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Impacts of exhaust gas cleaning systems (EGCS) discharge waters on planktonic biological indicators

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ABSTRACT

Exhaust Gas Cleaning Systems (EGCS), operating in open-loop mode, continuously release acidic effluents (scrubber waters) to marine waters. Furthermore, scrubber waters contain high concentrations of metals, polycyclic aromatic hydrocarbons (PAHs), and alkylated PAHs, potentially affecting the plankton in the receiving waters. Toxicity tests evidenced significant impairments in planktonic indicators after acute, early-life stage, and long-term exposures to scrubber water produced by a vessel operating with high sulphur fuel. Acute effects on bacterial bioluminescence (*Aliivibrio fischeri*), algal growth (*Phaeodactylum tricornutum, Dunaliella tertiolecta*), and copepod survival (*Acartia tonsa*) were evident at 10 % and 20 % scrubber water, while larval development in mussels (*Mytilus galloprovincialis*) showed a 50 % reduction at ~5 % scrubber water. Conversely, larval development and reproductive success of *A. tonsa* were severely affected at scrubber water concentrations ≤1.1 %, indicating the risk of severe impacts on copepod populations which in turn may result in impairment of the whole food web.

1. Introduction

Emissions of sulphur oxides (SO_x), nitrogen oxides (NO_x), carbon dioxide (CO_2), and particulate matter (PM) from international maritime transport are one of the primary sources of global air pollution (Gössling et al., 2021). Therefore, to mitigate the consequences of ship-derived SO_x, the International Maritime Organization (IMO) has introduced a stringent limit of 0.5 % maximum sulphur content in marine fuels since January 2020, which integrates the 0.1 % limit into force in sulphur emission control areas (SECAs) since 2015 (MARPOL Annex VI, Regulation 14.1.3). However, since IMO restrictions concern only atmospheric sulphur emissions, ships are still allowed to use high-sulphur fuels combined with an exhaust gas cleaning system (EGCS), commonly known as scrubber, to reduce SO_2 emissions into the atmosphere (IMO, 2015).

Different technologies were adopted for cleaning exhaust gases, but the most common system is the wet scrubber, in which water is sprayed on the exhaust to remove the SO_x (Teuchies et al., 2020; Ytreberg et al., 2019). Wet scrubbers can be further classified into closed-loop or open-loop scrubbers, but hybrid open/closed-loop systems are also available (Ytreberg et al., 2021b). In closed-loop scrubbers, freshwater pre-treated with sodium hydroxide is sprayed on exhaust gases, recirculated in the system, and finally treated to collect the particles and associated contaminants into a sludge that may be disposed of in port facilities (Lunde Hermansson et al., 2021; Teuchies et al., 2020). The discharge water (or scrubber water) volume produced by a closed-loop system is in the range of 100 m^3 per day on a medium-sized (12 MW) vessel (Lunde Hermansson et al., 2021). On the other hand, in open-loop scrubbers,

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seawater is pumped into the system to remove SO_x from the exhaust and is then discharged into surface waters without further cleaning at a rate of approximately 90 m^3 MWh^{-1} (Lunde Hermansson et al., 2021). The volume of scrubber water discharged by a medium-sized vessel equipped with open-loop scrubbers is thus estimated at 13,000 m^3 per scrubber unit per day (Ytreberg et al., 2019).

According to recent estimates (Lunde Hermansson et al., 2021), open-loop scrubbers are the most adopted solution for SO_x abatement, with a market share of 80 %, followed by hybrid systems (17 %) and closed systems (1.5 %). Consequently, considering the diffusion of open-loop scrubbers and the volume of scrubber water they may generate, large amounts of atmospheric pollutants are locally discharged to the sea without any atmospheric dispersion. Thus, an expected effect of open-loop scrubbers is that acidification and release of contaminants are concentrated along the shipping lanes (Claremar et al., 2017). Scrubber waters indeed contain considerable amounts of pollutants, which are either originating from the fuel itself, such as polycyclic aromatic hydrocarbons (PAHs) and metals such as lead (Pb) and vanadium (V), or by corrosion of piping, such as copper (Cu) and zinc (Zn), and leakage of antifouling agents (Koski et al., 2017; Lunde Hermansson et al., 2021). Recent studies evidenced that EGCS discharges may have detrimental effects on the planktonic communities of the receiving waters, but very little information is still available concerning the potential consequences of the discharge of large volumes of scrubber water on marine ecosystems (Koski et al., 2017; Ytreberg et al., 2021b).

Coastal waters are particularly vulnerable to scrubber water discharges since they are heavily trafficked and harbour high biodiversity. Furthermore, the most significant impact is expected on the planktonic organisms, regardless of whether they spend their entire life or part of their life cycle in water column (Thor et al., 2021). Marine plankton may be severely affected by scrubber water discharge, and both lethal and sub-lethal effects were reported on copepods and microalgae (Koski et al., 2017; Thor et al., 2021; Ytreberg et al., 2019, 2021a, 2021b). Since the productivity of aquatic ecosystems may be highly dependent on the lower trophic levels according to bottom-up ecosystem regulation (Aebischer et al., 1990; Irigoien et al., 2004), adverse effects on the plankton could have substantial consequences for the entire ecosystem's functioning.

To address the gap of knowledge on the potential consequences of the discharge of acidic EGCS effluents on marine plankton, we investigated the effects of the scrubber water from an open-loop system on a suite of planktonic bioindicators typical of nearshore environments, including holoplanktonic species and species spending a critical part of their life cycle in the water column. To this end, acute effects, impairment of larval development, and effects of chronic exposure on reproduction were evaluated on test species whose distribution range includes the North Adriatic Sea, such as the bacteria *Aliivibrio fischeri*, the microalgae *Phaeodactylum tricornutum* and *Dunaliella tertiolecta*, the copepod *Acartia tonsa*, and the mussel *Mytilus galloprovincialis*. The North Adriatic Sea was selected as a case study since it is a trafficked semi-enclosed basin located on the southern shore of the Baltic–Adriatic corridor. Here, the Adriatic gateway multi-port system is active (Notteboom, 2010), which consists of the Italian ports of Ravenna, Venezia, and Trieste and the non-Italian ports of Koper (Slovenia) and Rijeka (Croatia), and achieved a total container throughput of 2.6 million TEUs (twenty feet equivalent unit) in 2017 (Lupi et al., 2019). Furthermore, the North Adriatic Sea is included in the proposal for establishing a SECA area in the Mediterranean Sea, approved by the 78th session of the IMO's Marine Environment Protection Committee (MEPC 78) and expected to take effect from 1 July 2025. Consequently, the transit of vessels equipped with EGCS in the North Adriatic Sea is expected to increase significantly in the immediate future.

For comparison, the set of toxicity tests was also applied to the inlet water and ambient seawater collected in the North Adriatic Sea (NSW). Tests on inlet water were performed to obtain information on the effects of background contamination from the piping system (i.e., metals, antifouling agents, lubricants). In contrast, NSW was tested to identify possible background effects in the receiving water offshore the Port of Venice.

2. Materials and methods

2.1. Sampling

Scrubber water samples were collected from the outlet of the open-loop system (STI Marine, Seoul, South Korea) of the vessel LEO C (DANAOS) on 22/11/2021 in the Mediterranean Sea (36°26′21.86"N, 17°34′53.21″E), on the journey from Antwerp (Belgium) to Gebse (Turkey). The water was collected from the outlet pipe, close to the discharge point. Consequently, the sampled water can be assumed to be the same that the planktonic organisms would be exposed to close to the ship. At the time of sample collection, the ship used heavy fuel oil (HFO) with a sulphur content of approximately 2.4 %. Concurrently, the inlet water was collected from the seawater pump system onboard. The sampling point for the inlet water was located after the sea chest connected to the antifouling system. Samples for ecotoxicological test were collected directly into acid-washed 2-L amber glass bottles. Similarly, samples for PAHs analyses were collected into 1-L amber glass bottles, while samples for metals analysis (V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Cd, Pb, and U) were collected in acidified polyethene tubes (50 mL) and 40 mL amber glass vials (for Hg only). All samples were stored onboard at 4 °C in the dark until returning to Antwerp. Then, samples were shipped (overnight refrigerated shipping by delivery courier) to the laboratories for analysis, where they were kept at 4 °C in the dark until analyses.

North Adriatic natural seawater to be used in the experiments was collected in the Gulf of Venice, offshore from the Cavallino-Treporti peninsula (45°24′54.47"N, 12°27′46.44″E), an area with low maritime traffic. Water samples were collected into acid-washed 2-L amber glass bottles and shipped to the Ecotoxicology Laboratory of the Ca' Foscari University. Here, aliquots for chemical analysis (metals, Hg, and PAHs) were collected and stored as reported above and then shipped to the laboratories for analysis.

Before shipping, aliquots for metals and mercury (Hg) analysis were acidified (pH 1.7–1.8) by adding 2 μL high purity 10 M HCl per mL of sample.

2.2. Chemical analyses

Salinity, dissolved oxygen, and pH were measured using a HACH HQ40d multiprobe.

Metals were analysed at the Spanish Council for Scientific Research (CSIC) (Vigo, Spain) using an optimized methodology (González-Ortegón et al., 2019). First, acidified samples were allowed to rest for at least one week to recover any fraction of metals that might have been adsorbed onto the walls of the vials and release loosely bound and labile particulate metals. Then, samples were centrifuged to remove the particulate matter, and UV-oxidation was performed to break down complexing organic ligands in the samples. Metal analysis was finally performed using an Agilent 7900 Inductive Coupled Plasma-Mass Spectrometer (ICP-MS), following an automated preconcentration using a SeaFAST device. All data were reported in μg L^{-1}. The accuracy of the method and analysis was established using Near-shore Seawater Reference Material for Trace Metals (CASS 4, NRC-CNRC) with a range of recoveries higher than 95 % for all metals.

The 16 US EPA priority PAHs and 25 representative alkyl derivatives were analysed using an optimized methodology based on headspace solid-phase microextraction (HS-SPME), equipped with a polydimethylsiloxane (PDMS, 100 μm) fiber and using 10 mL sample volume. The SPME system was coupled to gas chromatography-tandem mass spectrometry (GC–MS/MS). The GC instrument used was a Trace 1300 Series GC (Thermo Scientific), with a Triplus RS autosampler, coupled to a TSQ 9000 Series, operated at a typical electron ionization

energy of 70 V. The GC was equipped with a 30 m Thermo F., TG-1710 MS column (0.25 mm i.d. x 0.5 μm film thickness). Samples were injected in splitless mode with 1.0 mL min^{-1} of helium as the carrier gas. The GC oven was programmed at 50 °C. These conditions were held for 1 min and then increased at a rate of 25 °C min^{-1} to 180 °C. The temperature was further increased to 280 °C at a rate of 6.5 °C min^{-1}, and this temperature was held for 5 min. Finally, 320 °C was reached at a rate of 10 °C min^{-1} and held for 8 min. System control and data acquisition were achieved with the TraceFinder software (Thermo Scientific). The identification of PAHs and the alkyl derivatives was made by Selected Reaction Monitoring (SRM) mode, monitoring two transitions per compound (from precursor ion to two specific fragment ions). Parent PAHs were quantified using the internal standard analytical approach, using their isotopically labelled analogues. For alkylated derivatives, representative standards to quantify different homologue groups were selected based on the approach by Yang et al. (2014) and using response factors relative to the internal standards. For quality assurance and quality control, extraction recoveries (spiking at 20 and 100 ng L^{-1}), limits of detection (LOD) and quantification (LOQ) were calculated. Recoveries for most compounds ranged from approximately 60 to over 100 %. The overall method precision, calculated as the relative standard deviation (%RSD) of the extraction recovery replicates, ranged from 1 to 13 %. LODs and LOQs ranged from 0.17 to approximately 1.7 ng L^{-1} and from 0.5 to 5 ng L^{-1}, respectively, and these values are satisfactory to ensure the detection of PAHs and alkyl derivatives in the samples. All data were reported in ng L^{-1}. The sum of PAHs (ΣPAHs) and alkyl-PAHs (Σalkyl-PAHs) were calculated using LOQ/2 and LOD/2 in place of data below LOQ and LOD, respectively.

