

Polycyclic aromatic compounds (PACs) in oily wastewater from shipping with a focus on scrubber water



Nordic Council
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1. Introduction

Environmental problems caused by oil spills from ships have been taken seriously and given considerable attention. Conscious action and decision-making by authorities has resulted in better vessels and improved operational procedures, and the number of marine oil spills has drastically decreased over the past decades (Statista 2023). Much less attention has been paid to the smaller but chronic spills of oily wastewater from shipping. The International Maritime Organisation (IMO) has accepted that ship-owners can continue to use heavy fuel oil (HFO) and still comply with the new sulphur reduction rules (MARPOL Annex VI) by installing scrubbers on-board the ships. In a scrubber, the combustion gases are washed with seawater or freshwater, depending on the design of the equipment. The contaminated wastewater contains, besides oil-related compounds, also metals, acidifying sulphur, eutrophication NO_x, and a range of combustion particles. It is discharged to the ocean, either without any pre-treatment (open-loop scrubbers) or with some treatment (closed-loop scrubbers). The use of scrubbers is an economically favourable alternative to switching to cleaner fuels, and since the number of ships equipped with scrubbers have increased dramatically over the past decade (from 242 in 2015 to 4737 in 2022), so have the volumes of discharged scrubber water. In a report from the International Council on Clean Transportation (ICCT) in 2021, the discharge of scrubber water from ships on a global basis was estimated at over 10 gigatons (Gt) annually (Osipova et al. 2021). Discharged volumes in the OSPAR region in 2020 were estimated at 622 million tonnes (OSPAR Commission 2022).

Scrubber water is continuously discharged when a ship is in operation and concentrations of the harmful components in the wastewater (scrubber water) will inevitably be chronically increased around shipping lanes. Among the most problematic contaminants in scrubber water are the polycyclic aromatic compounds (PACs). This is a group of chemicals comprising hundreds of compounds, among them the better known polycyclic aromatic hydrocarbons (PAHs), however chemical analysis of environmental samples is often limited to a set of 16 PAHs. The scientific literature on toxic effects of PACs on marine organisms is extensive but again there is a very limited set of PACs included in most of the studies (see Tables 6 and 7). Although knowledge on the mechanisms behind the toxicity of many PACs is still incomplete, we have today a fairly good overview of how effects are mediated in different organisms and which life stages are most sensitive (see section 7 *PAC fate and general toxicity*). Still, much of these scientific data are ignored in environmental risk assessments of PAC containing mixtures like scrubber water, and when determining threshold values for PAC concentrations in the environment. An example of the latter is the threshold values, or environmental

quality standards (EQS) of the EU Water Framework Directive, for which the last update was made in 2013. Many risk assessments today are based on the same standard protocols, using the same endpoints, the same species/life stages of species, the same exposure times, regardless of the contaminants being investigated. With this approach, there is a risk of overlooking the most sensitive endpoints when examining the toxicity of specific contaminants or mixtures of contaminants, and the consequences for marine ecosystems may be underestimated.

This report presents chemical and toxicity data on PACs detected in scrubber water. The chemical analysis includes an extended number of PACs, beyond the standard 16 PAHs, and information on the "fingerprint" of PACs that is indicative of this specific source is presented. Data show that when limiting analysis to the standard set of 16 PAHs, many other compounds are ignored. Some of them, e.g., the alkylated PAHs, occur in much higher concentrations than the "conventional" PAHs. The report presents results from an extensive review of research on PACs (mainly PAHs) toxicity on marine fish, invertebrates and algae show that effects occur at much lower concentrations than what is commonly detected using standardised protocols. All toxicity data are reported as values that can be applied in risk assessments, such as the lowest tested concentration that was significantly different from control (LOEC), the effective concentration inducing an effect in 50%, 20% or 10% of the exposed population (EC₅₀, EC₂₀, or EC₁₀). The information can be used in future attempts to estimate the impact of scrubber water and other oily wastewaters on the environment.

2. Aim of the report

The aim of the report is to contribute to an increased understanding of the impact of oily wastewaters on marine organisms and thereby make it easier for decision-makers to make decisions that ensure a good marine environment. Scrubber water from ships is selected as an example of oily wastewater that is discharged to the sea, and the focus is on PACs as these are identified as the most toxic oil fraction (Adams et al. 2014, Bornstein et al. 2014).

The report presents chemical data on PACs in scrubber water and a review of scientific data on toxicological effects of the detected compounds on organisms in a marine pelagic food web consisting of algae, zooplankton, and fish.

It is also discussed the need to include new scientific findings in risk assessments and not, as is often the case, solely base assessments on existing guidelines and threshold levels. The importance of putting the ecotoxicological data in an ecological context is also addressed. This is an often neglected step in risk assessments, but essential to understand the impacts that scrubber water can be expected to have on marine ecosystems.

Although emphasis of the report is on scrubber water, much of the information can be used for risk assessments of any oil or oil containing water that is discharged to an aquatic environment.

3. Background

3.1 Input of petroleum oil to the marine environment

Mineral oil is one of the most problematic groups of pollutants that marine organisms are confronting. It has been formed naturally over geological time spans and consists of mixtures of many thousands of organic compounds, and although many of them have the potential to be toxic, living organisms have developed physiological systems to cope with them (Goksoyr and Forlin 1992). However, the oil concentrations in the oceans today are many times higher than in pre-industrial times, and often higher than these defence systems can handle. This may lead to severe acute and chronic toxic effects.

In the 1970s and 80s there were numerous incidents of large accidental and intentional illegal oil spills at sea. Dramatic images of oil-covered birds and oil washed up on beaches were common in media. Names of some of the wrecked oil tankers, like Amoco Cadiz, Exxon Valdez and Prestige, are still familiar to many people because of the environmental disasters they caused. The attention has led to technical improvements of vessels, such as double hulls on ships, along with improved standard operating procedures. The efforts have had a positive effect on both accidental and illegal spills, and on a global basis the number has decreased from an average of 24.5 spills per year in the 1970s to 1.8 in the 2010s (Statista 2023). Instead, the most important input of mineral oil to the sea today comes from legal discharges of oil wastewaters from shipping and other maritime activities (Magnusson et al. 2018, Carpenter 2019, Lundberg 2021, HELCOM 2022). There is also ongoing leakage of stern tube oil from ships, and input of uncombusted fuel from ships and leisure boats (Johansson et al. 2020, Jalkanen et al. 2021). Until 2015 bilge water, the water that is gathered at the bottom of a ship and periodically discharged to the sea, was the most important source of oily wastewater from shipping, but since 2015 the IMO has allowed the use of scrubbers for scrubbing of the exhaust gases from ships running on heavy fuel oil (HFO), and this is now the dominating source of oil from shipping to the sea (Jalkanen et al. 2021, Ytreberg et al. 2022a). These releases of oily wastewater to the sea lead to increased base-line concentrations of oil components, primarily around ship lanes but over time also in larger sea areas surrounding the ship lanes. Oil from any future accidental or illegal oil spill will hence be added on top of these increased base-line concentrations.

3.2 Ship fuel and the IMO sulphur regulation

HFOs are the most widely used maritime fuels for vessels of all categories. HFOs are produced from heavier residuals from the crude oil refining processes and one of their main advantages over other marine fuels is that they are cheap. However, the downside is that they contain a wide spectrum of hazardous compounds and additional harmful components are formed when they are combusted. The most recognised hazardous components in the exhaust gases from combusted HFOs have been sulphur, with the acidifying effect it has when H_2SO_4 is formed in contact with the humidity in the air, and nitrogen oxides (NO_x), with their eutrophication properties (Stipa et al. 2007, Turner et al. 2018). The large quantities of particulate matter (PM) formed during combustion and present in exhaust gases, e.g., soot, ash, unburned organic carbon, sulphur particles, and elemental carbon (char), have also received considerable attention since many particles are of a size where they can cause damage to human lungs (Moldanova et al. 2009, Firlag et al. 2018).

To reduce these environmental threats, the IMO has passed regulations that originally involved a limitation of the sulphur content of ship fuel. Certain geographical areas regarded as particularly sensitive to acidification were designated as Sulphur Emission Control Areas (SECAs) where ship fuel, from the 1 January 2015, may contain a maximum concentration of 0.10% sulphur. The SECAs include the Baltic Sea, the North Sea and the English Channel, the United States, Canada, and the United States Caribbean Sea, and concerns all vessels over 300 gross tons (GT). A global limit for the maximum allowable sulphur content in ship fuel of 0.50% (from a previous maximum allowed level of 3.5%) was subsequently introduced on 1 January 2020 (MARPOL Annex VI, MEPC.280(70)). This regulated limitation of emission of sulphur in combustion gas is often referred to as the *IMO sulphur cap*. By switching to a low sulphur fuel also other hazardous components in the exhaust would be reduced. It is shown that emission factors of polycyclic aromatic hydrocarbons (PAHs, a group of PACs), oxy-PAHs and nitro-PAHs could be lowered by 97%, 77% and 73%, respectively, when using marine diesel oil (MDO) instead of HFO (Zhao et al. 2020). However, before the regulations came in place an alternative way of complying to the rules was presented. Instead of switching to low sulphur fuel, ships could install an onboard equipment, a *scrubber* (also called an Exhaust Gas Cleaning System, EGCS), where the exhaust gases are *scrubbed* with water, a process where much of the contaminants are captured in the wash water, or the *scrubber water* as it is also called.

There are two main varieties of scrubbers, open-loop and closed-loop. There are also hybrid scrubbers that may be operated in either an open or a closed mode. In open-loop scrubbers, the sea water is pumped onboard and sprayed on the exhaust gases. The wash water from the process, which contains most of the hazardous

components in the exhaust gases, is then released back to the sea. In closed-loop scrubbers, the exhaust gases are washed with freshwater in a recirculating system with a cleaning/treatment device. Even from the closed-loop scrubbers there is a discharge of the scrubber water to the sea, although the volumes are much smaller than from the open-loop ones. In a closed-loop scrubber, part of the content in the combustion gases is retained as sludge, which is temporarily stored on-board the ship but eventually deposited on land. Nevertheless, a substantial part of the hazardous components remain in the scrubber water that is released into the sea, and since scrubber water from closed-loop scrubbers has been recirculated, the pollutant concentrations are higher than in wash water from open-loop scrubbers (Thor et al. 2021). The sulphur in the exhaust gases has a pH reducing effect, which in an open-loop scrubber is regulated with the natural buffering capacity of the sea water used for scrubbing. In closed-loop scrubbers, certain chemicals, usually caustic soda (NaOH) or limestone (CaCO_3), are added to the water.

The choice to install scrubbers on-board ships instead of switching to cleaner fuel as a way to comply with the IMO sulphur cap is economically beneficial for the shipping companies. However, the economic gain has an environmental price. A consequence of the sulphur cap as it is formulated today is that a large part of the emissions of toxic compounds in the exhaust gases is transferred from the air to the sea. At the time when it was put in place, the focus of responsible authorities was exclusively on improving air quality and the terrestrial environment and no assessments of the consequences for marine ecosystems were made before this new source of pollution was introduced to the ocean (Hassellöv et al. 2020).

Scrubber water contains a considerable number of hazardous compounds. There are aliphatic and aromatic compounds from combusted and uncombusted HFO, combustion particles, residual lubrication oil from the engine, metals from the fuel oil itself, and metals released from the piping and the scrubber equipment during the scrubbing process (Kjølholt et al. 2012, Moldanova et al. 2011, (Hassellöv et al. 2020). The scrubbers remove up to 95% of the sulphur dioxide in the exhaust gas, which therefore ends up in the scrubber water instead and may contribute to local acidification of the seawater, particularly in heavily trafficked harbours and in brackish or estuarine water systems (Andersson et al. 2008, Turner et al. 2018, Yang et al. 2021). The scrubbing process is less effective at removing particulate matter (PM), measured as particles $\geq 2.5 \mu\text{m}$ ($\text{PM}_{2.5}$) for which only about 10% of the mass was reduced (Yang et al. 2021). The particles that are not greatly affected by the scrubber process consist mainly of sulphate, followed by organic carbon and elemental carbon.

3.3 Scrubber water and the marine environment

The first, and widely cited, reports on the impact the use of scrubbers have on the marine environment claimed that pollutant concentrations in discharged scrubber water are not alarmingly high and will rapidly dilute to concentrations well below toxic levels (Kjølholt et al. 2012, IMO; MEPC 74/INF.24 2018). However, recently there has been an increasing number of both reports and scientific articles showing that scrubber water is toxic to marine biota and either recommending stricter rules for where scrubbers could be used or advocating a complete scrubber ban (Hassellöv et al. 2020, Thor et al. 2021, Picone et al. 2023). Experimental studies have shown that low concentrations of scrubber water cause severe disruption of the early development of marine invertebrates, such as planktonic copepods (small crustaceans with a central position in all marine pelagic food webs), and in larvae of blue mussels, sea urchins and polychaetes (Thor et al. 2021, Magnusson and Granberg 2022, Picone et al. 2023). In some cases, significant toxic effects occurred already at concentrations as low as 0.001% scrubber water.

Vessels equipped with open-loop scrubbers discharge wastewater from the scrubbing process (scrubber water) to the ocean as long as the engine is running. In busy ship lanes and harbours, there is therefore a continuous input of this polluted water to the environment. The number of ships equipped with scrubbers has increased over the past decade. Between 2015 and 2022, the global number of vessels equipped with scrubbers increased from 242 to 4737 (Det Norske Veritas (DNV- GL)), and in the Baltic Sea the number increased from 178 in 2018 to 462 in 2020 (HELCOM 2021). The volumes of discharged scrubber water to the ocean, and also volumes of other ship related waste streams like bilge water, grey- and black water, have been calculated using the Ship Traffic Emission Assessment Model (STEAM), where input data on activity, position and technical information of most ships in operation are obtained via the automatic identification system (AIS) (Jalkanen et al. 2021). STEAM generates data on volumes of discharged scrubber water and the geographical location of the discharges. The Baltic Sea had 462 ships equipped with scrubbers in 2020, and estimations of the volumes of scrubber water generated and discharged show that they may be as high as 0.6 billion m³/year (Ytreberg et al. 2022a).

There is a clear risk that the scrubber water pollutants will be harmful to the Baltic ecosystems. Pelagic organisms are the primary target since they are found where the scrubber water is discharged, but benthic communities and sensitive coastal habitats near ship lanes will also be affected. Many stretches of shipping lanes in the Baltic Sea run close to coast lines or shallow sea areas, e.g., in the Gulf of Finland, south of Gotland, the Oresund Sound and the Belt areas. In all these areas there are zones which are important breeding and nursery grounds for fish,

stopover sites for huge numbers of migrating birds feeding on invertebrates in shallow water, areas of great importance for over wintering sea birds, etc. (Larsson 2012, Sundblad and Bergstrom 2014, Kraufvelin et al. 2018). To these ecosystems the dilution of scrubber water in the sea might not necessarily be enough to keep concentrations of potentially hazardous components at safe levels. As an example, embryos of haddock developed heart malformations after exposure to diluted crude oil where the concentration of total PAHs amounted to 0.1 µg/L, which corresponds to a dilution of around 0.001% scrubber water (Sorhus et al. 2023b).

