ZERO BRINE

D9.1 Report on environmental impacts from brine discharge

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FINAL

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[1] **R**=Document, report; **DEM**=Demonstrator, pilot, prototype; **DEC**=website, patent fillings, videos, etc.; **OTHER**=other

[2] **PU**=Public, **CO**=Confidential, only for members of the consortium (including the Commission Services), **CI**=Classified

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Abbreviations

AMBI	AZTI Marine Biotic Index
BACI	Before – After, Control – Impact
BEQI	Benthos Ecosystem Quality Index
BOPA	Benthic opportunistic polychaeta amphipoda index
DWP	Demineralized Water Plant
EQR	Ecological Quality Ratio
ES	Ecological Status
HELCOM	Helsinki Commission (Baltic Marine Environment Protection Commission)
ICPR	International Commission for the Protection of the Rhine
IEX	Ion Exchange
IMO	International Maritime Organization
MARPOL	International Convention for the Prevention of Pollution from Ships
MPC	Maximum Permissible Concentration
MSFD	Marine Strategy Framework Directive
OSPAR	Oslo and Paris Conventions (Convention for the Protection of the Marine Environment of the North-East Atlantic)
PAHs	Polycyclic Aromatic Hydrocarbons
POC	Particulate Organic Carbon
POPs	Persistent Organic Pollutants
PoR	Port of Rotterdam
RAP	Rhine Action Programme
RBD	River Basin District
RBMP	River Basin Management Plan
RO	Reverse Osmosis
SAC	Special Areas of Conservation of Natura 2000 Network
SPA	Special Protection Areas of Natura 2000 Network
SWB	Surface Water Body

WFD	Water Framework Directive 2000/60/EC
WWTP	Wastewater Treatment Plant

1 Executive summary

Population growth and economic development have led to an increased demand for drinking water. Besides this, climate change and environmental pollution are resulting in a decline of the quantity and quality of water resources. In this context, desalination of brackish and seawater is an effective and widely used process to address the need of drinking and industrial water in the world originating mainly from sea water or brackish water. However, this comes with environmental effects, both in terms of environmental impacts in the aquatic environment, and greenhouse gas emissions. The scope of this study is to explore the environmental impacts of the brine discharge from two Evides Demineralized Water Plants (DWPs) on the aquatic environment of the Port of Rotterdam.

The investigated area includes three sites, one in the vicinity of the EVIDES DWP Botlek in the Brittaniëhaven area, one in the vicinity of Evides DWP Maasvlakte in the Hartelkanaal area, and one in the Elbeweg area that was designated as Reference site. In total, 4 sampling surveys were performed, namely in September 2019, January 2020, July 2020 and September 2021. A total of 6 sampling stations have been established for benthic macroinvertebrates analysis supported by data about physicochemical and hydromorphological conditions. Biological quality descriptors (abundance A, species richness S, and Shannon's diversity H), biological quality indices (AMBI, BEQI, and BOPA), and statistics analysis were applied to objectively assess the effect of brine release on benthic fauna and quantify the ecological status of the investigated areas.

A remarkable diversity of taxa has been observed, enabling a detailed characterization of biological communities. The analysis of communities and species and comparison with other studies revealed a similar macrobenthic composition, although with lower abundance and diversity compared to the nearby North Sea. Interpretation of the impacts on patterns of benthic macroinvertebrates in terms of the brine loadings was attempted. This was challenging because the Port of Rotterdam is a complex estuarine ecosystem influenced by severe anthropogenic stress. Specifically, the Brittaniëhaven area is a heavily industrialized and disturbed section of the Rotterdam port, and a dead-end waterway which means that the environmental quality of the area is affected by several major anthropogenic sources. For that reason, Hartelkanaal area that receives brine from the second DWP Maasvlakte has also been studied with a priority given to this area as it is surrounded by less intense industrial activity. Therefore, it is less impacted in relation to Brittaniëhaven area and impacts directly from the brine can be better recognizable.

Both studied areas lack a baseline ecological assessment prior to the operation of the DWPs, and for this reason a Before – After, Control – Impact (BACI) approach cannot be applied. Therefore, it is difficult to prove with high confidence that an effect occurred due to the brine discharge or may have existed before the DWPs' operation. Quantifiable environmental benefits due to the operation of Plant

One cannot be noticeable for two reasons. Firstly, the percentage of the brine treated was less than 1% of the total EVIDES DWP Botlek brine discharge and secondly the technology was applied for a short period of time (less than 1%) in which the response of ecological parameters cannot be detectable.

It can be concluded that this study has established a baseline understanding of the environmental conditions in the vicinity of DWP Botlek and DWP Maasvlakte in the Port of Rotterdam and provides essential background information for the assessment of environmental benefits from the implementation of a large scale zero brine technology in the future.

2 Overview of the project

The ZERO BRINE project aims to facilitate the implementation of the Circular Economy package and the SPIRE roadmap in various process industries by developing necessary concepts, technological solutions and business models to redesign the value and supply chains of minerals and water while dealing with present organic compounds in a way that allows their subsequent recovery.

These resources will be recovered from saline impaired effluents (brines) generated by the process industry while eliminating wastewater discharges and minimizing the environmental impacts of brines from industrial operations (ZERO BRINE). ZERO BRINE brings together and integrates several existing and innovative technologies to recover products of high quality and sufficient purity to represent good market value.

A large-scale demonstration plant for the treatment of part of the brine effluent will be tested in the Energy Port and Petrochemical cluster of Rotterdam Port by using the waste heat from one of the factories in the port. The quality of the recovered products will be aimed to meet local market specifications. Additionally, three large-scale pilot plants will be developed in other process industries in Poland, Spain, and Turkey, providing the potential for immediate replication and uptake of the project results after its successful completion.

3 Objectives

This study aims to assess the ecological quality status of the Port of Rotterdam and in particular of Evides DWP Botlek in the Brittaniëhaven area and Evides DWP Maasvlakte in Hartelkanaal area due to brine discharge-related activities. This study focuses on the impacts of brine on benthic macroinvertebrates that are ideal bioindicators due to their sensitivity to water quality and inability to escape a disturbance once settled (Clark et al., 2018). Benthic macroinvertebrates also constitute a biological quality element in the European umbrella regulations for water systems, namely the Water Framework Directive 2000/60/EC (WFD) and the Marine Strategy Framework Directive 2008/56/EC (MSFD) for the assessment of the ecological quality status of a water body. The physical and chemical parameters of the aquatic environment and seabed were also studied as they are considered important for the thorough assessment of the aquatic environmental quality.

4 Introduction

The world's population is growing, and global water demand is increasing. Climate change is threatening global access to clean water and many areas are exposed to water-related risks (drought or flooding), while the marine environment is facing multiple, man-made stressors (Küpper and Kamenos, 2018). The natural resources crisis is one of the top risks by impact facing the planet (World Economic Forum, 2021). Desalination is considered a feasible, economic and increasingly common method to meet the water demand for drinking water purposes as well as industrial and agricultural uses. According to the 31st desalination inventory, which covers the period July 2017-June 2018, the total global installed desalination capacity stands at 97.4 million m^3/d.

However, currently desalination is far from being sustainable. Seawater and brackish water desalination discharge hypersaline brine that also contains several chemicals used throughout the different stages of the desalination process and concerns are raising about potential impacts on the aquatic environment. Moreover, most desalination plants are powered by burning fossil fuels, which contributes to the vicious cycle of climate change and causes water scarcity in the first place (Cornejo et al., 2014).

Macrobenthic organisms, examined in this study, are good ecological indicators to assess the effect of brine on the aquatic environment because they (i) are relatively sedentary and so unable to avoid deteriorating water / sediment quality, (ii) have relatively long-life spans, (iii) include diverse species with different tolerances to stress, and (iv) playing a vital role in cycling nutrients and materials between the underlying sediment and the overlying water column (Dauvin et al., 2007).

5 Legislative framework for the prevention of aquatic pollution applicable to the Port of Rotterdam

5.1 Water Framework Directive (WFD) 2000/60/EU

WFD 2000/60/EU was adopted in 2000 and covers territorial waters (out to 12 nautical miles) for aspects of chemical quality, and coastal waters (up to 1 nautical mile) for aspects of ecological quality.

Following an adaptive management approach, it establishes a six-year planning cycle, during which Member States prepare River Basin Management Plans that require the implementation of Programmes of measures to help achieve the Good Ecological and Chemical Status. The original target for achieving good status was 2015, but further deadlines are set for 2021 and 2027. For water bodies designated as heavily modified or artificial, the respective targets are good ecological potential and good chemical status. Good ecological potential is a different ecological objective that takes into account the physical modifications necessary to sustain specified human uses such as navigation. Another important part of the WFD is an extensive programme of monitoring of surface and groundwater bodies. The results of this monitoring are being used to assess achievement of the WFD objectives. The measures required to meet WFD objectives need to be summarised in a series of new "river basin management plans" (RBMP). The first RBMP was published in 2009 and the second in 2015. Actions taken aim to reduce marine pollution from land-based sources and to protect ecosystems in coastal and transitional waters, which are vital habitats for many marine species.

5.2 Stockholm convention on persistent pollutants (POPs)

According to the information provided on the official website of the Stockholm Convention on Persistent Organic Pollutants (POPs), this convention is a global treaty to protect human health and the environment from chemicals that remain intact in the environment for long periods, become widely distributed geographically, accumulate in the fatty tissue of humans and wildlife, and have harmful impacts on human health or on the environment. Given their long-range transport, no one government acting alone can protect its citizens or its environment from POPs. In response to this global problem, the Stockholm Convention, which was adopted in 2001 and entered into force in 2004, requires its parties to take measures to eliminate or reduce the release of POPs into the environment.

5.3 International convention for the prevention of pollution from ships (MARPOL)

According to the information provided on the official website of the International Maritime Organization (IMO), the International Convention for the Prevention of Pollution from Ships (MARPOL) is the main international convention covering prevention of pollution of the marine environment by ships from operational or accidental causes. The MARPOL Convention was adopted on 2 November 1973 at IMO. The Protocol of 1978 was adopted in response to a spate of tanker accidents in 1976-1977. MARPOL has been updated by amendments through the years. The Convention includes regulations aimed at preventing and minimizing pollution from ships - both accidental pollution and that from routine operations - and currently includes six technical Annexes. Special Areas with strict controls on operational discharges are included in most Annexes.

5.4 Ballast Water Management Convention

According to the information provided in the IMO official website, invasive aquatic species present a major threat to the marine ecosystems, and shipping has been identified as a major pathway for introducing species to new environments. Ballast Water Management Convention, adopted in 2004, aims to prevent the spread of harmful aquatic organisms and pathogens from one region to another, by establishing standards and procedures for the management and control of ships' ballast water and sediments. The Convention requires all ships to implement a ballast water management plan. All ships have to carry a ballast water record book and are required to carry out ballast water management procedures to a given standard. Parties to the Convention are given the option to take additional measures which are subject to criteria set out in the Convention and to IMO guidelines. On 8 September 2017, the Port State Control of the Port of Rotterdam enforced the regulations according to the procedures made by the Paris Memorandum of Understanding on Port State Control (Paris MoU).

Based on the overall IMO framework, the 21 Baltic and North-East Atlantic coastal states and the EU have developed and agreed in 2013 on a detailed joint harmonised procedure to define “low risk” routes, as well as other necessary steps in granting exemptions under regulation A-4 of the IMO Ballast Water Management Convention. This has been done as a joint venture between the two regional seas commissions HELCOM and OSPAR. The countries who have agreed to this approach within HELCOM include Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Poland, Sweden, and Russia. The countries who have agreed to this approach within OSPAR include Belgium, Denmark, Finland, France, Germany, Iceland, Ireland, Luxembourg, The Netherlands, Norway, Portugal, Spain, Sweden, Switzerland, and the United Kingdom. Also, the European Union is a member of both HELCOM and OSPAR.

6 Literature review of the impacts of brine on aquatic ecology

The magnitude of the brine impact on the aquatic environment depends on the physicochemical characteristics of the desalination brine, the discharge method, the hydrogeological factors such as bathymetry, waves, currents, depth of the water column (Sadhwani et al., 2005), and the ecological conditions of the ecosystem that receives the brine. The hydrogeological factors determine the extent of the mixing of the brine and therefore the geographical range of the impact (Einav et al., 2002). High energy oceanic coasts with parallel coastal currents have lower sensitivity to the effects of a desalination plant in comparison to poorly flushed environments with high biodiversity (Hopner and Windelberg, 1996).

Impacts of RO brine on the marine environment are mainly associated with the high concentration of salts, the release of chemicals used during the seawater pretreatment stage (such as antiscalants), and cleaning of the membranes (Sadhwani et al., 2005; Lattemann and Hopner, 2008). As brine has a higher density than the seawater, it sinks to the seabed, extends horizontally following the slope of the sea bottom bathymetry (Fernandez-Torquemada et al. 2005) and therefore an effect can be observed on benthic communities.

The sensitivity of species to the increase in salinity levels depends on the tolerance of given species. According to Clark et al. (2018), polychaetes and bryozoans showed high sensitivity while barnacles proliferated and dominated communities near the operating outfall. However, this was the result of the increased flow created by the high-pressure diffusers rather than hypersalinity or other potential stressors. The study of Belatouia et al. (2017) showed a detrimental effect on both the abundance and diversity of benthic communities close to the outfalls. Only some organisms were capable of surviving near the discharge (*Spionidae, Urothoe grimaldi, Paraonidae, Synchelidium haplocheles, Periculodes longimanus, Chamelea gallina,* Nemertea), but in very small abundances compared to control and impacted areas at 15 m depth. According to de-la-Ossa-Carretero et al. (2016), amphipods showed sensitivity to abrupt changes in salinity produced by concentrated brine effluent. However, they can tolerate a broader range of salinity than other osmoconformer organisms such as ehinoderms that are not able to regulate their osmotic pressure. Brine influence on echinoderms was studied by Fernandez-Torquemada et al. (2013) who observed that high salinities diminish echinoderm densities in affected brine areas. Del-Pilar-Ruso et al. (2007) showed that infaunal communities close to the brine outfall were dominated by nematodes. Polychaetes, molluscs, and crustaceans become more abundant with increasing distance from the discharge. According to Del-Pilar-Ruso et al. (2008), desalination activity caused a decrease in abundance, richness, and diversity in polychaete asseblages of the study area. Polychaete families showed different sensitivity levels with Amphaetidae being the most sensitive, followed by Nephtyidae and Spionidae. Syllidae and Capitellidae showed some resistance initially, while Paraonidae proved the least sensitive. Einav et al. (2002) mentioned that biota which have originated in the Pacific can cope more easily with an increase in salinity. Moreover, certain species are able to tolerate higher salinities after a period of accumulation. This study (references in Einav et al., 2002) also mentioned that the sensitivity of the invertebrates, mainly that of crabs, varies but in general it is found that long abdomen invertebrates are more sensitive to an increase in salinity than those with short abdomens. According to Mandelli (1975), brine appeared to enhance pathogenic fungus infection in the exposed oysters. The experiment of Chesher (1971), in which echinoderms, ascidians, gorgonian corals, and stone crabs were transplanted to site receiving effluents, showed that the echinoderms were the most sensitive. Survival improved when copper emissions were reduced.

7 Port of Rotterdam

7.1 Man-made estuarine environment

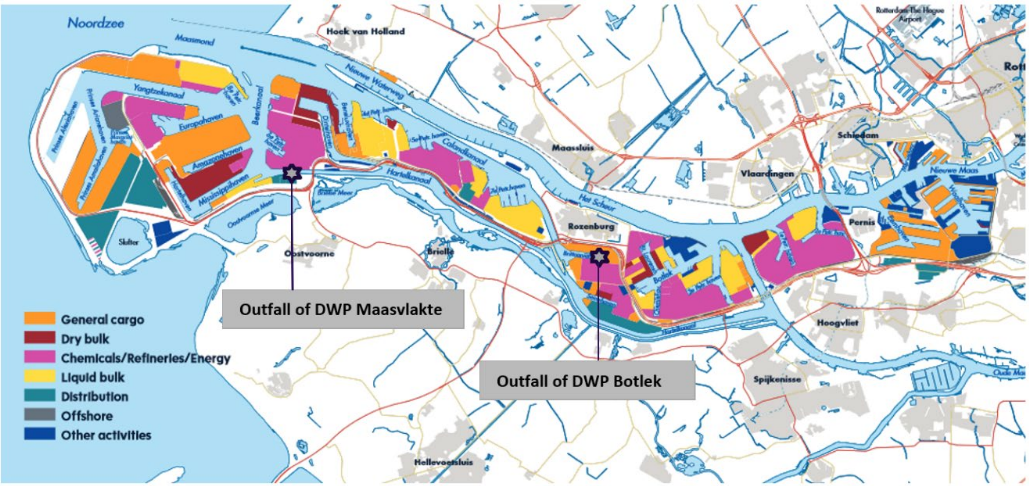
The Port of Rotterdam is situated in the estuary of the main branch of the river Rhine at a transition of freshwater to marine ecosystems. Before the Delta Project which was implemented after the storm surge of 1953 (Smits et al., 2006), the intertidal zone of the estuary consisted mostly of beaches, salt and brackish marshes, sand and mud flats, tidal creeks, immense fresh and brackish rush and reed beds and intertidal forests. In the northern part of the Rhine–Meuse estuary, many of these soft substrate ecotopes disappeared gradually with the development of the Port of Rotterdam between 1870 and 1970 (Paalvast 2002; Paalvast et al. 2012). Nowadays, the port of Rotterdam is a highly engineered estuarine environment and the only completely open access into the river Rhine is through the Rotterdam Waterway (Nieuwe Waterweg), the main navigation channel of the Port of Rotterdam (Fig. 1).

