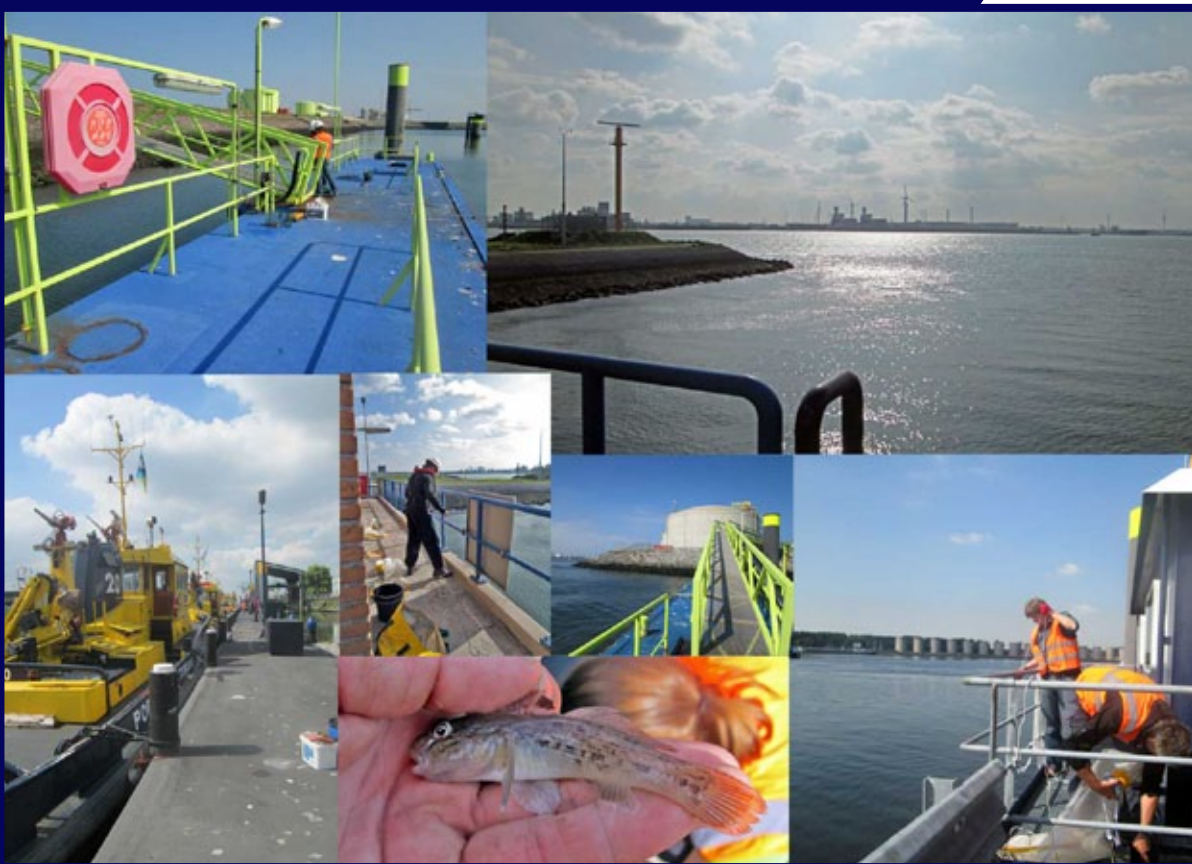


Port of Rotterdam survey and monitoring non-native species conform HELCOM/OSPAR protocol

Issued by: Bureau Risicobeoordeling & Onderzoeksprogrammering,
Nederlandse Voedsel- en Warenautoriteit, Ministerie van Economische Zaken



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GiMaRIS report 2014_31

Date:

November 2014

Report nr.:

GiMaRIS 2014_31

Title:

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The supporting organisations for the Rotterdam port survey project:

The Dutch Ministry of Economic Affairs (EZ/NVWA-BuRO)

The Dutch Ministry of Infrastructure and Environment (I&M-DGB)

The Dutch Ministry of Infrastructure and Environment-Rijkswaterstaat (I&M-RWS)

The Rotterdam Port Authority (HbR)

P&O Ferries

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1. Summary

In 2014 a non-native species survey was conducted in the port of Rotterdam, The Netherlands, following the joint HELCOM/OSPAR guidelines for the contracting parties of OSPAR and HELCOM on the granting of exemptions under the international convention for the control and management of ships' ballast water and sediments, regulation A-4. Next to providing an accurate list of non-native species present in the port of Rotterdam, the project aimed at providing comments and recommendations from the Rotterdam port sampling project to be considered for the amendment of the joint HELCOM/OSPAR port sampling methodology. In general the methods described in the HELCOM/OSPAR port survey protocol could be followed and proved to be very effective. For some habitats, like the underground water systems in ports and the littoral zone of the dike, the HELCOM/OSPAR protocol did not describe a monitoring method yet. In those cases methods were used that were not included in the protocol. For other habitats the HELCOM/OSPAR protocol describes two monitoring methods of which one was specified as the preferential one. In all cases the preferential method was used in the port survey and in some cases both methods. In total 229 species were recorded of which 32 were non-native. For various monitoring methods the species accumulation curves indicated that more sampling would have resulted in scoring several more species. Regardless of that result, the total list of non-native species recorded for the port of Rotterdam with all survey methods combined does provide a close to complete overview of the non-native species that were present in 2014, as virtually all of these species were recorded in at least two research areas, and within more than one sample.

2. Introduction

When large ships have unloaded their cargo or lost weight due to fuel and water consumption, they become unstable. To regain their balance they take in ballast water in ports where they unload and release the ballast water in ports where they pick up new cargo. This can pose a threat to the environment because bacteria, microbes, small invertebrates, eggs, cysts and larvae of various species can travel along within the ballast water. Some of these hitchhikers may settle, expand their population and have a significant impact on ecosystems where they are introduced. To minimize this risk the International Maritime Organisation (www.imo.org) has developed the Ballast Water Management Convention, a convention that describes demands for ships that are releasing their ballast water. In practice this means that ships have to install approved ballast water treatment systems onboard. The Convention, which has been signed already by many countries, will enter into force 12 months after the ratification by 30 States, representing 35 per cent of the world merchant shipping tonnage (www.imo.org).

In the convention the possibility of exemptions is included for shipping routes for which it can be proven that ballast water transports will not pose a threat to the environment. According to article 3(1) of the Ballast Water Management Convention, such exemptions can be given by parties, in waters under their jurisdiction, when they are:

- [a] granted to a ship or ships on a voyage or voyages between specified ports or locations; or to a ship which operates exclusively between specified ports or locations;
- [b] effective for a period of no more than five years subject to intermediate review;
- [c] granted to ships that do not mix Ballast Water or Sediments other than between the ports or locations specified in paragraph 1.1; and

- [d] granted based on the Guidelines on risk assessment developed by the Organization.

To be able to grant such exemptions in European waters a HELCOM and OSPAR task group on Ballast water Exemptions was formed in 2012 to develop a joint HELCOM/OSPAR Harmonized Protocol (JHP) for granting exemptions to contracting parties when the Ballast Water Convention comes into effect. In 2013, a detailed description of a port survey procedure was included in the joint HELCOM/OSPAR harmonized Protocol (JHP). The port survey procedure described in the JHP was strongly based on the results of the work undertaken in the Aliens 2 project.

In December 2013 the Netherlands promised to test the Joint HELCOM-OSPAR Harmonized Protocol in the port of Rotterdam. The aim of the Rotterdam project, was to evaluate the practical implementation of the port sampling protocol as described in the HELCOM/OSPAR protocol. This includes the collection, analysis and storage of the port survey data. The results and experiences gained during the Rotterdam port survey will be used to improve the quality of the port survey procedure described in the present joint HELCOM/OSPAR protocol.

The individual government departments and private companies that supported the Rotterdam port sampling project expect the results of the project to assist them in various ways including:

- [a] Providing relevant information for shipping companies with the desire of applying for an exemption to the Ballast Water Management Convention for one or more of their shipping routes.
- [b] Providing an updated overview of the presence and spread of non-native species in the port of Rotterdam for the stakeholders concerned.
- [c] Providing a dataset from a Dutch port to be included in the central OSPAR/HELCOM database for (target) species.

- [d] Studying the practical application of the OSPAR/HELCOM guidelines within ports in The Netherlands.
- [e] Gaining general experience for the Dutch stakeholders with conducting these kinds of projects.
- [f] Providing an overview list of non-native species for the port of Rotterdam that can be used as a baseline list (T_0 measurement) to be compared with non-native species lists resulting from similar Rotterdam port surveys that may be conducted after the ballast water convention will have entered into force.

2.1 Previous non-native species surveys in the port of Rotterdam

Relatively little is known about the species diversity in the main ports of the Netherlands in comparison to the other marine waters along the Dutch coast, i.e. the Delta area, the North Sea and the Wadden Sea, which are monitored on a continuous basis to comply with the European Water Framework Directive. Therefore the Dutch Ministry issued a rapid species assessment in the main ports of The Netherlands in 2007 to get an indica-

tion of the ecological status of these waters (Gittenberger, 2008). This assessment, including the port of Rotterdam, focused on species that settled on settlement plates deployed from floating docks because the highest species diversity in ports is in general found on floating docks. These artificial habitats are for example also specifically monitored on a regular basis along the North American coast (Hines & Ruiz, 2001; McIntyre *et al.*, 2013; Wells *et al.*, 2014) and Australian coasts (Connell & Glasby, 1999; Holloway & Connell, 2002). Floating docks tend to locally increase the diversity of native species (Connell, 2000, 2001; Glasby & Connell, 2001; Holloway & Connell, 2002) but also attract a relatively high diversity of non-native species (Glasby *et al.*, 2007; Tyrrell & Byers, 2007; Gittenberger *et al.*, 2010) increasing the stepping-stone role that ports form in the distribution of these species (Van der Weijden *et al.*, 2007).

The port survey project was continued for one year from 2007 to 2008 during which the species diversity was assessed every three months on ten plates per research site. In the port of Rotterdam the plates were deployed in the 8e Petroleumhaven close to the North Sea and in the Beneluxhaven, a part of the harbour that lies more inland (Fig. 1). Nineteen fouling species were recorded among which three non-natives: The calcareous tube-

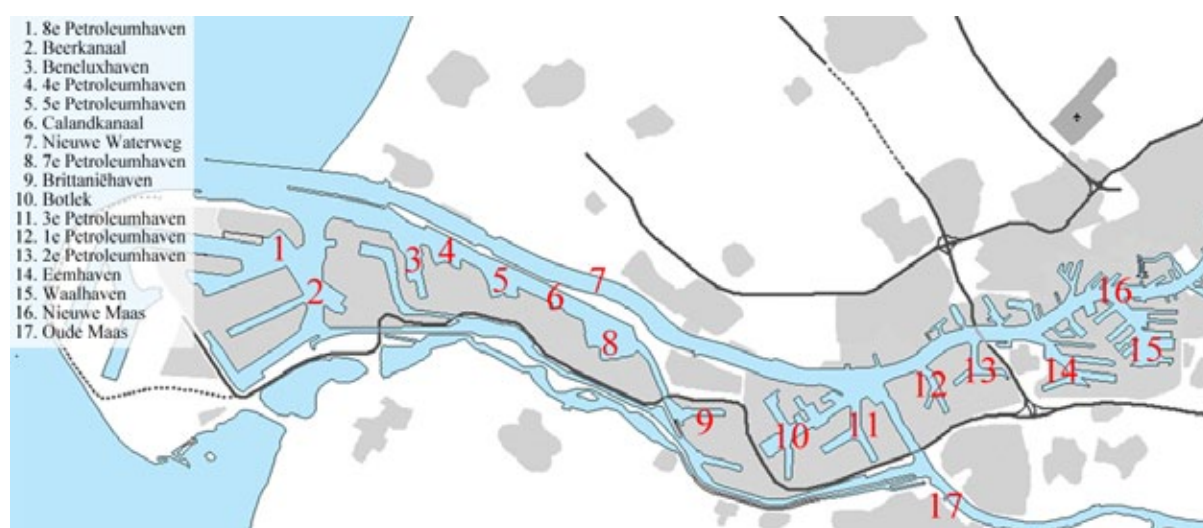


Fig. 1. The main harbours and water ways within the Port of Rotterdam.

worm *Ficopomatus enigmaticus* (Fauvel, 1923), the Asian shore crab *Hemigrapsus sanguineus* (De Haan, 1835) and the ascidian *Molgula manhattensis* (De Kay, 1843). None of these species dominated the fouling community. Furthermore the number of non-native species found in the port of Rotterdam was relatively low in comparison to the other ports along the Dutch coast like the port of Vlissingen where sixteen species were found on the settlement plates of which six were non-native (Gittenberger, 2008).

Since 2008 the monitoring project was continued by GiMaRIS and the settlement plates in the Dutch ports were renewed, and checked for species every three months. In addition to the main ports the number of research sites was expanded with various marina's. The data has since been used for various publications and reports varying from a risk analysis of hull fouling (Gittenberger *et al.*, 2011) to articles focusing on the succession of marine fouling communities over the seasons and years (Lindeyer & Gittenberger, 2011), the variation between fouling communities on different artificial structures in harbours (Gittenberger & van der Stelt, 2011), and the quantification of the competition for space between native and non-native fouling species (Gittenberger & Moons, 2011).

More recently Paalvast (2014) published his dissertation on an ecological study of the man-made estuarine environment of the port of Rotterdam. Hereby he focused on the distribution, settlement and growth of the non-native shipworm *Teredo navalis* Linnaeus, 1758, and its potential threat to the port's wooden structures under a selection of climate change scenarios. Although the species was unknown for the area until 2003, its presence in especially the western polyhaline harbours (Beer- and Calandkanaal) could be demonstrated with the aid of wooden panels that were deployed throughout the port. To study its potential impact, Paalvast (2014), did an extensive study on the habitats and their history within the port. As Rotterdam lies at the mouth of the Rhine-Meuse estu-

ary, a salinity gradient exists from almost fresh-water (about 0.5 to 1 ppt) in the eastern harbours the Eemhaven and Waalhaven to brackish waters (about 22 ppt) in the western part of the port close to the North Sea. These salinities further vary with depth depending on the direction of the tide and the amount of rainfall upstream. Concerning the substrate types and habitats available for species to settle, the port of Rotterdam underwent an enormous growth after the second world war to almost 4,000 ha in 1970 (Paalvast, 2014) and 5,257 ha in 2014. Because of the closure of the Brielsche Maas, and the development of the Botlek, Europoort and Maasvlakte harbour systems the soft estuarine ecotopes were reduced to 17 ha, less than 4 ‰ of the 4,745 ha in 1835 (Paalvast, 2014). The intertidal hard substrate on the other hand increased from 95 ha around 1940 till 338 ha in 1970, and the shoreline of the Noordrand consisted in 2008 of 344 km of hard substrate and only 1 km of soft substrate. Nowadays this part of the Rhine-Meuse estuary is considered completely petrified.

In a pilot project investigating ways to increase the diversity of species in the port of Rotterdam and thereby the local water quality, Paalvast *et al.* (2012) attached "pole hulas" and "pontoon hulas" (respectively hula skirt and raft like structures) to poles and pontoons in the port. Both structures were rapidly colonized after a few months by a variety of organisms, dominated by the mussel *Mytilus edulis* Linnaeus, 1758. In the dense layer of mussels a variety of mobile soft-bottom amphipods and young ragworms occurred including various non-native species: the ascidian *Molgula manhattensis* (De Kay, 1843), the brackish water barnacle *Amphibalanus improvisus* (Darwin, 1854), the slipper limpet *Crepidula fornicata* (Linnaeus, 1758), the amphipod *Monocorophium sextonae* (Crawford, 1937), the club tunicate *Styela clava* Herdman, 1881, the Asian shore crab *Hemigrapsus takanoi* Asakura & Watanabe, 2005 and the tanaidacean *Sinelobus stanfordi* (Richardson, 1901).

Within these studies about ten non-native species were recorded for the port of Rotterdam in total. Some sporadic records in literature of additional species, like the 1931 citing in the port described by Kamps (1937) of the Chinese mitten crab *Eriocheir sinensis* H. Milne Edwards, 1853, may have been missed. The low number of non-native species that is recorded in literature for the port of Rotterdam is probably due to the fact that few studies were done and that the studies that were done only focused on a few habitats.

3. Locations

The port of Rotterdam is the largest port of Europe covering about 5,257 ha. In the joint HELCOM/OSPAR port survey protocol it is indicated that a minimum of three research areas should be selected within a port and that these areas should be selected on the basis of the physical characters of the port and its waters. As is described in the previous paragraph a salinity gradient exists in the ports, which lies at the end of the Rhine-Meuse estuary and is influenced by the North Sea tides. To include habitats with various salinities and tidal current strengths and differences, it was decided to select research areas in the four regions marked in figure 2, i.e. between the North Sea entrance to the port with relatively high salinities and strong tidal influences, and the more inland part where the water is only slightly saline and the tidal difference minimal. Within these regions the exact research areas were selected aiming at including a large variety of structures on which different species communities are expected to be found, like the littoral and sub-littoral parts of a dike, floating docks, and pilings. The habitats on these structures that were searched for species are described in more detail in the next chapter. The research areas that were selected are from west to east, concentrated around the 8e Petroleumhaven,



Fig. 2. The four research areas that were selected in the Port of Rotterdam to represent the various habitats present that differ from each other in for example their salinities and the tidal influences. As the port lies at the mouth of the Rhine-Meuse estuary, a salinity gradient is present from ~22 ppt in research area 1 to ~0.5 ppt in research area 4.



Fig. 3. Research areas and sampling locations in the Port of Rotterdam. The different areas are displayed in more detail in Figs 4-11. The four selected research areas are concentrated around [1] the 8e Petroleumhaven (Figs 4-5), [2] the Beneluxhaven (Figs 6-7), [3] the Brittanniëhaven (Figs 8-9) and [4] the 1e Eemhaven (Figs 10-11).

the Beneluxhaven, the Brittanniëhaven and the 1e Eemhaven (Figs 1, 3). The exact locations that were monitored within these research areas are described in the next paragraphs.



Fig. 4. Sample locations 1-4. Maasvlakte Oil Terminal (MOT). Fire hydrants from which samples were taken.

3.1 Maasvlakte Oil Terminal (MOT)

At the Maasvlakte Oil Terminal four fire hydrants spread over the premises were selected for sampling the underground water system (Fig. 4; Table 1). This water system consists of various pipes that reach throughout the area and is used as a source of water in case of fire. The system is in open connection with the harbour water enabling larvae that are present in the harbour to enter. In general there is no to little current within the system, enabling the settlement of organisms in a sheltered area with relatively clear, i.e. sediment free, water. As there is little current, the salinity of the water in the system remains relatively constant. In most parts of the port of Rotterdam salinities vary strongly because of the tides and the varying amount of rainfall up river. In addition the water in the port tends to be relatively murky and tidal currents can be strong.

Table 1. Sample locations 1-4 (Fig. 4). Maasvlakte Oil Terminal. Description and geographical coordinates.

Loc.	Description	Geogr. coordinates
1	Fire hydranth FH-9151	N51 58.811 E4 2.917
2	Fire hydranth FH-9528	N51 58.410 E4 3.057
3	Fire hydranth FH-9624	N51 58.575 E4 3.592
4	Fire hydranth FH-9003	N51 58.045 E4 4.631

The environment in the underground water system, which can be described by its sheltered position, little to no currents, little to no sedimentation, and no direct sun light, is therefore unique in the port. As this may enable the settlement of species that can not settle elsewhere in the port, this habitat was included in the present inventory. Several times per year when the system is tested, the water pumps are turned on and water is flushed through the system.

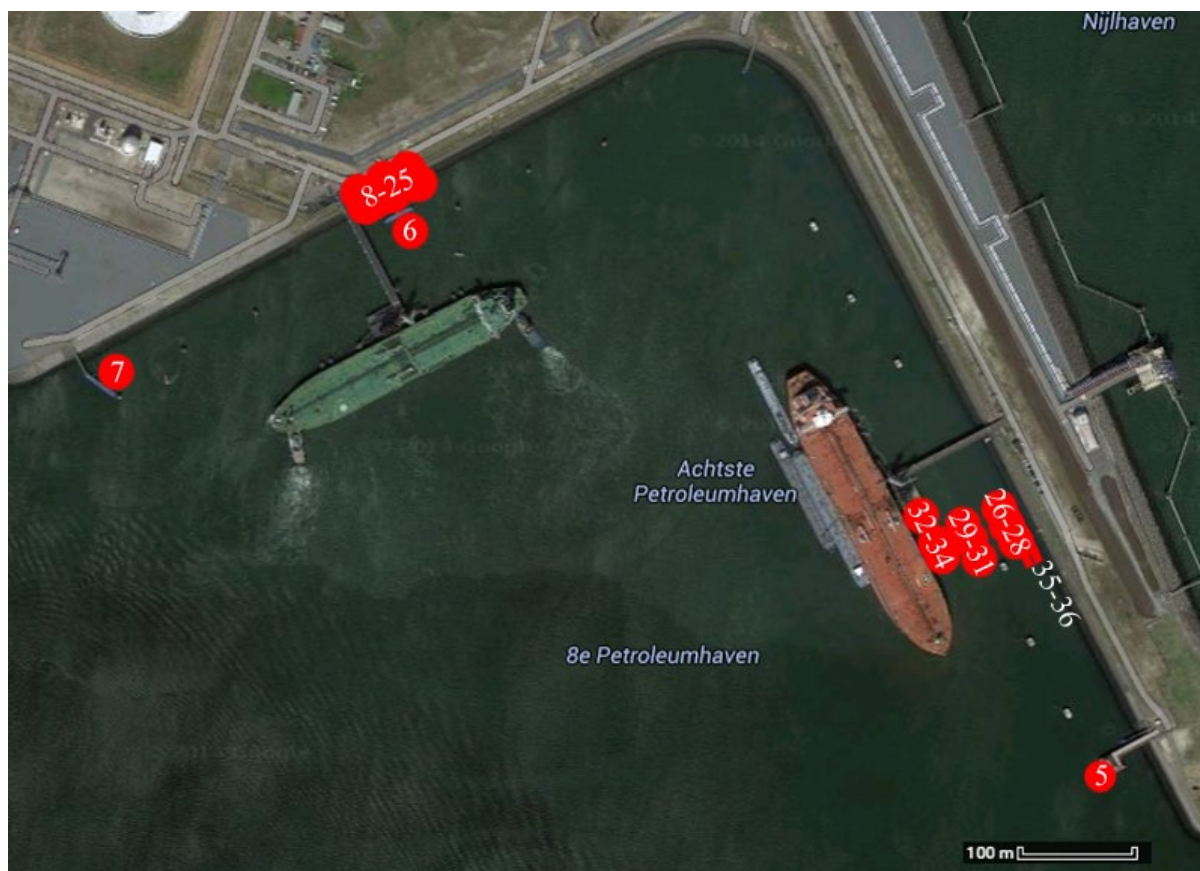


Fig. 5. Sample locations 5-36. 8e Petroleumhaven, Maasvlakte Oil Terminal (MOT). Littoral and sub-littoral locations from which samples were taken in the harbour.

3.2 8e Petroleumhaven

At the 8e Petroleumhaven samples were taken and searched for species at 31 sites (Fig. 5; Table 2). These sites included habitats in the littoral zone of the dike, the sub-littoral zone of the dike, on the floating docks, on the pilings, in the water column and on and in the sandy bottom. All of these habitats were assumed to have their own unique species communities as they have different environmental characteristics that may be important for the ability of species to settle and grow. Species that live in the littoral zone of the dike for example have to be able to survive being above water during low tide. In addition they are much more exposed to wave action than species that live in the sub-littoral zone. Species in the sub-littoral zone of the dike tend to be covered more by sediments however, and if the water is

murky the amount of sunlight is much less than in the littoral zone, especially with high tide. Sub-littorally much less sediment will fall on organisms that foul vertical structures like pilings, and organisms that foul floating objects like docks. The benefit for species of being on the floating dock, can be that they settle on a surface that is always submerged, but never far under the water line. The amount of sunlight that reaches an organism on the side of a floating dock therefore remains relatively constant, while the amount of sunshine that reaches organisms on pilings will significantly reduce with high tides when the water is murky. In the 8e Petroleumhaven samples all these habitats were searched for species following the HELCOM/OSPAR port survey protocol where possible.

Table 2. Sample locations 5-36 (Fig. 5). 8e Petroleumhaven, Maasvlakte Oil Terminal. Description and geographical coordinates.

Loc.	Description	Geogr. coordinates
5	Concrete jetty	N51 57.903 E4 4.649
6	Floating dock	N51 58.160 E4 4.107
7	Floating dock	N51 58.076 E4 3.884
8	Dike, littoral zone, <i>Fucus spiralis</i> zone	N51 58.161 E4 4.073
9	Dike, littoral zone, <i>Fucus spiralis</i> zone	N51 58.171 E4 4.100
10	Dike, littoral zone, <i>Fucus spiralis</i> zone	N51 58.180 E4 4.129
11	Dike, littoral zone, <i>Fucus vesiculosus</i> zone	N51 58.160 E4 4.073
12	Dike, littoral zone, <i>Fucus vesiculosus</i> zone	N51 58.169 E4 4.102
13	Dike, littoral zone, <i>Fucus vesiculosus</i> zone	N51 58.179 E4 4.130
14	Dike, littoral zone, tide pools	N51 58.159 E4 4.074
15	Dike, littoral zone, tide pools	N51 58.168 E4 4.104
16	Dike, littoral zone, tide pools	N51 58.177 E4 4.131
17	Dike, littoral zone, stones breakwater zone	N51 58.158 E4 4.075
18	Dike, littoral zone, stones breakwater zone	N51 58.167 E4 4.103
19	Dike, littoral zone, stones breakwater zone	N51 58.177 E4 4.132
20	Dike, sub-littoral zone, <i>Crassostrea gigas</i> (Pacific oyster) reef	N51 58.161 E4 4.098
21	Dike, sub-littoral zone, <i>Crassostrea gigas</i> (Pacific oyster) reef	N51 58.161 E4 4.098
22	Dike, sub-littoral zone, <i>Crassostrea gigas</i> (Pacific oyster) reef	N51 58.170 E4 4.122
23	Dike, sub-littoral zone, <i>Crassostrea gigas</i> (Pacific oyster) reef	N51 58.170 E4 4.122
24	Dike, sub-littoral zone, <i>Crassostrea gigas</i> (Pacific oyster) reef	N51 58.185 E4 4.166
25	Dike, sub-littoral zone, <i>Crassostrea gigas</i> (Pacific oyster) reef	N51 58.185 E4 4.166
26	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 58.008 E4 4.565
27	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 58.008 E4 4.565
28	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 58.008 E4 4.565
29	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 57.995 E4 4.524
30	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 57.995 E4 4.524
31	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 57.995 E4 4.524
32	Pillar, sub-littoral zone	N51 58.010 E4 4.501
33	Pillar, sub-littoral zone	N51 57.998 E4 4.510
34	Pillar, sub-littoral zone	N51 57.992 E4 4.515
35	Sub-littoral transect (50 m), 3 m depth, parallel to dike	N51 58.011 E4 4.580
36	Sub-littoral transect (50 m), 6 m depth, parallel to dike	N51 58.011 E4 4.580

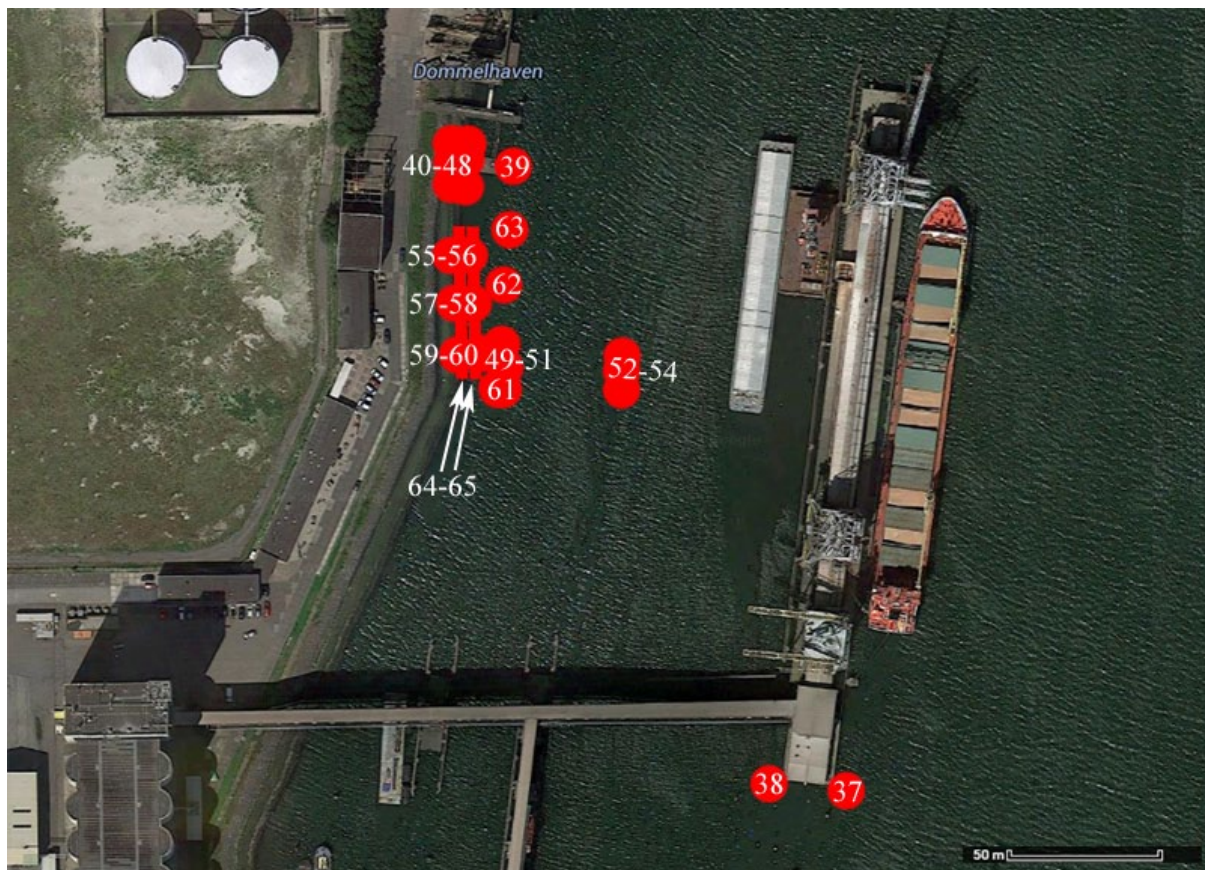


Fig. 6. Sample locations 37-65. Beneluxhaven, European Bulk Services (EBS). Littoral and sub-littoral locations from which samples were taken in the harbour.

3.3 Benelux haven

At the Beneluxhaven samples were taken and searched for species at 29 sites (Fig. 6; Table 3). These sites included habitats in the littoral zone of the dike, the sub-littoral zone of the dike, on the floating docks, on a concrete jetty, on the pilings, in the water column and on and in the sandy bottom. All of these habitats were assumed to have their own unique species communities as they have different environmental characteristics that may be important for the ability of species to settle and grow. Species that live in the littoral zone of the dike for example have to be able to survive being above water during low tide. In addition they are much more exposed to wave action than species that live in the sub-littoral zone. Species in the sub-littoral zone of the dike tend to be covered more by sediments however, and

if the water is murky the amount of sunlight is much less than in the littoral zone, especially with high tide. Sub-littorally much less sediment will fall on organisms that foul vertical structures like pilings, and organisms that foul floating objects like docks. The benefit for species of being on the floating dock, can be that they settle on a surface that is always submerged, but never far under the water line. The amount of sunlight that reaches an organism on the side of a floating dock therefore remains relatively constant, while the amount of sunshine that reaches organisms on pilings will significantly reduce with high tides when the water is murky. In the Beneluxhaven samples all these habitats were searched for species following the HELCOM/OSPAR port survey protocol where possible.

Table 3. Sample locations 37-65 (Fig. 6). Beneluxhaven, European Bulk Services (EBS). Description and geographical coordinates.

Loc.	Description	Geogr. coordinates
37	Concrete jetty	N51 57.004 E4 7.551
38	Concrete jetty	N51 57.026 E4 7.559
39	Floating dock	N51 57.168 E4 7.509
40	Dike, littoral zone, <i>Fucus vesiculosus</i> zone	N51 57.174 E4 7.507
41	Dike, littoral zone, <i>Fucus vesiculosus</i> zone	N51 57.163 E4 7.503
42	Dike, littoral zone, <i>Fucus vesiculosus</i> zone	N51 57.154 E4 7.503
43	Dike, littoral zone, stones breakwater zone	N51 57.174 E4 7.507
44	Dike, littoral zone, stones breakwater zone	N51 57.163 E4 7.503
45	Dike, littoral zone, stones breakwater zone	N51 57.154 E4 7.503
46	Dike, littoral zone, along low water line	N51 57.174 E4 7.507
47	Dike, littoral zone, along low water line	N51 57.163 E4 7.503
48	Dike, littoral zone, along low water line	N51 57.154 E4 7.503
49	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 57.125 E4 7.551
50	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 57.125 E4 7.551
51	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 57.125 E4 7.551
52	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 57.127 E4 7.506
53	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 57.127 E4 7.506
54	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 57.127 E4 7.506
55	Dike, sub-littoral zone	N51 57.156 E4 7.505
56	Dike, sub-littoral zone	N51 57.156 E4 7.505
57	Dike, sub-littoral zone	N51 57.147 E4 7.506
58	Dike, sub-littoral zone	N51 57.147 E4 7.506
59	Dike, sub-littoral zone	N51 57.127 E4 7.507
60	Dike, sub-littoral zone	N51 57.127 E4 7.507
61	Pillar, sub-littoral zone	N51 57.127 E4 7.512
62	Pillar, sub-littoral zone	N51 57.145 E4 7.516
63	Pillar, sub-littoral zone	N51 5.7155 E4 1.518
64	Sub-littoral transect (50 m), 0,5 m depth, parallel to dike	N51 57.127 E4 7.506
65	Sub-littoral transect (50 m), 4 m depth, parallel to dike	N51 57.127 E4 7.506

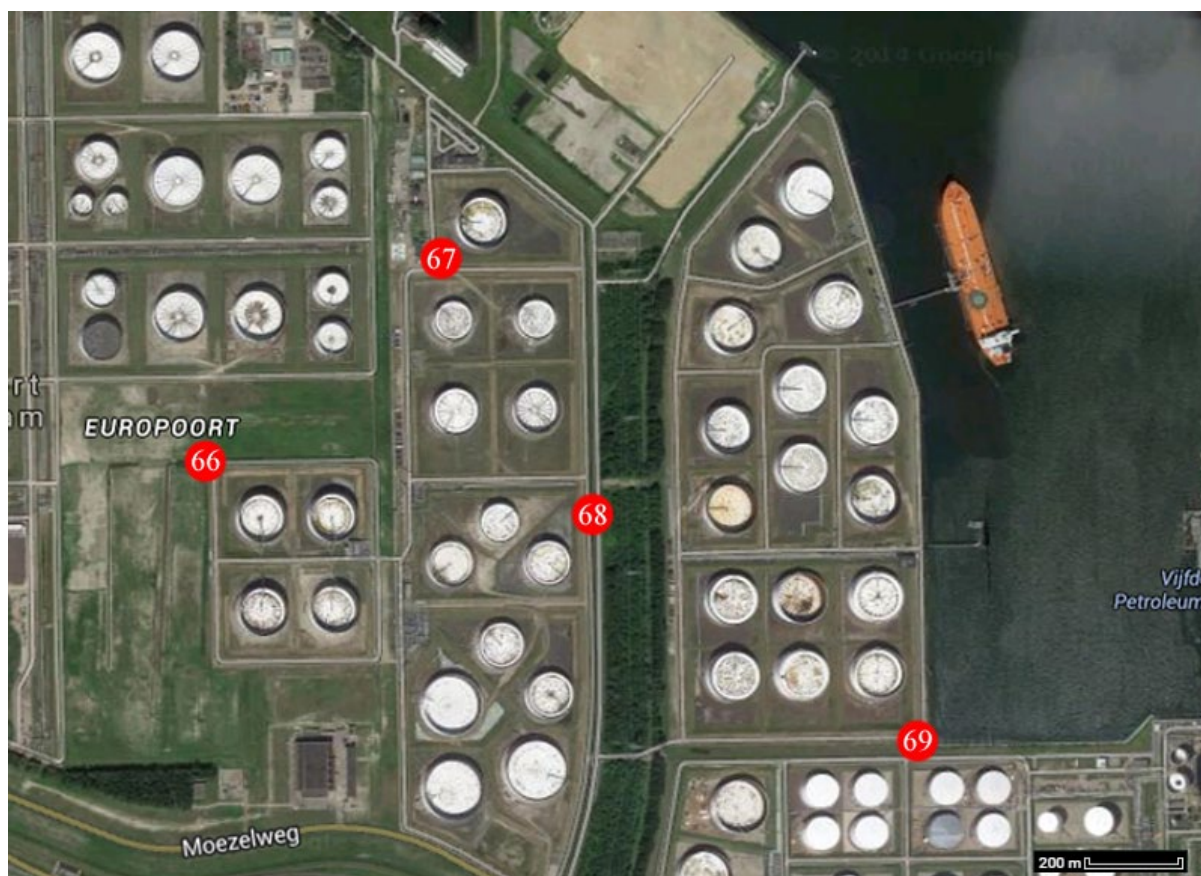


Fig. 7. Sample locations 66-69. Team Terminal. Fire hydranths from which samples were taken.

3.4 Team Terminal

At the Team Terminal four fire hydranths spread over the premises were selected for sampling the underground water system (Fig. 7; Table 4). This water system consists of various pipes that reach throughout the area and is used as a source of water in case of fire. The system is in open connection with the harbour water enabling larvae that are present in the harbour to enter. In general there is no to little current within the system, enabling the settlement of organisms in a sheltered area with relatively clear, i.e. sediment free, water. As there is little current, the salinity of the water in the system remains relatively constant. In most parts of the port of Rotterdam salinities vary strongly because of the tides and the varying amount of rainfall up river. In addition the water in the port tends to be relatively murky and tidal currents can be strong. The

Table 4. Sample locations 66-69 (Fig. 7). Team Terminal. Description and geographical coordinates.

Loc.	Description	Geogr. coordinates
66	Fire hydranth 02HY234	N51 56.568 E4 8.630
67	Fire hydranth 04HY112	N51 56.732 E4 8.908
68	Fire hydranth 07HY309	N51 56.540 E4 9.112
69	Fire hydranth 11HY243	N51 56.350 E4 9.540

environment in the underground water system, which can be described by its sheltered position, little to no currents, little to no sedimentation, and no direct sun light, is therefore unique in the port. As this may enable the settlement of species that can not settle elsewhere in the port, this habitat was included in the present inventory. Several times per year when the system is tested, the water pumps are turned on and water is flushed through the system.



