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Mytilus edulis and *Psammechinus miliaris* as bioindicators of ecotoxicological risk by maritime exhaust gas scrubber water

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ABSTRACT

Approximately 15 % of the global anthropogenic emissions of sulfur oxides (SOx) come from shipping. To meet sulfur emission regulations for marine vessels, many shipping companies have chosen to use exhaust gas cleaning systems (EGCS), commonly known as scrubbers. The resulting washwater from scrubbers contains various pollutants such as polycyclic aromatic hydrocarbons (PAHs), trace metals, and nitrates, is then directly discharged into the surrounding surface water, transferring potential atmospheric pollutants to the marine environment. The aim of this study was to investigate the impact of EGCS discharge water on blue mussels (*Mytilus edulis*) and sea-urchin (*Psammechinus miliaris*) embryos and larvae. Chronic toxicity tests were performed using a fertilization test and a larval development bioassay exposed to scrubber water dilutions (0.001, 0.01, 0.1, 1, 2, 5, 10, 20, 40 and 100 % of the original sample). Negative effects on fertilization success and larvae development in both species at very low concentrations were observed ($EC_{10} < 1$ %) indicating the severe impact of EGCS discharges on these species. EGCS effluents showed different effects depending on the species and life stages. Sea urchin embryos were more sensitive than the blue mussel embryos. However, blue mussel larvae were much more sensitive than sea urchin larvae. These results emphasize the potential toxic effects of direct exposure -not dietary- to scrubber water discharges on marine invertebrate. EGCS discharge limits are urgent to prevent further potentially irreversible damage to the marine environment.

1. Introduction

Marine shipping is expected to be responsible for 5–15 % of global anthropogenic SOx emissions contributing to the release of several airborne pollutants, such as particulate matter (PM), sulfur oxides (SOx), and nitrogen oxides (NOx) (Viana et al., 2014; Lindstad and Eskeland, 2016; Sofiev et al., 2018; Sokhi et al., 2021). The International Maritime Organization introduced Annex VI (air pollution) to the International Convention for the Prevention of Marine Pollution from Ships, which establishes limits to the sulfur emissions permitted from marine vessels (Endres et al., 2018). To comply with these limits, marine ships can adopt the use of low-sulfur fuel oil. However, due to the increased costs of these higher-quality fuels, many shipping companies opt for the use of exhaust gas cleaning systems (also known as 'scrubbers') that offer a viable solution at a lower economic cost (Endres et al., 2018; Teuchies et al., 2020). Scrubbers remove up to 95 % of SOx from the exhaust gas before they are emitted to the atmosphere (Andreasen and Mayer, 2007). They also reduce fresh and non-volatile ultrafine particle number (PN) and polycyclic aromatic hydrocarbons (PAHs) (Kuittinen et al., 2024) suggesting potential benefits in terms of air quality.

Scrubbers are engineered to function in either a closed or open mode. In the closed-loop system, more of the exhaust gas content is captured and retained onboard the ship, with the residue discharged in port, where it's later integrated into cement or asphalt (Endres et al., 2018; Teuchies et al., 2020). Conversely, the open-loop scrubber system utilize seawater to spray exhaust gases to remove SOx, and the resulting washwater is directly discharged into the surrounding surface water (Comer et al., 2020; Teuchies et al., 2020; Ytreberg et al., 2019). Considering that there was one vessel fitted with scrubbing systems in 2007, the number reached 4,737 b y 2022 and could surpassed 5,000 units by 2024 (Ship & Bunker, 2019), it could be expected that ships fitted with EGCS will increase marginally in the coming years. On a

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global scale, most ships equipped with scrubbers utilize the open-loop system (Endres et al., 2018; Teuchies et al., 2020), although in certain Sulfur Emission Control Areas (SECAs), closed and hybrid scrubbers are more prevalent.

Remnant washwater is discharged into the surrounding surface water at a flow rate between 200 and 500L/s for a 15 MW vessel from an open-loop scrubber, and only ~0.5–3L/s for a 15 MW vessel from a closed-loop scrubber due to the recirculation process (Teuchies et al., 2020). The washwater generated can contain a variety of pollutants, including PAHs, metals and nitrates, thus displacing potential atmospheric pollutants to the marine environment (USEPA, 2011; Lunde Hermansson et al., 2021). The chemical composition and concentrations of contaminants in discharged scrubber water vary based on factors such as the scrubber type, design, effectiveness in removing contaminants, fuel and lubricant oil composition, and the ship's operating conditions (Hassellöv et al., 2020). Several PAHs - such as anthracene, phenanthrene, naphthalene, fluorene and fluoranthene - have been identified in multiple studies (Magnusson et al., 2018; Schmolke et al., 2020), and may originate from the fuel itself or the fuel combustion process. Metals are an important component of scrubber water with the highest recorded concentrations reported as vanadium (V), zinc (Zn), copper (Cu) and nickel (Ni) (Teuchies et al., 2020). Vanadium and nickel originate from, and according to previous studies, correlate strongly with, the sulfur content of the used fuel (Hansen and Aalborg, 2012; Teuchies et al., 2020). Carnival corporation & PLC (2019) found similar metals in their washwater effluents, with additional metals such as lead (Pb) and chromium (Cr) present at lower concentrations.

Research examining the constituents of scrubber discharge indicates that employing scrubbers directly contradicts the objectives of the Water Framework Directive aimed at attaining a good environmental condition in European waters (Lunde Hermansson et al., 2021). Some preliminary studies have demonstrated that contaminants present in scrubber discharge water could induce biological effects at low concentrations causing lethal and sub-lethal effects (Koski et al., 2017; Magnusson et al., 2018; Ytreberg et al., 2019). Negative effects of scrubber water discharges on plankton have been reported with more toxic effects compared to single compounds (Koski et al., 2017). This indicates synergistic effects of the scrubber discharge water mixture, particularly with direct - not dietary - exposure to discharge water (Koski et al., 2017). If this is a common situation for organisms at the basal levels of the food web, it could have substantial consequences for the food web structure and ecosystem functioning.

For many aquatic species, early life stages are the most vulnerable to environmental stressors, and therefore embryos and larvae are commonly used for toxicity tests to determine the sensitivity of organisms to pollutants (Connor, 1972; Martin et al., 1981). Marine invertebrate larvaes are ideal test models due to their high sensitivity to pollutants, ecological importance, vital ecological niches, susceptibility to uptake of pollutants, and close connection with marine predators and human health. Embryos and larvae are less tolerant to toxic compounds than adults of the same species and therefore represent critical life stages for toxicity tests (Connor, 1972; Martin et al., 1981). For this reason, embryo-larval bioassays have proved to be very sensitive indicators of seawater contamination (Annamaria et al., 2005; Bay et al., 1983; Beaumont et al., 1987).

