

No. B 2319
December 2018

Scrubbers: Closing the loop

Activity 3: Task 2

Risk Assessment of marine exhaust gas scrubber water

Kerstin Magnusson, Peter Thor and Maria Granberg



Author: Kerstin Magnusson, Peter Thor and Maria Granberg
Funded by: Connecting Europe Facility (CEF) programme and Stena
Report number B 2318
ISBN 978-91-7883-017-6
Edition Only available as PDF for individual printing

© IVL Swedish Environmental Research Institute 2018
IVL Swedish Environmental Research Institute Ltd.
P.O Box 210 60, S-100 31 Stockholm, Sweden
Phone +46-(0)10-7886500 // www.ivl.se

This report has been reviewed and approved in accordance with IVL's audited and approved management system.

Preface

This is a project report covering exhaust gas emission measurements on Stena Britannica as part of the project Scrubbers: Closing the loop.

This report covers Task 2 Risk Assessment of marine exhaust gas scrubber water of Activity 3 (Integrated Life Cycle Balance) in the CEF funded project “Scrubbers – Closing the loop”. Together with this report the Activity is presented in:

- Scrubbers: Closing the loop; Activity 3. Summary; Environmental analysis of marine exhaust gas scrubbers on two Stena Line ships. IVL report B2317, by Winnes H., Granberg M., Magnusson K., Malmaeus M., Mellin A., Stripple H., Yaramenka K., and Zhang Y., 2018
- Scrubbers: Closing the loop; Activity 3. Task 1; Air emission measurements. IVL report B2318, by Winnes H., Fridell E., Moldanová J., Peterson K., and Salberg H., 2018
- Scrubbers: Closing the loop; Activity 3. Task 3; Cost benefit analysis. IVL report B2320, by Yaramenka K., Mellin A., Malmaeus M., and Winnes H., 2018
- Scrubbers: Closing the loop; Activity 3. Task 4; Evaluation of exhaust gas scrubber systems for ship applications in a system perspective. IVL report B2321, by Zhang Y and Stripple H., 2018

IVL Swedish Environmental Research Institute has been the leading organisation of the activity and has performed the studies in this report with support from representatives from the project partners Lloyd’s Register EMEA and Wärtsilä Sweden AB, and the project coordinator Stena UK Ltd.

We gratefully acknowledge our funders at European Commission/Connecting Europe Facility and the SIVL foundation, and the insightful and dedicated support from the project coordinator Stena, our partners Lloyd’s Register and Wärtsilä. Especially acknowledged are Andy Wright at LR who has supported with his specialist knowledge regarding air emissions measurements and Stian Aakre at Wärtsilä who has supported with technical knowledge of the system. The project coordinator has been much involved in the task with appreciated efforts from Per Stefenson and Björn Asplind at the Gothenburg office. Warm thanks also to the captains, chief engineers and crew on Stena Britannica for their support during emission measurements and water sampling, as well as the Stena Line personnel on shore in Hook of Holland for their assistance.

Table of contents

Summary	6
1 Introduction	7
2 Description of scrubber systems	7
2.1 Open loop scrubber system	7
2.2 Closed loop scrubber system.....	8
3 Legal framework	8
4 Analyses of scrubber water	9
4.1 Overview of sampled scrubber water	9
4.2 Chemical analyses	10
4.3 Microtox bioassay – testsystem	10
4.4 Zooplankton – test system	11
4.4.1 Test A- Closed loop EGSE 2017	13
4.4.2 Test B and C- Closed loop and open loop EGSE 2018	13
4.5 Blue mussel – test system	14
4.5.1 Test D- Closed loop EGSE 2017	15
4.5.2 Test E and F- Closed loop and open loop EGSE 2018.....	16
5 Efficiency of the scrubber water treatment system.....	17
5.1 Results from chemical measurements	17
5.1.1 Turbidity.....	17
5.1.2 Metals	18
5.1.3 Organic contaminants.....	19
5.1.4 Microtox bioassay	21
6 Effects on marine organisms	22
6.1 Results from ecological toxicity tests	22
6.1.1 pH and alkalinity of tested EGSE dilutions	22
6.1.2 Zooplankton- <i>Calanus helgolandicus</i>	24
6.1.3 Blue mussel (<i>Mytilus edulis</i>).....	27
6.2 Literature comparison of EGSE toxicity	29
7 Risk assessment of discharge of EGSE from scrubbers	32
7.1 Introduction to the risk assessment	32
7.2 Dilution of EGSE in recipient seawater	33
7.1 Risk assessment based on chemical analyses	34
7.2 Risk assessment based on results from toxicity tests	38
7.3 Summary of risk assessment	40
8 Conclusions.....	41
9 References	43

APPENDIX.....	46
Data from chemical analyses	46

Summary

In response to regulations on permitted levels of sulphur emissions from ships many ships have installed, or are planning to install, exhaust gas cleaning systems, so called scrubbers. The use of scrubbers makes it possible to continue to use high sulphur fuel oil on board. In the scrubbers, the exhaust gases are washed with a scrubber fluid aiming at reducing levels of sulphur dioxide. The fluid passes the exhaust gas once (“open loop scrubber”), or is recirculated (“closed loop scrubber”). The exhaust gas scrubber effluent water (EGSE) is discharged into the sea untreated or after passing through a water treatment system. The aim of the present project was to investigate the potential impact of the discharges from open and closed loop scrubbers on marine ecosystems.

Samples were taken of EGSE, ready to be discharged into the sea, from two ships with a closed loop scrubber system (Stena Britannica and Stena Transporter) and one with an open loop system (Stena Forerunner). The scrubber washwater on Stena Britannica and Stena Transporter is treated before discharge to the marine environment. Samples were collected at a number of stages from the process tank and along the scrubber water treatment process on Stena Britannica. Chemical analyses were carried out on a range of organic oil-related compounds and on metals.

The final water treatment step of the scrubber water was found to reduce most hydrocarbons by >99% and also a relatively high percentage of many metals. Still the acute toxicity of the water, as measured with Microtox bioassay method, was almost the same in the water leaving the treatment unit as in the water feeding in to it. This may be caused by the relatively low reduction of low molecular aromatic hydrocarbons, known for their acute toxicity, and by the high concentrations of Cu and Hg in effluent water compared to water feeding into the unit.

The EGSEs were tested for toxicity using experimental studies with field collected zooplankton of the species *Calanus helgolandicus* and bottom-dwelling blue mussels, *Mytilus edulis*. Zooplankton were found to be more sensitive than mussels, and significant toxic effects were found in dilutions of 0.04–0.1% of closed loop EGSE and 1.0 % of open loop EGSE. Since neither pH nor alkalinity (A_T) differed from the clean seawater at the effect concentrations, it was concluded that the observed effects on copepods were primarily caused by toxic compounds present in the EGSE rather than by acidification. It should be noted that in both closed and open loop exposures, the lowest tested concentration resulted in toxic effects on the juvenile copepods. Thus, it cannot be excluded that even lower concentrations would have been harmful to the tested zooplankton species.

The risk that the discharge of EGSE may pose on marine ecosystems was assessed by comparing estimated seawater concentrations of whole EGSE with threshold levels for EGSE derived from the toxicity tests. Seawater concentrations of individual chemicals in the discharged EGSE were compared to Environmental Quality Standards (EQS) for priority pollutants in the Water Framework Directive 2000/60/EC (WFD).

It was found that the toxicity of the estimated seawater concentrations of EGSE from both closed and open loop systems, when treating the discharged water as one unit, exceeded the threshold level derived from the toxicity tests on the zooplankton. This means that there is a risk that discharge of EGSE will have a negative effect on the marine pelagic ecosystems in the area around shipping lanes. In contrast, seawater concentrations of individual chemical components were in the range of 10^3 - 10^6 times lower than the EQS concentrations which shows that EGSE toxicity could

not be predicted simply by comparing the concentrations of specific chemicals to established threshold values.

1 Introduction

From January 1, 2015, ships operating in a Sulphur Emission Control Area (SECA) must comply with stricter IMO and EU policies on sulphur levels in marine fuels that significantly reduce emissions of sulphur oxides and particles to air in these areas. This entails using distillate fuels with 0.1% sulphur content in the SECA regions, or using a technology designed to reduce sulphur emissions to an equivalent level. Marine gas oil (MGO), liquefied natural gas (LNG) and heavy fuel oil (HFO) in combination with an exhaust gas scrubber of some kind are examples of options available, allowing ships to reach the higher SECA demands. Scrubbing the sulphur from the exhaust gasses while still using HFO has received increased attention as the less expensive option compared to using the cleaner MGO. Thus the number of scrubbers installed on board ships has increased substantially (den Boer & 't Hoen, 2015).

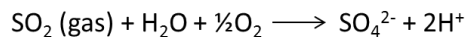
The aim of this investigation was to determine the ecological toxicity and environmental risks associated with releasing exhaust gas scrubber effluent (EGSE) into the sea. The main focus and the majority of tests described in this report were performed on water samples from the exhaust gas scrubber system on Stena Britannica. Samples were collected at strategic points along the scrubber water loop, and analysed for chemical contents and ecological toxicity. EGSE was also collected from Stena Forerunner and Stena Transporter and analysed for chemical contents and ecological toxicity. The combination of investigations allowed comparisons between EGSE from scrubber systems of different design.

2 Description of scrubber systems

Scrubber systems are generally composed of three parts; 1) the exhaust gas cleaning unit, 2) a wash water treatment unit, and 3) a sludge treatment unit. In the exhaust gas cleaning unit, the exhaust gas from the engine comes in close contact with water either by passing through a water spray or bubbling through water. The water can be either seawater, freshwater or both. In the cleaning unit SO_x is converted into sulphuric acid. The purpose of the wash water treatment unit is to remove solids and neutralize the pH of the wash water before release into the sea. During the removal of particles, many of the particle associated pollutants such as metals and larger polycyclic aromatic hydrocarbons will also be removed from the scrubber water. Sludge treatment consists of a storage container and the sludge is then handled on shore, i.e. sent for destruction or incorporated into, e.g. asphalt or concrete. This type of system is installed on Stena Britannica and Stena Transporter, while the system on Stena Forerunner is of an open loop scrubber type. The open loop scrubber does not include wash water treatment before discharge.

2.1 Open loop scrubber system

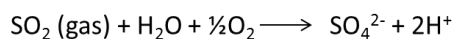
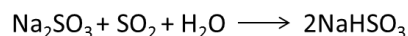
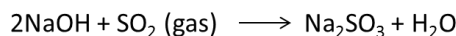
Open loop scrubbers utilize untreated seawater and thus use the natural alkalinity of the seawater to neutralize the sulphur from exhaust gases. In addition to capturing SO_2 , some of the NO_x may also be captured and released as nitrates with the EGSE. Chemical reactions are as follows:



The flow rates of seawater in open loop scrubbers must be very high in order to accommodate the chemical processes in the exhaust gas cleaning unit. The discharge of water from the open loop system from Stena Forerunner was estimated to be 350 m³ hour⁻¹.

2.2 Closed loop scrubber system

Closed loop scrubbers are not dependent on the alkalinity of the seawater for the cleaning process. Exhaust gasses are neutralized with caustic soda (NaOH), which is added to the process water in a recirculating system. The condition of the process water is continuously monitored. When the water reaches a defined limit value for density, a “bleed-off” is allowed from the system and exchanged with fresh seawater and NaOH. The bleed-off is subsequently treated in a special unit to remove particulates and neutralize the pH. Chemical reactions in the exhaust gas treatment unit are as follows:



The discharge rate of treated closed scrubber water to the recipient from Stena Britannica and Stena Transporter was estimated to 10 m³ hour⁻¹.

3 Legal framework

In resolution MEPC 259(68) the guidelines for implementation of MARPOL 73/78 Annex VI including the criteria for discharge of EGSE are stated. The values that must be monitored in the EGSE are pH, PAH, turbidity, temperature and total nitrogen.

- 1) Criteria for pH: The discharged EGSE should have a pH of no less than 6.5 at 4 m from the overboard discharge point with the ship stationary.
- 2) Criteria for PAH concentration: The concentration of the PAH compounds is measured as a toxic equivalence factor (TEF) named phenanthrene equivalence (PAH_{phe}). TEFs are used to express the toxicity of complex mixtures, and PAH_{phe} was designed to express the acute toxicity of 12 low molecular PAHs in sediment. Toxicity data from the PAHs derive from testing on a marine sediment living crustacean (Fisher, Law et al., 2011). Phenanthrene is used as a reference substance and given the TEF=1 and the TEF for the other 11 compounds are then related to how toxic they were in relation to phenanthrene. The criterion for EGSE is that the maximum continuous PAH concentrations must not exceed 50 µg·L⁻¹ PAH_{phe} above the PAH concentration in the inlet water.

- 3) Criteria for turbidity: The maximum continuous turbidity in EGSE should not be greater than 25 FNU (formazin nephelometric units) or 25 NTU (nephelometric turbidity units) or equivalent units, above the inlet water turbidity.
- 4) Criteria for nitrates: The scrubber water treatment system should prevent the discharge of nitrates beyond that associated with a 12% removal of NO_x from the exhaust, or beyond 60 mg·L⁻¹ normalized for EGSE discharge rate of 45 tons/MWh, whichever is greater.

