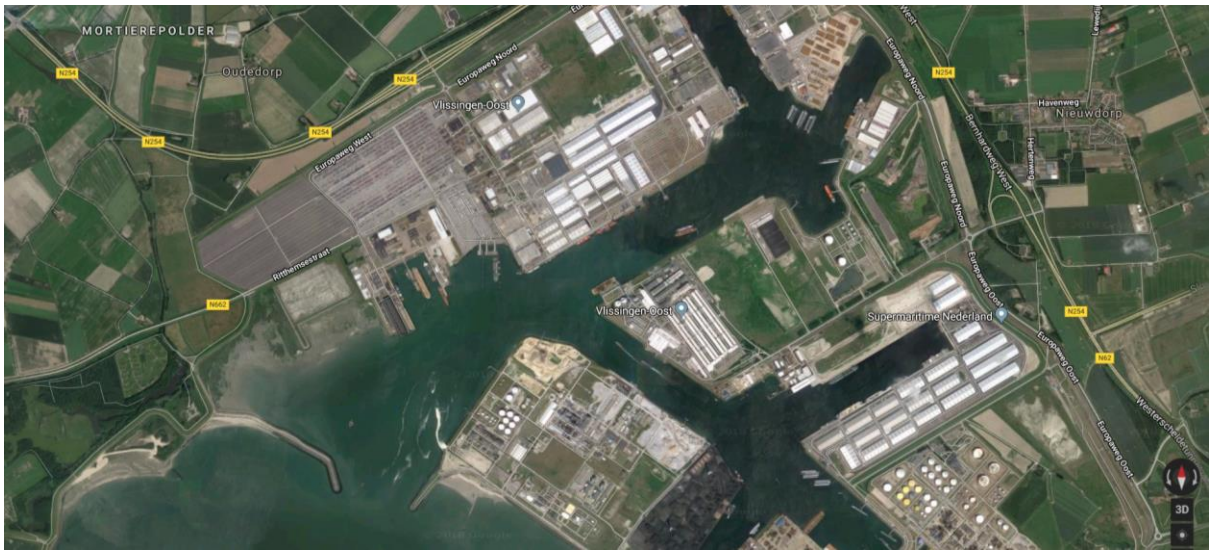
Environmental DNA Sloehaven

A multi-substrate metabarcoding approach for detecting non-indigenous species in a Dutch port



From	Status	Document	Pages
Berry van der Hoorn Arjan Gittenberger (GiMaRIS)	Final	Environmental DNA Sloehaven	43
Date	**Version**	**File name**	**Appendices**
29 December 2019	1.5	Sloehaven_291119.doc	5

Naturalis Biodiversity Center

Vondellaan 55
2332 AA Leiden

T 071 751 92 66
berry.vanderhoorn@naturalis.nl
www.naturalis.nl

Abstract

Commercial harbors and marinas are not only a hub for marine traffic, but also for vegetable and animal stowaways which are unintendedly shipped from one continent to another. This happens, among others, via the intake and discharge of ballast water and via hull fouling or biofouling, especially on niche areas like sea chests and rotors. Monitoring and early detection of non indigenous species is required for prevention and pathway management actions, but is expensive, time-consuming and labour-intensive. It requires a high level of taxonomic expertise and many larval and planktonic species are easily overlooked. In this study we used DNA metabarcoding to detect non-indigenous species (NIS) in the Sloehaven in Vlissingen. We collected water samples, sediment samples, bulk samples, fouling plates and scrape samples for environmental DNA analysis and compared the results of metabarcoding with the results of a recent morphology-based survey. We answered the research questions below:

Of which NIS are DNA barcodes publicly available at the Barcode of Life Database?
Of the 182 NIS recorded in the Netherlands, public databases contain the DNA barcodes for 112 (62%) species. Of the 30 non-indigenous species recorded during the conventional survey in the port of Vlissingen, barcodes are available for 27 species. For the remaining 3 non-indigenous species no such barcodes are available. These species concerned the cryptogenic sea-squirt Aplidium glabrum and the non-indigenous phytoplankton species *Biddulphia sinensis* and *Ethmodiscus punctiger*.

What is the added value of a metabarcoding approach to conventional monitoring?
The detection of the presence of the genetic material of a species in a port can provide an indication that living individuals of that non-indigenous species are present in the port. During the present study, on the basis of metabarcoding, the genetic material was detected of 53 species, which were not found during the conventional monitoring. It is uncertain however whether the genetic material detected originated from living organisms or for from dead material that washed into the port or was released in the port with ballast water and/or from hull fouling communities. In comparison, 200 species (including 22 NIS) were not detected with the metabarcoding approach, but were recorded with the conventional monitoring. For these species it can be concluded with certainty that living individuals were present in the port. During the conventional monitoring, only organisms that were clearly alive were analysed. However, it must be stated that the conventional monitoring was much more extensive covering many more habitats and samples than the DNA metabarcoding approach. In a direct comparison for two fouling plates, the living individuals of eight NIS were recorded based on their morphology, among which six NIS not recorded in the DNA-analyses, and seven NIS resulted from the DNA-analyses, among which five NIS of which no living individuals were recorded with the morphological analyses.

What is the added value specific for ecological or taxonomic groups like zooplankton, phytoplankton, macrobenthos, microbenthos and crustaceans?
As zooplankton often concerns animals in life stages during which species identification is difficult or even impossible on the basis of morphology because diagnostic characters are absent or unknown, metabarcoding has an added value in being able to identify these species. *Acartia tonsa* for example, concerns a non-indigenous species, known to be

present in zooplankton communities. The presence of this species was not detected during the conventional monitoring in the port of Vlissingen, although one could conclude on the basis of the eDNA-analyses that is was probably present. Similarly, eDNA methods can be very useful to detect the potential presence of phytoplankton species. In the port of Vlissingen the genetic material was detected of eight phytoplankton species that appear to be unknown to the Netherlands. Whether this genetic material originated from algae actually living in the port or from dead algae released into the port via ballast water, remains to be studied. Where it concerns macro- and, micro-benthos species, among which crustaceans, the metabarcoding analyses in the present study showed to be especially sensitive in recording the presence of genetic material from polychaetes and crustaceans, while missing the presence of for example most macro-algal and ascidian species.

What is the spatio and temporal variation of molecular communities, and can we use this variation to optimize sampling windows for NIS?
In the port of Vlissingen significantly different “molecular” communities were recorded when comparing samples taken [1] in spring and late summer, [2] at research areas A, B and C, and [3] in the water column from the water surface, just above the bottom, and in between. Based on this data it can be concluded that the season, geographical area, and sampling depth all have a distinct impact on the species that could be recorded by DNA metabarcoding. When optimizing sampling windows for NIS these parameters should therefore be taken into consideration.

What is the added value for early detection of NIS with DNA metabarcoding?
Metabarcoding can especially be used for the detection the genetic material of known non-indigenous species that one would expect to arrive. For this information to be valuable in the early detection of NIS, one should have a follow-up protocol in place to evaluate as soon as possible after the detection of the genetic material, whether living individuals of the non-indigenous species concerned are truly present. In ports, like Vlissingen, it is not unlikely that dead material from non-indigenous species is introduced into the port by ballast water or hull-fouling on the visiting vessels. The greatest added value of a DNA-based approach is in the NIS detection of planktonic species (zooplankton and phytoplankton) which are generally hard to identify. For settled (macrobenthos) species, conventional monitoring outperformes DNA metabarcoding of water samples for the present. An added value for this species group is in the DNA-based analysis of bulk samples (eg from fouling plates).

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Introduction

Commercial harbors and marinas are not only a hub for marine traffic, but also for vegetable and animal stowaways which are unintendedly shipped from one continent to another. This happens, among others, via the intake and discharge of ballast water and via hull fouling (López-Legentil et al. 2015, Keller et al. 2011, IUCN 2009). The habitats in harbors are the first NIS encounter when they embark and, consequently, where they are likely to settle (Gittenberger et al. 2011). From here they can spread to nearby suitable environments along the coast. In this way, harbors offer an important *stepping stone* between coastal areas.

The importance of port surveys for the detection of NIS is generally acknowledged (IUCN 2009). Besides the conventional deployed Rapid Assessment Surveys, metabarcoding of eDNA and bulk samples can offer an added value. Genetic analysis of the water column, the bottom sediment and extracted species from these substrates could generate information on species which are hard to detect and identify visually, like some groups of zooplankton, macrobenthos and fish. For freshwater monitoring there is a growing amount of publications with successful applications and refined techniques, rendered by the obligations from the European Water Framework Directive (Thomsen & Willerslev 2014, Valentini et al. 2015). Although research and applications in the marine realm are lacking behind (Taberlet et al 2018) there is some fast catching up in the detection of marine aquatic species and NIS (Leray & Knowlton 2016, Ardura et al. 2015, Zaiko et al. 2015).

An important difference compared to freshwater is the dilution in a much larger water volume and the influence of currents and tides on the spreading of DNA in marine environments (Borrell et al. 2017). For NIS in particular, there is a strong focus on detecting rare species which demands a special approach in filtering low abundance DNA reads.

Several regulations and conventions apply to the European and Dutch waters, among which the European Water Framework Directive (EWFD), the Marine Strategy Framework Directive (MSFD), the IMO Ballast Water Convention (IMO, 2004), and the EU regulation No. 1143/2014 on the prevention and management of the introduction and spread of invasive alien species (EU, 2014). We assume, as input for the implementation of these regulations, that monitoring data of alien species within ports will prove to be a valuable asset. Hereby an intergrated monitoring approach combining conventional methods with DNA-analyses may prove to be the most appropriate cost-effectiveness choice aiming at being able to detect a large variety of non-indigenous marine species at an early stage. This may aid the development, optimization and evaluation of marine alien species management measures like those obliged by the European Water Framework Directive and the worldwide IMO Ballast water convention.

The aim of this study is an exploration of the possibilities and limitations of metabarcoding in detecting marine biodiversity in harbors, with an emphasis on non-indigenous (NIS) species. This leads to the following research questions:

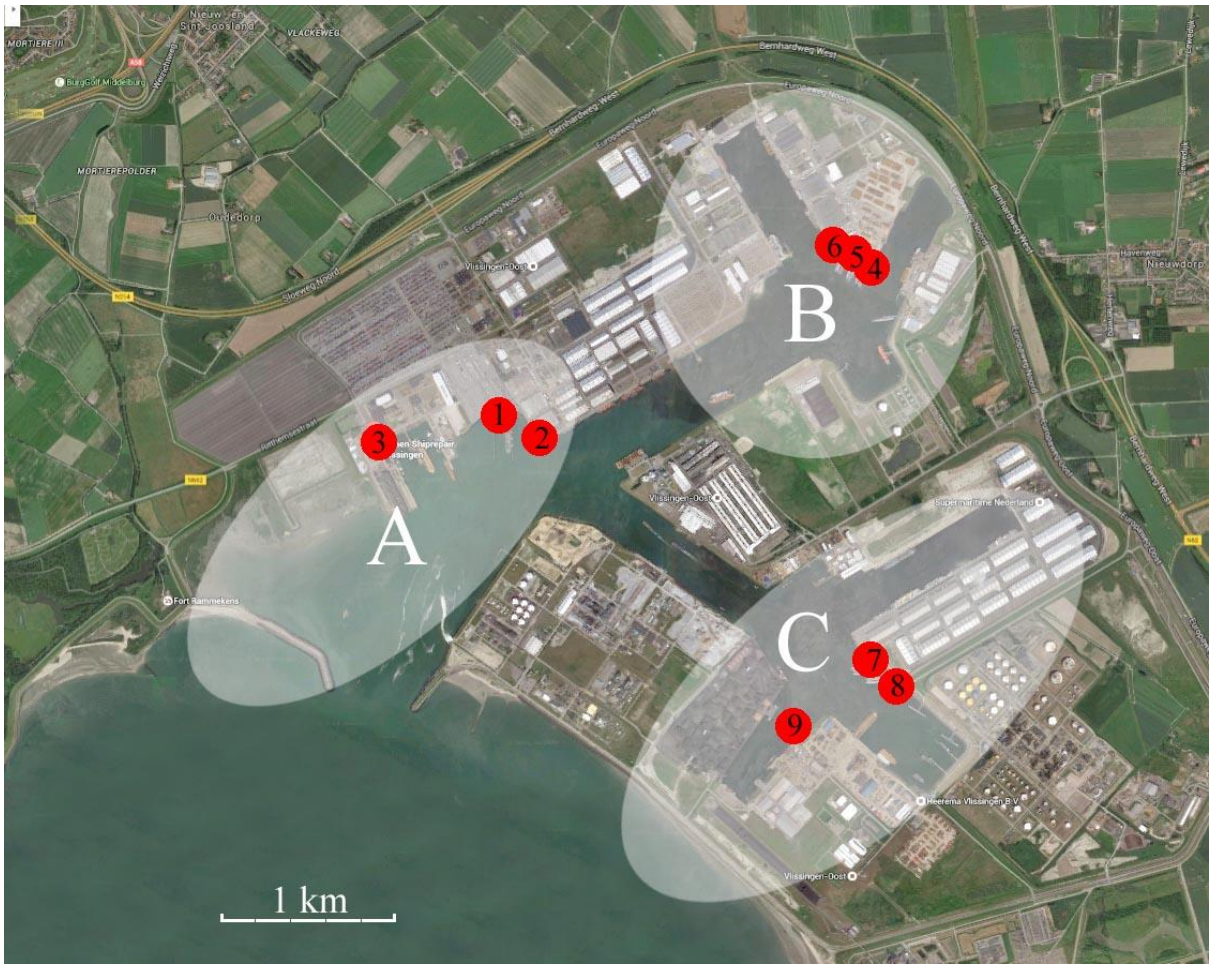
- Of which NIS are DNA barcodes publicly available at the Barcode of Life Database?
- What is the added value of a metabarcoding approach to conventional monitoring?
- What is the added value specific for ecological or taxonomic groups like zooplankton, phytoplankton, macrobenthos, microbenthos and crustaceans?
- What is the spatio and temporal variation of molecular communities, and can we use this variation to optimize sampling windows for NIS?
- What is the added value for early detection of NIS?