Despite the rapid rise in scrubber usage, the impact of scrubber effluent on the marine ecosystem remains uncertain (Lange and Markus, 2015). Prior to the decision, there was serious neglect as no risk assessments were conducted to evaluate the impact of the discharged washwater from the scrubbers on marine ecosystems. However, evidence now shows that adverse effects may be affecting marine organisms exposed to these discharges. Marine invertebrates play a crucial role in maintaining ecosystem functionality, with certain species identified as bioindicators due to their ecological significance, trophic interactions, well-characterized biology, and feasibility for collection and experimental investigation in both field and laboratory environments. Invertebrate larvae, alongside other taxa, constitute the foundation of marine food webs, often occupying key positions as primary producers and serving as essential energy sources within trophic networks. Due to their high vulnerability to pollutants during embryonic development and larval stages, these organisms are valuable for ecotoxicological studies (Annamaria et al., 2005; Morroni et al., 2023) and serve as bioindicators, as they spend a critical part of their life cycle in the water column. To test this hypothesis, we exposed blue mussel (*Mytilus edulis*) and sea urchin (*Psammechinus miliaris*) larvae to scrubber water from an open-loop EGCS and assessed its effects on their development.

This study provides essential data for evaluating the ecological risks associated with the increasing use of scrubbers, offering critical insights into their potential impact on marine larvae and contributing to a more comprehensive understanding of pollution-induced stress in marine ecosystems.

2. Materials and methods

2.1. Scrubber water samples

The composition of pollutants and their concentrations in scrubber water samples are expected to vary depending on the engine type, fuel used, and scrubber technology employed (Achten et al., 2024; García-Gómez et al., 2023). For this reason, two different scrubber water samples were used in this study.

2.1.1. DANAOS scrubber water

EGCS effluent samples were collected from the outlet of the open-loop system on board the container ship Catherine C (owned by DANAOS) during its journey from the North Sea to the Eastern Mediterranean (Jalkanen et al., 2024). The water was collected from the outlet pipe near the discharge point, ensuring that the sampled water was representative of what marine organisms would encounter close to the ship. Samples for ecotoxicological tests and metal analyses were collected directly into acid-washed 2-L amber glass bottles. One amber glass bottle and shipped to the Catalan Institute for Water Research (ICRA) (Spain) for PAHs analyses. All bottles were stored onboard at 4 °C in the dark. Then, samples were shipped (overnight refrigerated shipping by delivery courier) to the National Oceanographic Centre, Southampton (NOCS), England, for analysis, where they were kept at 4 °C in the dark until analyses.

2.1.2. Chalmers scrubber water

Natural seawater collected in the Gulf of Venice, in an area with low maritime traffic, was used to create laboratory-generated scrubber water effluents in a pilot-scale engine lab at the Department of Mechanics and Maritime Sciences, Chalmers University of Technology, Sweden (García-Gómez et al., 2023). Samples for PAHs analyses were collected into 1-L amber glass bottles and shipped to the Catalan Institute for Water Research (ICRA) (Spain). Samples for ecotoxicological tests and metal analyses were shipped to the NOCS and kept in acid-washed 2-L amber glass bottles at 4 °C in the dark until analyses.

2.2. Chemical analysis

Salinity, dissolved oxygen, and pH were measured using a HACH HQ 30d digital multimeter. PAHs and metals were measured in scrubber waters prior to the experiments. The filtration of water samples prior to the experiments can lead to the loss of some compounds that can remain adsorbed to the particulate matter present in the sample. Thus, PAHs analyses were carried out in filtered and unfiltered water samples. The 16 US EPA priority PAHs and 25 representative alkyl derivatives were analysed by ICRA using an optimized methodology following an internal protocol.

Scrubber waters were filtered (PTFE membrane filters, 0.45 μm), and metal analysis (V, Cr, Ni, Cu, Zn, As, Se, Cd, TI, and Pb) were carried out in both solution and filters at the NOCS. In brief, scrubber samples were transferred to glass vials, acidified with nitric acid and evaporated to dryness. Approx. 0.1–1 g of solid residue remained at this stage, indicating high salinity of the analysed samples. The solid residues for both type of samples (water and filters) were re-dissolved using 3 % (v/v) nitric acid solution and analysed using an Agilent8800 Triple Quad Mass Spectrometer, calibrated using NIST-traceable, mono-elemental standard solutions. The instrument was configured into an MS-MS mode, using the first set of quadrupoles as a mass filter to reduce interferences from other elements present in the sample solution. Additionally, helium gas was used as a collision gas to remove possible polyatomic interferences, and finally a second set of quadrupoles was used to analyse investigated elements. Signal suppression was monitored using internal standard additions (Sc, In and Re). The obtained correction factor was used to calculate the investigated elements' concentrations in the analysed samples.

2.3. Biological material

Ripe *M. edulis* were manually collected from natural populations around the Solent, UK, while avoiding areas near maritime ports. Mussels were cleaned of all encrusting epifauna and acclimated in the laboratory for up to one week in flow-through seawater tanks at 8 °C ± 1, with a salinity of 33.1 and a natural photoperiod (12:12 h). Adult *P. miliaris* were manually collected from Torquay, UK. After collection, the sea urchins were transported in insulated containers to the NOCS and acclimated for up to one week in flowing seawater at 10 °C ± 1, with a salinity of 33.2 and a natural photoperiod (12:12 h).

2.4. Fertilization assay on M. edulis and P. miliaris

Toxicity testing was carried out in the aquarium facilities of the NOCS. The tests were performed using an internal method based on ISO 17244 (ISO, 2015) for *M. edulis* and according to Environment Canada (2014) for *P. miliaris* (see Supplementary Table S3). Animals were induced to spawn by thermal shock and the best three females and males were chosen according to the eggs and sperm quality determined by visual inspection under an Olympus BH-2-RFCA microscope. Females with eggs that were not round, immature forms or debris were discarded. Sperm was collected "dry" from the gonopores of male sea urchins and was kept on melting ice until use (<1 h). After checking sperm motility, the sperm was exposed for 30 min to the different scrubber water dilutions. Then the oocytes were added and exposed for 30 min at room temperature. At the end of the incubation period, the 10 % neutral buffered formalin was added to block embryonic development at an early stage. At least 100 embryos from each replicate were scored for percentage fertilization based on the number of cells showing cleavage. The scrubber water dilutions tested 0.001, 0.01, 0.1, 1, 2, 5, 10, 20, 40 and 100 % of the original outlet scrubber discharge water. Five replicates were used for each dilution (see Supplementary Table S3). pH correction was made when necessary, at the beginning of the experiment.

2.5. Larval development test on M. edulis and P. miliaris

An internal protocol based on the standard ISO 17244 (ISO, 2015) was followed. The scrubber water dilutions tested 0.001, 0.01, 0.1, 1, 2, 5, 10, 20, 40 and 100 % of the original sample. Briefly, after checking the good quality of fresh eggs and sperm, and not later than 60 min for *M. edulis* or 2h for *P. miliaris* of spawning, active sperm from three males was added to the solution of fresh seawater containing eggs from three females at ambient temperature and with continuous aeration. Fertilization was accomplished at a ratio of between 100 and 200 sperm egg^{-1} in a measuring cylinder. Once polar bodies (for *M. edulis*) or fertilization ring (for *P. miliaris)* were detected at 20–30 min after fertilization, the activated egg suspensions at a density of 20–200 eggs cm^{-2} were distributed into flat-bottomed glass dishes or trays containing the test solutions. *M. edulis* fertilized eggs were kept undisturbed in the dark without aeration and food addition for up to 48 h at 16 °C. *P. miliaris* fertilized eggs were kept undisturbed in the dark without aeration and food addition for up to 72 h at 15 °C. After the incubation period a few drops of 10 % buffered formalin were added to each vessel to fix and preserve the larvae. Random samples of 100 larvae per replicate were counted. For *M. edulis* normal larvae (D-shaped) and abnormalities (malformed larvae and pre-larval stages) were recorded. *M. edulis* larvae were considered abnormal if they present at least shell abnormality, mantle abnormality, segmentation abnormalities, and/or empty shell. For *P. miliaris*, Pluteus larvae was considered as normal growth to distinguish it from any trochophore larvae, gastrulae, blastulae, morula, or split egg considered as larvae with abnormal development. pH correction was made when necessary, at the beginning of the experiment. Salinity, pH and dissolved oxygen were measured at the exposure's beginning (t = 0) before the inoculation of the zygotes. The acceptability of test results was based on negative control for a percentage of normal larvae ≥80 %. EC_{50} is calculated based on the percentage of normally developed larvae. Copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) is the recommended reference substance. The test concentrations were included in the range 0 μg/L to 100 μg/L of $CuSO_4 \cdot 5H_2O$. Five replicates were used for each dilution. The test was performed in triplicate, in three successive series, using three batches of fertilized eggs. Values of NOEC, LOEC, EC_{10}, and EC_{50} were calculated in each case.