Figure 1 Topography of the Rotterdam waterway in 1740 and 2020

Between 1960 and 1970 the pollution of the Port of Rotterdam was severely degrading the ecosystem, reducing biodiversity to a low number of pollution-tolerant species (Wolff, 1978). During more recent years, the pollution status of the Rhine and of many of its tributaries was distinctly improved due to the implementation of the Rhine Action Programme (RAP) introduced by the International Commission for the Protection of the Rhine (ICPR) in 1987, a year after the Sandoz chemical accident (ICPR. International 2021). Also, the European Parliament and the European Council adopted the WFD with the purpose to establish a framework for the protection of European waters. For artificial water bodies, like the Port of Rotterdam, the WFD stipulates that Member States shall protect and achieve good ecological potential and good chemical status by 2015, with extensions to 2021 and 2027, respectively.

The Port of Rotterdam is highly industrialized (Fig. 2). The total area of the Port of Rotterdam is 12,713 ha of which the land area is 7,903 ha and the water area is 4,810 ha. The total length is 42 km and the maximum water depth relevant to New Amsterdam Level is 24 m. In 2019, the Port of Rotterdam was Europe’s largest seaport. Shipping in the Port of Rotterdam is intensive. 29,491 seagoing vessels and 85,969 inland vessels visited the port of Rotterdam in 2019 (P.o.R. Authority, 2019). The main commercial activities are aggregates (sand, gravel etc.), ship repair, marine engineering, petroleum refining and product processing, roll-on/roll-of cargo transfer, chemical industry, general manufacturing, storage and packaging, refrigerated cargo and energy production. The main types of cargo handled are dry bulk, liquid bulk (non-oil), trade vehicles, perishable goods, petroleum/oil products, roll-on/roll-off and general cargo.

Figure 2 Distribution of activities in the Port of Rotterdam (Source: P.o.R Authority, 2019)

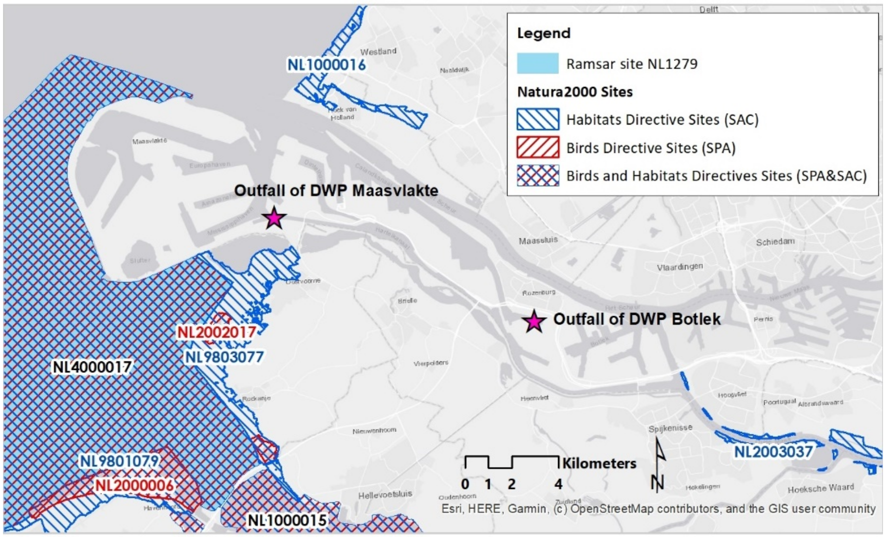


7.2 Environmentally designated areas

The port itself is not an environmentally designated area, however the environmentally sensitive character of the surrounding ecosystem is reflected by the presence of Natura 2000 network[3] sites and a Ramsar site that host numerous protected species. Specifically, the Natura 2000 sites close to the Port of Rotterdam (Fig. 3) are the SAC sites “Voornes Duin” NL9803077, “Solleveld & Kapittelduinen” NL1000016, and “Oude Maas” NL 2003037, the SPA site “Voornes Duin” NL2002017, and both SAC and SPA site “Voordelta” NL4000017 which is also designated as Ramsar site with site code NL1279. The latter is an extensive coastal wetland in the North Sea, characterized by shallow sandbanks, mudflats, salt meadows and embryonic dunes. The shallow mudflats are a very important spawning and nursery ground for migratory fish such as River lamprey *Lampetra fluviatilis* and Allis shad *Alosa alosa*. Moreover, common seals (*Halichoerus grypus*) and grey seals (*Phoca vitulina*) regularly use the site (European Environment Agency, 2021).

[3] Natura 2000 is the centerpiece of EU nature & biodiversity policy. It is an EU wide network of nature protection areas which aims to assure the long-term survival of Europe's most valuable and threatened species and habitats. It is comprised of Special Areas of Conservation (SAC) designated by Member States under the Habitats Directive 92/43/EEC, and incorporates Special Protection Areas (SPAs) designated under the Birds Directive 2009/147/EC.

Figure 3 Designated areas under the Natura 2000 network and Ramsar Convention in the vicinity of the Port of Rotterdam



8 Description of the DWPs and surrounding environment

EVIDES supplies high-quality demiwater from its DWP Botlek and DWP Maasvlakte to a large number of chemical and petrochemical companies in the Port of Rotterdam. Both DWPs are fed with fresh water from Brielse Meer, which is one of the branches of the river Maas. Brielse Meer is a major source of freshwater for agricultural irrigation on Voorne-Putten, maintaining water levels and greenhouse horticulture in the Westland area, but also industrial activity at Europoort/Botlek. In recent years – and particularly over the past two, relatively dry summers – it has become clear that the continuous supply of sufficient fresh water should not be taken for granted. Consequently, the three relevant authorities have drawn up a set of measures that will further improve the supply of fresh water from the key fresh water source of Brielse Meer (P.o.R. Authority, 2020). The raw water taken from Brielse Maas has a typical turbidity of 2 to 10 NTU, a typical conductivity of 500 to 850 µS/cm and approximate total hardness of 80 mg/L calcium.

DWP Botlek has been operating since December 2009, with a maximum capacity of 1,400 m^3/h demi water. It discharges the brine streams in the Brittaniëhaven area by a headwall on the slopes of the river above the water level. Close to EVIDES outfall, Huntsman WWTP and Wilmar Edible Oils are also discharging their effluent (Figure 4). DWP Maasvlakte has been operating since January 2018 and has maximum capacity of 800 m^3 demiwater per hour and discharges the brine in the Hartelkanaal area though two headwalls on the slopes of the river and above the water level.

Figure 4 Brine discharge points (A: Discharge point of DWP Botlek on the right and discharge point of another industrial facility on the left in Brittaniëhaven area, B: Discharge point of DWP Botlek in Brittaniëhaven area, C & D: two discharge points of DWP Maasvlakte in Hartelkanaal area)

In both DWPs, the purification process combines ion exchange technology (IEX) and membrane technology (RO). Two effluent streams are generated. The first effluent stream is generated from the IEX process, whilst the second effluent stream is generated by the RO process. IEX brine and RO brine effluents are discharged separately in compliance to the term set by the Dutch Water Authority Rijkswaterstaat. DWP Maasvlakte discharges 400-500 m^3/d IEX brine and 100-120 m^3/h RO brine. DWP Botlek discharges 1200 m^3/d IEX brine and 300 m^3/h RO Brine. For the Botlek case, where the ZERO BRINE project demonstration took place, the two brine effluents are as follows: (a) IEX brine: seven ion exchange vessels being regenerated every 20 hours (7 vessels * 141 m^3/vessel * 24 hours/day * 1/20 hours = 1,184 m^3/day); (b) Reverse Osmosis (RO) brine: 8 RO units in operation treating approx. 240 m^3 of river water per hour at 85% water recovery ratio, thus resulting in a combined RO brine effluent of approx. 300 m^3/h.

In the vicinity of DWP Maasvlakte in Hartelkanaal area, the main port's activities are freight distribution, chemicals/refineries/energy and liquid bulk. Specifically, the adjacent industrial facilities are (Fig. 5):

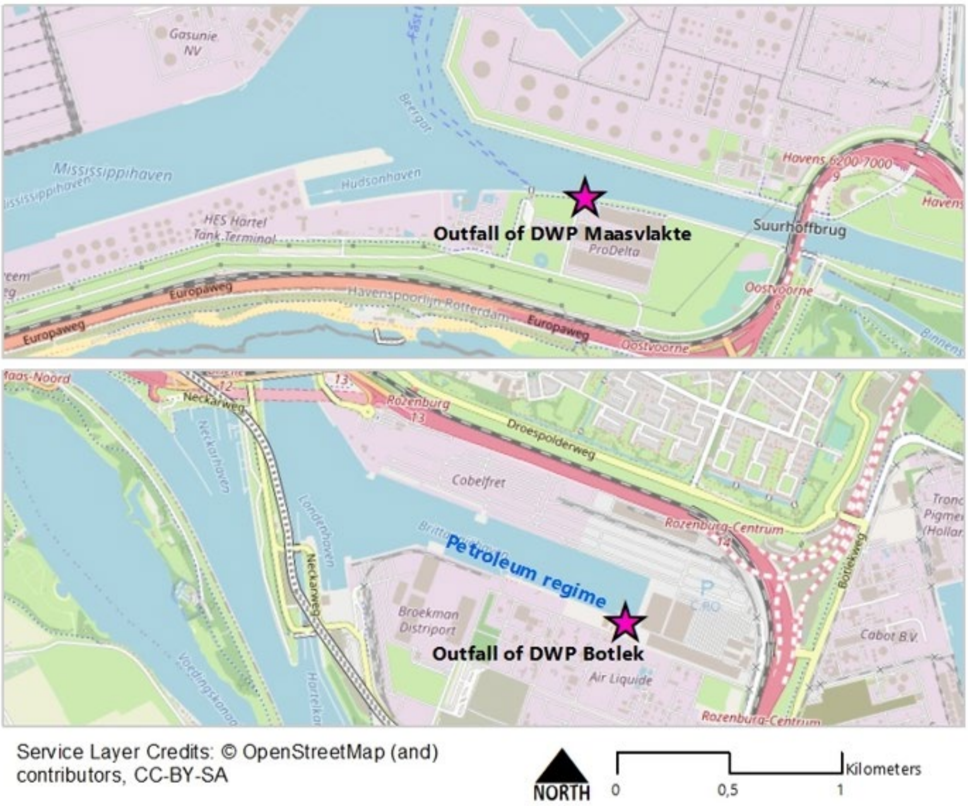
- BP refinery: Oil refinery, surface area 10500 sqm. The refinery currently uses hydrogen made from hydrocarbons in order to desulphurize petrochemical products. Replacing this entirely with green hydrogen produced from water using renewable energy could potentially result in a reduction of 350,000 tons of CO2 emissions per year based on current circumstances.
- HHTT: HES Hartel Tank Terminal, Storage point - 1.3 million cbm storage capacity for clean petroleum products (gasoline, diesel, gasoil and jet fuel) and biofuels
- FALCK: Industrial Fire Sevices, Emergency Services, Training Center
- PRODELTA: Development company, importer of cranes, trucks and bulldozers, parent company of ProDelta Real Estate, ProDelta Investments and Hovago Cranes

In the vicinity of the DWP Botlek in Brittaniëhaven area, the main port's activities are general cargo and chemicals, refineries and energy industries. Specifically, the adjacent industrial facilities (Fig. 5) are:

- CRO: Terminal (seaport), handling and dispatch of containers, trailers and vehicles
- RCT: Container/Tank container sale, repair, rental, lease, container deports/storage, inland container terminals
- RCC: Container/Tank container sale, repair, rental, lease
- INVISTA: Chemical manufacture (Polyamides (nylon 6.6))
- AIR LIQUIDE: Supply chain of Hydrogen-powered trucks, Transport of Hydrogen, Industrial gases and water (Carbon monoxide, hydrogen, syngas, oxogas), Steam and power
- DUKOR: Chemical Industry and Manufacturing, production of polypropylene, polyethylene and polyolefin products

- BROEKMAN: Logistics, Breakbulk terminal, Stevedoring, Warehousing and distribution
- BERTSCHI: Tank (container) transport, container deport and storage, supply chain management, inland container terminals
- HUNTSMAN: Chemical industry and manufacturing, main products - MDI, polyols, also steam and power

Figure 5 Physical and manmade environment in the vicinity of the DWPs

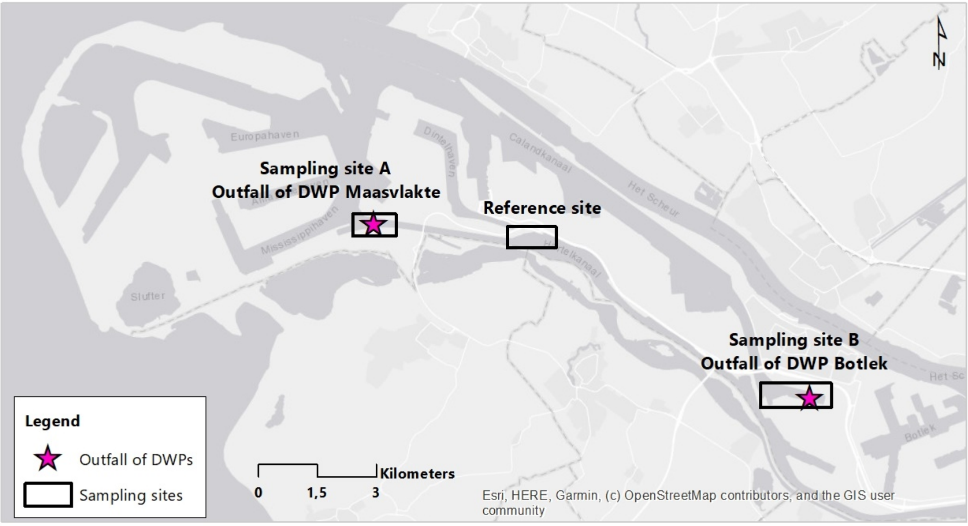


9 Methods

9.1 Study sites

Three sites were sampled within the framework of this study (Fig. 6): one in the vicinity of DWP Masvlakte in Hartelkanaal area (sampling site A), one in the vicinity of the DWP Botlek in Brittaniëhaven area (sampling site B), and one in Elbeweg area that was designated as Reference site.

Figure 6 Sampling sites



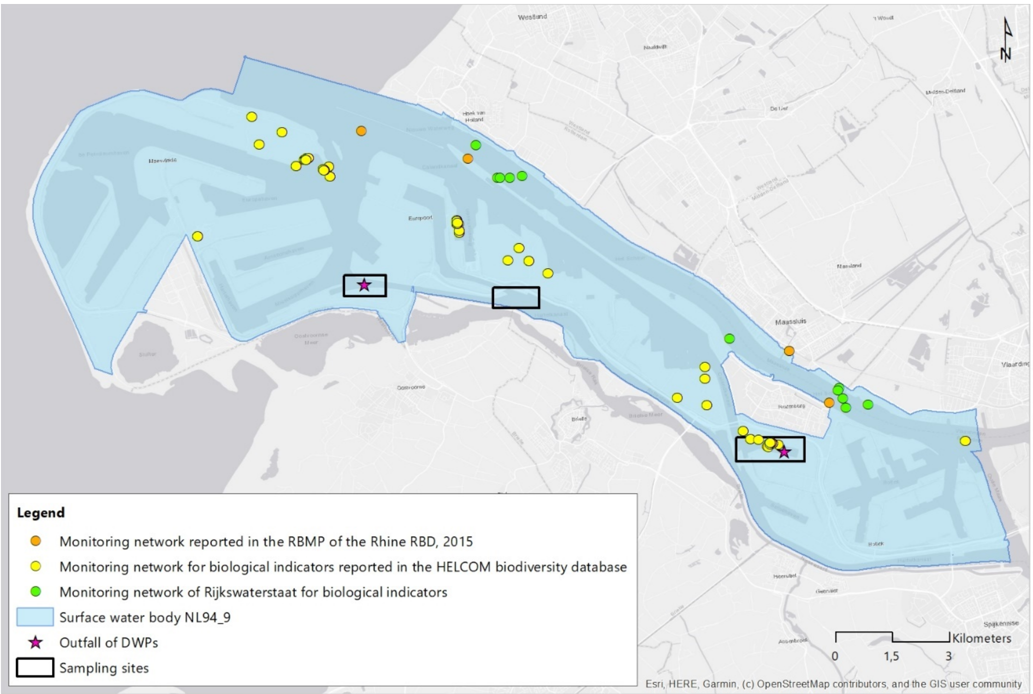
Due to being located on a waterway directly connected with the open North Sea, the Hartelkanaal has inherently variable salinities and current directions and receives effluent from the DWP Maasvlakte. This site is a well flushed environment influenced by currents that may dilute and disperse the brine discharge. The tide in the area ranges between 1-1.5 m. The seabed consists predominantly of *Crassostrea gigas* reef and the depth is around 6-8 m depending on tide conditions. Brittaniëhaven is a dead-end waterway, i.e. it has no river input and it is entirely marine. This area receives brine effluents from the DWP Botlek and effluents from Huntsman WWTP and Wilmar which is an oil refinery processing vegetable oil. This site can be characterized as an enclosed water body and a poorly flushed environment. The seabed consists mainly of silt and the depth reaches 10 m. The Reference site has naturally changing salinities and current directions due to tidal influence. The seabed there consists mainly of sand and the depth is around 9-13 m. In this study, it has been selected following the advice of the Port of Rotterdam Authority as a less-polluted site in the port.

9.2 Monitoring network

Based on the information provided WISE-WFD database for the RBMP 2015 (WISE-WFD, 2015) of the Rhine river basin district (RBD), that was conducted in the framework of the WFD, the study area is located within the River Basin District (RBD) coded NLRN and specifically in the Surface Water Body (SWB) coded NL94_9. The type of the SWB NL94_9 is classified transitional[4] and the category is classified artificial[5].

Data on benthic invertebrates, phytoplankton, macrophytes, fish and lamprey, and bacteria in the Port of Rotterdam are reported to the HELCOM Biodiversity database (2004-2020 – one measurement of 1982 is also included) and were taken into consideration for the evaluation of the results of this study. Similarly, Rijkswaterstaat monitors phytoplankton, diatomeae and macrozoobenthos in the Port of Rotterdam and the results from 2003 to 2019 were also considered. Some of the monitoring stations coincide with the sampling site B (Fig. 7).