2.3. Toxicity testing on scrubber water

Toxicity testing was carried out in the Ecotoxicology Laboratory at the Ca' Foscari University, Venice, Italy. The toxicity of scrubber and inlet water was assessed using five test species (*Aliivibrio fischeri*, *Phaeodactylum tricornutum*, *Dunaliella tertiolecta*, *Acartia tonsa*, and *Mytilus galloprovincialis*) and eight different test methods on a series of scrubber water concentrations ranging from 0.001 % to 40 %. The dilution ranges used for each toxicity test, the dilution media, and the measured endpoints are reported in Table 1. Inlet water and NSW were tested only as undiluted samples. NSW was assayed only in the tests with algae, mussels, and copepods (acute and early-life stage tests).

The Microtox® test was performed according to the ISO 11348 standard method (ISO, 2007) using an M500 Analyzer (Azur Environmental). The reactivation of lyophilised bacteria (BioLight Multi Reagent BIO2006, Aqua Science, Lot No. 10141120A, expiry 12/2022) was carried out by adding ultrapure distilled water (BioLight Recon BIO2001, Lot. No. 10030620, expiry 06/2023) to the acute reagent vial. During the bioassay, the bacterial dispersion was placed in the appropriate analyzer well at 4 °C. The test was run in triplicates. Inhibition of the natural bioluminescence (at 490 nm) of *A. fischeri* was measured after 5, 15 and 30 min of exposure to test solutions at 15 °C. A 20 ‰ NaCl solution was used as the control and dilution medium. A positive control test was performed using a $ZnSO_4$ solution as a reference toxicant following the procedure reported in the ISO 11348 standard method for liquid samples using freeze-dried bacteria (ISO, 2007). Acceptability criteria for the Microtox test included a 15 min-EC_{50} for the reference toxicant (as Zn) within the range 0.6–2.2 mg L^{-1} and bioluminescence in the control (I_0) bracketing the interval 80–110 % after 30 min of exposure.

The algal growth inhibition tests with the chlorophyte *D. tertiolecta* and the diatom *P. tricornutum* were carried out according to the ASTM E1218 standard guideline (ASTM, 2021). The algal clones used for testing scrubber and inlet water derived from in-house cultures maintained in Guillard's F/2 medium (Guillard and Ryther, 1962). In-house culturing conditions provided a temperature of 20 ± 1 °C and a 16:8 light-dark photoperiod. The test was performed in 250-mL glass Erlenmeyer flasks containing 40 mL of testing solution, using 4 replicates for each treatment. F/2 Guillard's medium was used as dilution water and control. The exposure was performed for 96-h at a temperature of 20 ± 1 °C under a 16:8 light-dark photoperiod. Salinity, pH and dissolved oxygen were measured at the exposure's beginning ($t = 0$) and end ($t =$ 96 h). A positive control with $K_2Cr_2O_7$ as a reference toxicant was performed to check for the sensitivity of the algal clones. Acceptability criteria for the test included 1) a daily growth rate (μ_r) in the control (Guillard's F/2 medium) >0.9 after 72-h for both *D. tertiolecta* and *P. tricornutum*, 2) a coefficient of variation (CV%) <7 % in the control,

Table 1
Summary of the test species and test methods used for assessing the toxicity of scrubber and inlet water. Inlet water was tested only as undiluted sample. NSW = North Adriatic Seawater.

Species	Test	Endpoint	Exposure time	Temperature	Replicates	Medium used as control and for diluting scrubber water	Scrubber water tested concentrations (%)
Aliivibrio fischeri	Microtox test	Bioluminesce inhibition	5, 15, and 30 min	15 °C	3	20 ‰ NaCl solution (ISO, 2007)	0.01, 0.1, 1, 2, 5, 10, 20, 40
Phaeodactylum tricornutum	Algal growth test	Algal growth rate (μ_r)	96 h	20 ± 1 °C	4	Guillard's F/2 (Guillard and Ryther, 1962)	5, 10, 20, 40
Dunaliella tertiolecta	Algal growth test	Algal growth rate (μ_r)	96 h	20 ± 1 °C	4	Guillard's F/2 (Guillard and Ryther, 1962)	5, 10, 20, 40
Mytilus galloprovincialis	Embryo-larval development test	Larval development	48 h	18 ± 1 °C	5	NSW	0.001, 0.01, 0.1, 1, 2, 5, 10, 20, 40
Acartia tonsa	Copepod acute test	Lethality	48 h	20 ± 1 °C	4	20 ‰ salinity medium (ISO, 2015b) 30 ‰ salinity medium (ISO, 2015b)	0.01, 0.1, 1, 2, 5, 10, 20, 40
	Copepod early-life stage test	Hatching (F_0)	5 days	20 ± 1 °C	6	20 ‰ salinity medium (ISO, 2015b)	0.001, 0.01, 0.1, 1, 2, 5, 10, 20, 40
		Larval mortality (F_0)					0.001, 0.01, 0.1, 1, 2, 5, 10, 20, 40
		Larval development (F_0)					0.001, 0.01, 0.1, 1, 2, 5, 10, 20, 40
		Egg production (F_0)	13 + 4 days	20 ± 1 °C	10		0.001, 0.01, 0.1, 1
	Copepod long-term exposure test	Hatching (F_1)	21 days for F_0 + 5 days for F_1	20 ± 1 °C	6	20 ‰ salinity medium (ISO, 2015b)	0.001, 0.01, 0.1, 1
		Larval mortality (F_1)					0.001, 0.01, 0.1, 1
		Larval development (F_1)					0.001, 0.01, 0.1, 1

and 3) an EC_{50} for $K_2Cr_2O_7$ within the range 17–62 mg L^{-1} and 20–36 mg L^{-1}, respectively for *D. tertiolecta* and *P. tricornutum*.

The larval development test with *M. galloprovincialis* was performed using wild individuals collected during their reproductive period close to the Malamocco artificial reef in the North Adriatic Sea (45°21′57.72"N, 12°20′38.86″E). Adults were used for testing after an acclimation period (5 days) in aquaria at a temperature of 10 °C, where they were fed with a mixture of three flagellates, the chlorophyte *Tetraselmis suecica* and the haptophytes *Tisochrysis lutea* and *Pavlova lutheri*. The test was performed using an internal method based on ISO 17244 (ISO, 2015a). Briefly, mussels were induced to spawn following thermal stimulation by alternating water baths at 18 °C and 28 °C every 30 min. Spawning females were individually isolated in 300 mL glass beakers and allowed to release their eggs for 30 min. Then the egg suspension was filtered through a 100 μm mesh sieve to remove impurities, faecal pellets and detritus. Males were placed into 150 mL crystallising dishes and allowed to spawn for 15 min before filtering the sperm-cell suspension by using a 32 μm mesh sieve. The motility of the sperm was checked under a dissecting microscope, and then the most motile sperms from at least 3 males were pooled and used for a fecundation trial by adding 1–2 mL of the filtered sperm-cell suspension to the eggs collected from each female, to obtain a 100:1 sperms:egg ratio. The early development stages (polar body appearance and first segmentation) were monitored under an inverted microscope (mod. DM-IL, Leica Microsystems, Wetzlar, Germania). Only the eggs collected from females displaying the highest fecundation rate were selected for the test and then collected and poured into a 1-L glass cylinder. Zygote density was determined by counting four times 100 mL aliquots, and then the fertilised eggs were added to the test solutions to obtain a final density of 100 zygotes cm^{-2} in 20-mL of testing solution. The exposure to scrubber water was initiated within 2-h from fertilisation. Incubations were performed in 30 mL crystallization vials at 18 °C for 48 h under dark conditions ($n = 5$). The larval development was quantified by counting 100 larvae per replicate under an inverted microscope by discriminating normally developed prodissoconch-I larvae (D-shaped larvae) from abnormal prodissoconch-I larvae and delayed larval stages (trochophore larvae, gastrulae) (ASTM, 2004). NSW filtered through a cellulose nitrate filter with 0.2-μm porosity was used as control and dilution water. Salinity, pH and dissolved oxygen were measured at the exposure's beginning ($t = 0$) before the inoculation of the zygotes. A positive control using Cu as a reference toxicant was performed to verify the sensitivity of the testing organisms. Acceptability criteria for the larval development test with *M. galloprovincialis* included a percentage of normally developed larvae in the control >80 % and an EC_{50} for the reference toxicant (Cu) within the range 15–32 μg L^{-1} (Volpi Ghirardini et al., 2005).

The specimens of *A. tonsa* used for testing were obtained from in-house cultures maintained as reported by Picone et al. (2021, 2022a, 2022b). Young adults and copepodite V stage (C—V) individuals were selected for the 48-h acute exposure test, according to ISO 14669 standard guide (ISO, 1999). The test was run in four replicates, using 30-mL crystallising dishes as experimental units. The motility of copepods was checked after 24-h and 48-h exposure to scrubber water, inlet water and NSW. Two different tests were performed, using copepods cultured at 20 ‰ and 30 ‰ salinity, respectively. The 20 ‰ and 30 ‰ salinity media were prepared according to ISO 16778 standard guide (ISO, 2015b) and used as dilution water and controls. Salinity, pH and dissolved oxygen were measured at the exposure's beginning ($t = 0$), after 24-h of exposure, and at the end of the test ($t = 48$-h). A positive control test using 3,5-dichlorophenol (3,5-DCP) as a reference toxicant was run for both specimens cultured at 20 ‰ and 30 ‰. Acceptability criteria for the acute test included a survival >90 % in the controls and an EC_{50} for 3,5-DCP within the range 0.5–1.5 mg L^{-1} (ISO, 1999).

The copepod early-life stage test with *A. tonsa*, also referred to as the larval development ratio test (LDR test), was performed according to the ISO 16778 standard guide (ISO, 2015b), modified according to Picone et al. (2021, 2022b). Briefly, the test started by adding a known number of newly released eggs collected from the in-house cultures (up to 80) to a 100 mL glass beaker containing 30 mL of test solution. The test was run in 6 replicates. The 20 ‰ salinity medium was used as a control and for dilution. Test vials were then maintained for five days in a thermostatic incubator (FOC 215E, Velp Scientifica, Milan, Italy) at 20 ± 1 °C, with a 16-h light 8-h dark photoperiod. On day 2, 30 mL of test solution were added to each beaker to refresh the medium. Larvae were fed on day-0 and day-2 with 100 μL of a concentrated mixture of *T. suecica*, *T. lutea* and *P. lutheri*. The exposure ended on day 5 when approximately 40 % of the larvae in controls should have completed the metamorphosis to the copepodite-I stage (C—I). The ratio of C—I larvae was first determined in one control's replicate, after exactly 5-d, by fixing the beaker's content with 0.5 mL of Lugol's solution, used to kill, stain, and preserve unhatched eggs, nauplii and copepodites. The test solution was then filtered through a mixed cellulose ester filter with gridlines (diameter 47-mm, porosity 0.45-μm), and all the larvae and unhatched eggs were counted under a dissecting microscope (Stemi SV 6, Zeiss). If the first control contained 40 % or more copepodites, the test was finished, and the other beakers' content was fixed with Lugol's solution. Otherwise, the test was run for additional two hours before another control was stained with Lugol's solution. Unhatched eggs, nauplii and copepodites recovered on the filter were counted to calculate the hatching ratio (H), the early-life stages mortality ratio (ELS_m) and the larval development ratio (LDR), namely the ratio between copepodites and total recovered larvae. Salinity, pH and dissolved oxygen were measured at the exposure's beginning ($t = 0$) and the end ($t = 120$-h). A positive control test using 3,5-dichlorophenol (3,5-DCP) as a reference toxicant was run to verify the quality of the biological material. Acceptability criteria for the LDR test included $H > 0.80$, $ELS_m < 0.30$, and $0.30 < LDR < 0.70$ in the controls, and then an EC_{50} for 3,5-DCP within the range 31–250 μg L^{-1} for the endpoint LDR (Picone et al., 2018).