Materials and methods

Study area and sampling

Field studies took place at Port Sloehaven, near the city of Vlissingen, in the Delta area of The Netherlands. The study area is divided into three research areas (A, B and C, map 1), previously selected for visual inventories of NIS according to the HELCOM/OSPAR protocol (HELCOM/OSPAR 2013). The research areas were chosen based on differences in their distance to the port entry and hence on differences in their exposure to the influence of currents and waves (Gittenberger et al. 2017a). For each research area water samples, sediment samples and bulk samples were collected. In addition, two scrape samples and two fouling plates were collected from research area A, location 3 (map 1).

Map 1: research areas A, B and C with water sample stations 1-9.



Water samples were collected at three stations per research area, in two different seasons. Sample dates were 20th of May 2016 and 5-9 September 2016. Two seasons were chosen to detect as much as possible seasonal species in the water column. At each station, a 2-liter Van Dorn water sampler from KC Denmark (www.kc-denmark.dk) was deployed to take a sample from the surface (~30 cm depth), from 1 meter and from there on at three meter intervals to the bottom, yielding a total of 88 water samples (appendix 1 & 2). From each sample environmental measurements were taken (appendix 1 & 2). Temperature, acidity and salinity were measured with the multimeter H19829 from Hanna instruments (www.hannainstruments.nl). Turbidity was measured with the portable turbidity meter HI93414 from Hanna instruments. One liter of water from each sample was stored in two 0.5-liter plastic bottles and transported to the laboratory of Naturalis Biodiversity Center.

Sediment and bulk samples were collected on 4 September 2017. From each station, three replicates were collected resulting in a total nine samples (map 1). A petite ponar (a modified Van Veen grab) with a content of 2.4 liter was used to sample the bottom. Three sediment subsamples of 5 ml were extracted from the sample surface of each grab, using a pointless syringe. Sediment subsamples were pooled and stored on 98% ethanol in a 50 ml tube. The rest of the grab content was sieved with consecutive mesh sizes of 6.35 mm, 1.7 mm and 0.5 mm. All organic material larger than 0.5 mm was collected and stored as a bulk sample on 98% ethanol in a weckpot.

Two fouling plates were collected on 26 October 2018 in research area A, location 3 (map 1). One plate was placed in Port Sloehaven in September 2015, the other one in December 2015, so both were deployed for circa three years at a depth of 1 meter. After collecting, each plate was stored in a bucket with seawater and transported to the laboratory to keep all organisms alive. Buckets were stored cold overnight and were processed the next day. Two sub-littoral scrape samples were collected from the floating pontoon on 26 October 2018, directly next to the location where the sampled fouling plates were deployed. Samples were taken using a paint scraper and stored in a bucket with seawater. All equipment used during this study was pre-cleaned with 10% chlorine to minimize contamination.

To compare the metabarcoding approach conducted in this study with the traditional approach, we used the results of the most recent port survey. This survey was carried out in 2016 by GiMaRIS as an assignment for the Netherlands Food and Consumer Product Safety Authority. An extensive array of methods was used to detect a maximum amount of NIS: sediment grabs, plankton nets, lobster traps, fouling plates, hand dredges, visual dike inspections and drop-down camera images. A total of 336 samples were taken from 149 different sample locations, yielding 220 identified species of which 30 were non-indigenous (Gittenberger et al. 2017a).

Sample processing and DNA extraction

Half a liter from each water sample was filtered the next day after collecting using resolvable polyethersulfone (PES) filters (diameter 47mm, pore size 0.4mu). Filters were stored in cetyltrimethyl ammonium bromide (CTAB) lysis buffer to break down cell tissue, before they were dissolved in chloroform:isoamyl alcohol or DNA isolation. The supernatant was purified by four precipitation steps.

Bulk samples were photographed prior to being processed, and species were visually identified for a direct comparison with DNA analysis. Stones and hard shells were removed before the samples were homogenized in a blender. DNA from 10 gram of organic material was extracted using DNeasy Blood Tissue Kit according to the manufacturer's instructions (MOBIO)

Sediment samples were homogenized using a mortar. DNA from 10 gram of sediment was extracted using the PowerMax Soil DNA Isolation Kit according to the manufacturer's instructions (MOBIO). DNA was purified afterwards using the PowerClean Pro DNA Clean-Up Kit (MOBIO) to mitigate inhibitory effects caused by organic substances.

Organic material was scraped off the fouling plates using a hammer and a chisel and was stored in a jar containing 98% ethanol. To collect motile organisms, the seawater was sieved at a 0.5 mm mesh size and added to the jar. Scrape samples were also sieved at a 0.5 mm mesh size. Processing was the same as with the bulk samples.

Library preparation and sequencing

Scrape samples and bulk samples from fouling plates were sequenced on a IonTorrent at Naturalis Biodiversity Center. Before sequencing, DNA was amplified during the initial PCR using uniquely labeled Ion Torrent-tailed primers. PCR products were checked and equimolar normalized and pooled with the Qiagilty pipetting robot (QIAgen). The pool was cleaned with a NucleoMag NGS Clean-up kit. A quality and quantity check was done using on the Bioanalyzer (Agilent) using a High Sensitivity chip. The equimolar pool was diluted according to target 10-30% of positive Ion Sphere Particles. The template preparation and enrichment was carried out with the Ion Touch 2 system, using the OT2 400 view kit (Life Technologies). The enriched Ion Sphere Particles were prepared for sequencing on a Personal Genome Machine (PGM) with the Ion PGM 400 view Sequencing kit as described in the protocol using a 318v2 chip.

All other samples were processes on a Ilumina MySeq at BaseClear. We used a two-step PCR approach. During the initial PCR step, DNA was amplified using Nextera-tailed primers. PCR products were checked and cleaned with a NucleoMag NGS Clean-up kit. During the index PCR step, samples were labeled with unique Nextera XT (Illumina) labeled primers. PCR products were checked and equimolar normalized and pooled with the Qiagilty pipetting robot (QIAgen). The pool was cleaned with a NucleoMag NGS Clean-up kit. A quality and quantity check was done using on the Bioanalyzer (Agilent) using a High Sensitivity chip. Sequencing of the library prepared pool was done on an Illumina Miseq PE 300 bp.

Tabel 1: CO1 primers (Leray et al. 2013)

Forward: mlCOIintF GGWACWGGWTGAACWGTWTAYCCYCC
Reverse: mlCOIintR GGRGGRTASACSGTTCASCCSGTSCC

Bioinformatic and data analysis

Deployment of metabarcoding for species detection and identification requires reliable DNA barcodes of all targeted species. The Barcode of Life Database BOLD (Ratnasingham & Hebert 2007) has been checked for the publicly availability of relevant marine species by creating a checklist for i) all marine species recorded in the Netherlands based on Bos et al. (2016) and ii) a checklist for all NIS recorded in the Dutch territorial waters of the North Sea, Wadden Sea and estuaries in the Delta area based on Bos et al. (2016) and Gittenberger (2017b). The progress reports and exports of BOLD are used for further analysis. As an extra the NIS species have been checked against the OSPAR Target Species List, submission date 9-9-2015, for relevance for the OSPAR/HELCOM agreement.

To get from RAW sequences to taxa lists, Illumina and IonTorrent data was processed using a custom bioinformatic pipeline in Galaxy developed by Naturalis Biodiversity Center. Paired-end sequences from the Illumina runs were merged using FLASH (Magoč & Salzberg, 2011) when the overlap was at least 10 basepairs and the error rate (mismatch) less than 20%. Primers were trimmed using Cutadapt (Martin, 2011). Quality filtering was done using FastQC (Andrews, 2010) with a cut-off value set at 20. Reads outside the length ranges 160 - 170 (12S) and 310 - 314 (CO1) were removed using PRINSEQ (Schmieder & Edwards, 2011). Reads were dereplicated (merging identical reads) using VSEARCH (Rognes et al., 2016). Chimeras were removed during clustering of DNA reads to moleculair operational taxonomic units (MOTUs) using UNOISE (Edgar, 2016). Clustering

For taxonomic assignment CO1 MOTUs were blasted (Camacho et al. 2009) against publicly available sequences at BOLD (Ratnasingham & Hebert 2007) and NCBI (Benson et al. 2013) using a matching value of at least 98% for assignment to species level. 12S MOTUs were blasted against MitoFish (Iwasaki et al. 2013) and NCBI with the same matching value. Species names were matched with WORMS (Horton et al., 2018) and all non-marine species were removed. Abundance was filtered by removing singletons.

Patterns in temporal and spational community turnover were analysed by recording sampling area, sampling date and sampling depth. As daylight, salinity, oxygen content and turbidity vay with water depth, different species communities are expected to be found at different depths. To test this hypothesis, the water samples were grouped as surface, pelagic and bottom samples. Surface water comprise of water samples up to 1 meter in depth, bottom samples of samples from about 1 meter above the bottom and pelagic samples concern all water samples taken in between the surface and bottom samples.

Biological and environmental data were imported in PRIMER-e (Clarke & Gorley 2015) for downstream statistical analysis. Species abundance was transformed to presence/absence data and converted to Jaccard resemblance matrices. Environmental data was standardized and converted to Eucledian distance resemblance distances.

Results

DNA Barcodes

A total number of 182 marine NIS recorded in the Netherlands were selected from Bos et al. (2017) and Gittenberger et al. (2017). BOLD contains specimen data for 120 (66%) of these species and DNA barcodes for 112 (62%). So 70 marine NIS recorded in the Netherlands (38%) are still lacking reference DNA barcodes for molecular identification of environmental samples. Table 1 comprises a concise overview. An extensive overview of specimens and DNA barcodes at species level is in appendix 3.

Table 1. Dutch marine NIS on BOLD: available specimens and DNA barcodes per phylum/class.

Phylum	***Class***	***Species***	***Species with specimen***	***Species with DNA Barcodes***	***Species to do***
Annelida	*Clitellata*	4	2	2	2
Annelida	*Polychaeta*	15	10	8	7
Arthropoda	*Branchiopoda*	1	1	1	0
Arthropoda	*Hexanauplia*	14	13	13	1
Arthropoda	*Insecta*	2	1	1	1
Arthropoda	*Malacostraca*	34	28	27	7
Arthropoda	*Merostomata*	1	1	1	0
Arthropoda	*Pycnogonida*	1	1	1	0
Bryozoa	*Gymnolaemata*	12	8	7	5
Bryozoa	*Phylactolaemata*	1	1	1	0
Chordata	*Actinopterygii*	7	7	7	0
Chordata	*Ascidiacea*	10	7	7	3
Cnidaria	*Anthozoa*	3	3	1	2
Cnidaria	*Hydrozoa*	5	3	3	2
Ctenophora	*Tentaculata*	1	1	1	0
Entoprocta		3	0	0	3
Mollusca	*Bivalvia*	18	16	14	4
Mollusca	*Gastropoda*	11	10	10	1
Mollusca	*Polyplacophora*	1	1	1	0
Nematoda	*Chromadorea*	2	0	0	2
Nemertea	*Palaeonemertea*	1	1	1	0
Platyhelminthes	*Rhabditophora*	2	1	1	1
Porifera	*Calcarea*	2	0	0	2
Porifera	*Demospongiae*	7	1	1	6
Myzozoa	*Dinophyceae*	2	0	0	2
Ochrophyta	*Phaeophyceae*	4	0	0	4
Chlorophyta	*Ulvophyceae*	3	2	2	1
Rhodophyta	*Florideophyceae*	13	0	0	13
Tracheophyta	*Magnoliopsida*	2	1	1	1
Total		***182***	***120***	***112***	***70***
Percentage			***66%***	***62%***	***38%***

NIS found exclusively in DNA analysis

A total of 53 species was detected with DNA analysis but not with conventional analysis during the OSPAR/HELCOM port survey (Gittenberger et al. 2017a). These species mostly concern polychaetes, small crustaceans (e.g. copepods) and unicellular pelagic algae. Possibly, the small pelagic stages of these species or DNA traces were recorded, explaining why they were not found during the conventional OSPAR/HELCOM port survey. Within this survey, zooplankton samples were collected and analysed, but most of the pelagic larval stages of species that were found within these samples did not show any diagnostic morphological characters that could be used to distinguish closely related species.