Fig. 8. Sample locations 70-73. 7e Petroleumhaven. Fire hydrants from which samples were taken.

3.5 7e Petroleumhaven

At the 7e Petroleumhaven four fire hydrants spread over the premises were selected for sampling the underground water system (Fig. 8; Table 5). This water system consists of various pipes that reach throughout the area and is used as a source of water in case of fire. The system is in open connection with the harbour water enabling larvae that are present in the harbour to enter. In general there is no to little current within the system, enabling the settlement of organisms in a sheltered area with relatively clear, i.e. sediment free, water. As there is little current, the salinity of the water in the system remains relatively constant. In most parts of the port of Rotterdam salinities vary strongly because of the tides and the varying amount of rainfall up river. In addition the water in the port tends to be relatively murky and tidal currents can be strong.

Table 5. Sample locations 70-73 (Fig. 8). 7e Petroleumhaven. Description and geographical coordinates.

Loc.	Description	Geogr. coordinates
70	Fire hydrant H61	N51 54.473 E4 12.350
71	Fire hydrant H210	N51 54.886 E4 13.031
72	Fire hydrant H267	N51 54.721 E4 13.013
73	Fire hydrant H24	N51 54.346 E4 13.015

The environment in the underground water system, which can be described by its sheltered position, little to no currents, little to no sedimentation, and no direct sun light, is therefore unique in the port. As this may enable the settlement of species that can not settle elsewhere in the port, this habitat was included in the present inventory. Several times per year when the system is tested, the water pumps are turned on and water is flushed through the system.

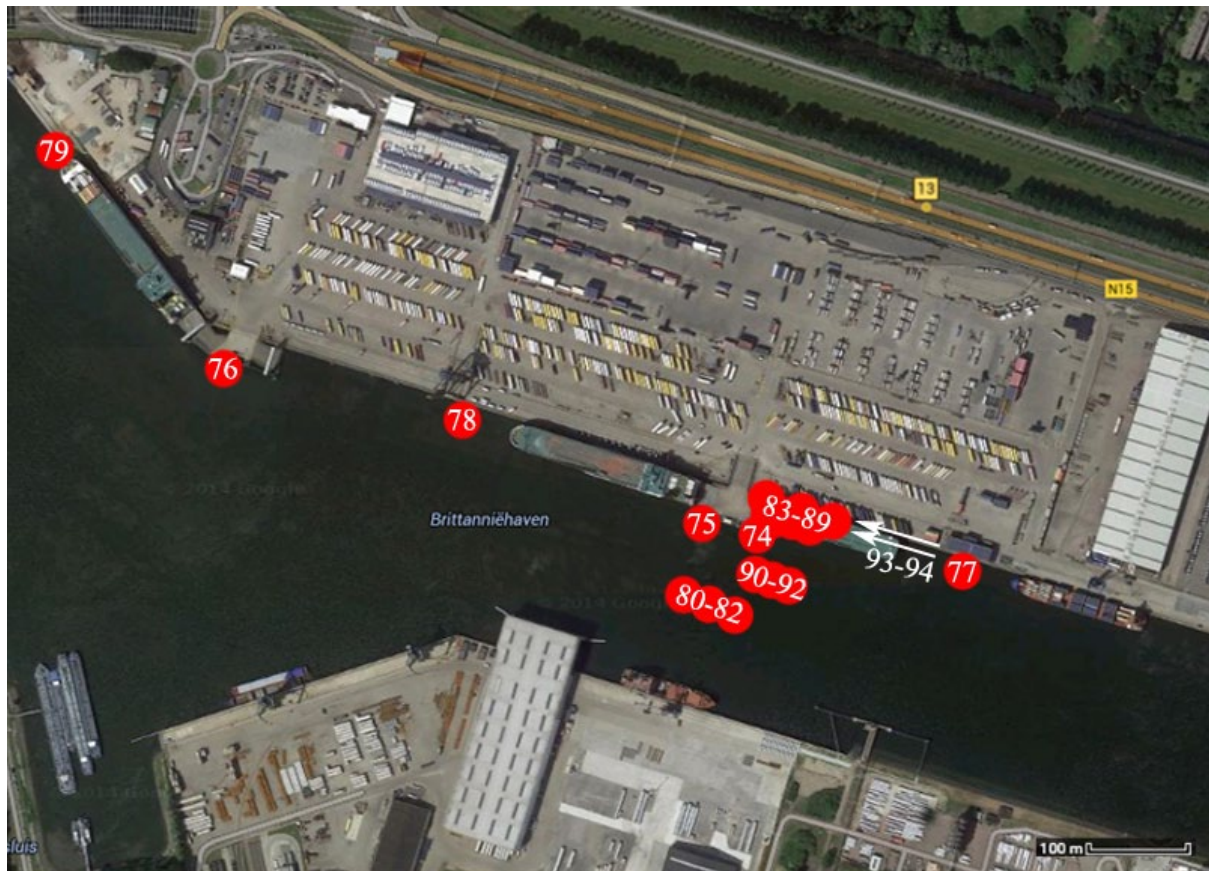


Fig. 9. Sample locations 74-94. Brittaniëhaven, CRO Ports Netherlands. Littoral and sub-littoral locations from which samples were taken in the harbour.

3.6 Brittaniëhaven

At the Brittaniëhaven samples were taken and searched for species at 20 sites (Fig. 9; Table 6). These sites included habitats on the floating docks, on the harbour wall and the pilings, in the water column and on and in the sandy bottom. All of these habitats were assumed to have their own unique species communities as they have different environmental characteristics that may be important for the ability of species to settle and grow. In the Brittaniëhaven samples of all these habitats were searched for species following the HELCOM/OSPAR port survey protocol where possible.

Table 6. Sample locations 74-94 (Fig. 9). Brittaniëhaven, CRO Ports Netherlands. Description and geographical coordinates.

Loc.	Description	Geogr. coordinates
74	Floating dock	N51 53.745 E4 14.403
75	Floating dock	N51 53.748 E4 14.395
76	Concrete jetty	N51 53.829 E4 13.978
77	Harbour wall	N51 53.726 E4 14.608
78	Harbour wall	N51 53.811 E4 14.161
79	Harbour wall	N51 53.951 E4 13.821
80	Bottom, sub-littoral, soft sediments	N51 53.708 E4 14.353
81	Bottom, sub-littoral, soft sediments	N51 53.705 E4 14.365
82	Bottom, sub-littoral, soft sediments	N51 53.699 E4 14.386
83	Floating dock, sub-littoral zone	N51 53.752 E4 14.426
84	Harbour wall and pillars, sub-littoral zone	N51 53.750 E4 14.483
85	Harbour wall and pillars, sub-littoral zone	N51 53.755 E4 14.459
86	Harbour wall and pillars, sub-littoral zone	N51 53.759 E4 14.432
87	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.731 E4 14.428
88	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.731 E4 14.428
89	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.731 E4 14.428
90	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.758 E4 14.440
91	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.758 E4 14.440
92	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.758 E4 14.440
93	Sub-littoral transect (50 m), 12 m depth, parallel to dike	N51 53.759 E4 14.431
94	Sub-littoral transect (50 m), 12 m depth, parallel to dike	N51 53.752 E4 14.473

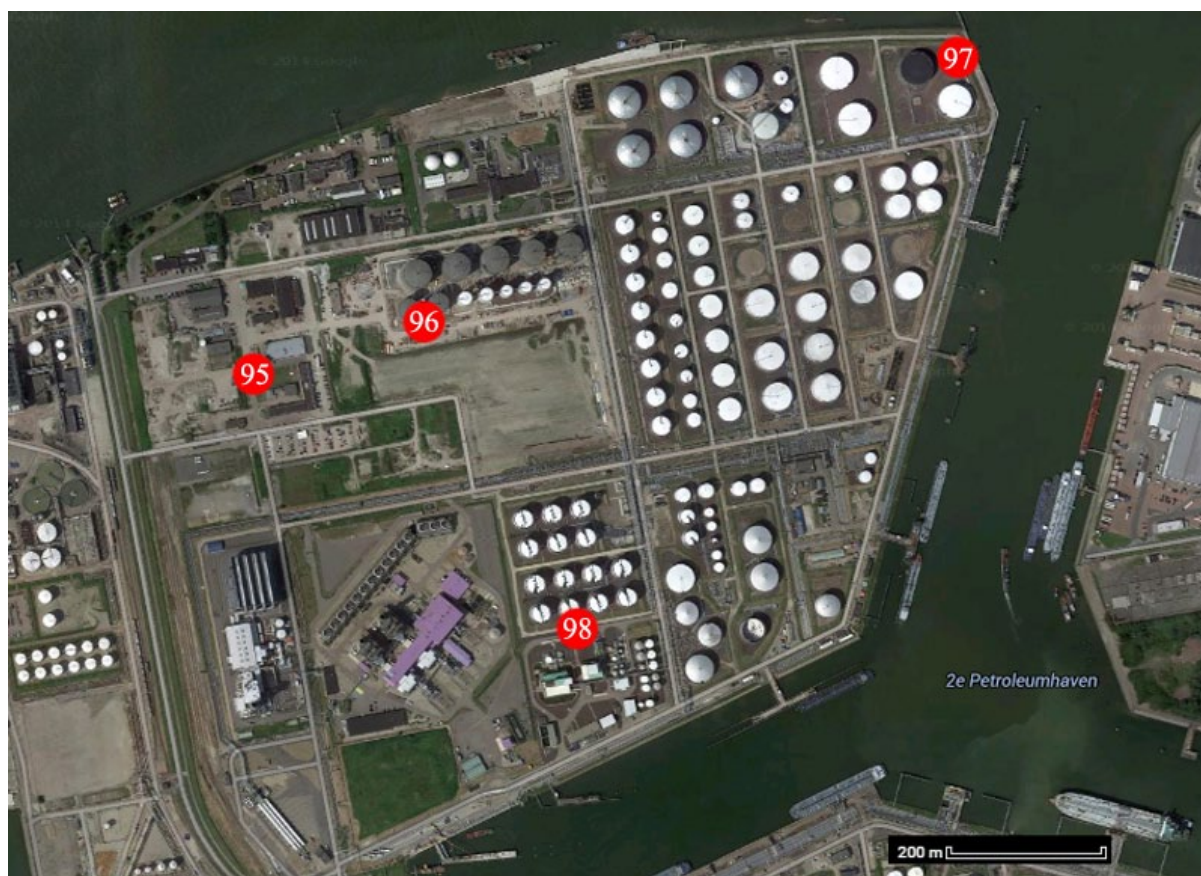


Fig. 10 Sample locations 95-98. NOVA Terminal. Fire hydrants from which samples were taken.

3.7 NOVA Terminal

At the NOVA Terminal four fire hydrants spread over the premises were selected for sampling the underground water system (Fig. 10; Table 7). This water system consists of various pipes that reach throughout the area and is used as a source of water in case of fire. The system is in open connection with the harbour water enabling larvae that are present in the harbour to enter. In general there is no to little current within the system, enabling the settlement of organisms in a sheltered area with relatively clear, i.e. sediment free, water. As there is little current, the salinity of the water in the system remains relatively constant. In most parts of the port of Rotterdam salinities vary strongly because of the tides and the varying amount of rainfall up river. In addition the water in the port tends to be relatively murky and tidal currents can be strong. The environment in the underground water sys-

Table 7. Sample locations 95-98 (Fig. 10). NOVA Terminal. Description and geographical coordinates.

Loc.	Description	Geogr. coordinates
95	Fire hydranth 221	N51 53.621 E4 21.126
96	Fire hydranth 808	N51 53.635 E4 21.318
97	Fire hydranth 179	N51 53.821 E4 21.929
98	Fire hydranth 484	N51 53.422 E4 21.471

tem, which can be described by its sheltered position, little to no currents, little to no sedimentation, and no direct sun light, is therefore unique in the port. As this may enable the settlement of species that can not settle elsewhere in the port, this habitat was included in the present inventory. Several times per year when the system is tested, the water pumps are turned on and water is flushed through the system.

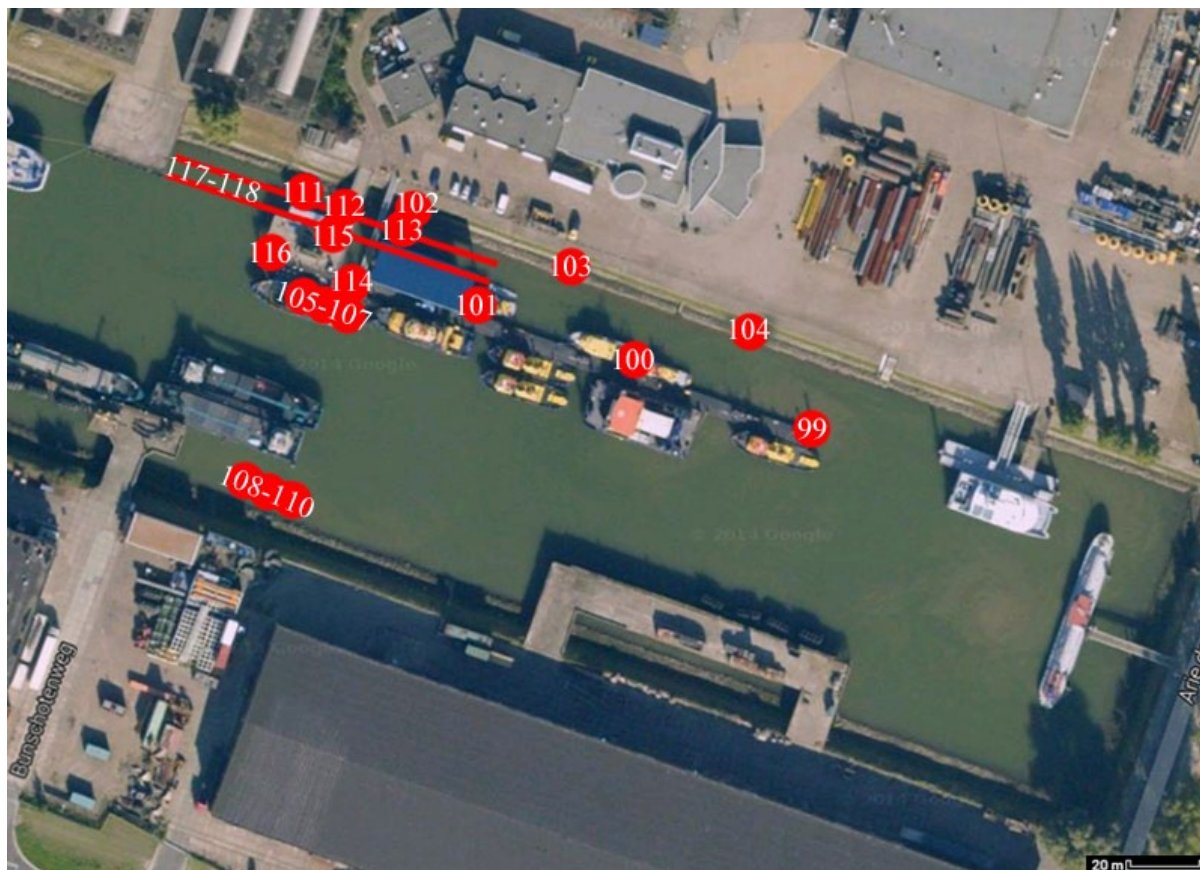


Fig. 11. Sample locations 99-118. 1e Eemhaven, Port of Rotterdam Authority. Littoral and sub-littoral locations from which samples were taken in the harbour.

3.8 1e Eemhaven

At the 1e Eemhaven samples were taken and searched for species at 20 sites (Fig. 11; Table 8). These sites included habitats in the littoral zone of the dike, the sub-littoral zone of the dike, on the floating docks, on the pilings, in the water column and on and in the sandy bottom. All of these habitats were assumed to have their own unique species communities as they have different environmental characteristics that may be important for the ability of species to settle and grow. Species that live in the littoral zone of the dike for example have to be able to survive being above water during low tide. In addition they are much more exposed to wave action than species that live in the sub-littoral zone. Species in the sub-littoral zone of the dike tend to be covered more by sediments however, and if the water is murky the amount

of sunlight is much less than in the littoral zone, especially with high tide. Sub-littorally much less sediment will sink onto organisms that foul vertical structures like pilings, and organisms on floating objects like docks. The benefit for species of being on the floating dock, can be that they settle on a surface that is always submerged, but never far under the water line. The amount of sunlight that reaches an organism on the side of a floating dock therefore remains relatively constant, while the amount of sunshine that reaches organisms on pilings will significantly reduce with high tides when the water is murky. In the 1e Eemhaven samples all these habitats were searched for species following the HELCOM/OSPAR port survey protocol where possible.

Table 8. Sample locations 99-118 (Fig. 11). 1e Eemhaven, Port of Rotterdam Authority. Description and geographical coordinates.

Loc.	Description	Geogr. coordinates
99	Floating dock	N51 53.329 E4 25.147
100	Floating dock	N51 53.337 E4 25.113
101	Floating dock	N51 53.344 E4 25.086
102	Dike, littoral zone	N51 53.362 E4 25.060
103	Dike, littoral zone	N51 53.362 E4 25.069
104	Dike, littoral zone	N51 53.356 E4 25.084
105	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.323 E4 25.029
106	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.323 E4 25.029
107	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.323 E4 25.029
108	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.349 E4 25.041
109	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.349 E4 25.041
110	Bottom, sub-littoral, soft sediments, start of 50 m transect perpendicular to dike	N51 53.349 E4 25.041
111	Dike, sub-littoral zone	N51 53.365 E4 25.032
112	Dike, sub-littoral zone	N51 53.363 E4 25.043
113	Dike, sub-littoral zone	N51 53.359 E4 25.057
114	Pillar, sub-littoral zone	N51 53.348 E4 25.051
115	Pillar, sub-littoral zone	N51 53.359 E4 25.044
116	Pillar, sub-littoral zone	N51 53.355 E4 25.028
117	Sub-littoral transect (50 m), 0.5 m depth, parallel to dike	N51 53.366 E4 25.027
118	Sub-littoral transect (50 m), 1.3 m depth, parallel to dike	N51 53.366 E4 25.027

4. Sampling methods

The HELCOM/OSPAR protocol aims at describing for each habitat that may be present in a port, which monitoring method should be used. The habitats that could be distinguished in the port of Rotterdam are illustrated in figures 12 and 13. Figure 12 hereby illustrates the situation in the Britanniëhaven. Just below the low water line the harbour wall discontinued and an overhang was created supported by pilings, as is illustrated in figure 12. At the other locations a dike was present sloping down to the bottom as is illustrated in figure 13. With the exception of the littoral zone and the underground water systems, the HELCOM/OSPAR protocol describes all of these habitats and for each of them the preferred species survey method. These methods were used during

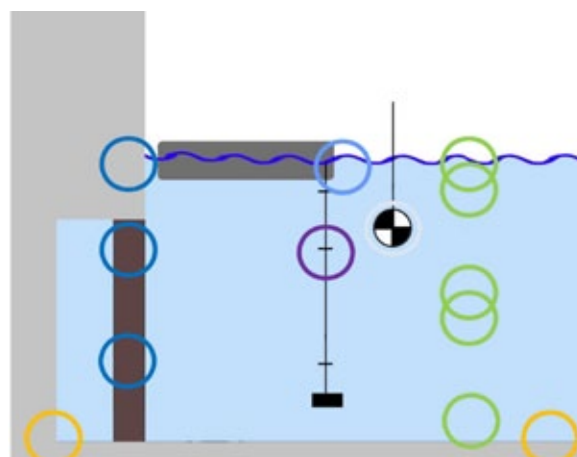


Fig. 12. Habitats sampled in the Port of Rotterdam. Dark blue: harbour wall and pillars in the sub-littoral zone; light blue: floating dock; purple: Fouling plates; orange: bottom; green: water column.

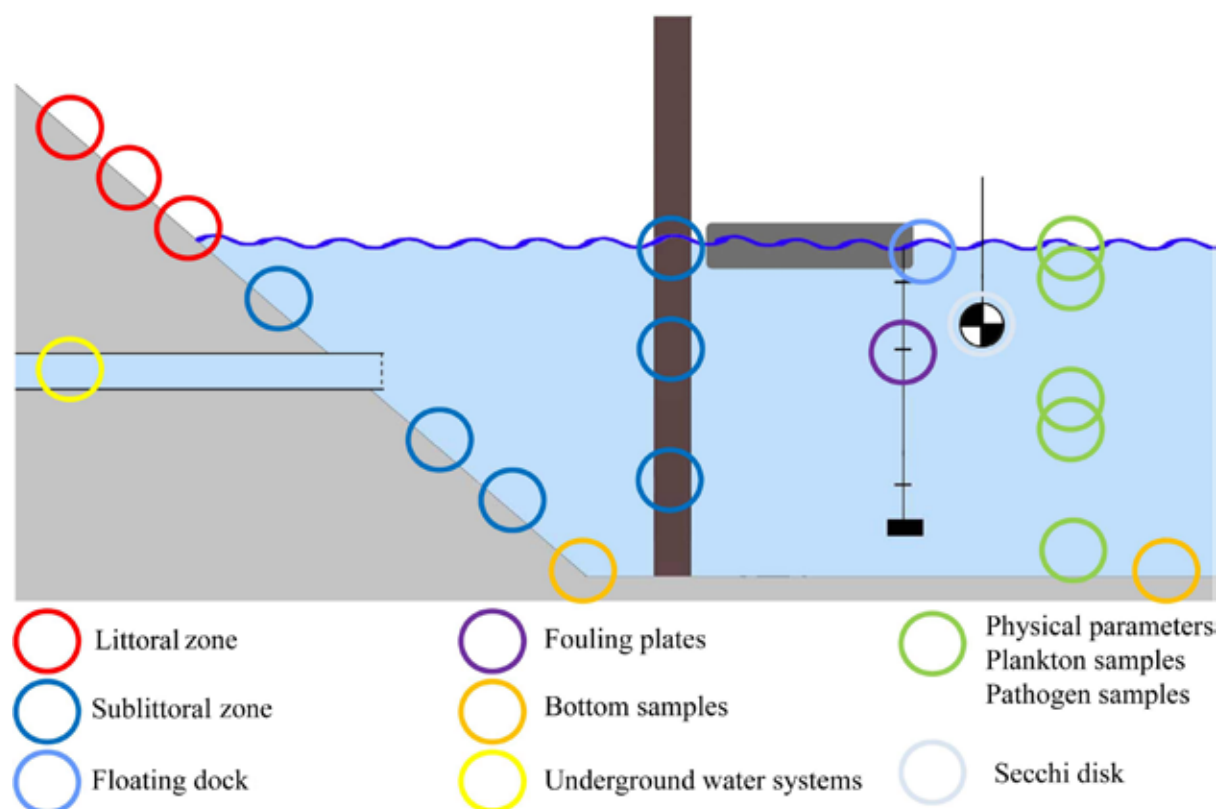


Fig. 13. Habitats sampled in the Port of Rotterdam. Red: dike in the littoral zone; dark blue: dike and pillars in the sub-littoral zone; light blue: floating dock; purple: fouling plate construction; orange: bottom; green: water column; yellow: underground water system. An indication of the methods used are given in the legends.

the Rotterdam Port survey to search each of the habitats. For the underground water systems a monitoring method was used that is not described in the HELCOM/OSPAR protocol. The method used for the survey of the littoral zone of the dike was based on the method with scuba-divers that is described in the HELCOM/OSPAR protocol for the sub-littoral zone of the dike. The turbidity of the water and the species diversity in the bottom and on the sub-littoral zones of the dike and pilings, were sampled with two methods, enabling a comparison of the efficiency and applicability of these sampling methods. In the following paragraphs each of the sampling methods that was used is described in more detail, including the habitat for which they were used, and whether or not they are the preferential method according to the HELCOM/OSPAR protocol for the habitat concerned.

As a quality check, the risk assessment tool under the HELCOM/OSPAR Harmonized Procedure on Exemptions under the Ballast Water Management Convention (http://jointbwmexemptions.org/ballast_water_RA , accessed 10-11-2014) calculates cumulative curves for each of the groups of organisms with the aim of evaluating if the number of samples taken represents the species present in the port (where the curve becomes asymptotic). These accumulation curves are hereby plotted without using a model that corrects for example for the presence of rare species. Models for estimating species diversity differ. They can be inaccurate depending on the species composition present, especially with low sample numbers. We therefor selected six models to calculate species accumulation curves on the basis of each of the sampling datasets. This includes both models that tend to overestimate and models that tend to underestimate the number species. The species accumulation curves were calculated in Primer-E 6.0 (Primer-E, 2007). Three of these models may provide an overestimation of the number of species as they specifically take into account and correct for the presence of a relatively high number of rare species: **Chao 2** - Chao's estima-

tor, based on the number of rare species, using just presence-absence data; **Jackknife 1** - Jackknife estimator based on species that only occur in one sample; **Jackknife 2** - Second order jackknife estimator. The other three methods assume that the abundances of the species within communities present are more equal, which may result in an under-estimation of the number of species, especially when sample numbers are low: **Bootstrap** - Bootstrap estimator based on proportion of quadrats containing each species; **Michaelis-Menton** - Curve fitted to observed S curve: $S(n) = S_{max} - BS(n)/n$; **UGE** - Calculated species accumulation curve according to Ugland, Gray and Ellingsen (2003).

4.1 Physical parameters

At each research area during the monitoring in both spring and summer the physical parameters temperature, pH, salinity and dissolved oxygen were measured at three locations, at least 15 m apart, at three meter intervals from 1 m depth to the bottom (Fig. 14). Additionally, at each of these depths the turbidity was measured in ntu with a turbidity meter. The water transparency was measured at each of the locations using a Secchi disk (30 cm in diameter).

The samples were taken at the various depths with a Van Dorn Water sampler from KC Denmark (Fig. 15). The temperature, pH, salinity (in ppt and PSU) and dissolved oxygen measurements were done with the multimeter HI9829 from Hanna instruments (Fig. 15). The turbidity was measured with the portable turbidity meter HI 93414 from Hanna instruments (Fig. 15).

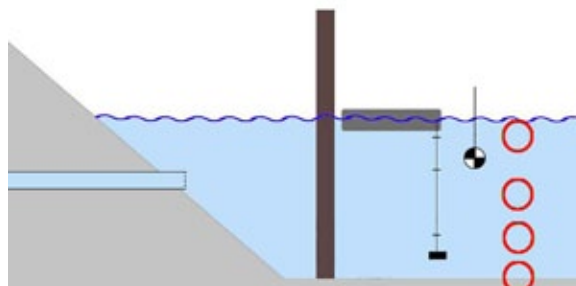


Fig. 14. Water samples were taken from the water surface (30 cm), 1 m, 4 m, 7 m, 10 m, 13 m, 16 m and the bottom.

At each research area the date of the inventory, the wind speed, wind direction, and the air temperature were noted. For each location within an area the start time of the inventory and the geographical coordinates were noted.



Fig.15. Equipment used for measuring water quality parameters. A. Multiparameter HI9829 of Hanna Instruments; B. Van Dorn Watersampler of KC Denmark; C. Portable Turbidity Meter HI 93414 of Hanna Instruments.

Using a turbidity meter (Fig. 15) for measuring the turbidity is not described as a method for measuring water transparency in the HELCOM/OSPAR protocol. This method was added here as it is an accurate, repeatable and objective measurement method of water transparency while the Secchi disk method that is indicated in the HELCOM/OSPAR protocol is a subjective measurement method that can give varying results among different observers. Except for the turbidity (in ntu) measurement, all measurements were done according to the HELCOM/OSPAR protocol.

4.2 Human pathogens

Each research area during the monitoring in both spring and summer was monitored for the presence of bacteria according to Regulation D-2 (Intestinal Enterococci, *Escherichia coli* and *Vibrio cholerae*). For these measurements a water sample of about 4 liters from approximately 30 cm depth (Fig. 16) was taken with a Van Dorn Water sampler (Fig. 15) in each of the areas. This water was used to assess the concentrations of *E. coli*, Enterococci and *Vibrio cholerae* present. This was done with the help of *E. coli*, Enterococci and *Vibrio* spp. specific growth media (Fig. 17) and conditions on the basis of undiluted water samples at first, and if necessary (when relatively high numbers of colonies were present) with dilutions of 1:1, 1:10 and 1:100. As *Vibrio cholerae*, the only *Vibrio* species that needs to be monitored according to the HELCOM/OSPAR port survey protocol, is known for its yellow colonies, *Vibrio* colonies with a yellow colour were identified to the species level with a MALDI-TOF MS analysis at the department of Medical Microbiology and Infectious Diseases of the Erasmus Medical Center in Rotterdam. Although not all *Vibrio* species can be identified by MALDI-TOF MS analysis, this method has been optimized to reliably identify *Vibrio cholerae* colonies including the pathogenic O1 and O139 strains on which one

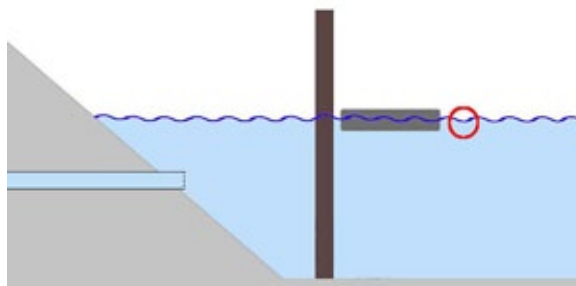


Fig. 16. A water sample from the water surface (30 cm) was tested for human pathogens.



Fig. 17. *Escherichia coli* counts were done with the TBX plate method (ISO 16649-2).

should focus according to Regulation D-2 Ballast Water Performance Standard of the “Ballast water management convention”.

All measurements were done according to the HELCOM/OSPAR protocol. Although this protocol only proposes to monitor the presence of Enterococci, *Escherichia coli* and *Vibrio cholerae* the MALDI-TOV analyses detected the presence of various other *Vibrio* species of which some may be considered non-native.

4.3 Plankton

As described in the HELCOM/OSPAR protocol samples for the plankton monitoring were taken in both spring and summer of 2014. The analyses of these samples were done by Koeman en Bijkerk BV. In total 12 samples were analysed in each season, i.e. spring and summer: 3 x a preserved phytoplankton sample from a water sample, 3 x an alive phytoplankton sample from a plankton net sample, 3 x a preserved zooplankton sample from a plankton net sample, and 3 x an alive larger zooplankton sample from a plankton net sample.

4.3.1 Phytoplankton

At each research area during the monitoring in both spring and summer, water samples were taken, following the HELCOM/OSPAR protocol to assess the phytoplankton diversity present. Samples for the phytoplankton monitoring have been collected with a Van Dorn Water sampler (Fig. 15). At each area samples have been collected by obtaining a 250 ml water sample pooled from three locations, at least 15 m apart. Samples (0.5 l) have been taken at each location at the surface (1 m depth) and at 5 m depth. Additionally, a concentrated vertical sample using a small hand held 20 µm plankton net, 250 mm wide and 500 mm deep, was taken (Fig. 18). Three tows, at least 15 m apart have been taken to ensure an adequate sample size. Haul and tow rates did not exceed 0.25 – 0.30 m/s. Clear, colourless iodine-proof bottles with tightly fitting screw caps have been used as containers. Samples have been preserved in acid Lugol solution (0.25 – 0.5 cm³/ 100 cm³ sample) and placed in a cooler for transport to the analysing laboratory. The analyses of the samples were done by Koeman and Bijkerk bv according to the HELCOM/OSPAR protocol.

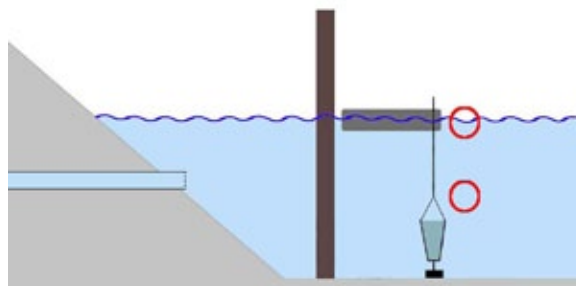


Fig. 18. Water samples that were taken from the water surface and at 5 m depth are checked for plankton.



Fig. 19. Plankton net for larger zooplankton.

4.3.2 Zooplankton

Vertical zooplankton samples were collected with a standard 100 µm mesh drop net, 500 mm wide and 2 m deep (Fig. 19). The samples were taken, following the HELCOM/OSPAR protocol. Three tows, at least 15 m apart have been conducted per area to ensure an adequate sample size. Tow rate was adjusted to approximately 1 m/s and the net was stopped 1 m before the bottom. Samples were placed in sample bottles and were preserved in either 96% ethanol or acid Lugol solution (0.25 – 0.5 cm³/ 100 cm³ sample) as instructed by the analyzing laboratory Koeman and Bijkerk BV. The analysis could not be done exactly according to the HELCOM/OSPAR protocol. Following the HELCOM protocol “All specimens should be identified and counted until one has reached 100 individuals of each of the three dominating taxonomic groups (excluding nauplii, rotifers and

tintinnids)”. With the high density of plankton in the samples and composition of the species in the samples taken in the port of Rotterdam, it would take several days per sample to reach 100 individuals of each of the three dominating taxonomic groups (excluding nauplii, rotifers and tintinnids). Instead all specimens were identified and counted until 100 individuals were reached for the dominating taxonomic group (excluding nauplii, rotifers and tintinnids). Each sample was searched for species for at least four hours.

4.3.3 Larger zooplankton including gelatinous species

Vertical “larger” zooplankton samples including gelatinous species were collected with a standard 500 µm mesh drop net, 500 mm wide and 2 m deep (Figs 18-19). Three tows, at least 15 m apart have been conducted per area to ensure an adequate sample size. Tow rate was adjusted to approximately 1 m/s and the net was stopped 1 m before the bottom. Larger zooplankton species, including gelatinous species, were examined by GiMaRIS immediately after collection without preservation. All larger zooplankton species, including gelatinous species, were digitally photographed with a 21.1 megapixel camera (Canon EOS 5D Mark II), in a tray with seawater, while still alive. The sampling and the analysis were done according to the HELCOM/OSPAR protocol.

4.4 Traps

Mobile epifauna, such as crabs and fish, were sampled in late summer at each site using light weight traps tethered to existing structures like pilings, buoys and docks (Fig. 20). Traps were baited with frozen fish (cod). As suggested in the HELCOM/OSPAR protocol two types of traps were used to sample mobile epifauna: Chinese crab traps and Gee's minnow traps (Fig. 21). Three traps of both trap types were deployed at each site for at least 48 h. The traps were weighted by attaching a brick to the frame. After retrieving the traps, the catch was identified in the field and/or placed in zipper storage bags for identification in the lab. The traps were deployed at the locations where also water samples were taken, and petit ponar grabs were conducted including a bottom sediment analysis, which can be used to describe the environment where the trap was deployed. The Chinese crab trap measured 63 cm x 42 cm x 20 cm, with 1.3 cm mesh netting. The Gee's minnow trap measured 45 x 22 x 22 cm, with 6 mm netting and a 8 cm mouth.

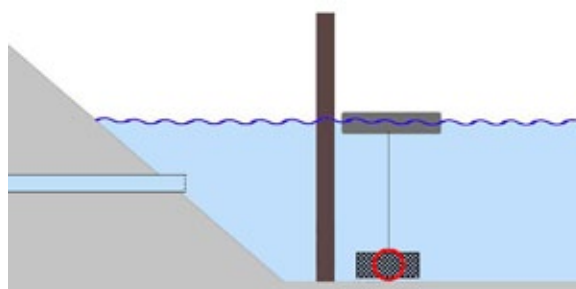


Fig. 20. Traps were attached to a dock or the harbour wall and weighted with a brick to place them on the bottom.



Fig. 21. The two trap types that were described in the HELCOM/OSPAR protocol. A: Chinese crab trap; B: Gee's minnow trap.

4.5 Fouling plates

In each of the four research areas three fouling plate units were deployed at least 15 m apart from each other on dock structures at locations where they are not disturbed by for example port traffic. The fouling plate units were constructed of approximately 11 m of rope (≈ 0.5 cm), three gray 15 cm x 15 cm PVC plates and a brick. Each plate was sanded briefly prior to the deployment to provide a more hospitable settling substrate for the organisms. A hole (≈ 0.5 cm) was drilled at the center of each plate for the rope. Plates were secured on the rope at set distances using knots secured with zipties. The plates were secured at 3 m, 5 m and 9 m distances measured from the beginning of the rope. Units were tied securely to the dock structures so that the first plate was submerged at approximately 1 m depth, the second plate at approximately 3 m and the third plate at approximately 7 m of depth (Figs 22-23). If the depth of a site was insufficient, the deeper plates were removed and the bricks were tied at suitable depth for the site. Fouling plate units were retrieved with the summer sampling. When the units were retrieved, they were carefully pulled on the dock to prevent losing organisms such as mobile epifauna. The whole units were placed on plastic sheets and the ropes and bricks were separated from the plates. The plates were photographed and placed in labeled resealable plastic bags with some sea water prior to transport. The bricks and ropes were packed in separate bags. All detached organisms were collected and placed into a separate labeled ziplock bags. Most organisms were photographed and identified in the field or in the laboratory directly after collecting them while they were still alive. The smaller algal specimens were preserved on 4% formaldehyde and identified in the laboratory at a later time. The settlement plate deployment and analysis could be conducted according to the HELCOM/OSPAR protocol at most locations. At some locations more than 80 kilograms of fouling attached itself to the settlement plate units (Fig. 23), making it virtually impossible to lift the units out of

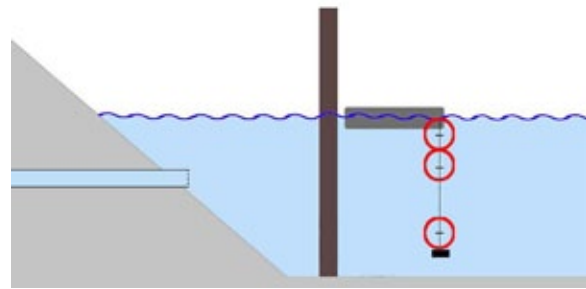


Fig. 22. Fouling plate constructions with a fouling plate construction at 1 m, 3 m and 7 m depth (when depth was sufficient) were deployed in the water.

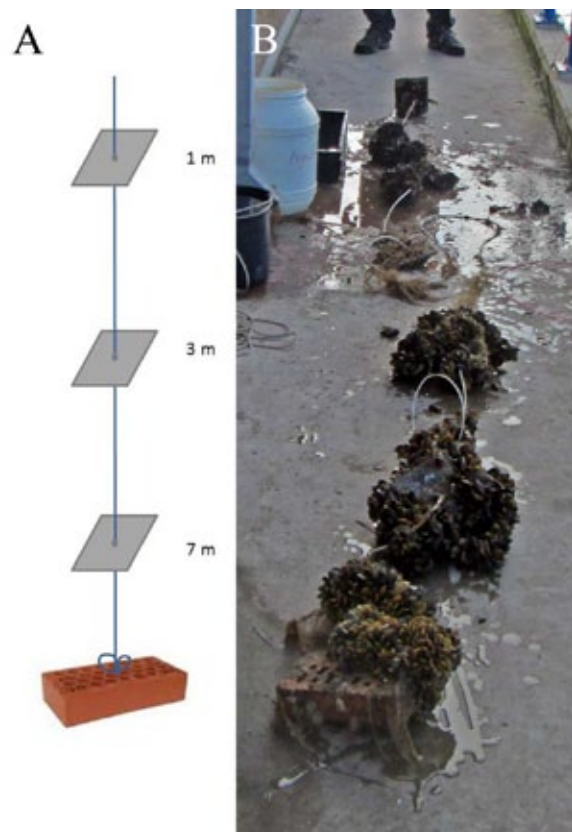


Fig. 23. Fouling plate construction. A: 15 x 15 cm fouling plates of grey PVC, hanging at various depths in the water, as illustrated in the HELCOM/OSPAR protocol; B: fouling plate construction retrieved after 4 month in the water.

the water however. It took three persons to lift these units out of the water and some of the fouling organisms fell off the units the moment they were lifted above the water.