2.6. Data analysis

Hypothesis-based toxicity data, namely the no-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC), were estimated using the one-way analysis of variance (ANOVA) and the Tukey's HSD *post hoc* test ($\alpha = 0.05$). The NOEC was designated as the highest tested concentration not statistically different from the control, while the LOEC was the lowest tested concentration statistically different from the control. Data normality and variance homoscedasticity were checked using Kolmogorov-Smirnov and Levene's tests based on medians, respectively. When normality and variance homoscedasticity conditions were not met, the Kruskal-Wallis non-parametric test on the ranks and Dunn's pairwise test were performed. Statistical analyses were performed using the software package IBM SPSS Statistics v.25. Dose–response curve (DRC) model was applied to derive effective concentrations 10 % (EC_{10}) and 50 % (EC_{50}). A log-normal distribution of the observed effects was assumed.

Correlations between ecotoxicological effects and pH of the tested concentrations were explored using the Spearman non-parametric correlation ($\alpha = 0.05$), using parameters

Measured at the beginning of the exposure (t0).

3. Results

The scrubber water from the DANAOS ship collected for the Solent case study presented higher concentrations for all the 16 PAHs and 13 AlkylPAHs analysed (see Supplementary Table S1 and Table S2) than for the Chalmers scrubber water. According to these results, PAHs and metals are lost after the filtration process because they remain adsorbed to the particulate matter present in the samples. For this reason, the scrubber water used for experimental treatments was unfiltered in all cases.

DANAOS scrubber water samples did not require any pH correction for the ecotoxicological tests. Chalmers scrubber water was tested with and without pH correction.

3.1. Effects of scrubber water on fertilization success

The scrubber water obtained from Chalmers and DANAOS produced sublethal negative effects on fertilization success in both species (Fig. 1, Table 1). In this test, the sea urchin embryos were more sensitive (NOEC = 0.1 %; LOEC = 1 %; EC_{10} = 0.12 %; EC_{50} = 4.31 %) than the blue mussel embryos (NOEC = 1 %; LOEC = 2; EC_{10} = 0.49 %; EC_{50} = 156 %) when exposed to DANAOS scrubber water. The same behaviour was observed when the test was performed with Chalmers scrubber water with or without pH correction. The sea urchin embryos were more sensitive (NOEC = 0.001 %; LOEC = 0.01 %) than the blue mussel embryos (NOEC = 0.1 %; LOEC = 1 %) when exposed to Chalmers scrubber water. When the pH of Chalmers scrubber water was corrected, the toxicity of the scrubber water decreased for both species (*M. edulis*: Without pH correction: EC_{50} = 40.89 %; with pH correction: EC_{50} = 85.32 %; *P. miliaris*: Without pH correction: EC_{50} = 1.2 %; with pH correction: EC_{50} = 1.49 %). Spearman's correlation showed a significant relationship between the percentage of unfertilized eggs with pH for *P. miliaris* (Spearman's R = −0.414, p = 0.017) but not for *M. edulis* (Spearman's R = −0.137, p = 0.448). Physicochemical parameters and fertilization success are reported in Supplementary Material Table S4.

3.2. Effects of scrubber water on larval development

The effects on early-life stages showed that larval of both species are sensitive to low concentrations of scrubber water (Fig. 2, Table 1). After 72 h exposure, some abnormalities observed in *P. miliaris* larvae included additional crossbarred body rod, missing or shorter arms, and apically crossed body rod (Fig. 3). In *M. edulis* hypertrophy of the mantle and hinge abnormality were the most common larvae abnormalities found after 72 h exposure to both scrubber waters (Fig. 4). In this case, blue mussel larvae were much more sensitive (NOEC = <0.001 %; LOEC = 0.001 %; EC_{10} = 0.27 %; EC_{50} = 9.27 %) than sea urchin larvae (NOEC = 0.01 %; LOEC = 0.1; EC_{10} = 0.15 %; EC_{50} = 1.54 %) when exposed to DANAOS scrubber water. The same behaviour was observed when the test was performed with Chalmers scrubber water with or without pH correction. The blue mussel larvae were more sensitive (NOEC = <0.001 %; LOEC = 0.001 %) than the sea urchin larvae (NOEC = 0.001 %; LOEC = 0.01 %) when exposed to Chalmers scrubber water without pH correction. When the pH of Chalmers scrubber water was corrected the toxicity of the scrubber water slightly decreased for both species.

Spearman's correlation did nor evidence a significant relationship between the percentage of abnormalities with pH for *P. miliaris* (Spearman's R = −0.174, p = 0.334) but evidence a significant moderately high correlation for *M. edulis* (Spearman's R = −0.405, p = 0.019). Physicochemical parameters and percentage of abnormalities (shell abnormality, mantle abnormality, segmentation abnormalities, and/or empty shell for *P. miliaris*, and D-shaped larvae, malformed larvae and pre-larval stages for *M. edulis*) are reported in Supplementary Material, Table S4.

4. Discussion

The exposure of *M. edulis* and *P. miliaris* embryos and larvae to different dilutions of scrubber water showed a concentration-dependent effect on all explored endpoints. The adverse effects on fertilization success and larvae development were observed even at the lowest dilutions of scrubber water, confirming that scrubber water could present adverse effects on the whole life cycle of the marine organisms. Similar results have been reported in other bivalves when exposed to scrubber water. Picone et al. (2023) found the larval development of *M. galloprovincialis* was significantly delayed by exposure to scrubber water under similar conditions to those used in this study. However, according to NOEC, LOEC and EC values reported in that study, *M. edulis* seems to be more sensitive to the exposure to scrubber water compared to *M. galloprovincialis*. In the same manner, *M. edulis* was more sensitive that *P. miliaris* larvae when exposed to DANAOS and Chalmers scrubber water.