Of the 53 species additionally recorded with DNA analysis (table 3), 20 concern species that are not represented in the Dutch Species Register, and therefore may concern species that have not or rarely been recorded in the Netherlands. Further research is necessary to investigate whether living individuals of these species are actually present and established in Sloehaven port.

For some of these species it is likely that they concern misidentifications of native species (see remarks in table 3), because the DNA-sequences of the native species may not be available in public DNA-databases used for identifications, or because the CO-I marker may not be variable enough to distinguish between native species and non-indigineous species scored.

For some of the species, correctly identified based on their DNA, no living individuals may be present in the port. Especially if ships do not exchange their ballast water on open sea, for example because they have a ballast water treatment system onboard. The DNA of organisms coming from far away may then be recorded in the port. The origin of this DNA may concern dead specimens, or living specimens (if the ballast water was not treated) of organisms that will be unable to settle because the environment is unsuitable. Concerning the latter, one may also record the living larval stages of species that occur in relatively warmer waters south to the Netherlands. Although these larvae may not be able to settle in the Netherlands, they can reach the Netherlands just by flowing along with the residual south to north current along the western European coast.

For the fouling plates we could make a direct comparison between visual identification and DNA analysis within our study. In the direct comparison between visual detection and DNA analysis of the fouling plates, 21 species were exclusively recorded by DNA analysis. Again, these species mostly concern annelids and crustaceans (appendix 4). The annelids concerned mostly soft bottom species which were never visually recorded on fouling plates. Among the crustaceans are four species of crabs which in general are easily identified by visual inspection. It might be possible that most of these records refer to stomach contents of, for example, tunicates that abundantly settled on the plates. The 5 NIS exclusively found with DNA analysis of fouling plates are from a wide range of species groups: a barnacle, a crab, a bryozoan, and two mollusks.

Table 2. Species exclusively found during DNA analysis. Species registered in the Dutch Species Register as NIS, are green highlighted. Species not registered in the Dutch Species Register are yellow highlighted.

	Species	***Phylum***	***Class***	***Remarks***
1	*Amphichaeta sannio*	Annelida	Clitellata	
2	*Tubificoides brownae*	Annelida	Clitellata	
3	*Tubificoides diazi*	Annelida	Clitellata	
4	*Arenicola defodiens*	Annelida	Polychaeta	
5	*Arenicola marina*	Annelida	Polychaeta	
6	*Ctenodrilus serratus*	Annelida	Polychaeta	
7	*Lagis koreni*	Annelida	Polychaeta	
8	*Magelona johnstoni*	Annelida	Polychaeta	
9	*Ophryotrocha puerilis siberti*	Annelida	Polychaeta	West European species; not in Dutch species register.
10	*Pherusa affinis*	Annelida	Polychaeta	Possible misidentification of the native species *Pherusa plumosa*; not in Dutch species register.
11	*Platynereis dumerilii*	Annelida	Polychaeta	
12	*Polydora cornuta*	Annelida	Polychaeta	
13	*Protodrilus adhaerens*	Annelida	Polychaeta	West European and Mediterranean species; not in Dutch species register.
14	*Scoloplos armiger*	Annelida	Polychaeta	
15	*Streblospio benedicti*	Annelida	Polychaeta	
16	*Acartia (Acanthacartia) tonsa*	Arthropoda	Hexanauplia	NIS (Settled 10-99 years according to Dutch Species Register)
17	*Acartia bifilosa*	Arthropoda	Hexanauplia	
18	*Acartia clausii*	Arthropoda	Hexanauplia	West European species; not in Dutch species register.
19	*Amphibalanus improvisus*	Arthropoda	Hexanauplia	NIS (Settled >100 years according to Dutch Species Register)
20	*Balanus balanus*	Arthropoda	Hexanauplia	NIS (Occasional import according to Dutch Species Register); May concern the native species *Balanus crenatus* as this species is common in the Sloehaven.
21	*Cyclops kikuchii*	Arthropoda	Hexanauplia	Freshwater species. Possible misidentification of a native Cyclops species; not in Dutch species register.
22	*Euterpina acutifrons*	Arthropoda	Hexanauplia	
23	*Nitokra spinipes*	Arthropoda	Hexanauplia	
24	*Paracalanus parvus*	Arthropoda	Hexanauplia	
25	*Temora longicornis*	Arthropoda	Hexanauplia	
26	*Zygomolgus dentatus*	Arthropoda	Hexanauplia	Korean species. Possible misidentification of the native species *Zygomolgus tenuifurcatus.*
27	*Mesopodopsis slabberi*	Arthropoda	Malacostraca	
28	*Pilumnus hirtellus*	Arthropoda	Malacostraca	
29	*Bugula neritina*	Bryozoa	Gymnolaemata	NIS (Settled <10 years according to Dutch Species Register). Possible misidentification of the NIS *Bugula stolonifera*. *B. neritina* is easily identified by its bright purple colonies, making misidentification based on morphology unlikely. B. stolonifera was repeatedly recorded in the Sloehaven based on morphology, but was not recorded based on its DNA.
30	*Tricellaria occidentalis*	Bryozoa	Gymnolaemata	Northeastern Pacific species, closely resembling Tricellaria inopinata, which is registered in the Dutch Species Register as a Pacific NIS (Settled 10-99 years).
31	*Bathycoccus prasinos*	Chlorophyta	Mamiellophyceae	Mediterranean Sea species; not in Dutch species register.
32	*Pseudoscourfieldia marina*	Chlorophyta	Pyramimonadophyceae	Not in Dutch species register, but known from the Netherlands (Veen et al., 2015)
33	*Pholis gunnellus*	Chordata	Actinopterygii	

34	*Pomatoschistus pictus*	Chordata	Actinopterygii	
35	*Chroicocephalus ridibundus*	Chordata	Aves	Concerns the black-headed hull (Kokmeeuw).
36	*Rattus norvegicus*	Chordata	Mammalia	Concerns the brown rat (Bruine rat).
37	*Sagartiogeton viduatus*	Cnidaria	Anthozoa	NW European species, which may also be present in the Netherlands, misidentified as the morphologically very similar native species *Sagartiogeton undatus.*
38	*Obelia bidentata*	Cnidaria	Hydrozoa	
39	*Calvadosia cruciformis*	Cnidaria	Staurozoa	NW Pacific species; not in Dutch species register.
40	*Ophiothrix fragilis*	Echinodermata	Ophiuroidea	
41	*Cerastoderma edule*	Mollusca	Bivalvia	
42	*Kurtiella bidentata*	Mollusca	Bivalvia	
43	*Magallana angulata*	Mollusca	Bivalvia	Although this species is considered to be "accepted" in the world register of marine species, many scientists (based on genetic studies) consider it to be a synonym of the Pacific oyster Magallana gigas, an NIS (Settled in the Netherlands 20-99 years)
44	*Heterocapsa rotundata*	Myzozoa	Dinophyceae	Occurring in W Europe; not in Dutch species register.
45	*Hubrechtella dubia*	Nemertea	Palaeonemertea	Northern European species (Sweden, Norway and Denmark); not in Dutch species register.
46	*Bellerochea polymorpha*	Ochrophyta	Bacillariophyceae	North American species; not in Dutch species register.
47	*Ethmodiscus punctiger*	Ochrophyta	Bacillariophyceae	NW Pacific species; not in Dutch species register.
48	*Thalassiosira nordenskioeldii*	Ochrophyta	Bacillariophyceae	Arctic species; not in Dutch species register.
49	*Pseudochattonella verruculosa*	Ochrophyta	Dictyochophyceae	Not in Dutch species register, but known from the Netherlands (Veen et al., 2015)
50	*Leathesia marina*	Ochrophyta	Phaeophyceae	
51	*Fibrocapsa japonica*	Ochrophyta	Raphidophyceae	NIS (Settled 10-99 years according to Dutch Species Register)
52	*Notocomplana koreana*	Platyhelminthes	Rhabditophora	NW Pacific species; not in Dutch species register.
53	*Protosuberites mereui*	Porifera	Demospongiae	Mediterranean species. Possibly a misidentification of the native species *Protosuberites denhartogi.*

NIS found in both DNA and conventional analysis

Only 16 species recorded during the conventional survey (Gittenberger et al. 2017a) were also recorded by DNA analysis (CO1 marker) of water, sediment, fouling plates and floating docks (table 3). Of these 16 species, 8 are non-indigenous (table 2, green highlighted).

The 8 NIS detected by both DNA and conventional analysis have in common that they all concern species that are widespread and occur in high densities in waters throughout the Netherlands (Gittenberger et al. 2010, Wolff 2005). The annelid *Ficopomatus enigmaticus* for example is the most abundant non-indigenous annelid fouling species in brackish waters throughout the Netherlands forming reefs up to at least 10 cm high with calcareous tubes sometimes completely covering the submerged parts of floating docks and piling (pers. obs. Gittenberger).

In the direct comparison between visual detection and DNA analysis of the fouling plates, we found 20 species based on visual inspection of the photographs of the plates, 24 species based on DNA analysis and an overlap of only 3 species, of which the tunicate *Botrylloides violaceus* and the barnacle *Austrominius modestus* are non-indigenous (appendix 4).

Table 3. Species identified in both eDNA and conventional analysis. NIS are green highlighted.

	Species	***Phylum***	***Class***	***Bulk_COI***	***Sediment_COI***	***Scrape_COI***	***SETL_COI***	***Water_COI***
1	*Ficopomatus enigmaticus*	Annelida	Polychaeta	0	0	1	0	1
2	*Nephtys hombergii*	Annelida	Polychaeta	1	1	0	0	0
3	*Austrominius modestus*	Arthropoda	Hexanauplia	0	0	1	1	0
4	*Caprella mutica*	Arthropoda	Malacostraca	0	1	0	0	0
5	*Carcinus maenas*	Arthropoda	Malacostraca	0	0	1	1	0
6	*Hemigrapsus sanguineus*	Arthropoda	Malacostraca	0	0	0	1	0
7	*Hemigrapsus takanoi*	Arthropoda	Malacostraca	0	0	1	0	0
8	*Pisidia longicornis*	Arthropoda	Malacostraca	1	0	0	1	0
9	*Micromonas pusilla*	Chlorophyta	Mamiellophyceae	0	0	0	0	1
10	*Botrylloides violaceus*	Chordata	Ascidiacea	1	0	1	1	0
11	*Spisula subtruncata*	Mollusca	Bivalvia	0	0	1	1	0
12	*Crepidula fornicata*	Mollusca	Gastropoda	1	0	0	0	0
13	*Magallana gigas*	Mollusca	Bivalvia	0	0	0	1	0
14	*Pseudo-nitzschia delicatissima*	Ochrophyta	Bacillariophyceae	0	0	0	0	1
15	*Pseudo-nitzschia pungens*	Ochrophyta	Bacillariophyceae	0	0	0	0	1
16	*Halichondria (Halichondria) panicea*	Porifera	Demospongiae	0	0	1	1	0
			Total	4	2	7	8	4
			Native	2	1	3	4	3
			Non-indigenous	2	1	4	4	1

NIS found exclusively in conventional analysis

A total of 216 species was morphologically identified during the conventional OSPAR/HELCOM port survey in the Sloehaven of Vlissingen (Gittenberger et al. 2017a). Of these species, 200 were not recorded with DNA analysis. The NIS of this list are presented in table 4, the full list is in appendix 5.

For some species groups it is obviously that they were not recorded with DNA analysis. Primers specific for macro-algae (Chlorophyta, Ochrophyta and Rhodophyta) were not used in the present study. Primers for selection of these groups are still under development and for some groups discussions are still ongoing in the scientific world, on the marker that they should target.

