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A baseline study of the occurrence of non-indigenous species in Danish harbours

Photo: Henrik Carl, University of Copenhagen

Den Europæiske Union
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REPORT

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Summary

We report the first ever nation-wide study of the occurrence of non-indigenous species in Danish harbours. The sampling was carried out using both conventional and biomolecular methods (eDNA). In total, 16 harbours were covered – Esbjerg and Aarhus, the two largest harbours in Denmark, with intensive sampling and 14 harbours with a reduced programme. 26 non-indigenous species were recorded using conventional sampling and 13 species were recorded using eDNA-based methods. Excluding overlapping records, we have recorded a total of 34 non-indigenous species in the 16 harbours studied. Based on the results, we conclude the following: 1) more non-indigenous species are found in the western parts of Denmark (North Sea region) then in the eastern parts (Baltic Sea), and 2) a few species previously unseen in Danish marine waters were recorded, i.e. the two bristle worms *Eteone heteropoda* (fam. Phyllodocidae) and *Streblospio benedicti* (fam. Spionidae). Further, we provide a proof-of-concept regarding the overarching objectives of the MONIS 1-3 projects and the eDNA-based test systems developed. The results constitute a baseline for future studies in Danish ports and other hotspot areas.

Four keywords	Fire emneord
1. Non-indigenous species	1. Ikke-hjemmehørende arter
2. Monitoring	2. Overvågning
3. Marine Strategy Framework Directive (MSFD)	3. Havstrategidirektivet (HSD)
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MONIS 4

A baseline study of the occurrence of non-indigenous species in selected Danish harbours

Clients:
Danish Fisheries Agency and
Danish Environmental Protection Agency

Preface

We report the outcome of the first nation-wide study of the occurrence of non-indigenous species in Danish harbours. The results represent a baseline for occurrence of non-indigenous species in 16 carefully selected Danish harbours.

The overarching aims of this study – named the MONIS 4 project, indicating it is the fourth phase of the project named 'Monitoring of non-indigenous species in Danish marine waters' (abbreviated to MONIS) – have been:

- to monitor non-indigenous species in 16 Danish harbours,
- to make use of both conventional methods and molecular methods, the latter targeting a total 18 species (originally 21, but during reporting three assays were found to produce potentially false positive results and thus considered inaccurate) using operational eDNA-based test systems,
- to assess the occurrence of non-indigenous species in the harbours and to report the data to relevant data hosts, both nationally and internationally, and
- to provide a proof-of-concept regarding the overarching objectives of the earlier phases of the MONIS project, especially the test systems developed so far for detection of selected non-indigenous species in Danish marine waters.

The activities and the reporting have been funded by the Danish Fisheries Agency and carried out in a close and constructive dialogue with the Danish Environmental Protection Agency.

Copenhagen, 8 September 2022

Jesper H. Andersen

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The front cover photo is provided by Henrik Carl, National History Museum Denmark.

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1 Introduction

According to the UN's International Maritime Organization (IMO), invasive aquatic species are one of the four greatest threats to the World's oceans, and invasive species are known to cause severe environmental, economic and public health impacts. Such unwanted visitors from foreign ecosystems have historically "hitchhiked" on transport of goods, foodstuffs and people around the Globe, but in the recent decades the increasing trade volumes and the overall globalisation trend, have led to a rapid increase in the detection rates of non-indigenous species[1] (NIS) in most countries.

In Denmark, a number of research studies have identified approx. 43 marine non-indigenous species with established populations in Denmark (Stæhr & Thomsen 2012; Madsen *et al.* 2014), while a total of 85 marine and coastal non-indigenous species has been reported since the 1990's (Stæhr *et al.* 2016). Marine invasive species may exert a considerable economic burden on maintenance of aquatic infrastructure and occasionally render fisheries, aquaculture and tourism unsustainable in affected areas. In the European Union (EU) it is estimated that between 12 and 20 billion € are lost annually due to the truly invasive species (albeit both terrestrial and aquatic).

International efforts to address the key transfer vectors of non-indigenous species have been underway over the last 20 years. Denmark is contracting party to the regional marine conventions HELCOM (www.helcom.fi) and OSPAR (www.ospar.org), where the import, transfer and monitoring of marine invasive species is followed closely, and where the main sources of non-indigenous species in the North Sea and Baltic Sea have been identified as shipping followed by aquaculture. In 2012, the 118 non-indigenous species in the Baltic Sea were attributed with about 50 % to shipping and about 25 % to aquaculture/stocking, with the remaining 25 % of unknown origin (HELCOM 2012). The contribution from shipping led the Member States of the IMO to adopt the Ballast Water Management Convention, which entered into force in September 2017. Lastly, but undoubtedly most importantly, the EU Marine Strategy Framework Directive (MSFD) requires EU Member States to establish 'good environmental status' for a range of environmental descriptors in an administrative process that should be completed by 2020. The non-indigenous species are included under the MSFD and the monitoring programme reported here forms an essential part of this obligation.

