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ZERO BRINE

D9.1 Report on environmental impacts from brine discharge

February 2022

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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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 port of Rotterdam, currently the biggest port in Europe, is one of the largest chemical and petrochemical clusters in Europe whose supply of distilled/deionized water is supplied from two demineralized water plants (DWPs) in Botlek and Maasvlakte. Both DWPs combine ion exchange technology (IEX) and membrane technology (RO) for the purification process. In this study, we assessed the ecological conditions near the brine outfalls focusing on benthic macrofaunal composition. The investigated area includes three sites, one in the vicinity of DWP Botlek in the Brittaniëhaven area, one in the vicinity of 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 and 6 sampling stations have been established for benthic macroinvertebrates analysis. 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.

The analysis of the communities and species recorded in the case studies revealed a similar macrobenthic composition, although with lower abundance and diversity compared to the nearby North Sea environment. Even when considering the invasive species present and the taxa Spionidae and Capitellidae indicating poor environmental quality, the present data confirm that the community established in our study area is comparable to similarly impacted areas elsewhere. Among the two study sites, the DWP Botlek outfall area, which is a dead-end waterway in contrast to the well flushed environment of DWP Maasvlakte outfall, was characterized by the lowest biodiversity values and the highest disturbance levels according to AMBI in conjunction to BEQI results, dominated by the opportunistic native species *Capitella capitata*, and *Varicorbula gibba*, and species typical for organic matter enrichment *Alitta succinea* (native), and Streblospio cf shrubsolii (native), and *Theora lubrica* (invasive). At DWP Maasvlakte, higher biodiversity values were associated with the well-established biogenic reef of the pacific oyster *Crassostrea gigas*, however only dead individuals were recorded at the time of surveys. Multivariate analysis showed low similarity between these areas, confirming the heterogeneity of the highly variable estuarine system in terms of natural and anthropogenic stress.

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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 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.

2 Objectives

This study aims to assess the ecological quality status near the outfalls of DWP Botlek in the Brittaniëhaven area, and DWP Maasvlakte in Hartelkanaal area due to brine discharge-related activities. We focused on benthic biodiversity since these are mostly sessile or burrowing animals of limited horizontal mobility, i.e., they cannot evade a local environmental stressor and their community composition and number is likely to reflect the environmental conditions at a given place. 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.

3 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).

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

4.1 Water Framework Directive 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.

4.2 Stockholm convention on persistent pollutants

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.

4.3 International convention for the prevention of pollution from ships

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.

4.4 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.

5 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 benthic macroinvertebrates to an increase in salinity levels depends on the tolerance of the 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.

6 Port of Rotterdam

6.1 Man-made estuarine environment

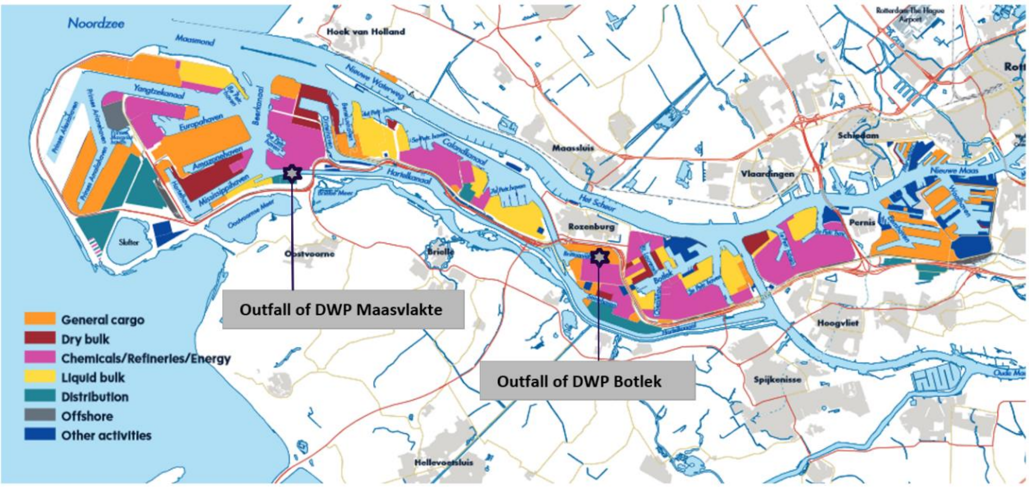
The Port of Rotterdam is situated in the estuary of the main branch of the river Rhine at a transition of freshwater to marine ecosystems. Before the Delta Project which was implemented after the storm surge of 1953 (Smits et al., 2006), the intertidal zone of the estuary consisted mostly of beaches, 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)

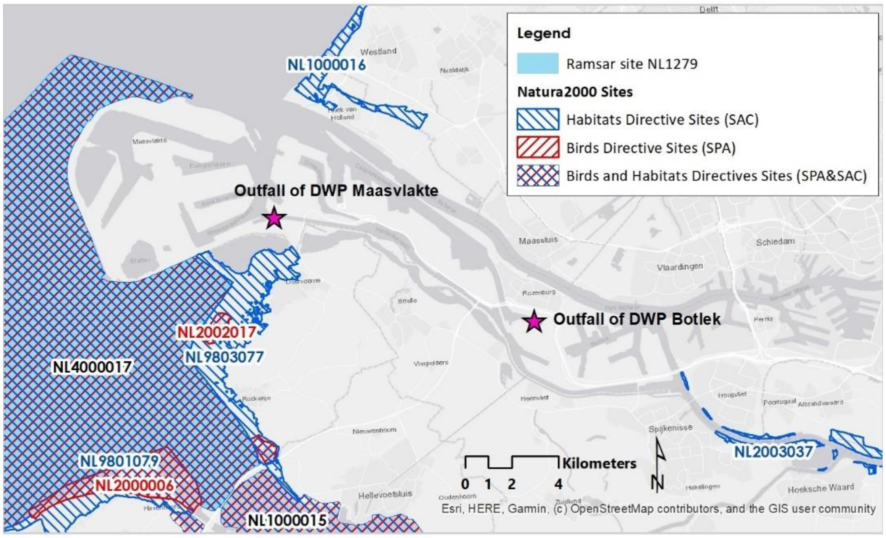


6.2 Environmentally designated areas

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 & Kapitelduinen" 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