The non-indigenous annelid *Neodexiospira brasiliensis*, which was recorded during the OSPAR/HELCOM survey but was missed in the DNA analyses (Table 2), concerns a widespread and common fouling species in the port. Its calcareous tubes are more sparsely distributed however and become only a few millimetres in size. It might be the size why it was not picked up by the DNA analyses but, more likely, the primers used were just not effective to detect this species.

More in general, NIS that belong to the annalids and arthropods appear to be relatively well represented within the DNA-analyses, while most of the (non-indigenous) species belonging to the ascidians (chordata in table 4) are missed. Ascidians are known for their low variability at the CO1 gene which makes it difficult to distinguish between species (Stefaniak et al. 2009). For the molluscs about half of the species are scored by both methods (appendix 4).

Based on the photographs of the two fouling plates, by visual inspection 17 species were recorded which were not detected with DNA analysis (Appendix 4). Most of these species belong to ascidians (tunicates) and cnidarians. Cindarians are known for their low variability at the CO1 gene which makes it difficult to distinguish between species.

Table 4. NIS identified during the conventional survey. Green highlighted are NIS that were also recorded within eDNA analyses. Orange highlighted are NIS for which it is assumed that the DNA analysis led to misidentification.

	Species	Phylum	Class	Probably misidentified as
1	*Ficopomatus enigmaticus*	Annelida	Polychaeta	
2	*Neodexiospira brasiliensis*	Annelida	Polychaeta	
3	*Austrominius modestus*	Arthropoda	Hexanauplia	
4	*Caprella mutica*	Arthropoda	Malacostraca	
5	*Hemigrapsus sanguineus*	Arthropoda	Malacostraca	
6	*Hemigrapsus takanoi*	Arthropoda	Malacostraca	
7	*Jassa marmorata*	Arthropoda	Malacostraca	
8	*Melita nitida*	Arthropoda	Malacostraca	
9	*Bugulina stolonifera*	Bryozoa	Gymnolaemata	*Bugula neritina*
10	*Smittoidea prolifica*	Bryozoa	Gymnolaemata	
11	*Tricellaria inopinata*	Bryozoa	Gymnolaemata	*Tricellaria occidentalis*
12	*Ulva australis*	Chlorophyta	Ulvophyceae	

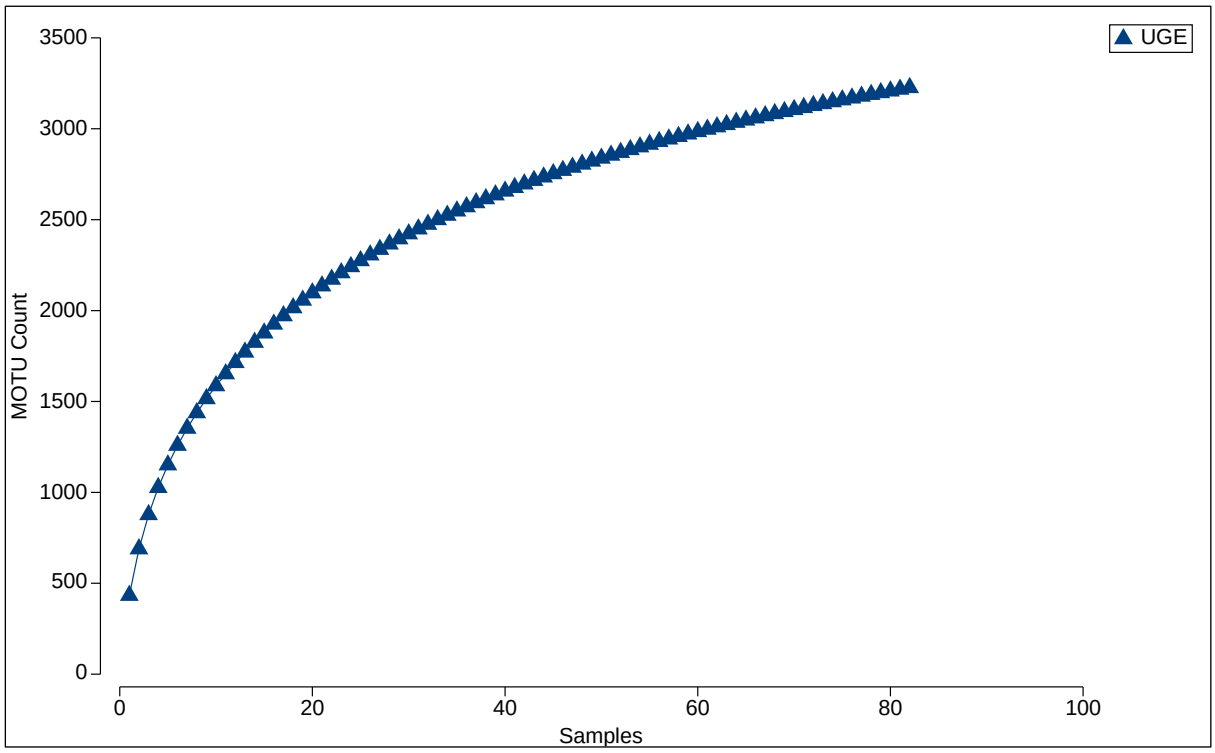
13	*Aplidium glabrum*	Chordata	Ascidiacea
14	*Botrylloides violaceus*	Chordata	Ascidiacea
15	*Didemnum vexillum*	Chordata	Ascidiacea
16	*Diplosoma listerianum*	Chordata	Ascidiacea
17	*Molgula manhattensis*	Chordata	Ascidiacea
18	*Perophora japonica*	Chordata	Ascidiacea
19	*Styela clava*	Chordata	Ascidiacea
20	*Diadumene cincta*	Cnidaria	Anthozoa
21	*Mnemiopsis leidyi*	Ctenophora	Tentaculata
22	*Magallana gigas*	Mollusca	Bivalvia
23	*Ensis leei*	Mollusca	Bivalvia
24	*Mya arenaria*	Mollusca	Bivalvia
25	*Crepidula fornicata*	Mollusca	Gastropoda
26	*Biddulphia sinensis*	Ochrophyta	Bacillariophyceae
27	*Ethmodiscus punctiger*	Ochrophyta	Bacillariophyceae
28	*Antithamnionella spirographidis*	Rhodophyta	Florideophyceae
29	*Dasysiphonia japonica*	Rhodophyta	Florideophyceae
30	*Melanothamnus harveyi*	Rhodophyta	Florideophyceae

Spatio and temporal variation of MOTUs in the Sloehaven

Sampling saturation

After quality filtering of CO1 DNA reads a total of 3029 molecular operational taxonomic units (MOTUs) remained wich were used for subsequent spatio and temporal analysis. MOTUs are DNA barcodes which are not necessarily linked to a taxon name (yet). A CO1 MOTU accumulation curve based on water samples demonstrates that even with the high number of 88 biological samples we still did not sample all MOTUs present in the Sloehaven, see figure 1. This means we could have missed rare MOTUs and therefore rare species, including NIS. There are several solutions to achieve higher number of MOTUs. Firstly, we could take more water samples in the Sloehaven. Secondly, we could try to optimize our lab procedures even further to obtain more MOTUs, by taking more DNA replicates (Lanzén et al. 2017), more PCR replicates (Alberdi et al. 2018) or aim for more sequencing depth (Smith & Peay 2014) by lowering the amount of samples in a run or bij switching from Illumina MySeq to a HiSeq sequencer.

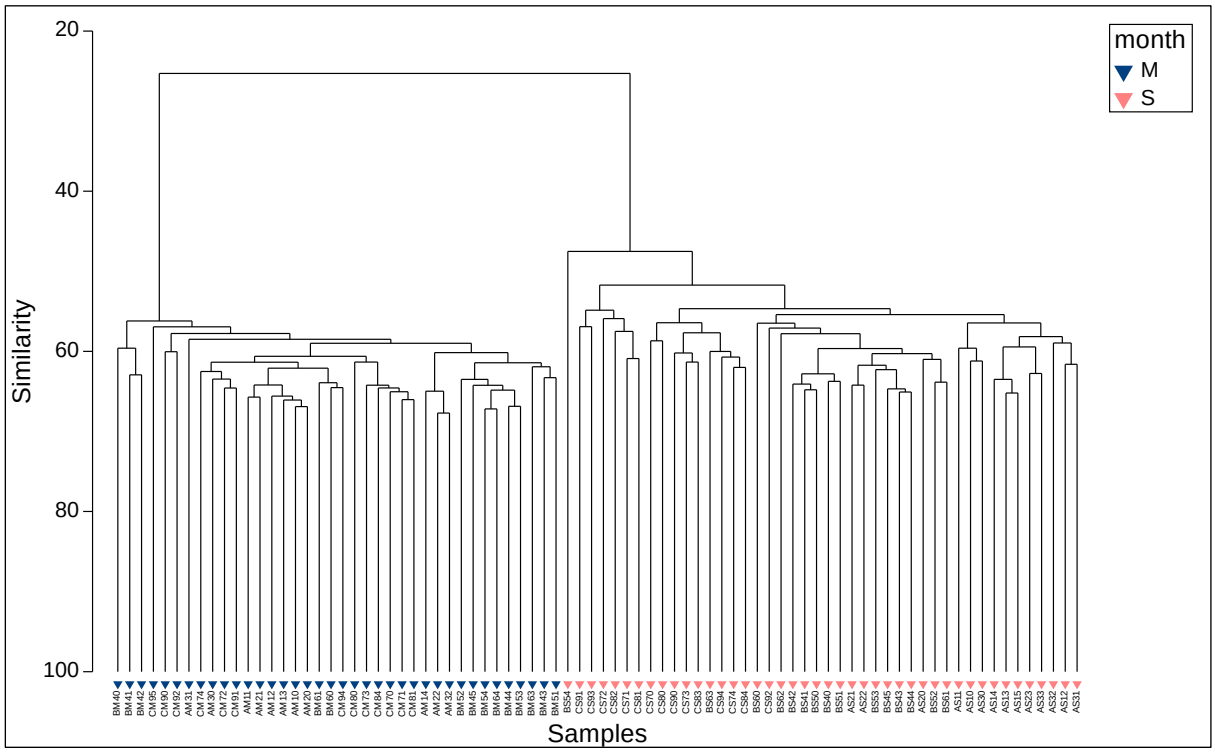
Figure 1. MOTU accumulation curve of the Sloehaven.



Effect of seasonality

There is a strong effect of seasonality on the composition of MOTU communities in the Sloehaven. This is demonstrated by the cluster analysis in figure 2, where the samples from May are clearly separated from September (SIMPROF $p<0.05$). Apparantly there is a high temporal turnover of species between spring and late summer in port Sloehaven, as is recently confirmed by an other study focusing on freshwater and marine ports (Chain et al. 2016). This means that sampling in only one season leads to the omission of MOTUs and hence species. A biological explanation for the differences might be that the larval stages of most marine species are in the water column in spring. The larvae in the Sloehaven may include species from warmer areas in Europe, to the south of the Netherlands, as there is a residual south to north current along the western European coastline. As for some of these species the Dutch waters are unsuitable for settlement, you only record them in their larval stages, i.e. in May and not in September.