Marine invertebrates play an important role in the functioning of marine ecosystems, contributing to key ecological processes such as nutrient cycling, habitat formation, and energy transfer. Certain species have been selected as bioindicators due to their ecological significance, trophic position, well-documented biology, and adaptability to both field and laboratory experiments (de Almeida Rodrigues et al., 2022;

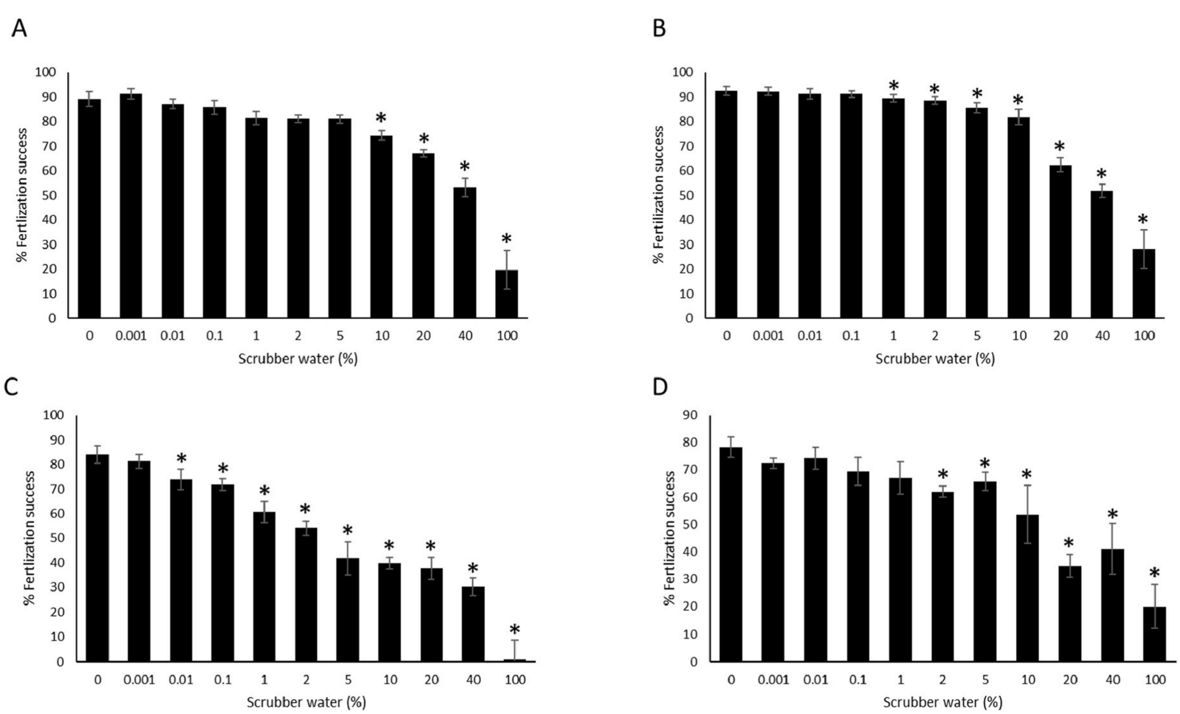

Fig. 1. Percentage of fertilization success in two species after exposure to scrubber water. *M. edulis* exposed to (A) Chalmers scrubber water and (B) Danaos scrubber water; *P. miliaris* exposed to (C) Chalmers scrubber water and (D) Danaos scrubber water. (*) indicates significant differences compared to control. Error bars designate standard deviations.

Table 1
Summary of the toxicity data.

Species	Endpoint	Scrubber water	pH corrected	Lowest % dilution tested	LOEC	NOEC	EC10	EC50
Mytilus edulis	Fertilization success	DANAOS	No	0.001	2	1	0.49	156
		Chalmers	No	0.001	1	0.1	0.58	40.89
		Chalmers	Yes	0.001	1	0.1	0.68	85.32
	Larvae development	DANAOS	No	0.001	0.001	<0,001	0.27	9.27
		Chalmers	No	0.001	0.001	<0,001	0.06	0.54
		Chalmers	Yes	0.001	0.001	<0,001	0.07	0.71
Psammechinus miliaris	Fertilization success	DANAOS	No	0.001	1	0.1	0.12	4.31
		Chalmers	No	0.001	0.01	0.001	0.12	1.2
		Chalmers	Yes	0.001	0.01	0.001	0.08	1.49
	Larvae development	DANAOS	No	0.001	0.1	0.01	0.15	1.54
		Chalmers	No	0.001	0.01	0.001	0.11	0.96
		Chalmers	Yes	0.001	0.1	0.01	0.11	1.42

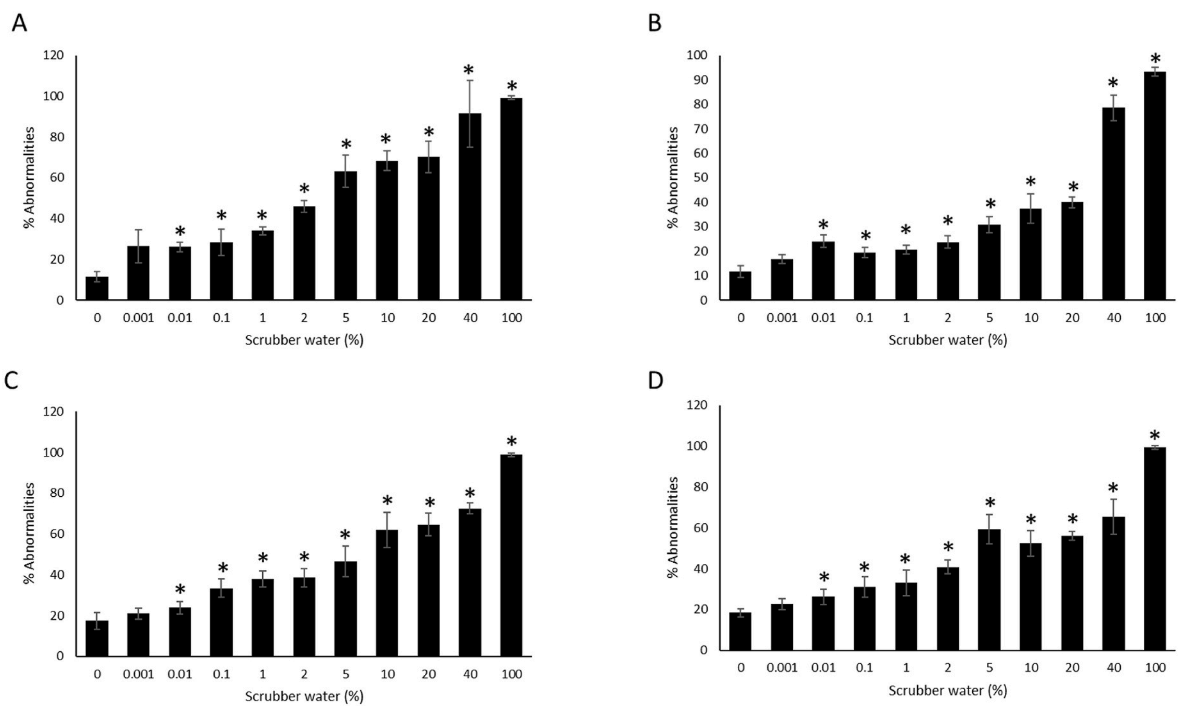

Fig. 2. Percentage of abnormal larvae in two invertebrate species after 72h exposure to scrubber water. *M. edulis* exposed to (A) Chalmers scrubber water and (B) Danaos scrubber water; *P. miliaris* exposed to (C) Chalmers scrubber water and (D) Danaos scrubber water. (*) indicates significant differences compared to control. Error bars designate standard deviations.