4.6 Sub-littoral scrape samples taken with scraping tools from shore

As described in the HELCOM/OSPAR protocol, scrape samples were taken from floating docks and pillings. With a paint scraper or an aluminum hand net on a 3 m long pole equipped with a scraping blade (Fig. 24), scrape samples were taken from the floating docks in each research area, and at low tide from the pilings where these could be reached from the docks (Fig. 25). For each scrape sample, the surface scraped was estimated and noted. All scrape samples were first placed in a plastic tray and photographed and identified in the field where possible. The remaining organisms were collected and preserved on either ethanol 96% (animals) or formaldehyde 4% (algae), and identified in the laboratory.

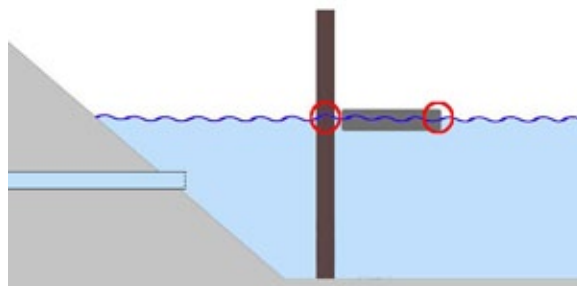


Fig. 25. Scrape samples were taken with a scrape net from floating docks and pillars.



Fig. 24. Scraping species from a pilar with a net on a pole, equipped with a scraping blade.

4.7 Dike fouling, littoral zone

During the summer monitoring the littoral zone of the dike, if present in a research area, was monitored at low tide.

A methodology for monitoring the littoral zone of the dike is not described in the HELCOM/OSPAR protocol. The method used in the port of Rotterdam was based on the monitoring method with scuba-divers that is described in the HELCOM/OSPAR protocol for the sub-littoral zone of the dike. In each clearly distinguishable littoral zone on the dike (Figs 26-27) three replicate 0.10 cm² quadrates, at least 15 m apart along a 45 meter horizontal transect, were digitally photographed. The area of each quadrate was scraped straight into zipper bags. All species were identified in the field when possible or else in the laboratory. Visual observations of additional species that were encountered in the transects in between the quadrate locations were noted.

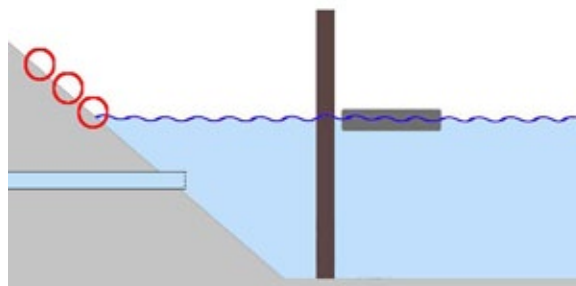


Fig. 26. Dike fouling in the littoral zone.



Fig. 27. Dike fouling in the sub-littoral zone.

4.8 Sampling with scuba-divers

According to the HELCOM/OSPAR protocol one should use scuba-divers for monitoring the species diversity in ports if diving is an option. The sampling by divers was organized by Bureau Waardenburg B.V. The dives were done in a team consisting of Bureau Waardenburg B.V., Joop Coolen and Duik- en bergingsbedrijf W.SMIT B.V.

4.8.1 Scrape sampling: Vertical structures

As described in the HELCOM/OSPAR protocol at least three pilings or similar structures (Fig. 28) per research area, at least 10-15 meter apart, were sampled by scuba divers during the summer monitoring. The selected pilings were vertically inspected and sampled. Where possible three replicate 0.10 m² quadrates were digitally photographed and scrape sampled at depths of 0.5 m, 3.0 m, 7.0 m and close to the bottom. The area of a quadrate was scraped straight into pre-labeled bags using a hand-held scraper tool, after taking the photo. The samples were placed in coolers and transported to the laboratory where they were identified. Although the samples could be taken in all research areas, the divers were unable to take the photographs of most of the quadrates because of the relatively murky waters in the port of Rotterdam.

4.8.2 Scrape sampling: Stone slope

As described in the HELCOM/OSPAR protocol similar scrape samples as were taken from the vertical structures, should also be taken from rocky shores and break waters (Fig. 29). At none of the locations the visibility allowed the scuba-divers however to make photographs of a quadrate or a similar surface of about 0.10 m². As the dikes were covered with oysters it was impossible for the scuba-divers to scrape a 0.10 m² surface.

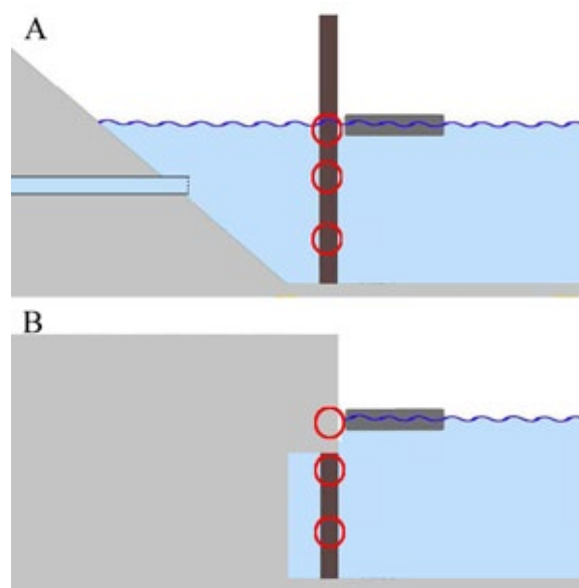


Fig. 28. Samples were taken by divers. Scrape samples were taken from [A] vertical pillars and [B] the harbour wall.

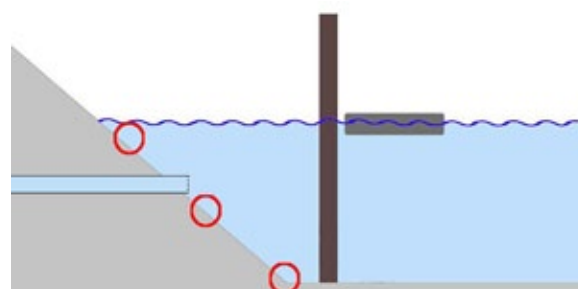


Fig. 29. Scrape samples taken by divers from the stone slope.

Instead, oysters and rocks in a surface area of about 0.10 m², were collected in a plastic bag and analyzed in the laboratory.

4.8.3 Transect observations

As described in the HELCOM/OSPAR protocol during the summer monitoring visual searches were conducted along 50 m transects along the dock/shore at several depths ranging from 0.5 m to the bottom to provide a visual idea of the bottom and record the presence of any non-native species including epifauna. In each research area at least two such transects were searched for species by scuba-divers. A video-camera on the helmet of the diver was used for recording. Because of a layer of silt and fine mud on the bottom the visibility at most locations was minimal however. Therefore most organisms could not be identified to the species level while diving and most of the video footage and camera images were inadequate for the identification of the species.

4.8.4 Visual observations during monitoring

As is described in the previous paragraph the bad visibility at most of the locations in the port of Rotterdam hampered visual observations made by scuba-divers and made it virtually impossible to make photographs and video footage that could aid the identification of the organisms that were encountered. Scuba-divers could therefore not record many visual observations as described in the HELCOM/OSPAR protocol.

4.8.5 Bottom sampling with hand corer

As described in the HELCOM/OSPAR protocol during the summer monitoring infauna was sampled (Fig. 30) on a 50 m transect perpendicular to the shore, by using benthic core tubes (0.025 m² hand corers). The corers were pushed to a depth of 20 to 25 cm if possible. Along the transect three inner cores (0 m at the transect) and three outer cores (50 m at the transect) were taken. They were transferred to 0.5 mm mesh bags and rinsed under water or transported to the surface for sieving, depending on the conditions. In most

research areas these samples could be taken, although a thick layer of oyster shells at the start of the transect in the Britanniëhaven made it impossible to get the corer into the ground. Instead a bag of oyster shells was collected at that site. All species were collected in bags and transferred in a cooler to the lab where most organisms could be identified while still alive. The rest of the organisms was preserved on either ethanol or formaldehyde and identified at a later time.

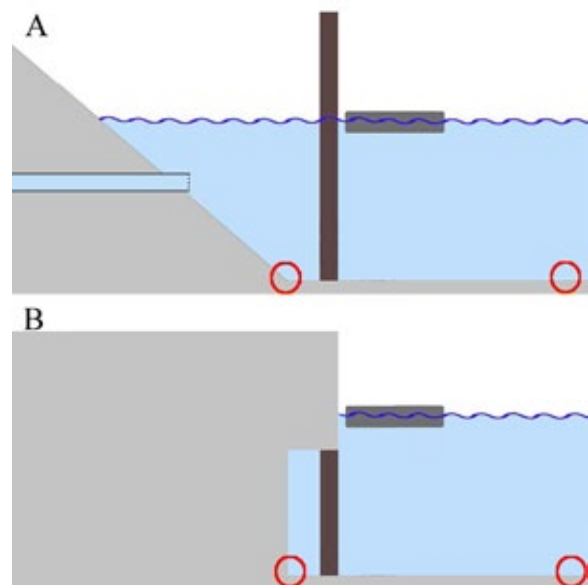


Fig. 30. Bottom samples taken by divers at the beginning and end of a 50m transect perpendicular to (A) the dike or (B) the harbour wall.

4.9 Petit ponar bottom sampling

As described in the HELCOM/OSPAR protocol during the summer monitoring three samples were taken in each research area at a distance of at least 15 m apart with a benthic grab operable from a dock, i.e. a petit ponar (Figs 30-32). Next to being suitable for soft sediments, the petit ponar is also suitable for taking samples in bottoms with pebbles and shells. In all research areas the samples could be taken. A thick layer of oyster shells along the shore in the Britanniëhaven made it impossible to operate the petit ponar from shore. Instead the petit ponar samples were taken there from a boat in the center of the harbour. The samples were sieved with a 0.5 mm sieve, and transferred to sample jars, after which the samples were analyzed in the laboratory. As the pe-

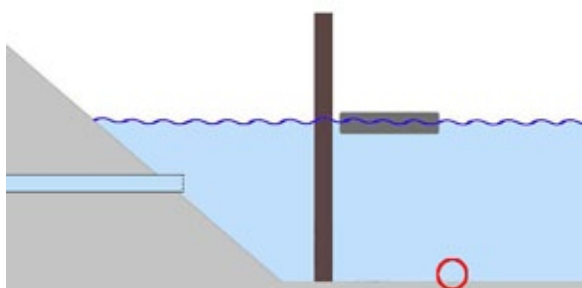


Fig. 31. Bottom samples were taken with a Petit Ponar grab (Fig. 32).



Fig. 32. The Petit Ponar, a modified Van Veen grab, suitable for samples of soft substrate bottoms as well as bottoms with hard substratum like pebbles and shells.



Fig. 33. The Geotech Sieve Analysis Field Kit to determine the exact composition of the bottom sediments.



Fig. 34. GiMaRIS design of cable with hand holds to easily lift the petit ponar out of the water by hand.

tit ponar is relatively heavy, it was attached to an iron cable, with handholds every half meter. With these handholds the petit ponar can easily be lifted out of the water by hand by one person without having to be very strong. Without the handholds this would have been very difficult because of the weight, especially when the grab is full. Each handhold on the cable was made by a stainless steel wire rope clamp, which was wrapped into a piece of neoprene fastened by cable ties and wrapped with duct tape (Fig. 34). From all bottom samples a 50 ml tube full of sediment was taken to the laboratory. There the sediment was dried and analyzed with a Geotech Sieve Analysis Field Kit (Fig. 33).

4.10 Hand dredge sampling

As an additional method to sample the sub-littoral zones of the harbour, a professional ‘Naturalists’ hand dredge was used, weighing 5 kg, with a 450 x 185 mm frame and a net bag with a 1 mm mesh size. During the summer monitoring three dredge samples per research area at a distance of at least 15 m from each other were taken. The dredge was deployed from the docks and pulled over the bottom over a distance of 10 meter (Figs 35-36). As the dredge can be heavy we suggest using the same cable with handholds as is described above in the case of the petit ponar (Fig. 34). The habitat on the bottom that is sampled with a hand dredge is most similar to the bottom habitat that is sampled by divers. Although the method is not described in the HELCOM/OSPAR protocol, dredges are commonly used in marine benthos monitoring and was therefore also used during the present inventory.

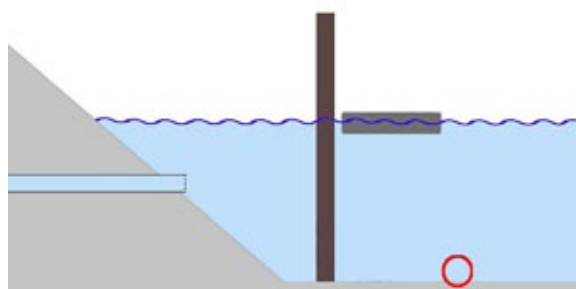


Fig. 35. Dredge samples were taken from a transect on the bottom along floating docks or the harbour wall.



Fig. 36. Sampling with a hand dredge.

4.11 Fire hydrant sampling

On the terrain of the majority of the larger companies (especially oil terminals) in the port of Rotterdam, a watersystem is present with underground pipes. These system are used as a source of water in case of fire as the drinking water supply will be inadequate for fires at these companies of which most store oil in huge quantities. These watersystems are in open connection with the port's water enabling larvae that are present in the port to enter (Fig. 37). In general there is little to no current within the system, enabling the settlement of organisms in a sheltered area with relatively clear, i.e. sediment free, water. As there is little current, the salinity of the water in the system remains relatively constant. In most parts of the port of Rotterdam salinities vary strongly because of the tides and the varying amount of rainfall up river. In addition the water in the port tends to be relatively murky and tidal currents can be strong. The environment in the underground water systems is unique in the port and was therefore sampled during the port survey. Although the HELCOM/OSPAR protocol does indicate that all port environments should be monitored, it does not specify the underground systems as a potential habitat and therefore does not describe the monitoring method that should be used. We have used the Pac-Bag® system of Corexeed B.V. (<http://www.corexeed.eu/en/>) and asked Corexeed B.V. to take samples for us out of at least four different hydrants spread over the terrain of four companies that lay close to the four research areas where

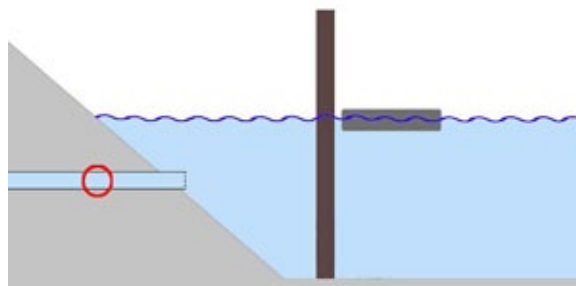


Fig. 37. Underground water systems.

the rest of the samples were taken in the port (Fig. 3). The Pac-Bag® system is a mesh bag that was specifically designed to be attached to a hydrant to take a sample of the organisms that live inside a water system (Fig. 38).



Fig. 38. To detect fouling organisms in the underground water systems, the water is flushed through the systems and sieved with a Pac-Bag® from coreXeed B.V.

5. Results

In total 257 samples were taken from 118 different locations in the Port of Rotterdam. In these samples a total of 225 species were identified to the species level (Table 9). The physical parameters measured and the species that were recorded within each of the monitoring methods in each of the research areas, are presented in the next paragraphs, which also describe whether or not the method description in the HELCOM/OSPAR

protocol could exactly be followed. The species that were scored in each of the samples taken, including the ones that could not be identified to the species level, can be found in the Appendix, i.e. Gamaris report 2014_32, including their abundances. Organisms that could not be identified to the species level concern for example algal specimens that miss the reproduction organs, which are diagnostic for the species, and visual observations of species that were not collected and could only be identified on the basis of their internal anatomy, for example sponges.

Table 9. The 225 species that were identified to the species level in the selected four research areas in the port of Rotterdam. Non-native species are highlighted.

Species	Authority	Group	Origin	Research area 1: 8e Petroleumhaven	Research area 2: Beneluxhaven	Research area 3: Brittaniëhaven	Research area 4: 1e Eemhaven
<i>Aglaothamnion hookeri</i>	(Dillwyn) Maggs & Hommersand	Algae	Native	1			
<i>Blidingia marginata</i>	(J.Agardh) P.J.L.Dangeard	Algae	Native				1
<i>Blidingia minima</i>	(Nägeli ex Kützinger) Kylin	Algae	Native	1			1
<i>Callithamnion corymbosum</i>	(Smith) Lyngbye	Algae	Native	1	1	1	
<i>Ceramium cimbrium</i>	H.E.Petersen	Algae	Native	1	1		
<i>Ceramium tenuicorne</i>	(Kützinger) Waern	Algae	Native	1	1		
<i>Chaetomorpha linum</i>	(O.F. Müller) Kützinger	Algae	Native	1			
<i>Dasydiphonia japonica</i>	(Yendo) H.-S. Kim	Algae	Non-native	1	1		
<i>Elachista fucicola</i>	(Vellay) J.E.Areschoug	Algae	Native	1			
<i>Erythrotrichia carnea</i>	(Dillwyn) J.Agardh	Algae	Native		1		
<i>Fucus spiralis</i>	Linnaeus	Algae	Native		1		
<i>Fucus vesiculosus</i>	Linnaeus	Algae	Native	1	1	1	
<i>Polysiphonia fucoides</i>	(Hudson) Greville	Algae	Native	1	1		
<i>Polysiphonia stricta</i>	(Dillwyn) Greville	Algae	Native	1	1		1
<i>Porphyra purpurea</i>	(Roth) C.Agardh	Algae	Native	1			
<i>Pylaiella littoralis</i>	(Linnaeus) Kjellman	Algae	Native	1	1		
<i>Rhizoclonium riparium</i>	(Roth) Harvey	Algae	Native	1			
<i>Ulva cf rigida</i>	C.Agardh	Algae	Native	1			
<i>Ulva compressa</i>	Linnaeus	Algae	Native	1			
<i>Ulva curvata</i>	(Kützinger) De Toni	Algae	Native	1	1	1	
<i>Ulva flexuosa</i>	Wulfen	Algae	Native		1		
<i>Ulva intestinalis</i>	Linnaeus	Algae	Native	1			
<i>Ulva linza</i>	Linnaeus	Algae	Native	1			1
<i>Ulva pertusa</i>	Kjellman	Algae	Non-native	1	1		

Species	Authority	Group	Origin	Research area 1: 8e Petroleumhaven	Research area 2: Beneluxhaven	Research area 3: Brittaniëhaven	Research area 4: 1e Eemhaven
<i>Ulva prolifera</i>	Müller	Algae	Native	1	1		1
<i>Micromonas pusilla</i>	(Butcher)	Algae	Native	1	1		1
<i>Pyramimonas longicauda</i>	L. Van Meel, 1969	Algae	Native		1	1	
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1	1	1	1
<i>Harmothoe imbricata</i>	(Linnaeus, 1767)	Annelida	Native		1		
<i>Lepidonotus squamatus</i>	(Linnaeus, 1758)	Annelida	Native	1	1	1	
<i>Neoamphitrite figulus</i>	(Dalyell, 1853)	Annelida	Native		1		
<i>Nereis pelagica</i>	Linnaeus, 1758	Annelida	Native	1	1	1	
<i>Polydora ciliata</i>	(Johnston, 1838)	Annelida	Native	1	1		
<i>Oikopleura (Vexillaria) dioica</i>	Fol, 1872	Appendicularia	Native	1	1	1	1
<i>Asciidiella aspersa</i>	(Müller, 1776)	Asciidiacea	Native	1	1	1	
<i>Botryllus schlosseri</i>	(Pallas, 1766)	Asciidiacea	Native	1	1	1	
<i>Ciona intestinalis</i>	(Linnaeus, 1767)	Asciidiacea	Native	1	1	1	
<i>Molgula manhattensis</i>	(De Kay, 1843)	Asciidiacea	Non-native	1	1	1	
<i>Styela clava</i>	Herdman, 1881	Asciidiacea	Non-native	1	1	1	
<i>Aeromonas veronii</i>	-	Bacteria	Native	1		1	
<i>Enterococci sp.</i>	Thiercelin & Jouhaud, 1903	Bacteria	n.a.	1	1	1	1
<i>Escherichia coli</i>	Castellani & Chalmers, 1919	Bacteria	Native	1	1		1
<i>Planktothrix agardhii</i>	(Gomont) Anagnostidis & Komárek, 1988	Bacteria	Native				1
<i>Vibrio alginolyticus</i>	Sakazaki, 1968	Bacteria	Native	1		1	
<i>Vibrio cf anguillarum</i>	-	Bacteria	Native			1	
<i>Vibrio cf brasiliensis</i>	Thompson <i>et al.</i> , 2003	Bacteria	Non-native			1	
<i>Vibrio cf vulnificus</i>	(Reichelt, et al, 1979) Farmer, 1980	Bacteria	Native			1	
<i>Vibrio parahaemolyticus</i>	Sakazaki, et al, 1963	Bacteria	Native	1		1	
<i>Alcyonidioides mytili</i>	(Dalyell, 1848)	Bryozoa	Native	1	1	1	
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native	1	1	1	
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Bryozoa	Native	1	1		
<i>Electra pilosa</i>	(Linnaeus, 1767)	Bryozoa	Native			1	
<i>Achnanthes brevipes</i>	C. Agardh, 1824	Chromista	Native				1
<i>Actinocyclus normanii</i>	(Gregory) Hustedt, 1957	Chromista	Native	1			1
<i>Actinoptychus octonarius</i>	(Ehrenberg) Kützing, 1844	Chromista	Native	1	1	1	
<i>Actinoptychus senarius</i>	(Ehrenberg) Ehrenberg, 1843	Chromista	Native	1	1	1	
<i>Actinoptychus splendens</i>	(Shadbolt) Ralfs, 1861	Chromista	Native		1		
<i>Apedinella radians</i>	(Lohmann) Campbell, 1973	Chromista	Native	1	1	1	
<i>Archaeperidinium minutum</i>	(Kofoid) Jörgensen, 1912	Chromista	Native			1	
<i>Asterionella formosa</i>	Hassall, 1850	Chromista	Native				1
<i>Asterionellopsis glacialis</i>	(Castracane) Round, 1990	Chromista	Native	1			
<i>Bacillaria paxillifera</i>	(O.F.Müller) T. Marsson, 1901	Chromista	Native	1	1		1

Species	Authority	Group	Origin	Research area 1: 8e Petroleumhaven	Research area 2: Beneluxhaven	Research area 3: Brittaniëhaven	Research area 4: 1e Eemhaven
<i>Cerataulina pelagica</i>	(Cleve) Hendey, 1937	Chromista	Native	1	1	1	
<i>Ceratoneis closterium</i>	Ehrenberg, 1839	Chromista	Native		1		1
<i>Chaetoceros affinis</i>	Lauder, 1864	Chromista	Native			1	
<i>Chaetoceros constrictus</i>	Gran, 1897	Chromista	Native	1	1	1	
<i>Chaetoceros curvisetus</i>	Cleve, 1889	Chromista	Native	1	1	1	
<i>Chaetoceros debilis</i>	Cleve, 1894	Chromista	Native	1	1	1	
<i>Chaetoceros didymus</i>	Ehrenberg, 1845	Chromista	Native	1	1	1	
<i>Chaetoceros pseudocurvisetus</i>	Mangin, 1910	Chromista	Native	1			
<i>Chaetoceros socialis</i>	H.S.Lauder, 1864	Chromista	Native	1	1	1	
<i>Coscinodiscus granii</i>	Gough, 1905	Chromista	Native			1	
<i>Coscinodiscus perforatus</i> var. <i>pavillardii</i>	(Forti) Hust., 1928	Chromista	Native		1		
<i>Coscinodiscus radiatus</i>	Ehrenberg, 1840	Chromista	Native	1	1	1	1
<i>Coscinodiscus wailesii</i>	Gran & Angst, 1931	Chromista	Non-native			1	
<i>Cylindrotheca gracilis</i>	(Brébisson ex Kützing) Grunow, 1882	Chromista	Native				1
<i>Cymatopleura librile</i>	(Ehrenberg) Pantocsek, 1902	Chromista	Native				1
<i>Dactyliosolen phuketensis</i>	(Sundström) Hasle, 1996	Chromista	Native	1			
<i>Delphineis minutissima</i>	(Hustedt) Simonsen	Chromista	Native			1	
<i>Dinophysis acuminata</i>	Claparède & Lachmann, 1859	Chromista	Native	1		1	
<i>Ditylum brightwellii</i>	(T.West) Grunow, 1885	Chromista	Native	1	1	1	1
<i>Eucampia zodiacus</i>	Ehrenberg, 1839	Chromista	Native	1	1		
<i>Gonyaulax digitale</i>	(Pouchet) Kofoed, 1911	Chromista	Native	1			
<i>Gonyaulax spinifera</i>	(Claparède & Lachmann) Diesing, 1866	Chromista	Native			1	
<i>Guinardia delicatula</i>	(Cleve) Hasle, 1997	Chromista	Native	1	1	1	
<i>Guinardia flaccida</i>	(Castracane) Peragallo, 1892	Chromista	Native	1	1	1	
<i>Guinardia striata</i>	(Stolterfoth) Hasle, 1996	Chromista	Native	1	1		
<i>Gyrodinium britannicum</i>	Kofoed & Swezy, 1921	Chromista	Native		1	1	
<i>Gyrodinium spirale</i>	(Bergh) Kofoed & Swezy, 1921	Chromista	Native			1	
<i>Gyrosigma fasciola</i>	(Ehrenberg) Griffith & Hen- frey, 1856	Chromista	Native	1			1
<i>Helicotheca tamesis</i>	(Shrubsole) M.Ricard, 1987	Chromista	Native		1		
<i>Heterocapsa minima</i>	A.J.Pomroy, 1989	Chromista	Native	1	1		
<i>Heterocapsa rotundata</i>	(Lohmann) G.Hansen, 1995	Chromista	Native				1
<i>Heterocapsa triquetra</i>	(Ehrenberg) F.Stein, 1883	Chromista	Native	1	1	1	
<i>Lauderia annulata</i>	Cleve, 1873	Chromista	Native	1	1	1	
<i>Leptocylindrus danicus</i>	Cleve, 1889	Chromista	Native	1	1	1	
<i>Leptocylindrus minimus</i>	Gran, 1915	Chromista	Native	1	1		

Species	Authority	Group	Origin	Research area 1: 8e Petroleumhaven	Research area 2: Beneluxhaven	Research area 3: Brittaniëhaven	Research area 4: 1e Eemhaven
<i>Leucocryptos marina</i>	(Braarud) Butcher, 1967	Chromista	Native			1	
<i>Lithodesmium undulatum</i>	Ehrenberg, 1839	Chromista	Native	1	1	1	
<i>Mediopyxis helysia</i>	Kühn, Hargreaves & Halliger, 2006	Chromista	Non-native	1			
<i>Melosira moniliformis</i>	(O.F.Müller) C.Agardh, 1824	Chromista	Native	1	1		
<i>Melosira varians</i>	C.Agardh, 1827	Chromista	Native				1
<i>Mesodinium rubrum</i>	(Lohmann, 1908)	Chromista	Native		1	1	
<i>Minutocellus scriptus</i>	Hasle <i>et al.</i> , 1983	Chromista	Native	1	1	1	
<i>Neoceratium fusus</i>	(Ehrenberg) Gomez et al 2010,	Chromista	Native	1	1	1	
<i>Neoceratium horridum</i>	(Gran) Gomez et al 2010,	Chromista	Native	1			
<i>Neoceratium lineatum</i>	(Ehrenberg) Gomez et al 2010,	Chromista	Native	1			
<i>Nitzschia sigma</i>	(Kützing) W.Smith, 1853	Chromista	Native			1	1
<i>Noctiluca scintillans</i>	(Macartney) Kofoid & Swezy, 1921	Chromista	Native	1	1		
<i>Oblea rotunda</i>	(Lebour) Balech, 1973	Chromista	Native	1	1		
<i>Odontella longicruris</i>	(Greville) Hoban, 1983	Chromista	Native	1	1	1	
<i>Odontella sinensis</i>	(Greville) Grunow, 1884	Chromista	Non-native	1	1	1	
<i>Oxytoxum mediterraneum</i>	Schiller	Chromista	Native			1	
<i>Paralia sulcata</i>	(Ehrenberg) Cleve, 1873	Chromista	Native	1	1	1	1
<i>Pleurosigma formosum</i>	W.Smith, 1852	Chromista	Native		1		
<i>Podosira stelligera</i>	(J.W.Bailey) A.Mann, 1907	Chromista	Native		1	1	
<i>Prorocentrum cordatum</i>	(Ostenfeld) Dodge, 1975	Chromista	Non-native	1	1	1	
<i>Prorocentrum micans</i>	Ehrenberg, 1834	Chromista	Native	1	1	1	
<i>Prorocentrum triestinum</i>	J.Schiller, 1918	Chromista	Native	1	1	1	
<i>Protoceratium reticulatum</i>	(Claparède & Lachmann) Butschli, 1885	Chromista	Non-native	1	1		
<i>Protoperidinium achromaticum</i>	(Levander) Balech, 1974	Chromista	Native	1	1		1
<i>Protoperidinium bipes</i>	(Paulsen) Balech, 1974	Chromista	Native	1	1		
<i>Protoperidinium claudicans</i>	(Paulsen) Balech, 1974	Chromista	Native	1	1	1	
<i>Protoperidinium conicum</i>	(Gran) Balech, 1974	Chromista	Native	1			
<i>Protoperidinium depressum</i>	(Bailey) Balech, 1974	Chromista	Native	1	1		
<i>Protoperidinium granii</i>	(Ostenfeld) Balech, 1974	Chromista	Native	1			
<i>Protoperidinium marie-lebouriae</i>	(Paulsen) Balech, 1974	Chromista	Native	1			
<i>Protoperidinium pellucidum</i>	Bergh, 1881	Chromista	Native	1	1		
<i>Protoperidinium pentagonum</i>	(Gran) Balech, 1974	Chromista	Native			1	
<i>Protoperidinium thorianum</i>	(Paulsen) Balech, 1974	Chromista	Native	1	1		
<i>Pseudo-nitzschia americana</i>	(Hasle) Fryxell, 1993	Chromista	Native			1	
<i>Pseudo-nitzschia delicatissima</i>	(Cleve) Heiden, 1928	Chromista	Native	1	1	1	
<i>Pseudo-nitzschia fraudulenta</i>	(Cleve) Hasle, 1993	Chromista	Native	1	1		

Species	Authority	Group	Origin	Research area 1: 8e Petroleumhaven	Research area 2: Beneluxhaven	Research area 3: Brittaniëhaven	Research area 4: 1e Eemhaven
<i>Pseudo-nitzschia pungens</i>	(Grunow) G.R.Hasle, 1993	Chromista	Native	1	1	1	
<i>Pyrocystis noctiluca</i>	Murray ex Haeckel, 1890	Chromista	Native	1			
<i>Rhaphoneis amphiceros</i>	(Ehrenberg) Ehrenberg, 1844	Chromista	Native	1			
<i>Rhizosolenia imbricata</i>	Brightwell, 1858	Chromista	Native	1	1	1	
<i>Rhizosolenia setigera</i>	Brightwell, 1858	Chromista	Native	1	1	1	
<i>Skeletonema costatum</i>	(Greville) Cleve, 1873	Chromista	Native	1	1	1	
<i>Skeletonema potamos</i>	(Weber) Hasle, 1976	Chromista	Native	1	1	1	1
<i>Thalassionema frauenfeldii</i>	(Grunow) Hallegraeff, 1986	Chromista	Native	1	1		
<i>Thalassiosira eccentrica</i>	(Ehrenberg) Cleve, 1903	Chromista	Native	1			
<i>Thalassiosira lacustris</i>	(Grunow) Hasle, 1977	Chromista	Native				1
<i>Thalassiosira nordenskiöldii</i>	Cleve, 1873	Chromista	Non-native			1	
<i>Thalassiosira rotula</i>	Meunier, 1910	Chromista	Native	1			
<i>Trigonum alternans</i>	(J.W.Bailey) A.Mann, 1907	Chromista	Native		1		
<i>Tryblionella coarctata</i>	(Grunow) D.G.Mann, 1990	Chromista	Native				1
<i>Aurelia aurita</i>	(Linnaeus, 1758)	Cnidaria	Native		1	1	
<i>Chrysaora hysoscella</i>	(Linnaeus, 1767)	Cnidaria	Native			1	
<i>Cordylophora caspia</i>	(Pallas, 1771)	Cnidaria	Non-native				1
<i>Hartlaubella gelatinosa</i>	(Pallas, 1766)	Cnidaria	Native	1			
<i>Hydractinia echinata</i>	(Fleming, 1828)	Cnidaria	Native	1			
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native	1	1	1	
<i>Nemopsis bachei</i>	L. Agassiz, 1849	Cnidaria	Non-native			1	
<i>Obelia dichotoma</i>	(Linnaeus, 1758)	Cnidaria	Native	1	1		1
<i>Obelia geniculata</i>	(Linnaeus, 1758)	Cnidaria	Native	1	1	1	
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native	1	1	1	1
<i>Sagartiogeton undatus</i>	(Müller, 1778)	Cnidaria	Native		1		
<i>Sarsia tubulosa</i>	(M. Sars, 1835)	Cnidaria	Native			1	
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native	1	1	1	1
<i>Austrominius modestus</i>	(Darwin, 1854)	Crustacea	Non-native	1	1	1	
<i>Balanus crenatus</i>	Bruguère, 1789	Crustacea	Native	1	1	1	
<i>Bosmina cf. longirostris</i>	(O. F. Müller, 1785)	Crustacea	Native	1			1
<i>Bosmina coregoni</i>	Baird, 1857	Crustacea	Native				1
<i>Cancer pagurus</i>	Linnaeus, 1758	Crustacea	Native	1			
<i>Caprella linearis</i>	(Linnaeus, 1767)	Crustacea	Native	1			
<i>Caprella mutica</i>	Schurin, 1935	Crustacea	Non-native		1	1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	1	1
<i>Corophium volutator</i>	(Pallas, 1766)	Crustacea	Native	1			
<i>Diaphanosoma mongolianum</i>	Uéno, 1938	Crustacea	Native				1
<i>Echinogammarus marinus</i>	(Leach, 1815)	Crustacea	Native	1			
<i>Echinogammarus stoereus</i>	(Reid, 1938)	Crustacea	Native				1

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Britanniëhaven	1e Eemhaven
<i>Gammarus locusta</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	1	
<i>Hemigrapsus sanguineus</i>	(De Haan, 1835)	Crustacea	Non-native	1	1	1	
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native	1	1	1	
<i>Jaera albifrons albifrons</i>	Leach, 1814	Crustacea	Native	1			
<i>Jassa marmorata</i>	Holmes, 1905	Crustacea	Non-native		1		
<i>Macropodia rostrata</i>	(Linnaeus, 1761)	Crustacea	Native	1			
<i>Melita hergensis</i>	Reid, 1939	Crustacea	Native	1			
<i>Microdeutopus gryllotalpa</i>	Costa, 1853	Crustacea	Native		1		
<i>Monocorophium acherusicum</i>	(Costa, 1853)	Crustacea	Native		1		
<i>Necora puber</i>	(Linnaeus, 1767)	Crustacea	Native	1			
<i>Neomysis integer</i>	(Leach, 1814)	Crustacea	Native				1
<i>Orchestia gammarellus</i>	(Pallas, 1766)	Crustacea	Native			1	
<i>Pagurus bernhardus</i>	(Linnaeus, 1758)	Crustacea	Native	1			
<i>Palaemon elegans</i>	Rathke, 1837	Crustacea	Native		1		
<i>Palaemon longirostris</i>	H. Milne Edwards, 1837	Crustacea	Native				1
<i>Pilumnus hirtellus</i>	(Linnaeus, 1761)	Crustacea	Native	1			
<i>Pisidia longicornis</i>	(Linnaeus, 1767)	Crustacea	Native	1			
<i>Podon leuckartii</i>	(Sars G.O., 1862)	Crustacea	Native	1	1	1	1
<i>Rhithropanopeus harrisi</i>	(Gould, 1841)	Crustacea	Non-native				1
<i>Semibalanus balanoides</i>	(Linnaeus, 1758)	Crustacea	Native	1	1		
<i>Beroë cucumis</i>	Fabricius, 1780	Ctenophora	Native			1	
<i>Mnemiopsis leidyi</i>	A. Agassiz, 1865	Ctenophora	Non-native	1	1	1	
<i>Asterias rubens</i>	Linnaeus, 1758	Echinodermata	Native	1		1	
<i>Ophiura ophiura</i>	(Linnaeus, 1758)	Echinodermata	Native	1			
<i>Abra alba</i>	(W. Wood, 1802)	Mollusca	Native	1			
<i>Corbicula fluminalis</i>	(O. F. Müller, 1774)	Mollusca	Non-native				1
<i>Corbula gibba</i>	(Olivi, 1792)	Mollusca	Native			1	
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1	1	1	
<i>Crepidula fornicata</i>	(Linnaeus, 1758)	Mollusca	Non-native		1	1	
<i>Dreissena bugensis</i>	Andrusov, 1897	Mollusca	Non-native				1
<i>Dreissena polymorpha</i>	(Pallas, 1771)	Mollusca	Non-native				1
<i>Littorina littorea</i>	(Linnaeus, 1758)	Mollusca	Native	1	1		
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1	1	1	1
<i>Nassarius reticulatus</i>	(Linnaeus, 1758)	Mollusca	Native		1		
<i>Physa acuta</i>	Draparnaud, 1805	Mollusca	Non-native		1		1
<i>Potamopyrgus antipodarum</i>	(Gray, 1843)	Mollusca	Non-native		1		1
<i>Rangia cuneata</i>	(G. B. Sowerby I, 1832)	Mollusca	Non-native				1
<i>Venerupis corrugata</i>	(Gmelin, 1791)	Mollusca	Native			1	
<i>Emplectonema neessi</i>	(Ørsted, 1843)	Nemertea	Native	1	1	1	

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Lineus longissimus</i>	(Gunnerus, 1770)	Nemertea	Native	1	1		
<i>Ciliata mustela</i>	(Linnaeus, 1758)	Pisces	Native	1			
<i>Neogobius cf. fluviatilis</i>	(Pallas, 1814)	Pisces	Non-native				1
<i>Neogobius melanostomus</i>	(Pallas, 1814)	Pisces	Non-native				1
<i>Perca fluviatilis</i>	(Linnaeus, 1758)	Pisces	Native				1
<i>Pholis gunnellus</i>	(Linnaeus, 1758)	Pisces	Native	1	1	1	
<i>Syngnathus acus</i>	Linnaeus, 1758	Pisces	Native	1	1		
<i>Trisopterus luscus</i>	(Linnaeus, 1758)	Pisces	Native	1	1	1	
<i>Leptoplana tremellaris</i>	(Müller, 1774)	Platyhelminthes	Native	1	1		
<i>Halichondria bowerbanki</i>	Burton, 1930	Porifera	Native	1	1	1	
<i>Halichondria panicea</i>	(Pallas, 1766)	Porifera	Native	1	1		
<i>Haliclona oculata</i>	(Pallas, 1766)	Porifera	Native	1			
<i>Spongilla lacustris</i>	(Linnaeus, 1759)	Porifera	Native				1
<i>Ebria tripartita</i>	(Schumann) Lemmermann, 1899	Protozoa	Native	1	1	1	

5.1 Water parameters

The date of the inventory, the wind speed and direction, and the air temperature during the inventories done in the spring bloom and the late summer of 2014 in the port of Rotterdam are presented for each of the four research areas in table 10. In each research area water samples for measuring water parameters were taken at three sample locations (Fig. 39). For each of these sample locations the geographical coordinates were noted (Tables 2, 3, 6, 8). At each location the depth of the bottom was noted and the water visibility in meters was measured with a Secchi disc in spring (Table 11) and summer (Table 12). In addition the water parameters [1] turbidity in ntu, [2] temperature, [3] pH, [4] salinity (in ppt and PSU), and [5] dissolved oxygen %, were measured in the water samples (Tables 11-12).