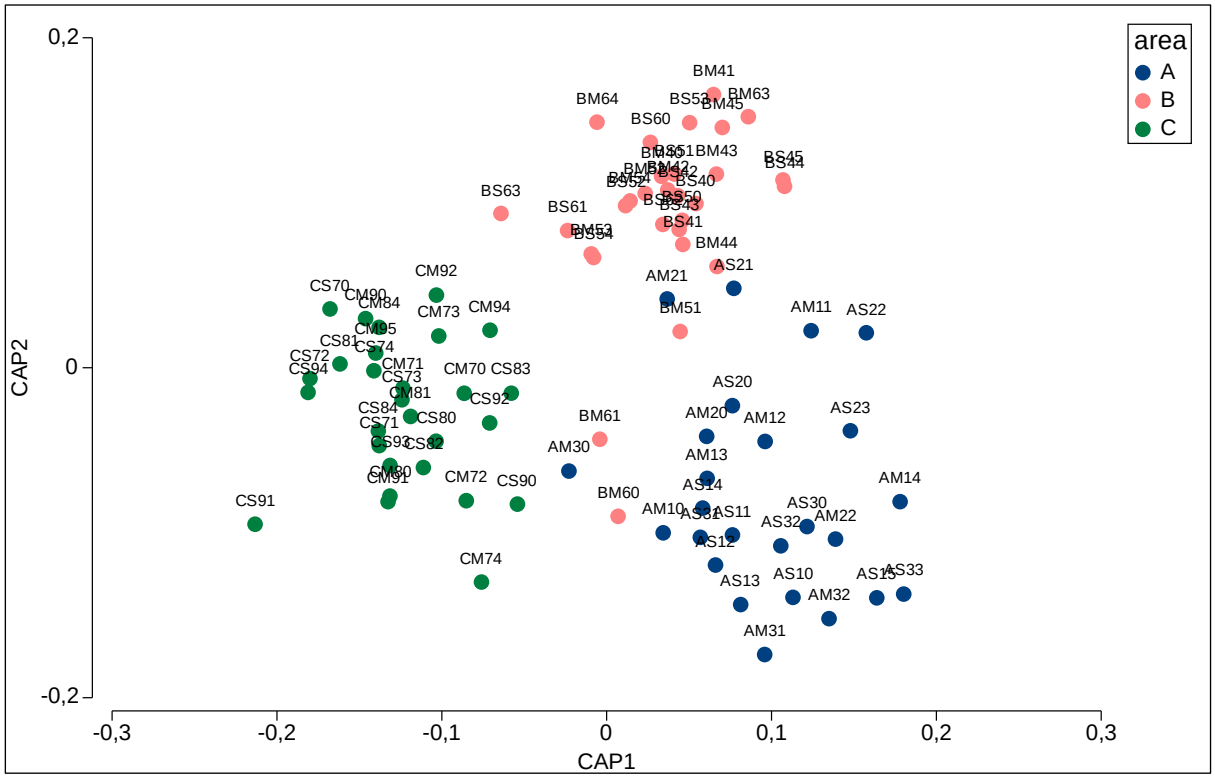
Figure 2: cluster analysis on seasonality of water samples in the Sloehaven



Effect of sampling area

Within the Sloehaven three different research areas were distinguished during the OSPAR/HELCOM survey (Gittenberger et al. 2017a). This is mandatory within the port survey protocol (HELCOM/OSPAR 2013) as it is expected that one may find different species communicities in these areas. They concern parts of the port that differ not only in the ships docking but also for example in the distance to the entrance of the port, which may result in varying current strengths and water salinities. The DNA-dataset supported the expectation that the species communities in these research areas differed significantly both in May and in September (Permanova, respectively p = 0.038 and p=0.001) as this was also illustrated by CAP-analysis (Fig. 3). This means when the aim is to detect as much as possible (non-indigenous) species it is necessary to sample at a relevant spatial scale, in this case in all the different ‘arms’ of the port, representing the three research areas (map 1).

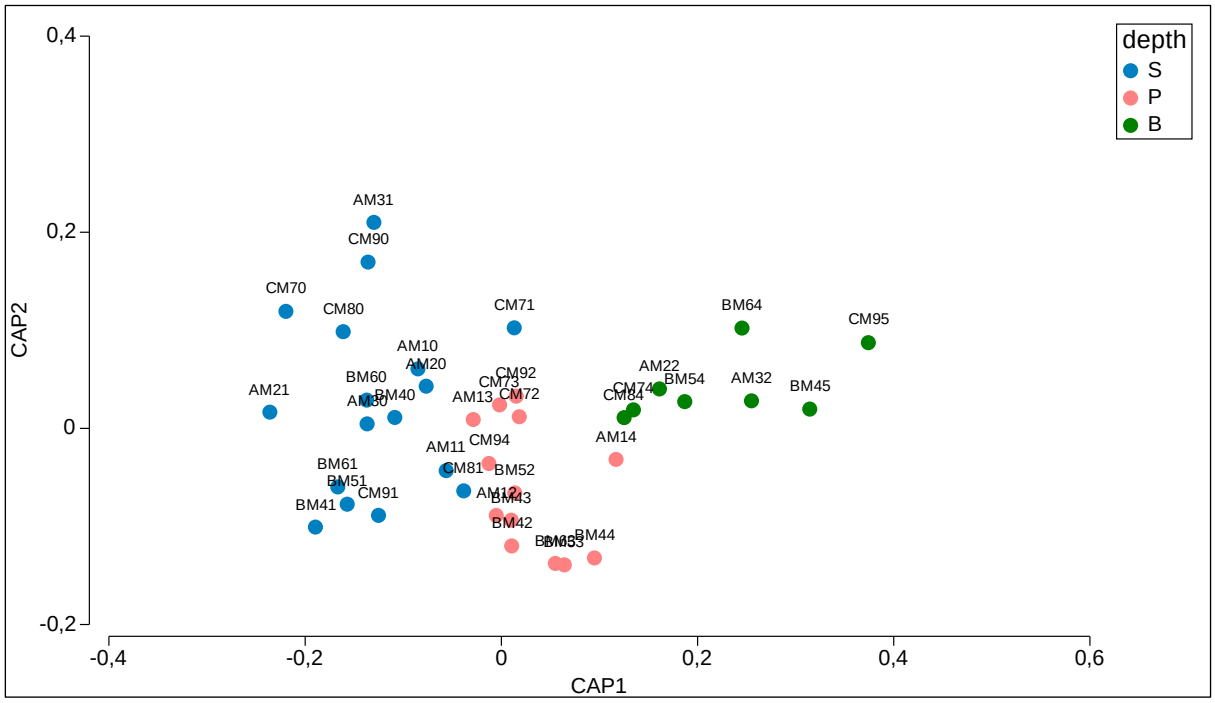
Figure 3: CAP analysis of water samples in three sample areas A, B and C of port Sloehaven



Effect of sampling depth

There is some effect of depth (surface, pelagic and bottom) on the occurrence of MOTU communities. For both seasons, May and September, communities of surface samples were siginificantly different from bottom samples (rep PERMANOVA p=0.001 and PERMANOVA p=0.001). For pelagic samples the patterns is less clear. In May there was a difference with bottom samples (PERMANOVA p=0.001) but not with surface samples (PERMANOVA p=0.303), suggesting there is no clear separation. In September the pelagic samples are not significantly different from both surface (PERMANOVA p=0.431) and bottom samples (PERMANOVA p=0.308). The May analysis is visualized by a CAP analysis (figure 4). Based on this result, one can conclude that it is necessary to analyse water samples from several depths when doing eDNA analyses aiming at detecting as many marine species as possible.

Figure 4: CAP analysis of water samples at different sampling depths in May, port Sloehaven. S = surface, P = pelagic, B = bottom.



Effect of environmental variables

Only for the September samples there was a significant relation with environmental parameters. The combination of turbidity, temperature and PH explained the patterns of the MOTUs in the samples best (BEST p<0.01).

Discussion and recommendations

Our study demonstrates that analysing environmental DNA and bulk DNA can complement the conventional OSPAR/HELCOM surveys for detecting NIS. A total of 53 species were detected with DNA analysis that were not detected with conventional sampling, of which five were NIS. Especially for annelids and arthropods the DNA analyses appear to perform well and give good indication of the presence of these species in life stages that are missed in the conventional survey. This probably concerns the early life stages that are present in the sediment or in the zooplankton community in the water column. These early life stages are missed within the traditional survey methodologies or, e.g. for some zooplankton species, may not show any obvious morphological characters that can be used to distinguish closely related species. Therefore, we advise to implement a sampling strategy that contains both visual identification and DNA analysis.

The efforts put into the conventional sampling compared to DNA sampling were far from equal. For example, during conventional sampling between 18 - 27 fouling plates and 16 scrape samples were analysed, compared to two of both for DNA analysis. Furthermore, the conventional sampling comprised methods not deployed for DNA analysis at all, like 45 dyke samples and 14 hand dredge samples. Increasing the number of biological samples would greatly improve the number of species detected by DNA analysis, as our results indicated that we did not capture the full range of MOTUs present at the study site. Differences in communities between sampling season, sampling area and sampling depth within port Sloehaven support the view of a sampling strategy that should cover both temporal and spatial variation. The reason why the spatial distribution of MOTUs in September could be explained by environmental variables, but the MOTU distribution in May could not, is unknown.

There are some considerations and methodological challenges to sort out before DNA analysis could function as a fully mature survey tool for NIS detection. Our inventory shows that DNA Barcodes at the international Barcode of Life Database BOLD are available for only 62% of the NIS recorded in the Netherlands. So 38% of the species cannot be detected or reliably indentified using (meta)barcoding. Like several previous studies conclude (Darling et al. 2017), we recommend to spend more time and money in collecting specimens of marine NIS and their congeneric species, to identify them by specialized taxonomists and to determine their DNA barcodes, in order to complete a reliable DNA reference database for future NIS biomonitoring.

Although some markers are called universal in literature, at present there are no markers that are truly useable to detect and identify all species in a sample at the same time. CO1 is the global standard to identify animals, and part of this gene is used as DNA marker in this study. CO1 is variable enough within most taxa to distinguish between species, like molluscs. CO1 Is the marker that is most present in reference libraries. However, many closely related cnidarian species, like corals and sea-anemones, cannot all be distinguished based on CO1 (Stefaniak et al. 2009). For ascidians, there are specific COI primers are available which were not used in the present study, but may resolve the underscoring of this group of species with DNA-analyses.

Naturalis is currently testing and evaluating the use of different universal primers in situ with the software package PrimerMiner (Elbrecht & Leese 2017a), with the preliminary conclusion that two new primers sets, BF2/BR2 (Elbrecht & Leese 2017b) and Leray XT (Wangensteen et al. in press) outperform the original Leray primerset mCOIintf/jgHCO used for the Sloehaven samples, in (theoretically) detecting a broader range of species. More research is needed to determine which NIS, and marine species in general, can be identified by which universal marker, to develop a multi-marker approach enabling the detection of (virtually) all species by DNA-analyses.

The turnaround time of 10 hours of extracellular DNA in seawater is short compared to the turnaround time of 29 – 93 days in sediments (Dell'Anno & Corinaldesi 2004). However, in both cases it is still possible that the recorded DNA signal comes from dead organisms floating in the water, leaking DNA, and therefore lasting much longer than extracellular DNA does. An alternative approach would be to analyse RNA as a better indicator for living organisms. At Naturalis and GiMaRIS, we haven't experimented with RNA analyses yet but this would be a logic option for future research.

In this study we applied community-based surveys by using a general marker to identify many species at the same time. The alternative is a targeted survey where species-specific primers are used to detect a single species or a single group of species of interest in a study area, which has been applied for the bivalve *Rangia cuneata*, among others (Adura et al. 2015). This methodology proved to be more sensitive and has the additional benefit that the amount of DNA could be determined using a ddPCR technique. In this way, differences in relative abundance between samples could be estimated. For some NIS of highly interest it could be a consideration to develop and deploy species-specific primers. As suggested in other studies, it could be useful to implement a two step approach, to do both a non-targeted community scanning for early warning combined with species-specific surveys for high priority species.

Conclusion

Based on the results of our research we conclude that analysis of environmental DNA in harbors can yield complementary information to a conventional monitoring approach, and it is advised to combine both types of surveys to obtain a maximum result = number of NIS. Especially for small and hard to identify species and life stages, such as plankton, DNA proves to be useful and could therefore lead to a more sensitive early warning system. We expect the role of metabarcoding in NIS detection to increase during the forthcoming years.

However, there are some challenges to resolve for the DNA methodology to mature. We present a shortlist of possible activities:

- All established and expected marine NIS and their close relatives should be collected, stored and be DNA sequenced, as a DNA reference and for future development of new, more effective DNA markers based full mitochondrial DNA analysis.
- Marine taxa, with an emphasis on NIS, should be analysed to define the uniqueness of their DNA profile compared to close relatives.
- It might be considered to experiment with targeted NIS detection, for high risk species. Targeted detection is much more sensitive and the amount of DNA can be determined accurately and compared between sites.
- New primer combinations are available that could greatly improve the results of NIS studies. However, this is an ongoing evolutionairy process. Especially for cnidarians and tunicates we might have to develop group specific primers. Different primers can be pooled into a primer cocktail before use.
- More research is required to separate living species from dead species by using RNA instead of DNA. Furthermore, for some specific high-risk species it might prove useful to determine the status of establishment, for example by using metabolomic techniques.

Acknowledgements

This study was commisioned by the Office for Risk Assessment and Research, Netherlands Food and Consumer Product Safety Authority, Utrecht, The Netherlands. Labwork was done by Frank Stokvis, Rob Pastoor, Elza Duijm and two BSc students Mejdy Kacem and Kevin Ocké. Bioinformatics was done bij Marten Hoogeveen.