The impact of shipping on the primary introductions of non-indigenous species to an area and on the secondary transfer from an initial location to a wider distribution is unquestionable. Ships may transport alien species in the ballast water and on their hull. While organisms associated with the hull may spread during the voyage, the paramount important time for the introduction of non-indigenous species is when the ship is moored in port and on anchorage. It is in the ports that the ship's ballast water is discharged, and with high densities of ships in ports this is also a first place to look for non indeginous species. Adding to that, ports are also typically providing a range of habitats including hard substrates, soft bottom and high energy vs. unexposed zones.

The non-indigenous species relevant for ports with respect to the Ballast Water Management Convention have been identifiec by Jensen (2013), and more recently the MONIS 1 report (Andersen *et al.* 2014) outlined a monitoring programme suited to the obligations of the MSFD with a high level of utilisation of existing monitoring activities and aimed at ports, other hotspots and baseline stations.

[1] *'Non-indigenous species' are often abbreviated and referred to as 'NIS'.*

1.1 Monitoring requirements and target species

The MSFD requires EU Member States to include non-indigenous species by way of the Descriptor 2 (D2) and to monitor the species identified. The Danish Nature Agency has established an Initial Assessment and targets regarding non-indigenous species (Danish Nature Agency 2013a, 2013b, 2014), as well as a technical background report compiled by the Danish Centre for Environment and Energy (DCE) at Aarhus University (Stæhr & Thomsen 2012). The Danish description of good environmental status for non-native species reads as follows:

> *The presence of non-native species that are invasive may not result in unacceptable direct or indirect effects on marine life. Danish environmental targets for trends in occurrence of non-indigenous species and their environmental effects are: (1) The risk of transporting non-native species via shipping will be reduced (criterion D2.1.1), and (2) the risk of transporting non-native species via fishing and aquaculture activities will be reduced (criterion D2.1.1). Indicator(s) for the environmental targets: (1) Screening of occurrence (abundance) of selected invasive species in risk areas, (2) monitoring/screening of the relationship between invasive species and native species in selected groups of species, and (3) the effect of invasive species where it can be registered (*ad hoc *basis).*

Marine non-indigenous species have not been subjected to a specific national monitoring programme in Denmark until the current efforts were initiated. Prior data generation on the presence of non-indigenous species in Denmark has been carried out through isolated campaigns, mainly for research purposes, and as by-products of other environmental monitoring such as the NOVANA programme and fish monitoring by DTU Aqua.

The previous study (MONIS 1) proposed a cost-effective monitoring programme for D2, Monitoring of Non-Indigenous Species in Danish Marine Waters (MONIS), considering existing monitoring activities and information (see Andersen *et al.* (2014) for details) with the following main scopes:

- taking the greatest possible advantage of existing monitoring activities,
- assessing the applicability of existing monitoring guidelines and of contemporary biomolecular technologies (i.e. barcoding/eDNA), and
- developing a proposal for a national D2 monitoring programme.

Amongst the six strategic objectives outlined for a monitoring programme of non-indigenous species in Danish marine waters were the following two, which are of specific relevance for the study undertaken and will be briefly introduced:

- the monitoring programme should be two-pronged with one based on conventional sampling via other existing sampling activities and one based on water sampling and eDNA-testing partly based on existing sampling of water (e.g. chlorophyll and nutroient concentrations), and
- the programme should include supposed hotspots, i.e. selected ports and cooling water outlets.

1.2 Conventional versus modern monitoring methodologies

The conventional methods of sampling are embedded in most monitoring activities in national and international programmes. These include various grab sampling methodologies and are key to the HELCOM and OSPAR Joint Harmonized Procedure (JHP), which also form the basis of the conventional sampling in the current study (HELCOM/OSPAR 2015). Andersen *et al.* (2014) suggested that, in comparison with conventional sampling and taxonomic determination, analysis of environmental DNA (eDNA) is a method with a strong potential to establish a comprehensive and cost-effective

routine monitoring programme of non-indigenous species and other species (Bohmann *et al*. 2014, Thomsen & Willerslev 2015, Agersnap *et al*. 2017, Robinson *et al*. 2018, Strand *et al*. 2019, Clusa *et al*. 2017, Thomas *et al*. 2020, Robinson *et al*. 2019, Simmons *et al*. 2015, Adrian-Kalchhauser & Burkhardt-Holm 2016). The method utilises DNA collected from the environment and species-specific test systems for selected species from the Danish Target Species List (TSL) (see Andersen *et al.* 2018 for details about the species-specific test systems developed).

