resources, 18(5): 927-939.
Not, F., J. del Campo, V. Balagué, C. de Vargas and R. Massana, 2009. New insights into the diversity of marine picoeukaryotes. PloS One, 4(9): e7143.
Nunes, B.S., F.D. Carvalho, L.M. Guilhermino and G. Van Stappen, 2006. Use of the genus Artemia in ecotoxicity testing. Environmental Pollution, 144(2): 453-462.
Nørgaard, K.B. and N. Cedergreen, 2010. Pesticide cocktails can interact synergistically on aquatic crustaceans. Environmental Science and Pollution Research, 17(4): 957-967.
Ojemaye, C.Y. and L. Petrik, 2019. Pharmaceuticals in the marine environment: A review. Environmental Reviews, 27(2): 151-165.
Packett, R., C. Dougall, K. Rohde and R. Noble, 2009. Agricultural lands are hot-spots for annual runoff polluting the southern great barrier reef lagoon. Marine Pollution Bulletin, 58(7): 976-986.
Payne, S.J., C.K. King, L.M. Zamora and P. Virtue, 2014. Temporal changes in the sensitivity of coastal antarctic zooplankton communities to diesel fuel: A comparison between single-and multi-species toxicity tests. Environmental Toxicology and Chemistry, 33(4): 882-890.
Persson, L., B.M. Carney Almroth, C.D. Collins, S. Cornell, C.A. de Wit, M.L. Diamond, P. Fantke, M. Hassellöv, M. MacLeod and M.W. Ryberg, 2022. Outside the safe operating space of the planetary boundary for novel entities. Environmental Science and Technology, 56(3): 1510-1521.
Picone, M., M. Russo, G.G. Distefano, M. Baccichet, D. Marchetto, A.V. Ghirardini, A.L. Hermansson, M. Petrovic, M. Gros and E. Garcia, 2023. Impacts of exhaust gas cleaning systems (egcs) discharge waters on planktonic biological indicators. Marine Pollution Bulletin, 190: 114846.
Reddy, C.M. and J.G. Quinn, 2001. The north cape oil spill: Hydrocarbons in Rhode island coastal waters and point judith pond. Marine Environmental Research, 52(5): 445-461.
Reuschenbach, P., M. Silvani, M. Dammann, D. Warnecke and T. Knacker, 2008. Ecosar model performance with a large test set of industrial chemicals. Chemosphere, 71(10): 1986-1995.
Rumschlag, S.L., M.B. Mahon, J.T. Hoverman, T.R. Raffel, H.J. Carrick, P.J. Hudson and J.R. Rohr, 2020. Consistent effects of pesticides on community structure and ecosystem function in freshwater systems. Nature Communications, 11(1): 6333.
Saage, A., O. Vadstein and U. Sommer, 2009. Feeding behaviour of adult Centropages hamatus (Copepoda, Calanoida): Functional response and selective feeding experiments. Journal of Sea Research, 62(1): 16-21.
Schnepf, E. and M. Elbrächter, 1992. Nutritional strategies in dinoflagellates: A review with emphasis on cell biological aspects. European Journal of Protistology, 28(1): 3-24.
Schulze, T., M. Ahel, J. Ahlheim, S. Aït-Aïssa, F. Brion, C. Di Paolo, J. Froment, A.O. Hidasi, J. Hollender and H. Hollert, 2017. Assessment of a novel device for onsite integrative large-volume solid phase extraction of water samples to enable a comprehensive chemical and effect-based analysis. Science of the Total Environment, 581: 350-358.

Serrana, J.M., Y. Miyake, M. Gamboa and K. Watanabe, 2019. Comparison of DNA metabarcoding and morphological identification for stream macroinvertebrate biodiversity assessment and monitoring. Ecological Indicators, 101: 963-972.
Shanks, A.L., 2009. Pelagic larval duration and dispersal distance revisited. The Biological Bulletin, 216(3): 373-385.
Sieburth, J.M., V. Smetacek and J. Lenz, 1978. Pelagic ecosystem structure: Heterotrophic compartments of the plankton and their relationship to plankton size fractions 1. Limnology and oceanography, 23(6): 1256-1263.