3.4 Polycyclic aromatic compounds (PACs) – one of the most toxic fractions of scrubber water

Classes of PAC

PACs are compounds with at least two benzene rings or benzene-like rings fused together. They include the better known polycyclic aromatic hydrocarbons (PAHs) but encompass also compounds with nitrogen, sulphur, or oxygen in the ring structure i.e., the heterocyclic PACs. In addition, PACs can be alkylated or contain different functional groups, allowing the PACs to include thousands of compounds. PACs without alkylation or functional groups are often referred to as *parent compounds* or *unsubstituted compounds*, while PACs with alkylation or functional groups are referred to as *substituted compounds* (Boehm 1964, Stout et al. 2015).

Unsubstituted PAHs receive most attention in risk assessments and in ecotoxicological research, but when alkylated PAHs are included in chemical analysis of environmental samples affected by oil spills, they are often found to occur at higher concentrations than the unsubstituted PAHs (Golzadeh et al. 2021, Grung et al. 2021). Alkylated PAHs can have one to several alkyl groups attached to the ring structure. The parent PAH and the corresponding alkylated PAHs form a "homologue series", where each compound group has a prefix indicating the number of carbon atoms in the attached alkyl groups. A CO-PAH is hence the parent PAH, C1-PAH is a PAH with one methyl group, a C2-PAH has two methyl groups or one ethyl group, a C3-PAH has three methyl groups, one methyl group and one ethyl group, or a propyl group, and C4-PAH may have various combinations of methyl-ethyl-, and propyl groups or a butyl group. The position of the alkylation(s) on the ring structure and the various combinations of alkyl groups generate numerous isomers of alkylated PAHs. Besides the alkylated PAHs there are PAHs with an oxygen or nitrogen containing functional group attached to the parent compound. Alkyl- and oxygen containing PAHs are abundant in petroleum and oil while nitrogen- and oxygen containing PAHs are formed during incomplete combustion (Zhao et al. 2020, Goto et al. 2021).

In the 1970s, a list of 16 PAHs, consisting of compounds with 2-6 aromatic rings, were selected by the United States Environmental Protection Agency (US EPA) to

be used when assessing risks to human health from drinking water (Andersson and Achten 2015a). The 16 US EPA PAHs were selected for three reasons: there were existing analytical standards available, they were known to occur in the environment, and they were known to be toxic. The intention was not that they should be used as a standard reference set for general risk assessment (Keith 2015). Yet this is the case for example in the EU Water Framework Directive where all PAHs that are regulated belong to the 16 US EPA PAHs (Directive 2013/39/EU). This practice has resulted in a situation where numerous PACs with potential toxic effects, such as alkyl-, oxygen-, and nitrogen containing PAHs, are not regulated at all.

Fate of PACs in the marine environment

PACs are hydrophobic, semi-volatile compounds with a high affinity for organic surfaces, including organic particles, living organisms, and biofilms. In general, hydrophobicity increases with increased number of aromatic rings and increased molecular weight. Alkylated PAHs are more hydrophobic than their corresponding parent PAHs, and the hydrophobicity increases with the degree of alkylation. Since particle affinity is directly correlated to hydrophobicity, most PACs compounds in the environment are extensively adsorbed to the surface of particles, primarily organic ones, and will be transported along with them. Factors like wind conditions, waves, sea currents and stratification of the sea water, will hence have a large influence on the spread of PACs from a point source.

The uptake of PACs and other organic pollutants by living organisms occurs simply by passive diffusion through the lipid-rich cell membranes. The uptake can be from the surrounding water over the gills or body surface, or over the gut epithelium when the animals consume PAC contaminated food. Through evolution, organisms have adapted a metabolic capacity to handle PACs and prevent them from accumulating in the body. Although this capacity is present in all living organisms, the metabolic efficiency differs considerably between groups of organisms, with invertebrates having a significantly lower capacity than vertebrates (Livingstone 1998). The PAC metabolites formed through the metabolic turnover are more water-soluble than the parent compounds and can therefore be excreted with urine (or similar, depending on the species). Many of the PAC metabolites are harmless, but there are also reactive metabolites formed in the process, and these are responsible for an important part of PAC toxicity. This means that a higher metabolic rate and exposure to higher PAC concentrations will generate more reactive metabolites and subsequently increase the risk of adverse effects. There are some data on trophic transfer (from prey to predator) of pyrene metabolites between invertebrate species (Carrasco Navarro et al. 2013). It is however not known how important this transfer is.

A consequence of the efficient metabolic turnover rate, especially by species at higher trophic levels is that PACs do not biomagnify in aquatic food webs. This is in contrast to halogenated organic contaminants like polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and dichlorodiphenyltrichloroethane (DDT) for which biomagnification is the result of slow metabolic turnover rates allowing concentrations to increase by each trophic level (Borgå et al. 2001, Takeuchi et al. 2009). The fairly efficient metabolism of PACs means that PAC concentrations in organisms do not correspond well with the exposure situation. A low PAC tissue concentration in an organism is not necessarily indicative of low PAC concentrations in the environment but instead the result of an efficient turnover rate. The lack of biomagnification was demonstrated very clearly in a Baltic Sea food chain, where 15 parent PAHs and 4 alkylated PAHs were analysed in seston (= plankton and organic particles), in blue mussels (*Mytilus edulis*) which feed exclusively on seston, and in gallbladder of young eiders (*Somateria mollissima*), which feed almost exclusively on blue mussels (Broman et al. 1990). All PAH concentrations were found to decrease when moving up the food chain, from seston to blue mussel to eider (Fig. 1). Similar results were found in a study where PAHs and PCBs were analysed in field-collected species of mussels, crustaceans, and fish from the innermost part of Tokyo Bay in Japan (Takeuchi et al. 2009). In this study the trophic position in the food web of each species was determined using a $\delta^{15}\text{N}$, a method well established to use for this application (Hobson and Welch 1992) and it was found that whereas the PCB concentration increased significantly with increased trophic level, the PAH concentration decreased (Fig. 2). Most studies on metabolism of PACs are carried out on PAHs, and there are limited data on if and how the metabolic activity differs between different groups of PACs.

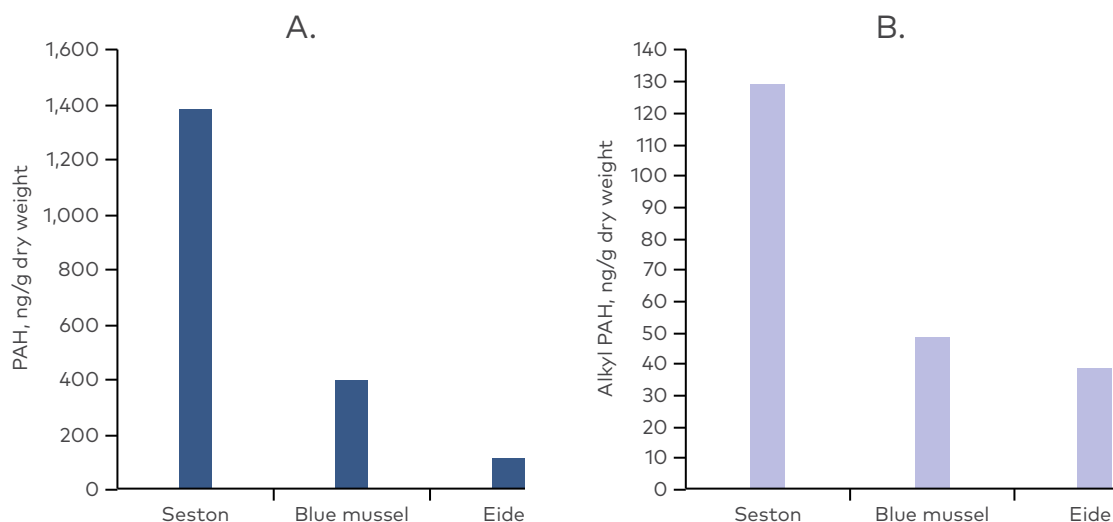


Figure 1 Concentration of unsubstituted and alkylated PACs in a Baltic marine food chain consisting of seston (=plankton/organic particles; n=10), blue mussel (n= 6), and juvenile eider (n= 3). A) Concentrations of 15 parent PAHs; B) Concentrations of 4 alkylated PAHs. Data obtained from Broman et al. 1990.

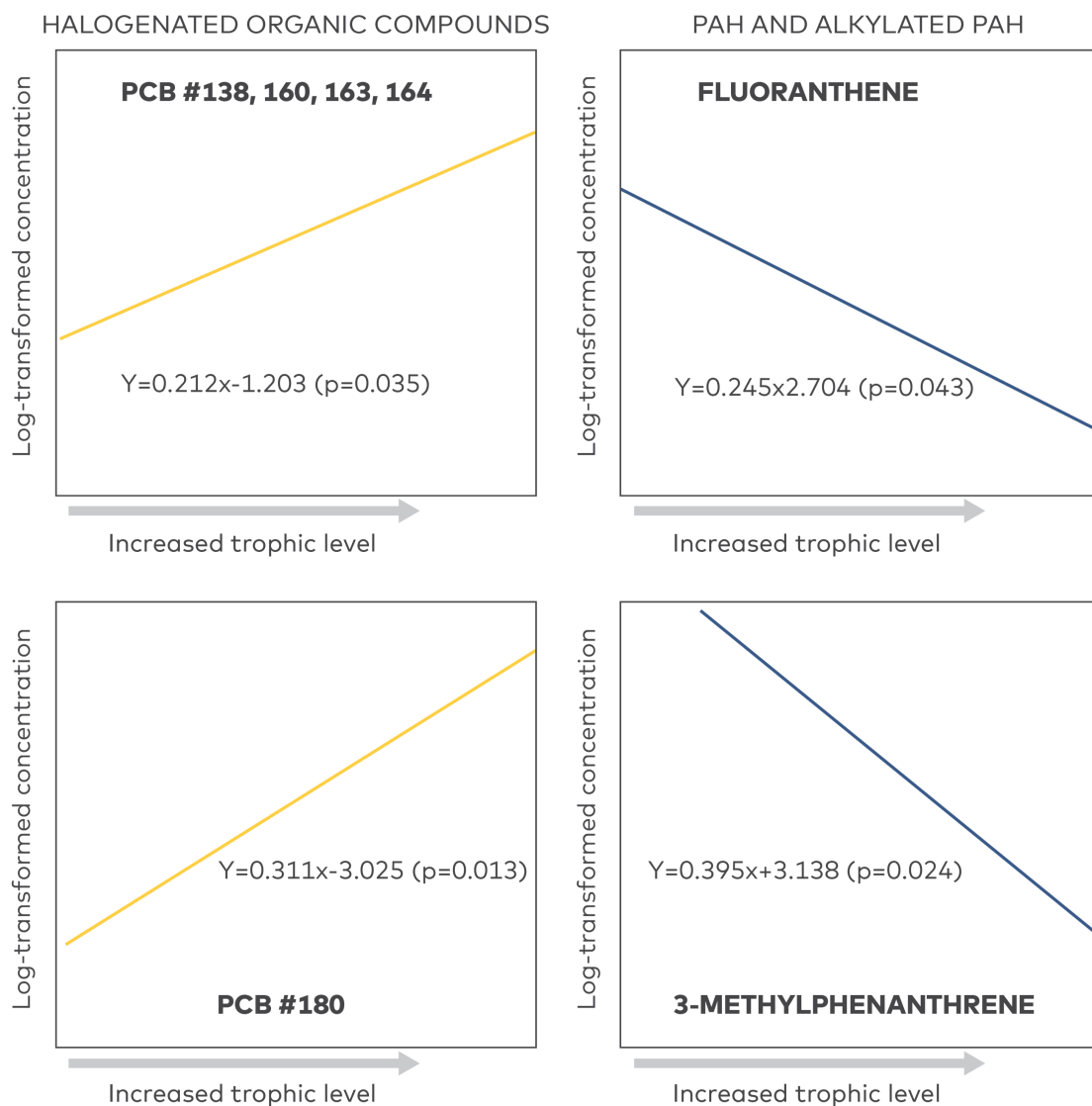


Figure 2 Concentrations of two PCBs, one PAH (phenanthrene) and one alkyl-PAH (3-methylphenanthrene) in organisms at different trophic levels (mussels, crustaceans, fish) collected from the innermost part of Tokyo Bay, Japan. Figures are redrawn from Takeuchi et al. 2009.

4. Results from chemical analysis of PACs in scrubber water

4.1 PACs detected in the scrubber water

Scrubber water was collected by crew members aboard two vessels equipped with open-loop scrubber systems (denoted vessel 1 and vessel 2). The water samples were collected at a location on the ship where the chemical composition was identical to that of the scrubber water discharged into the sea. Great care was taken to avoid any loss of compounds prior to the analysis. The samples were collected in acetone-washed glass containers to avoid adsorption of hydrophobic compounds to the container walls. The containers were sealed and kept dark and cool to avoid any photooxidation or evaporation of compounds.

The scrubber water samples were subsequently filtrated to separate particles from the water. The PACs from the water were extracted with pentane overnight through liquid-liquid extraction and the PACs from the particles on the filters were extracted with dichloromethane through pressurised liquid extraction on an ASE200 system. The extracts were analysed with gas chromatography – mass spectrometry (GC-MS) for quantification of selected parent and alkylated PACs, and with GC×GC-MS before and after derivatisation for a more extensive non-targeted screening of PACs and quantification of some of these PACs. The non-targeted screening is an explorative analysis with the aim to identify which compounds are in the sample. However, as the samples contain thousands of compounds, the compounds with the highest concentration are usually selected for identification. Therefore, not all compounds present in the sample are identified. In addition, a search for suspected compounds can be made to verify if they are present or not. For this type of analysis, only few standards are run for potential quantification of selected compounds. It is, therefore, not all detected compounds that can be quantified as the standards for these were not included in the analysis. Derivatization, in the context of GC, is the conversion of an analyte to a different form prior to analysis, typically to improve the volatility, stability, chromatographic properties, and separation of more polar compounds (Lin et al. 2008). Using derivatisation therefore improves the sensitivity and lowers the detection limit of polar compounds in GC. Here, silylation derivatization is used with the intention to derivatize and characterise polar hydroxy and carboxylic acids within the samples, e.g., naphthol and naphthoic acid.

PACs detected and quantified in the analysis of the scrubber water collected in the present study were, parent and alkylated PAHs (Table 1 and 2, Fig. 4), an S-heterocyclic PAC (dibenzothiophene), alkylated S-heterocyclic PACs (alkylated forms of dibenzothiophene and benzothiophene), parent and alkylated N-heterocyclic PACs (carbazole and alkylated carbazoles), and two O-heterocyclic PACs (dibenzofuran and 2-methyldibenzofuran) (Table 3, Fig. 4 and 5A). A summary of the total concentration of each of these groups is presented in Table 4.