At most of the research sites, the turbidity increased with depth, while the temperature and oxygen concentration decreased with depth. Within the more saline research areas, i.e. the 8e Petroleumhaven, Beneluxhaven, and Britanniëhaven (Figs 4, 6, 9), the salinity increased with depth as the freshwater that comes down the rivers into the port, tends to float on the more saline waters that come into the port at high tide.

The methods described in the HELCOM/OSPAR protocol were followed for measuring these water parameters with the exception of the turbidity in ntu. In addition to the turbidity measurement method indicated in the HELCOM/OSPAR protocol, i.e. the Secchi disc method, the turbidity at all sample depths was also measured with a HI 93414 turbidity meter of Hanna Instruments.

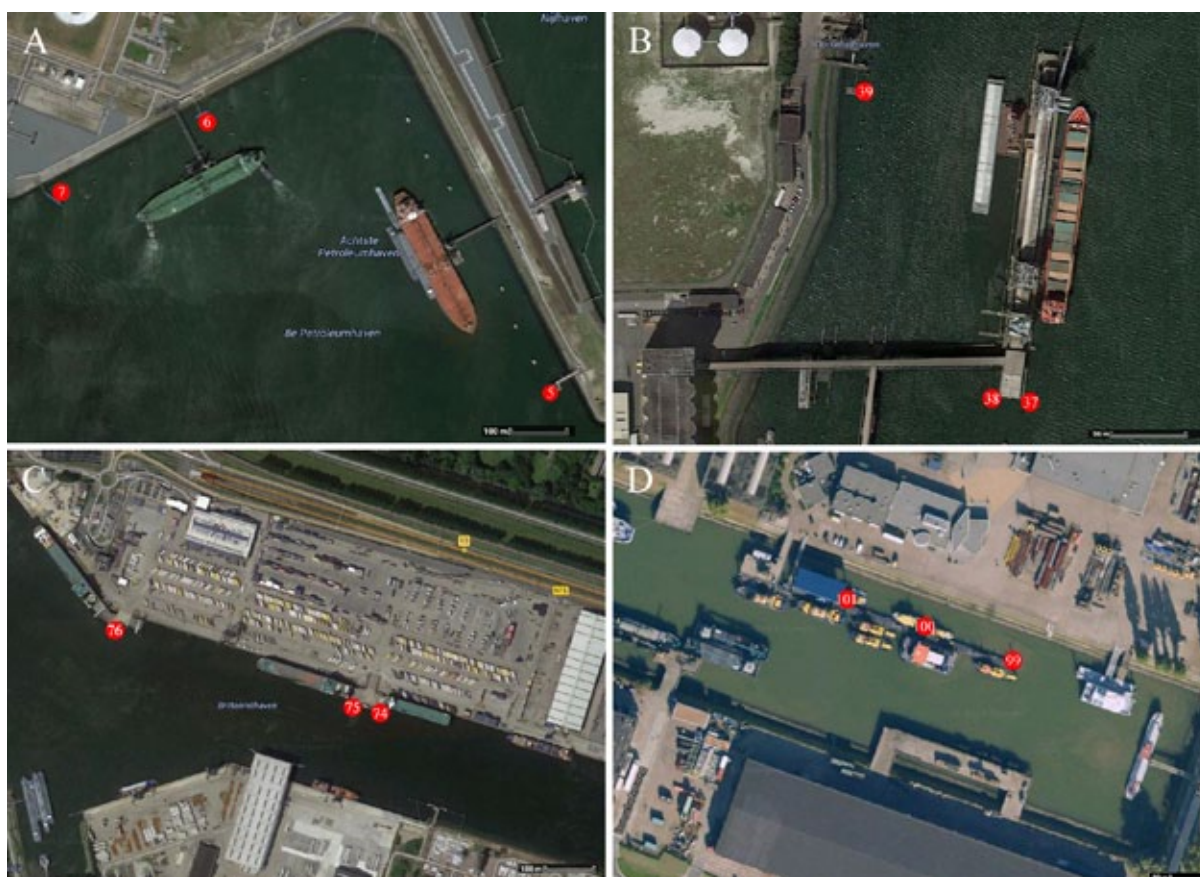


Fig. 39. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Britanniëhaven and [D] 1e Eemhaven where the watersamples were taken in both the spring and late summer period.

Table 10. Weather conditions at the four research areas (Fig. 39) during the sampling period in spring 2014 and the late summer 2014.

Area	Date	Wind Direction	Wind Speed	Air Temp
8e Petroleumhaven	20-5-14	SW	4 bft	20 °C
Beneluxhaven	21-5-14	SW	4-5 bft	18 °C
Brittanniëhaven	20-5-14	SW	3-4 bft	21 °C
1e Eemhaven	20-5-14	SW	4 bft	25 °C
8e Petroleumhaven	10-9-14	NO	3 bft	17 °C
Beneluxhaven	11-9-14	NNO	3 bft	18 °C
Brittanniëhaven	2-9-14	ENE	2 bft	21 °C
1e Eemhaven	2-9-14	ENE	2 bft	22 °C

Table 11. Water parameter measurements at each the research sites (Fig. 39; Table 10) during the sampling period in spring 2014. The Secchi measurements, which were done from the surface are noted in the “30 cm - rows”.

Area	Loc	Depth	Turbidity (ntu)	Secchi (m)	Water temp (°C)	pH	Salinity (ppt)	Salinity (PSU)	D.O. (%)
8e Petroleumhaven	5	30 cm	2.51	2.7	16,45	8,18	15,05	18,74	83,30
8e Petroleumhaven	5	1m	3.88	n.a.	15.82	8.24	15.08	18.78	75.80
8e Petroleumhaven	5	4m	4.47	n.a.	15.31	8.24	16.60	20.87	64.80
8e Petroleumhaven	5	7m	3.36	n.a.	14.38	8.34	21.33	27.50	64.60
8e Petroleumhaven	5	bottom (8.4m)	3.04	n.a.	14.38	8.34	21.36	27.54	64.10
8e Petroleumhaven	6	30cm	1.02	2.2	16.63	8.21	13.76	16.98	103.20
8e Petroleumhaven	6	1m	1.71	n.a.	15.94	8.22	16.70	21.00	107.80
8e Petroleumhaven	6	4m	1.39	n.a.	15.48	8.21	18.64	23.70	100.10
8e Petroleumhaven	6	bottom (6.0m)	2.75	n.a.	15.24	8.21	19.26	24.57	102.10
8e Petroleumhaven	7	30cm	2.32	2.0	17.27	8.22	14.41	17.86	107.90
8e Petroleumhaven	7	1m	1.06	n.a.	17.10	8.23	14.29	17.70	106.20
8e Petroleumhaven	7	4m	1.81	n.a.	16.54	8.23	16.75	21.08	104.90
8e Petroleumhaven	7	7m	2.33	n.a.	15.68	8.22	20.19	25.89	89.90
8e Petroleumhaven	7	bottom (7.2m)	2.09	n.a.	15.57	8.23	20.47	26.29	89.70
Beneluxhaven	37	30cm	2.60	1.8	16.40	8.28	13.98	17.29	82.20
Beneluxhaven	37	1m	1.93	n.a.	15.96	8.29	14.89	18.52	87.10
Beneluxhaven	37	4m	5.92	n.a.	15.30	8.24	18.23	23.12	82.60
Beneluxhaven	37	7m	3.71	n.a.	14.90	8.23	19.97	25.56	74.70
Beneluxhaven	37	10m	3.66	n.a.	14.60	8.28	21.87	28.26	73.40
Beneluxhaven	37	bottom (10.5m)	11.00	n.a.	14.59	8.28	21.58	27.85	76.20
Beneluxhaven	38	30cm	2.64	1.8	17.10	8.32	14.05	17.38	89.90
Beneluxhaven	38	1m	7.61	n.a.	17.12	8.30	14.20	17.58	87.60
Beneluxhaven	38	4m	7.56	n.a.	16.49	8.21	16.89	21.27	88.00
Beneluxhaven	38	7m	6.13	n.a.	15.76	8.23	19.51	24.93	81.90
Beneluxhaven	38	10m	7.88	n.a.	15.32	8.26	20.96	26.98	80.60
Beneluxhaven	38	13m	3.80	n.a.	14.87	8.28	22.02	28.49	74.50
Beneluxhaven	38	bottom (14.5m)	7.83	n.a.	14.51	8.29	22.11	28.61	77.90
Beneluxhaven	39	30cm	3.25	1.9	19.16	8.28	14.18	17.54	177.80
Beneluxhaven	39	1m	2.85	n.a.	18.85	8.30	14.03	17.34	158.20
Beneluxhaven	39	4m	2.44	n.a.	18.46	8.20	17.38	21.93	136.70
Beneluxhaven	39	bottom (5.5m)	4.48	n.a.	17.67	8.18	18.99	24.20	115.10
Brittanniëhaven	74	30cm	1.81	2.2	16.72	8.20	18.84	23.99	94.80
Brittanniëhaven	74	1m	1.73	n.a.	15.99	8.21	18.82	23.96	117.50
Brittanniëhaven	74	4m	2.27	n.a.	15.23	8.21	18.94	24.12	104.80
Brittanniëhaven	74	7m	2.37	n.a.	14.49	8.07	19.85	25.40	81.10
Brittanniëhaven	74	10m	2.23	n.a.	14.41	8.04	19.97	25.56	72.50
Brittanniëhaven	74	13m	1.78	n.a.	13.83	7.94	21.03	27.07	53.30
Brittanniëhaven	74	bottom (13.2m)	3.27	n.a.	13.84	7.92	18.81	27.10	98.50
Brittanniëhaven	75	30cm	1.90	2.2	15.71	8.24	18.81	23.94	98.70
Brittanniëhaven	75	1m	1.41	n.a.	15.69	8.23	18.82	23.95	102.00
Brittanniëhaven	75	4m	1.64	n.a.	15.92	8.17	18.95	24.15	99.50
Brittanniëhaven	75	7m	2.10	n.a.	15.11	8.10	19.54	24.96	82.20
Brittanniëhaven	75	10m	1.78	n.a.	15.01	8.02	20.06	25.71	75.80
Brittanniëhaven	75	13m	1.77	n.a.	14.00	7.94	21.05	27.09	62.90
Brittanniëhaven	75	bottom (13.2m)	1.44	n.a.	13.89	7.93	21.03	27.06	59.00

Area	Loc	Depth	Turbidity (ntu)	Secchi (m)	Water temp (°C)	pH	Salinity (ppt)	Salinity (PSU)	D.O. (%)
Brittanniëhaven	76	30cm	1.96	2.3	16.57	8.21	18.25	23.17	160.20
Brittanniëhaven	76	1m	1.93	n.a.	16.93	8.23	18.41	23.38	109.10
Brittanniëhaven	76	4m	1.75	n.a.	15.99	8.13	18.85	24.00	101.20
Brittanniëhaven	76	7m	3.41	n.a.	14.52	8.07	19.74	25.24	87.30
Brittanniëhaven	76	10m	1.63	n.a.	14.78	8.03	20.42	26.21	71.60
Brittanniëhaven	76	bottom (11.5m)	3.82	n.a.	14.08	8.00	20.56	26.40	69.50
1e Eemhaven	98	30cm	2.24	0.7	18.53	8.29	1.23	1.28	112.80
1e Eemhaven	98	1m	2.93	n.a.	18.24	8.28	1.25	1.29	115.80
1e Eemhaven	98	4m	8.24	n.a.	17.18	8.18	1.27	1.32	105.70
1e Eemhaven	98	bottom (4.5m)	17.80	n.a.	17.70	8.13	1.52	1.60	125.60
1e Eemhaven	99	30cm	3.39	0.7	18.48	8.28	1.23	1.27	141.80
1e Eemhaven	99	1m	4.00	n.a.	18.37	8.28	1.23	1.27	161.20
1e Eemhaven	99	4m	5.09	n.a.	17.64	8.23	1.24	1.29	151.90
1e Eemhaven	99	bottom (4.8m)	28.00	n.a.	18.05	8.24	1.25	1.30	286.90
1e Eemhaven	100	30cm	6.07	0.8	18.65	8.28	1.23	1.28	448.70
1e Eemhaven	100	1m	5.82	n.a.	18.34	8.28	1.23	1.27	169.10
1e Eemhaven	100	4m	7.47	n.a.	18.55	8.25	1.23	1.27	163.20
1e Eemhaven	100	bottom (4.5m)	10.60	n.a.	18.89	8.24	1.22	1.27	170.40

Table 12. Water parameter measurements at each the research sites (Fig. 39; Table 10) during the sampling period in the late summer of 2014.

Area	Loc	Depth	Turbidity (ntu)	Secchi (m)	Water temp (°C)	pH	Salinity (ppt)	Salinity (PSU)	D.O. (%)
8e Petroleumhaven	5	30cm	3,66	2.5	19,39	8,06	17,43	22,01	46,80
8e Petroleumhaven	5	1m	3,04	n.a.	19,28	8,13	17,88	22,63	48,80
8e Petroleumhaven	5	4m	2,36	n.a.	19,44	8,18	19,28	24,60	51,00
8e Petroleumhaven	5	7m	4,11	n.a.	19,46	8,17	20,09	25,75	53,10
8e Petroleumhaven	5	bottom (8.5m)	4,56	n.a.	19,09	8,14	20,92	26,93	54,50
8e Petroleumhaven	6	30cm	1,36	2.5	19,67	8,11	17,45	22,03	75,50
8e Petroleumhaven	6	1m	1,01	n.a.	19,68	8,12	17,56	22,19	84,60
8e Petroleumhaven	6	4m	1,92	n.a.	19,89	8,12	18,28	23,19	73,50
8e Petroleumhaven	6	bottom (6.0m)	2,91	n.a.	19,86	8,10	19,80	25,34	82,20
8e Petroleumhaven	7	30cm	2,18	2.0	20,24	8,13	16,95	21,33	100,90
8e Petroleumhaven	7	1m	5,15	n.a.	19,86	8,11	17,02	21,43	89,40
8e Petroleumhaven	7	4m	4,88	n.a.	19,64	8,12	18,31	23,24	94,40
8e Petroleumhaven	7	7m	3,73	n.a.	19,44	8,10	19,82	25,36	91,00
8e Petroleumhaven	7	bottom (7.5 m)	2,41	n.a.	19,33	8,10	20,91	26,91	90,50
Beneluxhaven	37	30cm	7,62	2.0	18,32	8,03	16,03	20,08	93,60
Beneluxhaven	37	1m	3,44	n.a.	18,25	8,06	16,11	20,19	93,10
Beneluxhaven	37	4m	5,64	n.a.	18,40	8,05	17,82	22,56	85,60
Beneluxhaven	37	7m	3,99	n.a.	18,31	8,06	20,18	25,88	83,90
Beneluxhaven	37	bottom (10.5 m)	15,70	n.a.	18,16	8,06	22,05	28,55	80,50
Beneluxhaven	38	30cm	4,40	2.1	17,86	8,06	15,59	19,47	76,70
Beneluxhaven	38	1m	8,27	n.a.	17,88	8,08	15,54	19,41	80,90
Beneluxhaven	38	4m	3,84	n.a.	18,17	8,07	18,59	23,64	78,10

Area	Loc	Depth	Turbidity (ntu)	Secchi (m)	Water temp (°C)	pH	Salinity (ppt)	Salinity (PSU)	D.O. (%)
Beneluxhaven	38	7m	3,93	n.a.	18,24	8,06	20,23	25,95	76,90
Beneluxhaven	38	10m	5,89	n.a.	18,23	8,07	21,96	28,42	74,10
Beneluxhaven	38	bottom (14.5 m)	5,62	n.a.	18,32	8,08	22,45	29,13	71,20
Beneluxhaven	39	30cm	3,99	2.0	18,34	8,03	16,18	20,29	95,20
Beneluxhaven	39	1m	5,79	n.a.	18,37	8,05	16,64	20,92	101,00
Beneluxhaven	39	4m	3,94	n.a.	18,82	8,03	18,00	22,80	98,30
Beneluxhaven	39	bottom (5.5 m)	4,27	n.a.	18,62	8,03	18,32	23,26	100,50
Brittanniëhaven	74	30cm	4,63	2.2	20,61	8,41	16,57	20,81	91,40
Brittanniëhaven	74	1m	3,07	n.a.	20,16	8,42	16,69	20,97	93,00
Brittanniëhaven	74	4m	2,64	n.a.	19,36	8,21	18,33	23,26	77,70
Brittanniëhaven	74	7m	2,43	n.a.	19,12	8,11	19,83	25,38	67,60
Brittanniëhaven	74	10m	1,58	n.a.	18,94	7,97	22,03	28,52	55,60
Brittanniëhaven	74	13m	2,33	n.a.	19,09	7,97	22,08	28,60	58,70
Brittanniëhaven	75	bottom (13.5m)	1,79	n.a.	18,94	7,97	22,94	29,84	61,10
Brittanniëhaven	75	30cm	4,18	2.3	19,82	8,37	17,27	21,78	98,30
Brittanniëhaven	75	1m	2,80	n.a.	19,75	8,34	17,51	22,12	105,50
Brittanniëhaven	75	4m	2,37	n.a.	19,42	8,19	18,47	23,46	81,40
Brittanniëhaven	75	7m	5,04	n.a.	19,28	8,09	19,84	25,40	79,50
Brittanniëhaven	75	10m	2,85	n.a.	19,11	7,96	21,68	28,03	64,10
Brittanniëhaven	75	bottom (13.5m)	3,88	n.a.	18,92	7,96	22,95	29,85	64,90
Brittanniëhaven	76	30cm	3,83	2.2	19,12	8,24	17,04	21,47	104,60
Brittanniëhaven	76	1m	1,50	n.a.	19,33	8,26	16,48	20,70	106,80
Brittanniëhaven	76	4m	2,64	n.a.	19,07	8,17	18,79	23,91	98,10
Brittanniëhaven	76	7m	1,86	n.a.	19,30	8,12	19,49	24,89	90,10
Brittanniëhaven	76	10m	3,49	n.a.	19,02	7,95	22,13	28,67	66,90
Brittanniëhaven	76	bottom (11.6m)	1,94	n.a.	19,19	7,96	22,34	28,96	62,60
1e Eemhaven	98	30cm	6,45	0.6	20,73	8,11	0,54	0,54	117,70
1e Eemhaven	98	1m	5,12	n.a.	19,97	8,09	0,59	0,59	112,20
1e Eemhaven	98	4m	12,60	n.a.	19,27	8,06	0,55	0,55	103,60
1e Eemhaven	98	bottom (4.5m)	70,70	n.a.	19,08	8,03	0,55	0,55	102,50
1e Eemhaven	99	30cm	8,93	0.6	19,34	8,03	0,55	0,55	100,50
1e Eemhaven	99	1m	13,50	n.a.	21,09	8,10	0,54	0,54	116,60
1e Eemhaven	99	4m	18,60	n.a.	20,77	8,08	0,55	0,54	115,00
1e Eemhaven	99	bottom (4.7m)	93,70	n.a.	20,65	8,05	0,53	0,52	113,10
1e Eemhaven	100	30cm	7,74	0.7	21,03	8,04	0,53	0,52	107,50
1e Eemhaven	100	1m	6,64	n.a.	20,49	8,11	0,53	0,52	108,50
1e Eemhaven	100	4m	15,70	n.a.	20,21	8,10	0,53	0,52	108,70
1e Eemhaven	100	bottom (4.5m)	75,90	n.a.	20,21	8,04	0,52	0,52	107,60

5.2 Human pathogens

The results of the human pathogen analyses as described by the HELCOM/OSPAR port survey protocol, i.e. based on Regulation D-2, are presented in table 13 (spring) and table 14 (summer). According to Regulation D-2 Ballast Water Performance Standard of the “Ballast water management convention” (International Maritime Organization; February, 2004) there should be less than 1 colony forming unit (cfu) per 100 ml of *Vibrio cholerae* (O1 and O139), less than 250 cfu per 100 ml of *Escherichia coli* and less than 100 cfu per 100 ml intestinal Enterococci in ballast water. These values were exceeded in the Beneluxhaven during the spring and summer monitoring, when respectively 130 cfu / 100 ml and 220 cfu / 100 ml intestinal Enterococci were recorded, and in the 8th Petroleumhaven during the summer monitoring, where 890 cfu / 100 ml were recorded (Tables 13-14).

In spring high numbers of *Vibrio* sp. were recorded in Brittanniëhaven and the 8th Petroleumhaven (Table 15). Most of the *Vibrio* colonies that grew on the growth media coloured yellow. As *Vibrio cholerae* is known for its yellow colonies, all *Vibrio* colonies on the growth media plates that appeared to have different morphologies (phenotype) were taken off the plates, grown over night on separate plates and analysed by MALDI-TOF MS to identify to the species. From these analyses it could be concluded that none of the *Vibrio* species concerned *Vibrio cholerae* (Table 16). During the summer monitoring *Vibrio* species were again recorded, both in the water and in mussels in the three more saline areas, i.e. the 8th Petroleumhaven, Beneluxhaven and Brittanniëhaven. In the water of the 1e Eemhaven no *Vibrio* spp were found. Although the morphologies of the colonies indicated the presence of several *Vibrio* species, none of the colonies matched the typical mor-

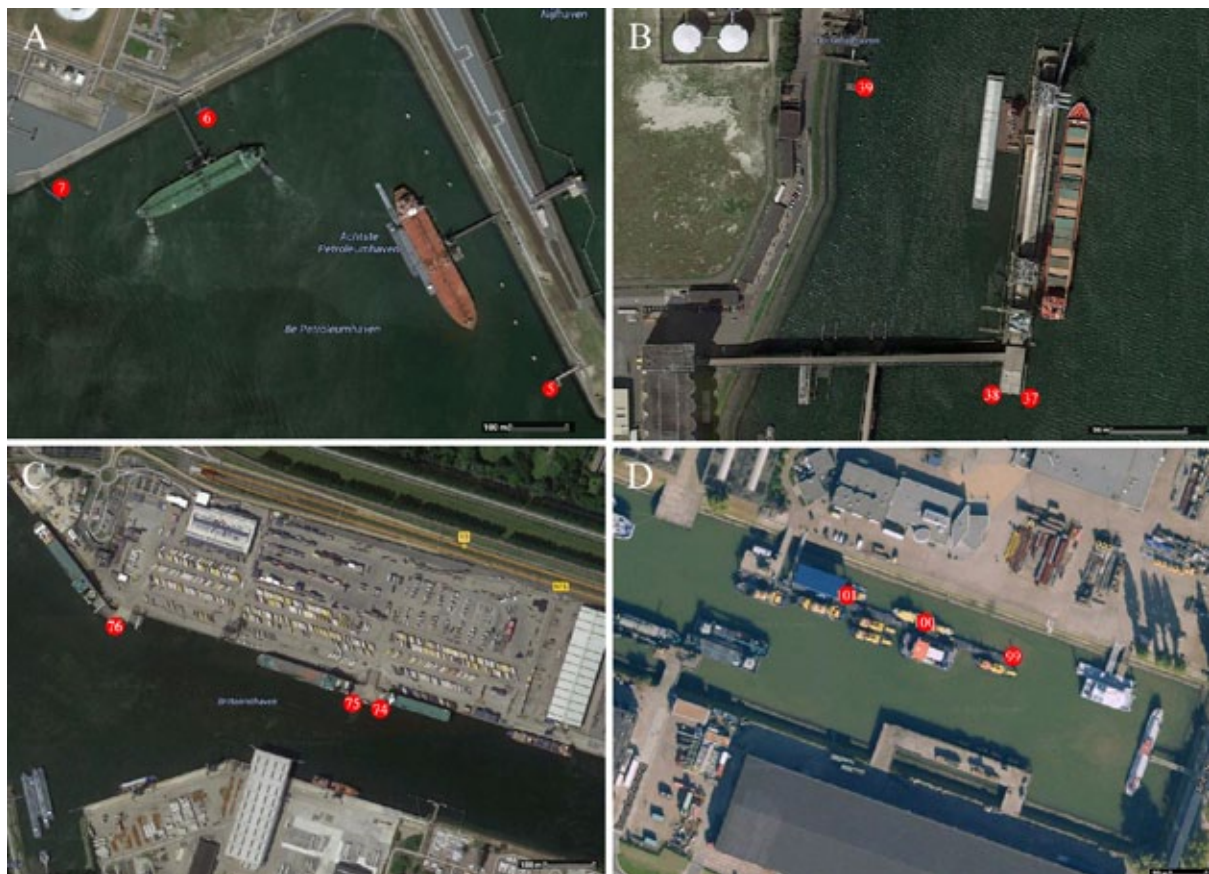


Fig. 40. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Brittanniëhaven and [D] 1e Eemhaven where the water samples were taken. The samples per location were combined and checked for human pathogens.

Table 13. Human pathogens in the four research areas during the sampling period in spring 2014.

Area	<i>E. coli</i>	<i>Enterococci</i>	<i>Vibrio cholerae</i>
8e Petroleumhaven	20 cfu/100 ml	0 cfu /100 ml	0 cfu /100 ml
Beneluxhaven	20 cfu /100 ml	130 cfu /100 ml	0 cfu /100 ml
Brittanniëhaven	0 cfu /100 ml	20 cfu /100 ml	0 cfu /100 ml
1e Eemhaven	0 cfu /100 ml	0 cfu /100 ml	0 cfu /100 ml

Table 14. Human pathogens in the four research areas during the sampling period in the late summer of 2014.

Area	<i>E. coli</i>	<i>Enterococci</i>	<i>Vibrio cholerae</i>
8e Petroleumhaven	10 cfu/100 ml	890 cfu /100 ml	0 cfu /100 ml
Beneluxhaven	50 cfu /100 ml	220 cfu /100 ml	0 cfu /100 ml
Brittanniëhaven	0 cfu /100 ml	10 ⁵ cfu /100 ml	0 cfu /100 ml
1e Eemhaven	20 cfu /100 ml	10 cfu /100 ml	0 cfu /100 ml

Table 15. *Vibrio* spp. concentrations in the four research areas in the port of Rotterdam during the sampling period in spring 2014.

	<i>Vibrio</i> spp. concentration
8e Petroleumhaven	2.84x10 ⁵ cfu/100 ml (based on a 1:1 dilution) 6.02x10 ⁵ cfu/100 ml (based on a 1:10 dilution) 5.60x10 ⁵ cfu/100 ml (based on a 1:100 dilution) of one a selected few concerned green colonies; the rest were yellow colonies
Beneluxhaven	0 cfu / 100 ml
Brittanniëhaven	3600 cfu / 100 ml of which 1000 cfu concerned green colonies and 2600 cfu yellow colonies
1e Eemhaven	0 cfu / 100 ml

phology of *Vibrio cholerae*, as was confirmed by MALDI-TOF MS and genetic (16S) analyses. The identification of the *Vibrio* species that were present is ongoing.

Although the HELCOM/OSPAR port survey protocol only requests the monitoring of *Vibrio cholerae*, all *Vibrio* species found during the spring time monitoring were identified as far as possible with the MALDI-TOF MS analysis (Table 16). All recorded species except for *Vibrio cf brasiliensis* and *Vibrio cf anguillarum* have been reported in literature to have caused human disease (Farmer & Hickman-Benner, 1992). Not all strains of these species are pathogenic however. It remains therefore uncertain whether the strains found in the port of Rotterdam were pathogenic. *Vibrio cf*

Table 16. *Vibrio* species identified with MALDI-TOF MS during the sampling period in the spring of 2014. Species names with the prefix “cf” (con forma) concern identifications that need to be validated as the MALDI-TOF MS analysis matched the *Vibrio* species studied with a known species in its database with a value < 2000, which is an indication that the identification may not be accurate.

8e Petroleumhaven	
<i>Vibrio alginolyticus</i>	yellow colonies
<i>Vibrio parahaemolyticus</i>	green colonies
<i>Aeromonas veronii</i>	yellow colonies
Brittanniëhaven	
<i>Vibrio alginolyticus</i>	yellow colonies
<i>Vibrio cf brasiliensis</i>	yellow colonies
<i>Vibrio cf vulnificus</i>	green colonies
<i>Vibrio cf parahaemolyticus</i>	green colonies
<i>Vibrio cf anguillarum</i>	yellow colonies
<i>Aeromonas veronii</i>	yellow colonies

brasiliensis concerns a recently described species, which appears to be only known from South American waters (Thompson *et al.*, 2003). As the MALDI-TOF analysis was inconclusive its identification should be checked by DNA-analysis before it can be concluded that this concerns a non-native *Vibrio* species for European waters. *Vibrio cf anguillarum* concerns a species that is already known from European waters. Pathogenic strains of this *Vibrio* species are known to cause large mortalities among fish and shellfish populations (Frans *et al.*, 2011).

5.3 Plankton

5.3.1 Phytoplankton

Both in the spring and in the summer sampling period phytoplankton samples were taken in each of the four research areas in the port of Rotterdam (Fig. 41). The species were identified and counted by Koeman & Bijkerk BV (Table 17). In these samples 99 species could be identified. 6 of these species were non-native (Gómez & Souissi, 2010; Gómez, 2008). All species were exclusively scored during the port survey with this monitoring method. The method could exactly be done according to the HELCOM/OSPAR protocol and proved to be a valuable asset. According to the species accumulation curves in Fig. 42, which did not become asymptotic yet after the 6 samples analyzed, a significant number of

additional species could have been found if more sampling was done. Most of the time analyzing the samples was spend on counting the numbers of native plankton species. We therefore suggest that in future monitoring the plankton samples should be analyzed qualitatively (presence/absence), enabling the analysis of a multitude of the number of samples in the same time that was now spend on the analysis of only one sample. With the same costs, that would yield a much better view of the plankton species diversity within the port.

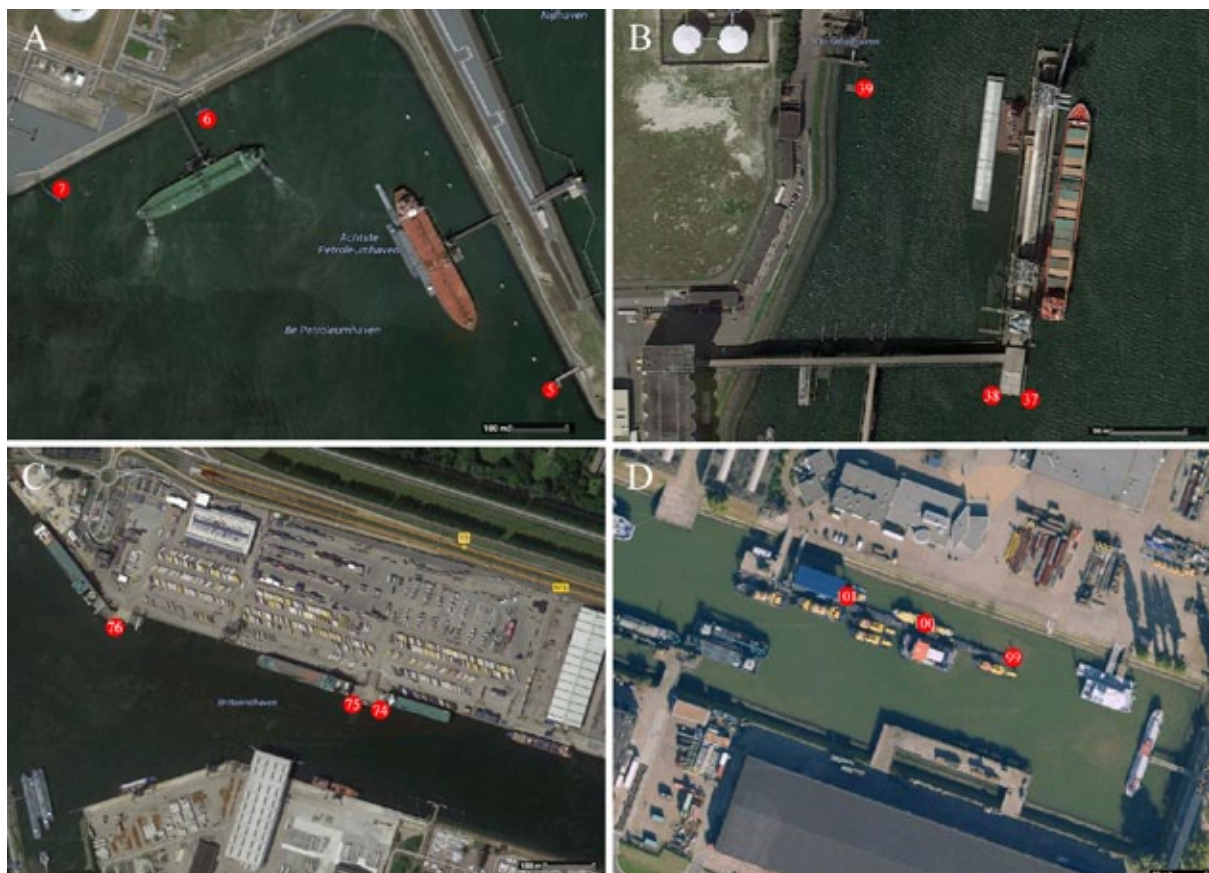


Fig. 41. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Brittaniëhaven and [D] 1e Eemhaven where the samples for the plankton were taken.