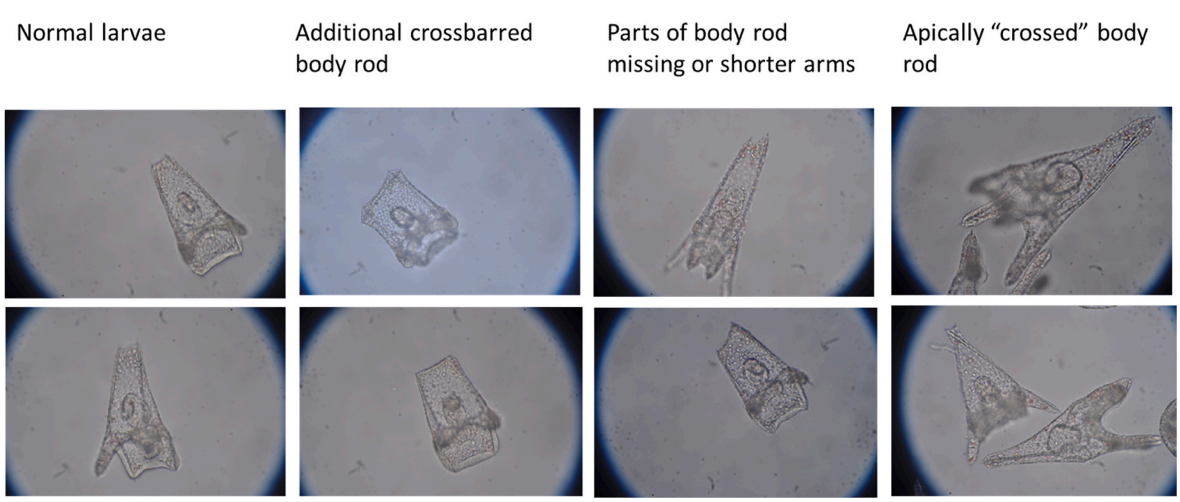

Fig. 3. Some abnormalities observed in *Psammechinus miliaris* larvae at 72 h exposure to scrubber water.

Fig. 4. Some abnormalities observed in *Mytilus edulis* larvae at 72 h exposure to scrubber water. Arrows: hypertrophy of the mantle. Arrowhead: hinge abnormality.

Vallaeys et al., 2017). These characteristics make them valuable for assessing environmental changes and anthropogenic impacts. Invertebrate larvae, along with other planktonic organisms, form the foundation of marine food webs, serving as a critical energy source for higher trophic levels, including fish and other marine predators. While they are not primary producers themselves, they play an essential role in the transfer of energy from primary producers (such as phytoplankton) to higher consumers, reinforcing the stability and productivity of marine ecosystems. This makes them important as they act as a food source to numerous aquatic organisms, which in turn are eaten by larger organisms further up the food chain and toxicants could be accumulated and transferred through biomagnification process at different trophic levels (Corsolini and Sarà, 2017).

Our findings confirmed that both blue mussels and sea urchins' larvae can be considered excellent model organisms for inclusion in ecotoxicological assessments due to their high ecological relevance, sensitivity to environmental stressors, and well-characterized life cycles (Boukadida et al., 2016, 2021; Morroni et al., 2023). Their widespread distribution in marine environments, ease of collection, and ability to be cultured under laboratory conditions make them ideal candidates for standardized toxicity testing. Furthermore, their early developmental stages (fertilization, cleavage, and larval formation) provide rapid and measurable endpoints to assess the effects of toxic substances on growth, survival, and morphological abnormalities (Annamaria et al., 2005; Morroni et al., 2023; Picone et al., 2023). The present study identified the toxic potential of scrubber water discharge resulting in abnormal development of marine invertebrates' larvae. On a larger scale, such as the increasing use and associated washwater discharge of scrubbers worldwide, such outcomes have the potential to massively impact marine food webs and subsequently marine ecosystems. Even at low concentrations, the discharge of scrubber washwater has the potential to effect marine organisms via the processes of bioaccumulation and biomagnification (Koski et al., 2017).

There is a differential sensitivity between species and life-stages to exposure to scrubber water. Sea urchin embryos seemed to be more sensitive to this exposure than blue mussel embryos. However, the pelagic larvae of *M. edulis* was more sensitive to exposure to scrubber water. Early life stages of fish and invertebrates have shown higher sensitivity when exposed to contaminants than adult organisms (Mohammed, 2013). For invertebrates, embryos may sometimes appear to be more sensitive than larvae. Therefore, the relationship between sensitivity and the life stage may vary by species. More comprehensive studies on gene expression, enzymatic activity, stress response pathways, and detoxification mechanisms are needed to identify the underlying factors driving species- and life stage-specific variations in susceptibility in response to scrubber water exposure. Further research incorporating transcriptomic, proteomic, and epigenetic analyses will be essential to unravel the biological pathways underlying these differential responses.

The discharge of scrubber water has the potential to impact various parameters of receiving waters, such as pH, dissolved oxygen levels, concentrations of trace elements, PAHs, and other contaminants. While the pH decrease in seawater from scrubber effluent occurs at a small scale, localized acidification will intensify, particularly in enclosed systems such as coastal lagoons and harbours (Lange and Markus, 2015). Furthermore, the repeated reduction in pH from the pulsed input of sulfuric acid from scrubber discharge, have been shown to significantly affect plankton communities (Ytreberg et al., 2019). Studies on scrubber water have demonstrated potential synergistic effects of contaminants and pH on copepods (Koski et al., 2017; Thor et al., 2021). To study the effect of scrubber water on different organisms it is necessary to consider relevant aspects such as the pH correction, which can change the behaviour of some compounds in scrubber water. Our findings show that the pH correction decreased the toxicity of the scrubber water. This was expected considering that a change in pH modifies the chemical form, solubility, and availability of some compounds present in the mixture.

In the same manner, the filtration of scrubber water prior to the experiments can lead to the loss of some compounds that can remain adsorbed to the particulate matter present in the samples. Contaminants in mixtures may be affected by bioavailability associated with sediments that creates a situation of major concern in areas affected by heavy maritime traffic (Merhaby et al., 2019; Valentina et al., 2021). Chemical sequestration is a well-known process occurring in sediments and soils that reduces the availability of hydrophobic compounds such as PAHs. Organic matter present in sediment has an important role in the first phase of the sorption process and it has been reported that small molecules are sequestered more rapidly than larger ones (Brion and Pelletier, 2005). Thus, removing particulate matter from environmental samples to carry laboratory exposure may introduce inaccuracies into the analysis and influence the extrapolation of potential effects associated with scrubber water exposure.

Scrubber water is known to comprise of a multitude of pollutants, including various PAHs, metals and nitrates, and it is highly acidic (USEPA, 2011). Concentrations of PAHs, alkylated derivatives and other pollutants have been reported from open and closed-loop operations on several ships (Achten et al., 2024; García-Gómez et al., 2023) showing that the discharge waters varied in concentration and can be associated with those of the fuels used, as they mainly originate in unburnt fuel (Achten et al., 2024). This study identified chrysene, fluorene, fluoranthene, naphthalene, phenanthrene and pyrene as the priority PAHs present in scrubber water from the Chalmers University system (see Supplementary Table S1). This matched well with other reports performed by García-Gómez et al. (2023) about the occurrence of PAHs in an open-loop scrubber system at the same University. However, this study detected a greater variety of PAHs, including alkylated PAHs, as well as higher concentrations in the scrubber water discharge from the DANAOS system. The variability observed between studies and scrubber waters in terms of pollutant types and concentrations further complicates the ability to formulate general conclusions regarding the ecological impacts of exposure to scrubber water discharges.