In addition, the following oxy-PAHs were detected: 9,10-anthracenedione (synonym: anthraquinone), naphthaldehyde (synonyms: 1-formylnaphthalene; 1-naphthalene carboxaldehyde), phenalene-1-one (synonyms: phenalenone, 1H-benzonaphthene-1-one), 9-hydroxyfluorene (synonym: fluorenone), and naphthol (synonyms: naphthalen-1-ol, hydroxynaphthalene) (Fig. 6). Anthraquinone was, however, only included in the analysis of the scrubber water from vessel 2.

Data on parent PAHs in the scrubber water from the present study, along with data from the literature compiled within the EU project EMERGE (Ytreberg et al. 2022b), are presented in Table 1. The parent PAHs quantified in the scrubber water from the present study included the 16 US EPA PAHs plus perylene and benzo[e]pyrene. Compiled data for scrubber waters in Ytreberg et al. (2022b) did not include alkylated PAHs.

In the scrubber water analysed in the present study, the total concentration of parent PAHs was 68.1 µg/L for vessel 1 and 18.0 µg/L for vessel 2. Naphthalene was the dominating congener, accounting for 40 – 45% of the concentration. The second highest concentration was found for phenanthrene (31 – 36%) followed by fluorene (7 – 11%) (Table 1). The total concentration of the 16 US EPA PAHs in scrubber water based on literature data from 49 – 64 ships was considerably lower than what was detected in the present study, namely 2.97 ± 0.06 µg/L (Ytreberg et al. 2022b). Still, the relative distribution of the three most abundant PAHs was the same with the highest concentration detected for naphthalene (45%), followed by phenanthrene (24%) and fluorene (8%).

The total concentration of alkylated PAHs amounted to 770 µg/L in scrubber water from vessel 1, and to 73 µg/L in water from vessel 2. Alkylated PAHs quantified in the present study included C1-C4 naphthalenes, C1-C3 fluorenes, C1-C4 phenanthrenes/anthracenes, C1-C2 fluoranthenes/pyrenes, and C1-C3 chrysenes (Table 2). The relative concentrations of alkylated PAHs were similar to that of the parent compounds, with C1-C4 naphthalenes amounting to 39 – 69% of the total concentration of alkylated PAHs, followed by C1-C4 phenanthrenes/anthracenes (16 – 35%), and C1-C3 fluorenes (11 – 13%). The alkylated PAHs made up in total 80 – 92% of the parent + alkylated PAHs (Table 4). Similar ratios, 74 – 85%, were found in another study of scrubber water from three vessels (Du et al. 2022).

Three homologue series of heterocyclic PACs were detected in the scrubber water, parent and alkylated forms of the S-heterocyclic dibenzothiophene, alkylated benzothiophene (no parent benzothiophene was detected), and parent and alkylated forms of N-heterocyclic carbazole (Table 3). For dibenzothiophene the alkylated analogues made up almost 90% of the sum of parent and alkylated analogues, 1.57 µg/L, and 13.14 µg/L, respectively. For the parent and alkylated carbazoles, concentrations of all alkylated groups were higher than that of the parent compound (Fig. 5).

The sum of all parent and alkylated PACs that were quantified are summarised in Table 4.

As mentioned above, five oxy-PAHs were detected by the non-target screening (Fig. 6). Of these, only naphthaldehyde was quantified reaching a concentration of 47.4 µg/L and 10.7 µg/L for vessel 1 and vessel 2, respectively.

Oxy- and nitro-PAHs are formed during combustion and several of them occur in higher concentrations than unsubstituted PAHs in the combustion gases (Zhao et al. 2020). The non-target screening of the scrubber water did not reveal any nitro-PAHs. The reason for this is not clear, but it has been shown that the scrubber is not efficient in capturing particles in the size category PM_{2.5} (Yang et al. 2021), and it is possible that the nitro-PAHs, since they are mainly formed during combustion, bind hard to particles that are emitted to air.

Table 1 Unsubstituted PAHs in scrubber water from open-loop scrubber systems. Data derive from Ytreberg et al. (2022b), and from scrubber water from two separate vessels analysed within the current project. Concentrations are presented as µg/L. ND= Not detected.

	Scrubber water literature data $X \pm 95\% \text{ CI}$ (µg/L; n= no of samples)	Scrubber water, vessel 1, present study (µg/L; n=1)	Scrubber water, vessel 2, present study (µg/L; n=1)
Naphthalene	2.76 ± 0.75 (n=55)	27.3	8.03
Acenaphthylene	0.13 ± 0.07 (n=63)	0.96	0.50
Acenaphthene	0.19 ± 0.07 (n=63)	2.72	0.35
Fluorene	0.46 ± 0.10 (n=63)	7.51	1.27
Phenanthrene	1.51 ± 0.30 (n=64)	24.7	5.53
Anthracene	0.08 ± 0.05 (n=63)	0.62	ND
Fluoranthene	0.16 ± 0.05 (n=63)	0.61	0.24
Pyrene	0.32 ± 0.12 (n=63)	1.73	0.94
Benzo[<i>a</i>]anthracene	0.13 ± 0.06 (n=64)	0.29	0.10
Chrysene	0.19 ± 0.07 (n=63)	1.01	0.63
Benzo[<i>b</i>]fluoranthene	0.04 ± 0.02 (n=63)	0.13	0.05
Benzo[<i>k</i>]fluoranthene	0.01 ± 0.01 (n=49)	0.03	ND
Benzo[<i>a</i>]pyrene	0.05 ± 0.02 (n=64)	0.08	0.02
Dibenzo[<i>a,h</i>]pyrene	0.03 ± 0.02 (n=63)	0.02	ND
Benzo[<i>g,h,i</i>]perylene	0.02 ± 0.01 (n=63)	0.11	0.15
Indeno [1,2,3- <i>c,d</i>]pyrene	0.07 ± 0.06 (n=63)	0.01	ND
Benzo[<i>e</i>]pyrene	Not analysed	0.19	0.16
Perylene	Not analysed	0.06	0.03
SUM PAH	2.97± 0.79 (n=35)*	68.1	18.0

* only 16 US EPA PAHs

Table 2 Alkylated PAHs in scrubber water from open-loop scrubber systems. The analyses were carried out on water from two separate vessels within the current project.

	Scrubber water, vessel 1 (µg/L)	Scrubber water, vessel 2 (µg/L)
Naphthalene	27.3	8.03
C1-naphthalenes	160	11.0
C2-naphthalenes	154	8.96
C3-naphthalenes	121	5.22
C4-naphthalenes	99.0	3.58
Sum alkylated naphthalenes	534	28.8
Fluorene	7.51	1.27
C1-fluorenes	24.4	2.32
C2-fluorenes	38.3	2.98
C3-fluorenes	34.1	2.65
Sum alkylated fluorenes	96.8	7.95
Phenanthrene + anthracene	25.3	5.53*
C1-phenanthrenes/anthracenes	50.4	7.93
C2-phenanthrenes/anthracenes	46.4	8.19
C3-phenanthrenes/anthracenes	20.5	6.98
C4-phenanthrenes/anthracenes	5.20	2.63
Sum alkylated phenanthrenes/anthracenes	122	25.7
Fluoranthene + pyrene	2.33	1.18
C1-fluoranthenes/pyrenes	4.21	2.77
C2-fluoranthenes/pyrenes	4.06	4.13
Sum alkylated fluoranthenes/pyrenes	8.23	6.90
Chrysene	1.01	0.63
C1-chrysenes	2.78	1.47
C2-chrysenes	4.08	1.54
C3-chrysenes	1.89	0.71
Sum alkylated chrysenes	8.75	3.72
SUM ALKYLATED PAH	770	73.1

* anthracene below the detection limit for vessel 2

Table 3 Sulphur and nitrogen heterocyclic PACs and their alkylated homologues in scrubber water from two separate vessels with open-loop scrubber systems. The analyses were carried out withing the current project.

	Scrubber water, Vessel 1 (µg/L)	Scrubber water, Vessel 2 (µg/L)
Dibenzothiophene	2.58	1.57
C1-dibenzothiophenes	6.25	2.89
C2-dibenzothiophenes	9.54	4.30
C3-dibenzothiophenes	11.0	5.95
Sum alkylated dibenzothiophenes	26.8	13.1
C1-benzothiophenes	2.39	2.44
C2-benzothiophenes	2.48	2.28
C3-benzothiophenes	10.6	1.34
C4-benzothiophenes	6.93	0.54
Sum alkylated benzothiophenes	22.4	6.61
Carbazole	0.12	0.10
C1-carbazoles	2.89	2.34
C2-carbarzoles	1.01	0.76
C3-carbazoles	0.56	0.40
C4-carbazoles	0.40	0.35
Sum alkylated carbazoles	4.98	3.85
Dibenzofuran	13.7	6.83
2-methyldibenzofuran	9.04	2.88

Table 4 Sum all PACs and alkylated PACs in scrubber water from two separate vessels with open-loop scrubber systems. The analyses were carried out within the current project (presented for individual congeners in Tables 1 – 3).

	Scrubber water, vessel 1 (µg/L)	Scrubber water, vessel 2 (µg/L)
ΣUnsubstituted PAH	68.1	18.0
ΣAlkylated PAHs	770	73.1
ΣUnsubstituted + alkylated PAH	838	91.1
ΣUnsubstituted S-heterocyclic PAC	2.58	1.57
ΣAlkylated S-heterocyclic PAC	49.1	19.7
ΣUnsubstituted + alkylated S-heterocyclic PAC	51.7	21.3
ΣUnsubstituted N-heterocyclic PAC	0.12	0.10
ΣAlkylated N-heterocyclic PAC	4.98	3.85
ΣUnsubstituted + alkylated N-heterocyclic PAC	5.10	3.95
ΣUnsubstituted O-heterocyclic PAC	13.7	6.83
ΣAlkylated O-heterocyclic PAC	9.04	2.88
ΣUnsubstituted + alkylated O-heterocyclic PAC	22.7	9.71

4.2 PAC fingerprint

The relative concentrations of individual PAC compounds, the PAC “fingerprint”, differ depending on whether the origin is petrogenic (oil, coal) or pyrogenic (combustion). These differences may be used in environmental forensics to identify sources of contamination (Yunker et al. 2002, Stout et al. 2015). Despite the fact that the scrubber water has been used to wash the gases of combusted HFO (pyrogenic origin), there are several characteristics in the PAC fingerprint more resembling a petrogenic than a pyrogenic profile. This might be caused by an incomplete combustion of the HFO and/or the presence of oil from other oil sources, e.g., lubrication oil, in the scrubber water (Teuchies et al. 2020).

Petrogenic PACs have been formed over geological times at relatively low temperatures whereas pyrogenic PACs derive from the incomplete combustion of organic matter at high temperatures for short periods of time. Two characteristics often used to separate petrogenic from pyrogenic PAC mixtures are i) the relative distribution between parent PACs and their alkylated forms, and ii) the relation between low molecular and high molecular PACs. In petrogenic mixtures, the relative concentrations of parent and alkylated forms of a PAC form a bell-shape with concentrations of PAHs with one and two carbon atoms in alkylated groups (C1 and C2-PACs), and sometimes also C3-PACs, being higher than concentrations of the unsubstituted parent PAH (Fig. 3) (Yunker et al. 2002, Stout et al. 2015). In mixtures of pyrogenic PACs, the high combustion temperatures have eliminated alkyl side chains so that there is a dominance of unsubstituted PACs (Fig.3).

PACs and alkylated PACs in scrubber water have the characteristics of petrogenic mixtures forming bell-shaped concentration patterns, with higher concentrations of alkylated than of parent compounds and have a predominance of low molecular weight PAHs (Figures 4A1, 4A2, and 4B; Table 1). Figure 4C shows the concentration of PAHs and alkylated PAHs in the HFO used by the vessel 1 and when comparing that to the pattern in scrubber water (Fig.4A and B) similarities can be observed. Concentrations of all C1 and C2-alkylated groups of compounds present in both scrubber water and HFO being higher than concentrations of the parent compounds. An indication of combustion is seen in the relative high concentration of acenaphthylene which is not commonly found in fuel (van Gelderen et al. 2021)

In scrubber water, parent and alkylated compounds of the S-heterocyclic PAC dibenzothiophene, the N-heterocyclic PAC carbazole, and the O-heterocyclic PAC dibenzofuran were detected at concentrations slightly lower but still at the same level as concentrations of phenanthrene, a PAC of similar molecular size (Tables 2 and 3). In addition, alkylated forms of the S-heterocyclic PAC benzothiophene were also detected at similar concentrations.

A summary of the composition of PACs typical for petrogenic and pyrogenic sources is presented in Table 5.