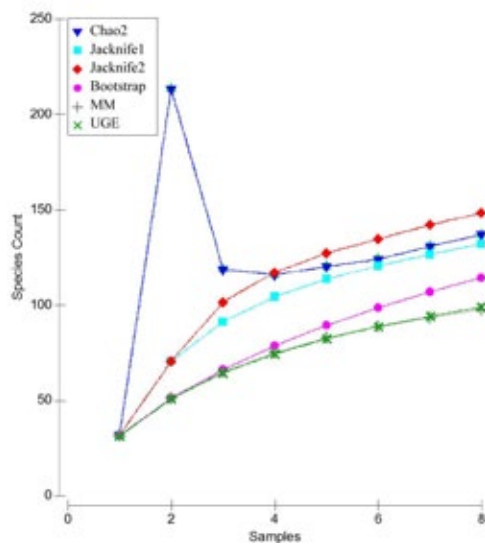


Fig.42. Six species accumulation curves were calculated based on the species found in the 8 Phytoplankton samples. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

Table 17. Phytoplankton in the four research areas during the sampling periods in spring and summer 2014. Non-native species are highlighted

Species	Authority	Group	Origin	8e Petroleumhaven		Beneluxhaven		Brittaniëhaven		1e Eemhaven	
				spring	summer	spring	summer	spring	summer	spring	summer
<i>Micromonas pusilla</i>	(Butcher) Manton & Parke, 1960	algae	Native	1	1	1	1			1	1
<i>Pyramimonas longicauda</i>	L. Van Meel, 1969	algae	Native				1		1		
<i>Planktothrix agardhii</i>	(Gomont) Anagnostidis & Komárek, 1988	Bacteria	Native								1
<i>Achnanthes brevipes</i>	C. Agardh, 1824	Chromista	Native								1
<i>Actinocyclus normanii</i>	(Gregory) Hustedt, 1957	Chromista	Native		1						1
<i>Actinopterychus octonarius</i>	(Ehrenberg) Kützing, 1844	Chromista	Native	1		1	1	1			
<i>Actinopterychus senarius</i>	(Ehrenberg) Ehrenberg, 1843	Chromista	Native	1			1	1			
<i>Actinopterychus splendens</i>	(Shadbolt) Ralfs, 1861	Chromista	Native				1				
<i>Apedinella radians</i>	(Lohmann) Campbell, 1973	Chromista	Native	1		1	1		1		
<i>Archaeoperidinium minutum</i>	(Kofoed) Jørgensen, 1912	Chromista	Native					1			
<i>Asterionella formosa</i>	Hassall, 1850	Chromista	Native								1
<i>Asterionellopsis glacialis</i>	(Castracane) Round, 1990	Chromista	Native		1						
<i>Bacillaria paxillifera</i>	(O.F.Müller) T.Marsson, 1901	Chromista	Native		1		1			1	1
<i>Cerataulina pelagica</i>	(Cleve) Hendey, 1937	Chromista	Native	1		1	1	1			
<i>Ceratoneis closterium</i>	Ehrenberg, 1839	Chromista	Native			1				1	
<i>Chaetoceros affinis</i>	Lauder, 1864	Chromista	Native					1			
<i>Chaetoceros constrictus</i>	Gran, 1897	Chromista	Native	1		1		1			
<i>Chaetoceros curvisetus</i>	Cleve, 1889	Chromista	Native	1	1	1		1			
<i>Chaetoceros debilis</i>	Cleve, 1894	Chromista	Native		1		1	1	1		
<i>Chaetoceros didymus</i>	Ehrenberg, 1845	Chromista	Native	1	1	1		1			
<i>Chaetoceros pseudocurvisetus</i>	Mangin, 1910	Chromista	Native		1						
<i>Chaetoceros socialis</i>	H.S.Lauder, 1864	Chromista	Native	1	1	1	1	1	1		
<i>Coscinodiscus granii</i>	Gough, 1905	Chromista	Native						1		
<i>Coscinodiscus perforatus</i> var. <i>pavillardii</i>	(Forti) Hust., 1928	Chromista	Native				1				
<i>Coscinodiscus radiatus</i>	Ehrenberg, 1840	Chromista	Native	1	1	1	1	1			1

Species	Authority	Group	Origin	8e Petroleumhaven		Beneluxhaven		Brittaniëhaven		1e Eemhaven	
				spring	summer	spring	summer	spring	summer	spring	summer
<i>Coscinodiscus wailesii</i>	Gran & Angst, 1931	Chromista	Non-native						1		
<i>Cylindrotheca gracilis</i>	(Brébisson) Grunow, 1882	Chromista	Native								1
<i>Cymatopleura librile</i>	(Ehrenberg) Pantocsek, 1902	Chromista	Native							1	
<i>Dactylosolen phuketensis</i>	(Sundström) Hasle, 1996	Chromista	Native	1							
<i>Delphineis minutissima</i>	(Hustedt) Simonsen	Chromista	Native					1			
<i>Dinophysis acuminata</i>	Claparède & Lachmann, 1859	Chromista	Native		1				1		
<i>Ditylum brightwellii</i>	(West) Grunow, 1885	Chromista	Native	1	1	1	1	1	1	1	
<i>Eucampia zodiacus</i>	Ehrenberg, 1839	Chromista	Native	1	1	1	1				
<i>Gonyaulax digitale</i>	(Pouchet) Kofoïd, 1911	Chromista	Native		1						
<i>Gonyaulax spinifera</i>	(Claparède & Lachmann) Diesing, 1866	Chromista	Native						1		
<i>Guinardia delicatula</i>	(Cleve) Hasle, 1997	Chromista	Native	1	1	1		1			
<i>Guinardia flaccida</i>	(Castracane) Peragallo, 1892	Chromista	Native	1	1	1	1		1		
<i>Guinardia striata</i>	(Stolterfoth) Hasle, 1996	Chromista	Native		1		1				
<i>Gyrodinium britannicum</i>	Kofoïd & Swezy, 1921	Chromista	Native				1		1		
<i>Gyrodinium spirale</i>	(Bergh) Kofoïd & Swezy, 1921	Chromista	Native						1		
<i>Gyrosigma fasciola</i>	(Ehrenberg) Griffith & Henfrey, 1856	Chromista	Native	1							1
<i>Helicotheca tamesis</i>	(Shrubsole) Ricard, 1987	Chromista	Native			1					
<i>Heterocapsa minima</i>	Pomroy, 1989	Chromista	Native	1	1		1				
<i>Heterocapsa rotundata</i>	(Lohmann) Hansen, 1995	Chromista	Native							1	
<i>Heterocapsa triquetra</i>	(Ehrenberg) F.Stein, 1883	Chromista	Native	1		1		1			
<i>Lauderia annulata</i>	Cleve, 1873	Chromista	Native	1	1	1		1			
<i>Leptocylindrus danicus</i>	Cleve, 1889	Chromista	Native	1	1	1	1	1	1		
<i>Leptocylindrus minimus</i>	Gran, 1915	Chromista	Native		1		1				
<i>Leucocryptos marina</i>	(Braarud) Butcher, 1967	Chromista	Native						1		
<i>Lithodesmium undulatum</i>	Ehrenberg, 1839	Chromista	Native		1		1		1		
<i>Mediopyxis heylsta</i>	Kühn, Hargreaves & Halliger, 2006	Chromista	Non-native	1							
<i>Melosira moniliformis</i>	(O.F.Müller) C.Agardh, 1824	Chromista	Native	1			1				
<i>Melosira varians</i>	C.Agardh, 1827	Chromista	Native								1
<i>Mesodinium rubrum</i>	(Lohmann, 1908)	Chromista	Native				1		1		

Species	Authority	Group	Origin	8e Petroleumhaven		Beneluxhaven		Brittaniëhaven		1e Eemhaven	
				spring	summer	spring	summer	spring	summer	spring	summer
<i>Minutoceclus scriptus</i>	Hasle, von Stosch & Syvertsen, 1983	Chromista	Native		1		1		1		
<i>Neoceratium fusus</i>	(Ehrenberg) Gomez, Moreira & Lopez-Garcia, 2010	Chromista	Native		1		1		1		
<i>Neoceratium horridum</i>	(Gran) F.Gomez, D.Moreira & P.Lopez-Garcia, 2010	Chromista	Native		1						
<i>Neoceratium lineatum</i>	(Ehrenberg) Gomez, Moreira & Lopez-Garcia, 2010	Chromista	Native		1						
<i>Nitzschia sigma</i>	(Kützing) Smith, 1853	Chromista	Native						1	1	1
<i>Noctiluca scintillans</i>	(Macartney) Kofoid & Swezy, 1921	Chromista	Native		1	1					
<i>Oblea rotunda</i>	(Lebour) Balech, 1973	Chromista	Native	1		1					
<i>Odontella longicruris</i>	(Greville) Hoban, 1983	Chromista	Native	1		1		1			
<i>Odontella sinensis</i>	(Greville) Grunow, 1884	Chromista	Non-native	1	1	1	1	1	1		
<i>Oxytoxum mediterraneum</i>	Schiller	Chromista	Native						1		
<i>Paralia sulcata</i>	(Ehrenberg) Cleve, 1873	Chromista	Native	1	1	1	1	1	1	1	
<i>Pleurosigma formosum</i>	Smith, 1852	Chromista	Native				1				
<i>Podosira stelligera</i>	(Bailey) A.Mann, 1907	Chromista	Native			1		1	1		
<i>Prorocentrum cordatum</i>	(Ostenfeld) Dodge, 1975	Chromista	Non-native		1		1		1		
<i>Prorocentrum micans</i>	Ehrenberg, 1834	Chromista	Native	1	1		1		1		
<i>Prorocentrum triestinum</i>	Schiller, 1918	Chromista	Native		1		1		1		
<i>Protoceratium reticulatum</i>	(Claparède & Lachmann) Butschli, 1885	Chromista	Non-native	1		1					
<i>Protoperidinium achromaticum</i>	(Levander) Balech, 1974	Chromista	Native	1		1				1	
<i>Protoperidinium bipes</i>	(Paulsen) Balech, 1974	Chromista	Native	1		1					
<i>Protoperidinium claudicans</i>	(Paulsen) Balech, 1974	Chromista	Native		1		1		1		
<i>Protoperidinium conicum</i>	(Gran) Balech, 1974	Chromista	Native	1	1						
<i>Protoperidinium depressum</i>	(Bailey) Balech, 1974	Chromista	Native		1		1				
<i>Protoperidinium granii</i>	(Ostenfeld) Balech, 1974	Chromista	Native	1							
<i>Protoperidinium marie-lebouriae</i>	(Paulsen) Balech, 1974	Chromista	Native		1						
<i>Protoperidinium pellucidum</i>	Bergh, 1881	Chromista	Native	1	1		1				
<i>Protoperidinium pentagonum</i>	(Gran) Balech, 1974	Chromista	Native						1		

Species	Authority	Group	Origin	8e Petroleumhaven			Beneluxhaven		Brittaniëhaven		1e Eemhaven	
				spring	summer	spring	spring	summer	spring	summer	spring	summer
<i>Protoperidinium thorianum</i>	(Paulsen) Balech, 1974	Chromista	Native	1				1				
<i>Pseudo-nitzschia americana</i>	(Hasle) Fryxell, 1993	Chromista	Native						1			
<i>Pseudo-nitzschia delicatissima</i>	(Cleve) Heiden, 1928	Chromista	Native	1	1	1	1	1	1	1		
<i>Pseudo-nitzschia fraudulenta</i>	(Cleve) Hasle, 1993	Chromista	Native	1	1	1	1	1				
<i>Pseudo-nitzschia pungens</i>	(Grunow) Hasle, 1993	Chromista	Native	1	1	1	1	1	1	1		
<i>Pyrocystis noctiluca</i>	Murray, 1890	Chromista	Native	1								
<i>Rhaphoneis amphicerus</i>	(Ehrenberg) Ehrenberg, 1844	Chromista	Native	1								
<i>Rhizosolenia imbricata</i>	Brightwell, 1858	Chromista	Native	1				1	1			
<i>Rhizosolenia setigera</i>	Brightwell, 1858	Chromista	Native	1	1	1	1	1	1			
<i>Skeletonema costatum</i>	(Greville) Cleve, 1873	Chromista	Native		1			1	1	1		
<i>Skeletonema potamos</i>	(Weber) Hasle, 1976	Chromista	Native	1		1	1		1	1	1	1
<i>Thalassionema frauenfeldii</i>	(Grunow) Hallegraeff, 1986	Chromista	Native		1			1				
<i>Thalassiosira eccentrica</i>	(Ehrenberg) Cleve, 1903	Chromista	Native	1								
<i>Thalassiosira lacustris</i>	(Grunow) Hasle, 1977	Chromista	Native									1
<i>Thalassiosira nordenskiöldii</i>	Cleve, 1873	Chromista	Non-native						1			
<i>Thalassiosira rotula</i>	Meunier, 1910	Chromista	Native		1							
<i>Trigonium alternans</i>	(J.W.Bailey) Mann, 1907	Chromista	Native					1				
<i>Tryblionella coarctata</i>	(Grunow) Mann, 1990	Chromista	Native								1	1
<i>Ebria tripartita</i>	(Schumann) Lemmermann, 1899	Protozoa	Native	1			1	1	1	1		

5.3.2 Zooplankton

Both in the spring and in the summer sampling period zooplankton samples were taken in each of the four research areas in the port of Rotterdam following exactly the HELCOM/OSPAR protocol (Fig. 41). The species were identified by Koeman & Bijkerk BV. As many of the larval “zooplankton” stages like the nauplii and pluteus stages of related species look exactly the same, only 5 species could be identified to the species level (Table 18). None of these species were non-native. All species were exclusively scored with this monitoring method during the survey. The analyses of the samples could not be done exactly according to the method suggested by the HELCOM/OSPAR protocol. The HELCOM protocol for counting zooplankton requires “All specimens to be identified and counted until one has reached 100 individuals of each of the three dominating taxonomic groups excluding nauplii, rotifers and tintinnids”. If this procedure would have been strictly followed in the Rotterdam survey, the analysis of one sample would have taken several days during which most time would have been lost in the counting of the individuals of native plankton species. During the port of Rotterdam survey all specimens in the zooplankton samples were counted until one had reached 100 individuals, excluding nauplii, rotifers and tintinnids.

As the species accumulation curves in Fig. 43 did not become asymptotic yet, it would have been beneficial to analyze more samples. Most of the time analyzing the samples quantitatively was spent on counting the numbers of native plankton species and larval “zooplankton” stages like the nauplii and pluteus stages, which do not show diagnostic characters enabling identification to the species level. As was also indicated for the phytoplankton analyses, many more samples could have been analyzed with the same costs involved if the samples would be analyzed qualitatively instead of quantitatively. Adding more samples would also yield a better overview of the species present.

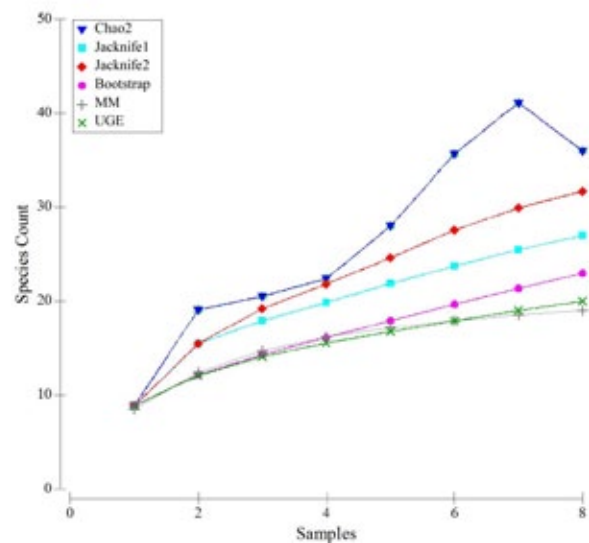


Fig.43. Six species accumulation curves were calculated based on the species found in the 8 Zooplankton samples. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

Table 18. Zooplankton from hauls with a 100 µm plankton net in the four research areas during the sampling periods in spring and summer 2014.

Species	Authority	Group	Origin	8e Petroleumhaven		Beneluxhaven		Brittaniëhaven		1e Eemhaven	
				spring	summer	spring	summer	spring	summer	spring	summer
Polychaeta	-	Annelida	n.a.	1	1	1	1	1	1	1	
Oikopleura (Vexillaria) dioica	Fol, 1872	Appendicularia	Native	1		1	1	1	1	1	
Balanidae nauplii	-	Crustacea	n.a.	1	1	1	1	1	1	1	
Bosmina cf. longirostris	(Müller, 1785)	Crustacea	Native	1							1
Bosmina coregoni	Baird, 1857	Crustacea	Native								1
Calanoidea copepodiet	-	Crustacea	n.a.	1	1	1	1	1	1	1	1
Calanoidea sp.1	-	Crustacea	n.a.		1						
Calanoidea sp.2	-	Crustacea	n.a.			1					
Copepoda nauplii	-	Crustacea	n.a.	1	1	1	1	1	1	1	1
Cyclopoida ♀ sp.	-	Crustacea	n.a.		1						
Cyclopoida copepodiet	-	Crustacea	n.a.		1		1	1	1		1
Diaphanosoma mongolianum	Uéno, 1938	Crustacea	Native								1
Harpacticoida	-	Crustacea	n.a.	1		1	1			1	
Orthona sp.	-	Crustacea	n.a.				1				
Podon leuckartii	(Sars, 1862)	Crustacea	Native	1			1	1	1	1	
Echinodermata pluteus	-	Echinodermata	n.a.	1							
Ophiopluteus	-	Echinodermata	n.a.	1							
Bivalvia veliger	-	Mollusca	n.a.	1		1		1	1	1	
Rotifera sp.	-	Rotifera	n.a.		1		1		1		1
Trichocerca sp.	-	Rotifera	n.a.				1		1		

5.3.3 Larger zooplankton including gelatinous species

Both in the spring and the summer period samples were taken with a 500 µm zooplankton net in each of the four research areas in the port of Rotterdam (Fig. 41). In these samples 8 species were scored (Table 19). Three of these species were non-native. Five were exclusively scored with this monitoring method, i.e. the cnidarians *Beroë cucumis*, *Chrysaora hysoscella*, *Nemopsis bachei*, *Sarsia tubulosa* and the shrimp *Palaeomon elegans*. Three of the species recorded were non-native, i.e. *Dreissena bugensis*, *Nemopsis bachi* (Fig. 44), and *Mnemiopsis leidyi*. The quagga mussel *Dreissena bugensis* may have ended up in the net when it accidentally scraped along the dock. The record of the medusa stage of the hydroid *Nemopsis bachi* during the spring monitoring in the Brittanniëhaven is nowadays a rare sighting for the Netherlands. It concerns a species native to the Atlantic coast of America, which was probably introduced in European waters by ship traffic over a century ago (Vervoort & Faasse, 2009). At the start of the 20th century it used to be an abundant species in the Netherlands in the Zuiderzee, but it disappeared when this water body was isolated from the Wadden

Sea, becoming a freshwater lake. In recent years it is only rarely recorded (Vervoort & Faasse, 2009). The larger zooplankton sampling and analysis could exactly be done according to the HELCOM/OSPAR protocol and proved to be a valuable asset during the inventory as five of these species were found exclusively with this method. The species accumulation curves were not calculated for this dataset as only one species was found in 7 out of the 8 samples that were taken. Based on these low species counts no indication can be given of how many species would have been found if more samples would have been taken.



Fig.44. The hydroid *Nemopsis bachei* found in the Brittanniëhaven.

Table 19. Gelatine zooplankton in the four research areas during the sampling periods in spring and summer 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven		Beneluxhaven		Brittaniëhaven		1e Eemhaven	
				spring	summer	spring	summer	spring	summer	spring	summer
<i>Aurelia aurita</i>	(Linnaeus, 1758)	Cnidaria	Native					1			
<i>Beroë cucumis</i>	Fabricius, 1780	Ctenophora	Native					1			
<i>Chrysaora hysoscella</i>	(Linnaeus, 1767)	Cnidaria	Native					1			
<i>Mnemiopsis leydii</i>	A. Agassiz, 1865	Ctenophora	Non-native		1		1	1	1		1
<i>Nemopsis bachei</i>	L. Agassiz, 1849	Cnidaria	Non-native					1			
<i>Sarsia tubulosa</i>	(M. Sars, 1835)	Cnidaria	Native					1			
<i>Palaemon elegans</i>	Rathke, 1837	Crustacea	Native			1					
<i>Dreissena bugensis</i>	Andrusov, 1897	Mollusca	Non-native							1	

5.4 Traps

5.4.1 Chinese crab trap

In the summer of 2014 in total 12 Chinese crab traps were deployed in the port of Rotterdam for about three days in each of the four research areas (Fig. 45). With the Chinese crab traps in total 21 species were recorded (Table 20). Two of these species were exclusively found in these traps and not scored with any other method in the port of Rotterdam, i.e. the common spidercrab *Macropodia rostrata* and the pipe fish *Sygnatus acus*. Most of the species were found in the 8e Petroleumhaven (Fig. 45).

Eight of the species were non-native. The method could exactly be done according to the OSPAR-HELCOM protocol and proved to be a valuable

asset during the inventory as two species were found exclusively with this method. In addition to being a valuable method for scoring fish and crab species, which were attracted by the bait as was expected a priori, these traps are apparently also very suitable for recording gelatinous species like the invasive ctenophore *Mnemiopsis leidyi*. Other unexpected species that were found include three algal species, among which one non-native, that had apparently washed into the trap with the currents, and several hydroids, sea-anemones and barnacles that had settled on the crabs in the trap. According to the species accumulation curves in Fig. 46, which did not become asymptotic yet after the 12 samples taken, a several additional species could have been found if more sampling was done.

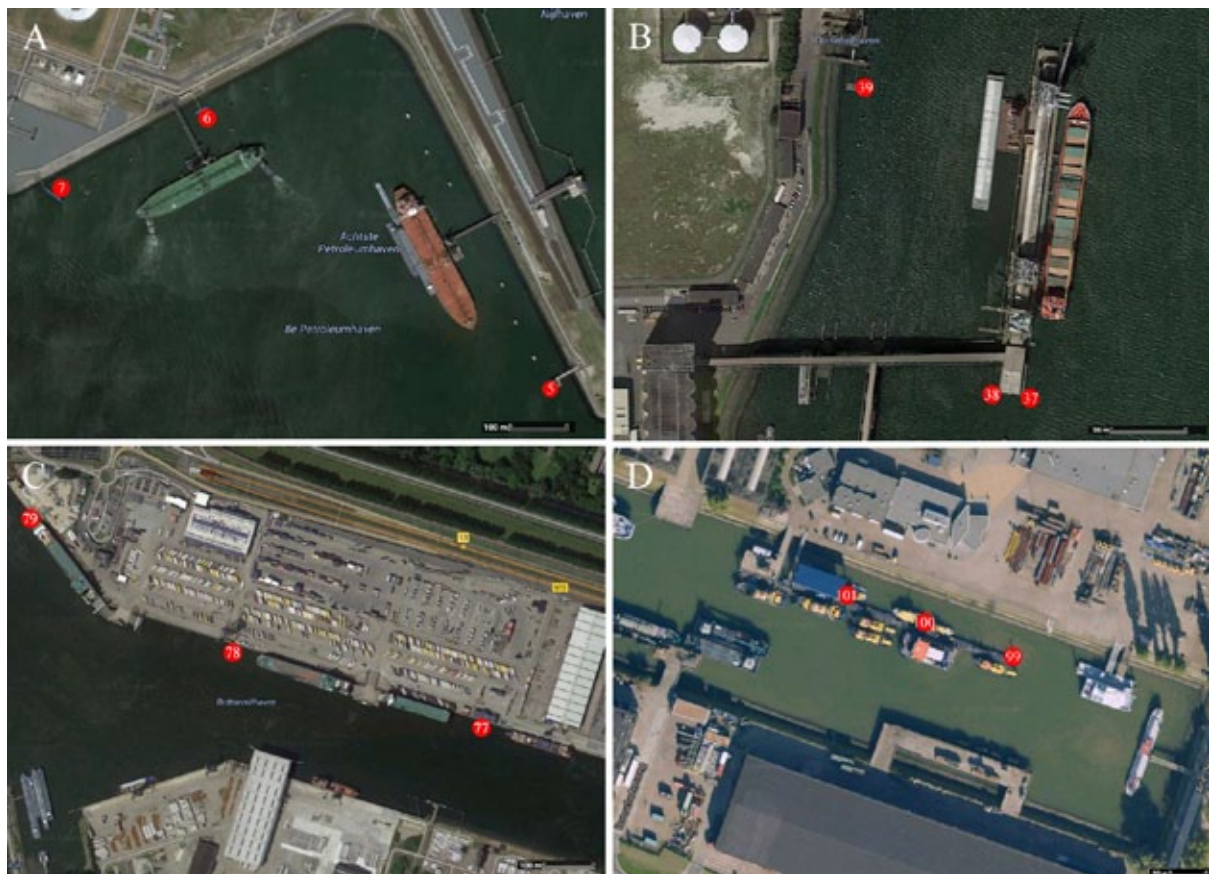
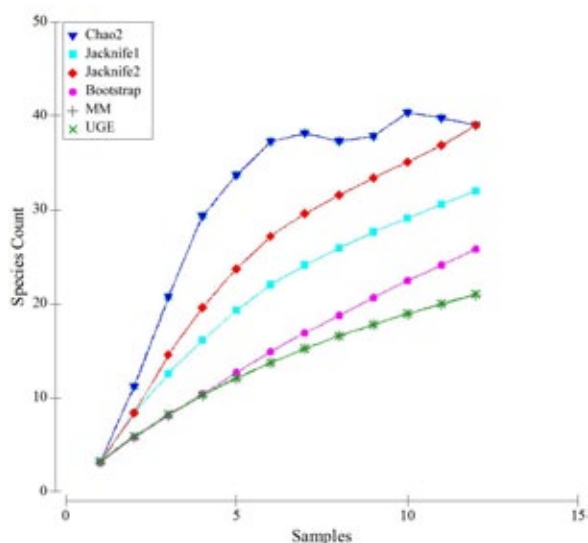


Fig. 45. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Brittaniëhaven and [D] 1e Eemhaven where both the Chinese crab trap and the Gee's minnow trap were deployed.



<< Fig.46. Six species accumulation curves were calculated based on the species found in the 12 Chinese crab traps. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

Table 20. Species found in the Chinese crab traps, that were deployed during the sampling period in the late summer of 2014 for at least 48 hours. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Ceramium tenuicorne</i>	(Kützinger) Waern	Algae	Native	1			
<i>Dasysiphonia japonica</i>	(Yendo) H.-S. Kim	Algae	Non-native	1			
<i>Ulva curvata</i>	(Kützinger) De Toni	Algae	Native	1			
<i>Ciona intestinalis</i>	(Linnaeus, 1767)	Ascidiacea	Native		1		
<i>Hartlaubella gelatinosa</i>	(Pallas, 1766)	Cnidaria	Native	1			
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native	1			
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native	1			
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native		1		
<i>Austrominius modestus</i>	(Darwin, 1854)	Crustacea	Non-native			1	
<i>Balanus crenatus</i>	Bruguière, 1789	Crustacea	Native	1			
<i>Cancer pagurus</i>	Linnaeus, 1758	Crustacea	Native	1			
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	1	
<i>Macropodia rostrata</i>	(Linnaeus, 1761)	Crustacea	Native	1			
<i>Palaemon longirostris</i>	H. Milne Edwards, 1837	Crustacea	Native				1
<i>Pisidia longicornis</i>	(Linnaeus, 1767)	Crustacea	Native	1			
<i>Rhithropanopeus harrisi</i>	(Gould, 1841)	Crustacea	Non-native				1
<i>Mnemiopsis leidyi</i>	A. Agassiz, 1865	Ctenophora	Non-native		1		
<i>Crepidula fornicata</i>	(Linnaeus, 1758)	Mollusca	Non-native			1	
<i>Neogobius melanostomus</i>	(Pallas, 1814)	Pisces	Non-native				1
<i>Syngnathus acus</i>	Linnaeus, 1758	Pisces	Native	1			
<i>Trisopterus luscus</i>	(Linnaeus, 1758)	Pisces	Native	1		1	

5.4.2 Gee's minnow trap

In the summer of 2014 in total 12 Gee's minnow traps were deployed in the port of Rotterdam for about three days in each of the four research areas (Fig. 44). With the Gee's minnow traps in total 16 species were recorded (Table 21).

Two of these species were exclusively found in these traps and not scored with any other method in the port of Rotterdam, i.e. the common hermit crab *Pagurus bernhardus* with the hydroid *Hydractinia echinata*, also known as snail fur, growing on the shell in which the hermit crab was housing. Most of the species, i.e. 13, were found in the 8e Petroleumhaven. Four of the species found in the Gee's minnow traps were non-native. The method could exactly be done according to the OSPAR-HELCOM protocol. In addition to being a valuable method for scoring fish and crab species as was expected a priori, these traps, like the Chinese crab trap, are apparently also very suitable for recording gelatinous species like the invasive stenophore *Mnemiopsis leidyi*. Like the Chinese traps, the Gee's minnow traps also caught drift algae and several sea-anemone and barnacle species that had settled on the crabs in the traps. According to the species accumulation curves in Fig. 47, which did not become asymptotic yet after the 12 samples taken, a significant number of additional species could have been found if more sampling was done. As the Chinese crab traps and the Gee's minnow traps actually showed a clear overlap in the species that were scored, one could combine the results. By doing so, the species accumulation curves would start to become asymptotic after 24 samples, indicative that most species that can be caught in baited traps were found during the port of Rotterdam survey.

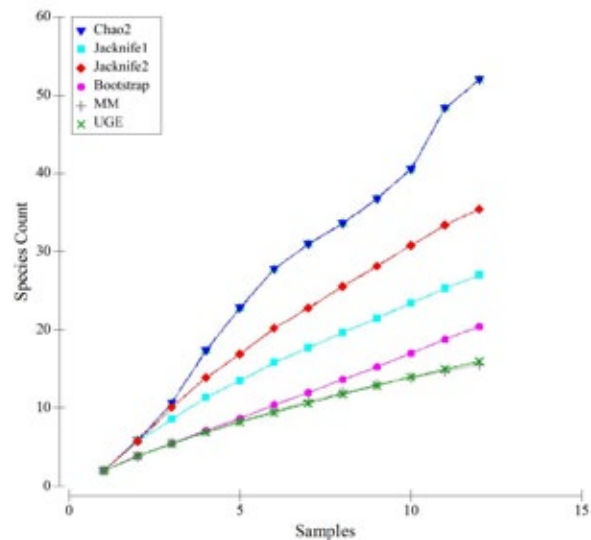


Fig.47. Six species accumulation curves were calculated based on the species found in the 12 Gee's minnow traps. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

Table 21. Species found in the Gee's minnow traps, that were deployed during the sampling period in the late summer of 2014 for at least 48 hours. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Ceramium tenuicorne</i>	(Kützinger) Waern	Algae	Native	1			
<i>Ulva curvata</i>	(Kützinger) De Toni	Algae	Native	1			
<i>Ciona intestinalis</i>	(Linnaeus, 1767)	Ascidacea	Native		1		
<i>Hydractinia echinata</i>	(Fleming, 1828)	Cnidaria	Native	1			
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native			1	
<i>Balanus crenatus</i>	Bruguière, 1789	Crustacea	Native	1			
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	1	
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native	1			
<i>Pagurus bernhardus</i>	(Linnaeus, 1758)	Crustacea	Native	1			
<i>Pisidia longicornis</i>	(Linnaeus, 1767)	Crustacea	Native	1			
<i>Rhithropanopeus harrisii</i>	(Gould, 1841)	Crustacea	Non-native				1
<i>Mnemiopsis leidyi</i>	A. Agassiz, 1865	Ctenophora	Non-native	1	1		
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native			1	
<i>Neogobius melanostomus</i>	(Pallas, 1814)	Pisces	Non-native				1
<i>Perca fluviatilis</i>	(Linnaeus, 1758)	Pisces	Native				1
<i>Trisopterus luscus</i>	(Linnaeus, 1758)	Pisces	Native		1	1	

5.5 Fouling plates

In the spring of 2014 in total 12 fouling plate constructions were deployed in the port of Rotterdam at each of the four research areas (Fig. 48). These constructions were retrieved in the late summer sampling period. With the fouling plate constructions 44 species were recorded (Table 22). Seven of these species were exclusively found with this method and not scored with any other method in the port of Rotterdam, i.e. the worm *Polydora ciliata*, the bryozoan *Electra pilosa*, the sea anemone *Sagartiogeton undatus*, the small crustaceans *Microdeutopus gryllotalpa* and *Monocorophium acherusicum*, the nemertean *Lineus longissimus* and the fivebeard rockling *Ciliata mustela*.

Most of the species, i.e. 37, were found in the 8e Petroleumhaven. Of the recorded species 11 were found to be non-native. The method could exactly be done according to the OSPAR-HELCOM protocol and proved to be a valuable asset during the inventory as seven species were found exclusively with this method.

According to the OSPAR-HELCOM protocol the organisms attached to all plates on a plate construction were supposed to be scraped off and scored together as one sample. Instead we scored the species present on each plate separately, both on the top and on the underside. By doing so 42 different records of separate species communities on plates were scored. The species accumulation curves in Fig. 49 start to become asymptotic around 45 to 50 species. As 44 species were found in total it can be concluded that most fouling spe-

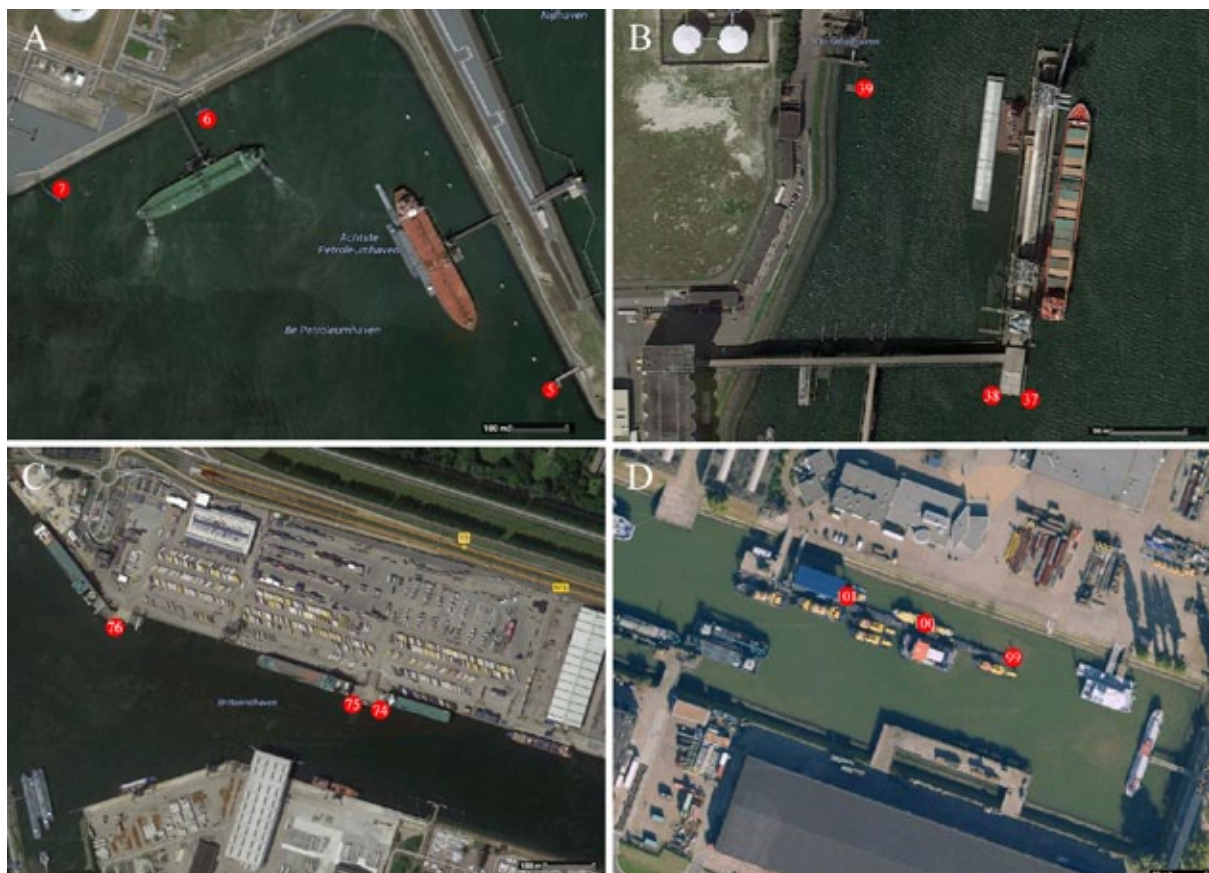


Fig. 48. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Britanniëhaven and [D] 1e Eemhaven where the fouling plate constructions were deployed in the spring period and retrieved in the late summer sampling period.

cies that could have been scored on the settlement plates, were scored. As some of the plate construction had attracted over 80 kilograms of fouling and were therefore hard to retrieve from the water without losing some of the fouling attached, it would be advisable to attach only one plate on a line instead of three as is recommended in the OSPAR-HELCOM protocol. This is also recommended as three plates on one line may influence each other, and scoring each plate separately will improve the accuracy of the method as is illustrated with the species accumulation curves.

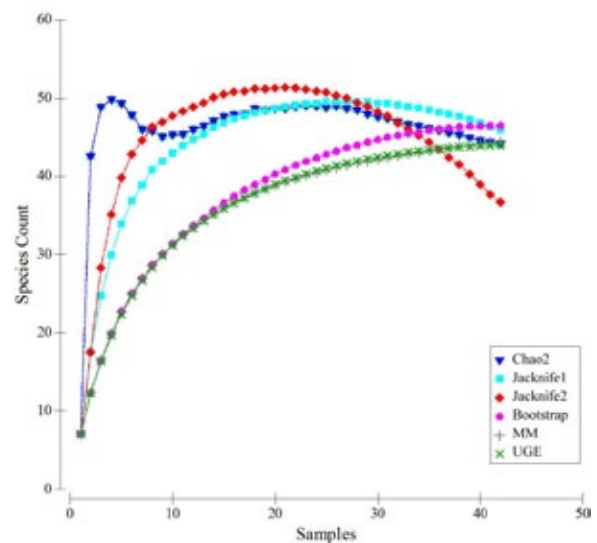


Fig.49. Six species accumulation curves were calculated based on the species found on the fouling plate constructions. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

Table 22. Species found on the fouling plates, that were deployed during the sampling period in spring and collected in the sampling period in the late summer of 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Callithamnion corymbosum</i>	(Smith) Lyngbye	Algae	Native			1	
<i>Ceramium tenuicorne</i>	(Kützinger) Waern	Algae	Native	1	1		
<i>Dasysiphonia japonica</i>	(Yendo) H.-S. Kim	Algae	Non-native	1			
<i>Fucus vesiculosus</i>	Linnaeus	Algae	Native			1	
<i>Ulva curvata</i>	(Kützinger) De Toni	Algae	Native	1		1	
<i>Ulva prolifera</i>	Müller	Algae	Native		1		
<i>Polydora ciliata</i>	(Johnston, 1838)	Annelida	Native	1	1		
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1		1	1
<i>Lepidonotus squamatus</i>	(Linnaeus, 1758)	Annelida	Native		1		
<i>Nereis pelagica</i>	Linnaeus, 1758	Annelida	Native	1		1	
<i>Ascidia aspersa</i>	(Müller, 1776)	Ascidacea	Native		1	1	
<i>Botryllus schlosseri</i>	(Pallas, 1766)	Ascidacea	Native	1	1	1	
<i>Ciona intestinalis</i>	(Linnaeus, 1767)	Ascidacea	Native		1	1	
<i>Molgula manhattensis</i>	(De Kay, 1843)	Ascidacea	Non-native	1	1	1	
<i>Styela clava</i>	Herdman, 1881	Ascidacea	Non-native	1	1	1	
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native	1		1	
<i>Electra pilosa</i>	(Linnaeus, 1767)	Bryozoa	Native			1	
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Bryozoa	Native	1			
<i>Cordylophora caspia</i>	(Pallas, 1771)	Cnidaria	Non-native				1
<i>Hartlaubella gelatinosa</i>	(Pallas, 1766)	Cnidaria	Native	1			
<i>Obelia geniculata</i>	(Linnaeus, 1758)	Cnidaria	Native			1	
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native	1	1	1	
<i>Sagartiogeton undatus</i>	(Müller, 1778)	Cnidaria	Native		1		
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native	1	1		1
<i>Balanus crenatus</i>	Bruguère, 1789	Crustacea	Native			1	
<i>Cancer pagurus</i>	Linnaeus, 1758	Crustacea	Native	1			
<i>Caprella mutica</i>	Schurin, 1935	Crustacea	Non-native		1	1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1			
<i>Gammarus locusta</i>	(Linnaeus, 1758)	Crustacea	Native	1		1	
<i>Hemigrapsus sanguineus</i>	(De Haan, 1835)	Crustacea	Non-native	1	1		
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native	1			
<i>Microdeutopus gryllotalpa</i>	Costa, 1853	Crustacea	Native		1		
<i>Monocorophium acherusicum</i>	(Costa, 1853)	Crustacea	Native		1		
<i>Necora puber</i>	(Linnaeus, 1767)	Crustacea	Native	1			
<i>Pilumnus hirtellus</i>	(Linnaeus, 1761)	Crustacea	Native	1			
<i>Pisidia longicornis</i>	(Linnaeus, 1767)	Crustacea	Native	1			
<i>Rhithropanopeus harrisi</i>	(Gould, 1841)	Crustacea	Non-native				1
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1	1		
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1	1	1	
<i>Lineus longissimus</i>	(Gunnerus, 1770)	Nemertea	Native	1	1		
<i>Ciliata mustela</i>	(Linnaeus, 1758)	Pisces	Native	1			
<i>Leptoplana tremellaris</i>	(Müller, 1774)	Platyhelminthes	Native	1	1		
<i>Halichondria bowerbanki</i>	Burton, 1930	Porifera	Native	1			
<i>Halichondria panicea</i>	(Pallas, 1766)	Porifera	Native	1			

5.6 Scrape samples

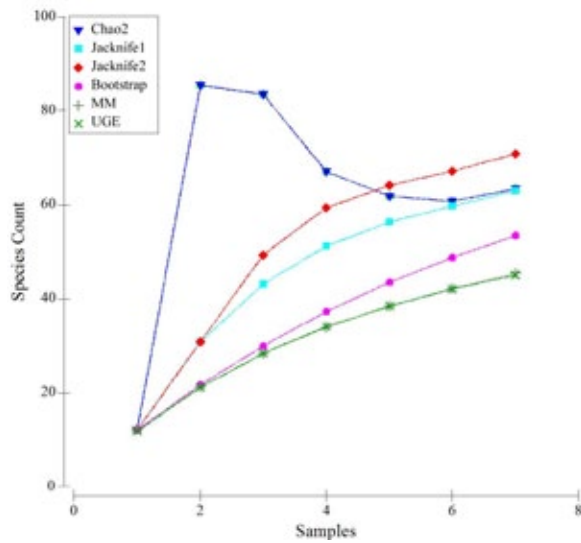
5.6.1 Floating docks

In the summer of 2014 in total seven scrape samples were taken from seven floating docks in the port of Rotterdam (Fig. 50). In these scrape samples a total of 44 species were recorded (Table 23). Four of these species were exclusively found with this method and were not scored with any other method in the port of Rotterdam, i.e. the algal species *Aglaothamnion hookeri* and *Ulva* cf. *rigida*, the scaleworm *Harmothoe imbricata* and the sponge *Spongilla lacustris*. Most of the species, i.e. 35, were found in the 8e Petroleumhaven. Of the species recorded in the scrape samples 14 were non-native. The method could exactly be done according to the OSPAR-HELCOM

protocol and proved to be a valuable asset during the inventory as four species were found exclusively with this method. The fact that the species accumulation curves in Fig. 51 have not become asymptotic yet, is an indication that a number of extra species could have been found with this method if more sampling was done.