Despite the low concentrations of most of the PAHs and metals analysed in Chalmers scrubber water, it showed a high potential to be toxic for both species, either for fertilization or the larval development. Exposure to multiple stressors has the potential to cause toxic effects, despite individual contaminants being below levels of concern (Beyer et al., 2014). For example, when testing the toxicity of scrubber effluent to copepods, none of the contaminants occurred at concentrations that could individually explain the toxic effects observed (Thor et al., 2021). Studies using similar species has shown some potential effects of particular contaminants present in scrubber discharge waters, such as vanadium or copper, that could be critical factors affecting marine invertebrates' larval development (Picone et al., 2023). However, scrubber water toxicity cannot be predicted by comparing the concentrations of individual pollutants to toxicity thresholds (Magnusson et al., 2018). Some contaminants have a similar mode of toxic action so assumption of dose (or concentration)-additive toxicity is typically made (Cassee et al., 1998). However, biota may exhibit unexpected responses to contaminant mixtures due to potential interactions such as agonisms and/or synergisms among individual contaminants, thereby altering the overall magnitude or character of toxicity (Cassee et al., 1998; De Zwart and Posthuma, 2005; Fleeger et al., 2007).

In addition to the effect of metal combination, there is an interactive effect between temperature and trace metals on early life stage of bivalves (Boukadida et al., 2016). It has been shown that combine exposure to environmentally realistic water temperature and metal pollution could affect tolerance limits and impair development and reproduction success (Boukadida et al., 2016). This represents an additional challenge under the assumption of increasing temperature of the sea in future scenarios related to the global warming.

To understand the full scrubber water toxicity we recommend studying the combined effects of chemicals under different environmental conditions and parameters such as pH and temperature. As phytoplankton have shown to be able to adapt to prolonged exposure to PAHs resulting in increased toxicity thresholds (Kottuparambil and Agusti, 2018), the inclusion of long-term studies to investigate the concept of adaptation to scrubber water exposure in different species is recommended.

Despite economic benefits, the discharge of scrubber water containing pollutants such as heavy metals and PAHs poses severe risks to marine ecosystems. For instance, in the Baltic Sea, the study estimates a societal damage cost of over €680 million between 2014 and 2022 due to marine ecotoxicity (Lunde Hermansson et al., 2024). The growing environmental concerns have led to increasing regulatory restrictions on scrubber use in specific regions, such as the Baltic Sea and certain ports, but the practice remains widespread globally due to economic pressures.

The current investigation offers additional evidence of the risk of using open-loop EGCSs as an environmentally sustainable approach for mitigating maritime sulfur emissions. The use of EGCS systems has relocated the issue from the atmosphere to the aquatic environment, and it has created an additional environmental stressor with the exposure of marine biota to increasing concentrations of toxicants such as heavy metals, PAHs, and alkylated PAHs. Consequently, efforts to decrease emissions from maritime transportation should no longer be pursued by equipping vessels with open-loop scrubbers. Instead, there is an imperative to transition towards alternative technologies that reduce the environmental impacts. Alternative strategies have been proposed to reduce emissions and mitigate the environmental risks associated with wet scrubbers (Sarbanha et al., 2023). These include the integration of exhaust gas cleaning with carbon capture via dry scrubbers using multifunctional sorbents, the implementation of CO_2 electrochemical cells on ships as an alternative to carbon storage, and the exploration of zero-emission fuels for ship propulsion.

5. Conclusions and recommendations

The increasing use of both closed- and open-loop scrubbers on marine vessels is leading to the discharge of large volumes of potentially toxic wash water, posing a significant threat to marine ecosystems. Given the significant environmental risks associated with scrubber water discharges, and the evident hazard for marine invertebrates, especially in the early life stages, there is an urgent need for stronger international regulations and enforcement mechanisms. Analysis of PAH distribution patterns confirms that unburnt petrogenic fuel is the primary source of PAHs in discharge water, emphasizing the need for a transition to cleaner fuels with low-PAH-content, such as MGO, LNG, or alternative clean fuels to minimize PAH emissions. Urgent investments in technological advancements, engine optimization, and combustion efficiency are needed, along with the establishment of standardized PAH monitoring programs and evidence-based discharge regulations to mitigate pollution risks. A more comprehensive policy framework under the International Maritime Organization (IMO) and other regulatory bodies would help ensure that scrubber discharge management aligns with global marine conservation efforts and emission reduction goals. The IMO, through MARPOL Annex VI, currently regulates sulfur emissions from ships, but scrubber discharge water remains largely unregulated or subject to inconsistent national and regional policies. Ship owners should transition to cleaner fuels and closed-loop scrubbers, policymakers must enforce stricter discharge limits, penalties for noncompliance, and monitoring requirements, and environmental agencies should expand water quality monitoring and assess long-term ecological impacts. To assess the cumulative effects of scrubber discharges in high-traffic areas and their long-term impacts, a multidisciplinary approach integrating field monitoring, ecotoxicological studies, chemical analyses, and ecosystem modelling is necessary. Without these proactive measures, continued scrubber discharge pollution could lead to rapid and potentially irreversible damage to marine ecosystems.

CRediT authorship contribution statement

Lina M. Zapata-Restrepo: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ian D. Williams:** Writing – review & editing, Resources, Project administration, Funding acquisition.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2025.107157.

Data availability

Data will be made available on request.

References

Achten, C., Marin-Enriquez, O., Behrends, B., Kupich, S., Lutter, A., Korth, R., Andersson, J.T., 2024. Polycyclic aromatic compounds including non-target and 71 target polycyclic aromatic hydrocarbons in scrubber discharge water and their environmental impact. Mar. Pollut. Bull. 208, 116790.

Andreasen, A., Mayer, S., 2007. Use of seawater scrubbing for SO_2 removal from marine engine exhaust gas. Energy Fuels 21 (6), 3274–3279.

Annamaria, V.G., Chiara, L., Alessandra, A.N., Alvise, B., His, E., Francesco, G.P., 2005. *Mytilus galloprovincialis* as bioindicator in embryotoxicity testing to evaluate sediment quality in the lagoon of Venice (Italy). Chem. Ecol. 21 (6), 455–463.

Bay, S.M., Oshida, P.S., Jenkins, K.D., 1983. A simple new bioassay based on echinochrome synthesis by larval sea urchin. Mar. Environ. Res. 8, 29–39.

Beaumont, A.R., Tserpes, G., Budd, M.D., 1987. Some effects of copper on the veliger larvae of the mussel *Mytilus edulis* and the scallop *Pecten maximus* (Mollusca, Bivalvia). Mar. Environ. Res. 21 (4), 299–309.

Beyer, J., Petersen, K., Song, Y., Ruus, A., Grung, M., Bakke, T., Tollefsen, K.E., 2014. Environmental risk assessment of combined effects in aquatic ecotoxicology: a discussion paper. Mar. Environ. Res. 96, 81–91.

Boukadida, K., Banni, M., Gourves, P.Y., Cachot, J., 2016. High sensitivity of embryo-larval stage of the Mediterranean mussel, *Mytilus galloprovincialis* to metal pollution in combination with temperature increase. Mar. Environ. Res. 122, 59–66.