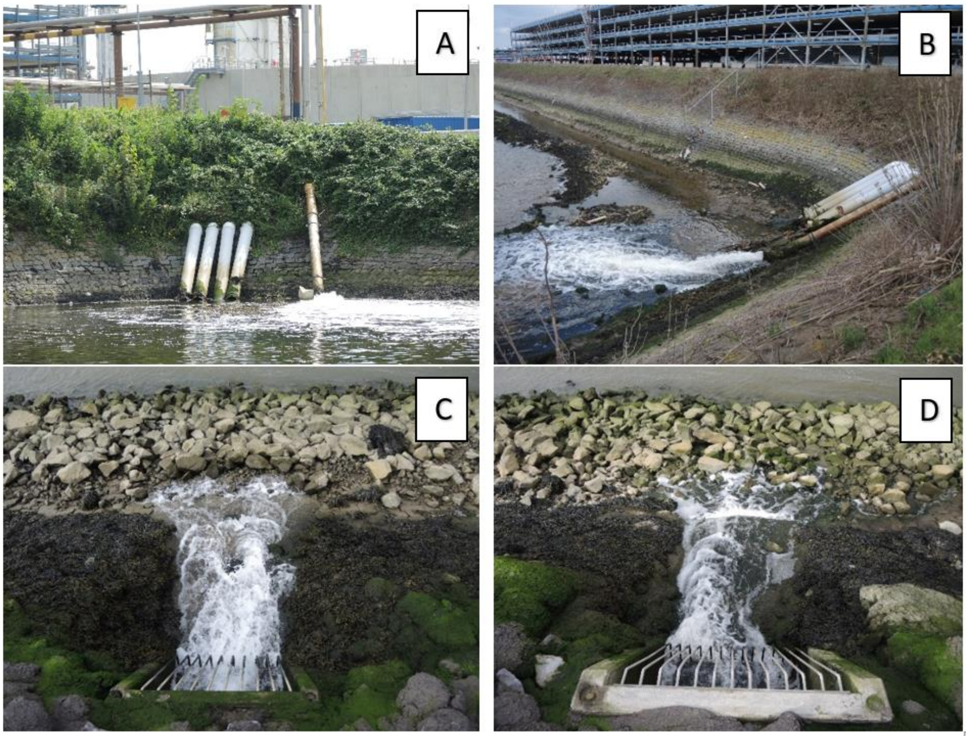


7 Description of the DWPs and surrounding environment

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. 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.

Figure 4: Brine discharge points (A & B: IEX and RO effluents from DWP Botlek, C: IEX effluent from DWP Maasvlakte, D: RO effluent from DWP Maasvlakte)



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, the main port's activities are freight distribution, chemicals/refineries/energy and liquid bulk. Specifically, the adjacent industrial facilities are (Fig. 5):

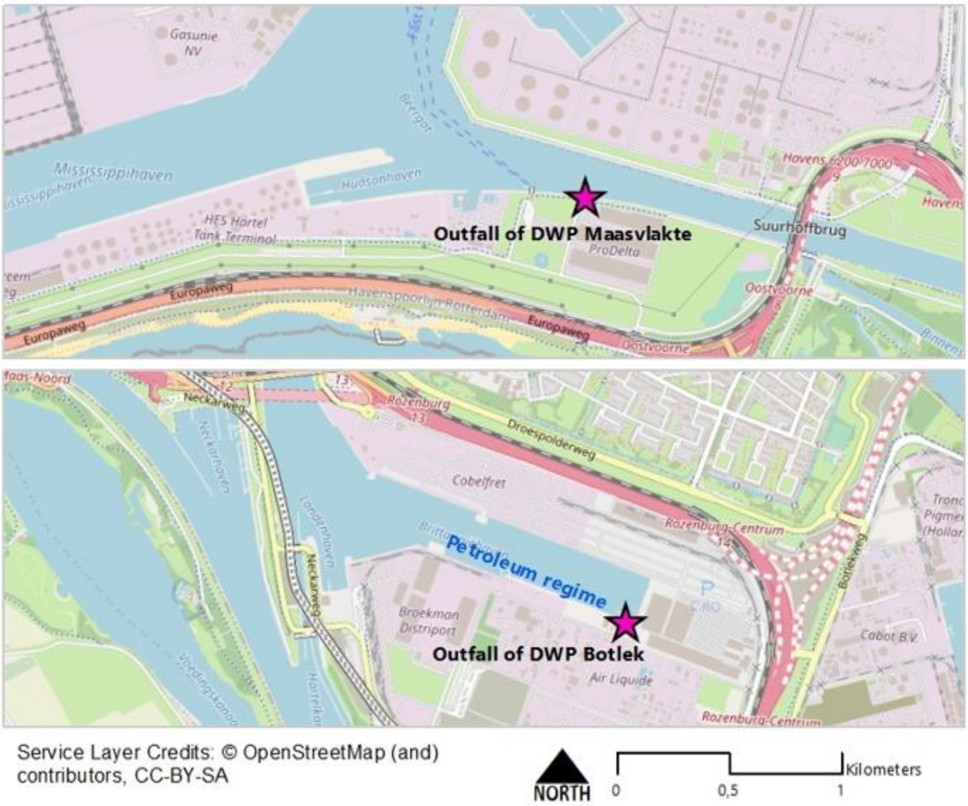
- BP refinery: Oil refinery, surface area 10500 sqm. The refinery currently uses hydrogen made from hydrocarbons in order to desulphurize petrochemical products. Replacing this entirely with green hydrogen produced from water using renewable energy could potentially result in a reduction of 350,000 tons of CO_2 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, 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))
- AIRLIQUIDE: 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

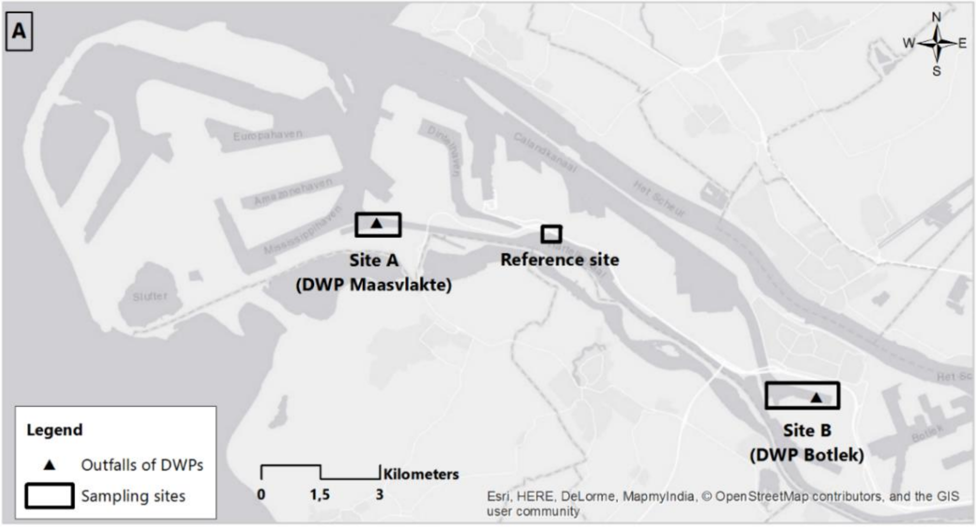


8 Methods

8.1 Study sites and sampling scheme

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 strong tidal currents 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 1.2-1.6 m. The seabed consists predominantly of *Crassostrea gigas* reef. Brittaniëhaven is a dead-end waterway, i.e. it has no river input and it is entirely marine. This area also receives effluent from chemical industry Huntsman Holland BV. This site can be characterized as an enclosed water body and a poorly flushed environment. The Reference site has naturally changing salinities due to tidal influence. In this study, this site was selected following the advice of the Port of Rotterdam Authority as a less-polluted site in the port.