Figure 7 (A) monitoring network reported in the RBMP of the Rhine RBD 2015, (B) monitoring network for biological indicators reported in the HELCOM Biodiversity database (C) monitoring network for biological indicators of Rijkswaterstaat. Only the monitoring stations within the SWB NL94_9 are shown.

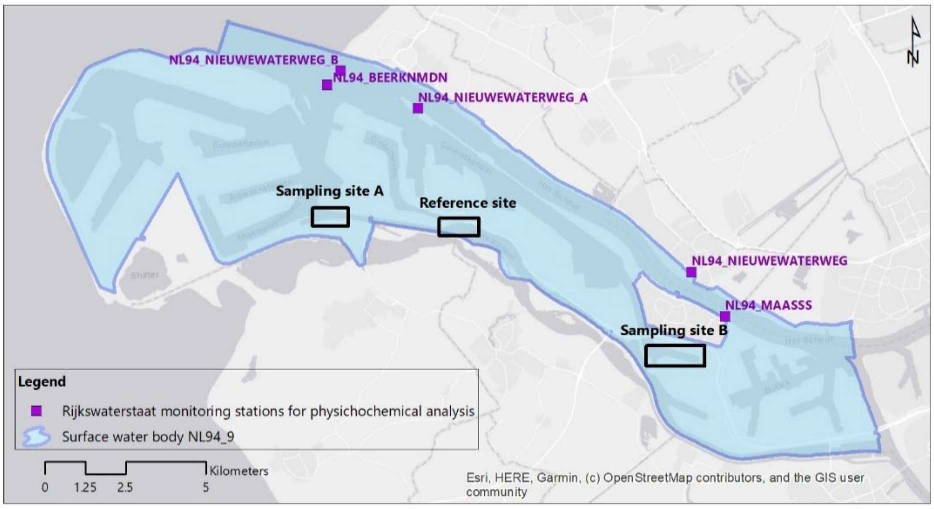


Apart from the biological indicators, Rijkswaterstaat monitors periodically the physical and chemical parameters in the Port of Rotterdam through a dense monitoring network with long timeseries. None of these monitoring stations coincide with the study sites (Fig. 8).

4 "Transitional" waters are bodies of surface water in the vicinity of river mouths which are partly saline in character as a result of their proximity to coastal waters, but which are substantially influenced by freshwater flows (WFD 2000/60/EC).

5 "Artificial" water body means a body of surface water created by human activity (WFD 2000/60/EC).

Figure 8 monitoring network of Rijkswaterstaat for physicochemical parameters. Only the monitoring stations within the SWB NL94_9 are shown.



9.3 Field sampling and laboratory analysis

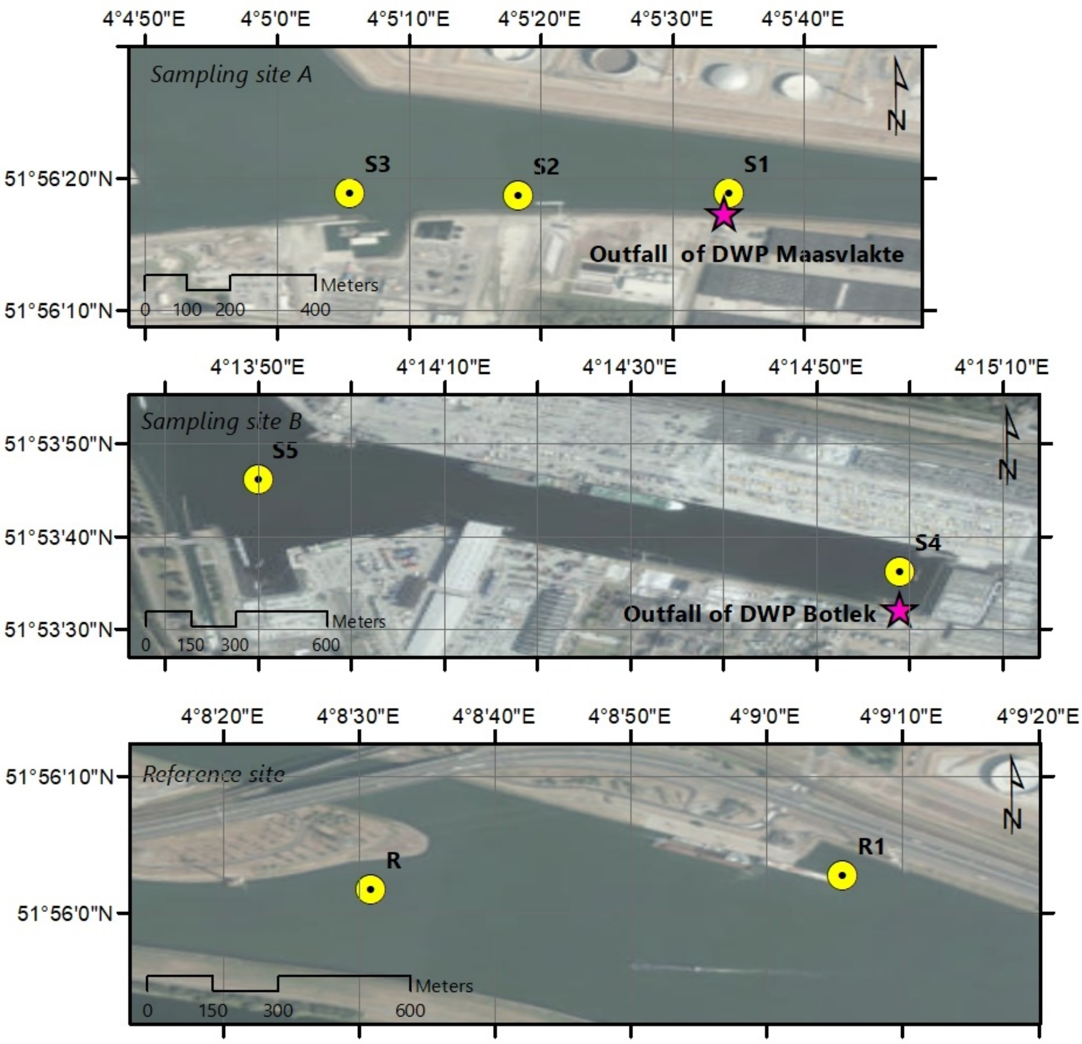
9.3.1 Benthic macroinvertebrates

In total, 4 sampling surveys were performed, namely in September 2019, January 2020, July 2020, and September 2021. The survey planned for April 2020 and then for April 2021 was cancelled due to the Covid-19 outbreak and finally performed in September 2021. The sampling survey in September 2019 was a reconnaissance survey for the design of the subsequent surveys. For this reason, some differences in the sampling scheme are observed in this survey in relation to the following surveys.

A total of 6 stations (Fig. 9) were selected and established for the benthic macroinvertebrates survey. The main characteristics of the sampling stations are presented in the Table 1. The stations were sampled aboard vessels of the Port of Rotterdam Authority (Tender during the 1st field survey and Surveyor 2 during the 2nd, 3rd, and 4th field surveys, respectively). Three replicates were collected at each sampling station using a Van Veen grab of 2L capacity. At each replicate, the Van Veen grab collected sediment twice, and the total volume collected were 4L. The sediment samples were sieved through a 1 mm mesh, stained with Rose Bengal and preserved in ethanol. In the laboratory, macrobenthic invertebrates were sorted, identified to the lowest taxonomic level possible, and counted. The type of bottom sediment in each station was defined based on the results of sediment fraction measurements %w/w (Table 8 – Appendix A).

Sampling was not easily applicable in the Brittaniëhaven because of the busy shipping traffic that generally observed in this area and during the surveys. Sampling in station 1 of Hartelkanaal was successfully performed even though the area consists of a thick layer of oyster shells that makes the sampling difficult.

Figure 9 Location of sampling station



Service layer credits: Source: ESRI, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community.

Table 1: Main characteristics of the sampled stations

Station	Coordinate (latitude / longitude) WGS 84	Depth (m)	Port sector	Bottom sediment	Surveys in which the stations were investigated
S1	X: 51°56'18.84"N, Y: 4°5'34.32"E	6-8 m	Hartelkanaal	Hard substrate consisting of *Crassostrea gigas* (Pacific oyster) reef	1st, 2nd, 3rd, 4th
S2	X: 51°56'18.66"N, Y: 4°5'18.34"E	6-8 m	Hartelkanaal	Mainly hard substrate consisting of *Crassostrea gigas* (Pacific oyster) reef mixed with soft substrate of mud	1st, 2nd, 3rd, 4th

Station	Coordinate (latitude / longitude) WGS 84	Depth (m)	Port sector	Bottom sediment	Surveys in which the stations were investigated
S3	X: 51°56'18.84"N, Y: 4°5'5.54"E	6-8 m	Hartelkanaal	Mainly hard substrate consisting of *Crassostrea gigas* (Pacific oyster) reef mixed with soft substrate consisting mostly of sand	1st, 2nd, 3rd, 4th
S4	X: 51°53'36.12"N Y: 4°14'59.04"E	6-8 m	Brittaniëhaven	Soft substrate, consisting mostly of silt	2nd, 3rd, 4th
S5	X: 51°53'46.08"N Y: 4°13'49.86"E	9-11 m	Brittaniëhaven	Soft substrate, consisting mostly of silt	3rd, 4th
R1	X: 51°56'2.76"N, Y: 4°9'5.64"E	13m	Hartelkanaal - Dolfijnweg	Soft substrate, consisting mostly of sand	1st
R	X: 51°56'1.72"N, Y: 4°8'30.93"E	9-11m	Hartelkanaal - Dolfijnweg	Soft substrate, consisting mostly of sand	1st, 2nd, 3rd, 4th

9.3.2 Water and sediment analysis

Water samples from S1, S4, and R were analysed for pH, EC, TSS, nutrients, a suite of heavy metals, and 16 PAHs. Water samples were collected from a depth of 1.5 m from the water surface and were stored in appropriate bottles and analysed in the laboratories of SGS Environmental Analytics B.V. (Steenhouwerstraat 15, 3194 AG Rotterdam, Netherlands) and C-MARK B.V (Munsterstraat 9, 7418 EV Deventer, Netherlands).

Sediment was also sampled as it is a major sink for contaminants such as trace metals that are not biodegradable and can accumulate over time and sediments may reach a level toxic to aquatic life. Specifically, samples from S3, S4, and R were analysed for Particulate Organic Carbon (POC) analysis, granulometric analysis, a suite of heavy metals and content of 16 PAHs. Sediment samples were collected with a van Veen grab and stored in appropriate bottles and analysed by the laboratory Eurofins (Gildeweg 42-48, 3771 NB Barneveld, Netherlands) and SGS Environmental Analytics B.V. (Steenhouwerstraat 15, 3194 AG Rotterdam, Netherlands).

The scope of water and sediment analysis was to investigate the environmental conditions and not to correlate the concentration levels of the parameters with the presence of the brine.

9.4 Statistical analysis and biotic indices

Differences between the multivariate species data set of each station were determined on square root transformed abundance data using the Bray-Curtis similarity measure. Community patterns were then visualised by non-metric multi-dimensional scaling (NMDS). Group average cluster analysis results on the same data were also overlaid on the NMDS ordination diagram. All statistical analyses were run using PRIMER 6.0 (PRIMER-e).

The AMBI index (Borja et al., 2000) was applied to classify the identified species into ecological categories, calculate the ecological quality ratio (EQR) and qualify the ecological status (ES) of the study area (Table 2). AMBI is a commonly used index and is for official use within the WFD as part of different multimetric indices in Portugal, the United Kingdom, Ireland, Denmark, Norway, and the Netherlands. AMBI has been tested in different geographic regions and has been proved to have large geographical coverage. AMBI shows responsiveness to various pressures (Borja et al., 2015) and is considered suitable for the pressures met in the study area such as chemical pollution (industrial discharges or presence of metals and organic compounds in water and/or sediment), dredging and sediment disposal (activity needed to maintain navigability in channels and harbours, creation of new harbours and disposal of sediments), harbours: presence of ports and normal activity, excluding dredging.

Table 2: AMBI values and classification (Borja et al. 2000, 2003)

<table>
<tr><th>Index value</th><th>Dominating ecological group</th><th>Benthic community health</th><th>Site disturbance classification</th><th>ES</th></tr>
<tr><td>0.0 ≤ AMBI ≤ 0.2</td><td rowspan="2">I-II</td><td>Normal</td><td rowspan="2">Undisturbed</td><td rowspan="2">High</td></tr>
<tr><td>0.2 < AMBI ≤ 1.2</td><td>Impoverished</td></tr>
<tr><td>1.2 < AMBI ≤ 3.3</td><td>III</td><td>Unbalanced</td><td>Slightly disturbed</td><td>Good</td></tr>
<tr><td>3.3 < AMBI ≤ 4.3</td><td rowspan="2">IV-V</td><td>Transitional to polluted</td><td rowspan="2">Moderately disturbed</td><td>Moderate</td></tr>
<tr><td>4.3 < AMBI ≤ 5.0</td><td>Polluted</td><td rowspan="2">Poor</td></tr>
<tr><td>5.0 < AMBI ≤ 5.5</td><td rowspan="2">V</td><td>Transitional to heavy pollution</td><td rowspan="2">Heavily disturbed</td></tr>
<tr><td>5.5 < AMBI ≤ 6.0</td><td>Heavily polluted</td><td rowspan="2">Bad</td></tr>
<tr><td>6.0 < AMBI ≤ 7.0</td><td>Azoico</td><td>Azoic</td><td>Extremely disturbed</td></tr>
</table>

Note: Group I: Species very sensitive to organic enrichment and present under unpolluted conditions. Group II: Species indifferent to enrichment, always present in low densities with non-significant variations with time. Group III: Species tolerant to excess organic matter enrichment. These species may occur under normal conditions; however, their populations are stimulated by organic enrichment. Group IV: Second-order opportunistic species, adapted to slight to pronounced unbalanced conditions. Group V: First-order opportunistic species, adapted to pronounced unbalanced situations. (Grall and Glemarec, 1997).

The Benthic Opportunistic Polychaetes and Amphipods (BOPA) index (Dauvin and Ruellet, 2007) was also applied. BOPA index results from the refinement of the polychaeta/amphipoda ratio (Gesteira and Dauvin, 2000). Accordingly, this index will be used to assign the estuarine communities into the five ES categories (Table 3) ranging from “High” to “Bad” where high is defined an area dominated by sensitive species, while bad an area dominated by opportunistic species.

Table 3: BOPA values and classification

Index value	Site disturbance classification	ES
0.00000 ≤ BOPA ≤ 0.06298	Unpolluted sites	High
0.04576 < BOPA ≤ 0.19723	Slightly polluted	Good
0.13966 < BOPA ≤ 0.28400	Moderately polluted	Moderate
0.19382 < BOPA ≤ 0.30103	Heavily polluted	Poor

Index value	Site disturbance classification	ES
0.26761 < BOPA ≤ 0.30103	Extremely polluted	Bad

BEQI was also examined as per the Decision 2018/229/EC which mentions that NL applies BEQI 2 for the evaluation of benthic macroinvertebrates in the context of the WFD 2000/60/EC.

Table 4: BEQI 2 values and classification

Index	Ecological quality ratio	
	High – good boundary	Good – moderate boundary
BEQI 2	0.80	0.60

10 Results and discussion

10.1 Brine characteristics

Table 6 (Appendix A) shows the average concentration of ions and organic matter at the effluent RO and IEX streams of DWP Botlek (Spanjer and Xevgenos, 2020). It can be assumed that the effluent characteristics of DWP Maasvlakte are similar to the DWP Botlek as the same IEX and RO process is applied to both plants except from TOC that is expected to be about 1 ppm higher than DWP Botlek because DWP Maasvlakte is fed with water that has more algae.

The IEX and RO effluent is characterized by its high salinity and density (negatively buoyant), ambient seawater temperature, low nutrient content, and elevated levels of chloride (Cl), TDS, bicarbonate (HCO^{-}_{3}) and sulphate (SO^{2-}_{4}). RO effluent also contains high organic content. As for heavy metal concentrations, the IEX effluent contains elevated levels of chromium (Cr), aluminum (Al), copper (Cu), zinc (Zn), barium (Ba) and lead (Pb). The RO effluent contains elevated levels of lithium (Li) and boron (B).

According to the national emission registration database (http://www.emissieregistratie.nl/), the following industrial companies have registered chloride releases within the Botlek area of Rotterdam Port: Air Products Nederland BV, Air Liquide, Akzo Nobel Chemicals BV (current name: NOBIAN), Almatis BV, Aluminium & Chemie Rotterdam BV, AVR NV (Rijnmond), Botlek VCM Plant, Borax NV, Biopetrol Rotterdam BV, Cabot BV, Cargill B.V., Climax Molybdenum BV, Den Hartogh Cleaning B.V., Ducor Petrochemicals B.V., EBS Terminal Laurenshaven, ExxonMobil Chemical Holland BV, Evonik Carbon Black Nederland B.V., Eurogen CV, Esso Nederland BV, Ecotechniek, Emerald Kalama Chemical Rotterdam BV, Enecal Energy VOF, ENCI B.V., Hexion UK Limited, Hoyer Nederland BV, Huntsman Holland BV, Invista Nederland BV, Kemira Rotterdam BV, J. den Breejen Ecotechniek VOF, Keppel Verolme BV, LBC Rotterdam BV, Lyondell Chemie Nederland BV, Lucite International Holland BV, Maas Silo BV, Nufarm BV, OTM Maritiem, Organik Kimya BV, Odfjell Terminals (Rotterdam) BV, Tronox pigments, Tankinstallatie Chemiehaven, Vopak and Van Leer.

In a comparison of the two wastewater streams for which the discharge points are presented in Figure 4, the following apply:

- Demineralized Water Plant of Evides: ~12,600 tons of chlorides per year (estimation). The estimate has been done considering approximately 25,000 mg/l present in the ion exchange brine (total chlorides discharged: 25,000 mg/l * 1,184,000 l/day * 365 days/year = 10,804 tons/year) and 700 mg/l present in the RO brine (total chlorides discharged: 700 mg/l * 300,000 l/h * 8760 h/year = 1840 tons/year);

- Huntsman BV: 24,470 tons of chlorides per year.