Boukadida, K., Mlouka, R., Clerandeau, C., Banni, M., Cachot, J., 2021. Natural distribution of pure and hybrid *Mytilus sp.* along the south Mediterranean and North-east Atlantic coasts and sensitivity of D-larvae stages to temperature increases and metal pollution. Sci. Total Environ. 756, 143675.

Brion, D., Pelletier, É., 2005. Modelling PAHs adsorption and sequestration in freshwater and marine sediments. Chemosphere 61 (6), 867–876.

Cassee, F.R., Groten, J.P., Bladeren, P.J.V., Feron, V.J., 1998. Toxicological evaluation and risk assessment of chemical mixtures. Crit. Rev. Toxicol. 28 (1), 73–101.

Comer, B., Georgeff, E., Osipova, L., 2020. Air Emissions and Water Pollution Discharges from Ships with Scrubbers. ICCT CONSULTING REPORT. November 2020. www.theicct.org. (Accessed 26 March 2021).

Connor, P.M., 1972. Acute toxicity of heavy metals to some marine larvae. Mar. Pollut. Bull. 3, 190–192.

Corsolini, S., Sarà, G., 2017. The trophic transfer of persistent pollutants (HCB, DDTs, PCBs) within polar marine food webs. Chemosphere 177, 189–199. https://doi.org/10.1016/j.chemosphere.2017.02.116.

de Almeida Rodrigues, P., Ferrari, R.G., Kato, L.S., Hauser-Davis, R.A., Conte-Junior, C. A., 2022. A systematic review on metal dynamics and marine toxicity risk assessment using crustaceans as bioindicators. Biol. Trace Elem. Res. 1–23.

De Zwart, D., Posthuma, L., 2005. Complex mixture toxicity for single and multiple species: proposed methodologies. Environ. Toxicol. Chem. 24, 2665–2676.

Endres, S., Maes, F., Hopkins, F., Houghton, K., Mårtensson, E.M., Oeffner, J., Quack, B., Singh, P., Turner, D., 2018. A new perspective at the ship-air-sea-interface: the environmental impacts of exhaust gas scrubber discharge. Front. Mar. Sci. 139. https://doi.org/10.3389/fmars.2018.00139.

Environment Canada, 2014. Reference Method for Measuring the Toxicity of Contaminated Sediment to Embryos and Larvae of Echinoids (Sea Urchins or Sand Dollars) 1/RM/58 July 2014. Science and Technology Branch Environment Canada.

Fleeger, J.W., Gust, K.A., Marlborough, S.J., Tita, G., 2007. Mixtures of metals and polynuclear aromatic hydrocarbons elicit complex, nonadditive toxicological interactions in meiobenthic copepods. Environmental Toxicology and Chemistry. Int. J. 26 (8), 1677–1685.

García-Gómez, E., Gkotsis, G., Nika, M.C., Hassellöv, I.M., Salo, K., Hermansson, A.L., et al., 2023. Characterization of scrubber water discharges from ships using comprehensive suspect screening strategies based on GC-APCI-HRMS. Chemosphere 343, 140296.

Hansen, J.P., Aalborg, A.L., 2012. Exhaust Gas Scrubber Installed Onboard MV Ficaria Seaways: Public Test Report, Environmental Project No. 1429. Miljøstyrelsen, Copenhagen.

Hassellöv, I.M., Koski, M., Broeg, K., Marin-Enriquez, O., Tronczynski, J., Dulière, V., et al., 2020. ICES Viewpoint background document: impact from exhaust gas cleaning systems (scrubbers) on the marine environment (Ad hoc). ICES Scientific Reports 2 (86), 40.

ISO, 2015. ISO 17244 Water Quality. Determination of the Toxicity of Water Samples on the Embryo-Larval Development of Japanese Oyster (*Crassostrea gigas*) and Mussel (*Mytilus edulis* or *Mytilus galloprovincialis*). ISO, p. 24, 17244:2015.

Jalkanen, Jukka-Pekka, Fridell, Erik, Kukkkonen, Jaakko, Moldanova, Jana, Ntziachristos, Leonidas, Grigoriadis, Achilleas, Moustaka, Maria, Fragkou, Evangelia, Tsegas, George, Maragkidou, Androniki, Sofiev, Mikhail, Hänninen, Risto, Grönholm, Tiia, Palamarchuk, Julia, Majamäki, Elisa, Winiwarter, Wilfried, Gueret, Samuel, Sokhi, Ranjeet S., Kumar, Saurabh, Alyuz Ozdemir, Ummugulsum, Kolovoyiannis, Vassilis, Zervakis, Vassilis, Mazioti, Aikaterini-Anna, Krasakopoulou, Evangelia, Hassellöv, Ida-Maja, Lunde Hermansson, Anna, Ytreberg, Erik, Williams, Ian, Hudson, Malcolm, Zapata, Restrepo, Lina, Hole, Lars, Robert, Aghito, Manuel, Breivik, Oyvind, Petrovic, Mira, Gross, Meritxel, Rodriguez-Mozaz, Sara, Neophytou, Maria, Monteiro, Alexandra, Russo, Michael A., Oikonomou, Fotis, Gondikas, Andreas, Marcomini, Antonio, Giubilato, Elisa, Calgaro, Loris, Jaakkola, Jouni J.K., Shiue, Ivy, Kiihamäki, Simo-Pekka, Broström, Göran, Hassellöv, Martin, Kaitaranta, Joni, Granberg, Maria, Kerstin, Magnusson, 2024. Environmental Impacts of Exhaust Gas Cleaning Systems in the Baltic Sea, North Sea, and the Mediterranean Sea Area. Finnish Meteorological Institute, Helsinki, Finland. https://doi.org/10.35614/isbn.9789523361898. https://helda.helsinki.fi/items/bb939453-8311-4e1e-88c7-4a3e8b9e80a3. http://hdl.handle.net/10138/569605, 978-952-336-189-8.

Koski, M., Colin, S., Stefan, T., 2017. Ecological effects of scrubber water discharge on coastal plankton: potential synergistic effects of contaminants reduce survival and feeding of the copepod Acartia tonsa. Mar. Environ. Res. 129, 374–385. https://doi.org/10.1016/j.marenvres.2017.06.006.

Kottuparambil, S., Agusti, S., 2018. PAHs sensitivity of picophytoplankton populations in the Red Sea. Environ. Pollut. 239, 607–616. https://doi.org/10.1016/j.envpol.2018.04.079.

Kuittinen, N., Timonen, H., Karjalainen, P., Murtonen, T., Vesala, H., Bloss, M., et al., 2024. In-depth characterization of exhaust particles performed on-board a modern cruise ship applying a scrubber. Sci. Total Environ. 946, 174052.

Lange, B., Markus, T., 2015. Impact of Scrubbers on the Environmental Situation in Ports and Coastal Waters. http://www.umweltbundesamt.de/sites/default/files/medien/378/publikationen/texte_65_2915_impacts_of_scubbers_on_the_envoronmental_situation_in_ports_and_coastal_waters.pdf. (Accessed 1 May 2024).

Lindstad, H.E., Eskeland, G.S., 2016. Environmental regulations in shipping: policies leaning towards globalization of scrubbers deserve scrutiny. Transport. Res. Transport Environ. 47, 67–76.