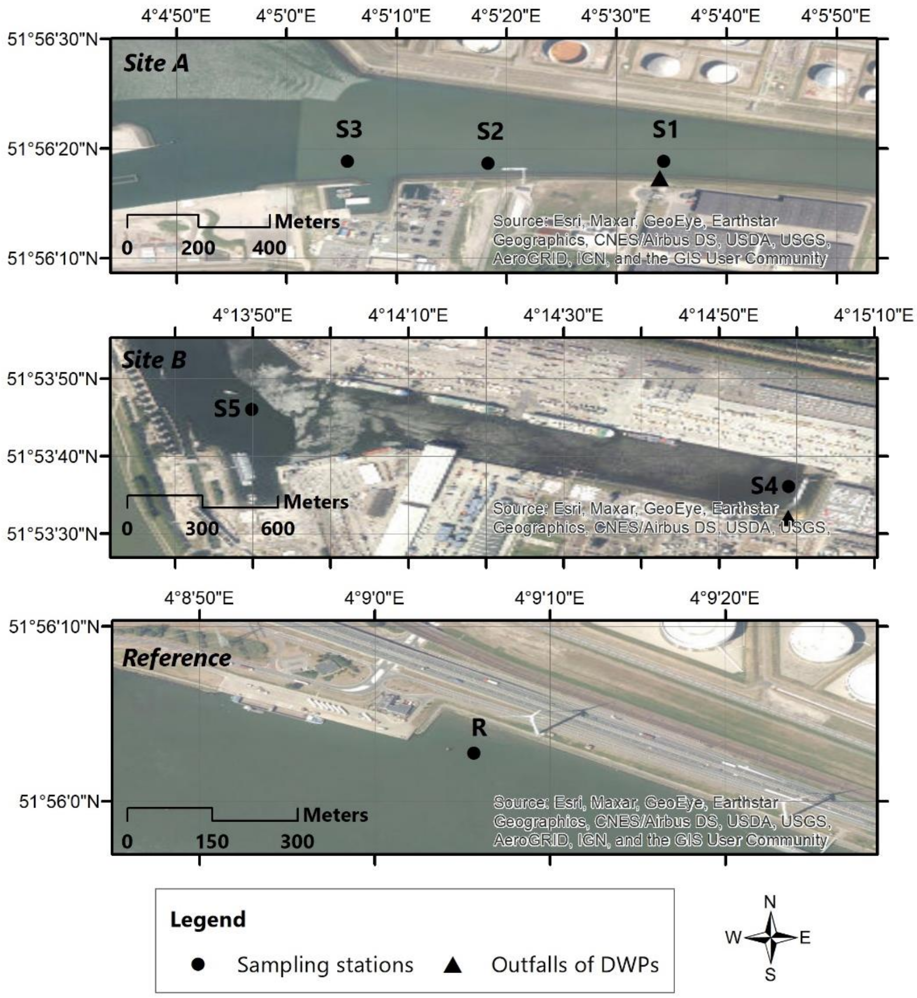
Annually, ~2,500 tons chloride (Cl-) / year (estimation) are discharged from DWP Maasvlakte and ~6,000 tons Cl- / year (estimation) from DWP Botlek. A significant amount of 22,460 tons Cl- / year (emission data for year: 2019) as well as 25 tons Iron (Fe) / year and 74 tons of Total Organic Carbon (TOC) / year are discharged in Brittaniëhaven close to the discharge point of DWP Botlek by the chemical industry Huntsman Holland BV which manufactures synthetic resins, plastics materials, and nonvulcanizable elastomers.

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. 7) were selected and established for the benthic macroinvertebrates survey. The main characteristics of the sampling stations are presented in the Table 1.

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 7: 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 (S1, S2, S3, S4, S5, and R) at the three sites (Site A, Site B, and R)

Site	Station	Coordinates (latitude / longitude) WGS 84	Port sector	Tide range (m)	Depth at low tide (m)	Water environment	Stream velocity	Water exchange	Bottom sediment	Surveys in which the stations were investigated
A	S1	51°56'18.84"N , 4°5'34.32"E	Hartelkanaal	1.2 – 1.6	6	Brackish water with strong tidal currents	Very fast flowing	High water exchange	*Crassostrea gigas* reef	1st, 2nd, 3rd, 4th
	S2	51°56'18.66"N , 4°5'18.34"E	Hartelkanaal	1.2 – 1.6	6	Brackish water with strong tidal currents	Very fast flowing	High water exchange	Hard substrate of *Crassostrea gigas* mixed with gravelly muddy sand (estimation)	1st, 2nd, 3rd, 4th
	S3	51°56'18.84"N , 4°5'5.54"E	Hartelkanaal	1.2 – 1.6	6	Brackish water with strong tidal currents	Very fast flowing	High water exchange	Gravelly muddy sand	1st, 2nd, 3rd, 4th
B	S4	51°53'36.12"N 4°14'59.04"E	Brittaniëhaven	0.5	6	Marine waterway with no to little currents	Slow flowing	Limited water exchange	Muddy sandy gravel	2nd, 3rd, 4th
	S5	51°53'46.08"N 4°13'49.86"E	Brittaniëhaven	0.5	8	Marine waterway with no to little currents	Slow flowing	Limited water exchange	Muddy sandy gravel (estimation)	3rd, 4th
Ref	R	51°56'1.72"N, 4°8'30.93"E	Hartelkanaal – Dolfijnweg	1.2 – 1.6	8	Brackish water with tidal currents	Fast flowing	Water exchange	Gravelly sand	1st, 2nd, 3rd, 4th

1st survey: September 2019
2nd survey: January 2020
3rd survey: April 2020
4th survey: September 2021