The long-term exposure of *A. tonsa* to scrubber and inlet water was performed according to Picone et al. (2022a). The test started on day-0 by adding 400–600 eggs released by the in-house laboratory cultures to each treatment (Table 1) in 1-L glass bottles. The bottles were then kept at 20 ± 1 °C, with a 16-h light 8-h dark photoperiod and under LED illumination. The food was provided three times per week during the renewal of test solutions. It was done as follows: 250-mL of test solution was siphoned off and substituted with the same volume of a freshly prepared solution, obtained by diluting appropriate aliquots of undiluted scrubber water with appropriate volumes of 20 ‰ salinity medium and algal mix (*T. suecica*, *T. lutea*, and *P. lutheri*) to obtain a food concentration > 6×10^4 cells mL^{-1}. Under test conditions, the eggs hatch within 24 h, the larvae reach the C—I stage after 5/6 days, and the adult stage is reached after 11 days. Finally, the onset of egg production is expected on day 12. On day 13, at least twelve females were collected from their treatment and individually placed into 15-mL glass vials filled with 10 mL of 20 ‰ salinity medium and 100 μL of the algal mix at 10^4 cell mL^{-1}. The exposure in the vials lasted 96-h, but the egg production was verified at 24-h intervals for three days by transferring the females to a new set of vials filled with 20 ‰ salinity medium and algal mix and then filtering the content through a cellulose nitrate filter (Ø 47 mm, porosity 0.45 μm) with gridlines. From day 18 to day 21, eggs produced by scrubber water treatments were collected from their respective culture, stored at 4 °C and then used to start an LDR test on the F_1 generation, according to the procedure reported above. The eggs were exposed only to the 20 ‰ salinity medium to assess the effects on the reproduction due to the exposure of parents (F_0) to scrubber water without superimposing the effects of F_1 exposure to scrubber water. Acceptability criteria for the long-term exposure test included an EC_{50} for 3,5-DCP within the range 31–250 μg L^{-1} for the endpoint LDR measured on the F_0 generation, mean egg production of at least 7 eggs $female^{-1}$ d^{-1} in the control (20 ‰ salinity medium), and the meeting of the following parameters in the control of the LDR test with generation

F_1: H > 0.80, ELS_m < 0.30, and LDR in the range 0.30–0.70.

2.4. Toxicity testing with A. tonsa on metals and PAHs

The acute test with *A. tonsa* was also used for assessing the individual toxicity of primary pollutants detected in the analysed scrubber water. The tests were performed to verify whether a single compound might cause the adverse effects observed in the scrubber water. The focus was on Co, Mn, Cu, Ni, V, and Zn as trace elements and fluorene, naphthalene, and phenanthrene concerning PAHs. The test concentrations bracketed those expected in the scrubber water dilutions and were: 0–1000 μg L^{-1} for Co, Mn and V; 0–250 μg L^{-1} for Cu; 0–200 μg L^{-1} for Ni; 0–600 μg L^{-1} for Zn; 0–1000 ng L^{-1} for fluorene; 0–6000 ng L^{-1} for naphthalene; 0–5000 ng L^{-1} for phenanthrene. The test method was the 48-h acute exposure test (ISO, 1999). The test was run in four replicates, using 30-mL crystallising dishes as experimental units. The 20 ‰ salinity medium was used as the control and dilution water.

All solutions were prepared from ACS reagent grade salts with purity >98 % ($CoCl_2 \cdot 6H_2O$, J.T. Baker; $MnCl_4 \cdot 4H_2O$, J.T. Baker; $CuSO_4 \cdot 5H_2O$, J.T. Baker; $NiCl_2$, Sigma-Aldrich; $ZnSO_4 \cdot 7H_2O$, Carlo Erba) or standard analytical solutions (NH_4VO_3 in 0.5 HNO_3, Supelco; fluorene 10 ng μL^{-1} in acetonitrile, Dr. Ehrenhofer; naphthalene 10 ng μL^{-1} in methanol, Dr. Ehrenhofer; phenanthrene 10 ng μL^{-1} in cyclohexane, Dr. Ehrenhofer).

2.5. Data analysis

Hypothesis-based toxicity data, namely the no-observed effect concentration (NOEC) and the lowest observed effect concentration (LOECs) were estimated using the one-way ANOVA and the Tukey's HSD post hoc test (α = 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. One-way ANOVA and the Tukey's HSD test were also used to check for differences among the tested treatments. Before running ANOVA, data normality and variance homoscedasticity were checked using Kolmogorov-Smirnov and Levene's tests based on medians, respectively. When data normality and variance homoscedasticity conditions were not met, the Kruskal-Wallis non-parametric test on the ranks and Dunn's pairwise test were performed. These statistical analyses were performed using the software package IBM SPSS Statistics v.25.

Effective concentrations 10 % (EC_{10}s), effective concentrations 20 % (EC_{20}s), and effective concentrations 50 % (EC_{50}s) were calculated using a statistical program for continuous response developed at the Technical University of Denmark (Christensen et al., 2009). A log-normal distribution of the observed effects was assumed.

Correlations between ecotoxicological effects and pH, dissolved oxygen and salinity of the tested concentrations were explored using the Spearman non-parametric correlation (α = 0.05), using parameters measured at the beginning of the exposure (t_0).

3. Results

3.1. Chemical analysis

The results of the analyses are summarised in Tables 2 and 3. Concerning metals, the scrubber process produced a marked increase in concentrations of V, Cr, Fe, Co, and Ni compared to the inlet water and NSW, while Zn increased substantially compared to inlet water but was comparable to the levels observed in NSW (49 μg L^{-1}). In particular, for V (348 μg L^{-1}) and Ni (99 μg L^{-1}), concentrations in scrubber water were 195 and 350-fold higher than in inlet water, respectively (Table 2). Mn, Cu, As, Cd, Pb, and U concentrations were higher in scrubber water than in inlet water, but the concentration increase was minimal for all these elements. As concern Cu, concentrations in scrubber water and NSW are in the same range, evidencing that coastal waters in the North Adriatic are enriched in Cu.

The scrubbing process largely increased the concentration of PAHs and alkyl-PAHs (Table 3). Concerning the 16 EPA PAHs, all compounds were below the method's LOQ or even LOD in the inlet water (Table 3), while the total 16 EPA PAH concentration in scrubber water was 13,690 ng L^{-1}, with naphthalene (7499 ng L^{-1}), phenanthrene (4815 ng L^{-1}), and fluorene (1000 ng L^{-1}) accounting for 85 % of the total PAHs concentration. The scrubber water concentrations of the PAHs anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, and indeno(1,2,3-*cd*)pyrene were all below the LOQ or LOD (Table 3). NSW evidenced traces of phenanthrene (5.3 ng L^{-1}) and fluoranthene (1.3 ng L^{-1}). Similarly to parent compounds, the alkyl-PAHs were below the LOQ or LOD in inlet water, while the total alkyl-PAHs concentration in scrubber water was almost three times the 16 PAH concentration, i.e., 36,612 ng L^{-1}. The alkyl-PAHs detected in the scrubber water are those derived from the most abundant PAHs in the scrubber water, i.e. the alkylated products of naphthalene and phenanthrene. Alkyl-PAHs were not measured in NSW.

3.2. Toxicity testing

3.2.1. Quality assurance / quality control (QA/QC)

Acceptability criteria for control and reference toxicants were met for all test procedures, as summarised in Table 4. These results guarantee the good quality of the biological material used for testing and the reliability of the toxicity data obtained for scrubber water, inlet water and NSW.

Table 2
Summary of trace element analyses performed on scrubber water, inlet water, and North Adriatic seawater (NSW). n.d. = not determined. LOQ^1 and LOD^1 refer to analyses on the inlet and scrubber water while LOQ^2 and LOD^2 to analyses on NSW.

Element	Concentration (μg L^{-1})						
	Inlet	Scrubber	LOD^1	LOQ^1	NSW	LOD^2	LOQ^2
V	1.79	348.28	0.005	0.015	0.73	0.005	0.0015
Cr	0.23	7.50	0.01	0.03	0.16	0.03	0.09
Mn	0.4	3.1	0.005	0.015	2.8	0.005	0.0015
Fe	3	109	0.01	0.03	4	0.01	0.03
Co	0.013	0.474	0.0001	0.0003	0.026	0.001	0.003
Ni	0.28	99.46	0.001	0.003	0.35	0.001	0.003
Cu	0.9	5.3	0.002	0.006	4.8	0.002	0.006
Zn	3	49	0.005	0.015	49	0.005	0.0015
As	1.51	2.02	0.02	0.06	0.94	0.05	0.15
Cd	0.007	0.032	0.0001	0.0003	0.007	0.0005	0.00015
Hg	n.d.	0.0018	0.0001	0.0003	0.0003	0.0001	0.0003
Pb	0.09	0.35	0.001	0.003	0.65	0.001	0.003
U	3.7	6.9	0.001	0.003	1.7	0.001	0.003

Table 3
Summary of the PAHs and alkyl-PAHs analyses performed on scrubber water, inlet water, and North Adriatic seawater (NSW). LOD = limit of detection; LOQ = limit of quantification; n.d. = not determined. LOQ^1 and LOD^1 refer to analyses on the inlet and scrubber water while LOQ^2 and LOD^2 to analyses on NSW. The sum of PAHs (ΣPAHs) and alkyl-PAHs (Σalkyl-PAHs) were calculated using LOQ/2 and LOD/2 in place of data below LOQ and LOD, respectively.