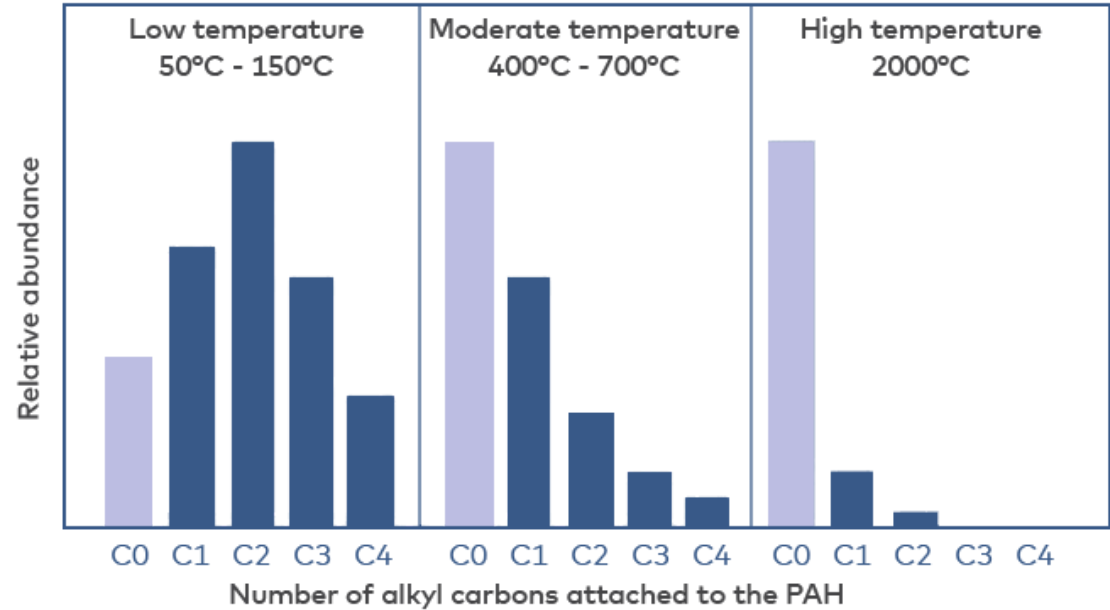
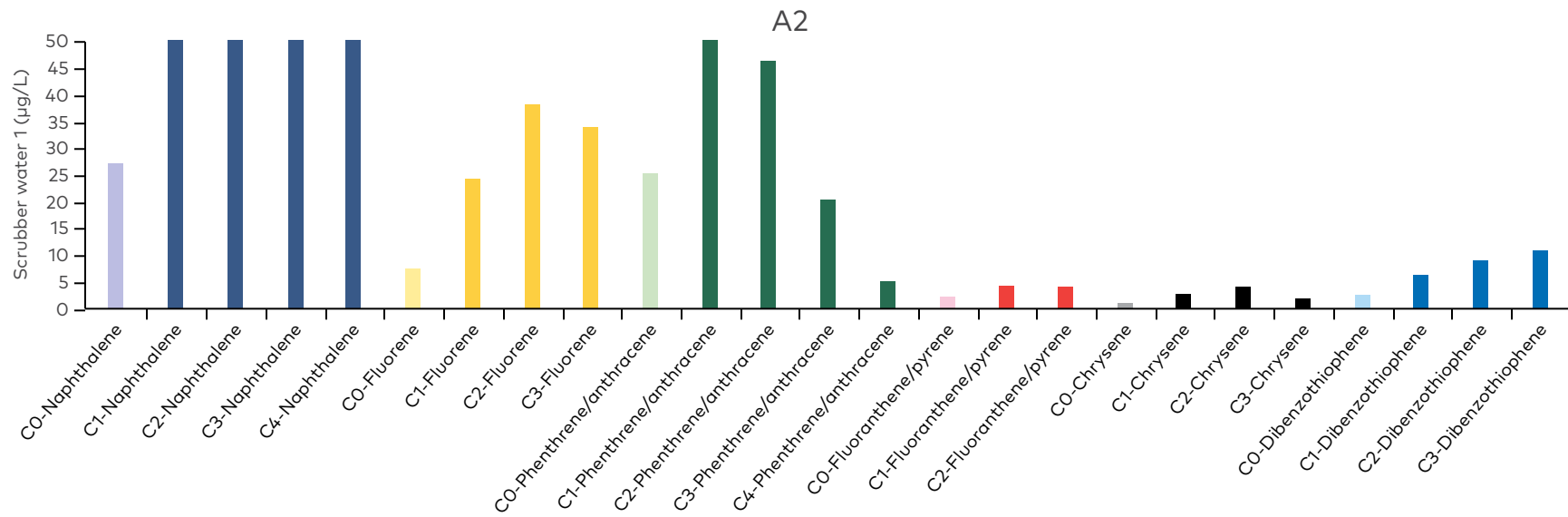
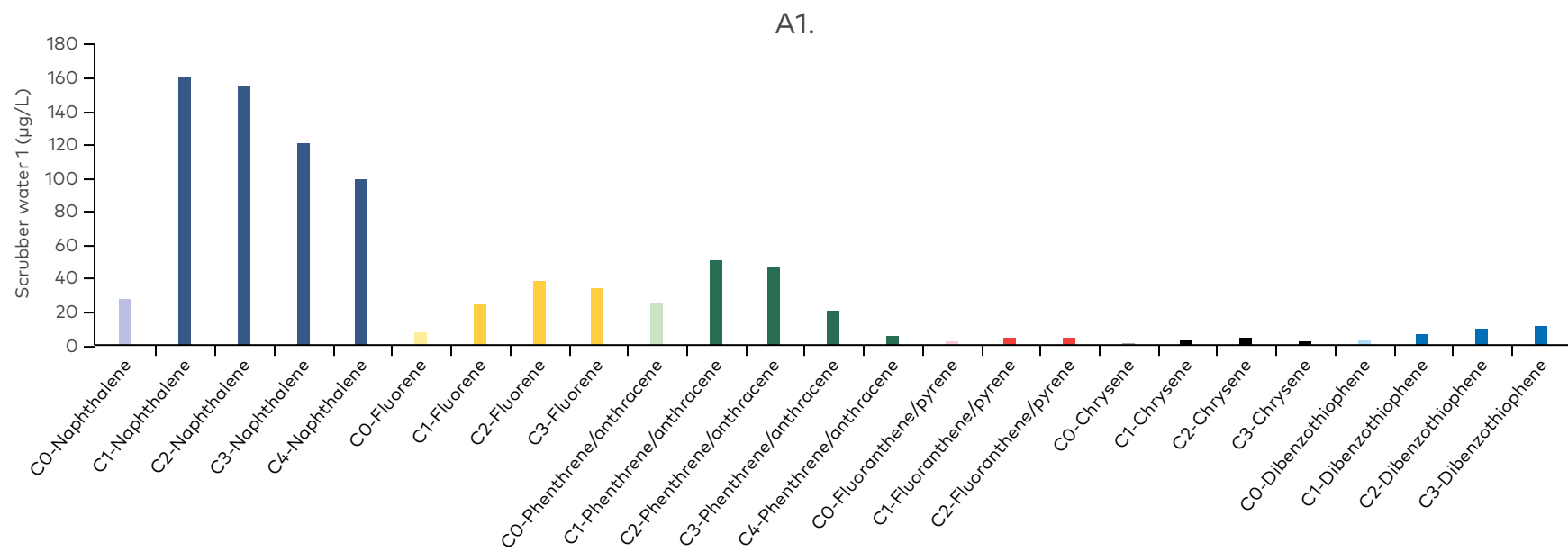


Figure 3 Schematic illustration of the relative abundance of alkyl carbons attached to PAHs formed at different temperatures. At low temperatures (petrogenic sources) the distribution is bell-shaped with a maximum concentration for C2 compounds. At moderate and high temperatures (pyrogenic sources), the highest concentration is found for the unsubstituted parent PAH. The figure is redrawn from Stout et al. 2015.

Table 5 Characteristics of PAC composition in a petrogenic versus a pyrogenic source (Kang et al. 2016, Zhao et al. 2020).

Petrogenic sources (e.g., non-combusted heavy fuel oil)	Pyrogenic sources (e.g., combusted heavy fuel oil)
• Dominance of low molecular weight PAHs • Higher prevalence of alkylated PAH	• Dominance of medium to high molecular weight PAH • Lower prevalence of alkylated PAH • Nitro-, oxy-, and hydroxy- PAH formed during photooxidation in air



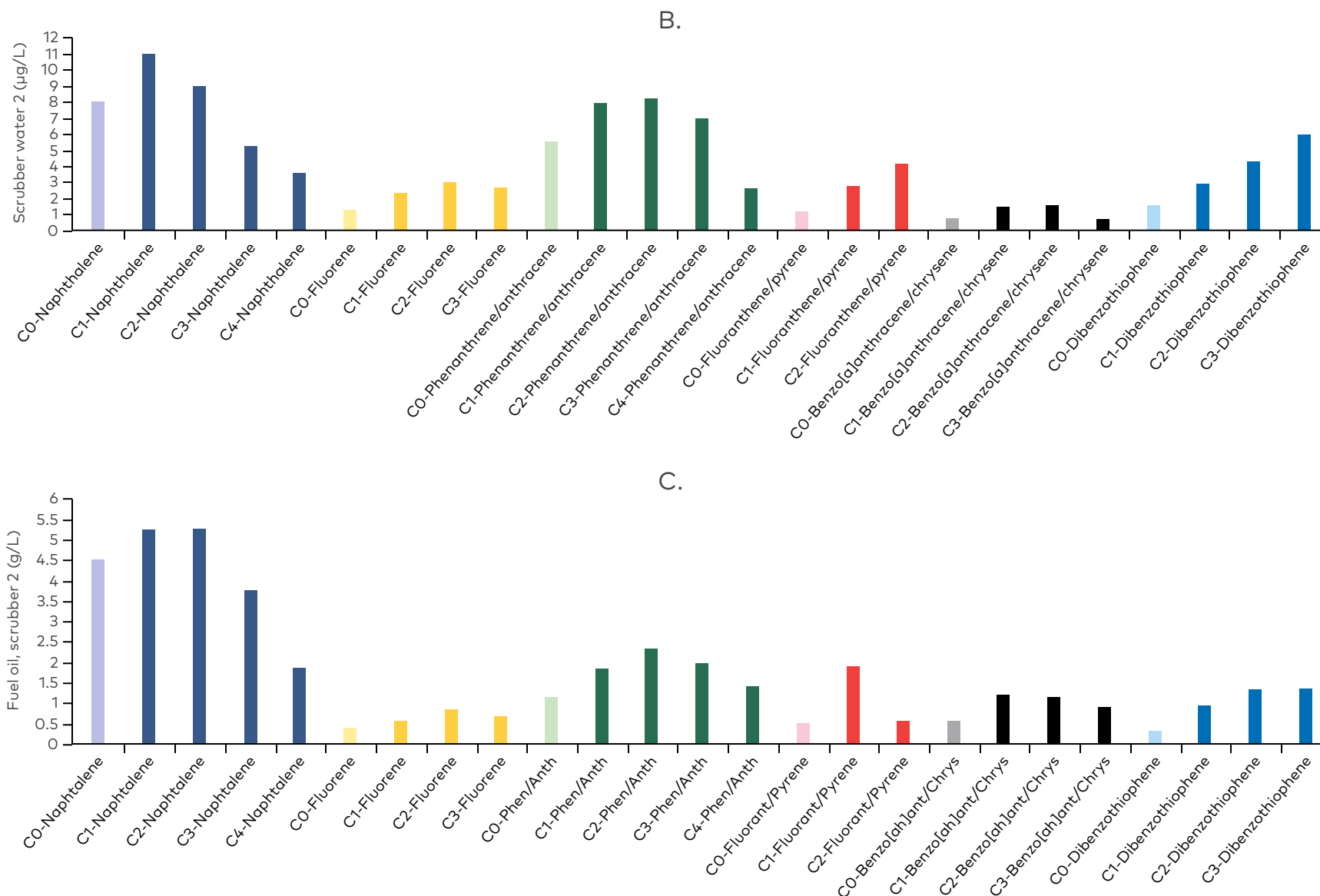


Figure 4 Concentrations of unsubstituted PAHs and dibenzothiophene, and their alkylated forms in scrubber water from two vessels with open-loop systems and in fuel oil. A1. Scrubber water 1 (from vessel 1); A2. Zoom of scrubber water 1; B. Scrubber water 2 (from vessel 2); C. HFO fuel used in vessel 2 at the time of the collection of the scrubber water. PAC concentrations in scrubber water are expressed as µg/L and concentrations in bunker oil as mg/L. Note the difference in scale of the y-axis.

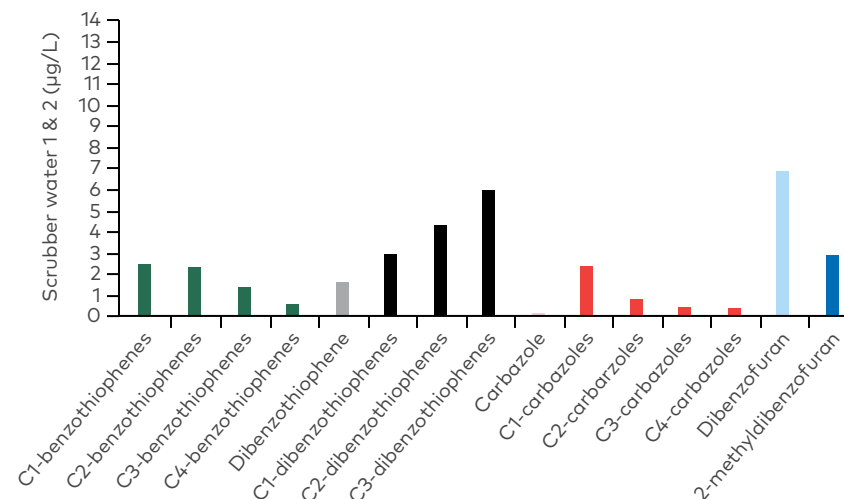
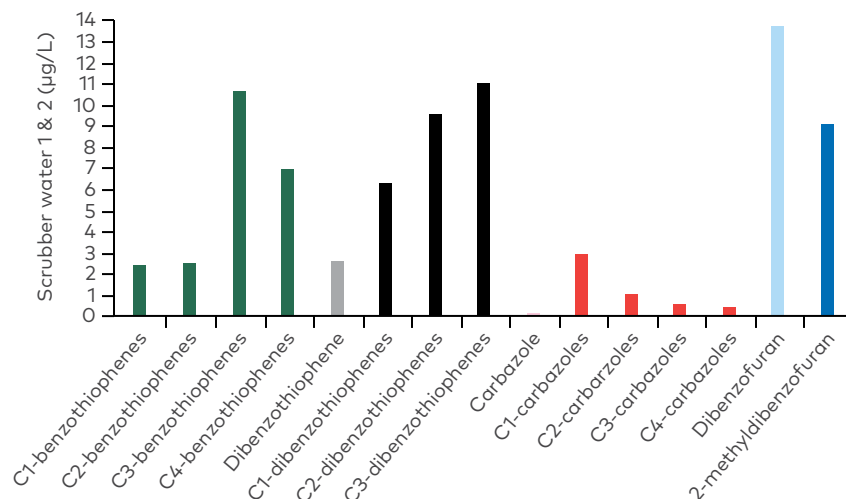


Figure 5 Scrubber water concentration of the S-heterocyclic PACs benzothiophene (only alkylated compounds), and parent and alkylated dibenzothiophene, parent and alkylated congeners of the N-heterocyclic PAC carbazole, and the O-heterocyclic compounds dibenzofuran and 2-methyldibenzofuran. Note that the dibenzothiophenes are also included in Fig. 4. Data derive from analysis of two separate vessels (vessel 1 and vessel 2)

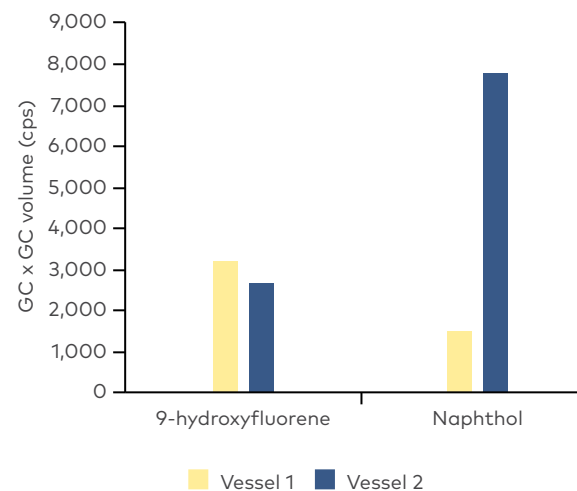
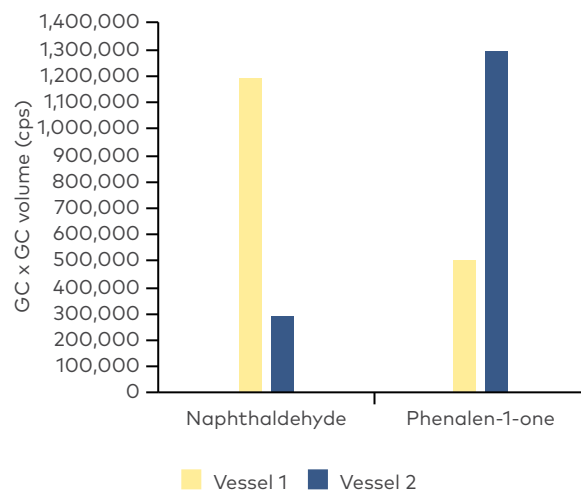


Figure 6 Compounds detected in scrubber water from the two vessels by non-target screening by GC×GC with derivatisation. Note that anthraquinone was detected but is not included in the figure as this was only analysed for vessel 2 without derivatisation.