10.2 Monitoring data

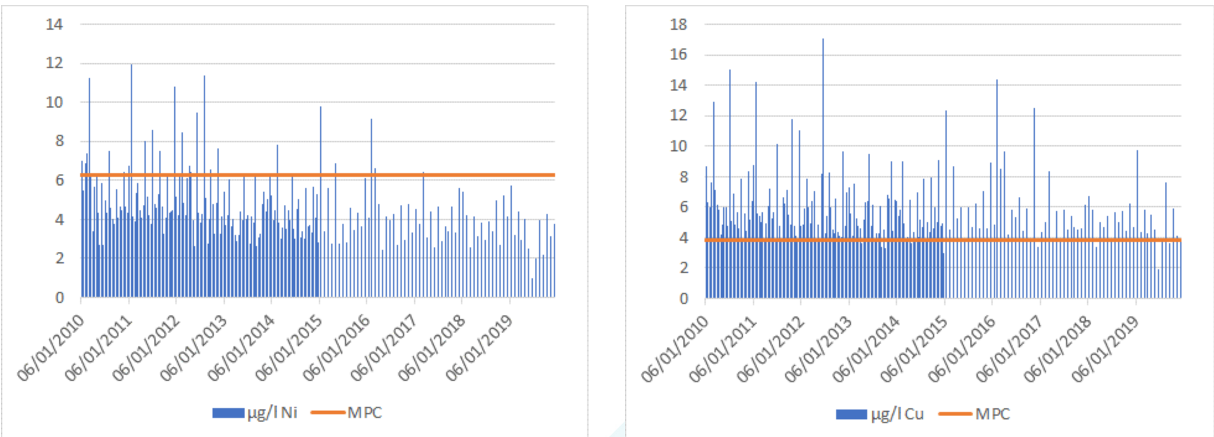
According to the WISE-WFD database for the RBMP 2015 (WISE-WFD, 2015), the ecological potential of the SWB NL94_9 was characterized as "moderate"[6] and the chemical status as "not good"[7]. Regarding the biological quality elements, phytoplankton, phytobenthos/macrophytes and macrozoobenthos were characterized as "good" ecological potential and the fish fauna with "moderate" ecological potential. In WISE-WFD 2015 database, it was stated that the goal of WFD for "good" ecological potential and "good" chemical status will not be achieved in 2021 but it is expected to be achieved beyond 2027. It was also stated that the most significant pressure is the introduced species and diseases (invasive alien species), and the most significant impact is the elevated temperature.

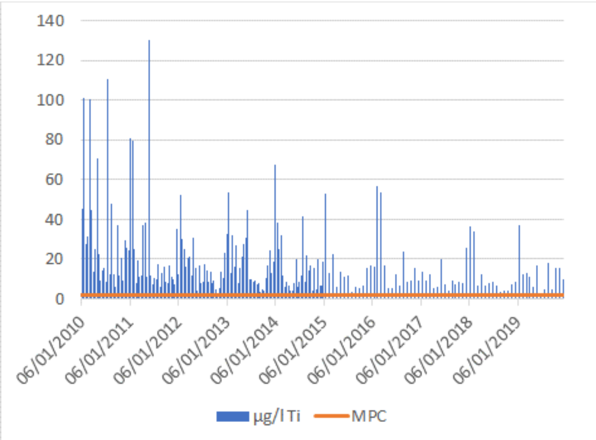
According to the benthic macroinvertebrates results of Rijkswaterstaat for the period 2003 – 2016 in the Port of Rotterdam (SWB NL94_9), 208 species and 77968 individuals have been recorded. In the HELCOM database for the year 2014, 82 species and 935 individuals have been recorded. Of those, 37 species and 187 individuals have been observed at the sampling site B where some monitoring stations of HELCOM exist. The species with the most individuals recorded in the Port of Rotterdam are *Sinelobus stanfordi* (non-native), *Apocorophium lacustre* (non-native), *Balanus improvisus* (native), *Balanus, Leptocheirus pilosus* (native), *Corophiidae* (native), *Sinelobus vanhaareni* (non-native), *Gammaridae, Corophium multisetosum* (native), *Melitidae* (native), *Gammarus tigrinus* (non-native), *Balanidae* (non-native), *Neomysis integer* (native), *Ficopomatus enigmaticus* (non-native), *Hediste diversicolor* (native), *Amphibalanus improvisus* (non-native), *Cyathura carinata* (native). For the site B, the species with the most individuals are *Balanus crenatus* (native), *Carcinus maenas* (native), *Conopeum reticulum*, *Crassostrea gigas* (non-native), and *Mytilus edulis* (native). It can be assumed that the non-native species have been introduced by shipping and ballast waters and that they can lead to competition with native biota.

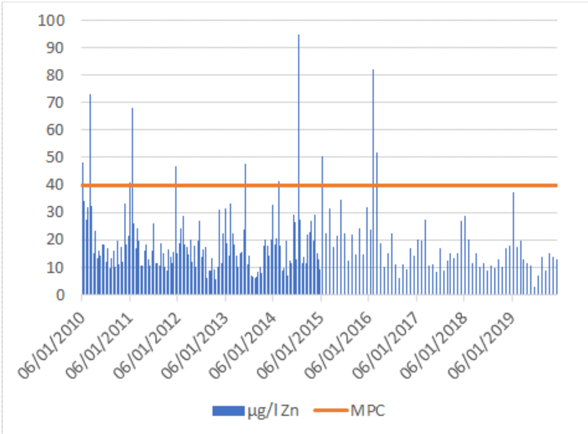
[6] "Moderate" ecological potential in terms of biological quality elements means that there are moderate changes in the values of the relevant biological quality elements as compared to the values found at maximum ecological potential. Whereas, "Good" ecological potential of biological indicators means that there are slight changes in the values of the relevant biological quality elements as compared to the values found at maximum ecological potential (WFD 2000/60/EC).
[7] "Not good" chemical status means that concentrations of priority substances exceed the relevant EQS established in the Environmental Quality Standards Directive 2008/105/EC (as amended by the Priority Substances Directive 2013/39/EU). EQS aim to protect the most sensitive species from direct toxicity, including predators and humans via secondary poisoning. A smaller group of priority hazardous substances were identified in the Priority Substances Directive as uPBT (ubiquitous (present, appearing or found everywhere), persistent, bioaccumulative and toxic). The uPBTs are mercury, brominated diphenyl ethers (pBDE), tributyltin and certain polyaromatic hydrocarbons (PAHs).

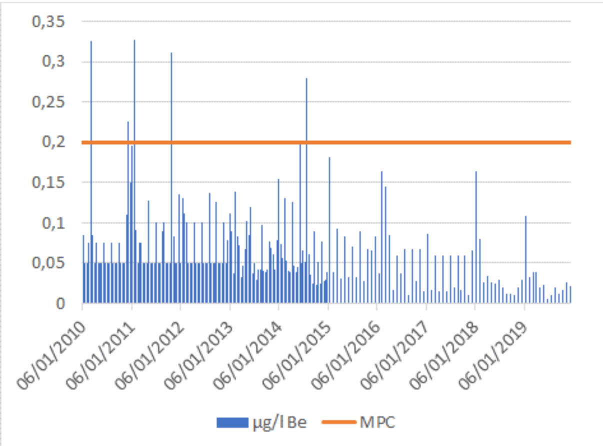
Based on the timeseries of the monitoring stations for BEERKNMDN and MAASSS metals provided by Rijkswaterstaat (Rijkswaterstaat, 2021) for the time period 2010 – 2019, and in comparison to the MPC and Target values set in the Staatcourant, The Netherlands, June 2000, it has been observed that cadmium (Cd), mercury (Hg),lead (Pb), chromium (Cr), arsenic (As), antimony (Sb), barium (Ba), cobalt (Co), molybdenum (Mo), selenium (Se), tin (Sn), vanadium (V) concentrations are below MCP but some of them above Target Value. Moreover, some measurements of copper (Cu), zinc (Zn), beryllium (Be) are above MPC as well as the most measurements of nickel (Ni) and thallium (Ti). As shown in Fig. 10, a slight improvement was observed in the last five years regarding the concentrations of metals exceeded the MPC. With regards to the PAHs measurements of BEERKNMDN and MAASSS monitoring stations for the time period 2010-2019 (Rijkswaterstaat, 2021) and compared to the MPC and Target values set in the Staatcourant, The Netherlands, June 2000, it can be concluded that measurements of napthalene, anthracene, phenanthrene, fluoranthene, chrysene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene and indeno(123-cd)pyrene are below the MPC however in some cases above the Target Value. One measurement of benzo(a)anthracene is above the MPC. As for the nutrients and eutrophication parameters measured from BEERKNMDN and MAASSS monitoring stations for the time period 2010 – 2019, P_T and N_T measurements exceed in some cases the MPC set in the Staatcourant, The Netherlands, June 2000. (Fig. 10). Improvement of the quality of the surface water in terms of P_T has been observed over the last five years but the situation for the concentrations of N_T is stable. It is noted that these are the most adjacent monitoring stations to the studied sites however none of them coincides with the study area. However, the results give an overview of the environmental baseline conditions in the Port of Rotterdam.

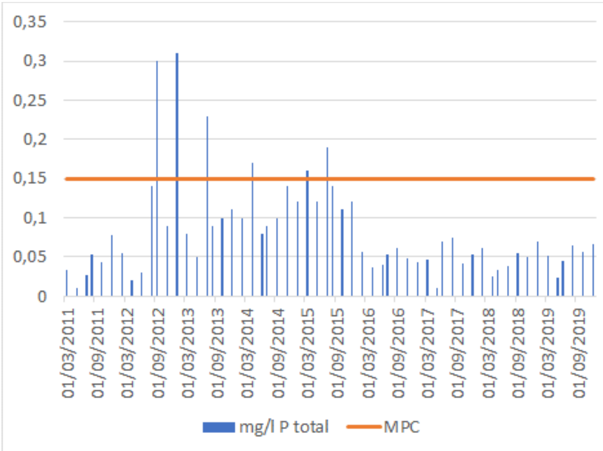
Figure 10 Timeseries of concentrations of metals and P_T, N_T (2010-2019, monitoring stations BEERKNMDN, MAASSS) that have been exceeded the MPC (Rijkswaterstaat, 2021)

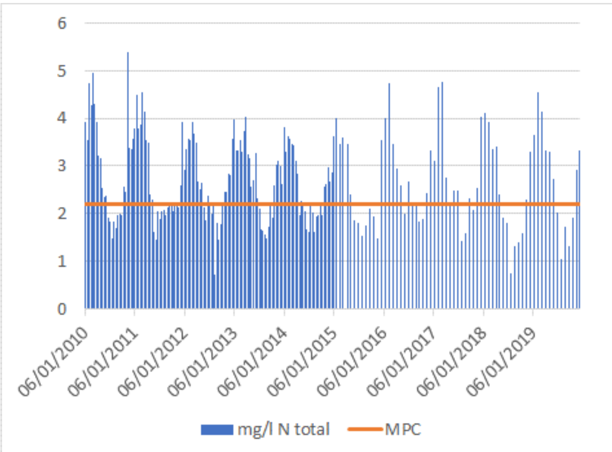












10.3 Water and sediment quality analysis results

The concentrations and levels of the parameters measured in the water samples in comparison to the Maximum Permissible Concentration (MPC) and Target Value set out in the Staatscourant, The Netherlands (June 2000), are presented in Table 7 (Appendix A). The results indicated that PAHs concentrations in seawater were below the detection limits of the method. Heavy metals were lower than the standard values set for the SWB NL94_9 by the WISE-WFD database for the Rhine RBMP 2015 and the MPC values and for the most cases below the Target Values.

Sediment analysis results in comparison to the MPC and Target Value are presented in Table 8 (Appendix A). The results indicated that the TOC, and Kjedahl nitrogen values are higher at S4 in comparison to S3 and R. This could be a result of the decomposition of the plants and animals or plankton and / or anthropogenic sources. This can also correlate with the reduced species richness to this station in comparison to the others as an overabundance of organic matter can lead to this due to due to oxygen depletion and buildup of toxic by-products (ammonia and sulphide) associated with the break-down of these materials (Hyland et. al 2005).

The results also indicated that metals and PAH concentrations in the sediment are lower than the MPC and for some cases also below the Target Values. These measurements also show that S4 and R have higher concentrations of metals and PAHs compared to S3, which indicates that these areas are more affected by anthropogenic activities and explains the presence of more pollution-tolerant benthic macroinvertebrates.

10.4 Benthic macroinvertebrates

10.4.1 Identification key points

The overall list of species identified during the four field surveys is presented in Table 09 of Appendix A. The number of individuals and species identified per replicate, sampling station and field survey are presented in the Tables 10, 11, 12 and 13 of Appendix A. Diversity and indices values per replicate, sampling station and field survey are presented in Table 14 of Appendix A.

During the January 2020 sampling, juveniles from the Semelidae Family were identified as *Abra nitida.* At the same time, Serpulidae individuals were found and identified as *Hydroides* sp. According to Bruyne et al. (2013), *Abra nitida* has been previously found in The Netherlands, although Faassee et al. (2019) have been unable to identify the molluscs as *Abra nítida* even when comparing them with *Abra nítida* specimens from an official collection for the area. The latter showed that all the specimens named before as *Abra nitida* should be reclassified as *Theora lubrica*, proposing that the presence of *A. nitida* in the area should be confirmed in future studies. So, we must assume that the individuals from this study identified initially as *Abra nitida* were probably *Theora lubrica*.

In the recent identification work for the September 2021 sampling, we found again organisms that looked initially like *Abra nitida*, but well-developed adult individuals we found later in the samples we could clearly see the characteristic internal ridge of *Theora lubrica,* confirming the initial hypothesis. Also, the presence of *Theora* agrees with the co-occurrence with *Hydroides sp*, specifically *Hydroides* *ezoensis*, also an invasive species like *Theora lubrica* coming from Pacific Ocean. *Hydroides* sp usually co-exists with *Ficopamatus enigmaticus*, with *F. enigmaticus* being usually more abundant (Faasse et al., 2020).

Hydroides ezoensis and *Theora lubrica* are usually found sharing habitat with other invasive species as *Mulinia lateralis*, *Ruditapes philippinarum* or the polychaete *Pseudpolydora paucibranchiata*. Reish (1955) pointed out the resistance of *P. paucibranchiata* to enrichments of organic matter and contaminated conditions. *T. lubrica* shows a similar behaviour, with a high capacity to tolerate low oxygen concentrations, showing a high fecundity and establishing itself in the community rapidly (Johnston, 2005).

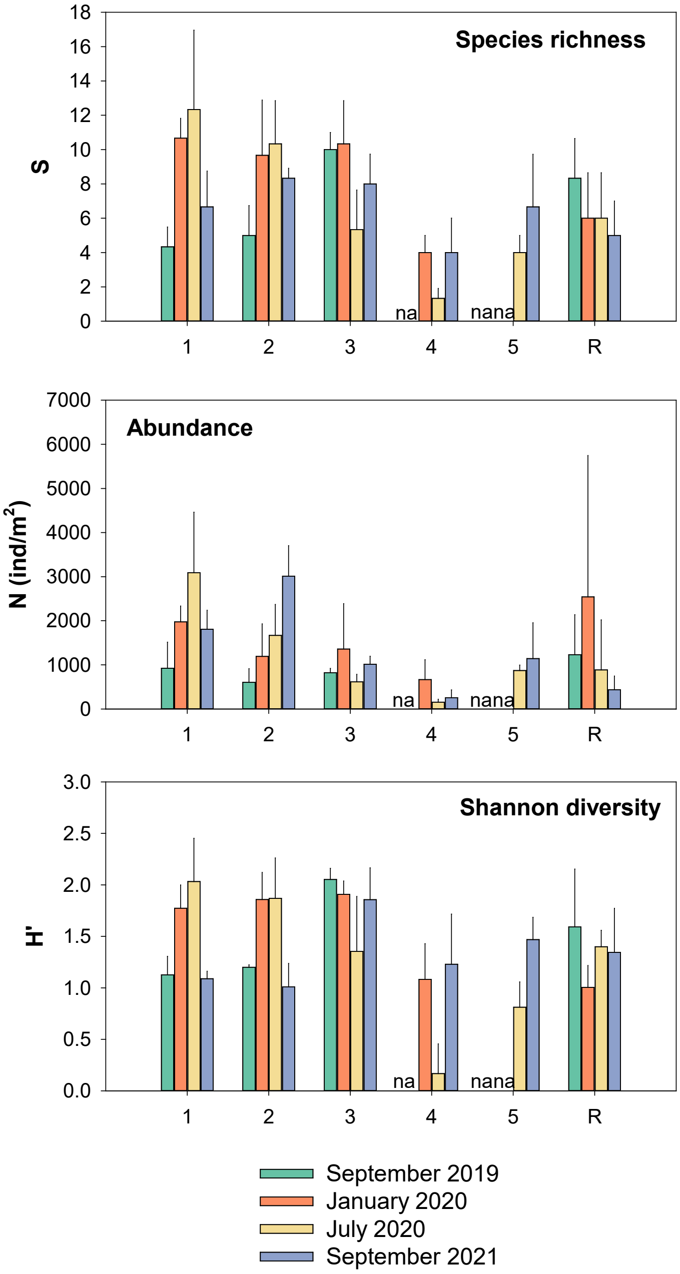
Further above, we mentioned *Mulinia lateralis* as a species usually founded together with *Theora lubrica*. In this sampling, we found juvenile organisms that we classified initially as *Spisula subtruncata*; however after consulting with different experts and in accordance with its sympatric occurrence with *T. lubrica* probably belong to *M. lateralis*. Studies in areas nearby (Klunder et al., 2019) have established its presence in coexistence with other species identified and collected in our job such as *Alitta succinea*. *Tharyx sp*., *Heteromastus filiformis* o *Corophium volutator*. Thus, although it is highly likely that the *M. lateralis* is present in the sampling area, this awaits to be unambiguously confirmed in future samples.