Chemicals		Concentration (ng L^{-1})						
		Inlet	Scrubber	LOD^1	LOQ^1	NSW	LOD^2	LOQ^2
PAHs	Naphtalene	< LOQ	7499	9.0	12.9	< LOQ	17	51
	Acenaphthylene	< LOQ	141	5.0	5.0	< LOQ	0.2	0.6
	Acenaphthene	< LOQ	592	5.0	5.0	< LOQ	0.1	2.6
	Fuorene	< LOD	1000	1.7	5.0	< LOQ	0.1	3.3
	Phenanthrene	< LOQ	4815	5.3	5.8	5.3	0.1	3.7
	Anthracene	< LOD	< LOQ	1.7	5.0	< LOD	0.1	0.3
	Fluoranthene	< LOD	139	2.6	3.2	1.6	0.1	0.6
	Pyrene	< LOD	701	1.7	5.0	< LOQ	0.1	0.7
	Benzo(*a*)anthracene	< LOD	89	2.0	3.8	< LOQ	0.2	0.7
	Chrysene	< LOD	236	1.1	1.4	< LOQ	0.1	0.4
	Benzo(*b*)fluoranthene	< LOQ	15	3.7	3.8	< LOD	0.2	0.6
	Benzo(*k*)fluoranthene	< LOQ	< LOQ	2.4	2.5	< LOD	0.5	1.5
	Benzo(*c*)phenanthrene	< LOD	< LOD	0.3	1.0	n.d.	n.d.	n.d.
	Benzo(*a*)pyrene	< LOD	< LOD	3.7	4.3	< LOD	0.7	2.3
	Indeno(1,2,3-*cd*)pyrene	< LOQ	< LOD	1.7	5.0	< LOD	0.5	1.8
	Dibenzo(*a*,*h*)anthracene	< LOQ	56	3.4	4.8	< LOD	0.4	1.2
	Benzo(*g*,*h*,*i*)perylene	< LOQ	111	3.4	4.8	< LOD	0.3	1.1
	ΣPAHs	**32.2**	**15,400**			**37.9**		
alkyl-PAHs	Naphthalene-2-methyl	< LOQ	4599	3.4	3.7	n.d.	n.d.	n.d.
	Naphthalene-1-methyl	< LOD	3573	1.4	1.9	n.d.	n.d.	n.d.
	Naphthalene-C2	< LOD	8461	0.4	1.1	n.d.	n.d.	n.d.
	Naphthalene-C3	< LOD	4025	0.3	1.0	n.d.	n.d.	n.d.
	Naphthalene-C4	< LOD	1334	0.3	1.0	n.d.	n.d.	n.d.
	Phenanthrene-C1	< LOD	4961	0.4	1.1	n.d.	n.d.	n.d.
	Phenanthrene-C2	< LOD	3405	0.3	1.0	n.d.	n.d.	n.d.
	Phenanthrene-C3	< LOD	1484	0.3	1.0	n.d.	n.d.	n.d.
	Phenanthrene-C4	< LOD	921	0.3	1.0	n.d.	n.d.	n.d.
	Fluorene-C1	< LOD	1859	0.3	1.0	n.d.	n.d.	n.d.
	Fluorene-C2	< LOD	1101	0.3	1.0	n.d.	n.d.	n.d.
	Fluoranthene-Pyrene-C1	< LOD	889	0.3	1.0	n.d.	n.d.	n.d.
	Σalkyl-PAHs	**5.9**	**36,612**			**n.d.**		

Table 4
Summary of the QA/QC parameters measured in controls and tests with the reference toxicants and their accordance with acceptability criteria. QA/QC results are reported as the arithmetic means of the experimental replicates ± standard deviation or effective concentration 50 % (EC_{50}). The numbers in brackets under the EC_{50} values represent the 95 % confidence intervals associated with the EC_{50}'s estimate.

Species	Test	Parameter	Acceptability criteria	QA/QC results
Aliivibrio fischeri	Microtox test	$I_{(0)}$ in control	80 % < $I_{(0)}$ < 110 %	$I_{(0)}$ = 90 ± 1 %
		15 min-EC_{50} for Zn	0.6–2.2 mg L^{-1}	1.23 mg L^{-1} (1.15–1.31)
Phaeodactylum tricornutum	Algal growth test	72 h-μ_r in control	μ_r > 0.9	μ_r = 1.26 ± 0.03
		CV(%) in control	CV(%) < 7	CV(%) = 2.1
		96 h-EC_{50} for $K_2Cr_2O_7$	20–36 mg L^{-1}	31 mg L^{-1} (27–36)
Dunaliella tertiolecta	Algal growth test	72 h-μ_r in control	μ_r > 0.9	μ_r = 0.90 ± 0.01
		CV(%) in control	CV(%) < 7	CV(%) = 0.9
		96 h-EC_{50} for $K_2Cr_2O_7$	17–62 mg L^{-1}	39 mg L^{-1} (37–40)
Mytilus galloprovincialis	Embryo-larval development test	D-shape larvae percentage in control	D-shape >80 %	D-shape = 84 ± 2 %
		48 h-EC_{50} for Cu	15–32 μg L^{-1}	17 μg L^{-1} (15–19)
Acartia tonsa	Copepod acute test	Survival (S) in control	S > 90 %	S = 100 %
		48 h-EC_{50} for 3,5-DCP	0.5–1.5 mg L^{-1}	0.53 mg L-1 (0.49–0.58)
	Copepod early-life stage test	H in control (F_0)	H > 0.80	H = 0.91 ± 0.01
		ELS_m in control (F_0)	ELS_m < 0.30	ELS_m = 0.11 ± 0.10
		LDR in control (F_0)	0.30 < LDR < 0.70	LDR = 0.65 ± 0.07
		5d-EC_{50} for 3,5-DCP (for F_0's LDR)	31–250 μg L^{-1}	63 μg L^{-1} (48–82)
	Copepod long-term exposure test	Egg production in control (F_0)	> 7 eggs $female^{-1}$ d^{-1}	8 eggs $female^{-1}$ d^{-1}
		H in control (F_1)	H > 0.80	H = 0.86 ± 0.09
		ELS_m in control (F_1)	ELS_m < 0.30	ELS_m = 0.17 ± 0.12
		LDR in control (F_1)	0.30 < LDR < 0.70	LDR = 0.47 ± 0.14
		5d-EC_{50} for 3,5-DCP (for F_0's LDR)	31–250 μg L^{-1}	63 μg L^{-1} (48–82)

3.2.2. Acute toxicity of scrubber water and inlet water

The toxicity data for scrubber water are summarised in Table 5, while physiochemical parameters and statistical analysis are reported in Supplementary Material (Tables 1S, 2S, 3S, and 4S). The bioluminescence of *A. fischeri* showed similar trends after 5, 15, and 30 min of exposure (Fig. 1, upper panel). Scrubber water significantly reduced the bioluminescence compared to the control (20 ‰ NaCl solution), only at a concentration of 20 % (LOEC), while differences compared to inlet water and NSW were significant only at the highest tested concentration of scrubber water (40 %). No significant differences were observed between inlet water and NSW after 5 min of exposure, but NSW showed inhibition of I_t compared to the inlet water and control after 15 min (Tukey's HSD test: $p < 0.001$) and 30 min of exposure (Tukey's HSD test: $p < 0.001$). The analysis of the effect-concentration curve confirmed that the onset of the toxic effects was at a concentration of approximately 20 % scrubber water (EC_{10}).

The inhibition of algal growth compared to the control (Guillard's F/2 medium) was significant at concentrations of 20 % and 40 % scrubber water for *D. tertiolecta* (Tukey's HSD test: $p = 0.003$) and only at 40 % scrubber water for *P. tricornutum* (Tukey's HSD test: $p = 0.025$) (Fig. 1, middle panel). No significant differences were observed between the control and NSW (Tukey's HSD test: $p = 0.062$ and $p = 0.451$, respectively, for *D. tertiolecta* and *P. tricornutum*) and between inlet water and NSW (Tukey's HSD test: $p = 0.231$ and $p = 0.286$, respectively, for *D. tertiolecta* and *P. tricornutum*). Conversely, inlet water showed a significantly lower growth rate compared to the control (Tukey's HSD test: $p < 0.001$ and $p = 0.025$, respectively, for *D. tertiolecta* and *P. tricornutum*). The analysis of the effect-concentration curve confirmed that effects on *D. tertiolecta* were observed at scrubber water concentrations lower than those causing an effect on *P. tricornutum* ($EC_{10} = 15$ % and $EC_{10} = 34$ %, respectively, for *D. tertiolecta* and *P. tricornutum*). The EC_{50}s were not calculable. Spearman correlation did not evidence a significant correlation between pH and growth rate for *D. tertiolecta* (Spearman's $R = 0.75$, $p = 0.055$) and *P. tricornutum* (Spearman's $R = 0.36$, $p = 0.423$).

The lethal effects of scrubber water on adult specimens of *A. tonsa* showed slight differences between specimens cultured at 20 ‰ and 30 ‰ salinity (Table 5 and Fig. 1, lower panel). NOECs and LOECs values evidenced that specimens cultured at 20 ‰ salinity (NOEC = 5 %; LOEC = 10 %) were more sensitive to scrubber water than individuals reared at 30 ‰ salinity (NOEC = 10 %; LOEC = 20 %). Similarly, survival in inlet water varied significantly with culturing salinity, being markedly lower for copepods cultured at 20 ‰ (50 ± 13 % survival) than for those reared at 30 ‰ (95 ± 5 % survival). Survival in NSW was comparable to that observed in controls at 20 ‰ and 30 ‰ salinity (Tukey's HSD test: $p = 0.999$ for both salinities). The Spearman correlation evidenced that the survival of the specimens cultured at 20 ‰ is negatively correlated with salinity (Spearman's $R = -0.63$, $p = 0.036$) and dissolved oxygen (Spearman's $R = -0.63$, $p = 0.036$) but positively correlated with pH (Spearman's $R = 0.67$, $p = 0.023$). Conversely, survival of adults reared at 30 ‰ salinity was correlated only with pH (Spearman's $R = 0.61$, $p = 0.046$).

3.2.3. Toxicity of scrubber water and inlet water to early-life stages

The larval development of *M. galloprovincialis* was significantly delayed by inlet water and scrubber water (Fig. 2). The percentage of normally developed D-shape larvae in inlet water was <50 % of the normal D-shape observed in the control (NSW water), and a significant reduction of D-shaped larvae in scrubber water was observed at concentrations as low as 1 %. No larvae had developed further than an early trochophore stage in the 10 % scrubber water concentration. At 20 % scrubber water, the development was blocked at the 4-cell stage, while at 40 % scrubber water, only the first polar body was observed. The effect-concentration curve was steep, and the EC_{50} was estimated at a 6 % scrubber water concentration. Spearman's correlation did not highlight any relationship between the percentage of D-shaped larvae with pH and salinity but evidenced a moderately high correlation (Spearman's $R = 0.62$, $p = 0.42$) with dissolved oxygen (range: 4.32–8.85 mg L^{-1}). Physicochemical parameters and statistical analysis are reported in Supplementary Material, Table 5S.

The hatching ratio of *A. tonsa* eggs (Fig. 3, upper panel) was significantly affected by scrubber water at concentrations of 20 % and 40 %. No differences were observed between the control and NSW (Tukey's HSD test: $p = 0.406$) and inlet water (Tukey's HSD test: $p = 0.999$).