1.2.1 Conventional sampling methods

A detailed description of the sampling methods employed in the study is given in Chapter 3. The conventional sampling protocol applied in the JHP is based on the CRIMP methods developed in Australia and aligned with the survey methods used in the HELCOM ALIENS-2 project, which was without Danish participation. Although the JHP is voluntary and was developed for the BWMC exemption regime, it is applied here (except for human pathogens) to ensure a well-established platform for comparison to other data[2]. General field sampling methodologies applied in JHP include standard methods of grab samples for water and sediments, plankton nets, traps, fouling plates, scrape poles and fish nets, which are applied in this study. In Aarhus Harbour and Esbjerg Harbour, the conventional sampling employed was an expanded JHP protocol and included diver transects both in a day and a night campaign, which was compared to eDNA. In addition, in the 16 eDNA ports night dive transects (fish, jellyfish, epifauna) were performed to inform the interpretation and crosscheck the results of eDNA-based results. Night dives have been shown to deliver very reliable results compared to other conventional methods and to be well correlated to the eDNA results for fish species.

1.2.2 Molecular methods

Molecular methods for identifying species rely on detecting eDNA occurring in a sample. The methodology is well established and similar to the individual identification techniques used in forensics and criminal investigations world-wide. The usefulness of a molecular technique in the monitoring context is that with the right tools the presence of a species' DNA can be determined in a simple environmental sample (Thomsen & Willerslev 2015). For aquatic species, the uncomplicated collection of a water sample combined with the amplification potential of polymerase chain reaction (PCR) assays for DNA allows for detection of a multitude of species based on residual DNA material (Thomsen *et al.* 2012a, Thomsen *et al.* 2012b, Sigsgaard *et al.* 2015, Knudsen et al. 2019). DNA enters aquatic ecosystems through a variety of mechanisms, including sloughing of external epidermal cells and natural secretions, sloughing of internal epidermal cells into faeces, and tissue residues following reproduction, moulting, injury, or predation.

In the current study, the PCR is quantitative (qPCR) and relies on the development of species-specific primers for non-indigenous developed during a previous study (MONIS 3 report; Andersen *et al.* 2018). This methodology allows a high degree of specificity and testing ofpossible overlap to other closely related species, which may not be non-indigenous species but native to Danish waters.

In the aquatic environment, eDNA has been shown to have persistency restricted to 1–2 weeks (Thomsen *et al.* 2012b). Therefore, positive detection of a target species via eDNA indicate a recent occupation or presence in the sampled area, when sampling relatively confined waterbodies such as harbours.

[2] *In the amended 2015 JHP, certain key requirements are changed: The 1 km criterion for a contiguous unit triggering new samples is changed to: "The division of a port in contiguous areas is independent of the distance between these areas and should be specified from case to case in close cooperation with the responsible administration", and the prior emphasis on use of divers is reduced to surface operated or deployed mechanisms (traps, video etc.).*

2 Methods

As part of this study of the occurrence of non-indigenous species in Danish harbours, a Sampling Portocol was compiled describing both the sampling programme and the methods used (Andersen *et al.* 2017). The following description of the monitoring network and the methods used is based on an updated version of the protocol. All sampling was conducted in 2017.

2.1 The NIS monitoring grid in Danish harbours

The eDNA sampling locations comprise 16 of the busiest Danish ports and include the main ferry ports and cargo ports, as they are anticipated hot spots for non-indigenous species:

1. Aarhus Harbour
2. Esbjerg Harbour
3. Aalborg Portland Harbour
4. Aalborg Harbour
5. Fredericia Harbour
6. Frederikshavn Harbour
7. Gedser Harbour
8. Grenå Harbour
9. Helsingør Harbour
10. Hirtshals Harbour
11. Kalundborg Harbour
12. Københavns Harbour
13. Køge Harbour
14. Odense Harbour
15. Rødby Ferry Port
16. Statoil Harbour (Kalundborg)

In two prioritised ports (Aarhus Harbour and Esbjerg Harbour), the eDNA samples and analysis were cross referenced with a comprehensive conventional sampling of plankton, soft and hard bottom communities and mobile epifauna (fish, crustaceans) in accordance with the JHP. These two ports are chosen because they are relatively busy and display different characteristics. Esbjerg Harbour is a fisheries and offshore service port exposed to the North Sea and in close proximity to the Wadden Sea. The port in Aarhus is a container hub located in the Kattegat/Baltic Sea area and also close to ecologically sensitive areas (Natura 2000 areas). The monitoring network is shown in **Figure 3.1**.