Fig. 50. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Brittaniëhaven and [D] 1e Eemhaven where scrape samples were taken from floating docks.



<< Fig.51. Six species accumulation curves were calculated based on the species found in the scrape samples taken from floating docks. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

Table 23. Species found in the scrape samples, collected from floating docks during de sampling period in the late summer of 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	8c Petroleumhaven	Beneluxhaven	Brittaniëhaven	1c Eemhaven
<i>Aglaothamnion hookeri</i>	(Dillwyn) Maggs & Hommersand	Algae	Native	1			
<i>Blidingia marginata</i>	(J.Agardh) P.J.L.Dangeard	Algae	Native				1
<i>Blidingia minima</i>	(Nägeli ex Kützing) Kylin	Algae	Native				1
<i>Callithamnion corymbosum</i>	(Smith) Lyngbye	Algae	Native	1		1	
<i>Ceramium tenuicorne</i>	(Kützing) Waern	Algae	Native	1	1		
<i>Cladophora sp</i>	-	Algae	n.a.				1
<i>Dasysiphonia japonica</i>	(Yendo) H.-S. Kim	Algae	Non-native	1	1		
<i>Polysiphonia stricta</i>	(Dillwyn) Greville	Algae	Native	1			1
<i>Ulva cf rigida</i>	C.Agardh	Algae	Native	1			
<i>Ulva curvata</i>	(Kützing) De Toni	Algae	Native	1			
<i>Ulva linza</i>	Linnaeus	Algae	Native				1
<i>Ulva pertusa</i>	Kjellman	Algae	Non-native	1			
<i>Ulva prolifera</i>	Müller	Algae	Native				1
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1			
<i>Harmothoe imbricata</i>	(Linnaeus, 1767)	Annelida	Native		1		
<i>Lepidonotus squamatus</i>	(Linnaeus, 1758)	Annelida	Native	1		1	
<i>Nereis pelagica</i>	Linnaeus, 1758	Annelida	Native		1	1	
<i>Botryllus schlosseri</i>	(Pallas, 1766)	Ascidacea	Native			1	
<i>Molgula manhattensis</i>	(De Kay, 1843)	Ascidacea	Non-native	1	1		
<i>Styela clava</i>	Herdman, 1881	Ascidacea	Non-native	1			
<i>Alcyonidioides mytili</i>	(Dalyell, 1848)	Bryozoa	Native		1	1	

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native	1	1	1	
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Bryozoa	Native	1	1		
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native		1	1	
<i>Obelia dichotoma</i>	(Linnaeus, 1758)	Cnidaria	Native				1
<i>Obelia geniculata</i>	(Linnaeus, 1758)	Cnidaria	Native	1	1	1	
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native	1		1	1
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native	1		1	1
<i>Austrominius modestus</i>	(Darwin, 1854)	Crustacea	Non-native	1			
<i>Caprella mutica</i>	Schurin, 1935	Crustacea	Non-native			1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native		1	1	
<i>Orchestia gammarellus</i>	(Pallas, 1766)	Crustacea	Native			1	
<i>Echinogammarus stoerensis</i>	(Reid, 1938)	Crustacea	Native				1
<i>Gammarus locusta</i>	(Linnaeus, 1758)	Crustacea	Native	1			
<i>Hemigrapsus sanguineus</i>	(De Haan, 1835)	Crustacea	Non-native	1		1	
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native	1	1	1	
<i>Palaemon longirostris</i>	H. Milne Edwards, 1837	Crustacea	Native				1
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1			
<i>Dreissena bugensis</i>	Andrusov, 1897	Mollusca	Non-native				1
<i>Dreissena polymorpha</i>	(Pallas, 1771)	Mollusca	Non-native				1
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1			
<i>Emplectonema neessi</i>	(Örsted, 1843)	Nemertea	Native	1		1	
<i>Pholis gunnellus</i>	(Linnaeus, 1758)	Pisces	Native	1			
<i>Spongilla lacustris</i>	(Linnaeus, 1759)	Porifera	Native				1

5.6.2 Sub-littoral scrape samples from pilings

With an aluminum hand net on a 3 m long pole equipped with a scraping blade (Fig. 24), scrape samples were taken at low tide from three pilings in the 1e Eemhaven (Fig. 52). Although the pilings in the 1e Eemhaven could easily be reached from the docks, the pilings in the other three research areas could not. There the pilings were surrounded by constructions that made it difficult to reach the surface under the low water line. In addition the tidal differences in the areas positioned closer to the North Sea were more extreme than in the 1e Eemhaven, which left a relatively short time window (at the lowest tide) to take the samples from the sub-littoral zone of the pilings from above water. For each scrape sample that could be taken, the surface scraped was estimated and

noted. All scrape samples were first placed in a plastic tray and photographed and identified in the field where possible. The remaining organisms were collected and preserved on either ethanol 96% (animals) or formaldehyde 4% (algae), and identified in the laboratory. In total six species were found in the scrape samples that were taken, of which three were non-native (Table 24). In the HELCOM/OSPAR protocol the scraping tool on a pole is described as a potential method to sample the fouling on pilings. The preferred method that is described in the HELCOM/OSPAR protocol is the scraping by scuba-divers. In the port of Rotterdam survey scraping by scuba-divers was also the most efficient method to sample this habitat.



Fig. 52. Sample locations in the 1e Eemhaven from where scrape samples of the pillars were taken.

Table 24. Species found in the scrape samples, collected from pillars during the sampling period in the late summer of 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	1e Eemhaven
<i>Polysiphonia stricta</i>	(Dillwyn) Greville	Algae	Native	1
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1
<i>Cordylophora caspia</i>	(Pallas, 1771)	Cnidaria	Non-native	1
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native	1
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1
<i>Echinogammarus storerensis</i>	(Reid, 1938)	Crustacea	Native	1

5.7 Dike fouling, littoral zone

In the summer of 2014 in total 24 scrape samples were taken from several littoral zones on the dike in three of the four research areas in the port of Rotterdam (Figs 53-54). In the Brittaniëhaven

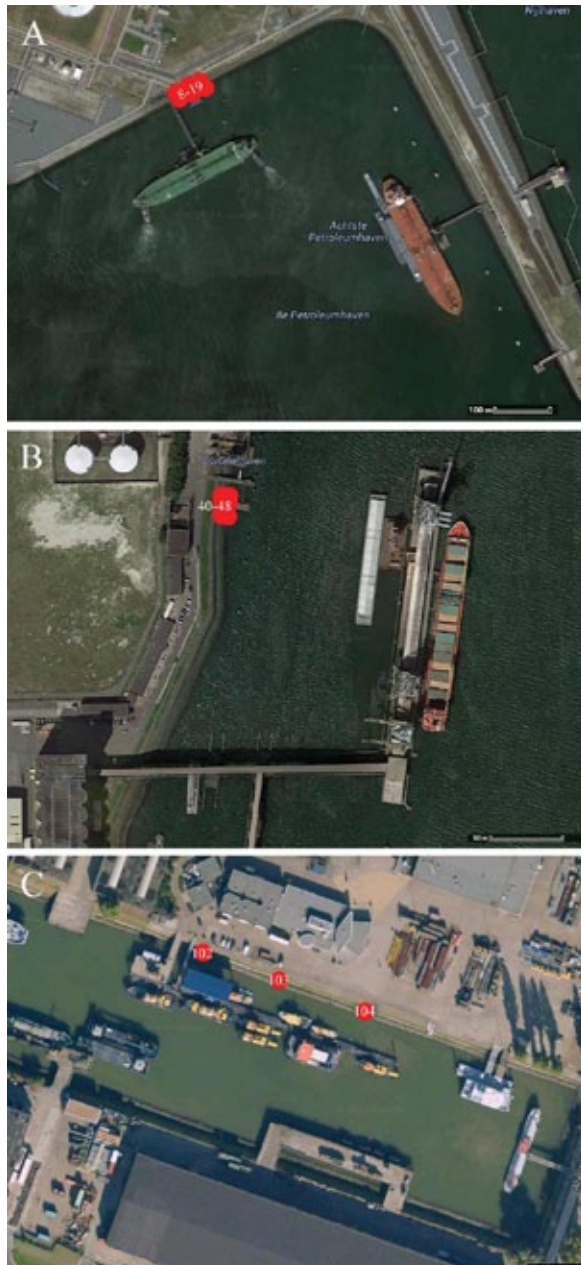


Fig. 53. Sample locations in the three research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven and [C] 1e Eemhaven where scrape samples were taken from the littoral zone of the dike.

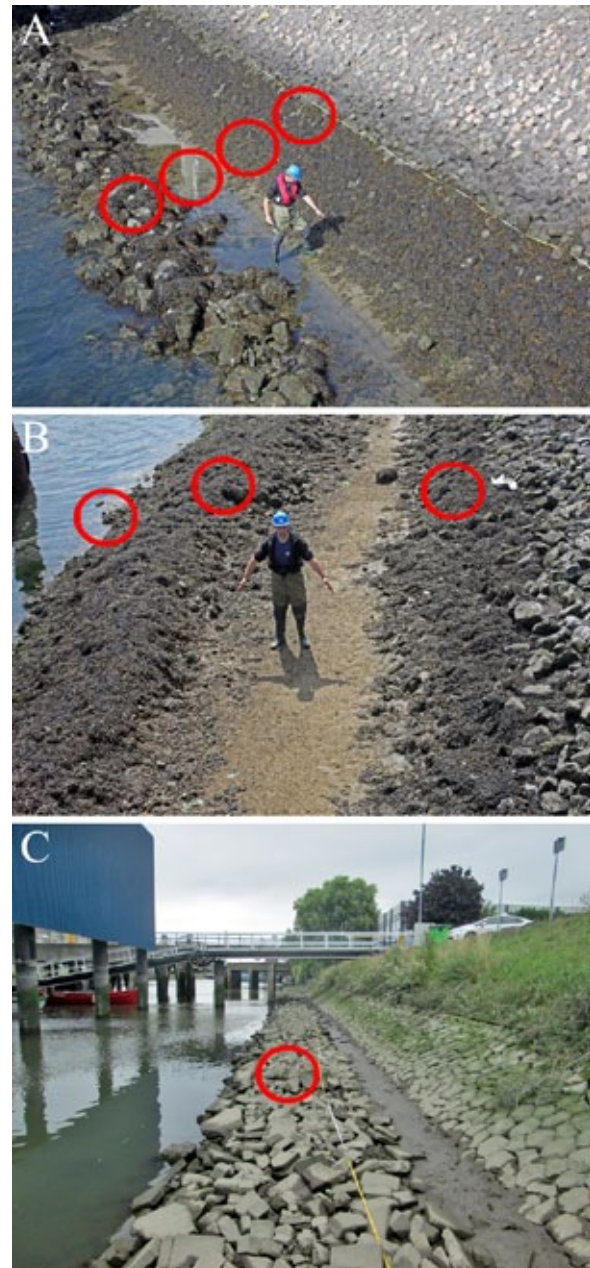


Fig. 54 Different littoral zones on the dike were distinguished in three research areas (Fig. 53). [A] 8e Petroleumhaven, from right to left, a *Fucus spiralis* zone, a *Fucus vesiculosus* zone, a tidal pool zone and a stone breakwater zone; [B] Beneluxhaven, from right to left, a *Fucus vesiculosus* zone, a stone breakwater zone and a zone along the low water line; [C] 1e Eemhaven, one littoral zone with virtually bare rocks.

Table 25. Species found on the transects in the littoral zone of the dike and around the low waterline during de sampling period in the late summer of 2014. This habitat was not present in the Britanniëhaven. Non-native species are highlighted.

Species	Authority	Group	Origin	8c Petroleumhaven	Beneluxhaven	1e Eemhaven
<i>Blidingia minima</i>	(Nägeli ex Kützing) Kylin	Algae	Native	1		1
<i>Callithamnion corymbosum</i>	(Smith) Lyngbye	Algae	Native	1	1	
<i>Ceramium cimbricum</i>	H.E.Petersen	Algae	Native	1	1	
<i>Ceramium tenuicorne</i>	(Kützing) Waern	Algae	Native	1	1	
<i>Chaetomorpha linum</i>	(O.F. Müller) Kützing	Algae	Native	1		
<i>Cladophora</i> sp	-	Algae		1		
<i>Dasysiphonia japonica</i>	(Yendo) H.-S. Kim	Algae	Non-native	1		
<i>Elachista fucicola</i>	(Velley) J.E.Areschoug	Algae	Native	1		
<i>Erythrotrichia carnea</i>	(Dillwyn) J.Agardh	Algae	Native		1	
<i>Fucus spiralis</i>	Linnaeus	Algae	Native		1	
<i>Fucus vesiculosus</i>	Linnaeus	Algae	Native	1	1	
<i>Polysiphonia fucoides</i>	(Hudson) Greville	Algae	Native	1	1	
<i>Polysiphonia stricta</i>	(Dillwyn) Greville	Algae	Native	1	1	
<i>Porphyra purpurea</i>	(Roth) C.Agardh	Algae	Native	1		
<i>Pylaiella littoralis</i>	(Linnaeus) Kjellman	Algae	Native	1	1	
<i>Rhizoclonium riparium</i>	(Roth) Harvey	Algae	Native	1		
<i>Ulva compressa</i>	Linnaeus	Algae	Native	1		
<i>Ulva curvata</i>	(Kützing) De Toni	Algae	Native		1	
<i>Ulva flexuosa</i>	Wulfen	Algae	Native		1	
<i>Ulva intestinalis</i>	Linnaeus	Algae	Native	1		
<i>Ulva linza</i>	Linnaeus	Algae	Native	1		
<i>Ulva pertusa</i>	Kjellman	Algae	Non-native		1	
<i>Ulva prolifera</i>	Müller	Algae	Native	1	1	
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1		
<i>Alcyonidioides mytili</i>	(Dalyell, 1848)	Bryozoa	Native		1	
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native		1	
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Bryozoa	Native		1	
<i>Obelia dichotoma</i>	(Linnaeus, 1758)	Cnidaria	Native	1	1	
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native	1	1	
<i>Austrominius modestus</i>	(Darwin, 1854)	Crustacea	Non-native	1	1	
<i>Jaera albifrons albifrons</i>	Leach, 1814	Crustacea	Native	1		
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	
<i>Echinogammarus marinus</i>	(Leach, 1815)	Crustacea	Native	1		
<i>Gammarus locusta</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	
<i>Hemigrapsus sanguineus</i>	(De Haan, 1835)	Crustacea	Non-native	1		
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native		1	
<i>Jassa marmorata</i>	Holmes, 1905	Crustacea	Non-native		1	
<i>Rhithropanopeus harrisi</i>	(Gould, 1841)	Crustacea	Non-native			1
<i>Semibalanus balanoides</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1	1	
<i>Littorina littorea</i>	(Linnaeus, 1758)	Mollusca	Native	1	1	
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1	1	

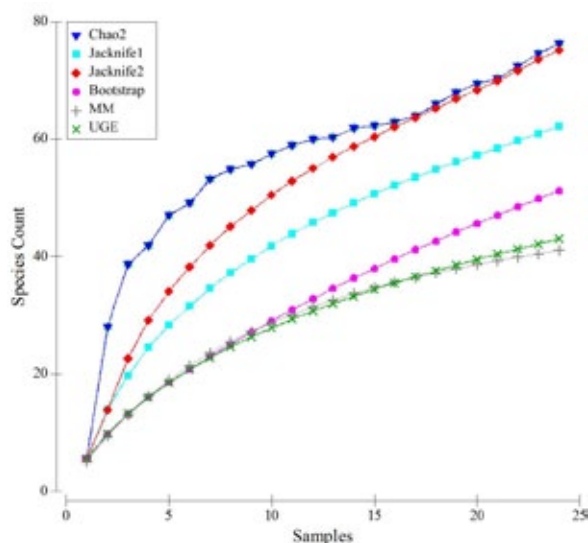


Fig.55. Six species accumulation curves were calculated based on the species found in the scrape samples taken from the littoral zone of the dike. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

these samples were not taken as a dike habitat was not present. In the scrape samples a total of 43 species were recorded (Table 25). Of these species 17 were exclusively found with this method and were not scored with any other method in the port of Rotterdam, i.e. the algal species *Ceramium cimbrium*, *Chaetomorpha linum*, *Elachista fucicola*, *Erythrotrichia carnea*, *Fucus spiralis*, *Polysiphonia fucoidea*, *Porphyra purpurea*, *Pyraliella littoralis*, *Rhizoclonium riparium*, *Ulva compressa*, *Ulva flexuosa* and *Ulva intestinalis*

and the crustaceans *Jaera albifrons*, *Caprella linearis*, *Echinogammarus marinus*, *Jassa marmorata* and *Semibalanus balanoides*. Of the species recorded 10 were found to be non-native, one of which, *Jassa marmorata*, was exclusively found during the port survey with this monitoring method. Most of the species, i.e. 37, were found in the 8e Petroleumhaven. Although this habitat and therefore the method was not specifically described in the OSPAR-HELCOM protocol, it proved to be a valuable asset during the inventory as 17 species were found exclusively with this method. The fact that the species accumulation curves in Fig. 55 have not become asymptotic yet, is an indication that a number of extra species could have been found with this method if more sampling was done. Additional species in this littoral dike habitat could also be found by a qualitative species assessment based on visual observations in the littoral zone (Table 26) without being restricted to the quadrates.

Table 26. Species found outside the quadrates in the littoral zone of the dike and around the low waterline during the sampling period in the late summer of 2014. This habitat was not present in the Britanniëhaven. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	1e Eemhaven
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Bryozoa	Native		1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native		1	
<i>Hemigrapsus sanguineus</i>	(De Haan, 1835)	Crustacea	Non-native	1		

5.8 Sampling with scuba-divers

5.8.1 Scrape sampling: Stone slope

In the summer of 2014 samples were taken by divers from the sub-littoral zone of the dike in the port of Rotterdam in three of the four research ar-

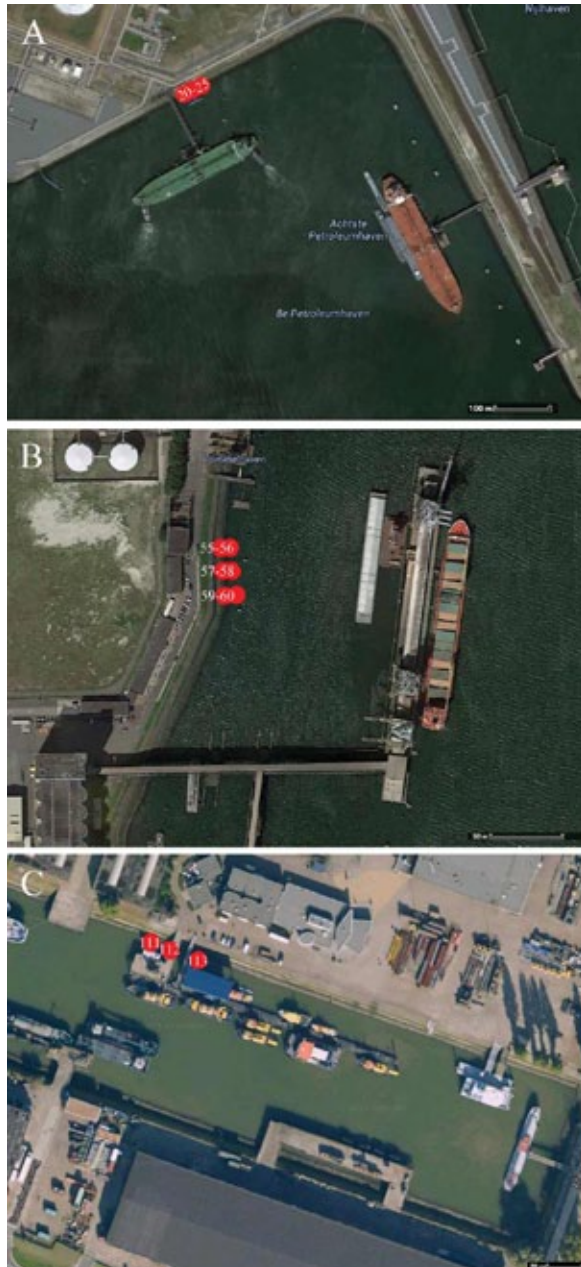


Fig. 56. Sample locations in the three research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven and [C] 1e Eemhaven where scrape samples were taken from the stone slopes in the sub-littoral zone.

reas (Fig. 56). This habitat was not sampled in the Britanniëhaven as there was not dike present (Fig. 9). Although the HELCOM/OSPAR protocol indicates that three replicate 0.10 m² quadrates should be digitally photographed and scrape sampled at depths of 0.5 m, 3.0 m, 7.0 m and close to the bottom, this could not be done. Photographs could not be taken because of the relatively murky waters in the port of Rotterdam, especially close to the bottom. The bottom could not be scraped off by the divers because of the oysters and rocks present. Instead oysters and rocks within about 0.10 m² were collected in separate bags. These samples were placed in coolers and transported to the laboratory where the species were identified. In the samples a total of 32 species were recorded (Table 27). None of these was found exclusively with this method. Most of the species, i.e. 29, were found in the 8e Petroleumhaven. Of all species scored 9 were non-native. The fact that the species accumulation curves in Fig. 57 have not become asymptotic yet, is an indication that several more species could have been found if more sampling was done.

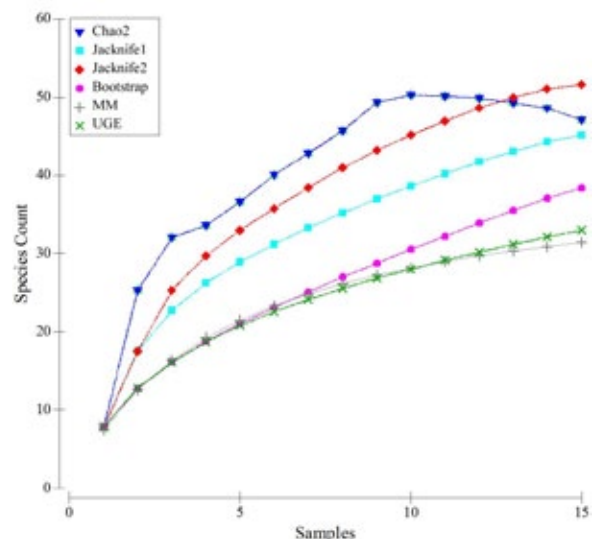


Fig.57. Six species accumulation curves were calculated based on the species found in the scrape samples taken from the sub-littoral zone of the stone slopes. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

Table 27. Species found in scrape samples taken by divers in the sub-littoral zone of the dike during the sampling period in the late summer of 2014. This habitat was not present in the Britanniëhaven. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	1e Eemhaven
<i>Ceramium tenuicorne</i>	(Kützinger) Waern	Algae	Uitheems	1		
<i>Dasyatis japonica</i>	(Yendo) H.-S. Kim	Algae	Non-native	1	1	
<i>Polysiphonia stricta</i>	(Dillwyn) Greville	Algae	Native			1
<i>Spirogyra</i> sp.	-	Algae	-			1
<i>Ulva pertusa</i>	Kjellman	Algae	Non-native	1	1	
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1	1	1
<i>Lepidonotus squamatus</i>	(Linnaeus, 1758)	Annelida	Native	1		
<i>Neoamphitrite figulus</i>	(Dalyell, 1853)	Annelida	Native		1	
<i>Nereis pelagica</i>	Linnaeus, 1758	Annelida	Native		1	
<i>Botryllus schlosseri</i>	(Pallas, 1766)	Ascidacea	Native	1		
<i>Styela clava</i>	Herdman, 1881	Ascidacea	Non-native	1	1	
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native	1	1	
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Bryozoa	Native	1	1	
<i>Hartlaubella gelatinosa</i>	(Pallas, 1766)	Cnidaria	Native	1		
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native	1		
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native	1		
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native			1
<i>Austrominius modestus</i>	(Darwin, 1854)	Crustacea	Non-native	1	1	
<i>Balanus crenatus</i>	Bruguière, 1789	Crustacea	Native	1	1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native		1	
<i>Corophium volutator</i>	(Pallas, 1766)	Crustacea	Native	1		
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native	1	1	
<i>Pisidia longicornis</i>	(Linnaeus, 1767)	Crustacea	Native	1		
<i>Rhithropanopeus harrisi</i>	(Gould, 1841)	Crustacea	Non-native			1
<i>Asterias rubens</i>	Linnaeus, 1758	Echinodermata	Native	1		
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1	1	
<i>Littorina littorea</i>	(Linnaeus, 1758)	Mollusca	Native		1	
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1	1	
<i>Emplectonema neessi</i>	(Ørsted, 1843)	Nemertea	Native		1	
<i>Pholis gunnellus</i>	(Linnaeus, 1758)	Pisces	Native		1	
<i>Leptoplana tremellaris</i>	(Müller, 1774)	Platyhelminthes	Native	1		
<i>Halichondria bowerbanki</i>	Burton, 1930	Porifera	Native	1	1	

5.8.2 Scrape sampling: Vertical structures

In the summer of 2014 in total 12 scrape samples were taken by divers from vertical structures in the sub-littoral zone in the port of Rotterdam in each of the four research areas (Fig. 58). In these scrape samples a total of 45 species were recorded (Table 28). Two of these species were exclusively found in these scrape samples and not scored with any other method in the port of Rotterdam, i.e. the mollusk *Venerupis corrugata* and the sponge *Haliclona oculata*. Most of the species, i.e. 39, were found in the 8e Petroleumhaven. Of all species recorded 12 were found to be non-native. All scrape samples could be taken following the HELCOM/OSPAR protocol. Because of the murky waters in the port of Rotterdam, the photographs of the quadrates that had to be taken according to the

protocol, could only be taken for a small selection of quadrates at the 8e Petroleumhaven. Most of the species accumulation curves in Fig. 59 start to become asymptotic (with the exception of Chao's model correcting for rare species), which is an indication that most species that could have been found with this method, were found and that adding more samples would not yield significantly more species.

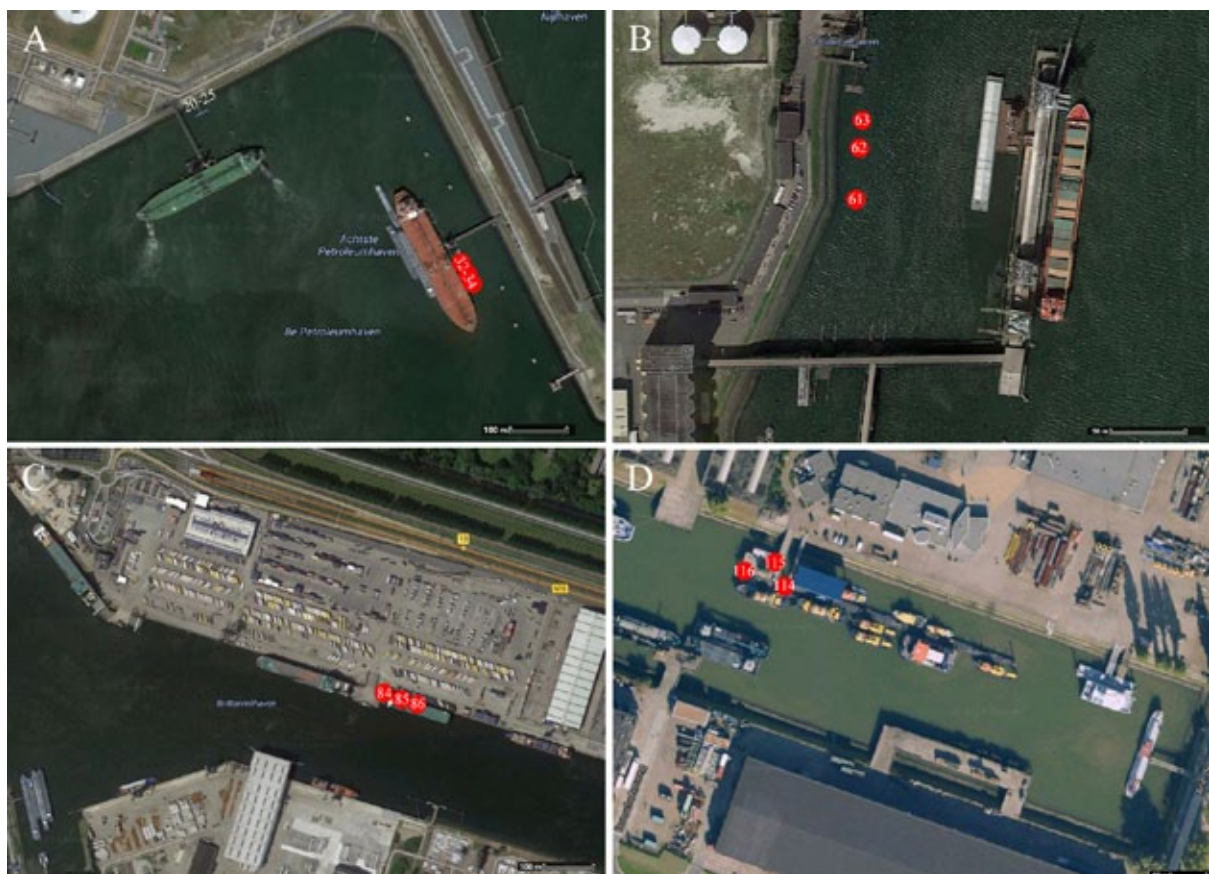


Fig. 58. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Britanniëhaven and [D] 1e Eemhaven where scrape samples from vertical structures in the sub-littoral zone were taken from by divers.

Table 28. Species found in scrape samples taken by divers in the sub-littoral zone of vertical structures, such as pillars, during the sampling period in the late summer of 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Bendixhaven	Brittaniëhaven	1e Eemhaven
<i>Ceramium tenuicorne</i>	(Kützting) Waern	Algae	Native	1			
<i>Dasyatisphonia japonica</i>	(Yendo) H.-S. Kim	Algae	Non-native	1	1		
<i>Fucus vesiculosus</i>	Linnaeus	Algae	Native		1		
<i>Polysiphonia stricta</i>	(Dillwyn) Greville	Algae	Native				1
<i>Spirogyra</i> sp.	-	Algae	-				1
<i>Ulva pertusa</i>	Kjellman	Algae	Non-native		1		
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1	1	1	1
<i>Lepidonotus squamatus</i>	(Linnaeus, 1758)	Annelida	Native		1	1	
<i>Neoamphitrite figulus</i>	(Dalyell, 1853)	Annelida	Native		1		
<i>Nereis pelagica</i>	Linnaeus, 1758	Annelida	Native	1	1	1	
<i>Ascidia aspersa</i>	(Müller, 1776)	Ascidacea	Native	1		1	
<i>Botryllus schlosseri</i>	(Pallas, 1766)	Ascidacea	Native		1		
<i>Ciona intestinalis</i>	(Linnaeus, 1767)	Ascidacea	Native	1		1	
<i>Molgula manhattensis</i>	(De Kay, 1843)	Ascidacea	Non-native		1		
<i>Styela clava</i>	Herdman, 1881	Ascidacea	Non-native	1	1	1	
<i>Alcyonidioides mytili</i>	(Dalyell, 1848)	Bryozoa	Native	1	1	1	
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native	1	1	1	
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Bryozoa	Native	1	1		
<i>Hartlaubella gelatinosa</i>	(Pallas, 1766)	Cnidaria	Native	1			
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native	1	1	1	
<i>Obelia dichotoma</i>	(Linnaeus, 1758)	Cnidaria	Native	1	1		1
<i>Obelia geniculata</i>	(Linnaeus, 1758)	Cnidaria	Native	1	1		
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native	1	1		1
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native				1
<i>Austrominius modestus</i>	(Darwin, 1854)	Crustacea	Non-native	1			
<i>Balanus crenatus</i>	Bruguière, 1789	Crustacea	Native	1	1	1	
<i>Cancer pagurus</i>	Linnaeus, 1758	Crustacea	Native	1			
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	1	
<i>Orchestia gammarellus</i>	(Pallas, 1766)	Crustacea	Native			1	
<i>Hemigrapsus sanguineus</i>	(De Haan, 1835)	Crustacea	Non-native	1	1	1	
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native	1	1	1	
<i>Necora puber</i>	(Linnaeus, 1767)	Crustacea	Native	1			
<i>Pilumnus hirtellus</i>	(Linnaeus, 1761)	Crustacea	Native	1			
<i>Pisidia longicornis</i>	(Linnaeus, 1767)	Crustacea	Native	1			
<i>Rhithropanopeus harrisi</i>	(Gould, 1841)	Crustacea	Non-native				1

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Asterias rubens</i>	Linnaeus, 1758	Echinodermata	Native	1		1	
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1	1	1	
<i>Crepidula fornicata</i>	(Linnaeus, 1758)	Mollusca	Non-native		1	1	
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1	1	1	
<i>Venerupis corrugata</i>	(Gmelin, 1791)	Mollusca	Native			1	
<i>Emplectonema neessi</i>	(Ørsted, 1843)	Nemertea	Native	1	1	1	
<i>Pholis gunnellus</i>	(Linnaeus, 1758)	Pisces	Native	1			
<i>Halichondria bowerbanki</i>	Burton, 1930	Porifera	Native	1	1	1	
<i>Halichondria panicea</i>	(Pallas, 1766)	Porifera	Native	1	1		
<i>Haliclona oculata</i>	(Pallas, 1766)	Porifera	Native	1			

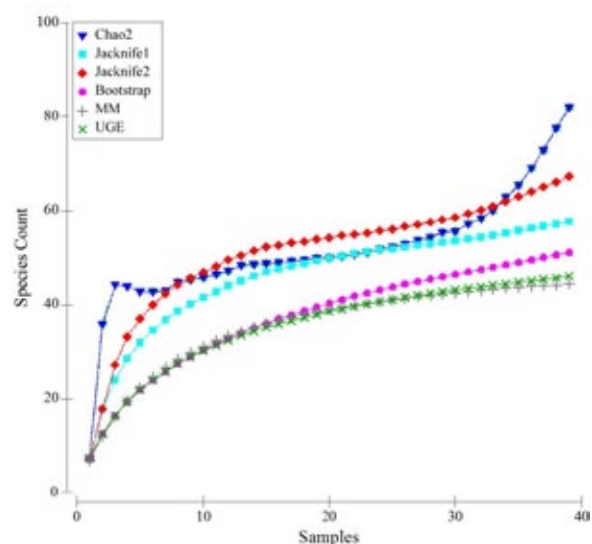


Fig.59. Six species accumulation curves were calculated based on the species found in the scrape samples taken from vertical structures in the sub-littoral zone. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

5.8.3 Transect observations

In the summer of 2014 in total 8 transects of 50 meters, parallel to the dike or the harbour wall were searched visually by divers in the port of Rotterdam (Fig. 60) following the HELCOM/OSPAR protocol. During these transects a total of 12 species was identified to the species level (Table 29). More species were seen, but could not be identified because the specimens could not be collected, photographed and/or videoed by the divers because of a layer of silt on the bottom that significantly decreased the visibility underwater. Of the species that were identified one was exclusively recorded by a visual observation during these transects and not scored with any other method in the port of Rotterdam, i.e. the non-native monkey goby *Neogobius fluviatilis*. The visual identification of this goby species, which closely resembles

several other goby species, could not be validated on the basis of a photograph, video, specimen or a record done with one of the other monitoring methods. This species is therefore referred to as *Neogobius* cf (con forma) *fluviatilis* in table 39. Of the species recorded 7 species were found to be non-native. The fact that the species accumulation curves in Fig. 60 have not become asymptotic yet, is an indication that several additional species could have been found if more sampling was done.

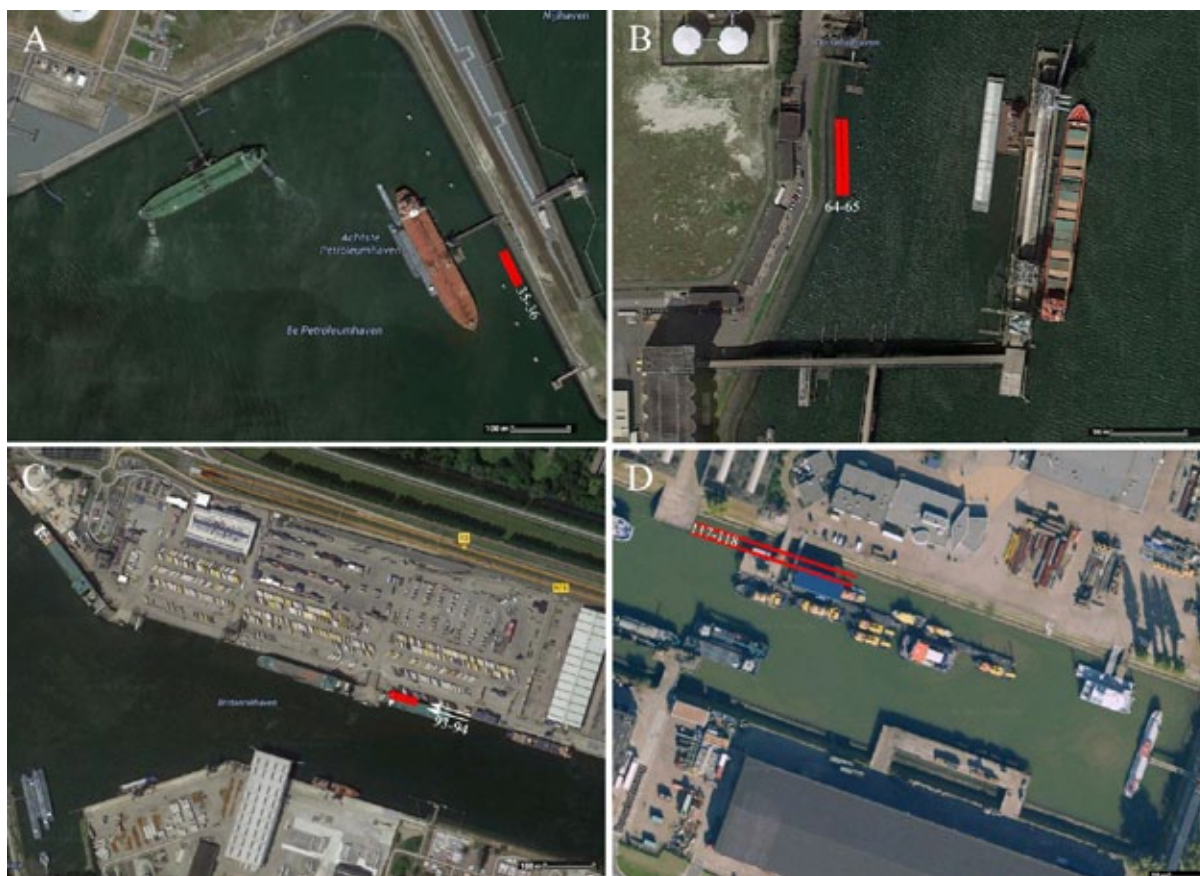


Fig. 60. Transects in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Britanniëhaven and [D] 1e Eemhaven where the mobile epifauna was scored by divers.