4 Analyses of scrubber water

4.1 Overview of sampled scrubber water

Water from two closed loop scrubber systems and one open loop system were sampled and analysed in September 2017 (Stena Britannica, closed loop) and in March 2018 (Stena Transporter, closed loop and Stena Forerunner, open loop) respectively. Samples from Stena Britannica were collected at a range of sites along the treatment process, including the sludge (Fig. 1). This sampling plan was designed to provide information on the efficiency of each exhaust gas cleaning step and also to determine the progression of the EGSE toxicity after each cleaning step (Table 1). The samples collected were; 1) process water, 2) incoming seawater (seawater in), 3) seawater leaving the heat exchanger (seawater out), 4) bleed-off treatment unit (BOTU) sludge, 5) pooled sludge from BOTU and decanting unit (DU), 6) bleed-off or BOTU feed, 7) effluent water (EGSE), and 8) water. Sampling was conducted for one engine load only, i.e. 70-75%.

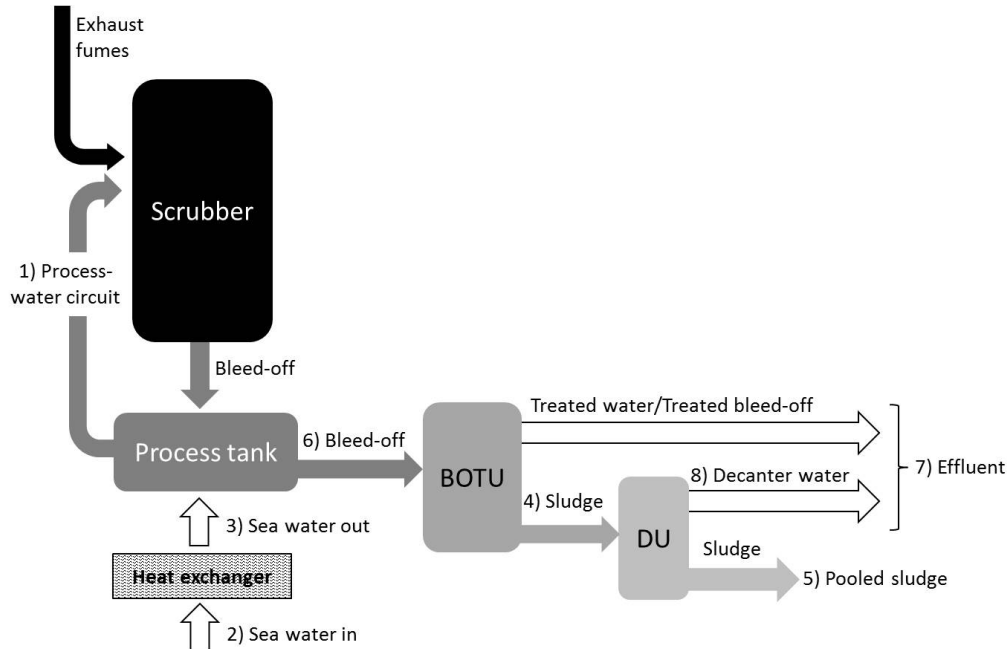


Figure 1. Simplified schematic of the closed loop scrubber system with bleed-off (BOTU) and decanting units (DU) as installed on “Stena Britannica”. The effluent water (EGSE) collects treated DU and BOTU water and is discharged overboard. Sample matrices are indicated with numbers

At the sampling in 2018 (Stena Transporter and Stena Forerunner) only EGSE, ready to be discharged into the sea, was collected. Chemical analyses and toxicity testing with Microtox ((ISO

11348-3)) were performed on all samples, whereas ecotoxicological tests with zooplankton *Calanus helgolandicus*) and blue mussels (*Mytilus edulis*) were performed only on EGSE from the scrubber systems of the three ships (Table 1)

Table 1. Overview of samples selected for chemical analyses (Chem), toxicity test with Microtox (TOX_{MT}) and toxicity tests with zooplankton and mussel (TOX_{Z&M}).

	Stena Britannica Closed loop Sept 2017	Stena Transporter Closed loop March 2018	Stena Forerunner Open loop March 2018
Process water (1)	Chem/ TOX _{MT}		
BOTU sludge (4)	Chem/ TOX _{MT}		
Bleed off/BOTU feed (6)	Chem/ TOX _{MT}		
Decanter water (8)	Chem/ TOX _{MT}		
EGSE (effluent water)(7)	Chem/ TOX _{MT} / TOX _{Z&M}	Chem/ TOX _{MT} / TOX _{Z&M}	Chem/ TOX _{MT} / TOX _{Z&M}
Sludge (5)	Chem		
Seawater in (2)	Chem/ TOX _{MT}		
Seawater out (3)	Chem/ TOX _{MT}		

4.2 Chemical analyses

Chemical analyses were carried out on all sampled fractions of scrubber water (denoted as Chem in Table 1). The samples were analysed for total hydrocarbon content fractionated into different size classes according to the number of carbon atoms. In samples from closed loop 2017 and open loop 2018 this fractionation was done separately on aliphatic and aromatic hydrocarbons, whereas the two categories were grouped together in the analyses of the sample from closed loop 2018. The samples were also analysed for 16 PAH and 11 alkylated PAHs. Analyses were done for the metals aluminium (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), zinc (Zn), vanadium (V) and mercury (Hg), and also of sulphur (S), Nitrate (NO₂-N), nitrite (NO₃-N), conductivity, pH, alkalinity and turbidity. Samples collected from Stena Britannica in 2017 were also analysed for HCB and dioxins. Full results from all chemical analyses are found in Appendix, Tables 1-4.

4.3 Microtox bioassay – test system

All water samples were tested for toxicity using a standard Microtox screening (SS-EN-ISO 11348-3:2008). In this bioassay the test organism is a species of bioluminescent marine bacteria (*Vibrio fischeri*) and reduction in bioluminescence after exposure to toxic compounds in test waters is used

as a response parameter. The test waters were added to the bacterial community in a range of dilutions and the dilution at which 20% and 50% inhibition of the fluorescence occurs was recorded and termed EC_{20} and EC_{50} , respectively.

4.4 Zooplankton – test system

Medium term (1-2 weeks) chronic effects of EGSE from closed- and open loop scrubber systems were investigated on the planktonic copepod *Calanus helgolandicus* (Fig. 2 left). The aim of these tests was to identify the lowest concentration of EGSE where a statistically significant effect on biota was detected. These tests thus complemented the acute toxicity tests represented by the Microtox bioassay.

The non-standard test species *Calanus helgolandicus* was chosen because of its high abundance and thus ecological relevance in the area where the studied ships operate. The species is ubiquitous in the Northern Atlantic where it dominates the zooplankton biomass and constitutes the main prey for planktivorous fish. Copepods go through a set of developmental stages, *i.e.* six nauplii (N) stages and five copepodite (C) stages, before the fertilized eggs become adult copepods (Fig. 2 right). Copepodite stages are hereafter referred to as juvenile stages.

Calanus helgolandicus were caught in the Gullmarsfjord, Sweden and immediately transferred to the Sven Lovén Centre - Kristineberg and kept cool (+8 °C) in seawater tanks with gentle aeration until tests were initiated. Juvenile stage CV dominated the *Calanus helgolandicus* populations in September 2017 and stage CIII in March 2018 and were thus used in the respective tests.

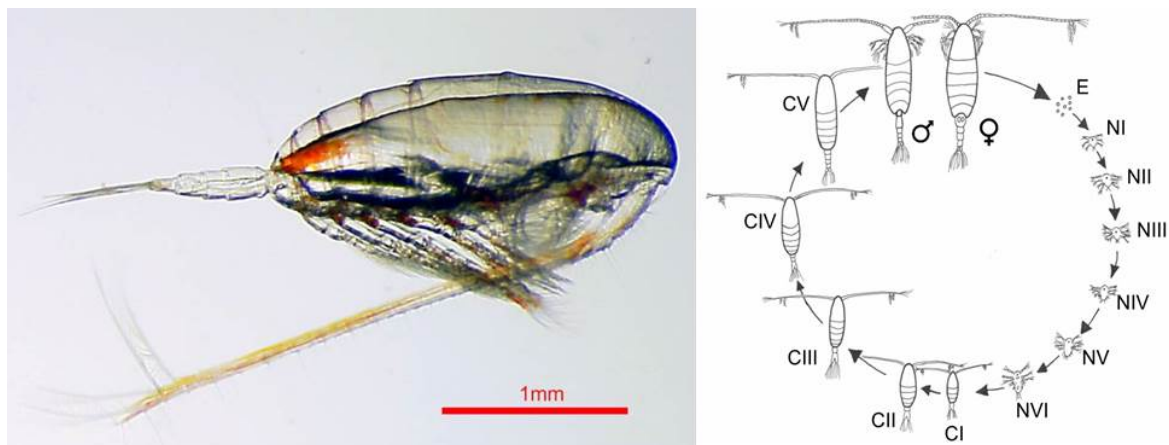


Figure 2. Side view of the copepod *Calanus helgolandicus* (left) and a schematic of its life cycle (right) with nauplii (N) and copepodite (C) stages. Photo: <https://www.sintef.no/project/calanus/graphics/calanus.jpg>.

Copepods were exposed to a range of EGSE concentrations (Table 2). The EGSE was mixed with filtered seawater in large glass flasks (Fig. 3 right). Microalgal paste (*Thalassiosira weissflogii*, Reed Mariculture) was used for food. Four replicate bottles with eight copepods in each were prepared for each exposure concentration. They were then incubated on a rotating plankton wheel (0.5 rpm) at +8 °C in darkness (Fig. 3 left) for a total of 7, 8 and 14 days respectively (Table 2). Exposure times were long and exposure concentrations were kept realistically low in order to detect chronic

toxicity effects. The duration of the tests were continuously adjusted in relation to the detected toxicity in order to obtain the best dataset possible. The following responses or endpoints were recorded, however, not all in all tests (Table 2).

Mortality rate is a toxic effect of EGSE exposure and was calculated as the ratio between dead and the total initially living copepods displayed as mortality rates (d^{-1}).

Feeding rate is a sensitive indicator of sublethal stress. Organisms may initially increase their feeding to compensate for a higher metabolic demand when trying to cope with pollutant stress (hormesis effect), or stop feeding completely as they become physically affected by the pollutants. Feeding rate was determined at the end of the toxicity tests by counting the number of microalgae before and after a specified feeding period.

Metabolic rate is directly linked to environmental stress and can show a similar hormesis effect as feeding rate at medium stress followed by a decrease when the stress becomes too high. Metabolic rate was determined by measuring respiration in individual copepods.

Lethargy is a measure of the narcotic effect that pollutants may have on organisms, which then become slower and less prone to feed. Lethargy was recorded through visual inspection of the copepod behaviour when changing the exposure water.

Stage development was measured as moulting, i.e. when the juveniles change state they need to shed their exoskeleton to be able to grow. The success of the moulting process can be affected by the pollutants and unsuccessful moulting result in death. Moulting was followed only in the CIII juveniles, i.e. from CIII to CIV, since the CV stage in *Calanus* species is very much prolonged and variable among individuals (Peterson, 2001).

Table 2. Exposure concentrations of EGSE (%) in the different tests of the two trials. Other test conditions and endpoints are also displayed.

Item	Test A Closed loop Stena Britannica September 2017	Test B Closed loop Stena Transporter March 2018	Test C Open loop Stena Forerunner March 2018
Control	0 %	0 %	0 %
Conc. 1	0.04 %	0.1 %	1 %
Conc. 2	0.2 %	0.5 %	5 %
Conc. 3	0.5 %	1 %	10 %
Conc. 4	1 %	2 %	40 %
Conc. 5	2 %	5 %	-
Conc. 6	5 %	-	-
Juvenile stage	CV	CIII	CIII
Exposure time (d)	7	8	14
Sampling times	Daily	daily	every second day

4.4.1 Test A- Closed loop EGSE 2017

Copepod **mortality** was recorded daily and the treatment water renewed. **Feeding rates** were estimated at the end of the experiment with freshly prepared treatment water of the different concentrations. Feeding on added microalgae was measured. **Metabolic rates** were measured during the last incubation day as respiration in individual copepods.

4.4.2 Test B and C- Closed loop and open loop EGSE 2018

Every day (every second day for CIII juveniles exposed to open loop EGSE) of the incubation period, **mortality** and **lethargic** copepods as well as number of shed exoskeletons from **moulting** juvenile CIIIs were counted and the treatment water renewed. **Feeding rates** were estimated at the end of the experiments with freshly prepared treatment water of the different concentrations and from the different scrubber types. Feeding on added microalgae was measured. **Metabolic rates** were measured during the last incubation day as respiration in individual copepods.



Figure 3. Investigations of EGSE on copepods (zooplankton). Left: the plankton wheel is running in the back while copepods are inspected under the stereoscope. Right: mixing EGSE at different concentrations prior to testing.

4.5 Blue mussel – test system

The ecotoxicological test systems with blue mussels (*Mytilus edulis*) consisted of two-litre aquaria (Fig. 4). The tests were designed to capture chronic effects of ecologically relevant concentrations of EGSE from the different scrubber systems. The aim of these tests was to identify the lowest concentration of EGSE where a statistically significant effect on biota was detected.

Field farmed blue mussels were used in the September 2017 trial while wild mussels collected at a pristine site in the vicinity of Tjärnö marine biological station on the Swedish west coast were used in the March 2018 trial. All mussels were kept in aquaria with running natural seawater for a week before use. Up to four individuals were placed together in each aquarium and four (September 2017) to five (March 2018) replicate aquaria were used for each tested concentration of diluted EGSE (Table 3). During the March 2018 test the mussels were continuously fed with microalgal paste (*Thalassiosira weissflogii*, Reed Mariculture). This was done in order to ensure best possible condition of the mussels during the long term exposures. This action is usually not required since food is naturally provided through the added seawater, and was not done in the 2017 test. The aquaria were aerated through submerged glass pipettes and exposure water was changed daily. The following endpoints were measured.