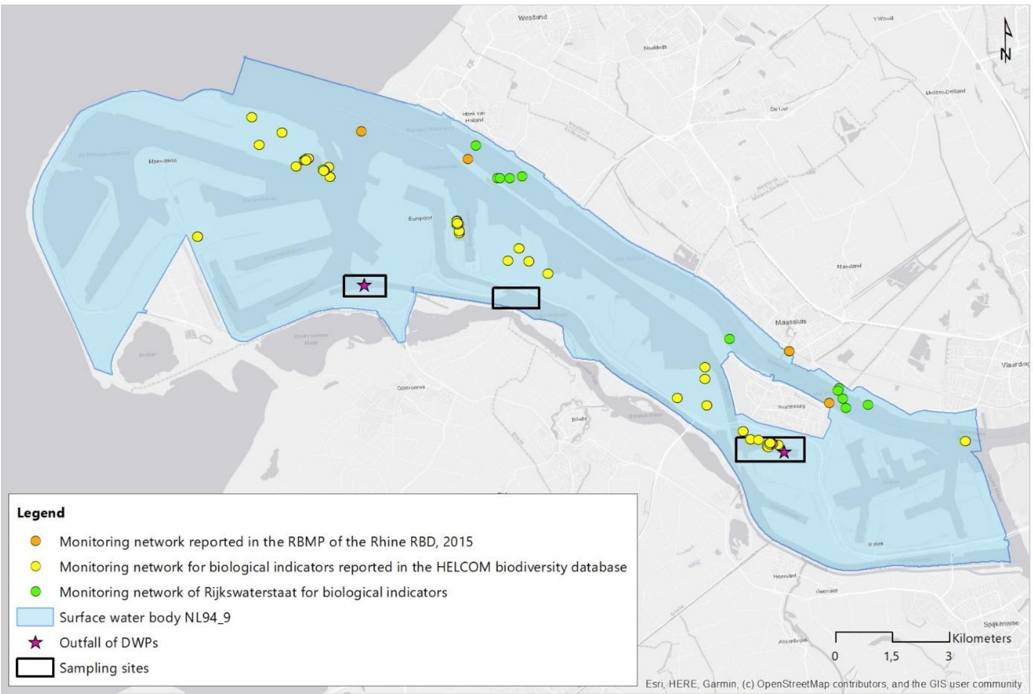
8.2 Monitoring network

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. 8).

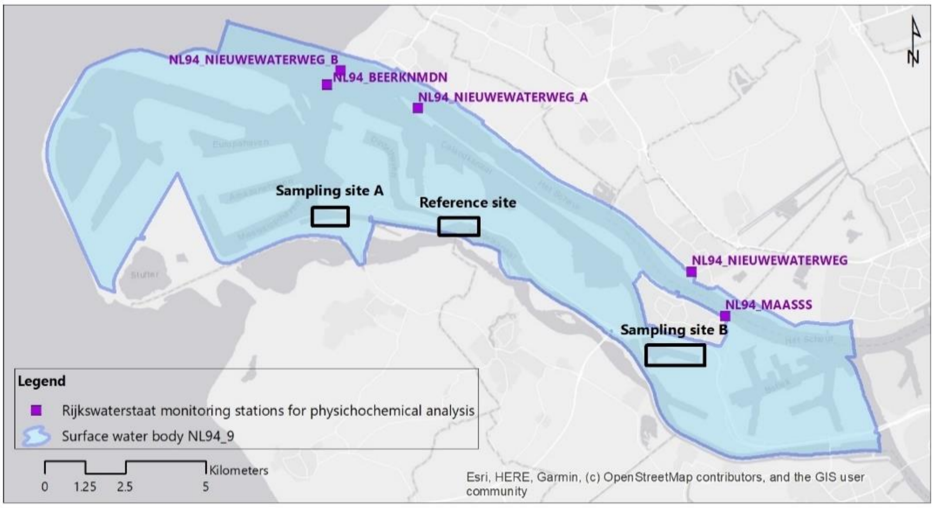
Figure 8: (A) monitoring network reported in the RBMP of the Rhine RBD 2015, (B) monitoring network for biological indicators reported in the HELCOM Biodiversity database (C) monitoring network for biological indicators of Rijkwaterstaat. Only the monitoring stations within the SWB NL94_9 are shown



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. 9).

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 9: Monitoring network of Rijkswaterstaat for physicochemical parameters. Only the monitoring stations within the SWB NL94_9 are shown



8.3 Field sampling and laboratory analysis

8.3.1 Benthic macroinvertebrates

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 sediment 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.

8.3.2 Water and sediment analysis

Water samples from S1, S4, and R were collected using an on-board pump from a depth of 1.5 m from the water surface. Water samples were analysed for pH, electrical conductivity (EC), total suspended solids (TSS), nutrients, sulfate (SO_4^{2-}), Total Organic Carbon (TOC), heavy metals, and polycyclic aromatic hydrocarbons (PAHs). All samples were analysed at an external laboratory (1st field survey: C-MARK B.V; last 3 field surveys (SGS Environmental Analytics B.V., Netherlands). Sediment samples were collected from S3, S4, and R with a van Veen grab and analysed for Total Organic Carbon (TOC) analysis, Kjedahl nitrogen, granulometric analysis, heavy metals, PAHs and Benzene, Toluene, Ethylbenzene and Xylenes (BTEX) at an external laboratory (1st field survey: SGS Environmental Analytics B.V.; last 3 surveys: Eurofins, Netherlands). It is noted that at Site A, sediment from S3 instead of S1 was collected as it was impossible to collect sediment at S1, as well as S2, due to the presence of a biogenic reef of *Crassostrea gigas*.

8.4 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)

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

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
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

9 Results and discussion

9.1 Brine characteristics

Table 6 (Appendix A) depicts 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), with a temperature of 17°C - 19°C, 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) compared to the intake water and the water bodies it is discharged into. The RO effluent contains elevated levels of lithium (Li) and boron (B).

9.2 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.