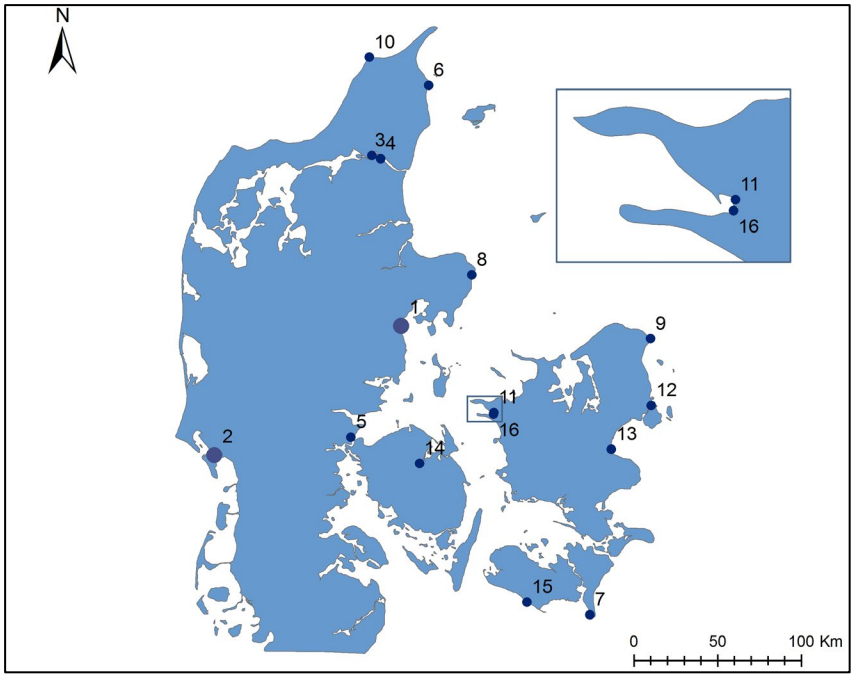

Figure 3.1: *Location of the 16 ports sampled for non-indigenous species.*

The sampling programme did not include the Port of Rønne on the island of Bornholm in the Baltic Sea. The sampling programme has a relatively even distribution across Danish marine water bodies.

2.2 Conventional methods - HELCOM-OSPAR Joint Harmonized Procedure

2.2.1 Plankton

Sampling of phyto- and zooplankton took place in Aarhus Harbour and Esbjerg Harbour, the two largest industrial harbours in Denmark. In each of the harbours, sampling was carried out in accordance with Annex 6 of the HELCOM COMBINE Manual for Marine Monitoring and the Joint Harmonised Procedure of OSPAR[3], at three sampling stations placed in different sections of the harbour. Sampling was performed twice, once in early summer (June) and once in late summer (September/October). All sampling took place from a boat.

2.2.1.1 Phytoplankton

A plankton net (10 µm mesh) (**Figure 3.2**) was lowered to the depth corresponding to the Secchi disc (**Figure 3.3**) measurement and carefully hauled (< 0.3 m/sec) to the surface. To collect all organisms into the sample bottle at the bottom of the net, the net was rinsed by raising and lowering it several times in the surface water while ensuring the upper ring was kept above the surface (sometimes, multiple hauls had to be carried out to collect sufficient material).

Figure 3.2: *Plankton net.*

Figure 3.3: *Secchi disk.*

Figure 3.4: *Water sampler.*

Water was collected with a water sampler (**Figure 3.4**) from four depths; the surface, 2.5 m, 7 m and 10 m (in shallow areas the deeper depths was omitted) and gently mixed in a bottle. A subsample of 100 ml was preserved in Lugol's solution (1 % final concentration).

After sampling, the plankton net was soaked for ca. 20 minutes in warm tap water, rinsed in tap water and dried. Identification of the taxa was carried out in light microscopy (Throndsen *et al.* 2003, Tomas 1996, Jensen & Moestrup 1998, Thomsen 1992, Berard-Terriault *et al.* 2000, Hoppenrath *et al.* 2009) and the enumeration according to the method of Utermöhl (1958). Algaebase.org has been used as taxonomic reference. Identification of phytoplankton in light microscopy has limitations as

[3] *With the exception from the COMBINE protocol that zooplankton is preserved in ethanol instead of formaldehyde.*

the resolution is too low to allow observation of morphological details that are important for the circumscription of many taxa that may need electron microscopy. In addition, many taxa have few morphological characters and cannot be identified by microscopy alone and may require molecular methods such as barcoding. New investigations of phytoplankton are changing the circumscription of established species and novel species are discovered and described continuously and even the classification is altered over time. Lastly, the experience of the phytoplankton analyst should be considered. Altogether these factors may make the identification of species complicated and sometimes ambiguous. We have in this study, however, followed the gudienelines and done the identification of species in the manner it has been done for decades.

2.2.1.2 Zooplankton

Two samples were taken at each station – one sample with fine (200 µm) net and one with coarse (500 µm) net. Sampling was performed by vertical hauls from near bottom to surface. The net was lowered to desired depth, and was slowly hauled towards the surface at ca. 0.5 m/s. Several hauls were combined in one sample, if the hauls were short and few animals were caught in each haul. Jelly plankton was identified alive. If the species could not be identified, a digital photo was taken for later identification.