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Appendix 1 Water sample parameters spring 2016

Area	*Sample loc.*	*Depth*	*Turbidity (ntu)*	*Water temp (°C)*	*pH*	*Salinity (ppt)*	*Salinity (PSU)*
A	5	0.3	3.28	9.9	8.26	25.73	33.90
A	5	1	3.5	9.9	8.27	25.82	34.03
A	5	3 (bottom)	142	9.8	8.23	25.78	33.98
A	19	0.3	1.83	9.9	8.19	26.00	34.30
A	19	1	3.2	9.9	8.25	25.92	34.18
A	19	4	3.82	9.9	8.27	25.96	34.23
A	19	5	3.91	9.9	8.27	25.98	34.28
A	19	7	4.67	9.8	8.27	26.22	34.63
A	19	8 (bottom)	5.62	9.8	8.26	26.18	34.57
A	29	0.3	2.28	9.9	8.28	25.80	34.01
A	29	1	2.22	9.9	8.27	25.72	33.89
A	29	4 (bottom)	38.8	9.9	8.27	25.76	33.95
B	63	0.3	1.41	9.9	8.26	25.53	33.61
B	63	1	1.57	9.9	8.26	25.48	33.54
B	63	4	2.24	9.9	8.27	25.53	33.62
B	63	5	3.18	9.8	8.27	25.64	33.77
B	63	6.5 (bottom)	7.66	9.8	8.25	26.36	34.83
B	71	0.3	1.27	9.9	8.27	25.55	33.65
B	71	1	1.96	9.9	8.27	25.49	33.55
B	71	4	2.34	9.9	8.26	25.66	33.81
B	71	5	2.8	9.8	8.26	25.73	33.90
B	71	6.5 (bottom)	2.54	9.8	8.25	25.87	34.12
B	82	0.3	4.55	9.9	8.24	25.37	33.38
B	82	1	5.66	9.9	8.25	25.46	33.52
B	82	4	2.34	9.9	8.27	25.46	33.51
B	82	5	2.59	9.8	8.27	25.66	33.80
B	82	7	2.14	9.8	8.26	26.33	34.80
B	82	9.5 (bottom)	4.79	9.8	8.27	27.34	36.30
C	96	0.3	2.32	9.9	8.24	25.76	33.96
C	96	1	2.41	9.9	8.25	25.81	34.02
C	96	4	2.6	9.9	8.24	25.83	34.05
C	96	5	2.96	9.9	8.24	25.78	33.98
C	96	6.2 (bottom)	5.09	9.8	8.23	25.76	33.95
C	114	0.3	2.49	9.9	8.26	25.80	34.00
C	114	1	3.35	9.9	8.26	25.81	34.03
C	114	4	2.23	9.9	8.25	25.77	33.96
C	114	5	2.14	9.8	8.25	25.75	33.95
C	114	7 (bottom)	5.46	9.8	8.24	25.81	34.04
C	124	0.3	1.73	9.9	8.21	25.61	33.75
C	124	1	1.4	9.9	8.23	25.78	33.99
C	124	4	2.61	9.9	8.23	25.84	34.07
C	124	5	2.97	9.8	8.22	25.89	34.16
C	124	7	3.5	9.8	8.22	26.29	34.74
C	124	7.5 (bottom)	5.41	9.8	8.22	26.41	34.92

Appendix 2 Water sample parameters autumn 2016

Area	*Sample loc.*	*Depth*	*Turbidity (ntu)*	*Water temp (°C)*	*pH*	*Salinity (ppt)*	*Salinity (PSU)*
A	19	0.3	3.00	21.0	7.90	20.49	26.26
A	19	1	3.72	21.0	8.05	20.63	26.46
A	19	4	3.81	21.0	8.08	20.77	26.65
A	19	7	4.60	20.8	8.10	20.78	26.68
A	19	10	4.83	20.6	8.10	21.13	27.18
A	19	13 (bottom)	5.36	20.5	8.10	21.88	28.25
A	29	0.3	2.69	20.9	8.08	21.19	27.26
A	29	1	3.46	20.9	8.08	21.14	27.19
A	29	4	3.83	20.8	8.09	21.27	27.37
A	29	6.5 (bottom)	20.40	20.5	8.10	21.53	27.74
A	5	0.3	6.00	21.1	8.12	20.88	26.82
A	5	1	7.12	21.0	8.13	20.85	26.77
A	5	4	6.98	20.8	8.13	20.92	26.87
A	5	5.5 (bottom)	71.50	20.0	8.11	20.99	26.98
B	82	0.3	2.52	21.0	8.12	21.00	26.98
B	82	1	4.85	21.0	8.13	20.99	26.96
B	82	4	2.15	20.8	8.11	21.06	27.06
B	82	7	4.61	20.3	8.11	21.33	27.45
B	82	10	9.79	20.0	8.13	22.05	28.49
B	82	10.5 (bottom)	46.90	20.0	8.16	22.16	28.64
B	71	0.3	3.03	21.0	8.11	21.03	27.01
B	71	1	3.74	21.0	8.11	20.94	26.89
B	71	4	2.31	20.8	8.09	21.01	27.00
B	71	7	4.03	20.5	8.09	21.21	27.29
B	71	9.5 (bottom)	49.10	20.4	8.09	21.79	28.12
B	63	0.3	3.03	21.0	8.12	21.09	27.11
B	63	1	5.80	21.0	8.11	21.03	27.03
B	63	4	8.85	20.8	8.12	21.03	27.03
B	63	6.5 (bottom)	15.90	20.5	8.10	21.39	27.54
B	96	0.3	4.82	22.0	8.08	21.14	27.18
B	96	1	2.79	22.0	8.08	21.07	27.08
B	96	4	3.03	21.8	8.07	21.09	27.11
B	96	7	6.67	21.5	8.08	21.37	27.52
B	96	8 (bottom)	22.50	21.0	8.07	21.58	27.82
C	114	0.3	1.55	21.5	8.08	21.06	27.08
C	114	1	2.76	21.5	8.04	21.10	27.13
C	114	4	2.96	21.0	8.07	21.15	27.21
C	114	7	3.94	21.0	8.08	21.46	27.64
C	114	8.5 (bottom)	23.84	21.0	8.07	21.53	27.74
C	124	0.3	1.92	21.0	8.08	21.20	27.28
C	124	1	1.99	21.0	8.05	21.17	27.23
C	124	4	2.37	20.8	8.07	21.17	27.23
C	124	7	3.76	20.8	8.06	21.27	27.37
C	124	8.7 (bottom)	8.53	20.5	8.07	21.36	27.49

Appendix 3 NIS species (n=182)

Recorded in the Netherlands. Number of specimens collected, DNA Barcodes available ob BOLD and presence (1) or absence (0) on the OSPAR Target Species List.

Species	*Phylum*	*Class*	*Specimen*	*DNA Barcodes*	*OSPAR*
Marionina southerni	Annelida	Clitellata	14	14	0
Monopylephorus parvus	Annelida	Clitellata	0	0	0
Hemibdella soleae	Annelida	Clitellata	0	0	0
Platybdella anarrhichae	Annelida	Clitellata	1	1	0
Syllidia armata	Annelida	Polychaeta	4	4	0
Alitta virens	Annelida	Polychaeta	65	59	0
Sabellaria spinulosa	Annelida	Polychaeta	3	3	0
Bispira polyomma	Annelida	Polychaeta	0	0	0
Branchiomma bombyx	Annelida	Polychaeta	0	0	0
Ficopomatus enigmaticus	Annelida	Polychaeta	5	0	1
Hydroides elegans	Annelida	Polychaeta	6	2	1
Neodexiospira brasiliensis	Annelida	Polychaeta	11	1	0
Pileolaria berkeleyana	Annelida	Polychaeta	0	0	0
Boccardia proboscidea	Annelida	Polychaeta	23	0	0
Boccardiella hamata	Annelida	Polychaeta	5	5	0
Boccardiella ligerica	Annelida	Polychaeta	0	0	0
Marenzelleria neglecta	Annelida	Polychaeta	31	22	1
Marenzelleria viridis	Annelida	Polychaeta	13	13	1
Polydora hoplura	Annelida	Polychaeta	0	0	0
Penilia avirostris	Arthropoda	Branchiopoda	44	22	0
Acartia (Acanthacartia) tonsa	Arthropoda	Hexanauplia	323	299	1
Eurytemora americana	Arthropoda	Hexanauplia	0	0	0
Mytilicola intestinalis	Arthropoda	Hexanauplia	5	5	0
Mytilicola orientalis	Arthropoda	Hexanauplia	2	2	0
Conchoderma auritum	Arthropoda	Hexanauplia	13	9	0
Conchoderma virgatum	Arthropoda	Hexanauplia	4	1	0
Lepas (Anatifa) pectinata	Arthropoda	Hexanauplia	17	17	0
Austrominius modestus	Arthropoda	Hexanauplia	19	10	1
Amphibalanus amphitrite	Arthropoda	Hexanauplia	163	161	0
Amphibalanus eburneus	Arthropoda	Hexanauplia	30	26	1
Amphibalanus improvisus	Arthropoda	Hexanauplia	50	37	0
Balanus balanus	Arthropoda	Hexanauplia	109	97	0
Megabalanus coccopoma	Arthropoda	Hexanauplia	6	6	0
Megabalanus tintinnabulum	Arthropoda	Hexanauplia	13	13	0
Telmatogeton japonicus	Arthropoda	Insecta	10	7	0
Prokelisia marginata	Arthropoda	Insecta	0	0	0
Caprella mutica	Arthropoda	Malacostraca	328	18	1
Chelicorophium curvispinum	Arthropoda	Malacostraca	47	24	0
Corophium multisetosum	Arthropoda	Malacostraca	15	13	0
Monocorophium acherusicum	Arthropoda	Malacostraca	42	30	0
Monocorophium sextonae	Arthropoda	Malacostraca	5	5	0
Monocorophium uenoi	Arthropoda	Malacostraca	0	0	0
Gammarus tigrinus	Arthropoda	Malacostraca	159	153	1
Ptilohyale littoralis	Arthropoda	Malacostraca	0	0	0

Jassa marmorata	Arthropoda	Malacostraca	157	144	0
Melita nitida	Arthropoda	Malacostraca	284	282	0
Incisocalliope aestuarius	Arthropoda	Malacostraca	0	0	0
Cryptorchestia cavimana	Arthropoda	Malacostraca	10	1	0
Platorchestia platensis	Arthropoda	Malacostraca	234	196	0
Palaemon macrodactylus	Arthropoda	Malacostraca	48	30	1
Palinurus elephas	Arthropoda	Malacostraca	96	88	0
Rhithropanopeus harrisii	Arthropoda	Malacostraca	258	256	1
Callinectes sapidus	Arthropoda	Malacostraca	60	50	1
Eriocheir sinensis	Arthropoda	Malacostraca	67	62	1
Hemigrapsus sanguineus	Arthropoda	Malacostraca	163	85	1
Hemigrapsus takanoi	Arthropoda	Malacostraca	33	29	1
Proasellus coxalis	Arthropoda	Malacostraca	63	62	0
Gnathia maxillaris	Arthropoda	Malacostraca	9	8	0
Idotea metallica	Arthropoda	Malacostraca	12	12	0
Idotea neglecta	Arthropoda	Malacostraca	7	6	0
Ianiropsis serricaudis	Arthropoda	Malacostraca	1	0	0
Janira maculosa	Arthropoda	Malacostraca	27	23	0
Limnoria quadripunctata	Arthropoda	Malacostraca	223	58	0
Uromunna	Arthropoda	Malacostraca	0	0	0
Cymodoce truncata	Arthropoda	Malacostraca	21	14	0
Dynamene bidentata	Arthropoda	Malacostraca	66	46	0
Hemimysis anomala	Arthropoda	Malacostraca	9	9	1
Neomysis americana	Arthropoda	Malacostraca	21	20	0
Sinelobus vanhaareni	Arthropoda	Malacostraca	0	0	0
Zeuxo holdichi	Arthropoda	Malacostraca	0	0	0
Limulus polyphemus	Arthropoda	Merostomata	17	13	0
Ammothea hilgendorfi	Arthropoda	Pycnogonida	2	2	0
Bugula neritina	Bryozoa	Gymnolaemata	160	142	0
Bugulina simplex	Bryozoa	Gymnolaemata	2	0	0
Bugulina stolonifera	Bryozoa	Gymnolaemata	18	9	0
Tricellaria inopinata	Bryozoa	Gymnolaemata	5	3	0
Fenestrulina delicia	Bryozoa	Gymnolaemata	0	0	0
Pacificincola perforata	Bryozoa	Gymnolaemata	0	0	0
Smittoidea prolifica	Bryozoa	Gymnolaemata	2	2	0
Arachnidium lacourti	Bryozoa	Gymnolaemata	0	0	0
Amathia gracilis	Bryozoa	Gymnolaemata	5	4	0
Amathia imbricata	Bryozoa	Gymnolaemata	2	2	0
Victorella pavida	Bryozoa	Gymnolaemata	3	1	0
Walkeria uva	Bryozoa	Gymnolaemata	0	0	0
Pectinatella magnifica	Bryozoa	Phylactolaemata	2	2	0
Poecilia reticulata	Chordata	Actinopterygii	176	107	0
Gobiusculus flavescens	Chordata	Actinopterygii	16	15	0
Neogobius melanostomus	Chordata	Actinopterygii	150	134	1
Trinectes maculatus	Chordata	Actinopterygii	16	11	0
Oncorhynchus kisutch	Chordata	Actinopterygii	209	196	0
Oncorhynchus mykiss	Chordata	Actinopterygii	461	429	0
Sebastes schlegelii	Chordata	Actinopterygii	22	21	0
Didemnum vexillum	Chordata	Ascidiacea	115	109	1
Diplosoma listerianum	Chordata	Ascidiacea	90	77	0
Aplidium glabrum	Chordata	Ascidiacea	0	0	0
Corella eumyota	Chordata	Ascidiacea	25	19	0