Based on the timeseries of the monitoring stations for BEERKNMDN and MAASSS metals provided by Rikjswaterstaat (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

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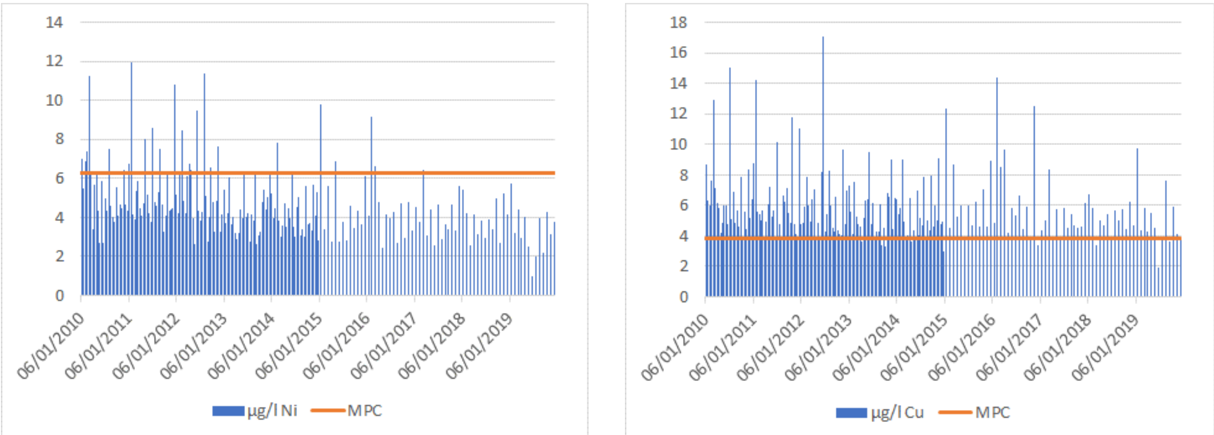
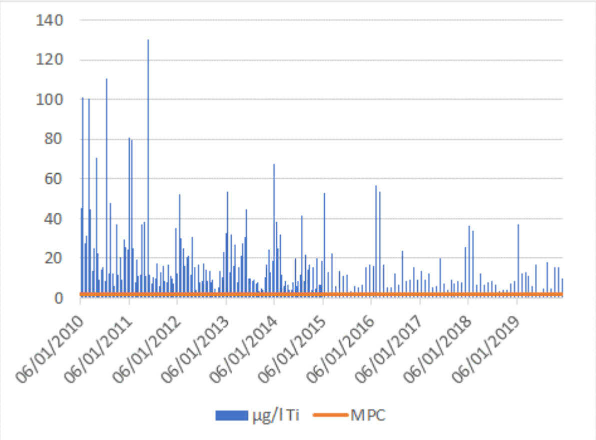
[6] “Moderate” ecological potential in terms of biological quality elements means that there are moderate changes in the values of the relevant biological quality elements as compared to the values found at maximum ecological potential. Whereas, “Good” ecological potential of biological indicators means 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).

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 PT, NT (2010-2019, monitoring stations BEERKNMDN, MAASSS) that have been exceeded the MPC (Rijkswaterstaat, 2021)




140
120
100
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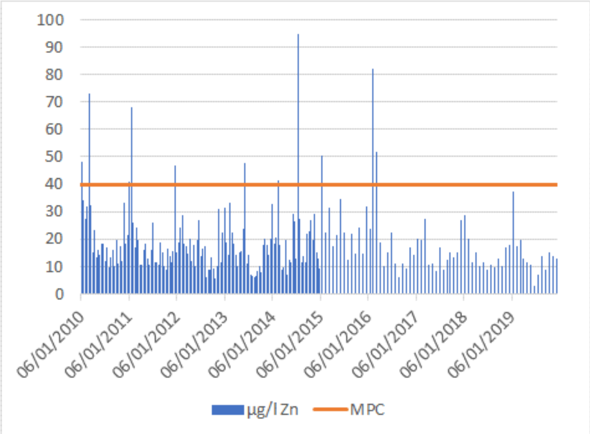
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µg/l Zn
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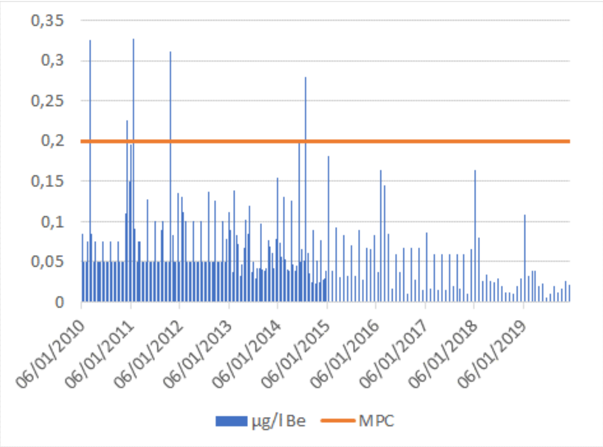
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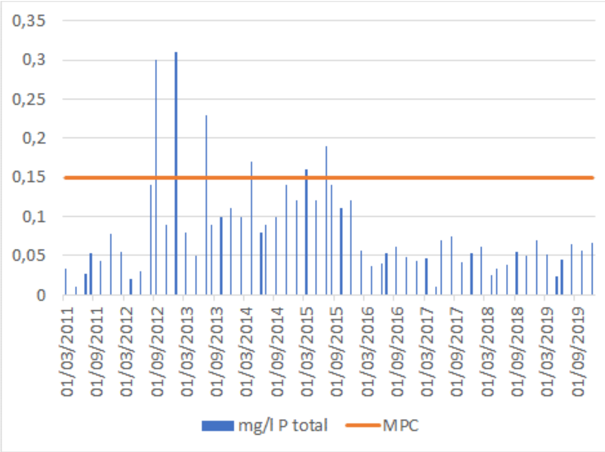
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mg/l P total
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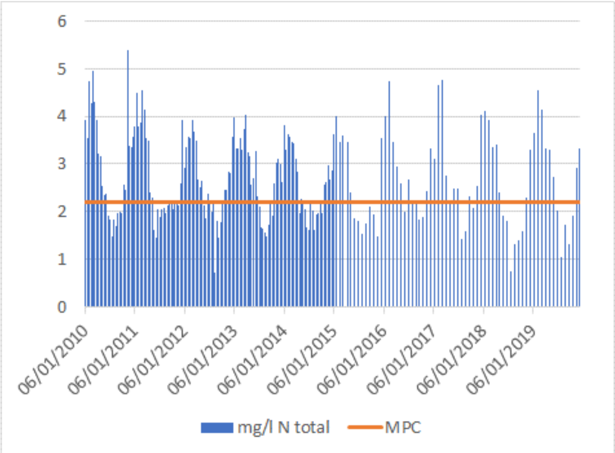
6
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06/01/2016
06/01/2017
06/01/2018
06/01/2019
mg/l N total
MPC

9.3 Water and sediment quality analysis results

The concentrations and levels of the parameters measured in the water and sediment samples in comparison to the Maximum Permissible Concentration (MPC) and Target Value set out in the Staatcourant, The Netherlands (June 2000), are presented in Tables 7 and 8 of the Appendix A. The results indicated that overall Site A had a low pollution impact while Sites B and Reference site showed an environment with higher influence from anthropogenic activities. The conditions at the latter were similar to those reported in other areas of the port of Rotterdam which are monitored regularly by Rijkswaterstaat.