The jelly plankton was removed from the sample after identification or photography and the samples was filtered again (in the plankton net or with a net of the same mesh size). The zooplankton was transferred into a sample bottle. The sample bottle was then filled with ethanol for preservation.

After sampling, plankton nets, weight, flow meter and line were disinfected by soaking 20 minutes in a Virkon S solution, or some alternative method (chlorine or 70 % ethanol).

2.2.2 Mobile epifauna

Mobile epifauna (crabs, prawns, snails, echinoderms, demersal fish) was collected using traps. Two types of traps were used, a standard crab trap 60 cm x 40 cm x 20 cm with 2.5 cm mesh netting and a 40 cm Gee minnow trap with 5 mm mesh netting (**Figure 3.5**). One trap of each type was deployed at each station, tethered together and lowered to the seabed at the front of the quay. Rope for retrieval was fixed to pins, chains etc just above sea level. The traps were baited with mackerel entrails (Aarhus) and herring fillets (Esbjerg).

Figure 3.5: *Crab trap (left) and Gees minnow trap (right) used for sampling of mobile epifauna in Aarhus and Esbjerg harbours.*

In each harbour, three trap stations were established in each of the three harbour areas (**Figure 3.6**). As far as possible, the traps were deployed at the same place or close to the place of the settling plates for fouling organisms. Coordinates and further description of each trap station are presented in **Annex 1**. The traps were deployed for two days (46–48 h). At trap retrieval, the catch of crabs, fish and other mobile fauna was identified as far as possible in the field, measured and weighed. Other, mostly smaller, specimens were fixed in ethanol and taken to the laboratory for identification, measured and weighed.