5. Development of PAH threshold values

Research on the effects of PACs may roughly (and without strict borders) be divided into a field more oriented towards applied research where the ultimate ambition is to define at what PAC concentrations toxic effects occur, and a field of more basic research where the main purpose is to understand underlying toxic mechanisms. Applied research forms the basis for design of standardised protocols for toxicity tests, which in turn are used in regulatory work such as in risk assessment or establishment of threshold values for PACs in the sea. Ideally, there would be ongoing communication between the two fields, so that when scientists in basic research discover previously unexplored endpoints that are more sensitive to PACs than those currently included in standardised protocols for toxicity testing, scientists in the applied field would ensure that the protocols are updated. If the applied research does not incorporate relevant findings from the basic research there is a risk that toxicity tests in the applied field will be performed not using the most sensitive species/life stages of species, not using the most sensitive endpoints, and maybe not using the relevant exposure times needed to detect effects. The risk is then that data obtained from the standardised tests will be used as a true description of the PAC toxicity whereas in fact there is a high risk that the toxicity will be underestimated.

Another problem when assessing the toxicity of PACs is that the focus today, both in research and in environmental legislation, is limited to the set of 16 US EPA PAHs. Currently, the only PACs regulated in any environmental legislation are selected from these 16 PAHs although it has been pointed out that this is far from sufficient if we want to understand the fate and predict toxicity of PACs in the environment (Andersson and Achten 2015b, Stout et al. 2015).

IMO has set threshold values on some of the components in scrubber water that must not be exceeded if the water is to be released into the sea and PAH is one of them (IMO; Resolution MEPC.340(77) 2021). The threshold concentration for PAHs is expressed as *phenanthrene equivalents* (PAH_{phe}) in the discharged scrubber water and is set to a maximum of 50 µg/L above the concentration in the surrounding water. The value should be normalised to a wastewater flow rate through the scrubber unit of 45 tons/MWh. It is not possible to trace which ecotoxicological data were used when setting the threshold value, and it is also worth noting that the value was the same in 2015 when the number of ships with scrubbers were around 250 as compared to close to 5000 in 2022 (Det Norske Veritas (DNV- GL)). The use of PAH_{phe} was suggested by for an estimation of the combined toxicity effect of multiple PAHs (Fisher et al. 2011), but such an approach

has been questioned by other scientists (Billiard et al. 2008, Le Bihanic et al. 2014a). PAH_{phe} was developed using the concept of toxic equivalency quotients (TEQs) which are applied to express the toxicity of complex mixtures of compounds. One of the compounds, in this case phenanthrene, is used as a reference and given the toxic equivalency factor (TEF) of 1, and all other compounds receive a TEF expressing their toxicity in relation to the reference substance. By multiplying the TEF with the concentration of each compound, a TEQ, in this case PAH_{phe} , is obtained. However, the use of toxic equivalents builds on the concept that all compounds included are toxic through the same mode of action but have different potency. This is the case for e.g., dioxins, but PAH toxicity occurs through several different modes of action. So, researchers active in the field of mechanistic studies on PAH toxicity strongly advice against the use of TEF and TEQ (Billiard et al. 2008, Le Bihanic et al. 2014a).

There are national and international threshold values for PAH in seawater which must not be exceeded if the sea area should be considered to have a good environmental status. The EU water framework directive has developed Environmental Quality Standards (EQS), which specify threshold levels for the maximum allowed concentrations (MAC), and the allowed annual average concentration (AA) of a set of 44 compounds, including 8 PAHs, in coastal sea water (Directive 2013/39/EU). The latest version of the EQS is from 2013, and no updates have been made since then. The eight PAHs with an EQS are: naphthalene, anthracene, fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-*c,d*]pyrene, and benzo[*g,h,i*]perylene, all of them belonging to the 16 US EPA PAHs.

6. PAC fate and general toxicity

PACs are considered to be the most toxic group of compounds in scrubber water, and there is an extensive amount of research going on covering effects and underlying mechanisms at genetic, molecular, cellular, and tissue levels, as well as effects that can be observed in studies on whole-organism or even at population and community levels. The PACs consist of hundreds of compounds, but only a few of them, mainly some of the 16 US EPA PAHs, receive attention in research or in legislation (e.g., development of standardised tests or development of threshold values for acceptable concentrations in the environment or in food for human consumption). Data on toxicity of alkylated PAHs, oxy- and nitro-PAHs, and on heterocyclic PACs are scarce. In a review on the effect of PAHs on reproduction in all animals, 75 relevant articles were found, and the total number of PAHs in the described experiments included five PAHs (i.e., benzo[a]pyrene, pyrene, phenanthrene, fluoranthene, and naphthalene) and one group of methylated PAHs (i.e., C2-naphthalene) (Bolden et al. 2017). Despite the limited knowledge on most PACs, it is clear that there is a significant difference in toxicity between individual compounds. There is also a difference in sensitivity between species and between different life stages of a species, with early development generally being the most vulnerable. Data show that early life stages of fish are very sensitive to PACs, and effects are often detected at lower concentrations than effects in marine invertebrates (Tables 6 and 7). Whether this really means that fish are more sensitive or whether it is a result of more research being done on vertebrates is not clear. Some toxic mechanisms occur only after the PAH molecule has been metabolised, and since vertebrates have a more efficient PAH metabolism this could partly explain the higher sensitivity.

The extensive research on PAH and other PACs is in sharp contrast to the limited data commonly referred to when assessing the toxic effects of scrubber water on marine ecosystems. These data generally derive from standardised tests/assays of a few PAHs with the most frequent endpoint being mortality in a population after 24 – 96 hours exposure to the toxicant (European Commission 2003). The same kind of tests also form the basis for developing pollutant threshold values, e.g., the EU environmental Quality Standards (EQS) (Directive 2013/39/EU). Threshold values only exist for a few PAH congeners and are rarely re-evaluated and updated according to new scientific findings. EQS for the EU priority pollutants have not been updated since 2013.

Sea water around the Nordic countries generally have pollutant levels well below all these EQSs, including those for the PAHs. Still, it is very clear that the marine ecosystems are not doing well, and the situation deteriorates rapidly with declining

populations of virtually all marine species in most sea areas. No area of the world's oceans is completely unaffected by man, and it is estimated that up to 40% is heavily affected (Halpern et al. 2008, Crain et al. 2009). The problem is multifaceted, and the role of PACs and other oil derived pollutants is not at all clear. It is however well documented that this group of compounds has a wide range of biological effects that may appear already after exposure to very low concentrations. In marine ecosystems these effects are best documented on early life stages of fish and include morphological deformations, cardiotoxicity, mutations and cancer, oxidative stress, endocrine disruption, impaired reproduction, mortality rates of embryos, reduced growth, circulatory failure, and fin erosion (Billiard et al. 2008, Hodson 2017, Wallace et al. 2020). It can therefore not be excluded that scrubber water, this new source of PACs to the ocean, could have an impact on the already hard-pressed coastal marine ecosystems.

Although there is an extensive scientific literature on PAH toxicity (data on other groups of PACs are scarce), knowledge is limited to a comparatively small number of individual compounds, and the toxicity is found to vary considerably between different groups of organisms. Research on the physiological mechanisms behind PAH toxicity has primarily been done on vertebrates, including fish. Studies on PAH in fish have been carried out for over half a century and developed into a large and well-established field of research, while data on toxicity on invertebrates and algae are more scattered. Therefore, when conducting toxicity studies to investigate the effects of scrubber water, there are a number of endpoints in fish known to be sensitive to PAHs present in scrubber water, while it is less clear what would be the most sensitive endpoints in invertebrates and algae. Available data on the effect of PAHs on invertebrates include studies on fertilisation, egg hatching, and early life stages, and although it has been shown that some life stages are more sensitive than others, the observations are rarely used for further studies on the underlying mechanisms. This limitation in knowledge on PAC toxicity on invertebrate poses a risk that toxicity studies on invertebrates are not carried out at the most sensitive life stages and that the risk scrubber water, and other oily wastewaters, pose to the marine environment is underestimated.

PAHs are metabolized more efficiently than halogenated organic pollutants (Takeuchi et al. 2009). However, in the metabolic process also reactive and potentially toxic metabolites are formed. This means that metabolism of PAHs is at the same time a detoxification process, by eliminating the PAHs, and a generator of toxic PAH metabolites. Formation of reactive metabolites is not the only toxic pathway, but studies in fish and other vertebrates have shown that PAH toxicity is caused by multiple mechanisms, varying between compounds, where important factors determining the toxic mode of action are e.g., the number of rings, number of alkyl carbons, and the steric composition of the compound (Hodson 2017, Incardona 2017). These multiple modes of action behind PACH toxicity make it difficult to theoretically predict the effect of PAHs mixtures.

Metabolism of PAHs is a two-step process. There is a phase I where the parent compound is metabolised to intermediate metabolites, and a phase II where these metabolites bind to conjugating enzymes and form water soluble conjugates with low toxicity that can be excreted (Varanasi 1989, Franco and Lavado 2019). Phase I is initiated when PAH molecules that have passed through the cell membrane, bind to, and thereby activate, the aryl hydrocarbon receptors (AhR) which is present in the cytosol (Fig. 7). The activated PAC-AhR complex controls the expression of a battery of genes in the DNA of the cells that encode for enzymes that convert PAH into water-soluble metabolites (phase II) (Nebert et al. 2004). One important family of PAH metabolising enzymes is the cytochrome P450 1A (CYP1A), and this and other enzyme systems are induced by many (but not all) PAHs. The phase I and phase II metabolic pathways prevent PAHs from accumulating, and the induction of CYP1A enzymes is a way for animals to adapt to situations of exposure to high PAH concentrations. However, phase I also generates the formation of reactive metabolites that may bind to cellular macromolecules and have a range of toxic effects, including mutations, cancer, immunotoxicity, teratogenic, and anti-estrogenic/anti-androgenic effects (Nebert et al. 2004, Bolden et al. 2017). So, a higher CYP1A production as a response to increased PAH/PAC exposure leads to an increased production of both harmless metabolites, and reactive metabolites that may cause different kinds of damage to the organism. From an ecotoxicological perspective it is therefore important to keep in mind that an increased PAH exposure will not necessarily lead to an increased bioaccumulation of PAHs (Fig. 1 and 2), but there will be an increased production of the toxic PAC metabolites.

The induction of CYP1A can be used in field monitoring of PAHs. Since these compounds are not bioaccumulating to any great extent, exposure to them can instead be estimated by measuring the CYP1A activity.

The AhR/CYP1A related mechanism of PAH toxicity is similar to that of dioxins and is mainly important for high molecular weight PAHs. Low molecular weight PAHs do not bind to the AhR but exert their toxicity through other pathways. Several of the low molecular weight (3-ringed) compounds are known to cause disturbance of cardiovascular function and craniofacial and body axis defects in fish, and the mechanism behind this is suspected to be an effect on the ion regulation in cells (Incardona et al. 2005, Incardona 2017).

Since at least part of the toxic effects of PAHs are linked to their metabolic conversion, species with a high metabolic capacity could be suspected to be more sensitive to these compounds than species with lower capacity. Invertebrates have a considerably lower metabolic turnover of PAHs than fish and other vertebrates (Livingstone 1998), and scientific data indicate that they also are less sensitive (see Tables 6 and 7 for references). However, it cannot be ruled out that this merely reflects the fact that there is much more research on effects of PAHs on fish than on invertebrates.

In addition to the specific toxic mechanisms mentioned above (through AhR activation and disturbance of ion channels), PAHs also have been claimed to have a non-specific mode of action, often referred to as "narcosis" (Di Toro et al. 2000). Narcosis is suggested to be caused by the mere presence of the hydrophobic PAC molecules dissolved in the cell membranes which would disturb normal cell processes and result in an anaesthetic or narcotic-like effect. With increasing molecular weight, PAH molecules become more hydrophobic, and this would lead to an increased narcotic effect. Lately the evidences for this form of narcotic effect have been questioned (Incardona 2017), although it cannot be ruled out that it exists.

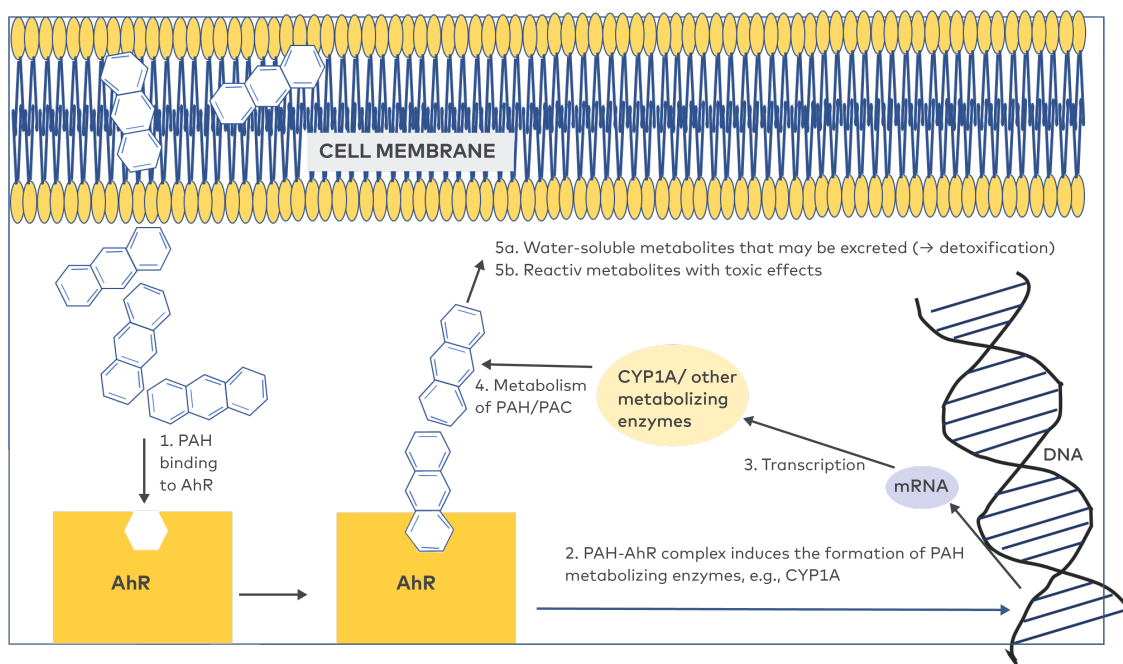


Figure 7 The metabolic turnover eliminates PAHs and limit the accumulation in the organism, but at the same time reactive metabolites with toxic effects are generated.