For the water, conductivity values recorded showed, as expected, a more brackish character for S1 and Ref - conductivity varied depending on the tidal stage when the sampling was performed and the season - than the more marine S4. Cadmium (Cd), Chromium (Cr), Nickel (Ni) concentrations in water column were lower than the environmental quality standards, MPC and target values. Copper (Cu) mean concentration exceeded the MPC and the target values with higher values recorded in S1 and R compared to S4. Lead (Pb) and Zinc (Zn) concentrations exceeded target values at S4. Concentrations of PAHs in seawater were below the detection limits of the method S4 had higher TDS values than S1 and R water which indicates impact of port activities on this enclosed water environment.

Total organic carbon (TOC), and Kjedahl nitrogen values in the sediment showed a typical range for the type of sediment found. Values were 2-5 times higher at S4 in comparison to S3 and R due to the finer grain size found in the former (30% silt and clay at S4 vs 5-13% at the other two stations). The organic matter in the sediment was relatively fresh, more so at stations S4 and R, as indicated by the C:N ratio (C:N ~9) suggesting that the organic matter sediments rapidly from the water column or is produced in situ due to the shallow depth. Slightly higher values for the heavy metals Cd, Cr, Hg, Ni, Pb, however below the limit values, were found in the sediment at S4 in the 3rd survey and for PAHs, specifically fluoranthene and pyrene compounds, in R in the 4th survey compared to the rest. Zn mean concentration was above the target value for S4. BTEX contamination of seabed sediment was observed in S4 due to toluene and o-xylene which can be associated with oil spills and industrial emissions.

9.4 Benthic macroinvertebrates

9.4.1 Key points of species identification

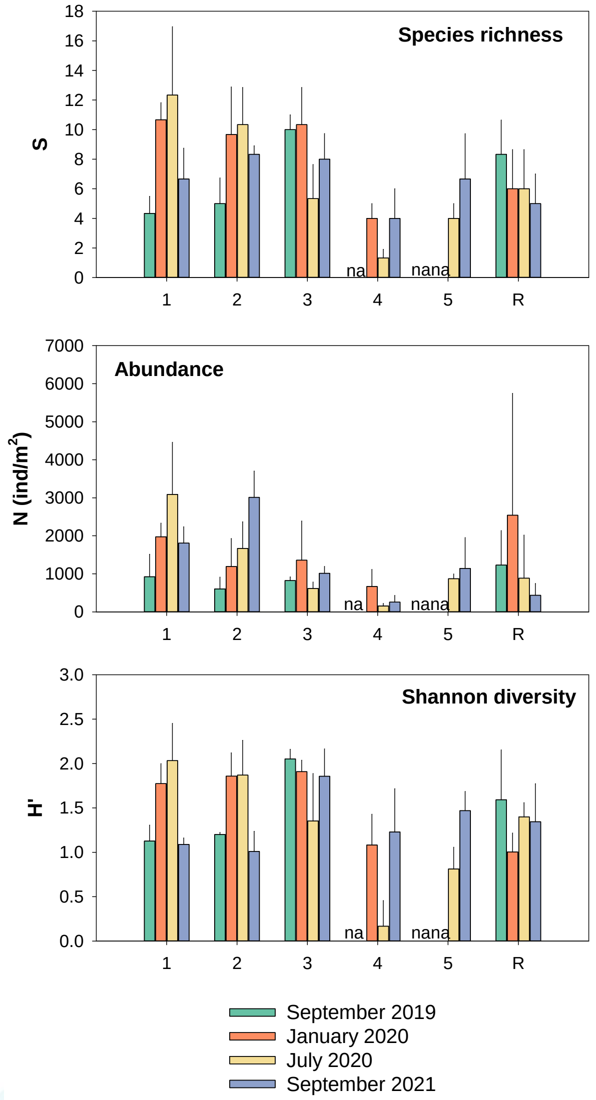
The overall list of species identified during the four field surveys is presented in Table 09 of Appendix A. The number of individuals and species identified per replicate, sampling station and field survey are presented in the Tables 10, 11, 12 and 13 of Appendix A.

During 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*. During 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. It is 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.

9.4.2 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. Diversity and indices values per replicate, sampling station and field survey are presented in Table 14 of Appendix A.