Byssus strength is a collective measure of the mussel's ability to form and attach byssus threads. It was measured using a digital dynamometer attached to the mussel with a clamp (Fig 4B). The maximum force required to detach the mussel was recorded. Byssus threads are essential for the survival of the mussel. They keep the animal attached to the substrate and thus enable ventilation and feeding (Fig. 4A). Byssus threads are produced in the byssus gland through an intricate process that requires energy. Pollutants can affect the biogeochemistry of the byssus production process as well as affect the energy available for byssus thread production. One day prior to the measurement all byssus threads were cut off with a knife without damaging the byssus gland. This forced the mussels to form new byssus threads and to reattach to the aquarium walls.

Hepatosomatic index is a measure of an organisms energy reserves but can also be a measure of chemical stress. It was determined by dissecting the mussels and separating the hepatopancreas from the rest of the mussel tissue (Fig 4D top). The wet weight of the tissue was recorded and the ratio between the hepatopancreas and the soft tissue was determined.

Cell viability assay was conducted on live haemolymph cells and measures their metabolic activity. The assay (CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (Promega)) detects living, but not dead cells and the signal generated is dependent on the degree of activation of the cells (Hernroth, 2003, Mosmann, 1983). Cell viability can be expected to initially increase to counteract the stress and then decrease as the stress increases. Cell viability is normalised to the protein content of each sample.

Table 3. Exposure concentrations of EGSE (%) in the experimental aquaria and other experimental conditions.

Item	Test D Closed loop Stena Britannica September 2017	Test E Closed loop Stena Transporter March 2018	Test F Open loop Stena Forerunner March 2018*
Control	0 %	0 %	0 %
Conc. 1	0.008 %	0.63 %	5 %
Conc. 2	0.04 %	1.25 %	-
Conc. 3	0.2 %	2.5 %	-
Conc. 4	1 %	5 %	-
Conc. 5	5 %	-	-
Mussel origin	Farmed	wild	wild
Food exposure	natural seawater only	microalgae	microalgae
Exposure time (d)	35	15	15
Endpoints	Byssus strength Hepatosomatic index	Byssus strength Hepatosomatic index	Byssus strength Hepatosomatic index Cell viability assay

*The volume of collected open loop EGSE was unfortunately so small that only one concentration aside from controls with seawater could be tested.

4.5.1 Test D- Closed loop EGSE 2017

Mussels from this test were sampled at the end of the test period after 35 days. The endpoints measured were **byssus strength**, and **hepatosomatic index**.

4.5.2 Test E and F- Closed loop and open loop EGSE 2018

Mussels from these tests were sampled after 15 days. The endpoints measured were **byssus strength**, **hepatosomatic index**, and **cell viability** (Test E only).

Statistical differences between treatments in the ecotoxicological toxicity tests were determined with PERMANOVA analyses on square root transformed data using the PRIMER-e software.

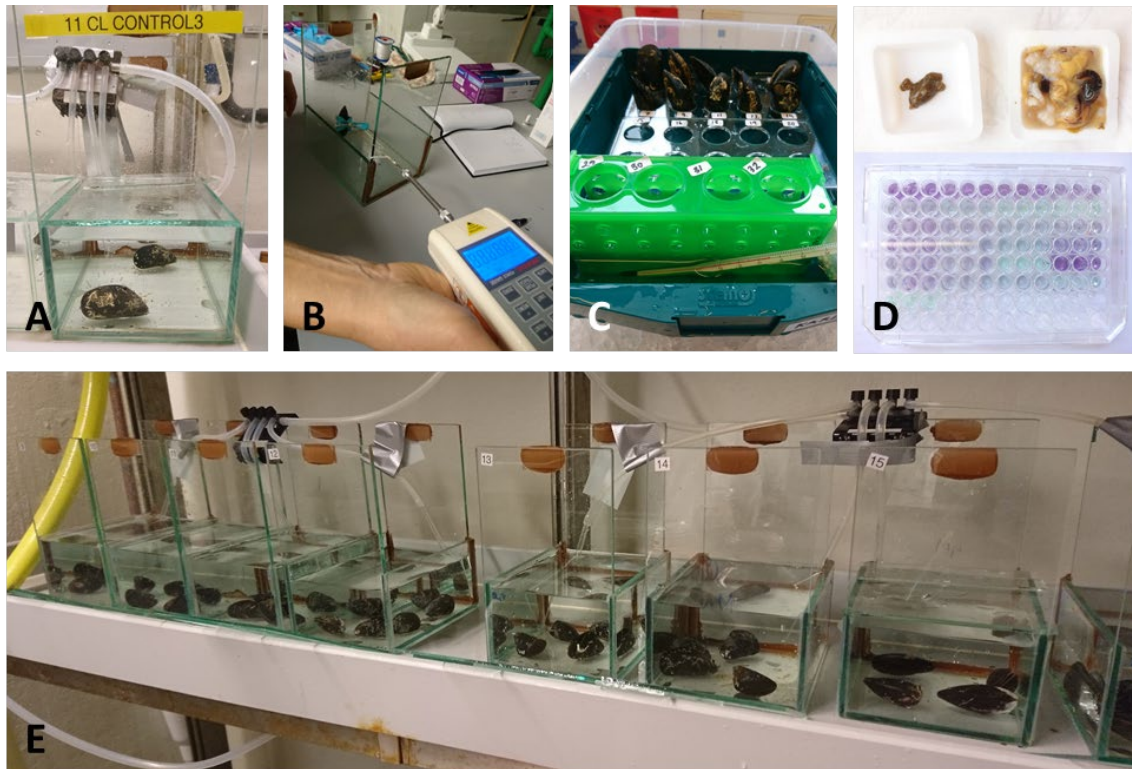


Figure 4. The test system for blue mussels and measurements of effects. A: experimental aquarium with blue mussel attached to the glass with byssus threads, B: measuring byssus strength with a digital dynamometer, C: mussels ready to be sampled for haemolymph and dissected, D top: dissected hepatopancreas (left) and remaining soft tissue (right), D bottom: microtiter plate with haemolymph cells exposed to a viable cell assay, E: setup of exposure aquaria.

5 Efficiency of the scrubber water treatment system

5.1 Results from chemical measurements

The efficiency of the treatment of the scrubber water in the BOTU was assessed by comparing data on turbidity, chemical analyses and toxicity testing with the Microtox assay on the water feeding into the BOTU with data on effluent water (EGSE). Analyses of BOTU feed water was only made from the sampling occasion in September 2017, on board Stena Britannica. Since data on the same parameters, turbidity, chemical content and Microtox toxicity, are available also for seawater at the same sampling occasion, these parameters are also presented.

Turbidity was reduced by 96% in the BOTU, and the heavier, more particle bound fractions of hydrocarbons were retained to a larger extent than lighter fractions. Although the BOTU treatment reduced total hydrocarbon by 97% and most metals by ~60 – 95% the concentrations of short chained aliphatic hydrocarbons and of Cu and Hg were actually higher in the EGSE than in the BOTU feed. This might explain why the acute toxicity, as measured by Microtox bioassay, did not change significantly after passage through the BOTU.

5.1.1 Turbidity

A reduction in turbidity of 96.4% was observed in the EGSE compared to the water feeding in to the BOTU. The turbidity is still higher than in seawater, 9.3 NTU in EGSE compared to below the detection limit of 2 NTU in seawater (Table 4).

Table 4. Concentrations of/values for turbidity, nitrite (NO₂-N), nitrate (NO₃-N), pH, alkalinity and also toxicity, measured as Microtox, in scrubber water before and after treatment in the BOTU. Note that for Microtox lower figures signify higher toxicity. Concentrations in seawater are included as a reference. NA=not applicable

Parameter	Process water	BOTU feed	Decanter water	EGSE	Seawater	Reduction efficiency (%)
Turbidity (NTU)	50	255	18.4	9.3	<2	96.4
pH	3.6 – 6.2	5.1	8	7.6	7.9	
Alkalinity (mmol·L ⁻¹)	-	0	13	6.0	2.5	
NO ₂ - * (mg N·L ⁻¹)	<30	<30	<30	49	<30	>-64.6
NO ₃ - * (mg N·L ⁻¹)	31	27	<1	<1	<1	>96
Microtox (EC50, 5 min)	6.7	13	30	15.5	>45	16.1

* High detection limit due to disturbance by high concentrations of chlorine and sulphate.

5.1.2 Metals

Concentrations of Cu and Hg were higher in EGSE compared to water feeding into the BOTU (Table 5). This is an unexpected finding and the reason for this increase in concentrations after the cleaning step is not known. A similar increase in Cu content from the fuel to the EGSE was also found in a previous study of scrubber systems (Kjølholt, Aakre et al., 2012). Also in their study the authors were unable to find an explanation to the increase.

Concentrations of Al, Cr, Ni and Zn were reduced by close to or >90% in the BOTU. The Zn concentration was below the detection limit of the analyses, 50 µg·L⁻¹. However, the Zn concentration in EGSE from Stena Transporter (also with a closed loop scrubber system) from 2018 was 46 µg·L⁻¹, which indicates that concentrations in Stena Britannica might have been close to the detection limit.

Table 5. Concentrations of metals and sulphur in scrubber water before and after treatment in the BOTU (BOTU feed and EGSE respectively). Negative/red figures indicate a higher concentration in the EGSE than in the BOTU feed. Concentrations in seawater are included as a reference.

	BOTU feed	EGSE	Seawater	Reduction efficiency (%)
Al ($\mu\text{g}\cdot\text{L}^{-1}$)	120 000	8 300	39	93.1
As ($\mu\text{g}\cdot\text{L}^{-1}$)	66	20	1.9	69.7
Cd ($\mu\text{g}\cdot\text{L}^{-1}$)	0.34	<0.2	0.11	>41.2
Cu ($\mu\text{g}\cdot\text{L}^{-1}$)	41	150	17	-265.9
Cr ($\mu\text{g}\cdot\text{L}^{-1}$)	90	9	<1.2	90.0
Ni ($\mu\text{g}\cdot\text{L}^{-1}$)	7 400	830	0.61	88.8
Pb ($\mu\text{g}\cdot\text{L}^{-1}$)	18	<6	0.098	66.7
V ($\mu\text{g}\cdot\text{L}^{-1}$)	27 000	9 800	3.7	63.7
Zn ($\mu\text{g}\cdot\text{L}^{-1}$)	1 200	<70	6.2	94.2
Hg ($\text{ng}\cdot\text{L}^{-1}$)	1.9	5.2	0.84	-173.7
S ($\text{mg}\cdot\text{L}^{-1}$)	22 000	19 000	1 100	13.6

5.1.3 Organic contaminants

Although total hydrocarbons were significantly reduced after passage through the BOTU, aliphatic hydrocarbons with short chains, between 8 and 12 carbon atoms, increased significantly during the BOTU treatment (Table 6). Molecules with >8 – 10 carbon atoms increased from <4 $\mu\text{g}\cdot\text{L}^{-1}$ to 49 $\mu\text{g}\cdot\text{L}^{-1}$, and molecules with >10 – 12 carbon atoms increased from 1 400 to 2 900 $\mu\text{g}\cdot\text{L}^{-1}$.

Overall, the heavy fractions of both aliphatic and aromatic hydrocarbons were reduced more efficiently than the lighter ones (Table 6). Aliphatic hydrocarbons with more than 16 carbon atoms (>C16) were reduced by > 99% in the BOTU. For those with shorter chains the reduction was less effective, >C12-C16 was reduced by 75% whereas the fraction >C8-C10 increased in concentration by 107% after the passage through the BOTU. The shortest aliphates, $\leq\text{C8}$ were below the detection limit in water both before and after the BOTU.

Aromatic compounds with three rings or more were reduced by 90% - >99% (Table 6 and Table 7). The fraction of aromatic compounds with >10 – 12 carbon atoms was reduced by only 27%. The only one of the 16 analysed PAHs with this number of carbon atoms is naphthalene, but since

naphthalene was reduced by 76% (Table 7) there must be one or more other small polycyclic aromatic compounds which were poorly eliminated.

Table 6. Concentrations of hydrocarbons in scrubber water before and after treatment in the BOTU ((BOTU feed and EGSE respectively). Aliphatic or aromatic hydrocarbons of different sizes are presented as fractions of different size, i.e. named after the numbers of carbon atoms in the compounds included (C₅, C₈, C₁₀ etc.). "Total hydrocarbon" is the sum of all these fractions. Concentrations in EGSE below the analytical detection limit were set both to the detection limit (max) and to zero /min). Concentrations in seawater are included as a reference.