7. Toxicity of PACs in scrubber water on pelagic organisms

A literature search was made on toxicity of PAHs and other PACs detected in scrubber water specifically on marine pelagic organisms. These organisms live in the free water masses, and therefore the ones that will encounter the highest concentrations of discharged scrubber water. The search hence included effects on fish, invertebrates that spend their entire life span in the pelagic (e.g., planktonic copepods), pelagic larval stage of invertebrates, and planktonic microalgae. Toxicity data on freshwater fish were also included to increase the data set. As already discussed, fish toxicity data are based on a better fundamental understanding of the mechanisms leading to the toxic effects, while invertebrate toxicity data are more often based on experiments where endpoints are not necessarily selected because they are known to be particularly sensitive to PAHs.

A common practice in applied ecotoxicology is to present data as the Lowest Observed Effect Concentration (LOEC), Effective Concentration 50%, 20%, or 10% (EC₅₀/EC₂₀/EC₁₀, the concentration of a toxicant having a significant toxic effect on 50%/20%/10% of the test population), or Lethal Concentration 50% or 10% (LC₅₀/LC₁₀, the concentration where 50%/10% of the test population dies). In this report data on LOEC have been selected as far as possible, and data on EC_{50/20/10} are only included when the study is of great interest, and no other data are available. Some data on mortality are included, but not when they derive from short term tests on adult individuals.

All groups of PAHs/PACs from which there are at least one compound identified in scrubber water have a heading below, even if toxicological data in many cases are scarce. The aim is not to present a resumé of the entire research field, but rather to highlight the most important findings relevant for understanding the risk of scrubber water and other oily wastewaters to the marine environment.

7.1 Toxicity of parent and alkylated PAHs

One of the most studied PAHs in terms of effects on fish embryo and fish larvae is the low molecular weight, 3-ringed compound phenanthrene, and this is also one of the PAHs that occurs at the highest concentration in scrubber water (Table 1 and 2). Embryos of the fish species marine medaka (*Oryzias melastigma*), exposed to low concentrations of phenanthrene for 28 days, developed a range of teratogenic effects (Table 6) (Zheng et al. 2020). Even at the lowest test concentration, 2 µg/L

of phenanthrene, there was a significant increase in occurrence of yolk sac- and pericardial edema of the embryos and the time for the eggs to hatch was prolonged (Table 6). At exposure to 10 µg/L of phenanthrene the hatching success of the eggs was reduced and there were significant deformations of the heart. It should be noted that these were the nominal concentrations of the pollutant, but analyses of the test water showed that the actual phenanthrene concentrations were at least 25% lower (Zheng et al. 2020).

Fertilised eggs of zebrafish (*Danio rerio*) exposed to phenanthrene in low doses for 96 hours were found to develop into adults with reduced reproductive functions. Males exposed to 0.089 µg/L developed fewer mature spermatozoa and females exposed to 0.89 µg/L developed fewer mature oocytes (Table 6) (Chen et al. 2021). When adult females, exposed as embryos to 0.009 µg/L (lowest test concentration), were mating with unexposed males, their egg production was significantly reduced compared to controls. Unexposed females mating with males exposed to 0.009 µg/L produced larvae with significantly increased mortality. Females exposed to 0.089 µg phenanthrene/L had a significantly reduced fertilisation success (Chen et al. 2021).

In another study the fish species marine medaka were also exposed as embryos to low doses of phenanthrene for 96 hours (F0 generation) and allowed to mate with unexposed males and females when they reached maturity. Significant malformations of the craniofacial cartilage of the next generation of larvae (F1 generation) appeared when the male had been exposed to the lowest test concentration, 0.009 µg/L, and the female to 0.089 µg/L (Table 6) (Zhang et al. 2022).

Recent research has shown that the cardiotoxic effects of phenanthrene on early life stages of cod (*Gadus morhua*) greatly depends on when during the cardiac development the exposure takes place (Sorhus et al. 2023a). Hatching of cod embryos to larvae occurs 12 days post fertilisation (dpf), and it was found that when the embryos/larvae were exposed to phenanthrene from 9 dpf to 3 days post hatching (6 days in all) more severe cardiac abnormalities were observed than when the embryos were exposed from 5 to 9 dpf.

4 and 5-ringed PAHs are also toxic to developing fish, and the effect of pyrene (4-ringed), which occurs in relatively high concentrations in the scrubber water (Table 1), is found to cause malformation of the fish heart during early development. However, symptoms are not identical to those of the 3-ringed PAHs and it is believed that the toxicity, in contrast to the 3-ringed PAHs, is linked to AhR activation (Incardona et al. 2004). A study was conducted comparing the effect of phenanthrene, pyrene and benzo[a]pyrene (a 3-ring, 4-ring and a 5-ring PAH) on developing larvae of the marine fish *Sebastiscus marmoratus* (Table 6) (Li et al. 2011). The fish larvae were exposed to the contaminants for 8 days after fertilisation, and the most sensitive endpoints were malformation of the spine of the larvae, which occurred after exposure to 1.0, 0.1 and 0.01 µg/L of phenanthrene,

pyrene and benzo[a]pyrene, respectively, and frequency of pericardial and yolk sac edema, which was detected after exposure to 1 µg/L pyrene or 0.01 µg/L benzo[a]pyrene. The lowest test concentration was 0.01 µg/L for all three compounds, and concentrations were nominal values, meaning that the actual exposure concentration could have been lower. Significant cardiotoxic effects were observed in another study on zebrafish embryos after exposure to 0.01 µg/L of pyrene (lowest test concentration) for 72 hours post fertilisation (Table 6) (Zhang et al. 2012).

Chronic exposure of zebrafish larvae to low doses of benzo[a]pyrene has been shown to have effects indicative of neurodegenerative diseases (Gao et al. 2015). After 230 days of exposure to 0.013 µg /L of benzo[a]pyrene (lowest test concentration) the swimming behaviour of the fish was significantly impaired. At 0.13 µg/L there was a significant reduction in brain weight to body weight ratio. There was no reduction in body weight between exposed and unexposed fish.

In scrubber water alkylated congeners make up around 80% of the sum of alkylated and parent PACs (Table 2). However, data on toxicity of alkylated PAHs are rare. There are studies showing that at least some alkylated PAHs are more toxic than the unsubstituted parent compound, and here phenanthrene/alkyl-phenanthrene is the most investigated homologue series (Turcotte et al. 2011, Donald et al. 2023). C1- and C2-phenanthrenes were found to be more embryotoxic (pericardial edema, yolk sac edema, hemorrhages and spinal deformities) to Japanese medaka (*Oryzias latipes*) than parent phenanthrene (C0) (Turcotte et al. 2011). EC₅₀ after 17 days exposure of the fish embryos was 96 µg/L for phenanthrene and 75 µg/L for C1-phenanthrene (just one C1-congener was tested). Three different C2-phenanthrenes were included (different positions of the alkyl groups), and their EC₅₀ were 39, 39, and 64 µg/L, respectively. EC₅₀ for C3-phenanthrene was > 36 µg/L and for C4-phenanthrene (retene) it was 61 µg/L (Turcotte et al. 2011). In a study of developmental toxicity of phenanthrene and alkyl-phenanthrenes, embryos of Atlantic haddock (*Melanogrammus aeglefinus*) were exposed for 72 hours, from 2 to 5 days after fertilisation to thirteen C1-, C2-, C3-, and C4-phenanthrenes (Donald et al. 2023). No obvious difference was observed between unsubstituted and alkyl-phenanthrenes, but all compounds induced one to several morphological defects or functional impairments as measured by a set of sixteen endpoints (e.g., yolk area, body axes deformity, eye deformities, jaw deformation, heart morphology).

In addition to studies on single PAC compounds, there are also studies on effects on early life stages of fish (e.g., survival, hatching delay, hatching success, allometry, developmental abnormalities, and DNA damage) following exposure to oil, consisting of complex mixtures of PAHs/alkyl-PAHs. The results clearly indicated that the concentrations and proportions of C1-phenanthrene and C1-anthracene were the main drivers of toxicity of the mixtures (Le Bihan et al. 2014a, Le Bihan et al. 2014b).

et al. 2014b). Strong indications that alkyl-phenanthrenes are the PAHs primarily responsible for the toxicity of crude oil to fish embryo have also been reported in other studies (Reviewed in Barron et al (2004)). Toxic effects on early life stages of fish after exposure to individual parent and alkylated PAHs are similar to what is seen after exposure to crude oil. However, the concentrations of individual PAHs causing these effects in experimental studies are much higher than their concentrations in a crude oil exposure giving rise to the same effect. The reason for this is most likely that the oil (and so also oily waters such as scrubber water) contains many more PACs with similar effects than those normally included in chemical analysis. It is also likely that mixtures of PACs are more toxic than the sum of each individual toxic compound due to synergistic effects.

The acquired knowledge of PAH toxicity in vertebrates is not reflected in research on the effect of PAHs on invertebrates. To fish, 3-ringed PACs, specifically phenanthrene and dibenzothiophene, are found to be among the most toxic compounds in an oil spill, causing cardiac toxicity and other malformations in early life stages. Since these compounds are among the PAHs/PACs occurring at the highest concentration in scrubber water they are of great interest in the understanding of scrubber water toxicity. Studies of these compounds in invertebrates and at relevant concentrations are however limited.

Exposing adult females of copepods of the species *Acartia tonsa* to phenanthrene, and to the 4-ringed PAHs fluoranthene and pyrene resulted in toxic effects on the number of eggs and the time to hatching of the eggs (Bellas and Thor 2007). Pyrene was the most toxic of the compounds with the lowest EC₂₀ being 22 µg/L for an effect on egg production, followed by fluoranthene (EC₂₀ = 46 µg/L) and phenanthrene (EC₂₀ = 124 µg/L) (Table 7).

In another study, the early life stages of a mussel (*Mytilus galloprovincialis*), a sea urchin (*Paracentrotus lividus*), and an ascidian (*Ciona intestinalis*) were exposed to naphthalene, fluorene, phenanthrene, fluoranthene, and pyrene, from the stage of newly fertilised eggs until a specific larval stage (20 – 48 hours depending on the species) (Bellas et al. 2008). The toxicity, measured as EC₁₀ (where 10% of the exposed population is affected) was not consistent between the PAH compounds and the species. Phenanthrene was most toxic to the mussel larvae, with an EC₁₀ of 29 µg/L, whereas fluoranthene was most toxic to the sea urchin larvae, with an EC₁₀ of 27 µg/L (Table 7). Thereafter followed pyrene with an EC₁₀ of 69 µg/L in sea urchin larvae, and 94 µg/L in mussels. Ascidian larvae appeared to be the least sensitive of the three species to phenanthrene, fluoranthene and pyrene, but more sensitive than the mussel and the sea urchin to naphthalene. Mussel larvae were found to be very tolerant to naphthalene with an EC₁₀ of 4035 µg/L (Table 7).

The effect of phenanthrene was tested on larval development rate of some marine invertebrate species. The nauplii (nauplii = the first larval stage), of the copepod species *Gladioferens pectinatus* were exposed for six days to phenanthrene, and

concentration causing a reduction in the larval development rate, measured as EC₂₀ (the concentration where 20% of an exposed population is affected), was determined to 12.6 µg/L of phenanthrene (Table 7) (Charry et al. 2019).

A series of experimental studies have been carried out to investigate the effect of pyrene on three key species in the Arctic pelagic ecosystems, the copepods *Calanus glacialis* and *C. hyperboreus*, which are truly Arctic, and *C. finmarchicus* which is a North Atlantic species, but all three species co-exist in the Arctic. All species build up large lipid reserves over the productive season and overwinter in resting stages, but whereas *C. hyperboreus* produces eggs from the lipid reserve during the winter season, *C. glacialis* and *C. finmarchicus* start producing eggs only after the onset of feeding in the spring.

Two separate long term studies were carried out where *C. glacialis* (Toxvaerd et al. 2018) and *C. hyperboreus* (Toxværd et al. 2019) were exposed to a single pulse dose of pyrene during their overwintering period, 103 days for *C. glacialis* and 88 days for *C. hyperboreus*. After exposure the animals were moved to clean water and supplied with food. Pyrene exposed individuals of both species had a significantly reduced capacity to rebuild new lipid reserves already at the lowest test concentration, 0.2 µg/L of pyrene. For *C. glacialis*, there was a concentration dependent decrease in survival of the animals from 0.2 to 60 µg/L of pyrene (Table 7) (Toxvaerd et al. 2018). A similar pattern was seen for the feeding rate (measured as the production of faecal pellets), and egg production, where there was a reduction already at 0.2 µg/L of pyrene. *C. hyperboreus* had no apparent reduction in egg production, but egg hatching success and feeding rate were both reduced at exposure to the lowest test concentration, 0.2 µg/L (Toxværd et al. 2019). The toxic effects observed in these two studies occurred at much lower concentration than in other short-term studies. This is probably because during a long-term exposure the compounds are accumulating in the lipids of the animals, and the exposure is hence not only from the external water. Data on *C. glacialis* was used to calculate LC₅₀ (after 103 days of pyrene exposure + 32 days recovery in clean water), and EC₅₀ at the same time for feeding rate (measured as faecal pellet production) and egg production rate. LC₅₀ was estimated to 5.6 µg/L, and EC₅₀ for both feeding rate and egg production to 0.2 µg/L (Table 7) (Toxvaerd et al. 2018).

There is no data on long-term exposure of *C. finmarchicus* to pyrene, but in a study where copepods were exposed to pyrene for 7 – 9 days, the feeding rate of adult *C. finmarchicus* was significantly reduced at a concentration of 20 µg/L of pyrene, whereas this concentration had no effect either on feeding rate or on egg hatching of *C. glacialis* (Hjorth and Gissel Nielsen 2011). The findings on *C. glacialis* are hence different from those in the long-term exposure study by Toxvaerd et al. (2018) where a reduced egg production and a reduced feeding rate was found already at 0.2 µg/L.

Effects on the egg hatching and naupliar development were investigated in *C. finmarchicus* and *C. glacialis* after exposure to pyrene from the stage of fertilised

egg throughout all naupliar stages (NI - NIV) (Grenvald et al. 2013). Increased naupliar mortality in both species *C. glacialis* occurred after exposure to 20 µg/L of pyrene (Table 7). No effect on egg hatching or hatching success was observed. This is in line with findings in Toxvaerd et al. (2018) where no effect on egg hatching was observed after exposure to pyrene for 103 days.