Finally, it should be noted that the adult specimens identified as *S. subtruncata* definitely belong to this species and not to *M. lateralis* as confirmed by the presence of transversal stripes in the lateral teeth.

10.4.2 Diversity and abundance

The identification and the posterior statistical analysis showed that the values of species richness and abundance of macrofauna are relatively low, with significant variations between stations and sites. This variation indicates the patchy distribution of the macrobenthic community and the need to increase the sampling effort to include more replicates. In addition, in many replicates less than 6 species (26 out of 63 replicates) or less than 500 ind m^{-2} (36 out of 63 replicates) were recorded. The maximum number of species found in a single replicate was 15, whereas the maximum number of individuals was 162 (3100 ind m^{-2}). Overall, the highest species richness and abundance was recorded at Stations in Hartelkanaal and was mostly related with sessile animals from hard substrate. In the replicates with the highest numbers, more than half of the individuals belonged to the Family Balanidae (barnacles) or other hard substrate bivalves (mussels), which probably grew on the *Crassostrea gigas* reef or fall off the ships. Diversity values are noticeably low, always below 2 and closer to 1 on many occasions, yet due to the lack of dominance of few species and the overall disturbance status of the sites.

Figure 11 Species richness, abundance and Shannon diversity index for each station on each sampling. Values represent means +/- SD (n=3). na: no samples available.

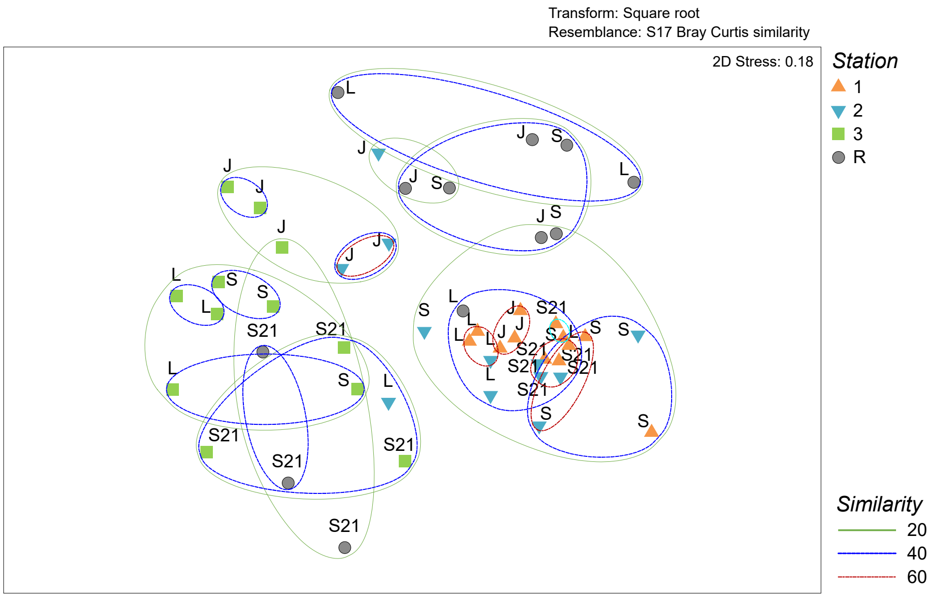


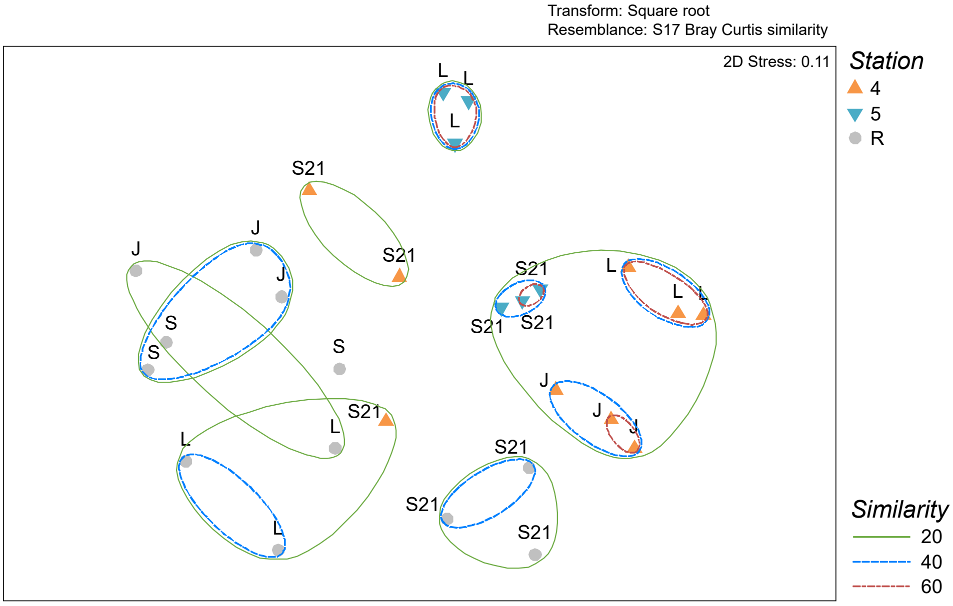
10.4.3 Multivariate analysis

In order to observe the similarities between stations and across time, a multivariate analysis based on the complete dataset was performed. Abundances were square root transformed to reduce the weight of the highly abundant taxa. Two separate analyses were performed for each location due to the differences in the substrate. The stress level of the ordination plot for the Hartelkanaal is relatively high which is reflected in the distortion of the superposed clustering of the same data. Overall, the similarity between replicate samples was quite low, usually between 40% and 20%. Only station 1 and station 4 showed on occasions similarities among replicates of >60%.

At Sampling Site A, replicates from each station were quite variable with at least one of the three replicate being less than 40% similar to the rest, highlighting the high heterogeneity of the system and the need for more replicates. The most homogenous group was that of Station 1 regardless of season with only one replicate being less than 40% similar to the rest. For Sampling Site B, stations are quite distinct, forming clearly different clusters.

Figure 12. nMDS ordination plot of the sampling stations in Sampling Site A, the Hartelkanaal (upper plot) and in sampling site B, the Brittaniëhaven (lower plot). The reference site has been included in each plot although the sediment type resembles more that of site B. nMDS is based on a Bray Curtis similarity index matrix between stations on square root transformed abundance data. Lines indicate the similarity between samples based on a group average linkage cluster analysis using the same matrix. S: September 2019, J: January 2020, L: July 2020, S21: September 2021.





10.4.4 AMBI index

For the calculations of the AMBI, hard substrate and epifaunal taxa were removed (Borja & Muxika, 2005). In addition, in some samples due to the low number of taxa (1 to 3) or individuals (less than 3 per replicate), the results should be considered with care as the robustness of the assessment is significantly reduced (Borja & Muxika, 2005). The results in the majority of the cases showed a slightly disturbed system and only stations 4 and 5 were characterised on occasions as moderately or heavily disturbed.

Figure 13. Contribution of the AMBI distinct ecological groups for the stations sampled on each sampling occasion. The contribution of groups IV and V, more tolerant to perturbations, only dominate in a few samples. na: no samples available.

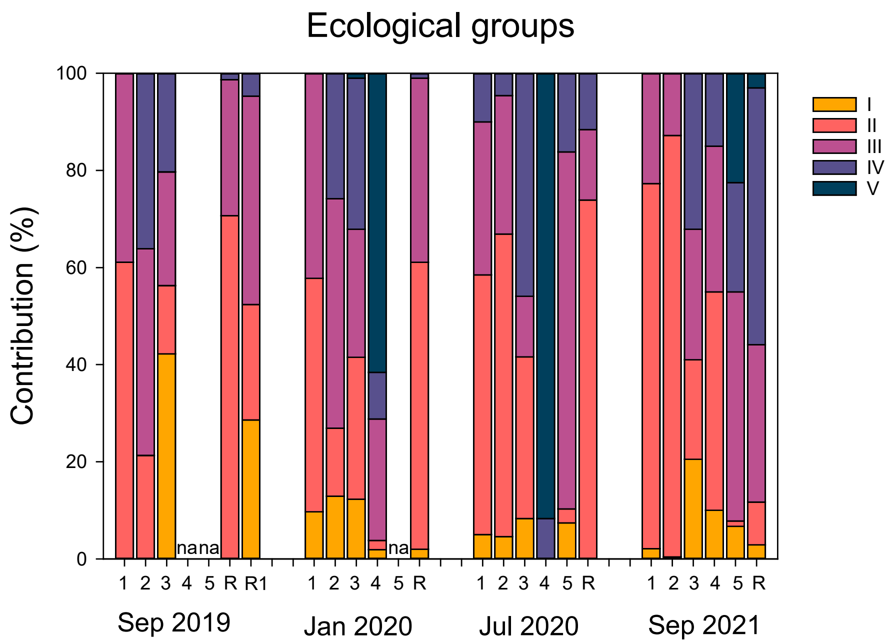
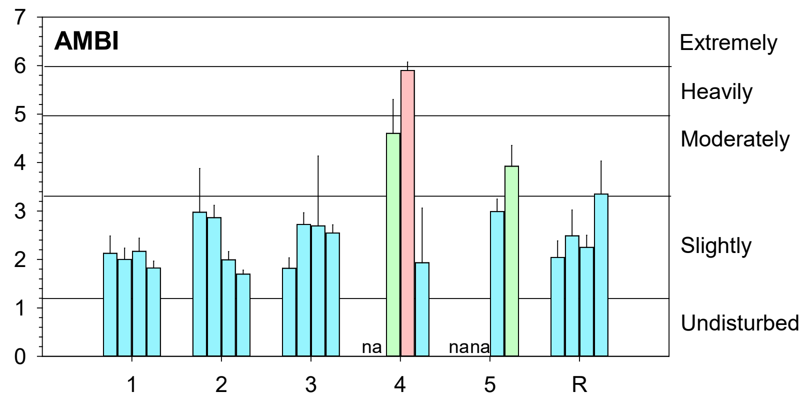


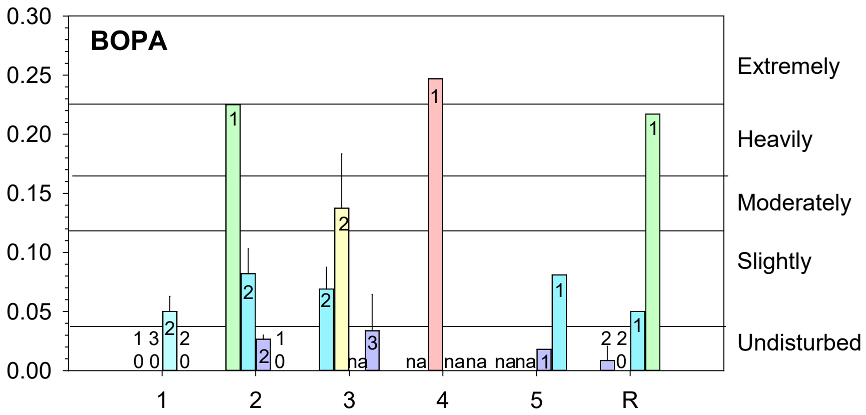
Figure 14 AMBI index for the sampled stations in the three surveys. The classification of ecological status for the sampling points according to the AMBI index is indicated on the right side. Values represent mean +/- SD (n=3). na: no samples available.



10.4.5 BOPA index

The calculation of the BOPA index presented several problems given that for many replicate samples it could not be calculated due to the fact that either the number of individuals were less than 20 or the sum of fA+fP was 0 (Dauvin & Ruellet, 2007; Subida et al., 2012). This resulted in only a few or no replicates being able to be used for the assessment.

Figure 15. BOPA index for the sampled stations in the three surveys. The classification of ecological status for the sampling points according to the BOPA index is indicated on the right side. Values represent mean +/- SD (n=3). na: no samples available or the BOPA index cannot be calculated. In some stations, the BOPA value was 0 as no opportunistic polychaetes of ecological groups 4 and 5 were found, although bivalves could be present. The numbers in the bars indicate the replicates that could be used to calculate the averages given the minimum requirements.



10.4.6 BEQI index

The BEQI index is the only index of the ones used here that is estimated at an ecotope level directly. Even though both sampling sites would be characterised as a sub-littoral transitional waters, they were analysed separately given the difference in the sediment substrate. However, only one reference site from within the disturbed environment of the harbour was available and this was more similar to Sampling Site A. The results for both locations assessed the status of the system as Good.

Table 5: BEQI index results

Sampling site	Similarity EQR	No. of species EQR	Density EQR	Final EGR	EQR NL	
A (St 1-3) Hartelkanaal	Moderate (0.49)	High (1)	Good (0.669)	0.72	0.766	Good
B (St 4-5) Brittaniëhaven	Poor (0.39)	Good (0.633)	High (0.824)	0.616	0.661	Good

10.4.7 Discussion

The overall disturbance status of the sites according to all indexes is characterised as slightly to moderately disturbed and the ecological status generally as Good, with the exception of Station 4 that showed the lowest values and worst ecological status. Although these indices show that the ecological status of most of the stations is Good, some important points have to be considered. First, these indices are designed for soft substrate systems and thus sessile macrofauna, epi- and hyper-fauna have to be excluded. When this was done here, both the species richness and abundance decreased due to the high abundance of barnacles and bivalves in the samples. Despite this, the disturbance levels in the samples that the indices could be applied to did not change dramatically. Second, the reference site is not an undisturbed and pristine site, a general problem for transitional waters even for sites unimpacted from anthropogenic activities. It was considered though as a suitable reference site following the advice of the Port of Rotterdam Authority for a less-polluted site in the port. However, its sediment composition does not really match either that of Site A nor of Site B, therefore the comparison, which provides better ecological status to the sites compared to the rest of the indices, should be taken with caution. Finally, it is interesting to note that the reference site was the site that showed the largest variability between replicates both within and between sampling cruises. Previous studies have shown variable effects in terms of the impacts of brine discharge on the benthic communities, often not being distinguishable from other environmental factors which confound any interpretations such as grain size distribution of the substrate, distance from source etc (Raventos et al., 2006; Riera et al., 2012; Lykkebo Petersen et al., 2019).

If we analyze in more detail the composition of the communities found in the area and we compare them with other studies, we recorded a similar macrobenthic composition, although with a lower abundance and diversity in comparison with nearby environments of the North Sea. Jensen et al. (1992), in a study in the Danish Wadden Sea, found results similar to those of Hojer (1990), and a similar species list to what we found based upon our samples. Those studies were focused on the "*Corophium*-bed" community, represented with species such as *Corophium volutator, Peringia* (Hydrobia) *ulvae, Macoma balthica*, *Mya arenaria, Hediste diversicolor* or *Heteromastus filiformis*. Most of these species, which essentially form part of the *Macoma balthica* Community, were found in our samples. Although the *Macoma balthica* Community is obviously characterized by the presence of *Macoma balthica*, this can be occasionally replaced by another bivalve, *Scrobicularia plana*, as occurred in some samples of our study. This is a community typical of areas with a different grain size to the one found here, but with a tendency towards fine and muddy sediments, which are often related to increased organic matter and to low oxygen and high hydrogen sulfide concentrations in the sediment. However, this community is important to improve and maintain healthy sediment conditions as many of its members (e.g., *Corophium volutator*, *Macoma balthica*, *Mya arenaria*, *Hediste diversicolor*) are important bioturbators reworking and oxygenating the sediment in the process (Michaud et al, 2006).

Other species typical of this community such as members of the Spionidae family and the polychaete *Capitella capitata* were also found. *C. capitata* is widely cited as an indicator species of pollution related to high concentrations of organic matter and environments contaminated with polycylic aromatic hydrocarbons (PAH) (Grassle & Grassle, 1974). However, according to Warren (1977) and Gray (1981), its presence in these areas is due to the opportunistic characteristic of the species which allows *C. capitata* to continuously re-populate disturbed areas rather than its tolerance to anoxia and hydrogen sulfide. In addition, in estuaries, *C. capitata* tends to show higher abundances in the more marine areas, rather than the brackish ones (Ysebaert et al., 1993), which agrees with what we found here; highest abundances were recorded at Station 4 which together with Station 5 are influenced only by marine water. Thus, the distribution of the intertidal macrozoobenthic species, like *Capitella capitata*, seems to be controlled mainly by salinity rather than sediment organic matter. This pattern has been observed in similar systems elsewhere (Wolff, 1973; Michaelis, 1983; Robineau et al., 1984; McLsky, 1987), although it should be taken into account that estuaries and port areas have their own particular physico-chemical conditions.

On the other hand, Ysebaert et al. (1993) related the absence in the brackish waters of the Schelde of typical species of this community, such as *Streblospio shrubsolii*, with pollution and anthropogenic disturbance. The same reasons have been used to explain the low penetration in the estuary of euryhaline species, such as *Hediste diversicolor* or *Corophium volutator* (Ysebaert et al, 1993). In our case, however, we found high abundances of *S. shrubsolii* at Site 2 and Site 3, as well as Site 5, which could indicate lower levels of contamination compared to historical data.

Several studies have found a higher complexity and biodiversity in polyhaline ecotopes than for mesohaline ecotopes. Thus, de Jong et al. (2015) found higher species richness along the Dutch coastal zone in front of the Port of Rotterdam. In contrast, Ysebaert et al. (1993, 2005) found lower richness but much higher abundances in the adjacent Schelde estuary. Wijnhoven et al. (2008) in a historical study of the inner Rhine-Meuse estuary found similar abundances and species richness and diversity as those found here; however, the sampling zone and community were more meso- to oligohaline.

In our case, the comparatively more diverse area was the brackish water site (Stations 1, 2 and 3), probably due to the position of the more marine sampling points (Stations 4 and 5) in our study in an enclosed area with no water renewal and within the estuarine section of the port. In addition, the micro-ecosystem associated to the *Crassostrea gigas* and *Mytilus edulis* reefs - both these species were found at Stations 1, 2 and 3 - increased the habitat diversity and the existence of microniches available for colonization, such as Cirripedia (barnacles) and other hard bottom species, a typical case of ecosystem engineering (Markert et al. 2010). Higher diversities in association with this type of reefs in the area, similar to the ones found here, have been described previously (van Broekhoven 2005; Christianen et al., 2018). Regarding the coexistence of M. edulis and C. gigas, several studies on the competition between the two species have shown that Crassostrea benefits from higher temperatures (Wrange et al., 2010). In our study, it is necessary to study the reason behind the larger abundances of *C. gigas* at Site 1, just under one of the outfalls.