	BOTU feed (µg·L ⁻¹)	EGSE (µg·L ⁻¹)	Seawater (µg·L ⁻¹)	Reduction efficiency (%)
Total hydrocarbon	211 960	7 103 _{max} /6 499 _{min}		96.9 _{max} /96.7 _{min} %
Fraction of different sizes of hydrocarbons :				
Aliphatic >C ₅ -C ₈	<4.0	<4.0	<4.0	-
Aliphatic >C ₈ -C ₁₀	<4.0	49	<4.0	<-1125%
Aliphatic >C ₁₀ -C ₁₂	1 400	2 900	<10	-107.1%
Aliphatic >C ₁₂ -C ₁₆	6 800	1 700	<10	75.0%
Aliphatic >C ₁₆ -C ₂₁	23 000	<100	<10	>99.6%
Aliphatic >C ₂₁ -C ₃₆	95 000	720	<30	99.2%
Aliphatic >C ₃₆ -C ₄₀	24 000	<100	<10	>99.6%
Aromatic >C ₁₀ -C ₁₂	860	630	<10	26.7
Aromatic >C ₁₂ -C ₁₆	4 900	500	<10	89.8
Aromatic >C ₁₆ -C ₂₁	16 000	<100	<10	>99.4
Aromatic >C ₂₁ -C ₃₆	40 000	<300	<30	>99.3

Table 7. Concentrations of 16 PAHs in scrubber water before and after BOTU treatment (BOTU feed and EGSE respectively). The reduction efficiency is measured as a percentage. Concentrations in seawater are included as a reference.

	BOTU feed	EGSE	Seawater	Reduction efficiency (%)
Naphtalene	18 000	4 400	<5,0	75.6
Acenaftylen	3 900	360	<1,0	90.8
Acenaften	35 000	2 100	<1,0	94.0
fluoren	49 000	3200	<1,0	93.5
fenanthren	520 000	10 000	<1,0	98.1
Anthracen	16 000	400	<1,0	97.5
Fluoranthen	99 000	220	<1,0	99.8
Pyrene	360 000	540	4.30	99.9
Benzo(a)anthracen	210 000	210	<1,0	99.9
Chrysen	400 000	330	<1,0	99.9
Benzo(b)fluoranthen	100 000	100	<1,0	99.9
Benzo(k)fluoranthene	21 000	70	<1,0	99.7
Benzo(a)pyrene	39 000	<100,0	<5,0	>99.7
Dibenzo(a,h)anthracene	17 000	<100,0	<5,0	>99.4
Benzo(g,h,i)perylene	76 000	<100,0	<5,0	>99.9
Indeno(1,2,3-c,d)pyrene	20 000	<100,0	<5,0	>99.5

5.1.4 Microtox bioassay

The Microtox assay (Microtox) is often used as a screening tool for measuring acute toxicity in whole effluents from various activities including industries and waste water treatment plants. It is a valuable tool when comparing the relative toxicity between different waters. In this case it was found that the toxicity of the process water was considerably more toxic than the water feeding in to the BOTU (Table 4, note that low figures indicate high toxicity). However, the treated scrubber water leaving the BOTU, was only slightly less toxic than the BOTU feed. So although the concentrations of most of the analysed compounds were reduced in the BOTU it did not have a large effect on the Microtox toxicity. One explanation may be that Microtox measures acute toxicity, and low molecular aromatics, >C10-C12, which are known to be acutely toxic, were only reduced by 26.7% in the BOTU (Table 9). High molecular aromatic hydrocarbons, which were reduced by >99%, generally lack acute toxicity but have more chronic effects like carcinogenicity.

The Microtox bioassay is also known to be particularly sensitive to certain metals, i.e. Cu and Hg which were both found in higher concentration in the water leaving the BOTU than in the BOTU feed (Codina, Pérez-García et al., 1993).

6 Effects on marine organisms

6.1 Results from ecological toxicity tests

Mortality rate in juveniles (copepodite) stage CV of the copepod *Calanus helgolandicus* was found to be the most sensitive indicator of all measured endpoints in all toxicity tests of EGSE toxicity. Statistically significant toxic effects were observed at 0.04% EGSE in the experiments with water from Stena Britannica (closed loop), and for copepodite stage CIII at 0.1% with water from Stena Transporter (closed loop) and 1.0% with water from Stena Forerunner (open loop). Neither pH nor alkalinity (A_T) was different from the control treatment at these EGSE concentrations, so we conclude that effects on copepod mortality (as well as all other measured physiological processes in the copepods) were not caused by acidification but were primarily due to the toxic effects of EGSE. It should be noted that in both closed and open loop exposure the lowest tested concentration resulted in toxic effects on the copepodites. Thus, it cannot be excluded that even lower concentrations would have been harmful to the tested zooplankton species.

Having the ability to quickly form strong and many byssus threads is crucial for the survival of the blue mussel (*Mytilus edulis*). Byssus strength was the only endpoint measured in blue mussels that showed a significant effect of the EGSE treatments. This effect was detected at a EGSE concentration of 1.25% and only in closed loop exposures from Stena Transporter (March 2018 trial).

6.1.1 pH and alkalinity of tested EGSE dilutions

The EGSE contains different sulphuric acids, which may compromise pH and/or alkalinity of the recipient water. The quantity of EGSE released from ships has been suggested to approach a magnitude that may contribute to ocean acidification in certain waters¹. While exhaust gas scrubbing removes atmospheric deposition to the ocean surface it merely acts as a more efficient mechanism for transferring acids to the ocean if these are not sufficiently neutralised before release (Hassellöv, Turner et al., 2013). pH and alkalinity was tested in this study in order to interpret the results from the toxicity tests (Table 8). No further chemical or oceanographic modelling of the fate and effect of released EGSE associated sulphuric acids into the ocean has been done within the framework of this study.

For all tested EGSE types, increasing concentrations of effluent had a clear depressing effect on pH. The closed loop EGSE depressed the seawater pH from ca 8.1 to ca. 7.94 (test A & D) and 7.45 (test B & E) at a 5% EGSE concentration. In comparison, global CO₂ driven ocean acidification is predicted to lower pH to ca. 7.7 at year 2100 if emissions continue unabated (Thor & Dupont, 2018). Interestingly, pH was much higher at the 25% concentration (Table 8) due to the neutralising agents added to the EGSE before release. But apparently the neutralisation power is lost already

¹ Ocean acidification is mainly driven by increased dissolution of CO₂ in seawater due to excessive fossil fuel burning.

when the EGSE is diluted to 5% due to the inherent carbonate buffering system of the sea water. The net effect is therefore a strong acidification of the sea water in the immediate vicinity of the vessel.

Total alkalinity (A_T) was unchanged with the closed loop EGSE and the seawater kept the buffering capacity at all concentrations. Thus, the pH of the seawater was restored to normal at an EGSE concentration of 0.5% (it is important to note that this buffering is not removing any released pollutants, it is just neutralising the acidification effect). For the open loop scrubber (test C & F), pH was depressed to as low as 7.75 at the 10% EGSE concentration and 7.16 at the 40% EGSE concentration (Table 8). These extreme levels correspond to an 8 fold increase in acidity at the 40% concentration. This strong increase in acidity was made possible by the very low alkalinity of the EGSE (at 40% EGSE concentration A_T was only $1\,011\ \mu\text{mole}\cdot\text{L}^{-1}$; Table 8).

Table 8. pH and total alkalinity (A_T) (\pm standard errors). Conc. indicates percentage dilution of EGSE in seawater tested. The waters and concentrations were prepared for the zooplankton tests. Bold numbers indicate concentrations corresponding to concentrations used in the blue mussel tests. Numbers in bold italic indicate concentrations approximate to those used in the blue mussel tests.

Test A & D Closed loop Stena Britannica September 2017			Test B & E Closed loop Stena Transporter March 2018			Test C & F Open loop Stena Forerunner March 2018		
Conc.	pH	A_T	Conc.	pH	A_T	Conc.	pH	A_T
%		$\mu\text{mole}\cdot\text{L}^{-1}$	%		$\mu\text{mole}\cdot\text{L}^{-1}$	%		$\mu\text{mole}\cdot\text{L}^{-1}$
0	8.060 ± 0.022	$2\,496 \pm 5$	0	8.073 ± 0.030	$2\,361 \pm 6$	0	8.073 ± 0.030	$2\,361 \pm 6$
0.04	8.061 ± 0.024	$2\,417 \pm 9$	0.1	8.052 ± 0.038	$2\,348 \pm 13$	1	8.031 ± 0.053	$2\,346 \pm 11$
0.2	8.027 ± 0.028	$2\,515 \pm 28$	0.5	<i>8.000 ± 0.030</i>	<i>$2\,366 \pm 24$</i>	5	<i>7.915 ± 0.057</i>	<i>$2\,177 \pm 3$</i>
0.5	8.012 ± 0.020	$2\,497 \pm 3$	1	<i>7.890 ± 0.029</i>	<i>$2\,294 \pm 1$</i>	10	7.748 ± 0.129	$2\,017 \pm 2$
1	<i>7.945 ± 0.044</i>	<i>$2\,498 \pm 6$</i>	2	<i>7.720 ± 0.035</i>	<i>$2\,191 \pm 16$</i>	40	7.163 ± 0.257	$1\,011 \pm 41$
2	7.940 ± 0.020	$2\,493 \pm 2$	5	<i>7.454 ± 0.008</i>	<i>$2\,241 \pm 23$</i>			
5	7.936 ± 0.003	-						
25	8.271 ± 0.005	-						

6.1.2 Zooplankton- *Calanus helgolandicus*

6.1.2.1 Mortality and lethargy

The copepods reacted strongly to the exposure to both closed loop and open loop EGSE. While there was no mortality in any of the control treatments, all copepods died within one day when exposed to the 5% concentration of closed loop EGSE and within 8 days when exposed to the 40% concentration of open loop EGSE.

The **mortality rate** from the different treatments was calculated and tested statistically among concentrations in order to find lowest effect concentration of EGSE. The lowest concentration where a significant effect on mortality rate was detected was 0.04% in Test A (closed loop 2017), 0.1% in Test B (closed loop 2018) and 1% in Test C (open loop 2018) (Table 9, indicated in red). All concentrations from Test A were not present in Tests B and C. Significant effects actually occurring at lower concentrations than those tested in B and C cannot be excluded.

Table 9. Mortality rates (\pm standard errors). Conc. is the tested concentration in % of EGSE mixed in seawater. CV and CIII indicate the juvenile life stages of the tested animals (e.g. CIII corresponds to copepodite stage three). Lower case italicised letters (*a-e*) indicate statistically equal groups. Red text indicates the lowest concentration where the mortality rate was statistically different from control.

Test A Closed loop Stena Britannica September 2017 CV			Test B Closed loop Stena Transporter March 2018 CIII			Test C Open loop Stena Forerunner March 2018 CIII		
Conc.	Mortality rate		Conc.	Mortality rate		Conc.	Mortality rate	
%	d ⁻¹		%	d ⁻¹		%	d ⁻¹	
0	0.0000 \pm 0.0000	<i>a</i>	0	0.000 \pm 0.000	<i>a</i>	0	0.0000 \pm 0.0000	<i>a</i>
0.04	0.0097 \pm 0.0029	<i>b</i>	0.1	0.100 \pm 0.009	<i>b</i>	1	0.0064 \pm 0.0023	<i>b</i>
0.2	0.0139 \pm 0.0029	<i>b</i>	0.5	0.154 \pm 0.010	<i>c</i>	5	0.0113 \pm 0.0011	<i>c</i>
0.5	0.0158 \pm 0.0056	<i>b</i>	1	0.178 \pm 0.014	<i>c</i>	10	0.0328 \pm 0.0032	<i>d</i>
1	0.0519 \pm 0.0059	<i>c</i>	2	0.162 \pm 0.009	<i>c</i>	40	0.1403 \pm 0.0151	<i>e</i>
2	0.0389 \pm 0.0064	<i>d</i>	5	>1	<i>d</i>			
5	>1	<i>e</i>						

Lethargy seemed to be an effect that occurred during the first few days of exposure and in particular with the closed loop EGSE. Lethargy was prominent during the first three days of exposure to the 2% closed loop EGSE concentration. Onwards, these juvenile copepods seemed to

recover somewhat and there was a significant decrease in lethargy during the remainder of the incubation period. There was no increased lethargy in exposures to open loop EGSE.

6.1.2.2 Stage development and moulting

Stage development, from juvenile stage CIII to CIV (Fig. 2) measured as moulting rate, was only measured in Tests B and C and was inhibited by EGSE from both closed and open loop systems. No shed exoskeletons were found in the three highest closed loop EGSE concentration treatments (1, 2, and 5%). Similarly, we found only one shed exoskeleton in the high concentrations of the open loop EGSE treatment (40 %) at the very first sampling and no subsequent moulting. Moreover, we observed several CIV juveniles showing signs of abnormal moulting with remains of old exoskeleton on the antennules and two CIV juveniles in the 5% and 10% open loop EGSE treatments showed malformed antennules.

Moulting rates decreased significantly from low to high concentrations for stage CIII juveniles exposed to EGSE from both the closed and the open loop system. It was significantly different from controls already at 0.1% EGSE in closed loop treatments and at 5% EGSE in open loop treatments as indicated in red in Table 10.

Table 10. Stage development expressed as moulting rates (\pm standard error) of *Calanus helgolandicus* stage CIII to stage CIV. Conc. indicates percentage mixing of EGSE in seawater. Lower case italicised letters (*a-d*) indicate statistically equal groups. Red text indicates the lowest concentration where moulting rates were statistically different from control.