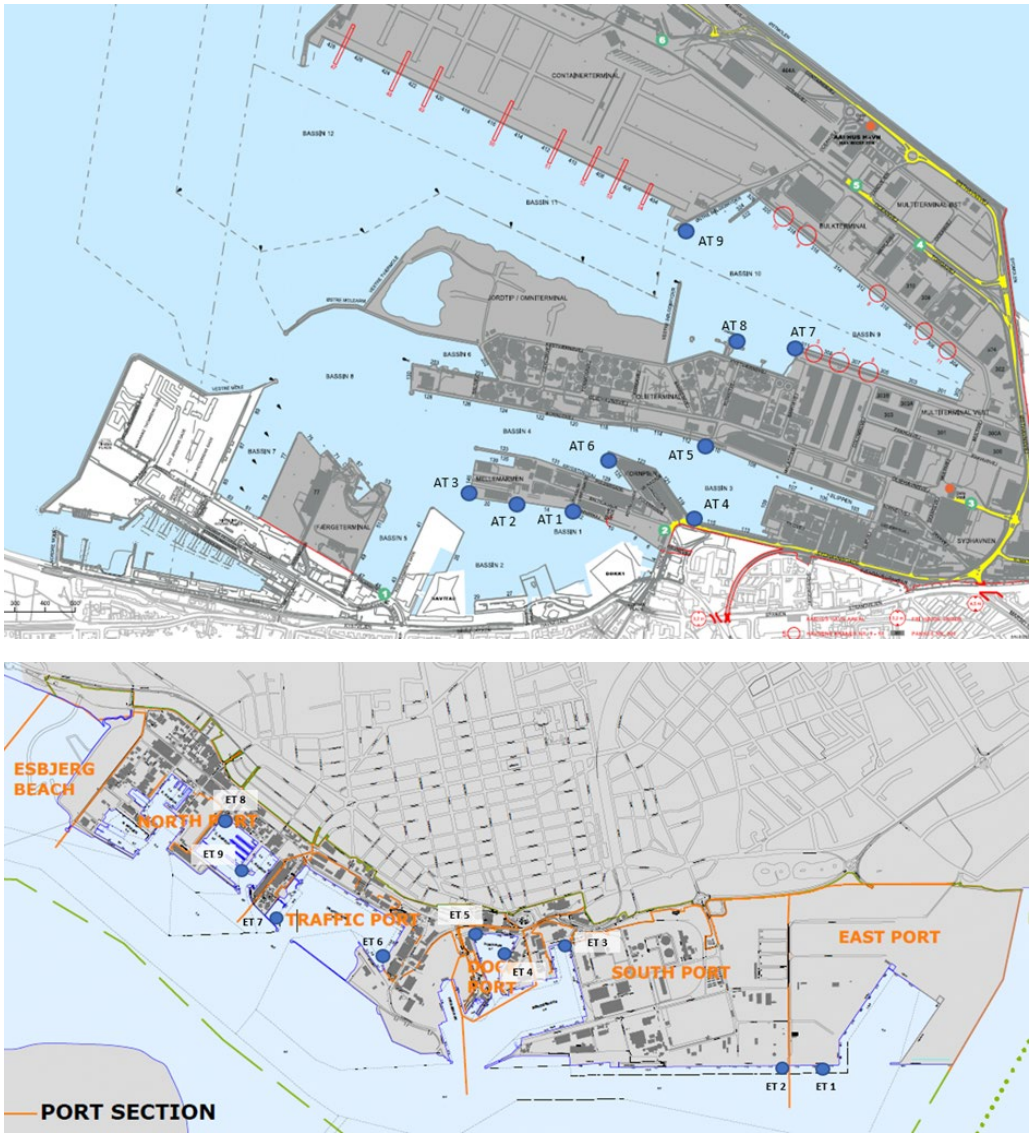

Figure 3.6: *Location of traps (blue spots) for sampling of mobile epifauna in Aarhus Harbour (top) and Esbjerg Harbour (bottom).*

2.2.3 Benthic infauna

Benthic infauna was collected using a 0.028 m^2 hand-operated van Veen grab (**Figure 3.7**). The grab penetrates to about 10 cm into the sediment. Supplementary material was taken using a Kajak corer to ensure material also from deeper strata in the sediment (**Figure 3.7**).

Figure 3.7: *Hand-operated van Veen grab (0.028 m^2) (left) and Kajak corer (inner diameter 5.5 cm) (right) used for sampling of sediment infauna.*

In each harbour, three grab stations were established in each of the three harbour areas (**Figure 3.8**). Each grab station consisted of three hauls with the grab and 1–2 corer hauls. The sediment catch was sieved on 0.5 mm screens. Large sieves (60 cm diameter) with perforated steel plates were used. The sieve residue was fixed in 4–6 % formaldehyde solution in seawater and taken to the lab for further processing. A visual description of the sediment (colour, smell, larger objects) was obtained before sieving. At each sampling station, one additional grab sample was taken and fixed in ethanol (96 %) in case of later molecular genetic analyses of selected species. Coordinates and further description of the samples are presented in **Annex 1**.

The samples were sorted and identified at NIVAs laboratory in Grimstad, Norway. All species were identified to the lowest possible taxonomic level. The analyses were carried out in accordance with NIVAs accredited routines for processing of quantitative soft bottom samples.

2.2.4 Sediment epifauna

Sediment epifauna (sessile and slow-moving organisms) was collected using a 40 cm wide light-weight hand operated dredge (**Figure 3.9**). One dredge sample was taken in each harbour area in both Aarhus and Esbjerg (**Figure 3.8**). The dredge was towed 30–50 m along the bottom. The dredge samples were taken simultaneously with the grab samples. The catch was sieved on 0.5 mm screens and fixed in 4–6 % formaldehyde solution in seawater for further processing in the lab. The samples were sorted and identified at NIVAs laboratory in Grimstad, Norway. Sorting and identification were following the same routines as the quantitative grab samples. Coordinates and further description of the samples are presented in **Annex 2**.

Figure 3.8: *Hand-operated dredge (40 cm x 20 cm) for collecting sediment epifauna.*