Table 5
Summary of the toxicity data

Species	Test	Endpoint	NOEC	LOEC	EC_{10}	EC_{20}	EC_{50}
Aliivibrio fischeri	Microtox test	Bioluminesce inhibition (5 min)	10 %	20 %	20 % (18 % - 22 %)	25 % (23 % - 26 %)	38 % (37 % - 39 %)
		Bioluminesce inhibition (15 min)	10 %	20 %	20 % (19 % - 22 %)	26 % (24 % - 27 %)	40 % (39 % - 41 %)
		Bioluminesce inhibition (30 min)	10 %	20 %	23 % (21 % - 25 %)	27 % (26 % - 29 %)	39 % (38 % - 40 %)
Phaeodactylum tricornutum	Algal growth test	Algal growth rate (μ_r)	20 %	40 %	34 % (15 % - 62 %)	n.c.	n.c.
Dunaliella tertiolecta	Algal growth test	Algal growth rate (μ_r)	10 %	20 %	15 % (10 % - 23 %)	31 % (26 % - 38 %)	n.c.
Mytilus galloprovincialis	Embryo-larval development test	Larval development	0.1 %	1 %	4.9 % (3.1 % - 8.0 %)	5.3 % (3.6 % - 7.9 %)	6 %
Acartia tonsa	Copepod acute test	Lethality (cultured at 20 ‰)	5 %	10 %	8 % (5 % - 11 %)	9 % (7 % - 11 %)	11 % (10 % - 12 %)
		Lethality (cultured at 30 ‰)	10 %	20 %	10 % (8 % - 14 %)	12 % (10 % - 16 %)	17 % (15 % - 19 %)
	Copepod early-life stage test	Hatching (F_0)	10 %	20 %	9 % (7 % - 12 %)	13 % (10 % - 16 %)	25 % (22 % - 30 %)
		Larval mortality (F_0)	10 %	20 %	9 % (8 % - 10 %)	10 % (9 % - 11 %)	13 % (11 % - 14 %)
		Larval development (F_0)	1 %	2 %	1.1 % (0.7 % - 1.6 %)	1.2 % (0.9 % - 1.6 %)	1.5 % (1.2 % - 1.9 %)
		Egg production (F_0)	The concentration-effect curve is U-shaped, with the lowest and significantly lower compared to control egg production observed at 0.01 % scrubber water				
	Copepod long-term exposure test	Hatching (F_1)	> 1 %	> 1 %	n.c.	n.c.	n.c.
		Larval mortality (F_1)	> 1 %	> 1 %	n.c.	n.c.	n.c.
		Larval development (F_1)	The concentration-effect curve is U-shaped, with the lowest and significantly lower compared to control LDR observed at 0.1 % scrubber water				

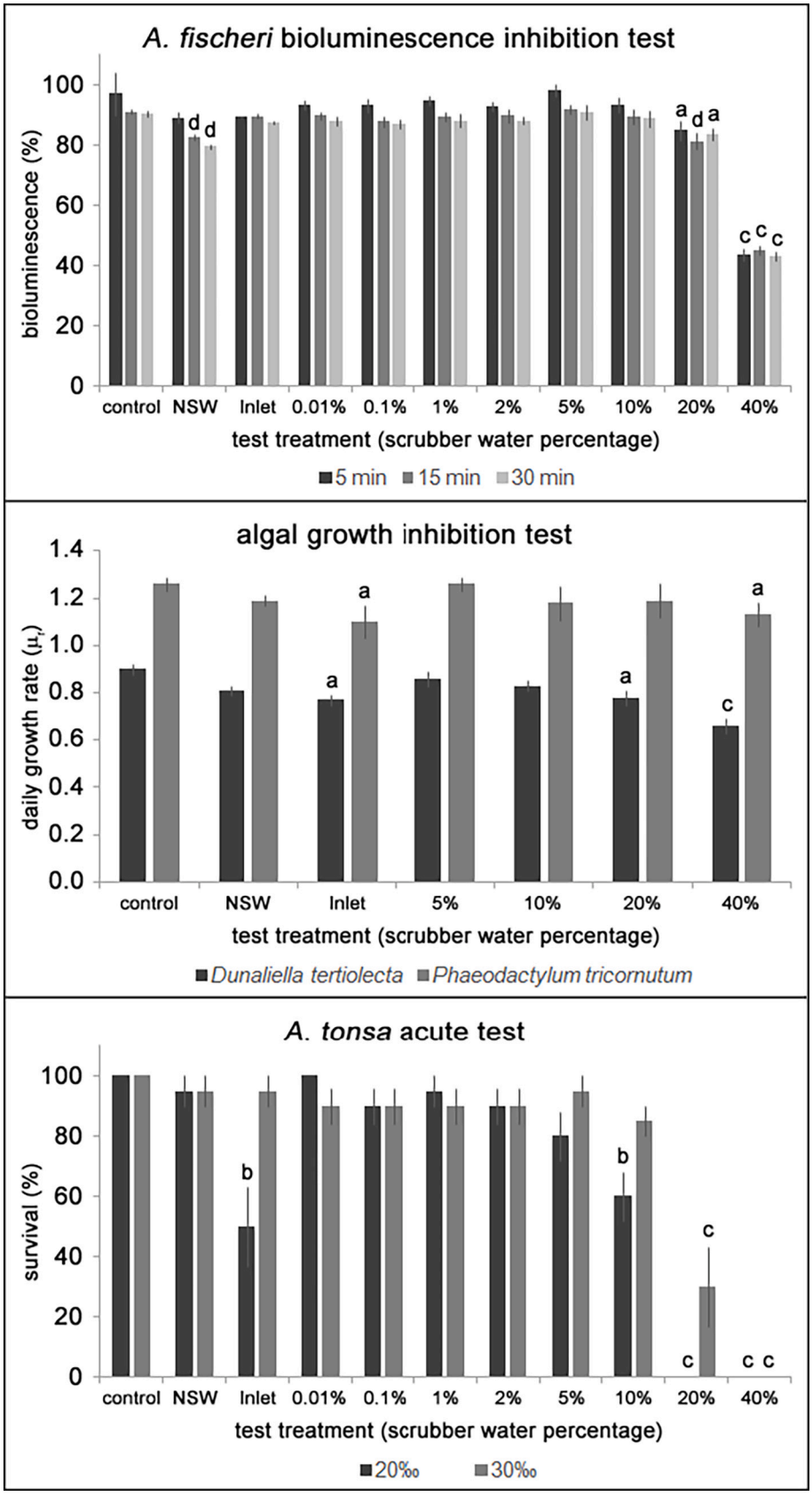

Fig. 1. Results of the acute tests with *A. fischeri* (upper panel), microalgae (middle panel), and *A. tonsa* (lower panel) performed on scrubber water, inlet water, and North Adriatic Sea water (NSW). Lower case letters indicate significant differences. a = treatments with response significantly lower than the control; b = treatments with response significantly lower than control and NSW; c = treatments with response significantly lower than control, NSW, and inlet water; d = treatments with response significantly lower than control and inlet water. Error bars designate standard deviations. The following media were used as controls (Table 1): 20 ‰ NaCl solution for *A. fischeri* (ISO, 2007); Guillard's F/2 medium for microalgae (Guillard and Ryther, 1962); 20 ‰ and 30 ‰ salinity media for *A. tonsa* (ISO, 2015b).

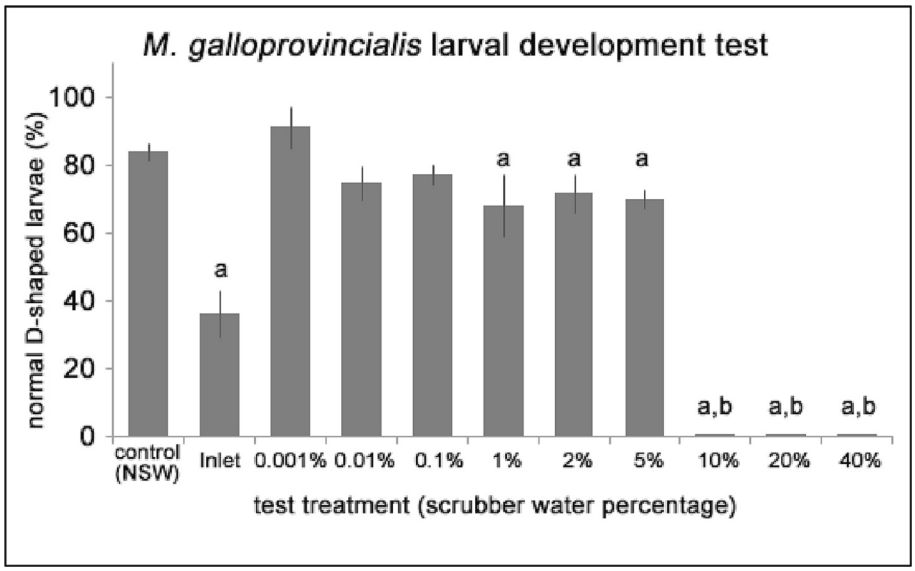

Fig. 2. Results of the early-life stage test with *M. galloprovincialis* performed on scrubber water, inlet water, and North Adriatic Sea water (NSW). a = treatments with response significantly lower than the control (NSW); b = treatments with response significantly lower than control and inlet water.

There were no differences between NSW and inlet water (Tukey's HSD test: $p = 0.866$). The hatching ratio was positively correlated with dissolved oxygen (Spearman's $R = 0.85$, $p = 0.001$; dissolved oxygen range: 8.55–9.31 mg L^{-1}) and negatively correlated with salinity (Spearman's $R = -0.71$, $p = 0.015$; salinity range: 21–30 ‰).

As observed for hatching, also the survival of the larval stages differed significantly from the control at scrubber water concentrations of 20 % and 40 %, where none of the larvae survived (Fig. 3, middle panel). The EC50% for scrubber water was estimated at 13 % for larval mortality. In addition, inlet water also provided a survival significantly lower than the control (Tukey's HSD test: $p < 0.001$). Conversely, no differences were observed between inlet water and NSW (Tukey's HSD test: $p = 0.079$). Larval survival was positively correlated with dissolved oxygen (Spearman's $R = 0.77$, $p = 0.005$; dissolved oxygen range: 8.55–9.31 mg L^{-1}) and negatively correlated with salinity (Spearman's $R = -0.82$, $p = 0.002$; salinity range: 21–30 ‰).

The larval development was the most sensitive endpoint measured in the early-life stage test with *A. tonsa* (Fig. 3, lower panel). A significant and severe delay in the LDR compared to control was observed at a concentration as low as 2 % scrubber water, so the EC_{50} was estimated at 1.5 % scrubber water. Inlet water also wholly inhibited the larval development of the copepods, while no differences were observed between the control and NSW (Tukey's HSD test: $p = 0.991$). The larval development showed a mild correlation with dissolved oxygen (Spearman's $R = 0.61$, $p = 0.048$).

Physicochemical parameters and statistical analysis for the test with early-life stages of *A. tonsa* are reported in Supplementary Material, Table 6S.

3.2.4. Long-term effects of scrubber and inlet water on A. tonsa

Egg production by F_0 generation showed a U-shaped trend (Fig. 4). At 0.001 % scrubber water, egg production was already affected, but the difference with the control (20 ‰ salinity medium) was not significant at $\alpha = 0.05$. The lowest egg production was obtained at 0.01 % scrubber water, then increased at 0.1 % and 1 % scrubber water concentrations (Fig. 4, panel A). However, egg production was lower in all treatments than in control (20 ‰ salinity medium). Inlet water also reduced daily egg production compared to the control condition. Due to the high variability observed among replicates, the egg production was significantly lower than control conditions only at 0.01 % scrubber water (Tukey's HSD test: $p = 0.010$). Consequently, NOEC and LOEC for egg production were not estimated.

The hatching ratio (Fig. 4, panel B) of generation F_1 was seemingly unaffected by the exposure to scrubber water and inlet water (one-way ANOVA: $F = 0.57$, $p = 0.724$). The early-life stage survival was lower in treatments than in control, suggesting a possible negative impact of scrubber water (Fig. 4, panel C). However, the statistical analyses highlighted that these differences were not statistically significant (one-way ANOVA: $F = 0.59$, $p = 0.709$), possibly due to the variability in the treatments (Supplementary Material, Table 8S). Conversely, the larval development of the F_1 generation showed a U-shaped trend (Fig. 4, panel D): LDR decreased almost linearly from the control to 0.1 % scrubber water concentration, then increased at 1 %. Albeit the variability of the LDR endpoint was noteworthy in the scrubber water treatments (Supplementary Material, Table 8S), the statistical analysis recognised a significant retard in the larval development at 0.1 % scrubber water compared to the control (Kruskal-Wallis test: $H = 20.9$, $p < 0.001$; Dunn's post hoc test: $p = 0.003$). Similarly, also inlet water caused a significant delay in the metamorphosis to the C—I stage (Dunn's post hoc test: $p = 0.039$).