Test B Closed loop Stena Transporter March 2018 CIII			Test C Open loop Stena Forerunner March 2018 CIII		
Conc.	Moulting rate		Conc.	Moulting rate	
%	d ⁻¹		%	d ⁻¹	
0	0.133 \pm 0.007	<i>a</i>	0	0.133 \pm 0.007	<i>a</i>
0.1	0.082 \pm 0.008	<i>b</i>	1	0.0751 \pm 0.0067	<i>a</i>
0.5	0.007 \pm 0.003	<i>c</i>	5	0.0505 \pm 0.0037	<i>b</i>
1	0.000 \pm 0.000	<i>c</i>	10	0.0481 \pm 0.0058	<i>b</i>
2	0.000 \pm 0.000	<i>c</i>	40	0.0119 \pm 0.0067	<i>c</i>
5	0.000 \pm 0.000	<i>d</i>			

6.1.2.3 Feeding rate and metabolic rate

Feeding rate in juvenile CIII copepods was significantly impacted by both closed and open loop EGSE during the March 2018 trial, while no effect was observed in juvenile stage CV copepods during the September 2017 trial. Feeding rates increased six-fold from 0% to 0.5% and 1% EGSE

and then decreased again in CIII copepods exposed to closed loop EGSE. Feeding rates in juvenile CIII copepods exposed to open loop water instead decreased three-fold from 1% EGSE and upwards compared to the control (Table 11).

This initial increase followed by a decrease in a measured physiological response as the exposure increases is typically observed in ecotoxicological tests and termed hormesis. A stressor may seem to positively affect an organism at a low concentration while the measured effect actually is the organism's way of compensating metabolically to cope with increased stress. This is what we see here.

Table 11. Feeding rate (\pm standard error). Conc. indicates percentage mixing of EGSE in seawater. Lower case italicised letters (*a-c*) indicate statistically equal groups. Red text indicates the lowest concentration where feeding rates are statistically different from the control.

Test A Closed loop Stena Britannica September 2017 CV			Test B Closed loop Stena Transporter March 2018 CIII			Test C Open loop Stena Forerunner March 2018 CIII		
Conc.	Feeding rate		Conc.	Feeding rate		Conc.	Feeding rate	
%	$\mu\text{gC} \cdot \mu\text{gC}^{-1} \text{d}^{-1}$		%	$\mu\text{gC} \cdot \mu\text{gC}^{-1} \text{d}^{-1}$		%	$\mu\text{gC} \cdot \mu\text{gC}^{-1} \text{d}^{-1}$	
0	0.3772 ± 0.0375	<i>a</i>	0	0.167 ± 0.081	<i>a</i>	0	0.1666 ± 0.0810	<i>a</i>
0.04	0.0901 ± 0.0757	<i>a</i>	0.1	0.520 ± 0.303	<i>b</i>	1	0.0320 ± 0.0307	<i>b</i>
0.2	0.2308 ± 0.1095	<i>a</i>	0.5	0.983 ± 0.359	<i>ab</i>	5	0.0366 ± 0.0034	<i>b</i>
0.5	0.2444 ± 0.1587	<i>a</i>	1	0.693 ± 0.000	<i>ab</i>	10	0.0628 ± 0.0150	<i>b</i>
1	0.2828 ± 0.0521	<i>a</i>	2	0.040 ± 0.007	<i>c</i>	40	-	
2	0.3309 ± 0.0272	<i>a</i>	5	-				
5	-							

Metabolic rates of stage CIII copepods were dramatically affected by closed loop EGSE during the March 2018 trial already at 0.5% exposure concentration, with a 6-fold increase from the control treatment to the 2% concentration (Table 12). No effect was observed either from open loop EGSE or during the March 2017 trial. Increased metabolic rate is a clear sign of stress and will negatively affect chances of survival and the ability to cope with additional stressors.

Table 12. Metabolic rate (\pm standard error). Conc. indicates percentage mixing of EGSE in seawater. Lower case italicised letters (*a-b*) indicate statistically equal groups. Red text indicates concentration where metabolic rates were statistically different from control.

Test A Closed loop Stena Britannica September 2017 CV			Test B Closed loop Stena Transporter March 2018 CIII			Test C Open loop Stena Forerunner March 2018 CIII		
Conc.	Metabolic rate		Conc.	Metabolic rate		Conc.	Metabolic rate	
%	$\mu\text{gC} \cdot \mu\text{gC}^{-1} \text{ d}^{-1}$		%	$\mu\text{gC} \cdot \mu\text{gC}^{-1} \text{ d}^{-1}$		%	$\mu\text{gC} \cdot \mu\text{gC}^{-1} \text{ d}^{-1}$	
0	0.0571 ± 0.0098	<i>a</i>	0	0.009 ± 0.001	<i>a</i>	0	0.0093 ± 0.0011	<i>a</i>
0.04	0.0508 ± 0.0052	<i>a</i>	0.1	0.017 ± 0.003	<i>a</i>	1	0.0041 ± 0.0018	<i>a</i>
0.2	0.0644 ± 0.0069	<i>a</i>	0.5	0.054 ± 0.004	<i>b</i>	5	0.0093 ± 0.0037	<i>a</i>
0.5	0.0210 ± 0.0030	<i>b</i>	1	0.167 ± 0.000	<i>c</i>	10	0.0064 ± 0.0009	<i>a</i>
1	0.0557 ± 0.0080	<i>a</i>	2	0.132 ± 0.029	<i>c</i>	40	-	
2	0.0376 ± 0.0023	<i>a</i>	5	-				
5	-							

6.1.3 Blue mussel (*Mytilus edulis*)

6.1.3.1 Byssus strength and hepatosomatic index

Having the ability to quickly form strong and many byssus threads is crucial for the survival of the mussel. Byssus threads form the holdfast needed for it to be able to feed and stay at a site where food is sufficiently prevalent, i.e. not fall into the deep. Strong byssus threads also prevent mussels from being pulled up and eaten by diving seabirds such as eider ducks. **Byssus strength** was the only endpoint measured in blue mussels that showed a significant effect of the EGSE treatments. This effect was detected at an EGSE concentration of 1.25% and upward only in closed loop exposures during the March 2018 trial (Fig. 5C).

Hepatosomatic index is the ratio between the weight of the hepatopancreas (liver) and the rest of the soft tissue of the mussel. The hepatopancreas can both increase and decrease in response to pollutant stress. There was no consistent effect pattern in the hepatosomatic index in any of the tests (Fig 5. B, D, F).

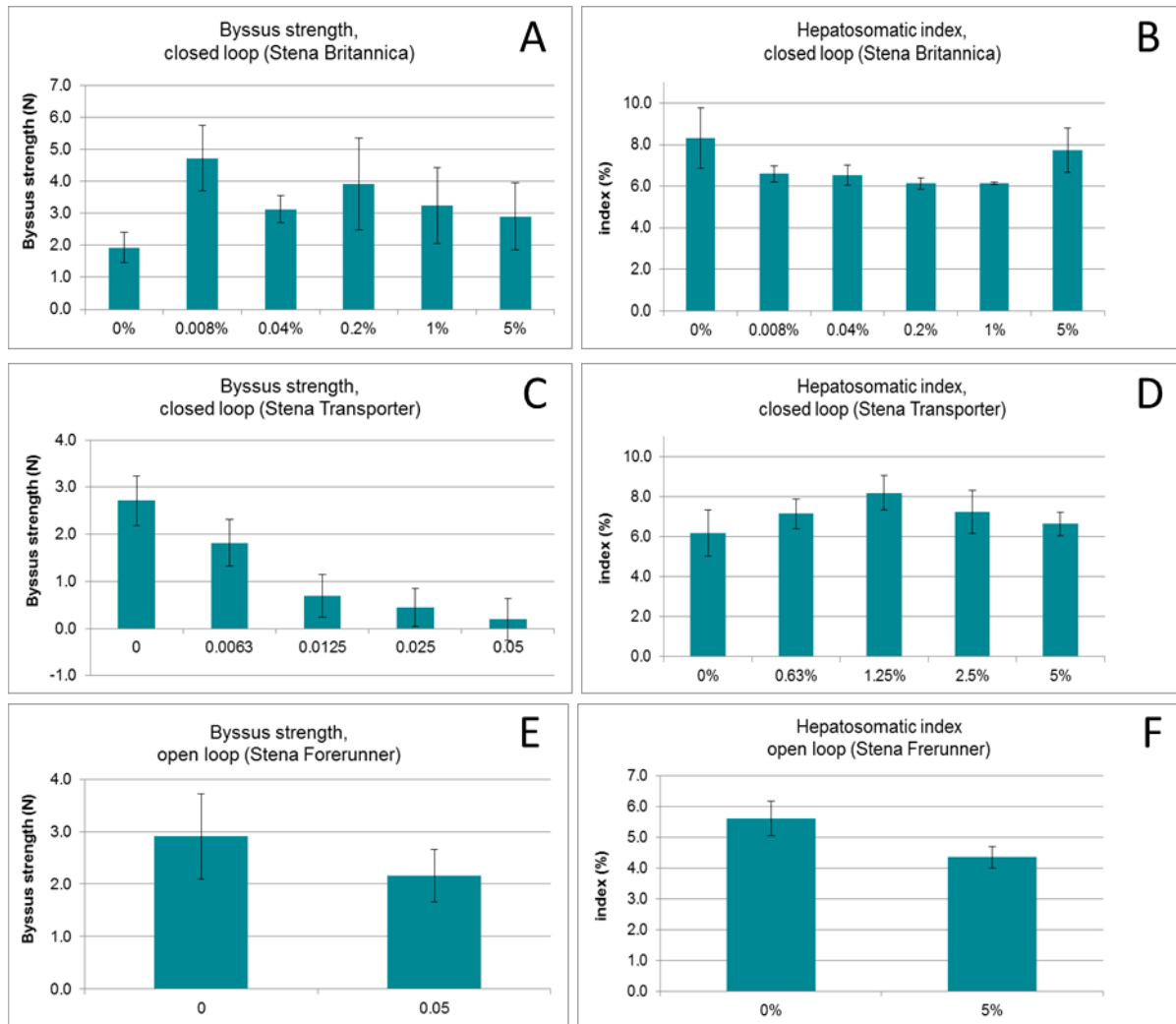


Figure 5. Byssus strength (graphs A, C and E) and hepatosomatic index (graphs B, D and F) of blue mussels measured at the end of the chronic exposures to different concentrations of EGSE. Average values \pm standard error.

6.1.3.2 Cell viability

Cell viability was measured on cells from the haemolymph (blood) of the mussels. These cells are known to react quickly to pollution stress. Although there was a clear trend in decreasing cell viability with increasing EGSE exposure (Fig. 6) it could not be determined statistically due to high individual variability. Cell viability was only measured on mussels exposed to closed loop EGSE during the 2018 trial.

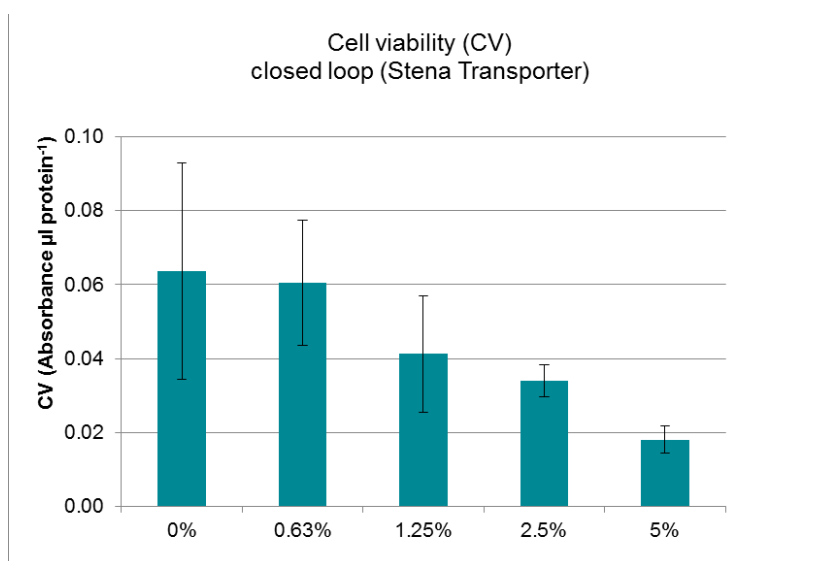


Figure 6. Average cell viability (\pm standard error) in haemolymph cells from blue mussels exposed to closed loop EGSE from Stena Transporter.

6.2 Literature comparison of EGSE toxicity

The toxicity of EGSE was expressed as the percentage of effluent water mixed into the exposure water having a toxic effect on the animals. However, by using the data from the chemical analyses, it was also possible to calculate the concentrations of the analysed compounds at the different dilutions. This calculation of chemical concentrations in the exposure water of the toxicity tests was only done for the dilution at the lowest detected toxic concentrations. These dilutions were 0.04% EGSE for Stena Britannica, 0.1% for Stena Transporter and 1.0% for Stena Forerunner. The results are shown in table 13. In an attempt to identify what specific compounds that might have been responsible for causing the toxic effects, the concentrations of all chemicals and groups of chemicals (total hydrocarbons, PAHs, alkylated PAHs and metals) in the exposure water were compared to toxicity data from the scientific literature. There are numerous studies on toxicity of hydrocarbons, and PAHs on species relevant for the present study, whereas studies on effects metals are less common. Studies on the toxic effects of mono aromatics and alkylated PAHs are very few. A brief overview of the outcome of the review is presented here.