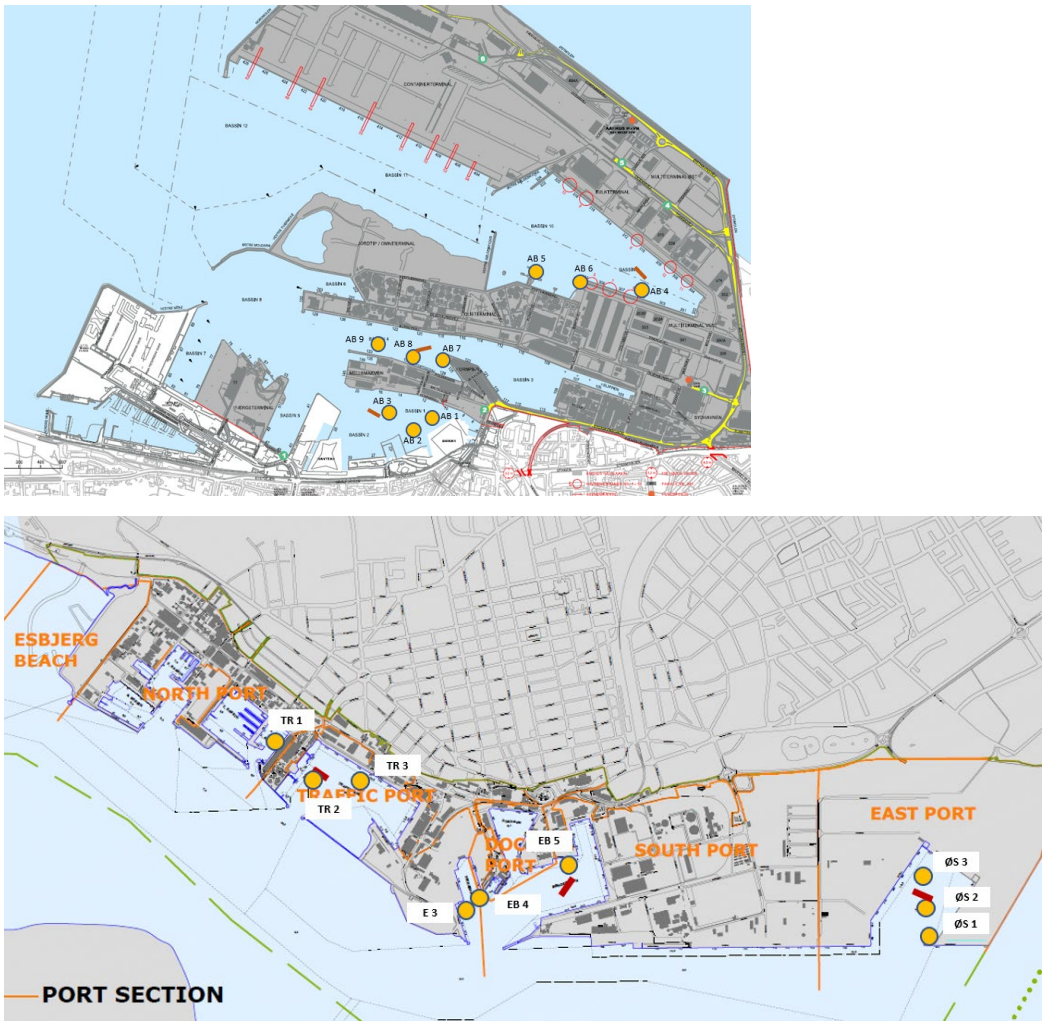

Figure 3.9: *Location of grab sampling stations (orange spots) and dredge stations (red bars) for soft bottom infauna and epifauna in Aarhus Harbour (top) and Esbjerg Harbour (bottom).*

2.2.5 Fouling organisms

Scraped samples were collected from nine different sites in each harbour (three in each of three survey locations). Each sample covered approximately 0.1 m^2 surface area and the samples were taken from close to the water surface to depths of <1 m. The distance between each sample was at least 10 m. The samples were collected using a scraper with a pocket-net attached to a pole which was

operated from a boat or from the dock. Sampled material was transferred from the net to a plastic tray with sea water and examined immediately. The sampling was performed in September 2017.

Each scraped sample was photographed. The abundance of the species was estimated according to a semi-quantitative scale: 1 = individual finding, 2 = 0-25 % coverage, 3 = 25-27 % coverage, and 4 = 75-100 % coverage. Species that could not be identified in the field or has not previously been recorded in Denmark was removed from the sample and brought to the laboratory for microscopical examination. These were preserved in 90 % ethanol.

In addition, selected substrates (floating bridges, fender constructions, ropes etc.) were examined for alien species using a 'rapid assessment survey' (RAS) technique. RAS is a time efficient method where submerged structures in marinas, such as pontoons, floats and pier elements, are examined for the presence of species that may be expected to occur ('target species'). The method is described in Minchin *et al.* (2007) and HELCOM-OSPAR (2015).

Fouling plates, sometimes also referred to as settlement plates, in PVC were used to examine colonization of alien species on artificial substrates. Fouling plates were deployed at nine sites (three in each of three survey locations) in each harbour. The plate units were constructed of three plates measuring 14 cm x 14 cm fixed on a polypropylene rope (**Figure 3.10**).

The plates had been sanded lightly with sandpaper on both sides to create a suitable surface for colonizing organisms. The position of each plate on the rope was secured with a knot above and below the plate and the length of the rope was adjusted so that the three plates achieved a depth of 1, 3 and 7 m when the unit was deployed in the water (at low tide). At sites where the water depth was less than 8 meters at low tide, the deepest plate was removed and the length of rope was adjusted accordingly. At the end of the rope a weight (a brick) was attached to weigh down the rig and ensure that the rope remained tight and achieved an approximately vertical position in the water column.

The plates were deployed in June and retrieved in September. Plates were carefully detached from the rope and photographed before they were placed in separate, pre-labeled zip-lock bags. Sufficient water was added in order to keep the material humid. Plates were kept in a cooler during transport to the laboratory.

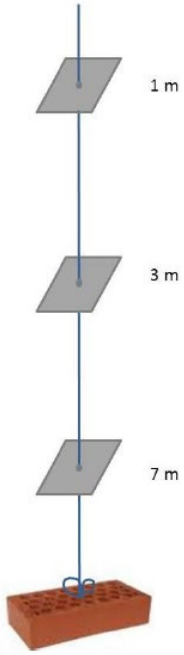

Figure 3.10: *Fouling plates.*

Species that could not be identified immediately or individuals to be saved for documentation were preserved in 90 % ethanol. If target species were observed on the settlement plates their coverage were estimated using the following semi-quantitative scale1 = individual finding, 2 = 0-25 % coverage, 3 = 25-27 % coverage, and 4 = 75-100 % coverage.