During the exposure of F_0 generation, salinity (one-way ANOVA: $F = 0.06$, $p = 0.993$) and pH (one-way ANOVA: $F = 1.74$, $p = 0.174$) did not vary significantly between control and scrubber water dilutions (Supplementary Material, Table 13S). Conversely, pH (one-way ANOVA: $F = 6.74$, $p < 0.001$) and salinity (one-way ANOVA: $F = 458$, $p < 0.001$) of inlet water differed significantly compared to control and scrubber water dilution all over the 18 days of exposure.

3.2.5. Effects of trace elements and PAHs on A. tonsa survival

None of the individually tested chemicals affected the survival of *A. tonsa* at the concentrations measured in the scrubber water. Copper was the trace element most toxic to *A. tonsa*, with 24 h-EC_{50} and 48 h-EC_{50} estimated at 194 µg L^{-1} and 75 µg L^{-1}, respectively. For Co, 48 h-NOEC and 48 h-EC_{50} were estimated at 500 µg L^{-1} and 736 µg L^{-1}, respectively. For V (one-way ANOVA: $F = 0.67$, $p = 0.654$), Zn (one-way ANOVA: $F = 1.63$, $p = 0.244$), Mn (one-way ANOVA: $F = 0.58$, $p = 0.684$), and Ni (one-way ANOVA: $F = 0.45$, $p = 0.808$), the survival of *A. tonsa* was not affected up to the highest tested concentration (600 µg L^{-1} for Zn; 1000 µg L^{-1} Mn and V; 200 µg L^{-1} for Ni). Similarly, also phenanthrene (one-way ANOVA: $F = 0.90$, $p = 0.427$), naphthalene (one-way ANOVA: $F = 1.21$, $p = 0.346$), and fluorene (one-way ANOVA: $F = 0.10$, $p = 0.756$) did not affect the survival up to 5000 ng L^{-1}, 6000

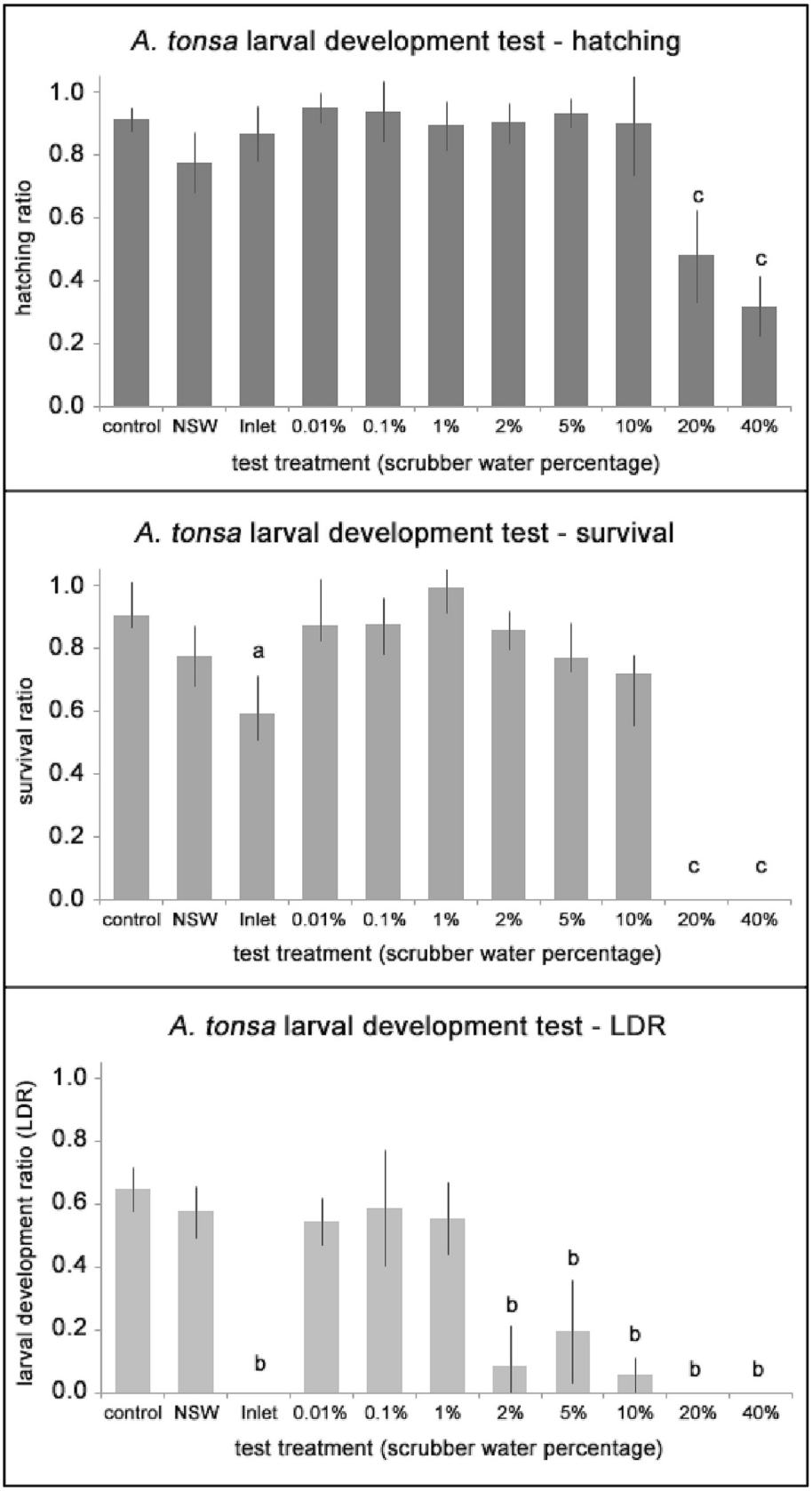

Fig. 3. Results of the larval development test with *A. tonsa*: hatching ratio (upper panel), early-life stages survival (middle panel), and larval development ratio (lower panel) performed on scrubber water, inlet water, and North Adriatic Sea water (NSW). Lower case letters indicate significant differences: a = treatments with response significantly lower than the negative control; b = treatments with response significantly lower than control (20 ‰ salinity medium; ISO, 2015b) and NSW; c = treatments with response significantly lower than control, NSW, and inlet water. Error bars designate standard deviations.

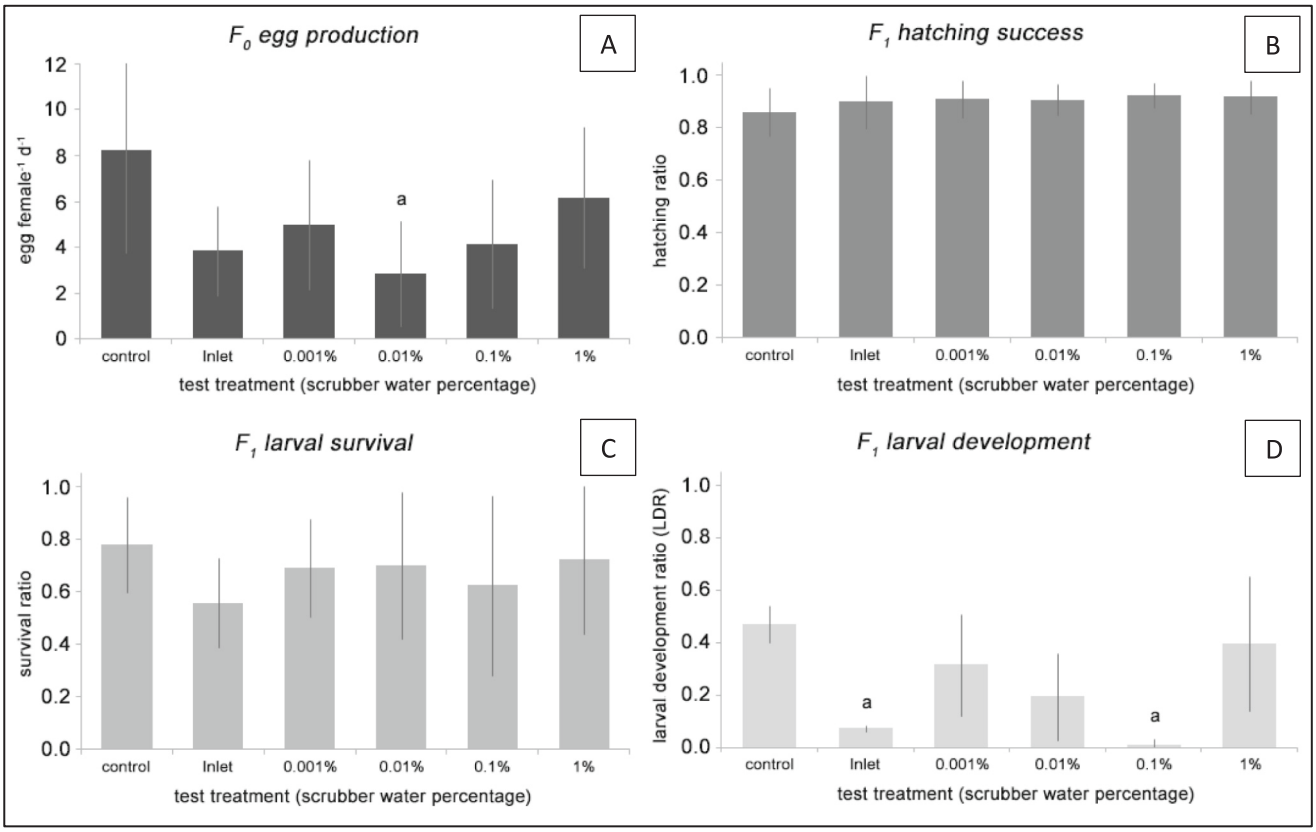

Fig. 4. Results of the long-term exposure test with *A. tonsa*: egg production by F_0 generation (panel A), hatching of F_1 generation (panel B), larval survival for F_1 (panel C), and larval-development of F_1 generation performed on scrubber water and inlet water. Lower case letters indicate significant differences: a = treatments with response significantly lower than the control (20 ‰ salinity medium; ISO, 2015b). Error bars designate standard deviations.

ng L^{-1}, and 1000 ng L^{-1}, respectively. Calculated NOEC, EC_{20}, and EC_{50} are reported in Supplementary Material – Table 11S.

4. Discussion

The different species and life stages used for testing responded differently to the exposure to the scrubber water, highlighting the need to perform multi-species and multi-endpoints research to characterise the possible adverse effects of EGCS discharge waters on the receiving waters. Furthermore, the exposure of planktonic organisms at different life stages to scrubber discharge water showed concentration-dependent effects on all explored endpoints, confirming that scrubber water may have adverse effects on the whole life cycle of the marine organisms.

Acute effects on bacterial bioluminescence, microalgal growth, and copepod survival were observed at relatively high scrubber water concentrations ($EC_{10} > 8$ %), conditions expected to be reached only in the immediate proximity of the discharge and for a limited period due to the dilution effect. Effects on copepod early-life stages occurred at scrubber water concentrations substantially lower than acute effects, with larval development emerging as the more sensitive endpoint ($EC_{10} = 1.1$ %), while hatching and early-life stage survival showed a sensitivity comparable to the acute test with *A. tonsa* ($EC_{10} = 9$ % both for hatching and early-life stage mortality). Similarly, the onset of effects on mussel development occurred at a scrubber water concentration lower than the acute effects ($EC_{10} = 4.9$ %). The long-term exposure of *A. tonsa* produced the lowest effect concentrations since both egg production by generation F_0 and the larval development of generation F_1 were significantly affected at a scrubber water concentration of <0.1 %. Nevertheless, effects on egg production by the parental group and offspring's larval development followed a U-shaped effect-concentration curve, impeding the calculation of toxicity data. This outcome could be an artefact due to the variability observed in the scrubber water treatments for both endpoints as well as the possible result of a trade-off for the allocation of the energy assimilated through the food among maintenance, somatic growth and reproduction in F_0 generation and maternal transfer of pollutants to F_1 generation (Klok et al., 2012). Further studies are needed to elucidate these trends, possibly by increasing the number of replicates for egg production and the F_1 larval development endpoints in an attempt to reduce the within-treatment variability and correctly assess NOEC and LOEC for scrubber water.