Studies on effects of alkylated PAHs on any invertebrate species are rare. One of the few available is a study on mortality of nauplii larvae of the brine shrimp, *Artemia parthenogenetica*, a common test species in standardised ecotoxicological experiments, where mortality rate was analysed after exposure to phenanthrene, C3-phenanthrene (3-methylphenanthrene), and C4-phenanthrene (retene/7-isopropyl-1-methyl phenanthrene), as well as anthracene and C2-anthracene (2-methylantracene) (Cong et al. 2021). The animals were also exposed to benzo[a]pyrene as a "reference" substance. No clear pattern on toxicity between parent and alkylated congeners was found. Whereas C4-phenanthrene (retene) was more toxic than the parent compound phenanthrene, C3-phenanthrene was less toxic. C2-anthracene was more toxic than anthracene, which was found to have no effect on the animals. Benzo[a]pyrene was by far the most toxic of all compounds. Expressed as LC₁₀ (death of 10% of the exposed population) after 7 days exposure, the sensitivity ranked as follows: benzo[a]pyrene (0.027 µg/L) > C4-phenanthrene (retene, 3.43 µg/L) > phenanthrene (9.76 µg/L) > C3-phenanthrene (69.6 µg/L) > C2-anthracene (87.2 µg/L) > anthracene (>402 µg/L) (Table 7).

Growth inhibition of phytoplankton is a frequently used endpoint when assessing the toxicity of compounds or mixtures of compounds, but data on toxic effects of individual PAHs on phytoplankton are rare. In a study of the effect of the PAHs fluoranthene and benzo[a]anthracene on the growth, measured as photosynthetic efficiency, of seven phytoplankton species, the sensitivity was found to vary largely between species, and it was not consistent between the two compounds (Ben Othman et al. 2012). Overall, fluoranthene was the more toxic, although the variation of the LOEC was large: between 16.1 and 161 µg/L for fluoranthene, and between 1.8 and 1825 µg/L for benzo[a]anthracene (Table 7). *Isochrysis galbana* was by far the most sensitive species to benzo[a]anthracene, with LOEC of 1.8 µg/L, however, this species was less sensitive to fluoranthene. *Nannochloris* sp. and *Picochlorum* sp. were the most sensitive species to fluoranthene (LOEC = 54.0 and 83.5 µg/L, respectively), and they were the second most sensitive to benzo[a]anthracene (LOEC = 57.6 µg/L).

EC₅₀ for growth inhibition of the algae *Raphidocelis subcapitata* was investigated for ten alkylated PAHs, four C1-, four C2-, and two C3-congeners of naphthalene, phenanthrene, anthracene, and pyrene (Kang et al. 2016). Values could only be determined for five of the tested compounds, and one of the C1-pyrene was found to be the most toxic to the algae with an EC₅₀ of 82 µg/L, followed by a C1-phenanthrene and a C1-anthracene (EC₅₀ = 280 and 300 µg/L, respectively) (Table 7).

Table 6 PAH toxicity to fish expressed as LOEC (lowest concentration with a statistically significant effect compared to control). "Test species" indicate the life stage that was exposed to the contaminant and effects were manifested in early life stages unless otherwise indicated.

PAH congener/ test species	Effect and LOEC	Reference
Phenanthrene Fish larvae	Exposure time 96 hours Fewer mature spermatozoa in adult male: 0.089 µg/L Fewer mature oocytes in adult female: 0.89 µg/L	(Chen et al. 2021)
Phenanthrene Fish larvae	Exposure time: 28 days Edema in yolk sac and cardium, delayed hatching: 2 µg/L Impaired hatching success: 10 µg/L	(Zheng et al. 2020)
Phenanthrene/Fish larvae	Exposure time : 8 days Increased mortality: 0.1 µg/L Increased frequency of dorsal curvature: 1.0 µg/L	(Li et al. 2011)
Phenanthrene/Fish larvae	Exposure time: 72 hours Morphological changes of retina: 3.56 µg/L Exposure time 7 days: Impaired phototactic response: 3.56 µg/L	(Huang et al. 2013)
Pyrene/Fish embryo	Exposure time: 72 hours Increased frequency of heart malformation: 0.01 µg/L*	(Zhang et al. 2012)
Pyrene/Fish larvae	Exposure time: 8 days Increased mortality: 1.0 µg/L Increased frequency of dorsal curvature: 0.1 µg/L Pericardial and yolk sac edema: 1.0 µg/L	(Li et al. 2011)
Benzo[a]pyrene/Fish larvae	Exposure time: 8 days Increased mortality: 0.1 µg/L Increased frequency of dorsal curvature: 0.01 µg/L* Pericardial and yolk sac edema: 0.01 µg/L*	(Li et al. 2011)
Benzo[a]pyrene/	Exposure time: 230 days Altered swimming behaviour: 0.013 µg/L* Reduced brain/body weight ratio: 0.13 µg/L	(Gao et al. 2015)
<i>Exposure of embryos in the F0 generation and effects analysed in the offspring (F1 generation) of exposed individuals</i>		
Phenanthrene Fish embryo/larvae	Exposure time: 72 – 96 hours Fewer eggs, increased larval mortality: 0.009 µg/L* Reduced fertilisation success: 0.089 µg/L	(Chen et al. 2021)
Phenanthrene/Fish embryo/ larvae	Exposure time: 72 hours Craniofacial malformation: 0.009 µg/L* Survival & dorsal malformation: 0.89 µg/L	(Zhang et al. 2022)

* Effects detected at the lowest test concentration.

Table 7 PAH toxicity to invertebrates expressed as LOEC, EC and LC values (only average values are presented).

Test species/PAH congener	Toxicity	Reference
<i>Acartia tonsa</i> (copepod) Phenanthrene Fluoranthene Pyrene	Egg production, EC ₁₀ 48h: 124 µg/L 46 µg/L 22 µg/L	(Bellas and Thor 2007)
<i>Acartia tonsa</i> (copepod) Phenanthrene Fluoranthene Pyrene	Egg hatching, EC ₁₀ 48h: 187 µg/L 41 µg/L 45 µg/L	(Bellas and Thor 2007)
<i>Mytilus galloprovincialis</i> (mussel) Naphthalene Phenanthrene Fluoranthene Pyrene	Larval development, EC ₁₀ , 48h: 4035 µg/L 29 µg/L >253 µg/L 94 µg/L	(Bellas et al. 2008)
<i>Paracentrotus lividus</i> (sea urchin) Naphthalene Fluorene Phenanthrene Fluoranthene Pyrene	Larval development, EC ₁₀ , 48h: 646 µg/L 492 µg/L 460 µg/L 27 µg/L 69 µg/L	(Bellas et al. 2008)
<i>Ciona intestinalis</i> (ascidian) Naphthalene Phenanthrene Fluoranthene Pyrene	Larval development, EC ₁₀ , 20h: 609 µg/L >427 µg/L >253 µg/L >129 µg/L	(Bellas et al. 2008)
<i>Gladioferens pectinatus</i> (copepod) Phenanthrene	Nauplii development, EC ₂₀ , 6 days: 12.6 µg/L	(Charry et al. 2019)
<i>Calanus finmarchicus</i> Pyrene	Naupliar mortality (exposure from egg to nauplii), LOEC: 20 µg/L	(Grenvald et al. 2013)
<i>Calanus finmarchicus</i> Pyrene	Feeding rate, 7 days exposure of adults, LOEC: 20 µg/L	(Hjorth and Gissel Nielsen 2011)
<i>Calanus glacialis</i> Pyrene	103 d exposure of adults, analysed 135 days after start of exposure, LC ₅₀ : 5.61 µg/L	(Toxvaerd et al. 2018)
<i>Calanus glacialis</i> Pyrene, single pulse dose at beginning of exposure	103 d exposure of adults, analysed 135 d after start of exposure, LOEC: Egg production: 0.2 µg/L Feeding rate: 0.2 µg/L Reduced rebuilding of lipid reserve: 0.2 µg/L	(Toxvaerd et al. 2018)
<i>Calanus hyperborealis</i> Pyrene, single pulse dose at beginning of exposure	LOEC, 88 d exposure of adults, analysed until 17 d after start of exposure: Egg hatching success: 0.2 µg/L Feeding rate: 0.2 µg/L Reduced rebuilding of lipid reserve: 0.2 µg/L	(Toxvaerd et al. 2019)

<i>Artemia parthenogenetica</i>	LC ₁₀ , 7 days	(Cong et al. 2021)
Phenanthrene	9.76 µg/L	
C3-phenanthrene	69.6 µg/L	
C4-phenanthrene (retene)	3.42 µg/L	
Anthracene	>402 µg/L	
C2-anthracene	87.2 µg/L	
Benzo[a]pyrene	0.027 µg/L	
Phytoplankton, Fluoranthene/ benzo[a]anthracene Fla/(b[a]a):	Fla/b[a]ant, Growth inhibition, LOEC 72h	(Ben Othman et al. 2012)
<i>Nannochloris sp.</i>	16.1/57.6 µg/L	
<i>Picochlorum sp.</i>	50.7/57.6 µg/L	
<i>Isochrysis galbana</i>	161/1.8 µg/L	
<i>Dunaliella tertiolecta</i>	50.7/182 µg/L	
<i>Chaetoceros muelleri</i>	161/576 µg/L	
<i>Phaeodactylum tricornutum</i>	161/1852 µg/L	
<i>Alexandrium catenella</i>	No sign. effect/no sign. effect	
<i>Raphidocelis subcapitata</i> , (phytoplankton)	Growth inhibition, EC ₅₀	(Kang et al. 2016)
C3 (1,4,5-methyl)-naphthalene	35–75 h (until steady state)	
C3 (2,4,5-methyl)-naphthalene	490 µg/L	
C1-phenanthrene	470 µg/L	
C1-anthracene	280 µg/L	
C1-pyrene	300 µg/L	
	82 µg/L	

* Effects detected at the lowest test concentration

7.2 Toxicity of oxy-PAH

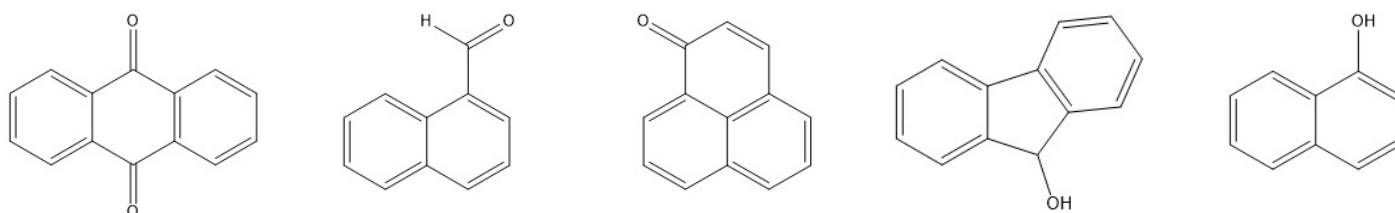


Figure 8 The oxy-PAHs detected in scrubber water from an open-loop system, from top left to right: 9,10-anthracenedione, naphthaldehyde, and phenalene-1-one, and from bottom left to right: 9-hydroxyfluorene and naphthol.

Five oxy-PAHs were detected in the non-target screening of scrubber water: 9,10-anthracenedione (synonym: anthraquinone), naphthaldehyde (synonyms; 1-formylnaphthalene; 1-naphthalene carboxaldehyde), phenalene-1-one (synonyms: phenalenone, 1H-benzonaphthene-1-one), 9-hydroxyfluorene (synonym: fluorenol), and naphthol (synonyms: naphthalen-1-ol, hydroxynaphthalene) (Fig. 8). As these were detected in the non-target screening, it was only possible to quantify naphthaldehyde. Field studies have revealed that concentrations of many oxy-PAHs are equal to, or may exceed, that of the corresponding unsubstituted PAH in collected samples of mussels and sediment (Brorström-Lunden et al. 2010, Layshock et al. 2010, De Witte et al. 2019).

Toxicity of these compounds is poorly investigated. In contrast to the parent PAHs, where genotoxicity is elicited by reactive PAH metabolites, the oxy-PAHs do not need enzymatic activation but can combine directly with DNA, RNA, and proteins once they have been taken up by an organism (Idowu et al. 2019, Qiao 2022). In vitro tests with eight commonly occurring oxy-PAHs, among them phenalene-1-one, showed that all compounds induce genotoxic effects at a similar or higher level than benzo[a]pyrene (McCarrick et al. 2019).

In another study, the non-genotoxic effects of 38 commonly occurring oxy-PAHs, including phenalene-1-one and 9-hydroxyfluorene and 9,10-anthracenedione, were tested on zebrafish embryos (Knecht et al. 2013). The embryos were exposed from 6 to 120 hours post fertilisation and a range of endpoints were analysed, including mortality, developmental progress, yolk sac edema, pericardial edema, morphology of the jaw, the eye and the body axes, fin malformation, pigment, swim bladder, and touch response. Eighteen of the tested oxy-PAHs had serious toxic effects and seventeen had moderate effects on one or several endpoints. Phenalene-1-one was among the most toxic compounds with severe effect on 8 endpoints and moderate effect on 5 endpoints at a concentration of 3,640 µg/L. 9-Hydroxyfluorene was slightly toxic at the highest test concentration, 18,200 µg/L. 9,10-Anthracenedione was among the least toxic of the tested compounds, with no effect on any endpoints, not even at the highest test concentration of 21,100 µg/L.

The toxicity of 9-hydroxyfluorene was tested on two freshwater species, the algae *Scenedesmus subspicatus* and the crustacean *Daphnia magna* and the EC₅₀s were found to be 710 µg/L and 240 µg/L, respectively (Sepic et al. 2003).

Toxicity of 1-naphthol has been tested in a few studies on five species of freshwater fish. LC₅₀ 96 hours was found to vary between 0.33 and 4.3 mg/L (Tilak et al. 1981, Mohana Rao et al. 1984).

7.3 Toxicity of heterocyclic PAC; S-PAC

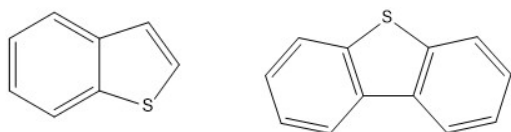


Figure 9 The two S-heterocyclic PACs benzothiophene (left,) and dibenzothiophene (right) detected in scrubber water from an open-loop system.

Two homologue series of sulphur-PACs were analysed in the scrubber water, benzothiophene and dibenzothiophene (Fig. 9). For benzothiophene, only alkylated compounds (C1-C4) were detected and for dibenzothiophene both the parent and

alkylated forms (C1-C3) were found (Tables 3 and 4, Fig. 4B and 5). Some toxic effects of dibenzothiophene resembles that of the 3-ringed PAH phenanthrene and is discussed also in the previous section, *8.1 Toxicity of unsubstituted PAH and alkylated PAH*. Like the 3-ringed PAH phenanthrene, dibenzothiophene is found to be embryotoxic to fish and cause malformation of spine, yolk sac edema, pericardial edema, and eye deformation (Incardona et al. 2004, 2005, (Rhodes et al. 2005). In an experiment with embryos of the Japanese medaka (*Oryzias latipes*), the lowest dibenzothiophene concentration producing a significant effect (LOEC), defined as a reduced number of normal larva (defined as larvae with insignificant malformations), was 25 µg/L (Rhodes et al. 2005). The LOEC for an alkylated dibenzothiophene (C2-dibenzothiophene) in the same study was higher, 200 µg/L.