Perophora japonica	Chordata	Ascidiacea	15	1	0
Molgula manhattensis	Chordata	Ascidiacea	58	46	0
Botrylloides violaceus	Chordata	Ascidiacea	0	0	0
Botryllus schlosseri	Chordata	Ascidiacea	489	445	0
Styela calva	Chordata	Ascidiacea	0	0	0
Styela clava	Chordata	Ascidiacea	70	59	0
Diadumene cincta	Cnidaria	Anthozoa	6	0	0
Diadumene lineata	Cnidaria	Anthozoa	22	14	0
Edwardsia claparedii	Cnidaria	Anthozoa	1	0	0
Pachycordyle navis	Cnidaria	Hydrozoa	0	0	0
Cordylophora caspia	Cnidaria	Hydrozoa	17	11	0
Moerisia inkermanica	Cnidaria	Hydrozoa	0	0	0
Blackfordia virginica	Cnidaria	Hydrozoa	25	25	0
Gonionemus vertens	Cnidaria	Hydrozoa	13	9	0
Mnemiopsis leidyi	Ctenophora	Tentaculata	17	1	1
Barentsia matsushimana	Entoprocta	Entoprocta incertae sedis	0	0	0
Barentsia mutabilis	Entoprocta	Entoprocta incertae sedis	0	0	0
Barentsia ramosa	Entoprocta	Entoprocta incertae sedis	0	0	0
Ensis leei	Mollusca	Bivalvia	0	0	1
Glycymeris glycymeris	Mollusca	Bivalvia	26	23	0
Rangia cuneata	Mollusca	Bivalvia	13	7	1
Dreissena polymorpha	Mollusca	Bivalvia	127	115	1
Mytilopsis leucophaeata	Mollusca	Bivalvia	19	18	1
Mya arenaria	Mollusca	Bivalvia	213	112	0
Psiloteredo megotara	Mollusca	Bivalvia	0	0	0
Teredo navalis	Mollusca	Bivalvia	44	0	0
Crassostrea virginica	Mollusca	Bivalvia	207	193	0
Magallana angulata	Mollusca	Bivalvia	0	0	0
Magallana gigas	Mollusca	Bivalvia	11	10	1
Anomia ephippium	Mollusca	Bivalvia	8	0	0
Pecten maximus	Mollusca	Bivalvia	13	4	0
Corbicula fluminalis	Mollusca	Bivalvia	7	7	0
Corbicula fluminea	Mollusca	Bivalvia	138	124	1
Mercenaria mercenaria	Mollusca	Bivalvia	227	221	0
Petricolaria pholadiformis	Mollusca	Bivalvia	8	5	0
Ruditapes philippinarum	Mollusca	Bivalvia	370	361	0
Calyptraea chinensis	Mollusca	Gastropoda	3	3	0
Crepidula fornicata	Mollusca	Gastropoda	85	70	1
Littorina compressa	Mollusca	Gastropoda	1	1	0
Potamopyrgus antipodarum	Mollusca	Gastropoda	316	300	0
Ocinebrellus inornatus	Mollusca	Gastropoda	15	11	0
Rapana venosa	Mollusca	Gastropoda	97	97	1
Urosalpinx cinerea	Mollusca	Gastropoda	56	46	0
Corambe obscura	Mollusca	Gastropoda	10	9	0
Calliostoma zizyphinum	Mollusca	Gastropoda	23	11	0
Steromphala cineraria	Mollusca	Gastropoda	0	0	0
Phorcus lineatus	Mollusca	Gastropoda	71	60	0
Leptochiton cancellatus	Mollusca	Polyplacophora	18	1	0
Anguillicoloides crassus	Nematoda	Chromadorea	0	0	0
Crassicauda boopis	Nematoda	Chromadorea	0	0	0
Cephalothrix simula	Nemertea	Palaeonemertea	13	13	0
Euplana gracilis	Platyhelminthes	Rhabditophora	8	1	0

Stylochus (Stylochus) flevensis	Platyhelminthes	Rhabditophora	0	0	0
Leucosolenia somesii	Porifera	Calcarea	0	0	0
Sycon scaldiense	Porifera	Calcarea	0	0	0
Chalinula loosanoffi	Porifera	Demospongiae	0	0	0
Haliclona (Rhizoniera) rosea	Porifera	Demospongiae	0	0	0
Haliclona (Soestella) xena	Porifera	Demospongiae	0	0	0
Celtodoryx ciocalyptoides	Porifera	Demospongiae	0	0	0
Mycale (Carmia) micracanthoxea	Porifera	Demospongiae	0	0	0
Hymeniacidon perlevis	Porifera	Demospongiae	42	39	0
Suberites massa	Porifera	Demospongiae	0	0	0
Alexandrium leei	Myzozoa	Dinophyceae	0	0	0
Karenia mikimotoi	Myzozoa	Dinophyceae	0	0	1
Corynophlaea verruculiformis	Ochrophyta	Phaeophyceae	0	0	0
Myriactula rivulariae	Ochrophyta	Phaeophyceae	0	0	0
Sargassum muticum	Ochrophyta	Phaeophyceae	0	0	0
Undaria pinnatifida	Ochrophyta	Phaeophyceae	0	0	1
Codium fragile	Chlorophyta	Ulvophyceae	71	7	0
Codium fragile subsp. fragile	Chlorophyta	Ulvophyceae	0	0	0
Ulva australis	Chlorophyta	Ulvophyceae	69	25	0
Antithamnionella spirographidis	Rhodophyta	Florideophyceae	0	0	0
Antithamnionella ternifolia	Rhodophyta	Florideophyceae	0	0	0
Dasya baillouviana	Rhodophyta	Florideophyceae	0	0	0
Dasysiphonia	Rhodophyta	Florideophyceae	0	0	0
Melanothamnus harveyi	Rhodophyta	Florideophyceae	0	0	0
Polysiphonia senticulosa	Rhodophyta	Florideophyceae	0	0	0
Anotrichium furcellatum	Rhodophyta	Florideophyceae	0	0	0
Colaconema dasyae	Rhodophyta	Florideophyceae	0	0	0
Acrochaetium catenulatum	Rhodophyta	Florideophyceae	0	0	0
Acrochaetium densum	Rhodophyta	Florideophyceae	0	0	0
Agardhiella subulata	Rhodophyta	Florideophyceae	0	0	0
Grateloupia turuturu	Rhodophyta	Florideophyceae	0	0	1
Lomentaria hakodatensis	Rhodophyta	Florideophyceae	0	0	0
Cotula coronopifolia	Tracheophyta	Magnoliopsida	16	4	0
Spartina townsendii var. anglica	Tracheophyta	Magnoliopsida	0	0	0
Total species (n-182)			***119***	***111***	***30***

Appendix 4 Visual and DNA analysis fouling plates

NIS green highlighted (n=13)

Species	*Phylum*	*Class*	*Morph*	*DNA*
Arenicola defodiens	Annelida	Polychaeta		1
Arenicola marina	Annelida	Polychaeta		1
Ctenodrilus serratus	Annelida	Polychaeta		1
Ophryotrocha puerilis siberti	Annelida	Polychaeta		1
Platynereis dumerilii	Annelida	Polychaeta		1
Amphibalanus improvisus	Arthropoda	Hexanauplia	1	
Austrominius modestus	Arthropoda	Hexanauplia	1	1
Balanus balanus	Arthropoda	Hexanauplia		1
Balanus crenatus	Arthropoda	Hexanauplia	1	
Cyclops kikuchii	Arthropoda	Hexanauplia		1
Euterpina acutifrons	Arthropoda	Hexanauplia		1
Nitokra spinipes spinipes	Arthropoda	Hexanauplia		1
Temora longicornis	Arthropoda	Hexanauplia		1
Carcinus maenas	Arthropoda	Malacostraca		1
Hemigrapsus sanguineus	Arthropoda	Malacostraca		1
Pilumnus hirtellus	Arthropoda	Malacostraca		1
Pisidia longicornis	Arthropoda	Malacostraca		1
Bugula neritina	Bryozoa	Gymnolaemata		1
Conopeum reticulum	Bryozoa	Gymnolaemata	1	
Cryptosula pallasiana	Bryozoa	Gymnolaemata	1	
Tricellaria inopinata	Bryozoa	Gymnolaemata	1	
Tricellaria occidentalis	Bryozoa	Gymnolaemata		1
Aplidium glabrum	Chordata	Ascidiacea	1	
Ascidiella aspersa	Chordata	Ascidiacea	1	
Botrylloides violaceus	Chordata	Ascidiacea	1	1
Ciona intestinalis	Chordata	Ascidiacea	1	
Didemnum vexillum	Chordata	Ascidiacea	1	
Diplosoma listerianum	Chordata	Ascidiacea	1	
Styela clava	Chordata	Ascidiacea	1	
Metridium dianthus	Cnidaria	Anthozoa	1	
Clytia hemisphaerica	Cnidaria	Hydrozoa	1	
Hartlaubella gelatinosa	Cnidaria	Hydrozoa	1	
Obelia dichotoma	Cnidaria	Hydrozoa	1	
Obelia geniculata	Cnidaria	Hydrozoa	1	
Obelia longissima	Cnidaria	Hydrozoa	1	
Calvadosia cruciformis	Cnidaria	Staurozoa		1
Ophiothrix fragilis	Echinodermata	Ophiuroidea		1
Magallana gigas	Mollusca	Bivalvia		1
Crepidula fornicata	Mollusca	Gastropoda		1
Leathesia marina	Ochrophyta	Phaeophyceae		1
Halichondria (Halichondria) panicea	Porifera	Demospongiae	1	1
		Total	***20***	***24***

Appendix 5 Species Sloehaven

An overview of the species recordedin de Sloehaven, divided to environmental DNA (CO1) and conventional methods, and to the substrates/samples they were found.

Species	Phylum	Class	COI	Maraholoav	Bulk COI	Sediment COI	Scrape COI	SETL COI	Water COI	Pathogens	Zooplankton	Phytoplankton	Gelatinaous Zooplankton	Crab cage	Fish cage	Hand dredge	Video	Dike	Scrape	SETL	Petit Ponar
Amphichaeta sannio	Annelida	Clitellata	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tubificoides brownae	Annelida	Clitellata	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tubificoides diazi	Annelida	Clitellata	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Arenicola defodiens	Annelida	Polychaeta	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Arenicola marina	Annelida	Polychaeta	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Capitellidae sp. CMC01	Annelida	Polychaeta	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ctenodrilus serratus	Annelida	Polychaeta	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Ficopomatus enigmaticus	Annelida	Polychaeta	1	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0
Lagis koreni	Annelida	Polychaeta	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Magelona johnstoni	Annelida	Polychaeta	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Nephtys hombergii	Annelida	Polychaeta	1	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Nereis pelagica	Annelida	Polychaeta	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Ophryotrocha puerilis siberti	Annelida	Polychaeta	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Owenia mitraria	Annelida	Polychaeta	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Pectinaria koreni	Annelida	Polychaeta	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pherusa affinis	Annelida	Polychaeta	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pherusa flabellata	Annelida	Polychaeta	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Platynereis dumerilii	Annelida	Polychaeta	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Polydora cornuta	Annelida	Polychaeta	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Protodrilus adhaerens	Annelida	Polychaeta	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Scoloplos armiger	Annelida	Polychaeta	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Serpula vermicularis	Annelida	Polychaeta	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1
Spirobranchus triqueter	Annelida	Polychaeta	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0
Spirorbis (Spirorbis) spirorbis	Annelida	Polychaeta	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Streblospio benedicti	Annelida	Polychaeta	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acartia (Acanthacartia) tonsa	Arthropoda	Hexanauplia	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Acartia bifilosa	Arthropoda	Hexanauplia	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Acartia clausii	Arthropoda	Hexanauplia	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Amphibalanus improvisus	Arthropoda	Hexanauplia	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Austrominius modestus	Arthropoda	Hexanauplia	1	1	0	0	1	1	0	0	0	0	0	1	0	0	1	1	1	1	1