The concentrations of total hydrocarbon in the lowest concentration of EGSE found to have a toxic effect were $2.84 \mu\text{g total hydrocarbons} \cdot \text{L}^{-1}$ in the water from Stena Britannica (closed loop), $1.96 \mu\text{g} \cdot \text{L}^{-1}$ from Stena Transporter (closed loop) and $3.8 \mu\text{g total hydrocarbons} \cdot \text{L}^{-1}$ in water from Stena Forerunner (open loop) (Table 13). There are many studies carried out on the effect of “total oil” on marine copepods. Total oil is often used as a synonym to total hydrocarbons. In several of the studies, toxic effects have been detected at concentrations only 5 - 10 times higher than the concentrations in the present study. The feeding rate, egg survival and stage development have been found to be affected in several species of copepods after exposure to crude oil ($10\text{-}20 \mu\text{g} \cdot \text{L}^{-1}$) (Avila, Bersano et al., 2010, Cowles, 1983, Jensen & Carroll, 2010). Effect on the life stage development means that the animal does not move from one stage to the next in the life cycle (see Fig. 2) and this was found to be one of the most sensitive parameters also in the present study. On a population level this is serious since it means that the animals do not become sexually mature and therefore cannot reproduce.

The swimming pattern of copepods has been found to be affected in the presence of total oil concentrations of $80 \mu\text{g oil} \cdot \text{L}^{-1}$ (Cowles & Remillard, 1983, Seuront, 2010). Effects on swimming behaviour of pelagic copepods were also seen after exposure to low and medium sized PAHs in concentrations of $40 \text{ ng} \cdot \text{L}^{-1}$ (Michalec, Holznere et al., 2013). The concentration of total PAH in the lowest toxic concentration with water from the open loop system was four times as high as this, $135 \text{ ng} \cdot \text{L}^{-1}$ (Table 13). PAH concentrations in water from the two closed loop systems were ten times lower than in the open loop system, $16 \text{ ng} \cdot \text{L}^{-1}$ (Stena Transporter) and $8.9 \text{ ng} \cdot \text{L}^{-1}$ (Stena Britannica), but still within the same range as the concentrations found to be toxic in the study by Michalec, Holznere et al (2013).

Metal concentrations in the exposure water at the lowest toxic concentrations were also compared to data from the literature although data on relevant species were quite limited. The reproduction of the marine copepod *Acartia tonsa* was found to be negatively affected by Zn, Cu and Ni in concentrations of 0.64 , 0.3 and $2.4 \mu\text{g} \cdot \text{L}^{-1}$ (Bielmyer, Grosell et al., 2006). These concentrations were exceeded for Zn in EGSE from Stena Forerunner (open loop) and for Ni in EGSE from Stena Transporter (closed loop) (Table 13).

The vertical migration patterns of *Daphnia magna* were found to be altered at chromium concentrations of $3.5 \mu\text{g} \cdot \text{L}^{-1}$, only ten times higher than what was found in the lowest toxic concentration of open loop water (Gutierrez, Gagneten et al., 2012)(Table 13). However, concentrations in the closed loop systems were 10 – 100 times lower than in the open loop system.

In conclusion it is likely that the toxicity has mainly been caused by the hydrocarbons (including the PAHs). Although several metals, e.g. Zn, Cu, Ni and Cr, were found in the exposure water in the present study at concentrations close to concentrations found to be toxic there were no consistency between the EGSE from the three ships. However, the lack of literature data on toxicity to zooplankton of several of the metals, mono aromatics and alkylated PAHs might have led to an underestimation of the toxicity of these groups of chemicals.

Table 13. Concentrations of chemicals in exposure water at the lowest concentration EGSE having a significantly toxic effect on marine zooplankton.

	Stena Britannica, 2017 Closed loop (0.04% EGSE)	Stena Transporter 2018 Closed loop (0.1% EGSE)	Stena Forerunner 2018 Open loop (1% EGSE)
Total hydrocarbon ($\mu\text{g}\cdot\text{L}^{-1}$)	2.84	1.96	3.88
Σ Mono aromatic hydrocarbons ($\text{ng}\cdot\text{L}^{-1}$)	1.4	not analysed	14.3
Σ 16PAH ($\text{ng}\cdot\text{L}^{-1}$)	8.9	16	135
Σ 11 alkylated PAH ($\text{ng}\cdot\text{L}^{-1}$)	55.2	not analysed	268
Al ($\mu\text{g}\cdot\text{L}^{-1}$)	3.32	1.10	1.80
As ($\mu\text{g}\cdot\text{L}^{-1}$)	0.008	0.010	0.024
Cr ($\mu\text{g}\cdot\text{L}^{-1}$)	0.004	0.022	0.310
Cu ($\mu\text{g}\cdot\text{L}^{-1}$)	0.060	0.032	0.140
Hg ($\text{ng}\cdot\text{L}^{-1}$)	0.002	0.001	0.065
Ni ($\mu\text{g}\cdot\text{L}^{-1}$)	0.332	4.40	0.320
Pb ($\mu\text{g}\cdot\text{L}^{-1}$)	< detection limit	0.00016	0.0063
V ($\mu\text{g}\cdot\text{L}^{-1}$)	3.92	13.0	0.840
Zn ($\mu\text{g}\cdot\text{L}^{-1}$)	< detection limit	0.046	0.820
S ($\text{mg}\cdot\text{L}^{-1}$)	7.60	22.0	12.0
NO ₂ -N ($\text{mg}\cdot\text{L}^{-1}$)	0.02	<detection limit	<detection limit
NO ₃ -N ($\text{mg}\cdot\text{L}^{-1}$)	<detection limit	0.018	0.0018

7 Risk assessment of discharge of EGSE from scrubbers

7.1 Introduction to the risk assessment

Ships equipped with an exhaust gas scrubber system will discharge EGSE into the sea more or less continuously during operation. In shipping lanes with intense traffic it is inevitable that potentially harmful components from the EGSE will occur in elevated concentrations compared to areas far away from any shipping activities. The main aim of the present study has been to investigate whether the discharged EGSE from the three ships included in the study run the risk of having harmful effects on the marine ecosystems in the area where the ships operate.

The risk assessment was done using two different approaches, one based on the results from the **chemical analyses** of the EGSE from the three ships (section 7.3) and another based on data from the **toxicity tests** carried out on the whole EGSEs (section 7.4). A dilution factor was used to calculate sea water concentrations of both individual chemical components and for whole EGSE in the area around the shipping lane (section 7.2).

In the first approach the calculated sea water concentrations of individual chemicals were compared to existing threshold concentrations from the IMO quality criteria for discharged EGSE (IMO Resolution MEPC.259(68) 2015 Guidelines for Exhaust Gas Cleaning Systems) and the environmental quality standards (EQS) from the EU Water Framework Directive (WFD, 2000/60/EC). In the second approach results from the toxicity tests were used to calculate threshold concentrations for whole EGSE from each of the three ships, i.e. how diluted the EGSE must be to ensure that it does not have a harmful effect on the marine environment. These threshold concentrations were then compared to the estimated seawater concentrations of whole EGSE. The assessments were performed for the discharge made during one crossing along the transect Hoek van Holland - Harwich. A detailed analysis of the effects of multiple crossings in busy ship lanes was not done.

7.2 Dilution of EGSE in recipient seawater

As EGSE is discharged from a moving ship it will quickly be diluted in the seawater. An important issue when estimating the risk a discharge may pose on the environment is to determine at what degree of dilution the assessment should be carried out. Should it be at the point where the discharged water meets the recipient water, or should it be at a distance from this point, and, in the latter case, at what distance?

In the present study we compare the concentrations of hazardous compounds in the EGSE with the EQS of the WFD. The WFD states that contaminated water should be treated with best available technology before being released into the recipient water, and preferably this should lead to a discharge where no EQS are exceeded (Article 10 of the WFD). Nevertheless the WFD accepts that the EQS may be exceeded in an area close to the point of release, an area called the *mixing zone* (Article 4 of Directive 2008/105/EC). It is clearly stated that the extent of the mixing zone should be restricted to the proximity of the point of discharge and that it should be of a size that ensures protection of the aquatic life and of the integrity of the whole water body. However, these guidelines focus primarily on mixing zones for discharges from a steady point source such as a waste water outlet, and they are not really applicable to discharges from moving ships.

When the mixing zone is discussed in the guidelines of the WFD it is made clear that a typical discharge is considered to be a plume of e.g. waste water from a pipe. The spreading on the surface is supposed to be rapid and the vertical mixing into the water column is considered to be taking 1-2 days if it is rapid (Technical guidelines for the identification of mixing zones pursuant to Art. 4(4) of the Directive 2008/105/EC). In the case of discharge of water from a moving ship the situation is very different as the vertical mixing is almost immediate due to the movement of the propellers. This means that the EGSE, (and other kinds of water discharged from ships), will quickly disappear from the surface and hence also away from the wind and waves that are the prime forces in horizontal dispersal. The rapid mixing will also move away the EGSE from the UV light of the sun, something that will seriously slow down the degradation of hydrocarbons in the discharge. It can hence be concluded that the rapid mixing down in the water column may lead to a local build-up of high concentrations of harmful compounds present in the discharged water.

In addition, we are dealing with discharges in a ferry lane where ships go back and forth along approximately the same transect several times per day. There is hence a substantial risk that pollutants building up in the mixing zone will spill over and have an impact also on the area outside the shipping lane itself. In this study we will therefore use the estimated seawater concentrations of hazardous compounds from the EGSE and of whole EGSE in the actual mixing zone to assess the environmental risk of the discharges

An approach specifically aiming at estimating the mixing of discharges of water from ships was proposed by a scientific advisory panel set out to assist the Alaska Department of Environmental Conservation in evaluating the effect of discharges of wastewater from cruise ships (Loehr, Atkinson et al., 2003). As a part of this work a dilution factor for discharges of wastewater from moving ships was formulated. The dilution factor was estimated based on presumptions that turbulence in the water is created both by the propellers and by the movement of the ship and the subsequent displacement of water. The water displacement is presumed to be related to the size of the ship. The following dilution factor was proposed by the Advisory Panel:

$$\text{Dilution factor} = \frac{\text{ship width} \times \text{ship draught} \times \text{ship speed}}{\text{discharge rate}} \quad (\text{equation 1})$$

where ship width (m), ship draft (m), ship speed in ($\text{m}\cdot\text{s}^{-1}$), and discharge rate ($\text{m}^3\cdot\text{s}^{-1}$).

Data on discharge rates of EGSE from ships with closed loop (Stena Britannica and Stena Transporter) and open loop (Stena Forerunner) scrubber water systems were obtained from Stena's personnel through personal communication with Björn Asplind in May 2018. The ship speeds were calculated as the distance between Hoek van Holland and Harwich divided by the time required for the crossings. The data for the factors included in the equation 1 and the calculated dilution factors for the three ships in this study are presented in Table 14.

Table 14. Data on ship size, ship speed, the discharge rate of EGSE and the calculated dilution factors for the three ships included in the present study.

	Ship width (m)	Ship draft (m)	Ship speed ($\text{m}\cdot\text{s}^{-1}$)	Discharge rate ($\text{m}^3\cdot\text{s}^{-1}$)	Dilution factor
Stena Britannica	32	6.65	8.72	0.0028	667 865
Stena Transporter	26.7	6.3	8.72	0.0028	527 921
Stena Forerunner	26.8	6.6	8.72	0.097	15 861

7.1 Risk assessment based on chemical analyses

The compounds analysed in the EGSEs from the three ships were total hydrocarbons (sometimes named total oil), 16 EPA PAHs, 11 alkylated PAHs, HCB and 17 dioxins/furans, 10 heavy metals and nitrate/nitrite. In addition analyses were made on turbidity, pH and alkalinity (see tables in the Appendix). The concentrations of these hazardous compounds were compared to the IMO regulations for EGSE discharged into the sea, and to the EQS for priority pollutants of the WFD. The IMO regulations apply only to the EGSE before dilution in the sea water whereas comparison to priority pollutant EQS' were done both for chemical concentrations in undiluted EGSE and after diluted in the seawater recipient. Seawater concentrations of the EGSE contaminants from the three ships were calculated by using the dilution factors in Table 14.

None of the IMO quality criteria for discharged EGSE were exceeded (Table 15). The criteria for PAH is set to phenanthrene equivalents (PAH_{PHE} , for details, see section 3) which was not possible to calculate from the analytical data obtained in the present study since not all the PAHs included in PAH_{PHE} were included in the chemical analyses of the EGSEs. However, the PAHs of the factor are all low molecular and light weight compounds, and since the concentration of the analysed 16 PAHs, which included several high molecular PAHs, was smaller than the threshold concentration for PAH_{PHE} , it was presumed that the PAH_{PHE} in the EGSEs also were within the limits of the quality criteria.

The EQS are threshold concentrations in surface waters derived for 45 priority pollutants, which must not be exceeded if a good chemical status of a water body is to be met (Directive 2013/39/EU). The EQS are set as annual average concentrations (AA-EQS) and for some substances also as a maximum allowable concentration that may only be exceeded for very short time periods (MAC-EQS). For priority polluting substances in discharges such as EGSE from ships, where there is a continuous supply to the seawater, a comparison to the AA-EQS should be most suitable.

Thirteen of the analysed organic compounds and metals in the EGSEs are classified as priority substances (Table 16), and in the undiluted EGSE from the three ships eight of them were found to occur in concentrations exceeding the AA-EQS: the PAHs naphthalene, anthracene, fluoranthene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(g,h,i)perylene, and also nickel (Ni). Five of the PAHs (anthracene, fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene), and nickel also exceeded the MAC-EQS in EGSE from one or more of the ships. After discharge and dilution of the EGSE in the seawater the concentrations of the 13 priority substances were 10^3 - 10^6 times lower than the EQS concentrations (data not shown).