The mechanism behind dibenzothiophene toxicity on fish embryos is independent of AhR activation and CYP1A induction, but in a study on the fish mummichog (*Fundulus heteroclitus*) an enhanced (synergistic) effect was found when the dibenzothiophene co-occurred with an AhR activating/CYP1A inducing compound (Wassenberg et al. 2005). Unlike in studies on zebrafish and Japanese medaka (Incardona et al. 2004, Rhodes et al. 2005), Wassenberg et al. (2005) found no effect of dibenzothiophene on the fish embryos when the exposure did not also include a CYP1A inducing substance.

Toxicity of several nitrogen, sulphur, and oxygen containing heterocyclic PACs, among them benzothiophene, were tested in a series of experiments on zebrafish embryos (Peddinghaus et al. 2012). The exposure lasted for 48 hours, starting 3 hours after fertilisation, and toxicity was measured as mortality. LC₅₀, based on nominal concentrations of the toxicants, was 12,980 µg/L.

7.4 Toxicity of heterocyclic PAC; N-PAC

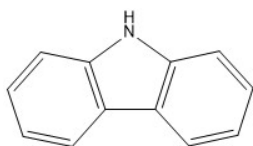


Figure 10 The N-heterocyclic compound carbazole detected in scrubber water from an open-loop system.

The N-heterocyclic compounds detected in the scrubber water were parent and alkylated (C1- C4) carbazoles (Tables 3 and 4, Fig. 5 and 10).

One of few studies on carbazole toxicity found mortality of zebra fish embryo, measured as LC₅₀ after 48 hours exposure, to be 1070 µg/L (Peddinghaus et al.

2012). In a study of the impact of eight N-heterocyclic PACs on the growth rate of a freshwater green algae, *Scenedesmus acuminatus*, no effect was observed for carbazole at the applied exposure concentration and over the exposure period of 96 hours ($EC_{10} > 400 \mu\text{g/L}$) (van Vlaardingen et al. 1996). Four other 3-ringed N-heterocyclic PACs were considerably more toxic: acridine, $EC_{10} = 0.32 \text{ mg/L}$, benzo[f]quinoline, $EC_{10} = 1.55 \text{ mg/L}$, phenanthridine, $EC_{10} = 5.24 \text{ mg/L}$, and benzo[h]quinoline, $EC_{10} = 6.65 \text{ mg/L}$.

Similar to dibenzothiophene the toxicity of carbazole to fish embryos is independent of AhR activation and CYP1A induction, but the toxicity is enhanced in the presence of another AhR activating/CYP1A inducing compound (Wassenberg et al. 2005). This synergistic effect was however found to be less pronounced than for dibenzothiophene.

7.5 Toxicity of heterocyclic PAC; O-PAC

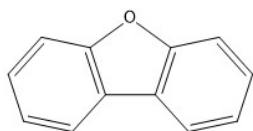


Figure 11 The O-heterocyclic compound dibenzofuran detected in scrubber water from an open-loop system.

Two O-heterocyclic compounds, dibenzofuran (Fig. 11) and 2-methyldibenzofuran were detected in scrubber water (Tables 3 and 4, Fig. 5).

There are to our knowledge no available data on aquatic toxicity of parent or alkylated forms of dibenzofuran in their original forms. However, exposure of PAHs in general to UV radiation in sunlight leads to the formation of reactive oxygen species that cause oxidative damage in biological systems (Arfsten et al. 1996). Toxicity of photoinduced dibenzofuran has been demonstrated in the marine bacteria *Vibrio fischeri*, manifested as inhibition in the bacterial bioluminescence (Shemer and Linden 2007).

8. Ecological implications of discharged scrubber water on the Baltic Sea ecosystem

When making risk assessments of discharges of a wastewater to the ocean it is essential to have access to good experimental ecotoxicological data in combination with expected (modelled or measured) pollutant concentrations in the recipient water. However, a third aspect that is crucial but almost completely neglected, is the need for ecological interpretation of the obtained information. What will the consequences be if a copepod species has a slightly reduced reproduction potential, or if the proportion of fish larvae with malformed hearts or spines increases? What are the effects on a larger scale, in specific food webs or entire ecosystems? These questions could be asked from a short-term human perspective - will there be consequences for marine species caught for human consumption? They could also be asked from the perspective of the marine organisms themselves. In a long-term perspective these two perspectives will of course always merge. The survival of humankind is dependent on the survival of the marine food webs.

It has been claimed in some reports that scrubber water and other wastewaters from shipping once discharged into the ocean will quickly be diluted to the point where they are harmless (Kjølholt et al. 2012, IMO; MEPC 74/INF.24 2018). Data from the present study indicate that this is far from the case. Several toxic compounds were detected in the scrubber water and with continuous discharge of scrubber water to the sea, it is likely that elevated concentrations of these can be expected along ship lanes. Long stretches of ship lanes run close to shorelines, not least in the Baltic Sea. These shallow waters are among the most productive areas of the ocean, a permanent home to numerous species of invertebrates and fish, and nursery ground for the early life stages of many pelagic fish species such as cod and herring. The shallow coastal areas in southern Baltic Proper where all ship traffic in and out of the Baltic Sea run close to land, also serve as stopover for vast amounts of migrating birds that also feed on marine species. Many coastal species like mussels, oysters, and crayfish are used for human food consumption and serve as food for other species consumed by humans. It is not unlikely that a continuous supply of PACs and other contaminants in scrubber water to these areas has an impact on these ecosystems.

It is also important to understand that the organisms in the pelagic zone of the ocean are not stationary, but often cross large areas of water, by passive dispersion driven by wind, waves, and sea currents or by active swimming. The ship lanes cut

like motorways through these areas where pelagic organisms drift or swim, many of them in their most sensitive embryonic or larval stages. Many of the invertebrate species have pelagic larval stages that last for several weeks. During that time, they can be transported long distances and are at great risk of crossing shipping lanes many times. The blue mussel (*Mytilus edulis*), a key stone species in most Scandinavian waters, stays as larvae for up to six weeks, and genetic analyses have shown that in the Baltic Sea the mussel larvae may disperse over either the entire Baltic Proper or the entire Bothnian Sea. The only barrier that cannot be crossed is the Åland archipelago (Larsson et al. 2017). Species spending their entire life span in the pelagic, such as the copepods that serve as a staple food are running the risk of drifting over ship lanes at any part of the life cycle. Copepods serve as staple food for herring and cod and declines in copepod populations have major impact on fish stocks (Beaugrand et al. 2003, Heath and Lough 2007, Stige et al. 2011). The Baltic cod reproduce at spawning sites in the central Baltic Proper, and as the fertilised eggs and newly hatched larvae are transported to the nursery areas by the coast (Bagge et al 1994), they cannot avoid crossing the busiest ship lanes in the Baltic Sea (HELCOM 2023. HELCOM Map and Data service. Layers: HELCOM AIS Shipping density maps. <https://helcom.fi/baltic-sea-trends/data-maps/>; (Bagge 1993) (Fig. 12). If the water in the shipping lanes is polluted enough there is a risk that they will serve as proper barriers for pelagic marine organisms and block the natural migration between sea areas.

When IMO has taken decisions to reduce the maximum allowed sulphur content in ship fuel, certain areas have been considered to be particularly sensitive to the air emissions and therefore been designated as SECAs within the MARPOL's Annex VI which deals with prevention of air pollution. It has been decided that the sulphur content in ship fuel in these areas could be no higher than 0.1% instead of 0.5% as the regulation is in non-SECAs. In practice, this means that the scrubber water produced in SECAs will be more polluted than water produced in non-SECAs due to a larger amount of scrubber water being discharged to the SECAs. The Baltic Sea is one of these SECAs, but the irony is that IMO also has designated the Baltic Sea as a Particularly Sensitive Sea Area (PSSA) (IMO Resolution MEPC.136(53), 2005) which is defined by IMO as "an area that needs special protection through action by IMO because of its significance for recognised ecological, socio-economic, or scientific reasons and which may be vulnerable to damage by international maritime activities" (IMO, Resolution A.982(24); IMO web page: [Particularly Sensitive Sea Areas \(imo.org\)](https://www.imo.org/en/About/Pages/Particularly-Sensitive-Sea-Areas.aspx)). So, while the IMO formulated the SECA classification aimed at protecting the terrestrial environment and human health, the PSSA classification aiming at protection of the marine environment has been ignored. Due to the brackish water conditions in the Baltic, the ecosystems are naturally sensitive. Here are fewer species which make the ecosystem functions less resilient than truly marine ecosystems where the biodiversity is much higher (Magnusson and Norén 2012). In addition, it is an enclosed sea in a highly

industrialised area and even before the introduction of scrubbers it was often claimed to be one of the world's most polluted sea areas (HELCOM 2018, Stockholm International Water Institute 2018). What the Baltic Sea ecosystems need is a removal of pollutant sources instead of introduction of new ones.

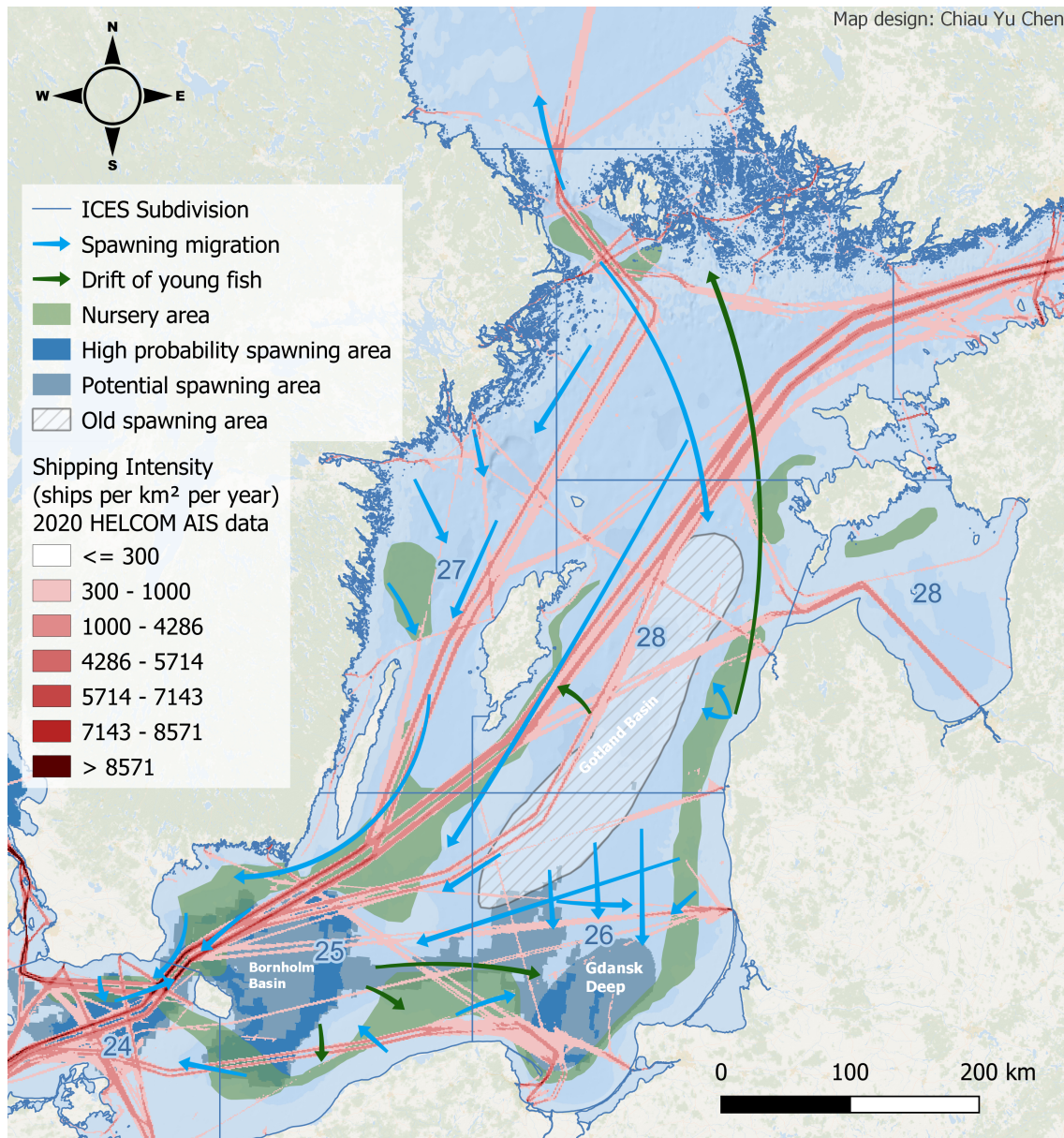


Figure 12 Shipping lanes (red lines) crossing spawning (dark blue) and nursery areas (green) for cod in the Baltic Proper. The colour intensity of the red lines indicates the number of ships per km², and year based on 2020 HELCOM AIS data.

9. Recommendations for the future

***Better communication between researchers, environmental agencies, and the shipping industry**

There is today a lack of communication between different scientific disciplines, and between scientists, the shipping industry, and authorities, which leads to situations where important existing research data, in this case data on PACs toxicity, are not being taken into account in decision-making. There is a great need for more efficient transfer of knowledge between researchers working on the toxic mechanisms of PACs and those conducting experiment aimed for risk assessments, and also between the scientific community, national and international environmental and transportation agencies, the shipping industry and other relevant stakeholders.

***Risk assessments of scrubber water should be based on tests with whole scrubber water, but selection of relevant endpoints should be based on chemical composition of the water and toxicity data on detected compounds**

Scrubber water contains a complex mixture of compounds that may be toxic by themselves but also interact with each other and cause synergistic effects. The best method to test the toxicity of scrubber water, and other complex wastewaters (sometimes also called whole effluent toxicity, WET), is therefore to perform tests on whole scrubber water. However, in order to select relevant and sensitive endpoints in the scrubber water tests, knowledge on what compounds the water contains and what toxic effects these compounds have is needed. Refinement of analytical methods so that a wider spectrum of PACs may be detected together with more basic research on toxicological effects of PACs on marine species to derive more refined LOECs and ECs is therefore crucial.

***Endpoints in ecotoxicological testing of scrubber water should be based on the most sensitive endpoints that have an impact on a population level**

Most standardised ecotoxicological protocols today are overly simplistic and there is a risk that the data generated underestimate the environmental risk of the test compounds/mixture of compounds. Endpoints in ecotoxicological tests used for risk assessment should be based on effects that are likely to have an impact on the population well-being, and the review of PAC toxicity presented in this report shows

that there are many physiological endpoints that meet this criterion and that are more sensitive than those commonly used today. Much interesting research is also done on a genetic or molecular level where the impact on the organism is still unknown. These findings may not be applicable for the applied ecotoxicological tests of today but may form the basis for even more sophisticated endpoints to be used in the future.

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