Silva, E., N. Rajapakse and A. Kortenkamp, 2002. Something from "nothing"− eight weak estrogenic chemicals combined at concentrations below NOECcs produce significant mixture effects. Environmental Science and Technology, 36(8): 1751-1756.
Smith, K.E. and M.S. McLachlan, 2006. Concentrations and partitioning of polychlorinated biphenyls in the surface waters of the southern Baltic Sea—seasonal effects. Environmental Toxicology and Chemistry: An International Journal, 25(10): 2569-2575.
Solaun, O., J.G. Rodríguez, I. Menchaca, E. López-García, E. Martínez, B. Zonja, C. Postigo, M.L. de Alda, D. Barceló and Á. Borja, 2020. Contaminants of emerging concern in the basque coast (in Spain): Occurrence and risk assessment for a better monitoring and management decisions. Science of The Total Environment: 142765.
Stoeck, T., D. Bass, M. Nebel, R. Christen, M.D. Jones, H.W. Breiner and T.A. Richards, 2010. Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Molecular Ecology, 19: 21-31.
Stoecker, D., M. Silver, A. Michaels and L. Davis, 1988. Obligate mixotrophy in Laboea strobila, a ciliate which retains chloroplasts. Marine Biology, 99: 415-423.
Stoecker, D.K., A. Taniguchi and A.E. Michaels, 1989. Abundance of autotrophic, mixotrophic and heterotrophic planktonic ciliates in shelf and slope waters. Marine Ecology Progress Series. Oldendorf, 50(3): 241-254.
Straub, D. and A. Peltzer, 2019. Nf-core/ampliseq. London: Zenodo.
Swadling, K.M. and N.H. Marcus, 1994. Selectivity in the natural diets of Acartia tonsa Dana (Copepoda: Calanoida): Comparison of juveniles and adults. Journal of Experimental Marine Biology and Ecology, 181(1): 91-103.
Swedish coast guard (2022). Kustbevakningens insatser avslutade efter oljeutsläppet vid Öckerö. [Accessed on 2023-03-24]. https://www.kustbevakningen.se/nyheter/kustbevakningens-insatser-avslutade-efter-oljeutslappet-vid-ockero/
Syberg, K., T. Backhaus, G. Banta, P. Bruce, M. Gustavsson, W.R. Munns Jr, R. Rämö, H. Selck and J.S. Gunnarsson, 2017. Toward a conceptual approach for assessing risks from chemical mixtures and other stressors to coastal ecosystem services. Integrated Environmental Assessment and Management, 13(2): 376-386.
Syberg, K., A. Elleby, H. Pedersen, N. Cedergreen and V.E. Forbes, 2008. Mixture toxicity of three toxicants with similar and dissimilar modes of action to Daphnia magna. Ecotoxicology and Environmental Safety, 69(3): 428-436.
Takishita, K., K. Kolke, T. Maruyama and T. Ogata, 2002. Molecular evidence for plastid robbery (kleptoplastidy) in Dinophysis, a dinoflagellate causing diarrhetic shellfish poisoning. Protist, 153(3): 293-302.
Tansel, B., C. Fuentes, M. Sanchez, K. Predoi and M. Acevedo, 2011. Persistence profile of polyaromatic hydrocarbons in shallow and deep gulf waters and sediments: Effect of water temperature and sediment–water partitioning characteristics. Marine Pollution Bulletin, 62(12): 2659-2665.

Teuchies, J., T.J. Cox, K. Van Itterbeeck, F.J. Meysman and R. Blust, 2020. The impact of scrubber discharge on the water quality in estuaries and ports. Environmental Sciences Europe, 32(1): 1-11.
Thor, P., M.E. Granberg, H. Winnes and K. Magnusson, 2021. Severe toxic effects on pelagic copepods from maritime exhaust gas scrubber effluents. Environmental Science and Technology, 55(9): 5826-5835.
Tornero, V. and G. Hanke, 2016. Chemical contaminants entering the marine environment from sea-based sources: A review with a focus on european seas. Marine Pollution Bulletin, 112(1-2): 17-38.
Tournadre, J., 2014. Anthropogenic pressure on the open ocean: The growth of ship traffic revealed by altimeter data analysis. Geophysical Research Letters, 41(22): 7924-7932.