Formally the chemical status of a water body, as defined by the threshold concentrations given by the EQS in the WFD, applies only to inland and coastal waters as far as 12 nautical miles (nm) from the coast, so from a legal aspect the directive does not have to be complied with in off shore waters. However, a substantial part of the distance between Hoek van Holland and Harwich is within 12 nm from the two coastlines and since the EQSs are derived from data from toxicological studies, the ecological risk assessment could still make use of them also when estimating environmental risks in off shore waters. This is particularly true when the focus is, as in the present study, on the pelagic community of marine organisms. The abundance of these species may even be higher in off shore areas than closer to land.

The estimated seawater concentrations of both priority pollutants and other EGSE chemicals were compared to toxicity data from the scientific literature and the concentrations of total hydrocarbons in the EGSE from Stena Britannica and Stena Forerunner were found to be around three orders of magnitude lower than the documented toxic effects on zooplankton (Table 17)(Cowles, 1983, Cowles & Remillard, 1983, Jensen & Carroll, 2010). Concentrations of the EGSE metals were also generally more the 1 000 times lower than reported toxic concentrations on marine zooplankton (Bielmyer et al., 2006). Only concentrations of Zn, Cu and Ni were closer to reported toxic concentrations. The seawater concentrations of Zn and Cu from the discharge from Stena Forerunner were 100 and 300 times lower than toxic concentrations and Ni concentrations from Stena Transporter discharge were estimated to be around 300 times lower than concentrations with documented toxic effects.

Table 15. IMO quality criteria for discharged EGCS for turbidity, pH, alkalinity and phenanthrene equivalence (PAH_{phe}) in EGSE from Stena Britannica and Stena Transporter (closed loop system) and Stena Forerunner (open loop system) (IMO Resolution MEPC.259(68) 2015 Guidelines for Exhaust Gas Cleaning Systems)

	Closed loop 2017 EGSE	Closed loop 2018 EGSE	Open loop 2018 EGSE	EGSE discharge criteria ¹⁾
Turbidity (NTU)	9.3	12.9	2.5	≤25
pH	7.6	6.9	?	≥6.5
PAH _{phe} (µg·L ⁻¹)	Not analysed, but sum 16 PAH= 21.9 µg·L ⁻¹	Not analysed, but sum 16 PAH= 16.0 µg·L ⁻¹	Not analysed, but sum 16 PAH= 13.5 µg·L ⁻¹	50

Table 16. Concentrations of priority pollutants from the Directive 2013/39/EU in EGSE from the three ships before dilution in the recipient water seawater. The data is compared to the AA-EQS (average annual environmental quality standard) and MAC-EQS (maximum allowable concentration) for the same compounds.

Priority substances (µg·L ⁻¹)	S.Britannica Closed loop	S.Transporter Closed loop	S.Forerunner Open loop	AA-EQS (µg·L ⁻¹)	MAC-EQS (µg·L ⁻¹)
Benzene	0.72	Not analysed	0.41	8	50
<u>Naphthalene</u>	<u>4.4</u>	<u>4.8</u>	<u>7.5</u>	2	130
<u>Anthracene</u>	<u>0.40</u>	<0.13	0.054	0.1	0.1
<u>Fluoranthene</u>	<u>0.22</u>	<u>1.49</u>	<u>0.39</u>	0.0063	0.12
<u>Benzo(a)pyrene</u>	<0.10	<u>0.014</u>	<u>0.017</u>	1.7 · 10 ⁻⁴	0.027
Benzo(b)fluoranthene	<u>0.10</u>	<u>0.11</u>	<u>0.068</u>		0.017
<u>Benzo(k)fluoranthene</u>	<u>0.07</u>	<u>0.023</u>	<u>0.024</u>		0.017
<u>Benzo(g,h,i)perylene</u>	<0.10	<u>0.031</u>	<u>0.040</u>		8.2 · 10 ⁻⁴
Hexachlorobenzene	<0.10	Not analysed	<0.03		0.05
Cd	<0.2	<0.5	<0.5	0.2	0.45-1.5
Hg	0.0052	0.0014	0.0065		0.07
<u>Ni</u>	<u>830</u>	<u>4 400</u>	<u>32</u>	8.6	34
Pb	<6	0.16	0.63	1.3	14



Table 17 Estimated concentrations of organic contaminants, metals and sulphur from EGSE in the mixing zone, after dilution in the seawater. ND = not detected (below the detection limit of the analyses). Note that different units are used for different compounds

	Stena Britannica Closed loop	Stena Transporter Closed loop	Stena Forerunner Open loop
Total hydrocarbon ($\mu\text{g}\cdot\text{L}^{-1}$)	0.011	0.0037	0.020
$\Sigma 16$ PAH ($\text{ng}\cdot\text{L}^{-1}$)	0.033	0.0303	0.85
$\Sigma 11$ alkylated PAH ($\text{ng}\cdot\text{L}^{-1}$) ¹⁾	0.21	not analysed	1.69
Al ($\text{ng}\cdot\text{L}^{-1}$)	12.4	2.1	11.34
As ($\text{ng}\cdot\text{L}^{-1}$)	0.03	0.019	0.15
Cd ($\text{ng}\cdot\text{L}^{-1}$)	ND	ND	ND
Cr ($\text{ng}\cdot\text{L}^{-1}$)	0.013	0.042	1.95
Cu ($\text{ng}\cdot\text{L}^{-1}$)	0.22	0.061	0.88
Ni ($\text{ng}\cdot\text{L}^{-1}$)	1.24	8.33	2.02
Pb ($\text{ng}\cdot\text{L}^{-1}$)	ND	0.0003	0.04
V ($\text{ng}\cdot\text{L}^{-1}$)	14.7	24.6	5.29
Zn ($\text{ng}\cdot\text{L}^{-1}$)	ND	0.087	5.17
Hg ($\text{pg}\cdot\text{L}^{-1}$)	0.0078	0.0027	0.41
S ($\mu\text{g}\cdot\text{L}^{-1}$)	28.45	41.67	75.66

7.2 Risk assessment based on results from toxicity tests

Threshold concentrations for EGSE in seawater were calculated for whole EGSE, treated as a unit, based on the results from the toxicity tests. Since the lowest concentration of EGSE found to have a toxic effect on the zooplankton differed between the three ships, a specific threshold level had to be calculated for each of them. Calculations were done by using the same approach as in the WFD when determining EQS for priority pollutants (European Commission, 2010). The EQS are derived from quality assured aquatic toxicity data and the procedure is that the lowest water concentration of a compound shown to have a toxic effect is selected. To this concentration a safety factor, referred to in the EU documentation as an assessment factor (AF), is applied in order to protect the

aquatic ecosystems. The safety factor can be between 10 and 10 000 depending on the amount of toxicity data available for a specific compound. If there is little available data the AF should be higher than if there is a substantial amount of data. In practice it means that the EQS concentrations are 10 to 10 000 times lower than the lowest toxic concentration found in experimental studies. The protocol for how to calculate an EQS is described in a special guidance document (European Commission Technical Report, 2011).

The threshold levels for the EGSE from Stena Britannica, Stena Transporter and Stena Forerunner were determined by taking the lowest toxic concentrations from the toxicity tests from each ship which were 0.04% EGSE for Stena Britannica, 0.1% for Stena Transporter and 1.0% for Stena Forerunner (Table 9) and then applying an assessment factor. Considering the limited amount of data available on EGSE toxicity, and also the fact that toxic effects were found in the lowest test concentration of all EGSEs, an assessment factor of 1 000 was selected. In table 18 is shown the lowest concentration of EGSE with a toxic effect on biota and also the threshold level obtained by adding an assessment factor of 1 000. The dilution of the EGSE in the toxicity tests is expressed both as the percentage in the exposure water since this was the unit applied in section 6, but also as mL EGSE per litre exposure water. The seawater concentrations of EGSE in the mixing zone of the three ships were determined by using the dilution factors derived from equation 1, and presented in Table 14.

The obtained EGSE concentrations were compared to the calculated threshold levels (Table 18). For all three ships the estimated seawater concentrations of discharged EGSE in the mixing zone were found to exceed the calculated threshold concentrations (Table 18). The difference between seawater and threshold concentrations was slightly higher for discharges from the open loop than from the closed loop systems. The seawater concentration of EGSE from Stena Forerunner was 6.3 times higher than the threshold concentration, $0.063 \text{ mL}\cdot\text{L}^{-1}$ compared to $0.01 \text{ mL}\cdot\text{L}^{-1}$. For Stena Britannica the seawater concentration was 3.8 times higher than the threshold concentration, $0.0015 \text{ mL}\cdot\text{L}^{-1}$ compared to $0.0004 \text{ mL}\cdot\text{L}^{-1}$ and for Stena Transporter it was 1.9 times higher than the threshold concentration, $0.0019 \text{ mL}\cdot\text{L}^{-1}$ compared to $0.01 \text{ mL}\cdot\text{L}^{-1}$.

The comparison between estimated seawater concentrations of EGSE and the threshold levels indicate that that water from the open loop system is more toxic than waters from the closed loop systems. However, in the experiments carried out in the present study, toxic effects on zooplankton were detected also in the lowest concentration of EGSE for both closed loop and the open loop system and it cannot be excluded that toxic effects would have appeared at even lower concentrations of EGSE in the exposure water.

Toxicity tests are often designed as standardized tests on animals reared in the laboratory. These animals are likely to be more tolerant to environmental stressors than animals in the field. In the present study all toxicity tests but the Microtox assay were done on animals collected in the field just before the start of the experiments. These animals are better at reflecting the actual response of a toxicant in the field than animals raised in a culture in the laboratory.

The toxicity studies carried out within the project were designed to have a high ecological relevance for the sea area of interest. The animals that were studied were benthic blue mussels (*Mytilus edulis*) and the planktonic copepod *Calanus helgolandicus*, and the later species was found to be the most sensitive to the EGSE. Planktonic copepods constitute the most obvious target group for discharges from ships since they live in the part of the sea where the discharges take place. They also possess a central and crucial position in marine food webs between microalgae and higher trophic levels like fish. Harmful effects on zooplankton will therefore have impacts also on e.g. fish stocks (Heath & Lough, 2007).

Table 18. The lowest concentrations of EGSE giving statistically significant toxic effect in the laboratory experiments (left column). These concentrations were used to calculate the threshold level for EGSE in seawater (middle column). The threshold levels were compared to the actual wash water concentrations in the seawater (right column). Data is presented as percentage (volume EGSE/volume seawater) and as mL EGSE·L⁻¹ seawater.

	Lowest toxic dilution (concentrations) of EGSE in toxicity tests	Calculated threshold level for EGSE in seawater	EGSE dilutions (concentrations) in seawater
Ship	% (mL·L ⁻¹)	% (mL·L ⁻¹)	% (mL·L ⁻¹)
S. Britannica closed loop	0.04 (0.4)	0.00004 (0.0004)	0.00015 (0.0015)
S. Transporter, closed loop	0.1 (1.0)	0.0001 (0.001)	0.00019 (0.0019)
S. Forerunner, open loop	1 (10.0)	0.001 (0.01)	0.0063 (0.063)

7.3 Summary of risk assessment

Results from the toxicity studies and ESGE discharge rates indicate that seawater concentrations of discharged EGSE from one crossing of one of the investigated ships are less than 1 000 times lower than the lowest concentration found to have a toxic effect. This was the case for discharges from both closed and open loop scrubber systems. A safety factor (assessment factor, AF) of 1 000 is commonly used when setting a threshold level based on toxicity tests. This means that the expected environmental concentration derived from EGSE discharge from a single ship are exceeding the threshold level.

This is in contrast to the picture obtained when estimating the environmental risk based on chemical data from the discharged EGSE. Several of the priority pollutants of the WFD were analysed in the EGSE, and the estimated seawater concentrations of these were all in the order of 10³- 10⁶ times lower than AA-EQS. It should be pointed out that the EQS values are derived from toxicity data to which an assessment factor is added. However, toxicity data on specific compounds present in the EGSE from the scientific literature are more comparable to the findings in our own toxicity studies. The estimated seawater concentrations of total hydrocarbons from discharged EGSE are around 1 000 times lower than concentrations found to be toxic to zooplankton. Seawater concentrations of Zn and Cu from EGSE from the open loop system and Ni from the closed loop system were only 100 - 300 times lower than documented toxic concentrations. From an ecotoxicological point of view it is highly relevant to base a risk assessment of a complex mixture such as EGSE on toxicity studies on the actual water itself rather than on chemical analyses of the water. One important reason is that only compounds suspected to be present in the water will be selected for chemical analyses. Even a qualified assessment of what compounds would be relevant to analyse might overlook toxicologically important compounds.

Another equally important aspect is that the sum of toxic effects exerted by all compounds in the EGSE may be dramatically different from the effect predicted when focusing on the compounds one at the time. Compounds in a mixture may have both cumulative additive modes of action (toxic effects of two or more compounds add to each other) and synergistic (toxic effect of two or more compounds is stronger than just the sum of them) (Faust, Altenburger et al., 2003, Pape-Lindstrom & Lydy, 1997).

In the WFD it is emphasized that when assessing the environmental risk of a discharge it is important to take into account also discharges from other sources to the area. So, when assessing the effect of discharged EGSE on marine ecosystems, all ships discharging EGSE in a given area, as well as discharges of pollutants from other sources, must be taken into account.