Table 29. Species that were seen by divers along 50 m transects parallel to the dike or harbour wall, during the sampling period in the late summer of 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Ciona intestinalis</i>	(Linnaeus, 1767)	Ascidacea	Native	1	1		
<i>Styela clava</i>	Herdman, 1881	Ascidacea	Non-native	1	1		
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	1	1
<i>Hemigrapsus sanguineus</i>	(De Haan, 1835)	Crustacea	Non-native	1	1	1	
<i>Mnemiopsis leidyi</i>	A. Agassiz, 1865	Ctenophora	Non-native	1	1	1	
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1	1	1	
<i>Dreissena bugensis</i>	Andrusov, 1897	Mollusca	Non-native				1
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native			1	
<i>Neogobius cf. fluviatilis</i>	(Pallas, 1814)	Pisces	Non-native				1
<i>Neogobius melanostomus</i>	(Pallas, 1814)	Pisces	Non-native				1
<i>Perca fluviatilis</i>	(Linnaeus, 1758)	Pisces	Native				1
<i>Pholis gunnellus</i>	(Linnaeus, 1758)	Pisces	Native			1	

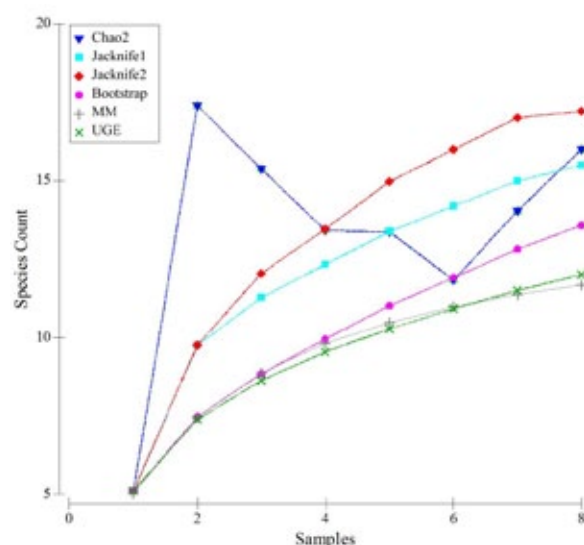


Fig.61. Six species accumulation curves were calculated based on the species observed during the 50 m transects parallel to the dike or harbour wall. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

5.8.4 Visual observations during monitoring

During the monitoring done by divers, several species were seen of which 11 could be identified to the species level (Table 30). More species were seen, but could not be identified because the specimens could not be collected, photographed and/or videoed by the divers mostly because of the murky waters. Of the species recorded 5 species were found to be non-native.

Table 30. Species that were seen and identified to a species level by divers during their sampling in the late summer of 2014. Several species groups were recorded that could not be identified to the species level. These groups are specified in the Appendix report (GiMaRIS report 2014_32). Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Ciona intestinalis</i>	(Linnaeus, 1767)	Ascidacea	Native			1	
<i>Styela clava</i>	Herdman, 1881	Ascidacea	Non native			1	
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native	1	1	1	
<i>Sagartia elegans</i>	(Dalyell, 1848)	Cnidaria	Native		1	1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	1	1
<i>Hemigrapsus sanguineus</i>	(De Haan, 1835)	Crustacea	Non native	1	1	1	
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non native	1	1		
<i>Mnemiopsis leidyi</i>	A. Agassiz, 1865	Ctenophora	Non native			1	
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non native	1	1	1	
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1		1	
<i>Pholis gunnellus</i>	(Linnaeus, 1758)	Pisces	Native			1	

5.8.5 Bottom sampling with hand corer

In the summer of 2014 in total 21 bottom samples were taken by divers with a hand corer in the port of Rotterdam in each of the four research areas, three at the start of a 50 m transect perpendicular to the dike or the harbour wall, and three at the end (Fig. 62). In these samples a total of 17 species were recorded (Table 31). Two of these species were exclusively found with this monitoring method and not scored with any other method in the port of Rotterdam, i.e. the serpent star *Ophiura ophiura* and the netted dog whelk *Nassarius reticulatus*. In total 5 non-native species were scored.

The fact that the species accumulation curves in Fig. 63 have not become asymptotic yet, is an indication that several more species could have been found if more sampling was done. The bot-

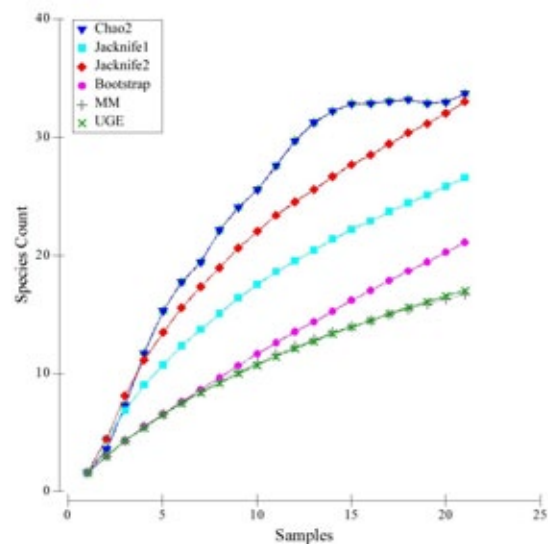


Fig. 63. Six species accumulation curves were calculated based on the species found in the bottom samples taken with a hand corer. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

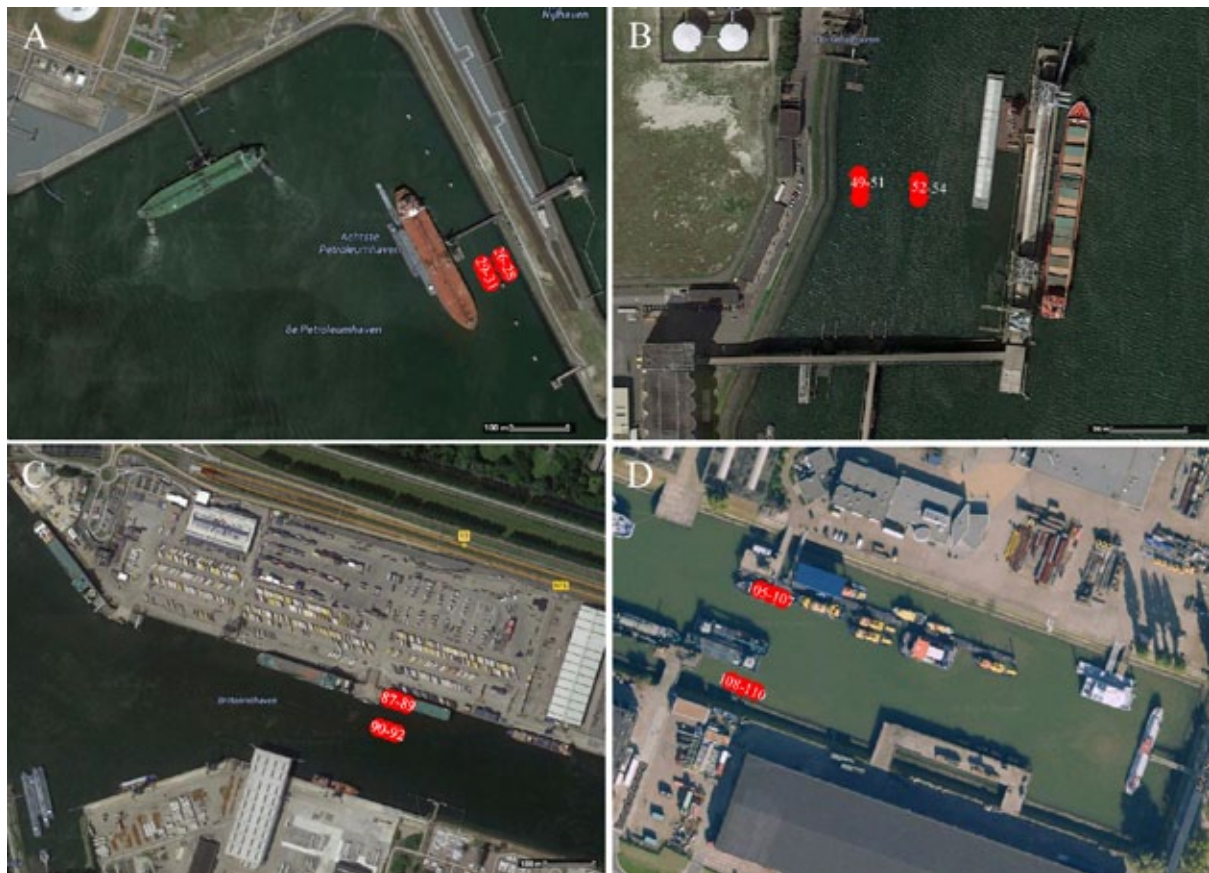


Fig. 62. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Brittaniëhaven and [D] 1e Eemhaven where the mobile epifauna was scored by divers.

tom samples taken with the petit ponar (see next paragraph) are similar to the ones taken with the hand corer. When combining these two datasets the species accumulation curves would probably start to become asymptotic. The sampling and analysis of this method could be done according to the OSPAR/HELCOM protocol in all research areas with the exception of the Brittaniëhaven, where the bottom corer samples could not be taken at the start of the transect because of a thick layer of oyster shells. Instead of taking bottom corer samples the scuba-divers collected oysters in separate bags, which were analyzed in the lab. The species in these samples were also found in the bottom corer samples taken at the 50 m point of the same transect and therefore didn't result in any additional records of species for the Brittaniëhaven.

Table 31. Species found in bottom samples taken by divers, during de sampling period in the late summer of 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Nereis pelagica</i>	Linnaeus, 1758	Annelida	Native		1	1	
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native			1	
<i>Hartlaubella gelatinosa</i>	(Pallas, 1766)	Cnidaria	Native	1			
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native			1	
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native	1		1	
<i>Austrominius modestus</i>	(Darwin, 1854)	Crustacea	Non-native			1	
<i>Balanus crenatus</i>	Bruguière, 1789	Crustacea	Native		1	1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native			1	1
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native	1			
<i>Rhithropanopeus harrisii</i>	(Gould, 1841)	Crustacea	Non-native				1
<i>Ophiura ophiura</i>	(Linnaeus, 1758)	Echinodermata	Native	1			
<i>Abra alba</i>	(W. Wood, 1802)	Mollusca	Native	1			
<i>Corbicula fluminalis</i>	(O. F. Müller, 1774)	Mollusca	Non-native				1
<i>Corbula gibba</i>	(Olivi, 1792)	Mollusca	Native			1	
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native			1	
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1		1	
<i>Nassarius reticulatus</i>	(Linnaeus, 1758)	Mollusca	Native		1		

5.9 Petit ponar bottom sampling

In the summer of 2014 in total 15 bottom samples were taken with a petit ponar in the port of Rotterdam in the four research areas (Fig. 64). In the Brittaniëhaven three attempts to take samples along the harbour wall failed because of a thick layer of oysters on the bottom (see previous paragraph). Therefore 3 samples were taken from a boat in the soft substratum in the center of the harbour (Fig. 64). In all samples together a total of 29 species were recorded (Table 32). One of these species was exclusively found in these samples and was not scored with any other method in the port of Rotterdam, i.e. the crustacean *Melita hergensis*. In total 10 non-native species were recorded with this monitoring method. The method could exactly be done according to the OSPAR/HELCOM protocol.

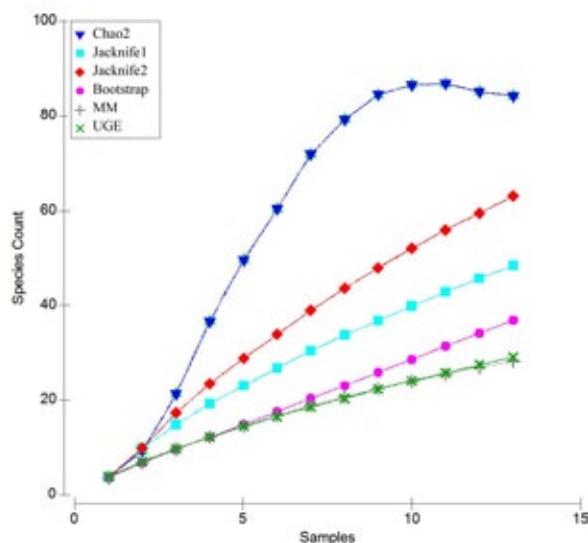


Fig.65. Six species accumulation curves were calculated based on the species found in the bottom samples taken with a petit ponar grab. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

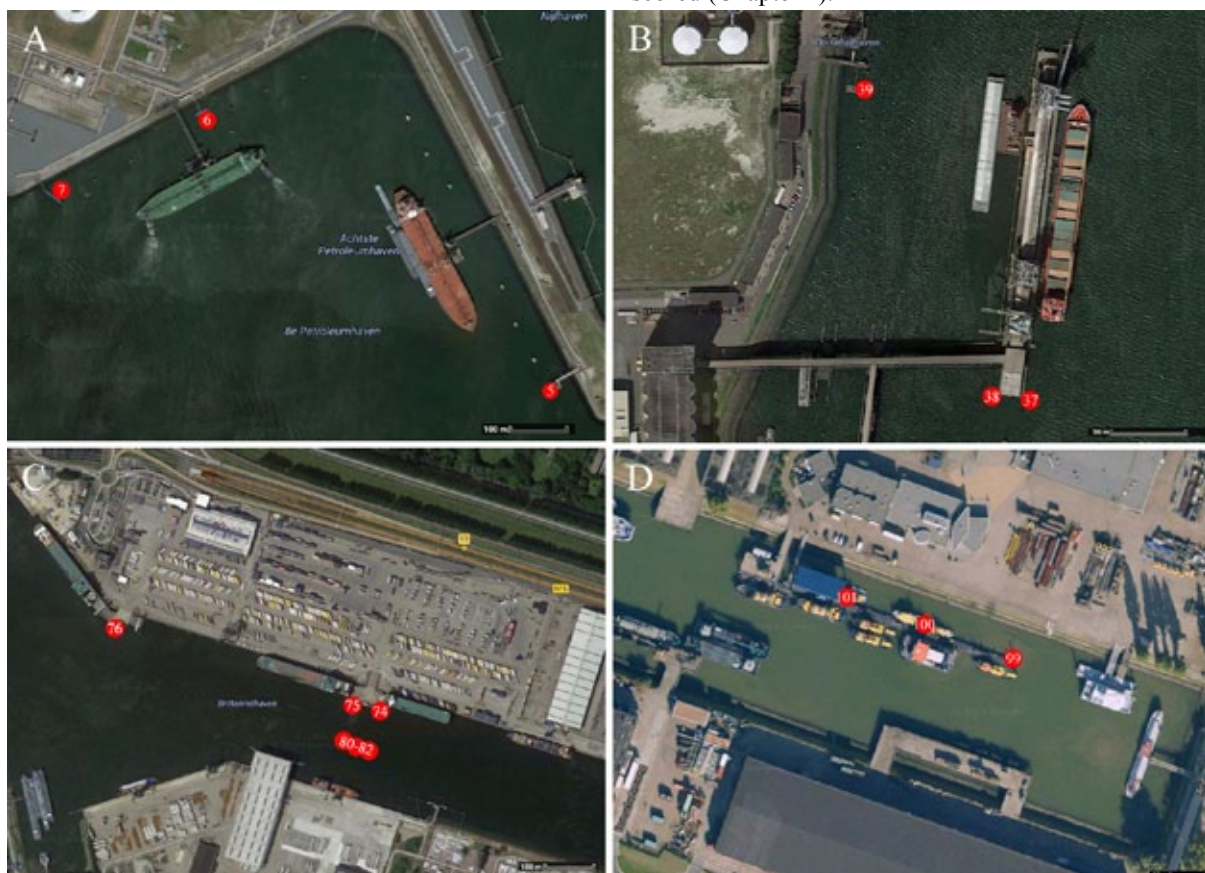


Fig. 64. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Brittaniëhaven and [D] Ie Eemhaven where aken with a petit ponar.

Table 32. Species found in bottom samples taken with a Petit Ponar grab, during de sampling period in the late summer of 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	Brittaniëhaven	1e Eemhaven
<i>Blidingia marginata</i>	(J. Agardh) P.J.L. Dangeard	Algae	Native				1
<i>Callithamnion corymbosum</i>	(Smith) Lyngbye	Algae	Native	1			
<i>Ceramium tenuicorne</i>	(Kützinger) Waern	Algae	Native	1	1		
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1			
<i>Lepidonotus squamatus</i>	(Linnaeus, 1758)	Annelida	Native	1			
<i>Nereis pelagica</i>	Linnaeus, 1758	Annelida	Native	1			
<i>Botryllus schlosseri</i>	(Pallas, 1766)	Ascidacea	Native	1			
<i>Molgula manhattensis</i>	(De Kay, 1843)	Ascidacea	Non-native	1			
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native	1	1		
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Bryozoa	Native	1			
<i>Cordylophora caspia</i>	(Pallas, 1771)	Cnidaria	Non-native				1
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native	1			
<i>Obelia dichotoma</i>	(Linnaeus, 1758)	Cnidaria	Native	1			
<i>Obelia geniculata</i>	(Linnaeus, 1758)	Cnidaria	Native	1			
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native		1		
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native	1	1		
<i>Austrominius modestus</i>	(Darwin, 1854)	Crustacea	Non-native		1		
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1			
<i>Corophium volutator</i>	(Pallas, 1766)	Crustacea	Native	1			
<i>Melita hergensis</i>	Reid, 1939	Crustacea	Native	1			
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native	1			
<i>Abra alba</i>	(W. Wood, 1802)	Mollusca	Native	1			
<i>Corbicula fluminalis</i>	(O. F. Müller, 1774)	Mollusca	Non-native				1
<i>Corbula gibba</i>	(Olivi, 1792)	Mollusca	Native			1	
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1			
<i>Dreissena bugensis</i>	Andrusov, 1897	Mollusca	Non-native				1
<i>Dreissena polymorpha</i>	(Pallas, 1771)	Mollusca	Non-native				1
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1	1		

Table 33. Bottom samples of the four research areas divided in different fractions. The fractions are highlighted from large to small until they represent (together) > 50 % of the sample.

	8e Petroleumhaven			Beneluxhaven			Brittanniëhaven			1e Eemhaven		
> 1.80 mm	9,4%	40,9%	32,3%	4,4%	1,5%	7,9%	2,2%	1,2%	1,6%	0,4%	0,3%	2,5%
1.80 - 0.50 mm	44,5%	20,5%	30,4%	34,2%	30,8%	61,2%	21,4%	15,2%	19,5%	14,3%	12,6%	15,9%
0.50 - 0.25 mm	28,2%	16,1%	19,9%	35,6%	38,8%	17,7%	54,4%	42,6%	36,2%	25,4%	56,9%	44,8%
0.25 - 0.06 mm	15,0%	19,8%	16,0%	21,9%	24,6%	8,5%	18,4%	35,5%	37,4%	44,5%	24,3%	30,3%
< 0.06 mm	2,8%	2,7%	1,4%	3,9%	4,2%	4,7%	3,6%	5,5%	5,4%	15,4%	5,8%	6,5%

The fact that the species accumulation curves in Fig. 64 have not become asymptotic yet, is an indication that several more species could have been found if more sampling was done. The bottom samples taken with the petit ponar are similar to the ones taken with the hand corer (see previous paragraph). When combining these two datasets the species accumulation curves would probably start to become asymptotic.

As described in the HELCOM/OSPAR protocol a sediments quality (grain size) analysis was done based on the samples taken with the bottom grab, i.e. the petit ponar (Table 33). From these analyses it could be concluded that the sediments are coarser, consisting of fine gravel to medium sand in the areas closer to the North Sea, i.e. the 8e Petroleumhaven, while the sediments in the inland part of the port, i.e. in the 1e Eemhaven, consist of a relatively high percentage of fine sand to silt (Fig. 66; Table 33).



Fig. 66. A: Bottomsample analyzed with the Geotech Sieve Analysis Field Kit; B-E: sieves; F: fine gravel (> 1.8 mm); G: coarse sand ($1.8-0.5$ mm); H: medium sand ($0.5-0.2$ mm); I: fine sand ($0.2-0.06$ mm); J: silt (<0.06 mm).

5.10 Hand dredge sampling

In the summer of 2014 in total 9 scrape samples were taken with a hand dredge in the port of Rotterdam in three of the four research areas (Fig. 67). This method was not easily applicable in the Britanniëhaven because of the busy shipping traffic during the monitoring. In these samples a to-

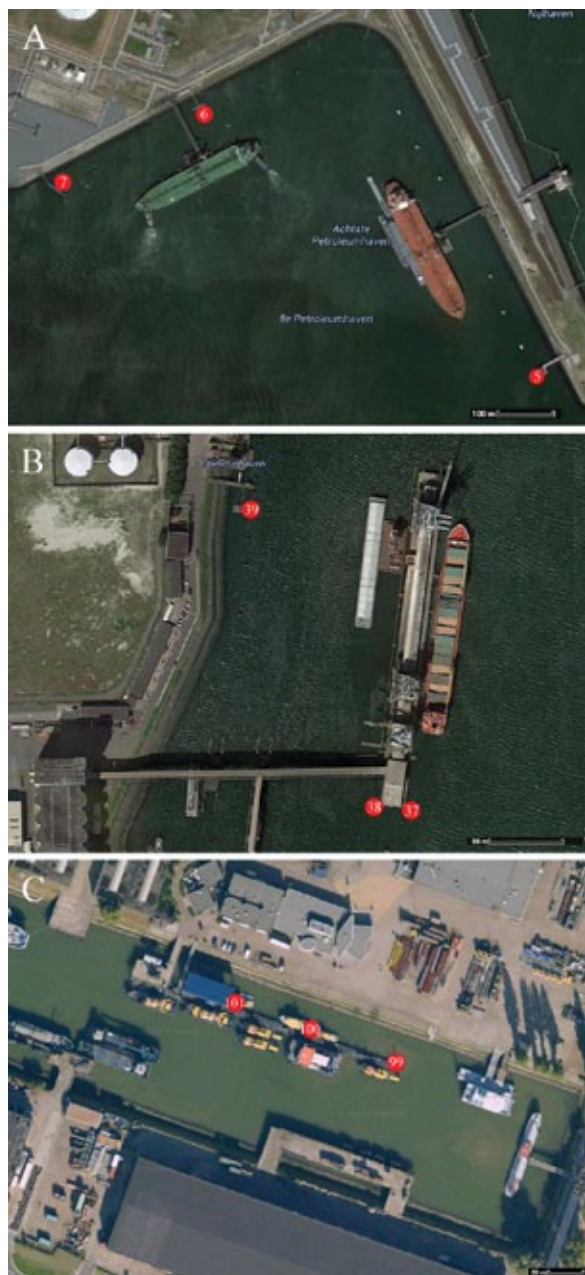


Fig. 67. Sample locations in the three research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven and [C] 1e Eemhaven where samples were taken with a hand dredge.

tal of 33 species were recorded (Table 34). Two of these species were exclusively found in these samples and not scored with any other method in the port of Rotterdam, i.e. the crustacean *Neomysis integer* and the non-native mollusk *Rangia cuneata*. Of all species 12 were non-native. Most of the species, i.e. 24, were found in the Beneluxhaven.

Although this method was not described in the HELCOM/OSPAR protocol, it proved to be a valuable asset during the inventory as three species were found exclusively with this method, including one non-native species.

The fact that the species accumulation curves in Fig. 68 have not become asymptotic yet, is an indication that several more species could have been found if more sampling was done.

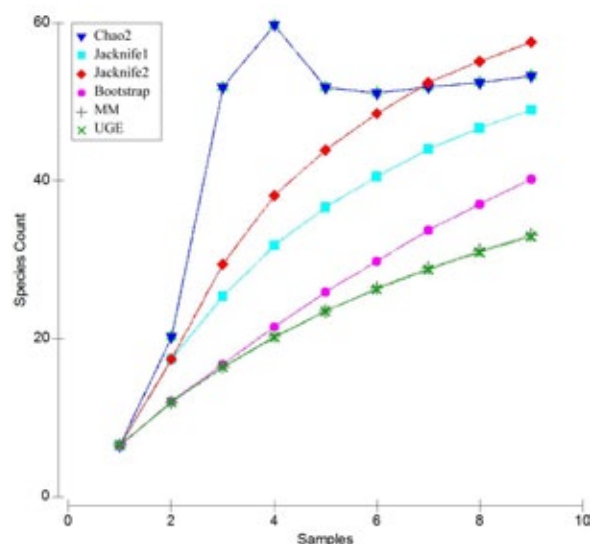


Fig.68. Six species accumulation curves were calculated based on the species found in the bottom samples that were taken with a hand dredge. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).

Table 34. Species found in the hand dredge samples, during de sampling period in the late summer of 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	8e Petroleumhaven	Beneluxhaven	1e Eemhaven
<i>Blidingia marginata</i>	(J.Agardh) P.J.L.Dangeard	Algae	Native			1
<i>Ceramium tenuicorne</i>	(Kützting) Waern	Algae	Native		1	
<i>Ulva pertusa</i>	Kjellman	Algae	Non-native		1	
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1	1	
<i>Neoamphitrite figulus</i>	(Dalyell, 1853)	Annelida	Native		1	
<i>Ascidella aspersa</i>	(Müller, 1776)	Ascidacea	Native	1	1	
<i>Botryllus schlosseri</i>	(Pallas, 1766)	Ascidacea	Native	1	1	
<i>Ciona intestinalis</i>	(Linnaeus, 1767)	Ascidacea	Native	1		
<i>Molgula manhattensis</i>	(De Kay, 1843)	Ascidacea	Non-native		1	
<i>Styela clava</i>	Herdman, 1881	Ascidacea	Non-native		1	
<i>Alcyonidioides mytili</i>	(Dalyell, 1848)	Bryozoa	Native	1	1	
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native	1	1	
<i>Cryptosula pallasiana</i>	(Moll, 1803)	Bryozoa	Native	1		
<i>Aurelia aurita</i>	(Linnaeus, 1758)	Cnidaria	Native		1	
<i>Metridium senile</i>	(Linnaeus, 1761)	Cnidaria	Native	1		
<i>Obelia geniculata</i>	(Linnaeus, 1758)	Cnidaria	Native		1	
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native		1	
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	Non-native	1	1	
<i>Balanus crenatus</i>	Bruguère, 1789	Crustacea	Native		1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1		
<i>Gammarus locusta</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	
<i>Hemigrapsus takanoi</i>	Asakura & Watanabe, 2005	Crustacea	Non-native	1	1	
<i>Neomysis integer</i>	(Leach, 1814)	Crustacea	Native			1
<i>Pisidia longicornis</i>	(Linnaeus, 1767)	Crustacea	Native	1		
<i>Rhithropanopeus harrisii</i>	(Gould, 1841)	Crustacea	Non-native			1
<i>Asterias rubens</i>	Linnaeus, 1758	Echinodermata	Native	1		
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1	1	
<i>Crepidula fornicata</i>	(Linnaeus, 1758)	Mollusca	Non-native		1	
<i>Dreissena bugensis</i>	Andrusov, 1897	Mollusca	Non-native			1
<i>Dreissena polymorpha</i>	(Pallas, 1771)	Mollusca	Non-native			1
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1	1	
<i>Rangia cuneata</i>	(G. B. Sowerby I, 1832)	Mollusca	Non-native			1
<i>Halichondria bowerbanki</i>	Burton, 1930	Porifera	Native		1	

5.11 Fire hydranth sampling

In the summer of 2014 in total 16 samples were taken from the underground water systems with a a Pack Bag. These samples were taken on the premises of four companies with underground water systems in open access to the harbour (Fig. 69). In these samples a total of 12 species were recorded (Table 35). Two of these species were exclusively found in these samples and not scored with any other method in the port of Rotterdam, i.e. the non-native mollusks *Physa acuta* and *Potamopyrgus antipodarum*. Of the species found 7 were non-native.

Although this method was not described in the HELCOM/OSPAR protocol, it proved to be a valuable asset during the inventory as two non-native species were found exclusively with this method.

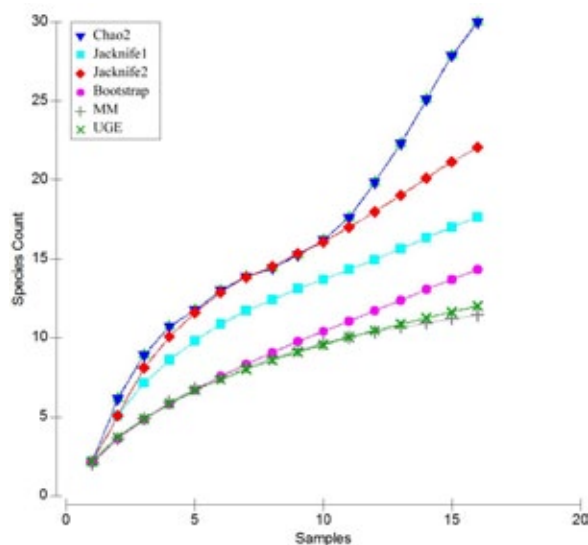


Fig.70. Six species accumulation curves were calculated based on the species found in the samples taken from the underground water systems. Three of these may provide an over-estimate (Chao 2 and Jackknife 1 & 2), and three may provide an under-estimate (Bootstrap, MM and UGE) of the number of species that could be scored (Chapter 4).



Fig. 69. Sample locations in the four research areas. [A] 8e Petroleumhaven; [B] Beneluxhaven; [C] Brittaniëhaven and [D] 1e Eemhaven where samples taken from the underground water systems.

The fact that the species accumulation curves in Fig. 70 have not become asymptotic yet, is an indication that several more species could have been found if more sampling was done.

Table 35. Species found in samples taken from the underground water systems, during de sampling period in the late summer of 2014. Non-native species are highlighted.

Species	Authority	Group	Origin	Research area 1: Maasvlakte Oil Terminal	Research area 2: 7e Petroleumhaven	Research area 3: 7e Petroleumhaven	Research area 4: Nova Terminal
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	Non-native	1	1	1	1
<i>Molgula manhattensis</i>	(De Kay, 1843)	Ascidacea	Non-native	1	1	1	
<i>Conopeum reticulum</i>	(Linnaeus, 1767)	Bryozoa	Native	1	1	1	
<i>Obelia longissima</i>	(Pallas, 1766)	Cnidaria	Native	1	1	1	1
<i>Balanus crenatus</i>	Bruguière, 1789	Crustacea	Native	1	1	1	
<i>Carcinus maenas</i>	(Linnaeus, 1758)	Crustacea	Native	1	1	1	1
<i>Rhithropanopeus harrisii</i>	(Gould, 1841)	Crustacea	Non-native				1
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	Non-native	1	1	1	
<i>Dreissena bugensis</i>	Andrusov, 1897	Mollusca	Non-native				1
<i>Mytilus edulis</i>	Linnaeus, 1758	Mollusca	Native	1	1	1	1
<i>Physa acuta</i>	Draparnaud, 1805	Mollusca	Non-native		1		1
<i>Potamopyrgus antipodarum</i>	(Gray, 1843)	Mollusca	Non-native		1		1

6. Discussion

6.1 Species diversity and the efficiency of monitoring methods in the port of Rotterdam

In total 229 species could be identified in the port of Rotterdam survey (Tables 9, 36). The diversity of species that was found within the port decreased with the distance to the North Sea, i.e. the numbers of species that were found from west to east were 152, 129, 104 and 56 in respectively the research areas the 8e Petroleumhaven, the Beneluxhaven, the Brittaniëhaven and the 1e Eemhaven (Table 36). This pattern was not found for the non-native species of which respectively 15, 19, 17 and 12 were recorded. Although for various monitoring methods the species accumulation curves presented in the previous chapter indicate that more sampling would have resulted in scoring more species, the list of non-native species recorded with all methods combined provides a close to complete overview of the non-native species that are present in the port of Rotterdam. The non-native species found in the three more saline research areas for example, were all recorded in at least two of the research areas, with the exception of one species in the Beneluxhaven: *Jassa marmorata* (Tables 37-38). Eight non-native species were uniquely found in the 1e Eemhaven. These species concern more typical freshwater species (Table 37) and were therefore not recorded in the other three research areas. This freshwater non-native species list is also concluded to be close to complete as all of the species were recorded in at least two different samples, with the exception of the goby *Neogobius cf. fluviatilis*, which was only observed once. In conclusion the list of non-native species that resulted from the survey of the port of Rotterdam includes close to all non-native species that could have been found in 2014 with the combination of survey methods used. Therefore this list provides an accurate overview of the non-native species that were present in 2014 in

the port of Rotterdam. It can be used as a baseline list (T_0 measurement) to compare with non-native species lists that may result from similar surveys in the future.

Most non-native species were found with at least two different monitoring methods, with the exception of the plankton and human pathogen species, which were only found with these methods. This can be explained by the fact that none of the other monitoring methods is suitable for scoring these species. Concerning the rest of the non-native species the monitoring methods “littoral zone of the dike scraping”, “hand dredge sampling”, “Fire hydranth sampling”, and “visual scuba-divers observations along transects” all scored non-native species that were uniquely recorded with (these) methods and were missed with all other survey methods. Of these four survey methods, only the “visual scuba-divers observations along transects” is described in more detail in the HELCOM/OSPAR protocol.

In figure 71 the habitats are shown, which could be monitored, i.e. sampled and analyzed, exactly as described in the HELCOM/OSPAR protocol. These concern the physical parameter measurements, the fouling plate analyses and the scraping of the floating docks. In addition to the Secchi disk measurements as described in the protocol the water turbidity was also measured with the turbidity meter of Hanna Instruments 93414, in each of the water samples that were taken for the physical parameters. This provides an accurate, repeatable and objective method of measuring turbidity, while Secchi disk measurements may differ slightly based on the different perceptions of the observers.

In figure 82 the habitats are shown where the sampling and/or the analysis method described in the HELCOM/OSPAR protocol could not exactly be followed. For the underground water systems and the littoral zone this can be explained by the fact that these habitats are not described in detail in the HELCOM/OSPAR protocol. Concerning the

Table 36. The number of native, non-native and uniquely recorded species per taxonomical group in the four research areas, that could be identified to the species level with each of the survey methods used in the port of Rotterdam survey.

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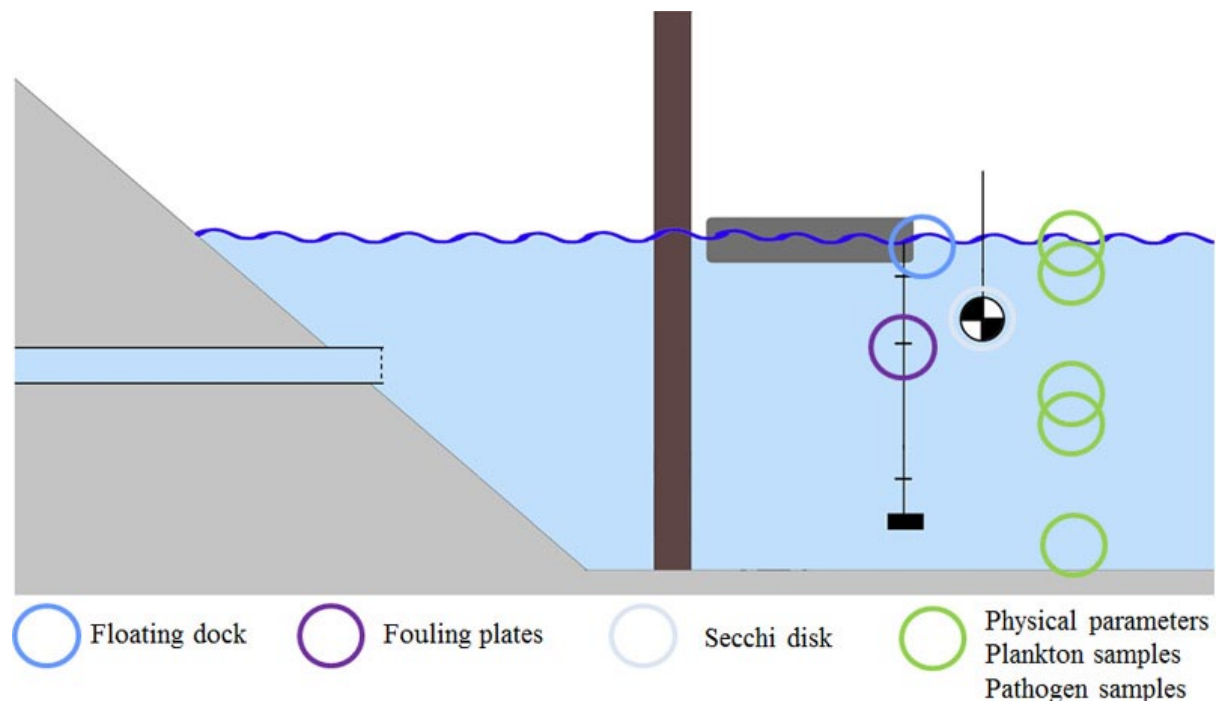


Fig 71. The habitats that were sampled and analyzed with the method described in the HELCOM/OSPAR protocol.