Lunde Hermansson, A., Hassellöv, I.M., Grönholm, T., Jalkanen, J.P., Fridell, E., Parsmo, R., et al., 2024. Strong economic incentives of ship scrubbers promoting pollution. Nat. Sustain. 1–11.

Lunde Hermansson, A., Hassellöv, I.-M., Moldanová, J., Ytreberg, E., 2021. Comparing emissions of polyaromatic hydrocarbons and metals from marine fuels and scrubbers. Transport. Res. Transport Environ. 97, 102912. https://doi.org/10.1016/j.trd.2021.102912.

Magnusson, K., Thor, P., Granberg, M., 2018. Scrubbers: Closing the Loop Activity 3: Task 2 Risk Assessment of Marine Exhaust Gas Scrubber Water. Retrieved from IVL Svenska Miljöinstitutet. website: https://urn.kb.se/resolve?urn=urn:nbn:se:ivl:diva-2835. (Accessed 9 April 2021).

Martin, M., Osborn, K.E., Billig, P., Glickstein, N., 1981. Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and Cancer magister larvae. Mar. Pollut. Bull. 12 (9), 305–308.

Merhaby, D., Rabodonirina, S., Net, S., Ouddane, B., Halwani, J., 2019. Overview of sediments pollution by PAHs and PCBs in Mediterranean basin: transport, fate, occurrence, and distribution. Mar. Pollut. Bull. 149, 110646.

Mohammed, A., 2013. Why Are Early Life Stages of Aquatic Organisms More Sensitive to Toxicants than Adults. New Insights into Toxicity and Drug Testing, pp. 49–62.

Morroni, L., Rakaj, A., Grosso, L., Flori, G., Fianchini, A., Pellegrini, D., Regoli, F., 2023. *Echinoderm* larvae as bioindicators for the assessment of marine pollution: sea urchin and sea cucumber responsiveness and future perspectives. Environ. Pollut. 335, 122285.

Picone, M., Russo, M., Distefano, G.G., Baccichet, M., Marchetto, D., Ghirardini, A.V., et al., 2023. Impacts of exhaust gas cleaning systems (EGCS) discharge waters on planktonic biological indicators. Mar. Pollut. Bull. 190, 114846.

Sarbanha, A.A., Larachi, F., Taghavi, S.M., Thiboutot-Rioux, M., Boudreau, A., Dugas, G., 2023. Mitigation of ship emissions: overview of recent trends. Ind. Eng. Chem. Res. 62 (4), 1707–1724.

Schmolke, S., Ewert, K., Kaste, M., Schöngaßner, T., Kirchgeorg, T., Marin-Enriquez, O., 2020. Environmental protection in maritime traffic – scrubber wash water survey. Final report. Baltic Marine Environment Protection Commission, p. 97.

Ship & Bunker, 2019. Projected Number of Vessels Fitted with Scrubbers Worldwide in 2020 and 2024 [Graph]. March 11, 2019. [Online]. Available 30 April 2024. https://www.statista.com/statistics/1099506/vessels-with-scrubbers-forecasts/.

Sofiev, M., Winebrake, J.J., Johansson, L., Carr, E.W., Prank, M., Soares, J., Vira, J., Kouznetsov, R., Jalkanen, J.P., Corbett, J.J., 2018. Cleaner fuels for ships provide public health benefits with climate tradeoffs. Nat. Commun. 9 (1). https://doi.org/10.1038/s41467-017-02774-9.

Sokhi, R.S., Singh, V., Querol, X., Finardi, S., Ttargino, A.C., Andrade, M.F., Pavlovic, R., Garland, R.M., Massagué, J., Kong, S., Baklanov, A., Ren, L., Tarasova, O., Carmichael, G., Peuch, V.H., Anand, V., Arbilla, G., Badali, K., Beig, G., Belalcazar, L. C., Bolignano, A., Brimblecombe, P., Camacho, P., Casallas, A., Charland, J.P., Choi, J., Chourdakis, E., Coll, I., Collins, M., Cyrys, J., da Silva, C.M., Di Giosa, A.D., Di Leo, A., Ferro, C., Gavidia-Calderon, M., Gayen, A., Ginzburg, A., Godefroy, F., Gonzalez, Y.A., Guevara-Luna, M., Haque, S.M., Havenga, H., Herod, D., Hõrrak, U., Hussein, T., Ibarra, S., Jaimes, M., Kaasik, M., Khaiwal, R., Kim, J., Kousa, A., Kukkonen, J., Kulmala, M., Kuula, J., La Violette, N., Lanzani, G., Liu, X., MacDougall, S., Manseau, P.M., Marchegiani, G., McDonald, B., Mishra, S.V., Molina, L.T., Mooibroek, D., Mor, S., Moussiopoulos, N., Murena, F., Niemi, J.V., Noe, S., Nogueira, T., Norman, M., Pérez-Camaño, J.L., Petäjä, T., Piketh, S., Rathod, A., Reid, K., Retama, A., Rivera, O., Rojas, N.Y., Rojas-Quincho, J.P., San José, R., Sánchez, O., Seguel, R.J., Sillanpää, S., Su, Y., Tapper, N., Terrazas, A., Timonen, H., Toscano, D., Tsegas, G., Velders, G.J.M., Vlachokostas, C., von Schneidemesser, E., Vpm, R., Yadav, R., Zalakeviciute, R., Zavala, M., 2021. A global observational analysis to understand changes in air quality during exceptionally low anthropogenic emission conditions. Environ. Int. 157, 106818. https://doi.org/10.1016/j.envint.2021.106818.

Teuchies, J., Cox, T.J.S., Van Itterbeeck, K., Meysman, F.J.R., Blust, R., 2020. The impact of scrubber discharge on the water quality in estuaries and ports. Environ. Sci. Eur. 32 (1). https://doi.org/10.1186/s12302-020-00380-z.

Thor, P., Granberg, M.E., Winnes, H., Magnusson, K., 2021. Severe toxic effects on pelagic copepods from maritime exhaust gas scrubber effluents. Environ. Sci. Technol. 55 (9), 5826–5835. https://doi.org/10.1021/acs.est.0c07805.

USEPA, 2011. Exhaust Gas Scrubber Washwater Effluent, p. 46. Available at: https://www3.epa.gov/npdes/pubs/vgp_exhaust_gas_scrubber.pdf. (Accessed 26 March 2021).

Valentina, P., Michele, M., Tommaso, G., Letizia, M., Laura, M.M., Francesca, M., et al., 2021. Sediment contamination by heavy metals and PAH in the *Piombino channel* (Tyrrhenian Sea). Water 13 (11), 1487.

Vallaeys, T., Klink, S.P., Fleouter, E., Le Moing, B., Lignot, J.H., Smith, A.J., 2017. Bioindicators of marine contaminations at the frontier of environmental monitoring and environmental genomics. Pharmaceuticals 57, 58.

Viana, M., Hammingh, P., Colette, A., Querol, X., Degraeuwe, B., Vlieger, I. de, van Aardenne, J., 2014. Impact of maritime transport emissions on coastal air quality in Europe. Atmos. Environ. 96–105.

Ytreberg, E., Hassellöv, I.M., Nylund, A.T., Hedblom, M., Al-Handal, A.Y., Wulff, A., 2019. Effects of scrubber washwater discharge on microplankton in the Baltic Sea. Mar. Pollut. Bull. 145, 316–324. https://doi.org/10.1016/j.marpolbul.2019.05.023.