The shipping intensity in the region around the route Hoek van Holland – Harwich is very high. In addition there are a lot of industrial activities taking place in the area and several large rivers with highly industrialized catchment areas flow into this part of the North Sea. All these factors add to the fact that OSPAR has stated that the coastal areas of France, Belgium, the Netherlands, Germany and Denmark have the poorest quality status in relation to hazardous substances in the whole North Sea (OSPAR, 2009).

In the present study the assessment is made from a short term perspective, one ship and one crossing, but in order to estimate concentrations of the continuous discharges of EGSE in a long term perspective, more advanced modelling of the dispersal of the scrubber water in the sea needs to be performed. This also something that is emphasized in the WFD the environmental risk of a discharge cannot be assessed on its own, but has to be considered along with other polluting sources in an area.

8 Conclusions

When using the chemical analyses of the EGSE to calculate seawater concentrations most of the pollutants were expected to be found in concentrations 10^3 - 10^6 times lower than EQS concentrations of the Water Framework Directive (WFD, 2008/105/EC). However, when estimated seawater concentrations of whole EGSE were compared to threshold concentrations calculated from the toxicity tests, they were found to be higher than the threshold levels. The EGSE hence seems to be more toxic to aquatic organisms than what could be predicted from the toxicity of the individual pollutants it contains. One explanation could be that important toxic components in the waters were not analysed. Another likely explanation is that the toxicity of the EGSE is the result of what is known as the “cocktail effect”, i.e. the joint effect of the mixture of all components in the discharged water is larger than the effect of the individual components.

No direct effects of the acidification by the released EGSE could be detected, but it was rather assumed that the effect was produced by the toxic pollutants.

A lowest toxic concentration could not be established for any of the tested EGSE since toxic effects on zooplankton were found in the lowest EGSE test concentration in each experiment, i.e. 0.04% and 0.1% EGSE in the tests with closed loop water and 1.0% ESG in the test with open loop water. It was therefore not possible to use the data from the experimental studies to compare the toxicity of the EGSE from the closed vs open loop systems.

The most sensitive of the tested species was the copepod *Calanus helgolandicus*. This is a zooplankton species and therefore the group of marine animals that are likely to be most affected by the discharge of contaminated water from ships. Zooplankton is a crucial link between



phytoplankton and higher trophic levels like fish and marine mammals. Toxic effects on zooplankton will therefore have serious consequences for the whole marine ecosystem.

9 References

- Avila TR, Bersano JGF, Fillmann G (2010) Lethal and sub-lethal effects of the water-soluble fraction of a light crude oil on the planktonic copepod *Acartia tonsa*. *Journal of the Brazilian Society of Ecotoxicology* 5: 19-25
- Bielmyer GK, Grosell M, Brix KV (2006) Toxicity of silver, zinc, copper, and nickel to the copepod *Acartia tonsa* exposed via a phytoplankton diet. *Environmental Science and Technology* 40: 2063-2068
- Codina JC, Pérez-García A, Romero P, de Vicente A (1993) A comparison of microbial bioassays for the detection of metal toxicity. *Archives of Environmental Contamination and Toxicology* 25: 250-254
- Cowles TJ (1983) Effects of exposure to sublethal concentrations of crude oil to the copepod *Centropages hamatus*. II Activity patterns. *Marine Biology* 78: 53-57
- Cowles TJ, Remillard JF (1983) Effects of exposure to sublethal concentrations of crude oil on the copepod *Centropages hamatus*. I Feeding and egg production. *Marine Biology* 78: 45-51
- den Boer E, 't Hoen M (2015) Scrubbers - An economic and ecological assessment. Delft, CE Delft, March 2015. In
- European Commission (2010) Technical guidelines for the identification of mixing zones pursuant to Art. 4(4) of the Directive 2008/105/EC In
- European Commission Technical Report (2011) Technical Report 2011-055. Common implementation strategy for the Water Framework Directive (2000/60/EC). Technical Guidance For Deriving Environmental Quality Standards no 27. In p 203.
- Faust M, Altenburger R, Backhaus T, Blanck H, Boedeker W, Gramatica P, Hamer V, Scholze M, Viggi M,
- Grimme LH (2003) Joint algal toxicity of 16 dissimilarly acting chemicals is predictable by the concept of independent action. *Aquatic Toxicology* 63: 43-63
- Fisher TT, Law RJ, Rumney HS, Kirby MF, Kelly C (2011) Towards a scheme of toxic equivalency factors (TEFs) for the acute toxicity of PAHs in sediment. *Ecotoxicology and Environmental Safety* 74: 2245-2251
- Gutierrez MF, Gagneten AM, Paggi JC (2012) Exposure to sublethal chromium and endosulfan alter the diel vertical migration (DVM) in freshwater zooplankton crustaceans. *Ecotoxicology* 21: 37-47
- Hassellöv I-M, Turner DR, Lauer A, Corbett JJ (2013) Shipping contributes to ocean acidification. *Geophysical Research Letters* 40: 2731-2736
- Heath MR, Lough RG (2007) A synthesis of large-scale patterns in the planktonic prey of larval and juvenile cod (*Gadus morhua*). *Fisheries Oceanography* 16: 169-185
- Hernroth B (2003) Factors influencing bactericidal activity of blue mussel (*Mytilus edulis*) haemocytes against *Salmonella typhimurium*. *Fish Shellfish Immunol* 14: 93-104
- IMO Resolution MEPC.259(68) 2015 Guidelines for Exhaust Gas Cleaning Systems In
- Jensen JK, Carroll JL (2010) Experimental studies of reproduction and feeding for two Arctic-dwelling *Calanus* species exposed to crude oil. *Aquatic Biology* 10: 261-271

Kjølholt J, Aakre S, Jørgensen C, Lauridsen J (2012) Assessment of possible impacts of scrubber water discharges on the marine environment. COWI, Danish Environmental Protection Agency, Environmental Project No. 1431. In

Loehr LC, Atkinson M, George K, Beegle-Krause CJ (2003) Using a simple dilution model to estimate wastewater contaminant concentrations behind moving passenger vessels.

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of immunological methods* 65: 55-63

OSPAR (2009) Assessment of trends and concentrations of selected hazardous substances in sediments and biota. Monitoring and Assessment Series CEMP assessment report: 2008/2009. In Trends and concentrations of selected hazardous substances in marine sediments and biota CEMP assessment report 2008/2009 OSPAR Commission, London Publication number 390/2009,

Pape-Lindstrom PA, Lydy MJ (1997) Synergistic toxicity of atrazine and organophosphate insecticides contravenes the response addition mixture model. *Environmental Toxicology and Chemistry* 16: 2415–2420
Peterson WT (2001) Patterns in stage duration and development among marine and freshwater calanoid and cyclopoid copepods: a review of rules, physiological constraints, and evolutionary significance. *Hydrobiologia* 453: 91-105

Seuront L (2010) Zooplankton avoidance behaviour as a response to point sources of hydrocarbon-contaminated water. *Marine and Freshwater Research* 61: 263-270
Technical guidelines for the identification of mixing zones pursuant to Art. 4(4) of the Directive 2008/105/EC
In

Thor P, Dupont S (2018) Ocean acidification. In: Salomon M, Markus T (eds) *Handbook on marine environment protection*. Springer, Berlin. In

APPENDIX

Data from chemical analyses

Appendix Table 1 Organic compounds (except PAHs and dioxins) in EGSE from Stena Britannica and Stena Transporter (closed loop system) and Stena Forerunner (open loop system).

Compound ($\mu\text{g}\cdot\text{L}^{-1}$)	St Britannica Closed loop	S Forerunner Open loop	Compound ($\mu\text{g}\cdot\text{L}^{-1}$)	S Transporter Closed loop
Total hydrocarbon	7 106	388	Total hydrocarbon	1 960
Benzene	0.72	0.41		-
Toluene	<0.40	0.22		-
1,3-xylen	0.95	0.20		-
1,4-xylen	0.55	0.20		-
1,2-xylen	0.40	0.20		-
Ethyl benzene	<0.40	0.20		-
Aliphatic>C5-C8	<4.0	90.5	Aliphatic/aromatic >C10-C12	363
Aliphatic>C8-C10	49.0	<10	Aliphatic/aromatic >C12-C16	1 030
Aliphatic>C10-C12	2 900	7.5	Aliphatic/aromatic >C16-C35	538
Aliphatic>C12-C16	1 700	33.5	Aliphatic/aromatic >C35-<C40	30.7
Aliphatic>C16-C21	<100	61.5		
Aliphatic>C21-C36	720	75		
Aliphatic>C36<C40	<100	7.5		
Aromatic>C10-C12	630	8.5		
Aromatic >C12-C16	500	16.5		
Aromatic>C16-C21	<100	6.5		
Aromatic>C21-C36	<300	<30		

Appendix Table 2 Polycyclic aromatic hydrocarbons, (PAHs) and HCB in EGSE from Stena Britannica and Stena Transporter (closed loop system) and Stena Forerunner (open loop system)., and in water from different stages of the treatment process from one of Stena Britannica.

Stena Britannica (CL)								S. Trans (CL)	S. Forer (OL)
(ng·L ⁻¹)	SW in	SW out	Proc. water	BOTU feed	Decant water	BOTU sludge	EGSE	EGSE	EGSE
Naphthalene	<5.0	<5.0	12 000	18 000	2 200	41 000	4 400	4 790	7 510
Ace- naphthylene	<1.0	<1.0	4 300	3 900	140	7 400	360	37	18
Ace- naphthene	<1.0	<1.0	40 000	35 000	1 200	110 000	2 100	454	113
Fluorene	<1.0	<1.0	31 000	49 000	3 100	160 000	3 200	1 380	815
Phenanthrene	<1.0	1.4	320 000	520 000	11 000	1 200 000	10 000	5 690	2 170
Anthracene	<1.0	<1.0	10 000	16 000	250	40 000	400	<132	<24
Fluoranthene	<1.0	<1.0	32 000	99 000	380	130 000	220	1 490	222
Pyrene	4.3	<1.0	100 000	360 000	1 000	420 000	540	1 470	63
Benzo(a) anthracene	<1.0	<1.0	55 000	210 000	430	200 000	210	231	14
Chrysene	<1.0	<1.0	110 000	400 000	700	380 000	330	278	39
Benzo(b) fluoranthene	<1.0	<1.0	21 000	100 000	160	80 000	100	108	17
Benzo(k) fluoranthene	<1.0	<1.0	4 600	21 000	57	21 000	70	23	<10
Benzo(a) pyrene	<5.0	<5.0	9 000	39 000	<100	33 000	<100	14	<10
diBenzo(ah) anthracene	<5.0	<5.0	3 400	17 000	<100	12 000	<100	12	<10
Benzo(ghi) pyrene	<5.0	<5.0	23 000	76 000	<100	64 000	<100	31	<10
Indeno(cd) pyrene	<5.0	<5.0	6 400	20 000	<100	16 000	<100	11	<10
Σ16 PAH							21 930	16 019	13 475
HCB							<100		<3

Appendix Table 3 Dioxins in EGSE from Stena Britannica (closed loop system).

Congener	pg·L ⁻¹
2378-TCDD	<0.91
12378-PeCDD	<1
123478-HxCDD	<2.3
123678-HxCDD	<2.3
123789-HxCDD	<2.3
1234678-HpCDD	<2.9
OCDD	<3.6
2378-TCDF	<2.7
12378-PeCDF	<1.4
23478-PeCDF	<2.9
123478-HxCDF	<1.6
123678-HxCDF	<1.6
123789-HxCDF	<1.6
234678-HxCDF	<1.6
1234678-HpCDF	<1.7
1234789-HpCDF	<1.7
OCDF	<3.1
TEQ-Lowerbound	0
TEQ-Upperbound	4.6

Appendix Table 4 Metals in EGSE from Stena Britannica and Stena Transporter (closed loop system) and Stena Forerunner (open loop system).

	Stena Britannica (CL)						S.Trans (CL)	S.Fore (OL)	
	SW in	SW out	Proc. water	BOTU feed	Decan. water	BOTU sludge	EGSE	EGSE	EGSE
As (µg·L ⁻¹)	1.9	1.8	74	66	19	330	20	9.8	2.4
Al (µg·L ⁻¹)	39	104	6 400	120 000	8 000	340 000	8 300	1 100	180
Cd (µg·L ⁻¹)	0.05	0.11	0.4	0.34	<0.2	0.67	<0.2	<0.5	<0.5
Cr (µg·L ⁻¹)	<1.2	53	89	90	<4	420	9	22	31
Cu (µg·L ⁻¹)	17	23	42	41	25	140	150	32	14
Hg (ng·L ⁻¹)	0.84	0.72	1.9	1.9	2.0	1.8	5.2	1.4	6.5
Ni (µg·L ⁻¹)	0.61	20	6 972	7 400	160	30 000	830	4 400	32
Pb (µg·L ⁻¹)	0.098	0.55	16	18	<5	51	<6	0.16	0.63
V (µg·L ⁻¹)	3.7	6.5	26 000	27 000	14 000	84 000	9 800	13 000	84
Zn (µg·L ⁻¹)	6.2	4.7	640	1 200	480	3 000	<70	46	82
S (mg·L ⁻¹)	1 100	1 100	23 000	22 000	20 000	20 000	19 000	22 000	1 200



IVL Swedish Environmental Research Institute Ltd.
P.O. Box 210 60 // S-100 31 Stockholm // Sweden
Phone +46-(0)10-7886500 // www.ivl.se