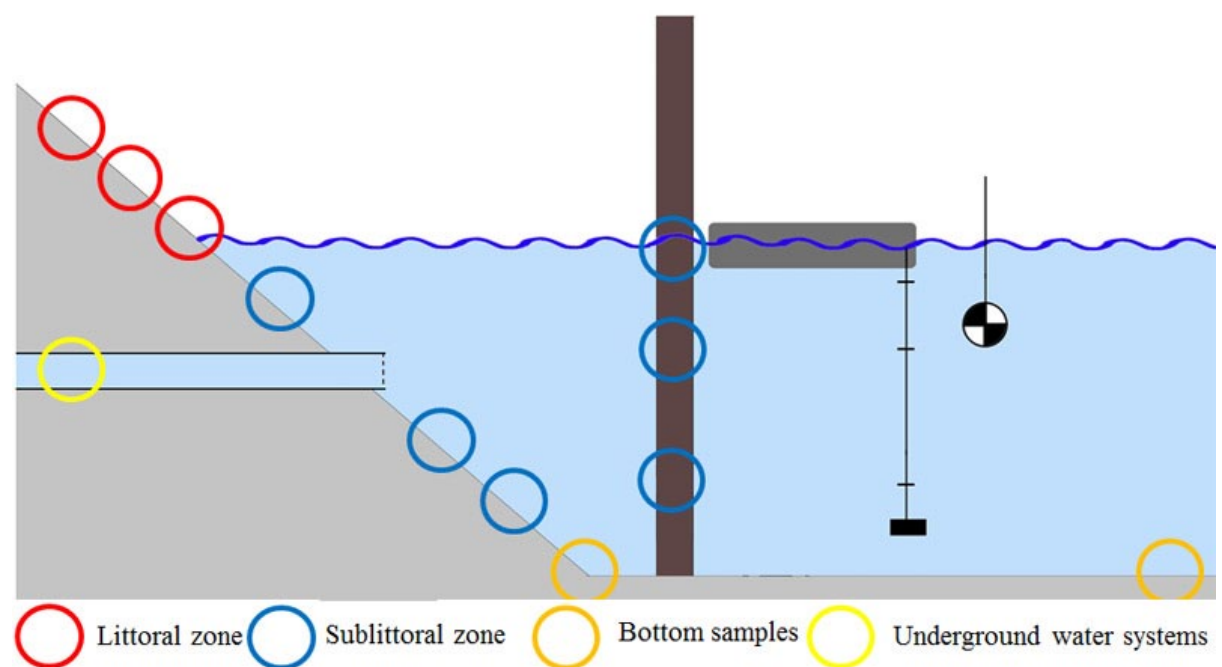


Fig 72. The habitats for which the sampling and/or the analysis method described in the HELCOM/OSPAR protocol could not exactly be followed.

Table 37. Non-native species in each of the four research areas, which are concentrated around [1] the 8e Petroleumhaven, [2] the Beneluxhaven, [3] the Brittanniëhaven and [4] the 1e Eemhaven.

Non-native species	Group	Research area 1: 8e Petroleumhaven	Research area 2: Beneluxhaven	Research area 3: Brittanniëhaven	Research area 4: 1e Eemhaven
<i>Dasysiphonia japonica</i>	Algae	1	1		
<i>Ulva pertusa</i>	Algae	1	1		
<i>Ficopomatus enigmaticus</i>	Annelida	1	1	1	1
<i>Molgula manhattensis</i>	Ascidacea	1	1	1	
<i>Styela clava</i>	Ascidacea	1	1	1	
<i>Vibrio cf brasiliensis</i>	Bacteria			1	
<i>Coscinodiscus wailesii</i>	Chromista			1	
<i>Mediopyxis helysia</i>	Chromista	1			
<i>Odontella sinensis</i>	Chromista	1	1	1	
<i>Prorocentrum cordatum</i>	Chromista	1	1	1	
<i>Protoceratium reticulatum</i>	Chromista	1	1		
<i>Thalassiosira nordenskiöldii</i>	Chromista			1	
<i>Cordylophora caspia</i>	Cnidaria				1
<i>Nemopsis bachei</i>	Cnidaria			1	
<i>Amphibalanus improvisus</i>	Crustacea	1	1	1	1
<i>Austrominius modestus</i>	Crustacea	1	1	1	
<i>Caprella mutica</i>	Crustacea		1	1	
<i>Hemigrapsus sanguineus</i>	Crustacea	1	1	1	
<i>Hemigrapsus takanoi</i>	Crustacea	1	1	1	
<i>Jassa marmorata</i>	Crustacea		1		
<i>Rhithropanopeus harrisii</i>	Crustacea				1
<i>Mnemiopsis leidyi</i>	Ctenophora	1	1	1	
<i>Corbicula fluminalis</i>	Mollusca				1
<i>Crassostrea gigas</i>	Mollusca	1	1	1	
<i>Crepidula fornicata</i>	Mollusca		1	1	
<i>Dreissena bugensis</i>	Mollusca				1
<i>Dreissena polymorpha</i>	Mollusca				1
<i>Physa acuta</i>	Mollusca		1		1
<i>Potamopyrgus antipodarum</i>	Mollusca		1		1
<i>Rangia cuneata</i>	Mollusca				1
<i>Neogobius cf fluviatilis</i>	Pisces				1
<i>Neogobius melanostomus</i>	Pisces				1

scrape samples that were to be taken by divers from quadrates along transects on the dike and on the pilings, these could only be taken from the pilings. From the dike the divers were unable to scrape of the fouling species as the surface was covered with oysters and rocks. Instead of scraping of the surface of a quadrate, oysters and rocks were collected in bags and analyzed in the laboratory. The HELCOM/OSPAR protocol indicates that the quadrates should be photographed before scraping. With the exception of several quadrates on pilings in the 8e Petroleumhaven, the divers were unable to make clear photographs of the quadrates because of the relatively murky waters in the port. The hand-corer samples that had to be taken by divers at 0 m and 50 m along a transect perpendicular to the shore, could be taken at all research areas with the exception of the Brittanniëhaven, where the samples could not be taken at the start of the transect because of a thick layer of oyster shells.

The HELCOM/OSPAR protocol recommends taking bottom samples both with a bottom grab like the petit ponar and by divers with a hand corer. Although the surface areas sampled are similar, i.e. 0.023 m² (petit ponar) and 0.025 m² (hand corer), 29 species were found in the 13 petit ponar grabs, while only 17 species were found in the 21 hand corer bottom samples. Part of this difference may be explained by organisms that were scared away by the divers.

Table 38. Non-native species found with the survey methods that were conducted in the port of Rotterdam.

Non-native species	Group	Phytoplankton	Zooplankton	"Larger zooplankton including gelatinous species"	Chinese crab trap	Gee's minnow trap	Fouling plates	Scrape samples, floating docks	Scrape samples, pillars	Dike fouling	"Sampling with scuba-divers; Scrape sampling: Stone slope"	"Sampling with scuba-divers; Scrape sampling: Vertical structures"	"Sampling with scuba-divers; Transect observations"	"Sampling with scuba-divers; Bottom sampling with hand corer"	Petit ponar bottom sampling	Hand dredge sampling	Fire hydrant sampling
<i>Dasysiphonia japonica</i>	Algae				1		1	1		1	1	1					
<i>Ulva pertusa</i>	Algae							1		1	1	1				1	
<i>Ficopomatus enigmaticus</i>	Annelida						1	1	1	1	1	1			1	1	1
<i>Molgula manhattensis</i>	Ascidacea						1	1				1			1	1	1
<i>Styela clava</i>	Ascidacea						1	1			1	1	1			1	
<i>Vibrio cf brasiliensis</i>	Bacteria																
<i>Coscinodiscus wailesii</i>	Chromista	1															
<i>Mediopyxis helysia</i>	Chromista	1															
<i>Odontella sinensis</i>	Chromista	1															
<i>Prorocentrum cordatum</i>	Chromista	1															
<i>Protoceratium reticulatum</i>	Chromista	1															
<i>Thalassiosira nordenskioeldii</i>	Chromista	1															
<i>Cordylophora caspia</i>	Cnidaria						1		1						1		
<i>Nemopsis bachei</i>	Cnidaria			1													
<i>Amphibalanus improvisus</i>	Crustacea				1		1	1	1	1	1	1			1	1	
<i>Austrominius modestus</i>	Crustacea				1			1		1	1	1		1	1		
<i>Caprella mutica</i>	Crustacea						1	1									
<i>Hemigrapsus sanguineus</i>	Crustacea						1	1		1		1	1				
<i>Hemigrapsus takanoi</i>	Crustacea					1	1	1		1	1	1		1	1	1	
<i>Jassa marmorata</i>	Crustacea									1							
<i>Rhithropanopeus harrisii</i>	Crustacea				1	1	1			1	1	1		1		1	1
<i>Mnemiopsis leidyi</i>	Ctenophora			1	1	1							1				
<i>Corbicula fluminalis</i>	Mollusca													1	1		
<i>Crassostrea gigas</i>	Mollusca						1	1		1	1	1	1	1	1	1	1
<i>Crepidula fornicata</i>	Mollusca				1							1				1	
<i>Dreissena bugensis</i>	Mollusca							1					1		1	1	1
<i>Dreissena polymorpha</i>	Mollusca			1				1							1	1	
<i>Physa acuta</i>	Mollusca																1
<i>Potamopyrgus antipodarum</i>	Mollusca																1
<i>Rangia cuneata</i>	Mollusca															1	
<i>Neogobius cf fluviatilis</i>	Pisces												1				
<i>Neogobius melanostomus</i>	Pisces				1	1							1				

6.2 Non-native species and target species in the port of Rotterdam

During the inventory in total 32 non-native species were encountered in the port of Rotterdam (Table 39). Respectively 15, 19 and 17 non-native species were found in the three more saline (about 20 ppt) research areas 1, 2 and 3, and only 12 non-native species were found in research area 4 where the waters are only slightly saline (about 0.5 - 1 ppt). The number of non-native species present in the various harbours and waterways of the port of Rotterdam could not specifically be linked to the ships traffic within the port in 2014 (Fig 73). Based on a selection of 22890 ships that have arrived in the port of Rotterdam between the January 1st and November 15th 2014, it can

be concluded that most of the ships in the port of Rotterdam, both the ones coming from Europe and the ones coming from other continents, i.e. 1031 ships, dock in research area 4. This is the most inland area that was studied during the present survey. The lowest number of non-native species (12) was found here. The lowest number of ships originating from other continents, i.e. 245 ships, was recorded for research area 2. There the highest number of non-native species (19) was recorded in the present survey.

To follow the Risk Assessment Tool under the HELCOM/OSPAR Harmonized Procedure on Exemptions under the Ballast Water Management Convention it has to be decided which of these species concern target species. HELCOM/OSPAR Target species are species for which the

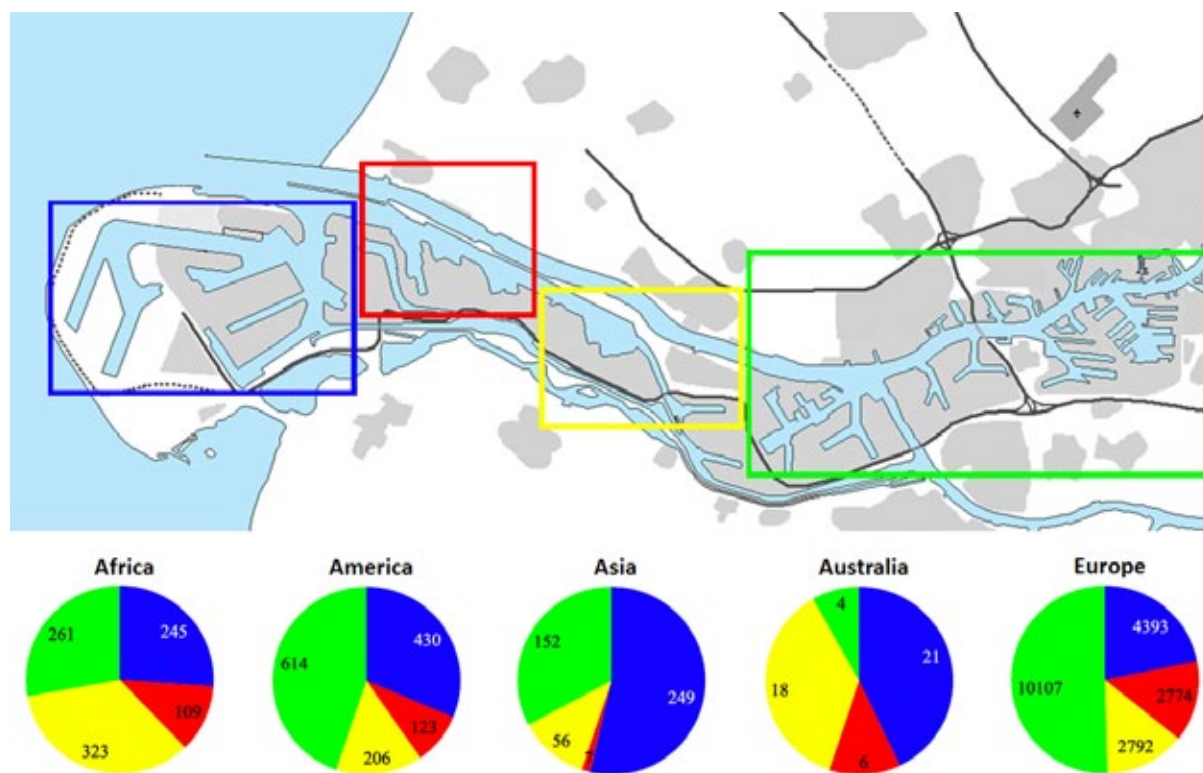


Fig 73 Blue: research area 1 (8e Petroleumhaven) with surrounding harbours; Red: research area 2 (Beneluxhaven) with surrounding harbours; Yellow: research area 3 (Brittaniëhaven) with surrounding harbours; Green: research area 4 (1e Eemhaven) with surrounding harbours.

Table 39. Non-native species recorded during the 2014 Rotterdam Port survey. Target species selection for the risk assessment tool under the HELCOM/OSPAR harmonized procedure on exemptions under the Ballast Water Management Convention. Species that are not on the HELCOM/OSPAR target species list yet, are highlighted. * In the HELCOM/OSPAR target species list these species are included under the names *Prorocentrum minimum* and *Hemigrapsus penicillatus*. The World Register of Marine Species (WoRMS) was used in this report as a source for the accepted names. We therefor consider *Prorocentrum cordatum* a synonym of *Prorocentrum minimum* and *Hemigrapsus penicillatus* (the records in Europe) as a synonym of *Hemigrapsus takanoi*.

Species	Authority	Group	Target in Baltic	Target in NE Atlantic
<i>Dasyatis japonica</i>	(Yendo) H.-S. Kim	Algae	YES	YES
<i>Ulva pertusa</i>	Kjellman	Algae	YES	YES
<i>Ficopomatus enigmaticus</i>	(Fauvel, 1923)	Annelida	YES	YES
<i>Molgula manhattensis</i>	(De Kay, 1843)	Ascidacea	YES	YES
<i>Styela clava</i>	Herdman, 1881	Ascidacea	YES	YES
<i>Vibrio cf brasiliensis</i>	Thompson et al., 2003	Bacteria	YES	YES
<i>Coscinodiscus wailesii</i>	Gran & Angst, 1931	Chromista	YES	YES
<i>Mediopyxis helysia</i>	Kühn, Hargreaves & Halliger, 2006	Chromista	YES	YES
<i>Odontella sinensis</i>	(Greville) Grunow, 1884	Chromista	YES	YES
<i>Prorocentrum cordatum</i> *	(Ostenfeld) Dodge, 1975	Chromista	YES	YES
<i>Protoceratium reticulatum</i>	(Claparède & Lachmann) Butschli, 1885	Chromista	YES	YES
<i>Thalassiosira nordenskiöldii</i>	Cleve, 1873	Chromista	YES	YES
<i>Cordylophora caspia</i>	(Pallas, 1771)	Cnidaria	YES	YES
<i>Nemopsis bachei</i>	L. Agassiz, 1849	cnidaria	YES	YES
<i>Amphibalanus improvisus</i>	(Darwin, 1854)	Crustacea	YES	YES
<i>Austrominius modestus</i>	(Darwin, 1854)	Crustacea	YES	YES
<i>Caprella mutica</i>	Schurin, 1935	Crustacea	YES	YES
<i>Hemigrapsus sanguineus</i>	(De Haan, 1835)	Crustacea	YES	YES
<i>Hemigrapsus takanoi</i> *	Asakura & Watanabe, 2005	Crustacea	YES	YES
<i>Jassa marmorata</i>	Holmes, 1905	Crustacea	YES	YES
<i>Rhithropanopeus harrisii</i>	(Gould, 1841)	Crustacea	YES	YES
<i>Mnemiopsis leidyi</i>	A. Agassiz, 1865	Ctenophora	YES	YES
<i>Corbicula fluminalis</i>	(O. F. Müller, 1774)	Mollusca	YES	YES
<i>Crassostrea gigas</i>	(Thunberg, 1793)	Mollusca	YES	YES
<i>Crepidula fornicata</i>	(Linnaeus, 1758)	Mollusca	YES	YES
<i>Dreissena bugensis</i>	Andrusov, 1897	Mollusca	YES	YES
<i>Dreissena polymorpha</i>	(Pallas, 1771)	Mollusca	YES	YES
<i>Physa acuta</i>	Draparnaud, 1805	Mollusca	YES	YES
<i>Potamopyrgus antipodarum</i>	(Gray, 1843)	Mollusca	YES	YES
<i>Rangia cuneata</i>	(G. B. Sowerby I, 1832)	Mollusca	YES	YES
<i>Neogobius cf fluviatilis</i>	(Pallas, 1814)	Pisces	YES	YES
<i>Neogobius melanostomus</i>	(Pallas, 1814)	Pisces	YES	YES

HELCOM and OSPAR Commissions have agreed upon that they may impair or damage the environment, human health, property and resources in a HELCOM/OSPAR area. Of the 32 non-native species encountered in the port of Rotterdam 23 are already considered target species in both the Baltic and the NE Atlantic by the HELCOM and OSPAR Commissions. The remaining nine species should probably also be considered target species as is explained below. Although various criteria, which are to be used by HELCOM MONAS and OSPAR BDC for the selection of target species, are described in the HELCOM/OSPAR protocol (Table 40), the exact selection protocol is not included in the HELCOM/OSPAR guidelines. Reviewing the list of species whose presence has been recorded in the HELCOM/OSPAR area and the list of target species provided in the HELCOM/OSPAR decision support tool (http://jointbwmexemptions.org/ballast_water_RA), it appears that every species that is non-native to the NE Atlantic is considered to be a target species by the HELCOM and OSPAR Commissions. The nine non-native species found in the port of Rotterdam that are not on the target species list yet are species that are non-native to the NE Atlantic. They are probably not included on the official HELCOM/OSPAR target species list, because they are not on the HELCOM/OSPAR list of species whose presence has been recorded in the HELCOM/OSPAR area. In addition all nine species would score high on several of the selection criteria set in the HELCOM/OSPAR guidelines. Based on the expert opinion of the first author and the literature mentioned below the criteria set in the HELCOM/OSPAR protocol (Table 40) were scored accordingly in Table 41. The nine species concern the bivalve *Corbicula fluminalis*, the gastropod *Physa acuta*, the fish *Neogobius fluviatilis*, the macro-algal species *Dasyatispongia japonica* and *Ulva pertusa*, the crustacean *Jassa marmorata*, the hydroid *Nemopsis bachei*, the phytoplankton species *Mediopyxis helysia* and the bacterial species *Vibrio cf brasiliensis*.

The bivalve *Corbicula fluminalis* concerns a species that is native in Asia and Africa, and introduced in South America and Europe. It was first reported in The Netherlands by Bij de Vaate and Greijdanus-Klass (1990), after which the species expanded its range within a few years throughout the Netherlands, entering both Belgium and Germany, after which it was spread to France and Switzerland (Wittenberger, 2006). Its introduction into The Netherlands was hypothesized to be by ballast water (Gittenberger & Janssen, 1998). *Physa acuta* is a small gastropod species commonly occurring in aquaria, which was originally thought to be native to the Mediterranean but was recently proven to be native to north America (Semenchenko *et al.*, 2008). It is a freshwater species, which can resist low salinities as becomes clear from its records in the port of Rotterdam. It is wide-spread throughout Europe, can locally become dominant and is able to survive under harsh conditions (Semenchenko *et al.*, 2008). The monkey goby *Neogobius fluviatilis* is one of the most successful fish invaders in inland Europe where it is found wide spread in recent decades (Copp *et al.*, 2005). Next to its natural distribution capacities through the channels it is hypothesized that this species is mainly transported by shipping as the gobies and their eggs may be taken into ballast water tanks (Ahnelt *et al.*, 1998; Biró, 1971). The macro-algal species *Dasyatispongia japonica* and *Ulva pertusa* are NW Pacific species, which are widespread in NW Europe. These species are known for their natural distribution capacities as spores, parts of the plants, and floating objects on which they grow can be transported with the water currents over large distances, after which they can settle and expand their populations, preferably in somewhat sheltered areas (Bjærke & Rueness, 2004; Gittenberger *et al.*, 2010; Gittenberger & Stegenga, 2012; Husa & Sjøtun 2006). Both hull fouling and ballastwater transports are commonly linked in literature to the distribution of these species (Sjøtun *et al.*, 2008). The amphipod *Jassa marmorata* is nowadays a cosmopolitan species, originating from the NW Atlantic coast (Gittenberger *et al.*, 2010). Locally,

Table 40. Selection criteria for target species according to the “Joint HELCOM/OSPAR Guidelines for the Contracting Parties of OSPAR and HELCOM on the granting of exemptions under International Convention for the Control and Management of Ships’ Ballast Water and Sediments, Regulation A-4” as described in agenda item 5 of the HELCOM/OSPAR meeting in Gothenburg, 24-28 June 2013.

	Low risk species=1	Medium risk species=2	High risk species=3
1. Dispersion potential or invasiveness	The species doesn’t spread in the environment because of poor dispersal capacities and low reproduction potential	Except when assisted by man, the species doesn’t colonise remote places. Natural dispersal rarely exceeds more than 1km per year. The species can however become locally invasive because of a <u>strong reproduction potential</u> .	The species is highly fecund, can easily disperse through active of passive means over distances > 1km/year and initiate new populations.
2. Colonisation of high conservation value habitats	Populations of the non-native species are restricted to habitats of no conservation value (e.g. harbour constructions as quay walls or bank and shoreline stabilisation or pipes for cooling systems)	Populations of the non-native species are usually confined to habitats with a low or a medium conservation value and may occasionally colonise high conservation value habitats	Non-native species often colonise high conservation value habitats, these are all biotopes where endangered species can be found. Most of the sites of a given habitat are likely to be readily colonized by the NIS when source population are present in the vicinity and makes therefore a potential threat for red-listed species.
3. Adverse impacts on native species	Data from invasion history suggest that the negative impact on native population is negligible	The non-native species is known to cause local changes (<80%) in population abundance, growth or distribution of one or several native species, especially among common and ruderal species. This effect is usually considered as reversible.	The development of the non-native species often cause local severe (>80%) population declines and the reduction of local species richness. At a regional scale, it can be considered as a factor precipitating (rare) species decline. Those non-native species form long-standing populations and their impacts on native biodiversity are considered as <u>hardly reversible</u> .
4. Alteration of ecosystem functions	The impact on ecosystem processes and structures is considered as negligible.	The impact on ecosystem processes and structures is moderate and considered as easily reversible. Temporary modification of water and sediment properties (e.g. algae which can be removed such as Lemna) or decrease of the rate of colonisation of open habitats by species which build barriers.	The impact on ecosystem processes and structures is strong and difficult to reverse e.g. food web disruption (<i>Crassostrea gigas</i>) or habitat destruction (<i>Eriocheir sinensis</i>).
5. Effects on human health	Data from invasion history suggest that the species has weak toxic effects and no treatment is necessary	Data from invasion history suggest that the species has moderate symptoms, easily treated, no permanent damage	Data from invasion history suggest that the species has negative impact on human health, permanent damage or death
6. Effects on natural resources (e.g. fisheries)	Data from invasion history suggest that negative impact on natural resources is negligible	Data from invasion history suggest that the species has only slight negative impact on natural resources and is restricted only on <u>single locations</u>	Data from invasion history suggest that the species causes serious loss on aquaculture or fisheries harvest
7. Effects on property (e.g. cooling systems)	Data from invasion history suggest that the negative impact on property negligible	Data from invasion history suggest that the species has only slight negative impact on property and this is restricted only on <u>single locations</u>	Data from invasion history suggest that the species has high negative impact on property at many locations
8. Dispersed by ballast water or sediments	Invasion without BW, but target species now found in the harbour with the chance to dispersed further by BW	Dispersal via BW and other possibilities	Dispersal mainly by BW or are already found in BW or Sediments

Table 41. Score-sheets for the target species selection criteria (Table 40) as specified in the “Joint HELCOM/OSPAR Guidelines for the Contracting Parties of OSPAR and HELCOM on the granting of exemptions under International Convention for the Control and Management of Ships’ Ballast Water and Sediments, Regulation A-4” as described in agenda item 5 of the HELCOM/OSPAR meeting in Gothenburg, 24-28 June 2013.

	1	2	3	4	5	6	7	8	total
<i>Corbicula fluminalis</i> (Mollusca)	3	2	2	2	1	1	2	2	15
<i>Physa acuta</i> (Mollusca)	3	2	2	2	1	1	1	2	14
<i>Jassa marmorata</i> (Crustacea)	3	2	1	2	1	1	1	3	14
<i>Nemopsis bachei</i> (Hydrozoa)	3	1	1	2	1	1	1	3	13
<i>Neogobius fluviatilis</i> (Pisces)	3	2	2	1	1	1	1	2	13
<i>Dasysiphonia japonica</i> (Algae)	3	2	1	2	1	1	1	2	13
<i>Ulva pertusa</i> (Algae)	3	2	1	2	1	1	1	2	13
<i>Mediopyxis helysia</i> (Chromista)	3	2	1	2	1	1	1	3	14
<i>Vibrio cf brasiliensis</i> (Bacteria)	3	2	1	2	1	1	1	3	14

especially on floating objects and hard substrata close to the surface, the species can become very dominant occurring with thousands of individuals close together (Gittenberger *et al.*, 2010). It is likely that it uses ships for its distribution as it found especially abundant in harbour systems and it has a preference for areas with strong currents (Faasse & Van Moorsel, 2000; Gittenberger *et al.*, 2010). The medusa stage of the hydroid *Nemopsis bachei* was locally found in high densities in the port of Rotterdam during the spring 2014 monitoring. The native area of this species lies along the NW Atlantic coast from where it was probably introduced into Europe by shipping over a century ago (Vervoort & Faasse, 2009). *Nemopsis bachei* is relatively rarely found in recent years but at the start of the 20th century it used to be an abundant species in the Netherlands in the Zuiderzee. It disappeared there when this water body was isolated from the Wadden Sea, becoming a freshwater lake. In recent years it is only rarely found in The Netherlands (Vervoort & Faasse, 2009). The species *Mediopyxis helysia* concerns a diatom species that was described as new to science from clones found in 2003 in the North Sea, northern Wadden Sea and the Gulf of Maine. Seven years after its first occurrence, it contributed up to almost 50% of the biovolume of the diatoms during a diatom spring bloom in the western Wadden Sea, showing its potential to become a dominant species (Loebela *et al.*, 2013).

It is likely that this planktonic species was aided in its distribution by ballast water transports. A bacterial species that was provisionally identified with the Maldi-tov method as *Vibrio cf brasiliensis*, was described from the south American coast of Brasil (Thompson *et al.*, 2002). As the HELCOM/OSPAR protocol only obliged the monitoring of *Vibrio cholerae*, the identification of *Vibrio cf brasiliensis* was not further checked. If the record in the port of Rotterdam does concern *V. brasiliensis*, this is species was most probably introduced in Europe with ballast water.

7. Recommendations

7.1 Comments and suggestions about abundance measurements

In many of the monitoring methods described in the HELCOM/OSPAR protocol it is indicated that the abundance of species must be measured. This is not always done consistently, leaving the researchers several possible methods to measure abundance, varying from 1-5 categories, to numbers of individuals, biomass, etc.

As abundance measurements tend to be extremely time consuming and costly, they do not seem beneficial to the protocol's goal of recording non-native species in a cost-effective manner.

We would suggest to analyze all samples by recording the presence of both native and non-native species in the samples. The time and effort saved by this process enables one to significantly increase the number of sampling locations in a port, giving a much more reliable view of the number of non-native species present in the port area.

It is for example possible to collect and to analyze up to five times more plankton samples in the same time needed to collect and to analyze one sample using the HELCOM/OSPAR protocol.

As a general approach to measure abundance of species in ports, one can calculate per species in what percentage of all samples searched, it occurred in the port. This "abundance" measure provides a measure of species spread in the port, which may be more important for the risk of species intake through "ballast water" than the number of individuals or the biomass of a species that happens to be locally present at the time of monitoring. This approach to measuring abundance, i.e. species spread in a port, is less also dependent

on seasonal and temporal fluctuations in the size of species communities and individual biomasses.

Focusing on scoring abundance instead of "all non-native species present in the port", the HELCOM/OSPAR protocol recommends scoring species within quadrates. Outside of the quadrates one should also note "visual observations". That is vaguely described in the protocol. We would suggest that each habitat, if possible, is visually searched for at least 30 minutes (by one person or 15 minutes with two persons) for as many species as possible.

7.2 Comments about physical parameter measurements

The HELCOM/OSPAR protocol suggests the use of the Secchi disk for turbidity measurement. The use of a Secchi disk is a subjective way of measuring and allows different observers to provide different results based on their perceptions. In the Rotterdam port sampling, we used a Secchi disk with a 30 cm diameter which is a common practice. Some researchers use other sizes however. Unfortunately, the HELCOM/OSPAR protocol does not specify the diameter size of the Secchi disk.

While it would be good to specify the diameter size of the Secchi disk, we are recommending that a more accurate instrument should be suggested. Using a turbidity meter like the Hanna Instruments 93414 for measuring the turbidity in ntu in each of the water samples that are collected anyways for measuring physical water parameters, is an accurate, repeatable and objective method of measurement.

7.3 Comments about human pathogen monitoring

The HELCOM/OSPAR protocol proposes to monitor the presence of only Enterococci, *Escherichia coli* and *Vibrio cholerae*. This approach is limited and therefore does not create the opportunity for recording other *Vibrio* species which may be non-native and pathogenic to humans.

During the MALDI-TOF analyses in the present study, the presence of various potentially non-native *Vibrio* species not included in the HELCOM/OSPAR list were recorded. These species may have been introduced by ballast water. We are suggesting that a larger number of *Vibrio* species be included in the protocol for analysis.

7.4 Comments about water sampling

The depths at which water samples need to be collected according to the HELCOM/OSPAR protocol for physical parameters, phytoplankton and human pathogens, seem to be somewhat chaotic. The depths need to be synchronized, to create a more logical and scientific approach while saving time. The present protocol suggests that water samples need to be taken at 30 cm, 1m, 4m, 5m, 7m, 10m, etc., and just above the bottom.

- 1) For the physical parameters water samples need to be taken at 1 m and every three meters from there on, including one sample above the bottom.
- 2) Samples for the phytoplankton monitoring have to be taken at 1 m depth and at 5 m depth.
- 3) Samples for the human pathogen monitoring have to be taken at the surface (30 cm depth).

To save time and to standardize the monitoring depths, we are suggesting that the water sampling

should be done at 30 cm, 3m, and every three meters after that, in addition to a sample just above the bottom. A more synchronized sampling could be done as follows:

- 1) For the physical parameters water samples need to be taken at the surface (30 cm) and every three meters from there on, including one sample above the bottom.
- 2) Samples for the phytoplankton monitoring have to be taken at 1 m depth and at 6 m depth.
- 3) Samples for the human pathogen monitoring have to be taken at the surface (30 cm depth).

7.5 Comments about infauna sampling

Based on our experience, we suggest the use of the Petit Ponar for the collection of infauna samples.

In the Rotterdam port survey, we used the Petit Ponar to collect decent samples from a relatively large variety of sediment types including muddy and sandy sea beds that are covered with pebbles and shells. The proposed hand corer bottom sampler to be used by the scuba-divers showed extreme limitations in use as is explained below.

The petit ponar is relatively heavy, but can be operated by one person when a cable with handholds is attached. The instrument was attached to an iron cable, with handholds every half meter. With these handholds the petit ponar was easily lifted out of the water. Without the handholds this would have been very difficult because of the weight, especially when the grab is full. Each handhold on the cable was made by a stainless steel wire rope clamp, which was wrapped into a piece of neoprene fastened by cable ties and wrapped into duct tape. Based on our experience more than 50 petit ponar samples could be taken by one person per day.

In the 13 samples taken with the petit ponar at the port of Rotterdam, 29 species were found. In the 21 hand corer bottom samples that were taken by the scuba-divers 17 species were found. This difference cannot be explained by the sampling surface areas of the two instruments: 0.023 m² (petit ponar) and 0.025 m² (hand corer). Part of the difference may be explained by species that were scared away by the divers.

Since more species were recorded in the 13 petit ponar samples as opposed to 21 hand corer samples taken by divers, we recommend the inclusion of the petit ponar for infauna sampling in the HELCOM/OSPAR protocol and omit the hand corer samples which are relatively expensive to take.

7.6 Comments on plankton samples

The HELCOM protocol for counting zooplankton requires “All specimens to be identified and counted until one has reached 100 individuals of each of the three dominating taxonomic groups excluding nauplii, rotifers and tintinnids”. If this procedure was strictly followed in the Rotterdam survey, the analysis of one sample would have taken several days. Time would have been lost in the counting of the individuals of native plankton species.

The number of species found in the plankton samples from the Rotterdam port indicate that a large number of planktonic species were missed due to the sampling method, i.e. not enough samples were taken.

We suggest that this number of samples could be increased (x5) without raising the cost of the survey if the species are qualitatively scored per sample.

“Abundances of planktonic species” can vary extremely between regions but also between days or

weeks. The variability at different times and locations certainly makes comparisons between ports extremely unreliable. We therefore do not recommend the use abundances (e.g. numbers/liter) of planktonic species in comparisons between ports, as is suggested in for example the joint A-4 risk assessment algorithm in the HELCOM/OSPAR protocol.

7.7 Comments about fouling plates

In the HELCOM/OSPAR protocol, the proposed size for fouling plates is 15x15cm. We recommend a standardization of the plate size with those used in other projects and in similar situations around the world.

A plate size of 14 x 14 cm is recommended instead of the 15 x 15 cm. This size (14 x 14 cm) is used as a standard in continuous three monthly fouling plate monitoring studies in The Netherlands, including the port of Rotterdam, since 2007. The same size was used in Europe wide fouling community studies like MarPACE (part of the European Marbef project). This fouling plate size (14x14cm.) was originally chosen in The Netherlands in 2006 as the Smithsonian Marine Invasions laboratory was then already using these plates and are still deploying them along both sides of the North American continent and in Hawaii for continuous monitoring of (non-native) fouling species.

The analysis of the plates could follow the methods described in Lindeyer and Gittenberger (2011), Ruiz *et al.* (2006) and Hines and Ruiz (2000).

The use of more than one fouling plate on one line could be difficult to manage in cases where the fouling of organisms becomes extremely heavy and difficult to lift out of the water.

We recommend the deployment of only one fouling plate per line. This reduces the weight of the fouling plate and prevents the dropping off of fouling species during removal of the plate from the water. In the Rotterdam port survey, several lines were used. On some lines more than 80 kg of fouling organisms settled on the plates within six months.

7.8 Comments about monitoring the littoral zone in ports

The HELCOM/OSPAR protocol did not include a description of the monitoring of the littoral zone. This habitat is part of the port ecosystem and it is extremely crucial for the habitation of new species. In the port of Rotterdam 17 species were exclusively found in this habitat. We therefore suggest that this habitat is included in the HELCOM/OSPAR monitoring protocol. Usually several littoral zones are clearly distinguishable, because of the presence of certain algal species, tidal pools or substrate types like either pebbles or larger stones and rocks. Each in the field clearly distinguishable zone should be photographed and searched for species in three replicate 0.10 cm² quadrates, at least 15 m apart along a 45 meter horizontal transect. The area of each quadrate should be scraped straight into zipper bags. Species have to be identified in the field when possible or else in the laboratory. Visual observations of additional species should be noted including mobile epibenthic species encountered in the vicinity of the transects in between the quadrate locations.

7.9 Comments about monitoring the sub-littoral zone in ports

A methodology for monitoring the benthos and epifauna in the sub-littoral zone of the dike and bottom of the port, without using scuba-divers is not clearly described in the HELCOM/OSPAR protocol.

We suggest that this habitat is monitored with a hand dredge that can be used from a dock or from a vessel to scrape over the bottom. One can use the professional 'Naturalists' hand dredge of NHBS, weighing 5 kg, with a 450 x 185 mm frame and a net bag with a 1 mm mesh size. Of course one has to note the distance that the dredge is pulled over the bottom. In the port of Rotterdam a dredging distance of 10 meter was used. As the dredge can be heavy we suggest using the same cable with handholds as is described above in the case of the petit ponar. The habitat on the bottom that is sampled with a hand dredge is most similar to the bottom habitat that is sampled by divers. In the port of Rotterdam three more non-native species were found on the bottom with the hand dredge than with the divers along the bottom transects.

7.10 Comments about monitoring the underground water systems present in larger ports as a source of water for fire extinguishing purposes.

In virtually all larger ports like Rotterdam, waters supply systems with underground pipes are installed for the purpose of fire disasters in petroleum storage tanks, tankers, and harbours. These water systems are in open connection with the water in the sea port providing convenient habitat for the habitation of some native and non-native organisms. These underground water pipes provide shelter for organisms from strong water currents, tidal differences and sudden changes in water temperatures and salinities. There is little to no

sedimentation, and no direct sun light. In the port of Rotterdam, most of the species present in these water pipes are non-native. Two of the non-native species found were only recorded in this habitat. As this is an easily overlooked habitat that can harbour non-native species that cannot be found elsewhere in the port, we would suggest including it in the HELCOM/OSPAR protocol. One can attach a mesh bag to a hydranth to take a sample of the species inside the water system. The Pac-Bag® system (<http://www.corexceed.eu/en/>) is one of such sampling bags specifically designed for taking samples from underground water systems through hydranths.

7.11 Comments about scuba-diving

It should be more clearly indicated in the HELCOM/OSPAR protocol whether the use of scuba-divers is an optional method or not.

Scuba-diving is expensive and labour intensive. Diving is sometimes difficult under extreme conditions like strong currents or bad visibilities. It is possible to dive under such conditions with the necessary precautions, procedures and equipment but with high financial costs (~17.000 euros for two days of diving).

Instead of indicating in the protocol that one must include scuba-divers for visual observations and making photos/video if diving is an option, one could indicate that one should only include scuba-divers for visual observations and photos/video if the water transparency is expected to be more than 1.5 meter at all sites both at the surface and above the bottom. The greatest benefits of using scuba-divers are that they can do visual observations, make underwater video and photos, and can collect organisms that they can visually see.

In the port of Rotterdam scuba-diving was possible and samples could be taken, but because of

a layer of silt on the bottom and murky waters photos were only possible at some locations, the video footage was in general too blurry to identify species, and when the bottom was touched by the divers, visibility decreased considerably hampering visual observations.

7.12 Number of sampling sites

The number of sampling sites as suggested in the HELCOM/OSPAR protocol is too few. A clear picture of the port situation is therefore difficult to ascertain. The species accumulation curves based on the species encountered in the Rotterdam port show an example of this sample size limitation. We recommend that the sample size selection should be in relationship with the size of the sea port area.

7.13 General comment

Suggestion to save reference material, such as photos or preserved specimens, for each species encountered in the samples, should be included in the HELCOM/OSPAR protocol. If the photos are stored in the general database managed by HELCOM/OSPAR, they can be made openly available for researchers doing port surveys, aiding them in the identification of species.

7. Literature

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