Scrubber water discharge may affect different aspects of the receiving waters, including pH, dissolved oxygen saturation, trace element, PAHs, and other contaminant concentrations. Consequently, determining the exact cause of the observed concentration-dependent effects in toxicity tests is hard and exploring all the possible mechanistic interpretation of the observed effects is out of the scope of the present work. The adverse effects observed in the long-term test with copepods and embryotoxicity test with mussels indicate that toxicity is probably due to pollutants deriving from the scrubbing process since pH, dissolved oxygen and salinity did not vary significantly among treatments. However, the synergistic effects of pollutants and the physico-chemical properties of scrubber water should also be considered (Koski et al., 2017; Thor et al., 2021). On the other hand, the impairments caused by inlet water on copepods and mussels indicate that leaching from the antifouling systems and piping corrosion might contribute to toxicity (Lunde Hermansson et al., 2021).

4.1. Effects of scrubber water on planktonic indicators

4.1.1. Acute effects

The lethality test with adult *A. tonsa* was the most sensitive acute test used for screening for scrubber water toxicity. The resulting outcome is

consistent with the data reported by Koski et al. (2017), who observed increased mortality at a concentration of 10 % scrubber water and almost total mortality at a concentration of 30 % scrubber water in adult *A. tonsa*.

Metals and PAHs in the scrubber water (Tables 2 and 3) were present at concentrations lower than the effect concentrations found in single compound tests reported for *Acartia* sp. (summarised in Supplementary Material, Table 9S). Therefore, none of these contaminants, taken individually, can be the cause of the measured effects. On the other hand, the concentration of alkylated PAHs in the scrubber water is significantly higher than that of unsubstituted parent PAHs (Table 3; Thor et al., 2021), and their toxicity is still largely unexplored, although there are indications that they are more toxic than the parent PAHs (Cong et al., 2021; Wassenaar and Verbruggen, 2021). In the case of copepods, however, the mortality of *Oithona davisae* was observed at concentrations of alkylated PAHs at least two orders of magnitude higher than those measured in the scrubber water in the present study, with the more toxic alkylated compounds being C2-phenanthrene (48 h-EC_{50} = 103 μg L^{-1}) and C2-naphthalene (48 h-EC_{50} = 617 μg L^{-1}) (Barata et al., 2005).

Consequently, available information indicates that lethal effects on *A. tonsa* are likely due to synergistic effects of multiple factors rather than single compounds. The factors involved include toxicants and physicochemical properties of scrubber water, such as pH and salinity. Potentiation and synergisms were already observed in testing metals and organic pollutants mixtures with copepods (Fleeger et al., 2007; Forget et al., 1999; Verriopoulos and Dimas, 1988), while the correlation between copepod mortality and salinity and pH was evident from our data. Furthermore, studies on scrubber water have already suggested synergistic effects on copepods due to contaminants and pH (Koski et al., 2017; Thor et al., 2021).

Contaminants in scrubber water were present at individual concentrations far below those known to cause adverse effects as reported in the literature for the Microtox test and microalgae (Supplementary Material, Tables 10S and 11S). Consequently, low toxicity was an expected outcome. However, the sensitivity of *P. tricornutum* and *D. tertiolecta* to Cu reported in a few studies seems to be an exception to this trend, suggesting that Cu might have played a role in the observed low effects on microalgae (72 h-LOEC = 1.5 μg L^{-1} for *P. tricornutum*; 72 h-NOEC = 8 μg L^{-1} for *D. tertiolecta*) (Levy et al., 2007, 2008). The observed effect is indeed attributable to the interaction of multiple chemicals, including trace elements, PAHs, and nutrients. At the same time, the lack of a correlation with physicochemical properties indicated a negligible effect of pH on the growth rate of *P. tricornutum* and *D. tertiolecta,* in agreement with previous studies reporting a minor impact on the microplankton community (Ytreberg et al., 2019).

4.1.2. Effects on early life stages

For mussels, a contaminant of primary concern in the scrubber water was V, which was detected at a concentration 7-fold higher than the LOEC (50 μg L^{-1}) reported for larvae of *Crassostrea gigas* (Fichet and Miramand, 1998). Thus, assuming a linear decrease of contaminants with scrubber water dilution, the extrapolated V concentration exceeded the EC_{50} for *C. gigas* yet at 20 % of scrubber water (~ 70 μg L^{-1}), suggesting that V had the potential to affect the larval development of *M. galloprovincialis* severely. Copper was also detected in the undiluted scrubber water at concentrations matching the adverse effect levels for mussels (Beiras and Albentosa, 2004; Boukadida et al., 2016); nevertheless, its contribution to the overall effects was probably minimal compared to V, also taking into consideration sample dilution. Other trace elements were detected in the scrubber water at concentrations lower than the adverse effect levels reported for bivalves (Supplementary Material, Table 12S), suggesting that they are not critical factors for larval development, pending potential synergistic effects. Additive effects of binary mixtures of trace elements have already been reported for larval development (see His et al., 1999). Conversely, very little is known concerning the interaction of trace elements with PAHs and alkyl-PAHs.

Regarding copepods, the effect of scrubber water is much more marked for larval development than for hatching and larval survival. This outcome was expected since the larval development to the C—I stage is a more sensitive endpoint in *A. tonsa* than hatching and early-life survival (Picone et al., 2021, 2022b; Wollenberger et al., 2003, 2005). Contaminants such as trace metals and PAH are known to affect different traits in copepods which may lead to retarded development. As an example, in *Tigriopus japonicus*, exposure to metals such as Cu, Cd, As and Mn upregulated the expression of *GST* genes coding for the endogenous glutathione-S-transferase (GST) isozymes, which play a key role in the antioxidant defence and in the phase II detoxification of organic pollutants (Lee et al., 2007, 2008). Similarly, the exposure of copepods to crude oil water accommodated fraction (WAF) induced the activity of the antioxidant enzymes (GST, glutathione reductase and catalase) and the expression of different genes coding for cytochrome P450 (CYP450) superfamily (Han et al., 2014; Hansen et al., 2009; Soloperto et al., 2022), resulting in dysfunctional development and reproduction (Han et al., 2014). These data suggest that the increased costs for somatic maintenance (i.e., antioxidant defence) and detoxification (i.e., induction of GST and CYP450), needed to maintain homeostasis, may limit the energy budget available for growth (Kooijman, 2010). Furthermore, exposure to WAF of *T. japonicus* also modulated the expression of chitinase, chitin deacetylase, and nuclear receptor family genes, suggesting that WAF-associated PAHs and alkylated PAHs can cause moult retardation through the down-regulation of the chitin metabolic pathway (Hwang et al., 2017). In particular, PAHs and alkylated PAHs concentrations in the WAF tested by Han et al. (2014) and Hwang et al. (2017) are comparable to those we observed in the scrubber water, confirming the plausibility of these mechanisms of action. Impaired development in copepods is also often reported as a consequence of reduced feeding rates or reduced assimilation from food (Klok et al., 2012). Reduced feeding and assimilation cause slower growth and delayed development in copepods since the molting process strictly depends on attaining a critical structural weight (Carlotti and Sciandra, 1989). Several contaminants have been documented to inhibit feeding, including Cd (Xu et al., 2001), PAHs (Barata et al., 2002; Saiz et al., 2009), and alkylated PAHs (Saiz et al., 2009). Furthermore, feeding inhibition generally occurs at a toxicant concentration lower than lethal effects for nauplii (Barata et al., 2002; Saiz et al., 2009). Consequently, it is more likely that the effect of scrubber water is an inhibition of larval development rather than increased mortality of the nauplii or an effect on the hatching rate, also in consideration that eggs used for testing were produced in a non-contaminated medium, and then the lipid reserves needed for fuelling embryos and nauplii are also presumed to have been free from contaminants that may affect hatching and survival. Anyhow, further research is warranted to elucidate the biochemical and physiological mechanisms underlying the reduction of the larval development we observed in *A. tonsa* exposed to scrubber water, also taking into consideration that the adverse effects are most probably due to synergisms between chemical and physico-chemicals features of the sample.

The observed correlations with physicochemical parameters indicate that dissolved oxygen and salinity may have affected larval survival and early development. Nonetheless, salinity and dissolved oxygen might have been factors driving toxicity only at the highest tested concentrations (i.e., 20 % and 40 % scrubber water). Conversely, effects measured at lower concentrations (i.e., < 10 % scrubber water) appear to be due to toxicants since salinity and dissolved oxygen are within the range observed in control.

4.1.3. Effects on reproduction and F_1 generation

Toxicant effects on copepod reproduction usually involve increased costs for egg production and impairments in the energy budget, which include reduced assimilation from feeding and digestion, increased costs

for somatic growth, and increased maintenance costs, including detoxification and antioxidant defence (Klok et al., 2012). In several studies, a reduction in egg production in copepods, including *A. tonsa*, was attributed to inhibited food uptake due to exposure to PAHs (Hjorth and Nielsen, 2011; Jensen et al., 2008; Krause et al., 2017), alkylated PAHs (Ott et al., 1978), and trace elements (Hook and Fisher, 2001). However, egg production reductions due to grazing inhibition were generally observed at concentrations higher than those expected in scrubber water dilutions. As an example, egg production in *Acartia* sp. was inhibited by 50 % after exposure to 1 nM Hg (~ 0.2 μg L^{-1}) and 5 nM Cd (~ 0.56 μg L^{-1}) (Hook and Fisher, 2001), namely concentration 110-fold (Hg) and 17-fold (Cd) higher than levels measured in the undiluted scrubber water (Table 2). Similarly, the feeding rate and egg production of the arctic copepods *Calanus finmarchicus* and *C. glacialis* were significantly inhibited by pyrene at 100 nM (~ 20.2 μg L^{-1}) (Hjorth and Nielsen, 2011; Jensen et al., 2008), a concentration considerably higher than the total PAH concentration measured in the undiluted scrubber water (Table 3). Conversely, exposure to pyrene of *C. finmarchicus* at levels not affecting feeding (0.01–10 nM) provided a significant but not dose-dependent inhibition of the egg production, with the highest significant reduction observed at 0.1 nM and no significant effects observed at 1 nM (Hjorth and Nielsen, 2011). This latter outcome is consistent with our test results and suggests that PAHs in scrubber water could potentially interfere with egg production. On the other hand, a study on WAF evidenced a lack of significant effect on egg production by *A. tonsa* also in treatments with total PAHs concentrations similar to or even higher than those measured in the undiluted scrubber water (Hafez et al., 2021), suggesting that effects of PAHs on *Acartia* reproduction tend to be evident at relatively high concentrations.

However, the mechanisms behind the observed effect remain unclear, and different hypotheses may be formulated. Firstly, PAHs and alkylated PAHs may induce narcosis in copepods (Barata et al., 2005; Ott et al., 1978), impairing feeding behaviour, energy income, and egg production (Jensen et al., 2008; Klok et al., 2012). Secondly, egg production may have been impaired by a direct effect of metals, PAHs and their metabolites on the maturation of the gonads or on the oogenesis (Hook and Fisher, 2001; Jensen et al., 2008). Thirdly, the copepods underwent toxicant-induced oxidative stress during the exposure, with consequent damage to the organs and/or increased maintenance costs (i.e., the energy required for enzyme synthesis and homeostasis), which led to dysfunctional reproduction (Han et al., 2014). Fourthly, PAHs and alkylated PAHs may impact mating behaviour and mating success in copepods by suppressing the ability of males to detect female pheromone trails, follow trails, and successfully track females (Seuront, 2011).