US EPA, 2022. Operation manual for the ecological structure-activity relationship model (ECOSAR) Class Program. Available from: https://www.epa.gov/tsca-screening-tools/ecological-structure-activity-relationships-program-ecosar-operation-manual
Ushakov, S., D. Stenersen, P.M. Einang and T.Ø. Ask, 2020. Meeting future emission regulation at sea by combining low-pressure egr and seawater scrubbing. Journal of Marine Science and Technology, 25(2): 482-497.
Utermöhl, H., 1958. Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 tabelle und 15 abbildungen im text und auf 1 tafel. Internationale Vereinigung für theoretische und angewandte Limnologie: Mitteilungen, 9(1): 1-38.
Uusitalo, L., J.A. Fernandes, E. Bachiller, S. Tasala and M. Lehtiniemi, 2016. Semi-automated classification method addressing Marine Strategy Framework Directive (MSFD) zooplankton indicators. Ecological Indicators, 71: 398-405.
Vanryckeghem, F., S. Huysman, H. Van Langenhove, L. Vanhaecke and K. Demeestere, 2019. Multi-residue quantification and screening of emerging organic micropollutants in the belgian part of the north sea by use of speedisk extraction and q-orbitrap hrms. Marine pollution bulletin, 142: 350-360.
Vedachalam, S., N. Baquerizo and A.K. Dalai, 2022. Review on impacts of low sulfur regulations on marine fuels and compliance options. Fuel, 310: 122243.
VISS (Vatteninformationssystem Sverige), 2023a. Status classification of Askeröfjorden for the second cycle 2016-2021. [Accessed on 2023-03-30]. Available at: https://viss.lansstyrelsen.se/Waters.aspx?waterMSCD=WA16499529
VISS (Vatteninformationssystem Sverige), 2023b. Status classification of Hake fjord for the second cycle 2016-2021. [Accessed on 2023-03-30]. Available at: https://viss.lansstyrelsen.se/Waters.aspx?waterMSCD=WA55040263
VISS (Vatteninformationssystem Sverige), 2023c. Status classification of Askeröfjorden for the second cycle 2016-2021. [Accessed on 2023-03-30]. Available at: https://viss.lansstyrelsen.se/Waters.aspx?waterMSCD=WA69972288
Walter, T.C.; Boxshall, G. (2023). World of Copepods Database. [Accessed on 2023-03-08] https://www.marinespecies.org/copepoda
Wang, Z., G.W. Walker, D.C. Muir and K. Nagatani-Yoshida, 2020. Toward a global understanding of chemical pollution: A first comprehensive analysis of national and regional chemical inventories. Environmental Science and Technology, 54(5): 2575-2584.
Winebrake, J.J., J. Corbett, E. Green, A. Lauer and V. Eyring, 2009. Mitigating the health impacts of pollution from oceangoing shipping: An assessment of low-sulfur fuel mandates. Environmental Science and Technology, 43(13): 4776–4782
Worm, B. and J.E. Duffy, 2003. Biodiversity, productivity and stability in real food webs. Trends in Ecology and Evolution, 18(12): 628-632.

Yanagihara, M., K. Hiki and Y. Iwasaki, 2022. Can chemical toxicity in saltwater be predicted from toxicity in freshwater? A comprehensive evaluation using species sensitivity distributions. Environmental Toxicology and Chemistry, 41(8): 2021-2027.
Yarimizu, K., S. Sildever, Y. Hamamoto, S. Tazawa, H. Oikawa, H. Yamaguchi, L. Basti, J.I. Mardones, J. Paredes-Mella and S. Nagai, 2021. Development of an absolute quantification method for ribosomal RNA gene copy numbers per eukaryotic single cell by digital pcr. Harmful Algae, 103: 102008.
Ytreberg, E., K. Hansson, A.L. Hermansson, R. Parsmo, M. Lagerström, J.-P. Jalkanen and I.-M. Hassellöv, 2022. Metal and PAH loads from ships and boats, relative other sources, in the Baltic Sea. Marine Pollution Bulletin, 182: 113904.
Zhou, L., D. Fan, W. Yin, W. Gu, Z. Wang, J. Liu, Y. Xu, L. Shi, M. Liu and G. Ji, 2021. Comparison of seven in silico tools for evaluating of Daphnia and fish acute toxicity: Case study on Chinese priority controlled chemicals and new chemicals. BMC bioinformatics, 22(1): 1-31.