11 Conclusions

This study has established a baseline understanding of the environmental conditions in the vicinity of DWP Botlek and DWP Maasvlakte in the Port of Rotterdam and provides essential background information for the assessment of environmental benefits from the implementation of large-scale ZERO BRINE technology in the future.

A remarkable diversity of taxa has been observed, enabling a detailed characterization of biological communities, which constitutes a significant asset considering how little published literature exists about the unique system of the Port of Rotterdam.

We recorded a similar macrobenthic composition, although with a lower abundance and diversity in comparison with nearby environments of the North Sea. Even when we consider the invasive species present and the negative biodindicators (Spionidae and Capitellidae Families) in the area, the present data confirm that the community established in our study area is comparable to what has been observed in similarly impacted areas previously (Reise et al, 1994 among other studies). Therefore, it is necessary to continue monitoring periodically the area to detect any potential impact of the desalination plant in the long term.

It is necessary to study the reason behind the larger abundances of *C. gigas* at Site 1, just under one of the outfalls. Temperature measurements and other physico-chemical variables would help understand its distribution in this area, since the temperatures recorded in the area are lower than the 20º C that *C. gigas* needs for the recruitment of its larvae (Wrange et al., 2010). More data on the current velocities, winds and turbulence in Site 1 would also help explaining the presence of *C. gigas* presence as they have shown to determine the conditions necessary for its initial establishment and growth to a full-scale reef (Markert et al. 2010; Kluijver & Leewis, 1994).

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13 References

A. Belatouia, H. Bouabessalam, O. R. Hacene, J. A. de-la-Ossa-Carretero, E. Martinez-Garcia, J. L. Sanchez-Lizaso, 2017. Environmental effects of brine discharge from two desalination plants in Algeria (South Western Mediterranean), doi:10.5004/dwt.2017.20812

A. Borja, J. Franco, V. Pérez, 2000. A Marine Biotic Index to Establish the Ecological Quality of Soft-Bottom Benthos Within European Estuarine and Coastal Environments, Marine Pollution Bulletin 40(12) p. 1100-1114.

A. Borja, I. Muxica, and F. Franco, 2003. The application of a Marine Biotic Index to different impact sources affecting soft-bottom benthic communities along European coasts, Marine Pollution Bulletin 46, p. 835–845.

A. Borja, S. L. Marin, I. Muxika, L. Pino, J. G. Rodriguez, 2015. Is there a possibility of ranking benthic quality assessment indices to select the most responsive to different human pressures? Marine Pollution Bulletin 97, p. 85–94.

W. van Broekhove, 2005. Macrofaunal diversity on beds of the Pacific oyster (Crassostrea gigas) in the Oosterschelde estuary. RIVO-Netherlands Institute for Fisheries Research.

Bruyne, R.H. de, van Leeuwen, S., Gmelig Meyling, A.W. & Daan, R. (eds), 2013. Schelpdieren vanhet Nederlanse Noordzeegebied Ecologische atlasvan de mariene weekdieren (Mollusca): 1-414. Tirion Uitgevers/Stichting Anemoon, Utrech/Bennebroek

R. Chesher, 1971. Biological impact of a large-scale desalination plant at Key West, Florida, in Elsevier Oceanography Series p. 99-164.

M.J.A. Christianen, W. Lengkeek, J.H. Bergsma, J.W.P. Coolen, K. Didderen, M. Dorenbosch, F.M.F. Driessen, P. Kamermans, E. Reuchlin-Hugenholtz, H. Sas, A. Smaal, K.A. van den Wijngaard, & T.M. van der Have, 2018. Return of the native facilitated by the invasive? Population composition, substrate preferences and epibenthic species richness of a recently discovered shellfish reef with native European flat oysters (Ostrea edulis) in the North Sea, Marine Biology Research, 14:6, 590-597, DOI: 10.1080/17451000.2018.1498520.

G. F. Clark, N. A. Knott, B. M. Miller, B. P. Kelaher, M. A. Coleman, S. Ushiama, E. L. Johnston, 2018. First large-scale ecological impact study of desalination outfall reveals trade-offs in effects of hypersalinity and hydrodynamics, Water Research 145, p. 757-768.

P.K. Cornejo, M.V.E. Santana, D.R. Hokanson, J.R. Mihelcic, Q. Zhang, 2014. Carbon footprint of water reuse and desalination: a review of greenhouse gas emissions and estimation tools, Journal of Water Reuse and Desalination 4(4) p. 238-252.

J.C. Dauvin, T. Ruellet, 2007. Polychaete/amphipod ratio revisited, Marine Pollution Bulletin 55, p. 215–224.

J.C. Dauvin, T. Ruellet, N. Desroy, A.L. Janson, 2007. The ecology quality status of the Bay of Seine and the Seine estuary: Use of biotic indices, Mar. Pollut. Bull. 55, p. 241–257

J.A. de-la-Ossa-Carretero, Y. Del-Pilar-Ruso, A. Loya-Fernández, L.M. Ferrero-Vicente, C. Marco-Méndez, E. Martinez-Garcia, F. Giménez-Casalduero, J.L. Sánchez-Lizaso, 2016. Bioindicators as metrics for environmental monitoring of desalination plant discharges, Marine Pollution Bulletin 103, p. 313–318.

Y. Del-Pilar-Ruso, J.A. De-la-Ossa-Carretero, F. Gimenez-Casalduero, J.L. Sanchez-Lizaso, 2007. Spatial and temporal changes in infaunal communities inhabiting soft-bottoms affected by brine discharge, Marine Environmental Research 64(4) p. 492-503.

Y. Del-Pilar-Ruso, J.A. De-la-Ossa-Carretero, F. Gimenez-Casalduero, J.L. Sanchez-Lizaso, 2008. Effects of a brine discharge over soft bottom Polychaeta assemblage, Environmental Pollution 156(2) p. 240-250.

Dutch Ministry of Transport, Public Works and Water Management, RIZA Institute for Inland Water Management and Wastewater Treatment (H. Warmer,R. van Dokkum), 2002. Water pollution control in the Netherlands, Policy and Practice 2001.

R. Einav, K. Harussi, D. Perry, 2002. The footprint of the desalination processes on the environment. Desalination 152, p. 141–154.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for community action in the field of water policy. Off. J. Eur. Communities 2000.

European Commission, 2008. Directive 2008/56/EC of the European Parliament and of the Council of of 17 June 2008 establishing a framework for community action in the field of marine environmental policy.

European Commission, 2008. Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council.

European Environment Agency, 2021. Natura 2000 Network Viewer. Available from: https://natura2000.eea.europa.eu/.

M.A. Faasse, H. Gheerardyn, C. Morys, T. van Haaren, T. Ysebaert, R. Nijland, 2019. The non-indigenous window shell Theora lubrica. Gould, 1861 (Bivalvia: Cardiida: Semelidae) in the delta area of the Netherlands. – Basteria, 83 (1-3): 52-58. Leiden.

Y. Fernandez-Torquemada, J.M. Gonzalez-Correa, J.M., J.L. Sanchez-Lizaso, 2013. Echinoderms as indicators of brine discharge impacts. Desalination Water Treat. 51, p. 567-573.

Y. Fernandez-Torquemada, J.L. Sanchez-Lizaso, J.M. Gonzalez-Correa, 2005. Preliminary results of the monitoring of the brine discharge produced by the SWRO desalination plant of Alicante (SE Spain), Desalination 182, p. 395–402.

J.F. Grassle, J.P. Grassle, 1974. Opportunistic life histories and genetic systems in marine benthic polychaetes, Journal of Marine Research 32(2) p. 253-284.

J.S. Gray, 1981. The ecology of marine sediments. An introduction to the structure and function of benthic communities. Cambridge: Cambridge University Press. 185.

J.L.G. Gesteira, J.C. Dauvin, 2000. Amphipods are Good Bioindicators of the Impact of Oil Spills on Soft-Bottom Macrobenthic Communities, Marine Pollution Bulletin 40(11) p. 1017-1027.

HELCOM. HELCOM Biodiversity Database. 2004-2020; Available from: https://maps.helcom.fi/website/biodiversity/.

T. Hopner, J. Windelberg, 1996. Elements of environmental impact studies on the coastal desalination plants. Desalination 108, p. 11–18.

J. Hyland, L. Balthis, I. Karakassis, P. Magni, A. Petrov, J. Shine, O. Vestergaard, R. Warwick, 2005. Organic carbon content of sediments as an indicator of stress in the marine benthos, Marine Ecology Progress Series, Vol. 295: 91–103, 2005

ICPR. International 2021; Available from: https://www.iksr.org/.

IMO official website, https://www.imo.org/en/

K.T. Jensen, K.N. Mouritsen, 1992. Mass mortality in 2 common soft-bottom invertebrates, Hydrobia ulvae and Corophium volutator - The possible role of trematodes, Helgolãnder Meeresuntersuchungen 46(3) p. 329-339.

O.R. Johnston, 2005. Distribution and biology of the marine invasive bivalve Theora lubrica (Semelidae): 1-213. Thesis, University of Canterbury, New Zealand

M. F. de Jong, M. J. Baptist, H. J. Lindeboom, P. Hoekstra, Relationships between macrozoobenthos and habitat characteristics in an intensively used area of the Dutch coastal zone. – ICES Journal of Marine Science, 72: 2409–2422.

M.J. de Kluijver, R.J. Leewis, 1994. Changes in the sublittoral hard substrate communities in the Oosterschelde estuary (SW Netherlands), caused by changes in the environmental parameters, Hydrobiologia 282(1) p. 265-280.

L Klunder, M Lavaleye, LK Schaars, R Dekker, S Holthuijsen, HW van der Veer. 2019. Distribution of the dwarf surf clam Mulinia lateralis (Say, 1822) in the Wadden Sea after first introduction. BioInvasions Records 8(4): 818–827,

F.C. Küpper, N.A. Kamenos, 2018. The future of marine biodiversity and marine ecosystem functioning in UK coastal and territorial waters (including UK Overseas Territories) – with an emphasis on marine macrophyte communities, Botanica Marina 61(6) p. 521-535.

S. Lattemann, T. Hopner, 2008. Environmental impact and impact assessment of seawater desalination, Desalination 220(1-3) p. 1-15.

K. Lykkebo P., K, N. Heck, B.G. Reguero, D. Potts, A. Hovagimian, A. Paytan, 2019. Biological and Physical Effects of Brine Discharge from the Carlsbad Desalination Plant and Implications for Future Desalination Plant Constructions. Water, 11, 208.E. Mandelli, 1975. The effects of desalination brines on Crassostrea virginica (Gmelin), Water Research 9 (1975) p. 287-295.

A. Markert, A. Wehrmann, I. Kröncke, 2009. Recently established Crassostrea-reefs versus native Mytilus-beds: differences in ecosystem engineering affects the macrofaunal communities (Wadden Sea of Lower Saxony, southern German Bight), Biological Invasions 12 p. 15-32.D.S. McLusky, 1987.

Intertidal habitats and benthic macrofauna of the Forth estuary, Scotland, Proceedings of the Royal Society of Edinburgh. Section B. Biological Sciences 93(3-4) p. 389-399.

H. Michaelis, 1983. Intertidal benthic animal communities of the estuaries of the rivers Ems and Weser. Ecology of the Waden Sea. Vol. 1.

E. Michaud, G. Desrosiers, F. Mermillod-Blondin, B. Sundby,G. Stora, 2006. The functional group approach to bioturbation: II. The effects of the Macoma balthica community on fluxes of nutrients and dissolved organic carbon across the sediment–water interface, Journal of Experimental Marine Biology and Ecology 337(2) p. 178-189.

P. Paalvast, 2002. Historische ontwikkeling estuariene ecotopen in de Noordrand van het benedenrivierengebied. In opdracht van het Rijksinstituut voor Kust en Zee/RIKZ. Ecoconsult, rapportnr.

P. Paalvast, B. K. van Wesenbeeck, G. van der Velde, M. B. de Vries, 2012. Pole and pontoon hulas: An effective way of ecological engineering to increase productivity and biodiversity in the hard-substrate environment of the port of Rotterdam, Ecological Engineering 44, p. 199– 209.

N. Phillips, C.M. Smith, C.W. Morden, 2001 An effective DNA extraction protocol for brown algae. Phycological Research 49, p. 97-102.

P.o.R. Authority, 2020. Funding Awarded to Brielse Meer project.

P.o.R. Authority, 2019. Highlights of 2019 Annual Report, Rotterdam, Netherlands.

N. Raventos, E. Macpherson, A. García-Rubiés, 2006. Effect of brine discharge from a desalination plant on macrobenthic communities in the NW Mediterranean. Marine Environmental Research. Volume 62, Issue 1, July 2006, Pages 1-14.

K. Reise, E. Herre, M. Sturm, 1994. Biomass and abundance of macrofauna in intertidal sediments of Königshafen in the northern Wadden Sea, Helgolãnder Meeresuntersuchungen 48(2) p. 201-215.

D.J. Reish, 1955. The relation of polychaetous annelids to harbor pollution. — Public Health Reports 70: 1168-1174.

R. Riera, F. Tuya, E. Ramos, M. Rodríguez, O. Monterroso, 2012. Variability of macrofaunal assemblages on the surroundings of a brine disposal. Desalination Volume 291, 2 April 2012, Pages 94-100.

Rijkswaterstaat, 2021. Rijkswaterstaat Water Info portal; Available from https://waterinfo.rws.nl/#!/nav/index/.

B. Robineau, J. Marchand, 1984. Distribution des communautes macrobenthiques des substrats meubles de l'estuarie interne de la Loire (France) Journal Recherche Océanographique 9 p. 146-149.

J.J. Sadhwani, J.M. Veza, C. Santana, 2005. Case studies on environmental impact of seawater desalination, Desalination 185, p. 1–8

A.J.M Smits, P.H. Nienhuis, H.L.F., Saeijs, 2006. Changing estuaries, changing views. Hydrobiologia 565, p. 339–355.

H.J. Spanjer, D. Xevgenos, ZEROBRINE (Grant Agreement no. 730390) - D2.2 Wastewater characterization: Physiochemical analyses of the wastewater composition of WP2, Ref.

Ares(2018)4649581 - 11/09/2018. 2018, European Commission under the Horizon 2020 programme: Delft, Netherlands.

Stockholm convention on persistent pollutants, official website, http://www.pops.int/

L. M. Warren, 1977. The ecology of Capitella capitata in British waters. Journal of the Marine Biological Association of the United Kingdom, 57 (1): 151-159.

S. Wijnhoven, W. Sistermans, H. Hummel, 2008. Historic developments in macrozoobenthos of the Rhine–Meuse estuary: From a tidal inlet to a freshwater lake estuarine, Coastal and Shelf Science Volume 76, Issue 1, 1 January 2008, Pages 95-110.

WISE, WISE-WFD database, 2015. Available from: http://cdr.eionet.europa.eu/nl/eu/wfd2016/

W.J. Wolff, 1978. The degradation of ecosystems in the Rhine. In: Holdgate, M.W., Woodman, M.J. (Eds.), The Breakdown and Restoration of Ecosystems. Plenum Press, New York, p. 167–187.

W.J. Wolff, 1999. The estuary as a habitat. An analysis of data on the soft-bottom macrofauna of the estuarine area of the rivers Rhine, Meuse and Scheldt, Zool. Verh. Leiden 126 p. 1-242.

World Economic Forum, 2021, Global Risks 2021, 16th Edition

A.L. Wrange, J. Valero, L.S. Harkestad, Ø. Strand, S. Lindegarth, H.T. Christensen, P. Dolmer, P.S. Kristensen,S. Mortensen, Massive settlements of the Pacific oyster, Crassostrea gigas, in Scandinavia, Biological Invasions 12(5) (2010) p. 1145-1152.

T. Ysebaert, M. Fettweis, P. Meire, M. Sas, Benthic variability in intertidal soft-sediments in the mesohaline part of the Schelde estuary Hydrobiologia, 540 (2005), pp. 197-216.

T. Ysebaert, P. Meire, D. Maes, J. Buijs, 1993. The benthic macrofauna along the estuarine gradient of the Schelde estuary, Netherland Journal of Aquatic Ecology 27(2) p. 327-341.