Further studies are needed to explore these possible pathways of toxicity. In addition, the contribution of toxicants not included in the chemical analysis should be considered, including soot and other combustion particles present in large concentrations in EGCS discharges (den Boer and 't Hoen, 2015) and might have toxic effects (Wu et al., 2017). Finally, the potential effect of pH was also considered. A pH-induced effect on egg production has been observed in copepods, with increasing reproductive success with decreasing pH (0.2–0.4 units) compared to ambient/control conditions (Engström-Öst et al., 2014; Fitzer et al., 2012). In our experiments, however, differences in average pH among the treatments were not significant, and the lowest egg production was observed at the concentration with the lowest pH (0.01 %), leading to the conclusion that a pH-driven effect on *A. tonsa* egg production was improbable.

Regarding the effects on F_1 generation, exposure to low concentrations of scrubber water significantly affected the larval development, while hatching and early-life stage survival were not, or only marginally, affected. Since the eggs produced by the F_0 generation were not exposed to scrubber water and the larvae were left to develop in a non-contaminated medium, the toxic effects are a consequence of parental exposure to scrubber water. Parents exposed to toxicants accumulate contaminants and their metabolites in their lipid reserves and, during vitellogenesis, into the eggs, resulting in a maternal transfer of body burdens to the offspring (Jensen and Carroll, 2010; Niehoff, 2007). Since the earlier naupliar stages do not feed, they depend entirely on the reserve obtained by their mother. Consequently, the contaminants associated with the reserves become available and may affect hatching, naupliar survival, and larval development (Klok et al., 2012). In particular, the inhibition of hatching success due to the exposure of the parental generation to PAHs and trace elements has often been observed (Hjorth and Nielsen, 2011; Hook and Fisher, 2001; Jensen and Carroll, 2010; Krause et al., 2017). Similarly, also exposure to WAF affected the hatching success in copepods (Hafez et al., 2021; Hansen et al., 2017; Olsen et al., 2013). In particular, Hafez et al. (2021) observed the inhibition of hatching after the exposure of *A. tonsa* to a WAF dilution (7 %) obtained from a commercial marine gas oil characterized by a total PAHs concentration of 14,739 ± 4080 ng L^{-1}, very close to the ΣPAHs measured in the undiluted scrubber water (Table 3). The authors, however, reported that the reduced hatching success was significant only in treatments causing a loss of total lipid levels in F_0 generation, suggesting that the toxic effect might be due to the lack of lipids in the eggs rather than to a maternal transfer of contaminants. The low larval mortality we observed in scrubber water was consistent with tests performed on WAFs (Faksness et al., 2022).

The lack of significant effects on hatching success and early-life stage survival in *A. tonsa*, also taking into consideration the variability of the data, suggests that burdens transferred from the mother to the egg during the exposure to scrubber water were too low to disrupt egg maturation and maintenance in the offspring but high enough to increase the costs for growth and generate a delay in the development to the C—I stage. This outcome is not surprising since the larval development to the C—I stage, as previously stated, is the more sensitive endpoint measured in tests with early-life stages of *A. tonsa* (Picone et al., 2021, 2022b; Wollenberger et al., 2003, 2005).

The detrimental effects on copepod reproduction and offspring development are the scrubber water's more ecologically relevant impacts observed in the present study. Due to the crucial role of copepods in the marine food webs (Turner, 2004; Williams et al., 1994), and in particular, of *Acartia* species for the coastal area of the Adriatic Sea (Camatti et al., 2019; Vidjak et al., 2006), adverse effects on the recruitment of new generations may reduce the abundance of *Acartia.* Then, this would have major consequences on the whole food web structure and functioning in the affected areas due to the altered availability of food for the upper trophic levels, including invertebrates and planktivorous fishes. Furthermore, the toxicant accumulated in the tissues of copepods may be transferred to the higher trophic levels. Copepods are indeed a food resource for fish larvae and planktivorous adult fish, including species commercially and recreationally relevant (Turner, 2004). Consequently, the transfer along the food web of scrubber water contaminants might also expose fish of ecological and economic relevance to the metal and PAH-induced toxic effects.

The increased larval development observed at 1 % scrubber water compared to the more diluted treatments at 0.01 %, and 0.1 % was unexpected. Nevertheless, this outcome is consistent with the higher, although not significantly different, egg production observed at 1 % compared to the other scrubber water dilutions (Fig. 4). Possible explanations might be a physiological reaction to low levels of stressors or a reduced feeding rate in the parental generation, which generated a lower accumulation of contaminants in the reserves. This latter hypothesis is, however, unlikely since during the long-term exposure of generation F_0 to the scrubber water PAHs had the time to reach the steady state between the lipids of parents and the surrounding medium. In any case, the absence of data on feeding rate does not allow us to corroborate this hypothesis.

4.2. Inlet water effects on planktonic indicators

The reason for testing the chemical composition and toxicity of the inlet water is to distinguish the sources of pollution. In this case, the aim is to understand the impact of pollution derived from the piping system, i.e., various metals and antifouling agents, on the different species and life stages.

In the acute tests, inlet water caused a significant and marked effect on copepods reared at 20 ‰ salinity, while effects were very low on algae and absent on copepods reared at 30 ‰ salinity (Fig. 1). Contaminant concentrations in inlet water were very low compared to adverse effect concentrations for *Acartia*, so effects due to trace elements and PAHs, also considering potential synergisms, are unlikely. In the case of algae, only Cu might have played a role in the observed effects due to the concentration approximating LOEC and NOEC reported in certain papers (Supplementary Material, Tables 2S and 3S). Effects on copepod cultured at 20 ‰ salinity significantly correlated with physicochemical parameters, suggesting that copepod mortality was substantially influenced by salinity, pH, and dissolved oxygen. Nevertheless, when copepods reared at 30 ‰ salinity were used in the toxicity test, the correlation between mortality and salinity was lacking, and effects on copepod survival disappeared, indicating that salinity was the driving factor addressing the toxicity of inlet water for copepods reared at 20 ‰ salinity. This outcome agrees with Svetlichny and Hubareva (2014), who observed that *A. tonsa* reared at 18 ‰ have an upper salinity tolerance boundary at about 30 ‰. In contrast, in copepods reared or acclimated at 30 ‰, the ability to osmoregulate may be shifted to higher salinities (Svetlichny and Hubareva, 2014).

The high salinity of inlet water is probably also the cause of the severe delay observed in the larval development of *A. tonsa*. Feeding, production and respiration rates in *A. tonsa* tend to be higher at intermediate salinities (10 ‰ to 20 ‰) and rapidly decrease at salinities ≥30 ‰ (Calliari et al., 2006). A reduction in feeding rate at a salinity of 30 ‰ or higher generates a decrease in the energy assimilated by the copepods and, consequently, a lower energy budget available for growth (Klok et al., 2012). Furthermore, to cope with the increasing salinity of the medium, euryhaline species such as *A. tonsa* behave as either iso- or hypo-regulator, performing both isosmotic intracellular regulation of volume (IIR) and anisosmotic extracellular osmoregulation (AER) (Rivera-Ingraham and Lignot, 2017). Nevertheless, AER is an energetically costly process due to the high quantity of ATP consumed to maintain the ion gradients (Sokolova et al., 2012). Consequently, the budget for growth is further reduced, considering that maintenance of structural elements takes precedence over growth and reproduction (Klok et al., 2012). Similarly, the lower egg production and the delayed development of F_1 generation observed after the long-term exposure of the parental generation to inlet water are consistent with a salinity-driven adverse effect on the energy budget of *A. tonsa*, which reduced the resources allocable for reproduction and growth of the offspring.

The hypothesis of salinity as a primary stressing agent is also supported by the low contaminant concentrations detected in the inlet water, which led to exclude that the inhibition of the larval development may be due to the analysed pollutants. For example, Cu is one of the most toxic trace elements toward *A. tonsa* larval development, which provided a NOEC = 50 μg L^{-1} (Picone, unpublished data), but its concentration in inlet water was too low to elicit detrimental effects (0.9 μg L^{-1}). However, effects due to non-measured contaminants cannot be a priori excluded, including those due to chemicals released from marine growth protection systems (MGPS) used to prevent biofouling in internal seawater systems. The most commonly employed MGPS are sacrificial anodic copper dosing systems, which release Cu, Fe, and Al, and chlorine-based dosing systems, which generate sodium hypochlorite (Grandison et al., 2011). However, other options exist which use different kinds of chemical biocides with the potential to affect marine life, including hydrogen peroxide, iodine, chlorine dioxide, ferrate ion, peracetic acid, and bromine (Grandison et al., 2011).

The toxicity of inlet water to the larval development of *M. galloprovincialis* corroborates the hypothesis that non-measured biocides may be involved. Trace element concentrations in inlet water were too low to elicit adverse effects on mussels (Supplementary Material Table 12S), and salinity is not a factor affecting larval survival and development in mussels at the testing conditions (T = 20 °C) (Brenko and Calabrese, 1969; His et al., 1989).

5. Conclusions

The scrubbing process generates acidic effluents characterized by high concentrations of metals (V and Ni in particular), PAHs, alkylated PAHs and possibly other contaminants not measured here, with the potential to exert adverse effects on the plankton communities of the receiving water. However, since single contaminants were generally below the adverse effect levels for the planktonic bioindicators, the toxicity of EGCS discharge water was likely due to synergistic effects of the chemical mixture and physicochemical properties of the scrubber water, including potentiation and additivity. Nevertheless, for many contaminants of potential concern, such as V and alkylated PAHs, there is the need to increase the dataset of adverse effects levels for the individual contaminants since for many endpoints (i.e., larval development with both bivalves and copepods and effects on reproduction of copepods) such information is not available in the literature.

Acute effects on plankton were observed at scrubber water concentrations higher than 8 %. Although dilution of scrubber water is expected to occur after discharge, the exposure concentrations may be relevant in shipping lanes close to the point of discharge where 100 % scrubber water is released to the sea. In contrast, significant effects on reproduction and offspring development of copepods were detected at low scrubber water concentrations (< 1 %). This impact of EGCS discharges is potentially critical since it affects a crucial component of the marine food web, possibly leading to impairments on the whole food web due to resource restriction for planktivorous invertebrates, fishes, and their larval stages.

The present study provides further evidence that EGCSs operating in an open-loop mode are not an environmentally sustainable solution to reduce maritime sulphur emissions. Although engineered to reduce the impact of sulphur emission from HFO in the atmosphere, EGCS merely move the problem from the atmosphere to the hydrosphere, increasing the exposure of the marine biota to toxicants (i.e., V, PAHs, and alkylated PAHs). Consequently, reducing emissions from maritime transport should not be any further sought by allowing the use of HFO in vessels equipped with open loop scrubbers, but there is a need to shift toward less impacting technological alternatives.

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Original draft preparation. **Mira Petrovic:** Investigation. Resources. **Meritxell Gros:** Investigation. Resources. **Elisa Garcia:** Investigation. Resources. **Elisa Giubilato:** Resources. **Loris Calgaro:** Resources. **Kerstin Magnusson:** Conceptualization. Writing-Original draft preparation. **Maria Granberg:** Conceptualization. Writing-Original draft preparation. **Antonio Marcomini:** Supervision. Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Marco Picone reports financial support was provided by European Union.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.114846.

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