Balanus balanus	Arthropoda	Hexanauplia	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Balanus crenatus	Arthropoda	Hexanauplia	0	1	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1
Cyclops kikuchii	Arthropoda	Hexanauplia	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Euterpina acutifrons	Arthropoda	Hexanauplia	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Nitokra spinipes	Arthropoda	Hexanauplia	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Paracalanus parvus	Arthropoda	Hexanauplia	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Semibalanus balanoides	Arthropoda	Hexanauplia	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Temora longicornis	Arthropoda	Hexanauplia	1	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Zygomolgus dentatus	Arthropoda	Hexanauplia	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Caprella mutica	Arthropoda	Malacostraca	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Carcinus maenas	Arthropoda	Malacostraca	1	1	0	0	1	1	0	0	0	0	0	1	1	0	1	1	1	1	1
Crangon crangon	Arthropoda	Malacostraca	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Echinogammarus stoerensis	Arthropoda	Malacostraca	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Hemigrapsus sanguineus	Arthropoda	Malacostraca	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0
Hemigrapsus takanoi	Arthropoda	Malacostraca	1	1	0	0	1	0	0	0	0	0	0	1	1	0	0	1	1	1	0
Jassa marmorata	Arthropoda	Malacostraca	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1
Macropodia rostrata	Arthropoda	Malacostraca	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Melita nitida	Arthropoda	Malacostraca	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Mesopodopsis slabberi	Arthropoda	Malacostraca	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Necora puber	Arthropoda	Malacostraca	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0
Palaemon longirostris	Arthropoda	Malacostraca	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Pilumnus hirtellus	Arthropoda	Malacostraca	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Pisidia longicornis	Arthropoda	Malacostraca	1	1	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0
Porcellana platycheles	Arthropoda	Malacostraca	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1
Alcyonidioides mytili	Bryozoa	Gymnolaemata	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Bugula neritina	Bryozoa	Gymnolaemata	1	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Bugulina stolonifera	Bryozoa	Gymnolaemata	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Conopeum reticulum	Bryozoa	Gymnolaemata	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1
Cryptosula pallasiana	Bryozoa	Gymnolaemata	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Electra pilosa	Bryozoa	Gymnolaemata	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Scrupocellaria scruposa	Bryozoa	Gymnolaemata	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	1	
Smittoidea prolifica	Bryozoa	Gymnolaemata	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Tricellaria inopinata	Bryozoa	Gymnolaemata	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Tricellaria occidentalis	Bryozoa	Gymnolaemata	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Bathycoccus prasinos	Chlorophyta	Mamiellophyceae	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Mantoniella squamata	Chlorophyta	Mamiellophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Micromonas pusilla	Chlorophyta	Mamiellophyceae	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Cymbomonas tetramitiformis	Chlorophyta	Pyramimonadophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pseudoscourfieldia marina	Chlorophyta	Pyramimonadophyceae	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pyramimonas longicauda	Chlorophyta	Pyramimonadophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ulva australis	Chlorophyta	Ulvophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Ulva curvata	Chlorophyta	Ulvophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Ulva intestinalis	Chlorophyta	Ulvophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Ulva prolifera	Chlorophyta	Ulvophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Atherina presbyter	Chordata	Actinopterygii	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Gobius niger	Chordata	Actinopterygii	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Holocentrus adscensionis	Chordata	Actinopterygii	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Lutjanus mahogoni	Chordata	Actinopterygii	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Pholis gunnellus	Chordata	Actinopterygii	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pomatoschistus microps	Chordata	Actinopterygii	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Pomatoschistus pictus	Chordata	Actinopterygii	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oikopleura (Vexillaria) dioica	Chordata	Appendicularia	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Aplidium glabrum	Chordata	Ascidiacea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Ascidiella aspersa	Chordata	Ascidiacea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Botrylloides violaceus	Chordata	Ascidiacea	1	1	1	0	1	1	0	0	0	0	0	0	0	0	1	0	1	1	0
Botryllus schlosseri	Chordata	Ascidiacea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Ciona intestinalis	Chordata	Ascidiacea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Didemnum vexillum	Chordata	Ascidiacea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0
Diplosoma listerianum	Chordata	Ascidiacea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Molgula manhattensis	Chordata	Ascidiacea	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	
Perophora japonica	Chordata	Ascidiacea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Styela clava	Chordata	Ascidiacea	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1
Chroicocephalus ridibundus	Chordata	Aves	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Rattus norvegicus	Chordata	Mammalia	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mesodinium rubrum	Ciliophora	Litostomatea	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Actinia equina	Cnidaria	Anthozoa	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Diadumene cincta	Cnidaria	Anthozoa	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Metridium senile	Cnidaria	Anthozoa	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1
Sagartia elegans	Cnidaria	Anthozoa	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Sagartia troglodytes	Cnidaria	Anthozoa	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Sagartiogeton undatus	Cnidaria	Anthozoa	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1
Sagartiogeton viduatus	Cnidaria	Anthozoa	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lovenella clausa	Cnidaria	Hydrozoa	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Obelia bidentata	Cnidaria	Hydrozoa	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Obelia dichotoma	Cnidaria	Hydrozoa	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Obelia longissima	Cnidaria	Hydrozoa	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	1
Cyanea lamarckii	Cnidaria	Scyphozoa	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Calvadosia cruciformis	Cnidaria	Staurozoa	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Leucocryptos marina	Cryptophyta	Cryptophyta incertae sedis	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Mnemiopsis leidyi	Ctenophora	Tentaculata	0	1	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	0	1
Pleurobrachia pileus	Ctenophora	Tentaculata	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Planktothrix agardhii	Cyanobacteria	Cyanophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ophiothrix fragilis	Echinodermata	Ophiuroidea	1	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Ophiura albida	Echinodermata	Ophiuroidea	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0

Ophiura ophiura	Echinoderma ta	Ophiuroidea	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Abra alba	Mollusca	Bivalvia	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Cerastoderma edule	Mollusca	Bivalvia	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Corbula gibba	Mollusca	Bivalvia	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Ensis directus	Mollusca	Bivalvia	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Kurtiella bidentata	Mollusca	Bivalvia	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Limecola balthica	Mollusca	Bivalvia	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Magallana angulata	Mollusca	Bivalvia	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Magallana gigas	Mollusca	Bivalvia	1	1	0	0	1	1	0	0	0	0	0	0	0	1	1	1	1	1	1
Mya arenaria	Mollusca	Bivalvia	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Mysella bidentata	Mollusca	Bivalvia	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Mytilus edulis	Mollusca	Bivalvia	0	1	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1
Ruditapes decussatus	Mollusca	Bivalvia	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Spisula subtruncata	Mollusca	Bivalvia	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Crepidula fornicata	Mollusca	Gastropoda	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0
Goniodoris castanea	Mollusca	Gastropoda	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Littorina littorea	Mollusca	Gastropoda	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Littorina obtusata	Mollusca	Gastropoda	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Patella vulgata	Mollusca	Gastropoda	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
Peringia ulvae	Mollusca	Gastropoda	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Tritia reticulata	Mollusca	Gastropoda	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1
Actiniscus pentasterias	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Akashiwo sanguinea	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Archaeperidiniu m minutum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Dinophysis acuminata	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gonyaulax spinifera	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gymnodinium galeatum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gymnodinium verruculosum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gyrodinium spirale	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Heterocapsa lanceolata	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Heterocapsa minima	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Heterocapsa rotundata	Myzozoa	Dinophyceae	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Katodinium glaucum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Nematopsides vigilans	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Noctiluca scintillans	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Peridinium achromaticum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Prorocentrum micans	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Prorocentrum triestinum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Protoperidinium bipes	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Protoperidinium claudicans	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Protoperidinium conicum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0

Protoperidinium depressum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Protoperidinium excentricum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Protoperidinium leonis	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Protoperidinium marie-lebouriae	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Protoperidinium ovatum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Protoperidinium pentagonum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Protoperidinium steinii	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Protoperidinium subinerme	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Protoperidinium thorianum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Torodinium robustum	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Warnowia polyphemus	Myzozoa	Dinophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Lineus longissimus	Nemertea	Anopla	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Hubrechtella dubia	Nemertea	Palaeonemertea	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Actinoptychus octonarius	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Actinoptychus senarius	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Asterionellopsis glacialis	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Asteroplanus karianus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Bacillaria paxillifera	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Bacteriastrum hyalinum	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Bellerochea polymorpha	Ochrophyta	Bacillariophyceae	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Biddulphia rhombus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Brockmanniella brockmannii	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Cerataulina pelagica	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Cerataulus radiatus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Ceratoneis closterium	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chaetoceros affinis	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chaetoceros curvisetus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chaetoceros danicus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chaetoceros debilis	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chaetoceros didymus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chaetoceros pseudocurvisetus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chaetoceros socialis	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chaetoceros subtilis	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Chaetoceros teres	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Coscinodiscus concinnus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0

Coscinodiscus perforatus var. pavillardii	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Coscinodiscus radiatus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Cymatosira belgica	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Dactyliosolen fragilissimus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Dactyliosolen phuketensis	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Delphineis minutissima	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ditylum brightwellii	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Ethmodiscus punctiger	Ochrophyta	Bacillariophyceae	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eucampia zodiacus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Grammatophora marina	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Guinardia delicatula	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Guinardia flaccida	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Guinardia striata	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gyrosigma balticum	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Helicotheca tamesis	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Lauderia annulata	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Lennoxia faveolata	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Leptocylindrus danicus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Leptocylindrus minimus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Lithodesmium undulatum	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Melosira moniliformis	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Membraneis challengeri	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Meuniera membranacea	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Minutocellus scriptus	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Navicula distans	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Neocalyptrella robusta	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Nitzschia lorenziana var. incerta	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Odontella longicruris	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Odontella sinensis	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Odontella turgida	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Paralia sulcata	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Plagiogrammopsis vanheurckii	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pleurosigma formosum	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Podosira stelligera	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pseudo-nitzschia americana	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pseudo-nitzschia delicatissima	Ochrophyta	Bacillariophyceae	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0

Pseudo-nitzschia fraudulenta	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pseudo-nitzschia pseudodelicatissima	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pseudo-nitzschia pungens	Ochrophyta	Bacillariophyceae	1	1	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0
Pseudo-nitzschia seriata	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pseudopodosira westii	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Rhaphoneis amphiceros	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Rhizosolenia imbricata	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Rhizosolenia setigera	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Roperia tesselata	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Skeletonema costatum	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Thalassionema nitzschioides	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Thalassiosira eccentrica	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Thalassiosira gravida	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Thalassiosira nordenskioeldii	Ochrophyta	Bacillariophyceae	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thalassiosira punctigera	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Trigonium alternans	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Tryblionella coarctata	Ochrophyta	Bacillariophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Dinobryon faculiferum	Ochrophyta	Chrysophyceae	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Pseudochattonella verruculosa	Ochrophyta	Dictyochophyceae	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ascophyllum nodosum	Ochrophyta	Phaeophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Fucus serratus	Ochrophyta	Phaeophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Fucus spiralis	Ochrophyta	Phaeophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Fucus vesiculosus	Ochrophyta	Phaeophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	1	0
Hincksia granulosa	Ochrophyta	Phaeophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Hincksia sandriana	Ochrophyta	Phaeophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Leathesia marina	Ochrophyta	Phaeophyceae	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Fibrocapsa japonica	Ochrophyta	Raphidophyceae	1	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Leptoplana tremellaris	Platyhelminthes	Rhabditophora	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Notocomplana koreana	Platyhelminthes	Rhabditophora	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Halichondria (Halichondria) bowerbanki	Porifera	Demospongiae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Halichondria (Halichondria) panicea	Porifera	Demospongiae	1	1	0	0	1	1	0	0	0	0	0	1	0	1	1	1	0	0	0
Haliclona (Haliclona) oculata	Porifera	Demospongiae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Protosuberites mereui	Porifera	Demospongiae	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Escherichia coli	Proteobacteria	Gammaproteobacteria	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Vibrio alginolyticus	Proteobacteria	Gammaproteobacteria	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Porphyra umbilicalis	Rhodophyta	Bangiophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0

Aglaothamnion roseum	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Antithamnionella spirographidis	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Ceramium cimbricum	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0
Ceramium virgatum	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Chondrus crispus	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Dasysiphonia japonica	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Hypoglossum hypoglossoides	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Neosiphonia harveyi	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Polysiphonia elongata	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Polysiphonia fucoides	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Polysiphonia stricta	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0
Pterothamnion plumula	Rhodophyta	Florideophyceae	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0