14 Appendix A:

Table 6: Average effluent inorganic and organic concentrations of IEX and RO streams at DWP Botlek

Parameter	Unit	IEX_EXP01	IEX_EXP02	IEX_EXP03	IEX_EXP04	RO_EXP01	RO_EXP02	RO_EXP03	RO_EXP04
Sodium (Na)	mg/L	1703	7974	8145	6307	845	1202	959	1056
Magnesium (Mg)	mg/L	1248	1337	1069	1414	0.17	2.17	0.07	0.06
Potassium (K)	mg/L	236	228	321	257	13.4	14.3	0	18.3
Calcium (Ca)	mg/L	6523	8538	7211	7038	0.52	3.34	2.16	2.30
Silica (SiO2)	mg/L	1.97	0	0	0	42	38	28	16
Iron (Fe)	mg/L	0	4.13	0.49	0.25	0	0.30	0.02	0.02
Strontium	mg/L	25	42	35	40	2.85	0	8.15	8.18
Titanium (Ti)	µg/L	0.00	17.04	31.99	41.60	1.19	0	0	0
Vanadium (V)	µg/L	84.57	274	0.58	0.00	5.38	4.72	0.05	0.16
Chromium (Cr)	µg/L	13.77	154	40.0	6.14	1.81	4.09	11.3	5.10
Arsenic (As)	µg/L	15.31	0	1.76	2.38	1.01	0	0.99	2.01
Selenium (Se)	µg/L	3.63	0.66	43.7	28.23	0.69	1.75	8.27	7.35
Lithium (Li)	µg/L	119	363	64.3	114	45.8	83.3	49.9	93.5
Boron (B)	µg/L	20	67	1807	2223	122	123	183	98
Aluminum (Al)	µg/L	0.14	1020	4.32	2447	0.70	2.70	0.06	0.06
Manganese (Mn)	µg/L	10.21	226.81	0	0	0	0	0	0.45
Cobalt (Co)	µg/L	0	88.98	4.86	2.35	0	2.92	1.61	1.81
Nickel (Ni)	µg/L	205	2858	82.4	3.63	9.02	13.6	20.1	22.1
Copper (Cu)	µg/L	34.16	59.52	0	60.45	12.9	0	51.2	7.54
Zinc (Zn)	µg/L	103	156	173	44.6	18.0	0	71.6	36.3
Molybdenum (Mo)	µg/L	1.27	13.81	7.61	0.37	9.31	10.7	7.63	12.7
Silver (Ag)	µg/L	0.04	11.12	17.98	18.21	0.15	0	0.83	0.99
Cadmium (Cd)	µg/L	0.35	0	14.19	12.22	0.01	0	0.04	0.03
Antimony (Sb)	µg/L	0.59	22.8	0	0	1.56	1.77	1.26	1.87
Barium (Ba)	µg/L	3554	4919	4436	5279	0.60	0	4.62	3.10
Thallium (Tl)	µg/L	0.52	0	0	0	Not measured	Not measured	Not measured	Not measured
Lead (Pb)	µg/L	0.03	220	502	424	0.16	7.10	3.63	4.25
Chloride (Cl)	mg/L	17821	31305	28569	26440	514	1122	704	846
Nitrate (NO3)	mg/L	43.7	22.9	51.9	30.2	39.4	7.32	53.4	22.4
Phosphate (PO4)	mg/L	1.78	0.29	0.02	0.72	0	2.93	0.03	0.05
Bicarbonate (HCO3)	mg/L	143	140	115	109	871	863	947	955
Sulphate (SO4)	mg/L	149	212	124	77	371	335	271	320
Total dissolved solids (TDS)	mg/L	27874	49772	45614	41683	2696	3591	2966	3237
POC	(µg/L C)	Not measured	Not measured	Not measured	Not measured	38	18	60	-82
DOC	(µg/L C)	Not measured	Not measured	Not measured	Not measured	9460	13750	12275	11500
TOC	(µg/L C)	Not measured	Not measured	Not measured	Not measured	9498	13800	12325	11425
Electrical conductivity (EC)	mS/cm	43.4	80.25	76.4	69.6	3.22	4.03	3.30	4.09
Averaged pH	-	7.26	7.08	6.86	6.66	9.8	8.81	8.87	8.79

EXP01: Sampling period 12-11-2017 to 27-12-2017 (for RO taken separately on 14-02-2018), EXP02: Sampling period 14-03-2018 to 28-03-2018, EXP03: Sampling period 03-04-2018, EXPO4: 11-07-2018.

Table 7: Measured values of physico - chemical parameters, nutrients, metals, and PAHs in water samples in comparison in comparison to the MPC and Target Value (Ref. Dutch Ministry of Transport, Public Works and Water Management, 2002 - Staatscourant, The Netherlands, June 2000). ne: not examined, (z): the value of z/kv : the value of 0.4 mg P/l holds for sandy sediments. The value of 3.0 mg P/l holds for clay-based and peaty soils the value of 2 mg N/l holds for sandy soils, the value of 10 mg N/l holds for clay-based and peaty soils.

		1st sampling survey		2nd sampling survey			3rd sampling survey			4th sampling survey					
Parameters	Unit	S1	R	S1	S4	R	S1	S4	R	S1	S4	R	Target Value	MPC	RBMP Rhine 2015
pH		7.9	7.9	7.9	7.9	8.1	8.2	8.3	8.5	7.4	7.1	7.3		6.5-9	
EC	µS/cm	18000	23000	8500	>13000	5600	>13000	>13000	>13000	10000	33000	2600			
NH_4^+	mg/l	<0.2	<0.2	<0,06	0.10	0.064	0.24	0.24	0.21	<0.2	0.2	<0.2			
NO_2^-	mg/l	<0.3	<0.3	0.066	0.099	0.066	0.13	0.23	0.099	<0.3	0.68	<0.3			
NO_3^-	mg/l	<0.75	<0.75	12	8.8	12	4.2	1.8	4.3	1.2	<0.75	11			
Kjeldahl nitrogen	mgN/l	ne	ne	ne	ne	ne	ne	ne	ne	<0.5	1.1	0.9			
PO_4^{3-}	mg/l	0.077	0.073	<0.05	0.12	0.06	0.13	0.03	0.14	<0.1	0.19	<0.1			
P_T	mgP/l	ne	ne	ne	ne	ne	ne	ne	ne	<0.15	<0.15	<0.15	0.05 (z)	0.15 (z)	
As	µg/l	ne	ne	ne	ne	ne	<2	2.3	<2	1.6	2.7	1.6	1.3	32	
Cd	µg/l	ne	ne	ne	ne	ne	<0.1	<0.1	<0,1	<0.2	<0.2	<0.2	0.4	2	1
Cr	µg/l	ne	ne	ne	ne	ne	<1	<1	<1	<1	<1	<1	2.4	84	
Fe	µg/l	ne	ne	ne	ne	ne	96	170	77	2.4	2.6	2.1			
Cu	µg/l	ne	ne	ne	ne	ne	21	5.9	22	<0.05	<0.05	<0.05	1.1	3.8	
Pb	µg/l	ne	ne	ne	ne	ne	<1	6.8	<1	<2	<2	<2	5.3	220	14
Ni	µg/l	ne	ne	ne	ne	ne	1.3	1.5	1,4	<3	<3	<3	4.1	6.3	34
Zn	µg/l	ne	ne	ne	ne	ne	21	31	12	<10	<10	<10	12	40	
Naphthalene	µg/l	ne	ne	ne	ne	ne	<0.05	<0.05	<0.05	<0.1	<0.1	<0.1	0.01	1.2	130
Phenanthrene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.003	0.3	
Anthracene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.0008	0.08	0.1
Fluoranthene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.005	0.5	0.12
Benz[a]anthracene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.0003	0.03	
Chrysene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.009	0.9	
Benzo[k]fluoranthene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.002	0.2	
Benzo[a]pyrene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.002	0.2	0.027
Benzo[ghi]perylene	µg/l	ne	ne	ne	ne	ne	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.005	0.5	
Indeno[1,2,3-cd]pyrene	µg/l	ne	ne	ne	ne	ne	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.004	0.4	
Acenaphthene	µg/l	ne	ne	ne	ne	ne	<0.05	<0.05	<0.05	<0.1	<0.1	<0.1			
Acenaphthylene	µg/l	ne	ne	ne	ne	ne	<0.05	<0.05	<0.05	<0.1	<0.1	<0.1			
Dibenz[a,h]anthracene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02			
Fluorene	µg/l	ne	ne	ne	ne	ne	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05			
Pyrene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02			

Table 8: Measured values of physical parameters, metals, and PAHs in sediment samples in comparison to the MPC and Target Value (Ref. Dutch Ministry of Transport, Public Works and Water Management, 2002 - set in the Staatscourant, The Netherlands, June 2000)

		3rd sampling survey			4th sampling survey			TARGET VALUE **(dry matter) Long term**	MPC **(dry matter) Short term**
Parameters	Unit	S3	S4	R	S1	S4	R		
Dry matter	% (w/w)	79.2	48.3	77.0	69.2	55.3	65.6		
Total Organic Carbon (TOC)	g/kg dm	<5.0	15	8.7	6.5	35	6.7		
Kjedahl nitrogen	mgN/kgdm	ne	ne	ne	605	4180	800		
Fraction < 2000 µm	% (w/w)	87.0	61.5	89.1	ne	ne	ne		
Fraction < 1000 µm	% (w/w)	86.8	61.2	89.0	ne	ne	ne		
Fraction < 500 µm	% (w/w)	86.5	60.9	89.0	ne	ne	ne		
Fraction < 250 µm	% (w/w)	81.2	59.6	87.9	ne	ne	ne		
Fraction < 125 µm	% (w/w)	28.3	46.6	23.0	ne	ne	ne		
Fraction < 63 µm	% (w/w)	1.3	30.4	4.7	ne	ne	ne		
Fraction < 45 µm	% (w/w)	6.0	27.5	4.1	ne	ne	ne		
Fraction < 16 µm	% (w/w)	4.6	18.7	2.9	ne	ne	ne		
Fraction < 2 µm, gravimetric	% (w/w)	3.1	9.9	<2.0	ne	ne	ne		
Metals									
Arsenic (As)	mg/kg dm	<5.0	11	<5.0	4.6	5.4	8.3	29	55 #
Cadmium (Cd)	mg/kg dm	<0.40	0.45	<0.40	0.21	0.88	0.28	0.8	12 #
Chromium (Cr)	mg/kg dm	12	31	16	16	19	17	100	380 #
Copper (Cu)	mg/kg dm	<5.0	43	6.5	7.8	14	8.7	36	73
Iron (Fe)	mg/kg dm	5700	21000	4800	7800	9700	10000		
Mercury (Hg)	mg/kg dm	<0.10	0.18	<0.10	0.08	0.10	0.11	0.3	10 #
Nickel (Ni)	mg/kg dm	5.8	17	5.9	7.8	8.2	10	35	44
Lead (Pb)	mg/kg dm	<10	22	<10	14	14	20	85	530 #
Zinc (Zn)	mg/kg dm	35	160	28	57	140	80	140	620
Polycyclic Aromatic Hydrocarbons (PAHs)*									
Napthalene	mg/kg dm	<0.010	0.029	<0.010	<0.02	0.08	0.03	0.001	0.1
Acenapthylene	mg/kg dm	<0.010	<0.010	<0.010	<0.02	<0.02	<0.02		
Acenapthene	mg/kg dm	<0.010	0.022	<0.010	<0.02	0.05	0.04		
Fluorene	mg/kg dm	<0.010	0.018	<0.010	<0.02	0.06	0.05		
Phenanthrene	mg/kg dm	0.017	0.075	0.012	0.03	0.19	0.16	0.005	0.5
Anthracene	mg/kg dm	0.011	0.031	<0.010	<0.02	0.04	0.07	0.001	0.1
Fluoranthene	mg/kg dm	0.041	0.17	0.026	0.07	0.23	0.45	0.03	3
Pyrene	mg/kg dm	0.036	0.16	0.022	0.06	0.20	0.36		
Benzo(a)anthracene	mg/kg dm	0.026	0.066	0.017	0.04	0.09	0.22	0.003	0.4
Chrysene	mg/kg dm	0.030	0.059	0.019	0.04	0.09	0.21	0.1	11
Benzo(b)fluoranthene	mg/kg dm	0.042	0.13	0.024	0.06	0.11	0.30		
Benzo(k)fluoranthene	mg/kg dm	0.017	0.048	0.011	0.03	0.05	0.13	0.02	2
Benzo(a)pyrene	mg/kg dm	0.024	0.065	0.013	0.04	0.08	0.22	0.003	3
Dibenzo(ah)anthracene	mg/kg dm	<0.010	<0.010	<0.010	<0.02	<0.02	0.04		
Benzo(ghi)perylene	mg/kg dm	0.022	0.083	0.012	0.04	0.06	0.15	0.08	8
Indeno(123cd)pyrene	mg/kg dm	0.022	0.073	0.016	0.03	0.06	0.16	0.06	6
PAH 10 VROM (sum)	mg/kg dm	0.21	0.70	0.13	0.32	0.97	1.8		
PAH 16 EPA (sum)	mg/kg dm	0.29	1.0	0.17	0.44	1.4	2.6		

**no soil type correction for sandy sediments (organic matter < 10%)*
#value = intervention value

Table 9: Species list

Abra alba
Abra nitida
Actinia equina
Alitta (Neanthes) cf succinea
Ampelisca brevicornis
Amphibalanus improvisus
Anomia ephippium
Aphelochaeta marioni
Ascidiidae sp.
Asterias rubens
Austrominius modestus
Balanus cf crenatus
Capitella capitata
Carcinus maenas
Cardiidae juv.
Chaetozone gibber
Chaetozone setosa
Varicorbula gibba
Corophium volutator
Cossura longocirrata
Crassostrea (Magallana) gigas
Crepidula fornicata
Cyathura carinata
Dreissena polymorpha
Ensis cf leei
Ericthonius punctatus
Ficopomatus enigmaticus
Gammaridae
Gastrosaccus spinifer
Glycera tridactyla
Hediste diversicolor
Hemigrapsus takanoi
Heteromastus filiformis
Hydroides ezoensis
Hydroides sp. *
Lagis koreni
Lanice conchilega
Laonome kroyeri
Magelona filiformis
Melita hergensis
Metridium senile VAR pallidus
Monocorophium acherusicum ♀
Mya arenaria
Mytilus edulis
Nephtys cirrosa
Nephtys hombergii
Nepthys kersivalensis
Nereis longissima
Nereis zonata
Orbinia latreillii
Oxydromus flexuosus
Palaemon longirostris
Perinereis cultrifera
Peringia (Hydrobia) ulvae
Phaxas pellucidus

Pherusa plumosa
Pholas dactylus
Phyllodoce lineata
Platynereis dumerilii
Polydora ciliata
Pseudopolydora paucibranchiata
Rhithropanopeus harrisii
Ruditapes philippinarum
Sagartiidae sp.
Scoloplos armiger
Scrobicularia plana
Sinelobus stanfordi
Sphenia sp.
Spiophanes bombyx
Spisula subtruncata
Streblospio cf shrubsolii
Tellina (Fabulina) fabula
Tellina tenuis
Tharyx cf killariensis
Theora lubrica
Tritia (Nassarius) reticulata
Varicorbula gibba
Venerupis corrugata
Websterinereis glauca

Table 10: Benthic macrofauna results at sampling stations during the 1st sampling survey (September 2019)

				PORT OF ROTTERDAM, FIRST SAMPLING SURVEY, SEPTEMBER 2019													
TAXA				**NATIVE / NON-NATIVE**	**S1**	**S1**	**S1**	**S2**	**S2**	**S2**	**S3**	**S3**	**S3**	**R**	**R**	**R**	**TOTAL**
Annelida	Class Polychaeta	Capitellidae	*Heteromastus filiformis*	Native					1		1					1	3
		Cirratulidae	*Chaetozone gibber*	Native							2						2
			Tharyx cf killariensis	Native					16			4	5				25
		Cossuridae	*Cossura longocirrata*	Native								1					1
		Nephtyidae	*Nephtys hombergii*	Native							2		1				3
		Nereididae	*Alitta (Neanthes) cf succinea*	Native						1							1
			Nereis zonata	Native												5	5
			Websterinereis glauca	Native											1		1
		Ophelidae	*Orbinia latreillii*	Native												1	1
		Orbiniidae	*Scoloplos sp.**	Native							2	1					3
		Phyllodocidae	*Phyllodoce lineata*	Native								1	1			1	3
		Serpulidae	*Ficopomatus enigmaticus*	Non-native		5	1	4	1					1		1	13
		Spionidae	*Spiophanes bombyx*	Native								1					1
		Terebellidae	*Lanice conchilega*	Native								1					1
Arthropoda	Orden Amphipoda	Corophiidae	*Corophium volutator*	Native										9	7	1	17
			Monocorophium acherusicum ♀	Native	1	4			1	5	1						12
		Ischyroceridae	*Ericthonius punctatus*	Native									1				1
		Melitidae	*Melita sp.*♀*	Native												4	4
	Order Decapoda	Varunidae	*Hemigrapsus takanoi*	Non-native	3	1		1								4	9
		Panopeidae	*Rhithropanopeus harrisii*	Non-native										2	1		3
	Order Isopoda	Anthuridae	*Cyathura carinata*	Native												1	1
	Order Sessilia	Balanidae	*Amphibalanus improvisus*	Non-native		16	18	1	1	7				42	3		88
			Balanus cf crenatus	Native			6				1		1				8
Mollusca	Class Bivalvia	Cardiidae	*Cardiidae juv.*	Native							1	4	1	2		1	9
		Dreissenidae	*Dreissena polymorpha*	Non-native											1		1
		Mactridae	*Spisula subtruncata*	Native								1	4			1	6
		Myidae	*Mya arenaria*	Non-native										2	2		4

				PORT OF ROTTERDAM, FIRST SAMPLING SURVEY, SEPTEMBER 2019													
TAXA				**NATIVE / NON-NATIVE**	**S1**	**S1**	**S1**	**S2**	**S2**	**S2**	**S3**	**S3**	**S3**	**R**	**R**	**R**	**TOTAL**
		Mytilidae	*Mytilus edulis*	Native	3	11	1	2	2	2	4						25
		Ostreoidea	*Crassostrea (Magallana) gigas*	Non-native			2		2								4
		Pharidae	*Ensis cf leei***	Non-native							1						1
		Pholadidae	*Pholas dactylus*	Native											1		1
		Tellinidae	*Tellina (Fabulina) fabula*	Native								4					4
			Tellina tenuis	Native									1				1
	Class Gastropoda	Hydrobiidae	*Peringia (Hydrobia) ulvae*	Native										1			1
		Nassariidae	*Tritia (Nassarius) reticulata (reticulatus)*	Native								1					1
Cnidaria	Class Anthozoa	Actiniidae	*Actinia equina*	Native							6	5	4				15
Number of individuals					7	37	28	8	24	15	21	24	19	59	16	21	279
Number of species					3	5	5	4	7	4	10	11	9	7	7	11	36

* broken animals or females
**recently renamed, previously named *Ensis americanus*

Table 11: Benthic macrofauna results at sampling stations in the 2nd sampling survey (January 2020)