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

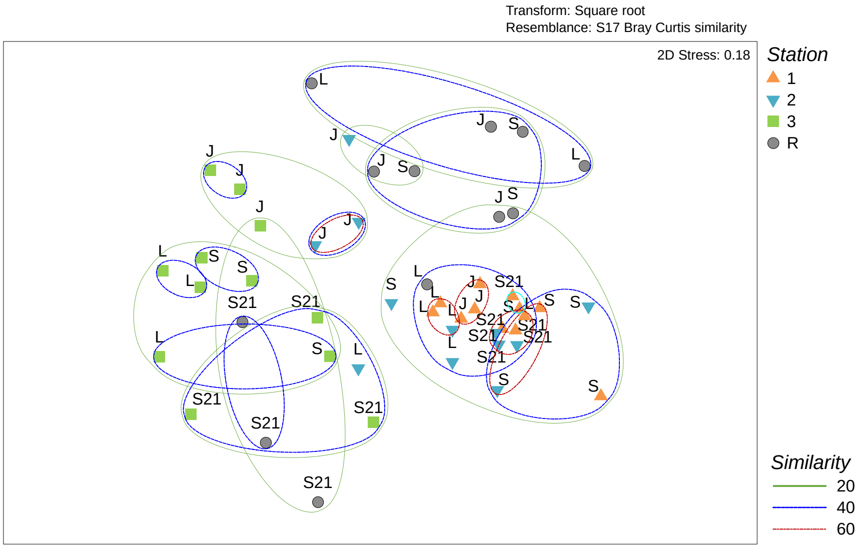


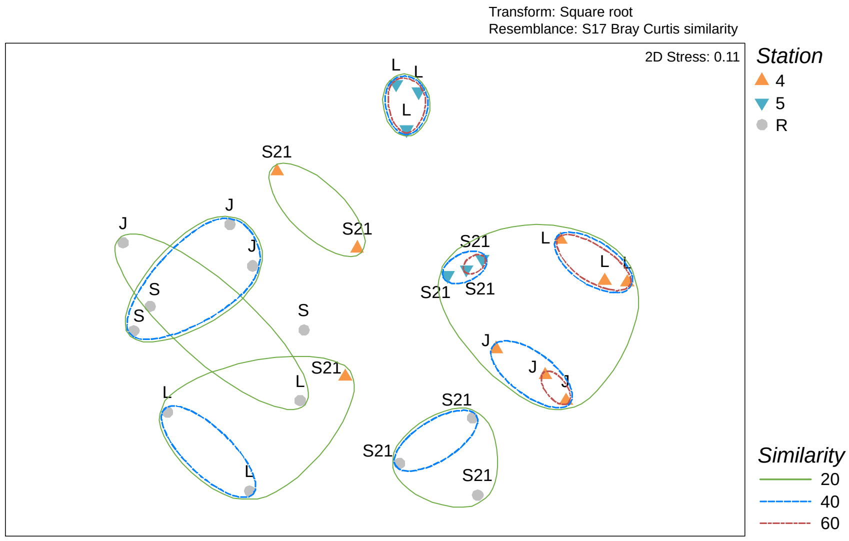
9.4.3 Multivariate analysis

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





9.4.4 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

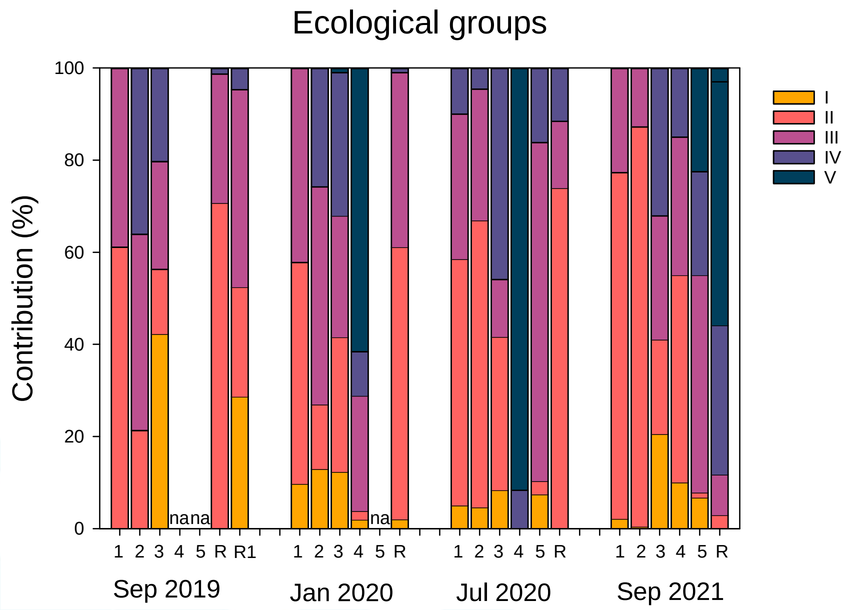
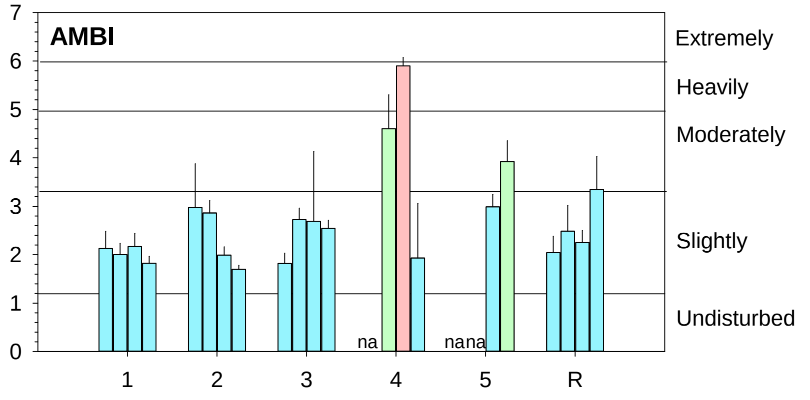


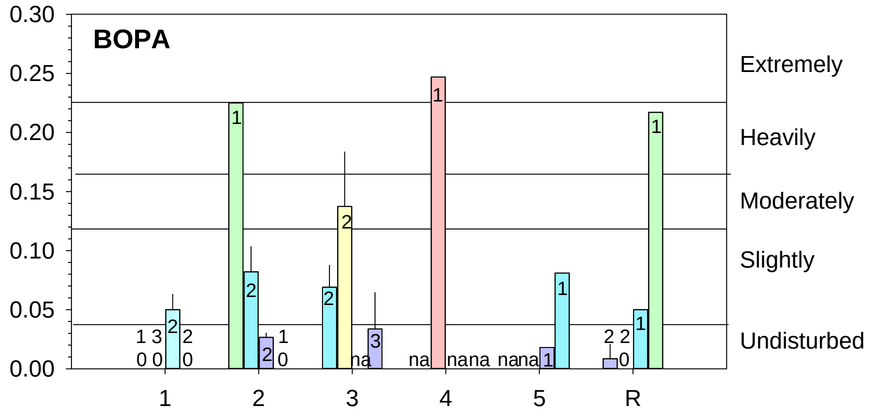
Figure 14: AMBI index for the sampled stations in the three surveys. The classification of ecological status for the sampling points according to the AMBI index is indicated on the right side. Values represent mean +/- SD (n=3). na: no samples available



9.4.5 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



9.4.6 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

9.4.7 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 (PAHs) (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). The reef was most developed at S1 and was shared with *Mytilus edulis.* During the samplings, all the oysters collected were dead, while most individuals of *M. edulis* attached on them were alive. 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.

10 Conclusions

This study has established an understanding of the environmental conditions near the DWP Botlek and DWP Maasvlakte in the Port of Rotterdam and provides essential information for the assessment of environmental benefits from the implementation of a 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.

Long-term monitoring of physicochemical variables and hydrodynamic conditions is required to understand the patterns influencing species composition as both studied sites lack a baseline ecological assessment and are simultaneously affected by multiple anthropogenic and natural stressors.

Regarding *Crassostrea gigas* reef at the outflow of the DWP Maasvlakte, it is expected that a number of conditions have co-occurred for its successful establishment. However, their mortality needs further investigation in terms of key environmental variables such as temperature, salinity, and chlorophyll a, as well as toxicological investigations.