				PORT OF ROTTERDAM, SECOND SAMPLING SURVEY, JANUARY 2020																
TAXA				**NATIVE / NON-NATIVE**	**S1**	**S1**	**S1**	**S2**	**S2**	**S2**	**S3**	**S3**	**S3**	**S4**	**S4**	**S4**	**R**	**R**	**R**	**TOTAL**
Annelida	Class Polychaeta	Capitellidae	*Heteromastus filiformis*	Native						3			1							4
			*Capitella capitata*****	Native									1	22	8	2				33
		Cirratulidae	*Chaetozone gibber*	Native				2			3	1			1					7
			Chaetozone setosa	Native								2								2
			Tharyx cf killariensis	Native				12	6		13	8	2		2					43
		Hesionidae	*Oxydromus flexuosus*	Native												1				1
		Magelonidae	*Magelona filiformis*	Native							1									1
		Nephtyidae	*Nephtys hombergii*	Native				1				1	1						1	4
		Nereididae	*Alitta (Neanthes) cf succinea*	Native										7	3	3				13
			Platynereis dumerilii	Native	1	1	4	2	1									1		10
		Orbiniidae	*Scoloplos sp.**	Native				1												1
		Phyllodocidae	*Phyllodoce lineata*	Native				1	4											5
		Sabellaridae	*Laonome kroyeri*	cf*							11		7					2		20
		Serpulidae	*Ficopomatus enigmaticus*	Non-native	5	1	2													8
			*Hydroides sp.**	Native	2	1	2													5
		Spionidae	*Polydora ciliata*	Native							2		1	1		1				5
			Streblospio cf shrubsolii	Native			1	18	9		21	1	1						1	52
		Terebellidae	*Lanice conchilega*	Native							1									1
Arthropoda	Orden Amphipoda	Corophiidae	*Corophium volutator*	Native						1							18	41	7	67
			Monocorophium acherusicum	Native		1			1											2
		Melitidae	*Melita hergensis*	Native	1	5	4	7	2	1								4		24
	Order Decapoda	Palaemonidae	*Palaemon longirostris*	Native	1															1

				PORT OF ROTTERDAM, SECOND SAMPLING SURVEY, JANUARY 2020															
TAXA				**NATIVE / NON-NATIVE**	**S1**	**S1**	**S1**	**S2**	**S2**	**S2**	**S3**	**S3**	**S3**	**S4**	**S4**	**S4**	**R**	**R**	**R**	**TOTAL**
		Panopeidae	*Rhithropanopeus harrisii*	Non-native														1		1
		Varunidae	*Hemigrapsus takanoi*	Non-native	1															1
	Order Isopoda	Anthuridae	*Cyathura carinata*	Native													1	3		4
	Order Mysida	Mysidae	*Gastrosaccus spinifer*	Native					1	1		6								8
	Order Sessilia	Austrobalanidae	*Austrominius modestus*	Non-native			2													2
		Balanidae	*Amphibalanus improvisus*	Non-native	35	21	12	2	3								3	108	2	186
			Balanus cf crenatus	Native		2														2
	Order Tanaidacea	Tanaididae	*Sinelobus stanfordi****	Non-native		1														1
Mollusca	Class Bivalvia	Anomiidae	*Anomia ephippium*	Native					1											1
		Corbulidae	*Varicorbula gibba*	Native						1							2			3
		Mactridae	*Spisula subtruncata*	Native							6	2			1					9
		Myidae	*Mya arenaria*	Non-native									1							1
		Mytilidae	*Mytilus edulis*	Native	5	5	13											1		24
		Ostreoidea	*Crassostrea (Magallana) gigas*	Non-native	8	6	4											1		19
		Pharidae	*Ensis cf leei***	Non-native							1									1
			Phaxas pellucidus	Native							1									1
		Semelidae	*Abra nitida*	Native									1							1
		Tellinidae	*Tellina tenuis*	Native							1									1
		Veneridae	*Ruditapes philippinarum*	Non-native				1	3	4	2	2							1	13
	Class Gasteropoda	Calyptraeidae	*Crepidula fornicata*	Non-native		2	1	1	2											6
		Nassariidae	*Tritia (Nassarius) reticulata*	Native							3									3
Cnidaria	Class Anthozoa	Actiniidae	*Actinia equina*	Native	3	1		1					1							6
Number of individuals					62	47	45	49	33	11	66	23	17	30	15	7	24	162	12	603
Number of species					10	12	10	12	11	6	13	8	10	3	5	4	4	9	5	44

* broken animals or females
** recently renamed, previously named as *Ensis americanus*
*** Van Haaren et al (2009) identified this specie in The Netherlands and Belgium
**** common in harbour areas with hydrocarbon enrichment (Fauna Iberica, CSIC España) actually is considered as instability indicator
***** refer to Paragraph 10.4.1 Identification key points
cf* recently studies are reviewing data because they suggest most of the registers for *Laonome kroyeri* are in fact *Laonome xeprovala*

Table 12: Benthic macrofauna results at sampling stations in the 3rd sampling survey (July 2020)

				PORT OF ROTTERDAM, THIRD SAMPLING SURVEY, JULY 2020																		
TAXA				NATIVE / NON-NATIVE	S1	S1	S1	S2	S2	S2	S3	S3	S3	S4	S4	S4	S5	S5	S5	R5	R5	R5	TOTAL
Annelida	Class Polychaeta	Capitellidae	*Heteromastus filiformis*	Native	3		2		2													2	9
			Capitella capitata	Native										4	2	5							11
		Cirratulidae	*Aphelochaeta marioni*	Native	2		3			3		3										2	13
			Chaetozone gibber	Native	1		1				3											1	6
			Tharyx cf killariensis	Native	7		5			1	3	13								1		1	31
		Flabelligeridae	*Pherusa plumosa*	Native							1												1
		Glyceridae	*Glycera tridactyla*	Native					1														1
		Nephtyidae	*Nephtys cirrosa*	Native													1						1
			Nephtys hombergii	Native				1			2	2	5										10
		Nereididae	*Hediste diversicolor*	Native																		2	2
			Nereis longissima	Native							1		1										2
			Nereis zonata	Native	4	2	6																12
			Perinereis cultrifera	Native	1			1	1	1													4
		Orbiniidae	*Scoloplos armiger*	Native							1	1	1										3
		Pectinariidae	*Lagis koreni*	Native														1					1
		Phyllodocidae	*Phyllodoce sp.**	Native					1														1
		Serpulidae	*Ficopomatus enigmaticus*	Non-native	11	4	4															3	22
		Spionidae	*Polydora ciliata*	Native																		1	1
			Streblospio cf shrubsolii	Native													16	19	15				50
		Terebellidae	*Lanice conchilega*	Native					6		4												10
Arthropoda	Orden Amphipoda	Corophiidae	*Corophium volutator*	Native																	1		1
			Monocorophium acherosicum♀	Native	5		1		1	7													14

					PORT OF ROTTERDAM, THIRD SAMPLING SURVEY, JULY 2020																		
TAXA				**NATIVE / NON-NATIVE**	**S1**	**S1**	**S1**	**S2**	**S2**	**S2**	**S3**	**S3**	**S3**	**S4**	**S4**	**S4**	**S5**	**S5**	**S5**	**R5**	**R5**	**R5**	**TOTAL**
		Melitidae	*Melita hergensis*	Native	8		4		1	1													14
	Order Decapoda	Carcinidae	*Carcinus maenas*	Native					1														1
		Varunidae	*Hemigrapsus takanoi*	Non-native		5	2														1		8
	Order Sessilia	Austrobalanidae	*Austrominius modestus*	Non-native	12	8	6	20		12												14	72
		Balanidae	*Amphibalanus improvisus*	Non-native	41	25	21	8		25												31	151
			Balanus cf crenatus	Native	8		1	2		4													15
Mollusca	Bivalvia	Corbulidae	*Corbula gibba*	Native										1			4	3	3				11
		Mactridae	*Spisula subtruncata*	Native													3						3
		Myidae	*Mya arenaria*	Non-native																3	2		5
		Mytilidae	*Mytilus edulis*	Native	9	5	9	3	5	7											1		39
		Ostreoidea	*Crassostrea (Magallana) gigas*	Non-native	6	2	4	4		2													18
		Pharidae	*Ensis cf leei***	Non-native													1						1
		Semelidae	*Scrobicularia plana*	Native			1		1											1	1		4
		Veneridae	*Ruditapes philippinarum*	Non-native																1			1
	Class Gasteropoda	Calyptraeidae	*Crepidula fornicata*	Non-native	2				1														3
Echinodermata	Asteroidea	Asteriidae	*Asterias rubens*	Native					2														2
Cnidaria	Class Anthozoa	Actiniidae	*Actinia equina*	Native					4				4						1				9
		Metridiidae	*Metridium senile VAR pallidus*	Native				1			3							1					5
Number of individuals					120	51	70	40	27	63	18	19	11	5	2	5	25	24	19	6	6	57	568
Number of species					15	7	15	8	13	10	8	4	4	2	1	1	5	4	3	4	5	9	40

*Broken animals or females, **recently renamed, previously named as *Ensis americanus*
Bryozoa colonial of *Conopeum seurati* (maybe death), its usually identified with *Ficopomatus, Amphibalanus y Austrominius*
There is a possible presence of *Halichondria panicea* in Site 3, but there are doubts because of the ethanol effect
Oligochaetas presence, belonging to Genus *Tubificoides* in sites 1, 2 and 3

Table 13: Benthic macrofauna results at sampling stations in the 4th sampling survey (September 2021)

				PORT OF ROTTERDAM, 4TH SAMPLING SURVEY, SEPTEMBER 2021																			
TAXA				**NATIVE / NON-NATIVE**	**S1**	**S1**	**S1**	**S2**	**S2**	**S2**	**S3**	**S3**	**S3**	**S4**	**S4**	**S4**	**S5**	**S5**	**S5**	**R**	**R**	**R**	**TOTAL**
Annelida	Class Polychaeta	Capitellidae	*Heteromastus filiformis*	Native									3					2	1	12	3	1	22
			Capitella capitata	Native													7	5	8	1			21
		Cirratulidae	*Tharyx cf killariensis*	Native								2									1		3
		Nephtyidae	*Nepthys cirrosa*	Native															1				1
			Nephtys hombergii	Native							2				2					1	2		7
			Nepthys kersivalensis	Native							8	4	3							2			17
		Nereididae	*Alitta (Neanthes) succinea*	Native	2			3											1	4	1	2	13
			Nereis zonata	Native	1																		1
		Orbiniidae	*Scoloplos armiger*	Native																	1		1
		Serpulidae	*Ficopomatus enigmaticus*	Non-native				1	1														2
			Hydroides ezoensis	Non-native				1	3	1													5
		Spionidae	*Polydora ciliata*	Native																	1		1
			Pseudopolydora paucibranchiata	Non-native									1						2				3
			Streblospio shrubsolii	Native															2				2
Arthropoda	Order Amphipoda	Ampeliscidae	*Ampelisca brevicornis*	Native							1												1
		Corophiidae	*Monocorophium acherusicum*	Native	1				3														4
		Gammaridae	Gammaridae**	Indet.										1									1
		Melitidae	*Melita hergensis*	Native	1	1			1														3
		Varunidae	*Hemigrapsus takanoi*	Non-native	1		2			1													4
	Order Sessilia	Austrobalanidae	*Austrominius modestus*	Non-native	3		4	7	2	7													23
		Balanidae	*Amphibalanus improvisus*	Non-native	32	21	41	51	46	81		4	4	2									282
			Balanus cf crenatus	Native			2	1	5	3			6	4									21

				PORT OF ROTTERDAM, 4TH SAMPLING SURVEY, SEPTEMBER 2021																			
TAXA				NATIVE / NON-NATIVE	S1	S1	S1	S2	S2	S2	S3	S3	S3	S4	S4	S4	S5	S5	S5	R	R	R	TOTAL
Mollusca	Class Bivalvia	Anomiidae	*Anomia ephippium*	Native		1																	1
		Corbulidae	*Varicorbula gibba*	Native							11	4	4	1	2		4	3	8				37
		Mactridae	*Spisula subtruncata*****	Native							1		1			1	1	2	1				7
		Myidae	*Mya arenaria*	Non-native												1							1
			Sphenia sp.	Indet.																		1	1
		Mytilidae	*Mytilus edulis*	Native	1	11	9	2	2	3				2	2								32
		Ostreoidea	*Crassostrea (Magallana) gigas*	Non-native	3	3	1	3	4	1													15
		Semelidae	*Abra alba*	Native								1	3										4
			*Theora lubrica****	Non-native									2		1		5	5	29				42
		Tellinidae	*Fabulina (Tellina) fabula*	Native															1				1
		Veneridae	*Ruditapes philippinarum*	Non-native																	1		1
			Venerupis corrugata	Native							3		3					1					7
	Class Gastropoda	Calyptraeidae	*Crepidula fornicata*	Non-native						2													2
Chordata	Class Ascidiacea	Ascidiidae	Ascidiidae sp.*	Indet.										1									1
Cnidaria	Class Anthozoa	Actiniidae	*Actinia equina*	Native							2	5											7
		Sagartiidae	Sagartiidae sp.*	Indet.								1											1
Number of individuals					45	37	59	69	67	99	28	21	30	11	7	2	17	18	54	20	10	4	598
Number of species					9	5	6	8	9	8	7	7	10	6	4	2	4	6	10	5	7	3	38

*Broken animals or females
**Without uropods 3
*** Refer to Paragraph 10.4.1 Identification key points
Presence of bryozoa colonial of *Conopeum seurati* (maybe death), its usually identified with *Ficopomatus, Amphibalanus* and *Austrominius*. We also saw *Cryptosula pallasiana.*

Table 14: Diversity and indices values per replicate

Station	Month	Repl.	S	N	H'(loge)	AMBI	fp+fA	BOPA2
1	Sep.21	1	9	45	1.16	2.40	0.044	0.000
1	Sep.21	2	5	37	1.08	0.00	0.027	0.000
1	Sep.21	3	6	59	1.02	7.00	0.000	
2	Sep.21	1	8	69	1.01	3.00	0.000	
2	Sep.21	2	9	67	1.23	2.57	0.060	0.000
2	Sep.21	3	8	99	0.78	3.00	0.000	
3	Sep.21	1	7	28	1.58	2.48	0.036	0.000
3	Sep.21	2	7	21	1.80	3.27	0.100	0.041
3	Sep.21	3	10	30	2.19	2.78	0.148	0.060
4	Sep.21	1	6	11	1.64	2.50	0.091	
4	Sep.21	2	4	7	1.35	3.00	0.000	
4	Sep.21	3	2	2	0.69	0.75	0.000	
5	Sep.21	1	4	17	1.23	4.41	0.412	
5	Sep.21	2	6	18	1.66	3.75	0.389	
5	Sep.21	3	10	54	1.51	3.61	0.204	0.081
R	Sep.21	1	5	20	1.16	3.83	0.650	0.217
R	Sep.21	2	7	10	1.83	3.45	0.500	
R	Sep.21	3	3	4	1.04	2.63	0.250	
1	Jul.20	1	15	120	2.24	2.86	0.217	0.041
1	Jul.20	2	7	51	1.55	3.00	0.000	
1	Jul.20	3	15	70	2.31	3.20	0.229	0.059
2	Jul.20	1	8	40	1.52	2.25	0.000	
2	Jul.20	2	13	27	2.29	2.14	0.148	0.029
2	Jul.20	3	10	63	1.80	3.23	0.190	0.024
3	Jul.20	1	8	18	1.96	3.00	0.333	
3	Jul.20	2	4	19	0.94	4.11	0.842	
3	Jul.20	3	4	11	1.16	1.93	0.000	
4	Jul.20	1	2	5	0.50	5.70	0.800	
4	Jul.20	2	1	2	0.00	6.00	1.000	
4	Jul.20	3	1	5	0.00	6.00	1.000	
5	Jul.20	1	5	25	1.09	2.70	0.000	
5	Jul.20	2	4	24	0.71	3.26	0.042	0.018
5	Jul.20	3	3	19	0.63	3.25	0.000	
R	Jul.20	1	4	6	1.24	2.50	0.167	
R	Jul.20	2	5	6	1.56	2.25	0.167	
R	Jul.20	3	9	57	1.40	4.17	0.123	0.050
1	Jan.20	1	10	62	1.52	2.25	0.016	0.000
1	Jan.20	2	12	47	1.86	1.17	0.128	0.000
1	Jan.20	3	10	45	1.94	1.91	0.089	0.000
2	Jan.20	1	12	49	1.86	2.93	0.429	0.097
2	Jan.20	2	11	33	2.12	2.83	0.273	0.067
2	Jan.20	3	6	11	1.59	3.14	0.455	

Station	Month	Repl.	S	N	H'(loge)	AMBI	fp+fA	BOPA2
3	Jan.20	1	13	66	2.01	2.61	0.273	0.105
3	Jan.20	2	8	23	1.76	3.00	0.478	0.170
3	Jan.20	3	10	17	1.95	2.72	0.294	
4	Jan.20	1	3	30	0.68	5.25	0.767	0.247
4	Jan.20	2	5	15	1.29	4.70	0.733	
4	Jan.20	3	4	7	1.28	3.86	0.429	
R	Jan.20	1	4	24	0.82	3.14	0.750	0.000
R	Jan.20	2	9	162	0.96	2.71	0.278	0.000
R	Jan.20	3	5	12	1.23	2.85	0.583	
1	Sep.19	1	3	7	1.00	3.00	0.143	
1	Sep.19	2	5	37	1.33	3.00	0.108	0.000
1	Sep.19	3	5	28	1.04	7.00	0.000	
2	Sep.19	1	4	8	1.21	7.00	0.000	
2	Sep.19	2	7	24	1.21	4.42	0.750	0.225
2	Sep.19	3	4	15	1.17	3.00	0.333	
3	Sep.19	1	10	21	2.07	2.85	0.190	0.056
3	Sep.19	2	11	24	2.15	2.37	0.208	0.082
3	Sep.19	3	9	19	1.94	2.04	0.316	
R	Sep.19	1	7	59	1.01	2.79	0.153	0.000
R	Sep.19	2	7	16	1.63	2.59	0.438	
R	Sep.19	3	11	21	2.13	1.88	0.286	0.017

In red are the samples/stations that do not comply with the minimum requirements for the robust estimation of the corresponding index AMBI or BOPA.