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13 Appendix A: Supplementary data

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 - Staatcourant, The Netherlands, June 2000). ne: not examined,

		1st sampling survey September 2019		2nd sampling survey January 2020			3rd sampling survey April 2020			4th sampling survey September 2021					
Variables	**Unit**	**S1**	**R**	**S1**	**S4**	**R**	**S1**	**S4**	**R**	**S1**	**S4**	**R**	**MPC**	**Target value**	**River Basin Manageme nt Plan - Rhine 2015**
Tide		Low tide	Low tide	Low tide	High tide	Low tide	High tide	High tide	High tide	High tide	High tide	Low tide			
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			
SO_4^-	mg/l	ne	ne	ne	ne	ne	930	2000	830	ne	ne	ne			
TDS	mg/l	15000	11900	8100	25500	4800	ne	ne	ne	ne	ne	ne			
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			
TOC	mg/l	ne	ne	ne	ne	ne	ne	ne	ne	3.0	3.7	3.3			
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	0.15 (z[8])	0.05 (z)	
Heavy metals															
Cd	µg/l	ne	ne	ne	ne	ne	<0.1	<0.1	<0,1	<0.2	<0.2	<0.2	2	0.4	1
Cr	µg/l	ne	ne	ne	ne	ne	<1	<1	<1	<1	<1	<1	84	2.4	
Fe	µg/l	ne	ne	ne	ne	ne	96	170	77	<50	130	110			
Cu	µg/l	ne	ne	ne	ne	ne	21	5.9	22	2.4	2.6	2.1	3.8	1.1	
Pb	µg/l	ne	ne	ne	ne	ne	<1	6.8	<1	<2	<2	<2	220	5.3	14
Ni	µg/l	ne	ne	ne	ne	ne	1.3	1.5	1,4	<3	<3	<3	6.3	4.1	34
Zn	µg/l	ne	ne	ne	ne	ne	21	31	12	<10	<10	<10	40	12	
PAHs															
Naphthalene	µg/l	ne	ne	ne	ne	ne	<0.05	<0.05	<0.05	<0.1	<0.1	<0.1	1.2	0.01	130
Phenanthrene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.3	0.003	

[8] 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

Variables	Unit	1st sampling survey September 2019		2nd sampling survey January 2020			3rd sampling survey April 2020			4th sampling survey September 2021					
		S1	R	S1	S4	R	S1	S4	R	S1	S4	R	MPC	Target value	River Basin Management Plan - Rhine 2015
Anthracene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.08	0.0008	0.1
Fluoranthene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.5	0.005	0.12
Benz[a]anthracene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.03	0.0003	
Chrysene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.02	<0.02	<0.02	0.9	0.009	
Benzo[k]fluoranthene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.2	0.002	
Benzo[a]pyrene	µg/l	ne	ne	ne	ne	ne	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.2	0.002	0.027
Benzo[ghi]perylene	µg/l	ne	ne	ne	ne	ne	<0.02	<0.02	<0.02	<0.02	<0.02	<0.02	0.5	0.005	
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	0.4	0.004	
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 Staatcourant, The Netherlands, June 2000)

Parameters	Unit	3rd sampling survey April 2020			4th sampling survey September 2021			MPC (dry matter)	Target value (dry matter)
		S3	S4	R	S3	S4	R	Short term	Long term
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		
C:N					12.5	9.76	9.77		
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)	7.3	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		
Heavy metals									
Cadmium (Cd)	mg/kg dm	<0.40	0.45	<0.40	0.21	0.88	0.28	12 #	0.8
Chromium (Cr)	mg/kg dm	12	31	16	16	19	17	380 #	100
Copper (Cu)	mg/kg dm	<5.0	43	6.5	7.8	14	8.7	73	36
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	10 #	0.3
Nickel (Ni)	mg/kg dm	5.8	17	5.9	7.8	8.2	10	44	35
Lead (Pb)	mg/kg dm	<10	22	<10	14	14	20	530 #	85
Zinc (Zn)	mg/kg dm	35	160	28	57	140	80	620	140
Polycyclic Aromatic Hydrocarbons (PAHs)*									
Napthalene	mg/kg dm	<0.010	0.029	<0.010	<0.02	0.08	0.03	0.1	0.001
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	0.5	0.005
Anthracene	mg/kg dm	0.011	0.031	<0.010	<0.02	0.04	0.07	0.1	0.001
Fluoranthene	mg/kg dm	0.041	0.170	0.026	0.07	0.23	0.45	3	0.03
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	0.4	0.003
Chrysene	mg/kg dm	0.030	0.059	0.019	0.04	0.09	0.21	11	0.1
Benzo(b)fluoranthene	mg/kg dm	0.042	0.130	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	2	0.02
Benzo(a)pyrene	mg/kg dm	0.024	0.065	0.013	0.04	0.08	0.22	3	0.003
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	8	0.08
Indeno(123cd)pyrene	mg/kg dm	0.022	0.073	0.016	0.03	0.06	0.16	6	0.06
PAH 10 VROM (sum)	mg/kg dm	0.210	0.700	0.130	0.32	0.97	1.80		
PAH 16 EPA (sum)	mg/kg dm	0.290	1.000	0.170	0.44	1.40	2.60		
Volatile Aromatics									
benzene	mg/kg dm	ne	ne	ne	<0.05	<0.05	<0.05		
toluene	mg/kg dm	ne	ne	ne	<0.05	0.21	<0.05		
ethylbenzene	mg/kg dm	ne	ne	ne	<0.05	<0.05	<0.05		
o-xylene	mg/kg dm	ne	ne	ne	<0.05	<0.05	<0.05		
p- and m-xylene	mg/kg dm	ne	ne	ne	<0.05	0.06	**<0.06		
xylenes	mg/kg dm	ne	ne	ne	<0.10	<0.10	<0.11		
total BTEX	mg/kg dm	ne	ne	ne	<0.20	0.30	<0.20		

**no soil type correction for sandy sediments (organic matter < 10%)*

#value = intervention value

***Increased detection limit due to a high moisture content.*

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

TAXA				NATIVE / NON-NATIVE	PORT OF ROTTERDAM, FIRST SAMPLING SURVEY, SEPTEMBER 2019												
					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)

TAXA				NATIVE / NON-NATIVE	PORT OF ROTTERDAM, SECOND SAMPLING SURVEY, JANUARY 2020															
					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

		TAXA		NATIVE / NON-NATIVE	PORT OF ROTTERDAM, SECOND SAMPLING SURVEY, JANUARY 2020															
					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)

TAXA				NATIVE / NON-NATIVE	PORT OF ROTTERDAM, THIRD SAMPLING SURVEY, JULY 2020																		TOTAL
					S1	S1	S1	S2	S2	S2	S3	S3	S3	S4	S4	S4	S5	S5	S5	R5	R5	R5	
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

TAXA				NATIVE / NON-NATIVE	PORT OF ROTTERDAM, THIRD SAMPLING SURVEY, JULY 2020																		TOTAL
					S1	S1	S1	S2	S2	S2	S3	S3	S3	S4	S4	S4	S5	S5	S5	R5	R5	R5	
		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.