2.3 Conventional methods – fish

Sampling of fish was based on standard methods (section 3.3.1) as well as snorkeling (section 3.3.2).

2.3.1 Standard methods

In all 16 harbours, the fish communities were sampled using gillnets and fyke-nets (**Figure 3.11**). In each harbour, one gill-net and one fyke-net were deployed in each of up to three activity sections: 1) a section with industrial activity (transporting, shipping, ferrying), 2) a section with yachting activity (recreational boating), and 3) a section with fishing activity (fishing boats and fishing industry). This resulted in a maximum deployment of 3 gillnets and 3 fyke-nets per harbour. Harbours, with only one or two activity sections represented, were only sampled in these sections.

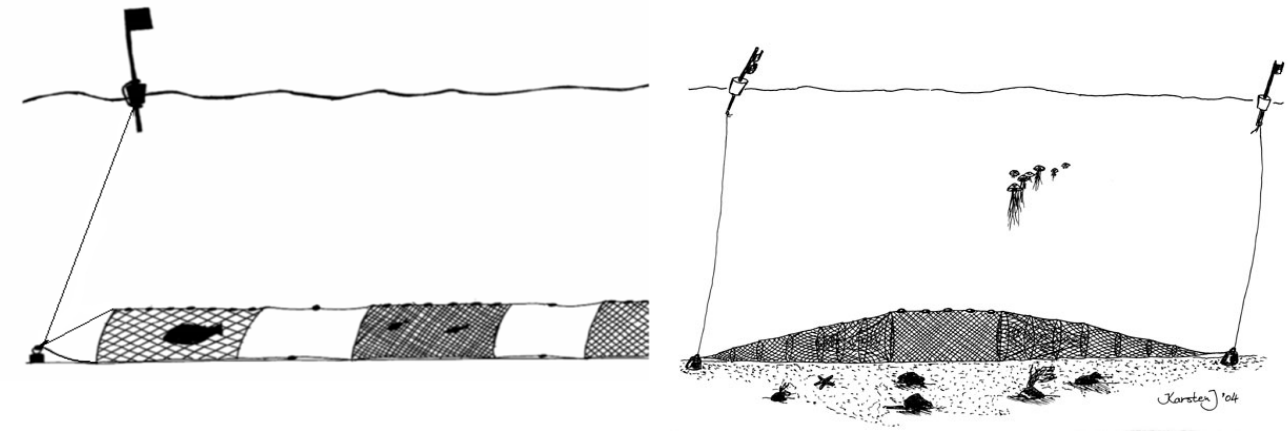

Figure 3.11*: Left: Multi mesh gillnets (illustration by Hansen 2012). Right: Fyke Nets (Illustation by [http://dyk.nu/pages/nettyper.htm)](http://dyk.nu/pages/nettyper.htm)*

Seven different panels were used to make up a gillnet series. The panels, each measuring 1.5 m in height, had the following mesh sizes: 11.0 14.3, 18.6, 24.2, 31.4, 53.1 and 89.7 mm. The lengths of each panel were 2.95, 5.30, 5.70, 5.70, 6.00, 11.60 and 11.60 m. Each panel was separated by about 1 m rope, resulting in gillnets of around 55 m in length.

A fyke-net consisted of two cod-ends, mesh size of 18 mm and 42 cm in height, separated by a 6.5 m leader. If there was no space for deploying the gillnet and fyke-net in extension of each other, they were deployed separately, but in close proximity of each other.

The harbours were sampled in summer from the 21st of August to the 14th of September. Gears sat 12 hours overnight. They were deployed in the evening around 20 PM and retrieved the following day again around 8 AM. As far as possible, deployment was done parallel to, and 5-10 meters from a stony pier. All caught fish were identified to species and measured (total length rounded down to the nearest cm). Species difficult to identify were either photographed or frozen for later identification in the laboratory. Invasive species specimens were photographed collectively in one sample and frozen. Any crabs caught in the gill-nets or fyke-nets were also species identified, counted and measured.

2.3.2 Snorkeling

As far as possible, snorkeling was carried out along a pier or the like, along an approximately 500 m long transect (**Figure 3.12**) (see Sigsgaard *et al.* 2017). If conditions inside the harbours made snorkeling impossible, for example poor visibility, snorkeling was moved to the outer side of the pier. Initially, snorkeling was done in one direction in shallow water near the pear and subsequently at a slightly deeper depth (up to 5 m) in the opposite direction. During these investigations, fish associated with both hard and soft bottom were registered. The swimming speed was adjusted so the entire investigation was completed within 1 hour.

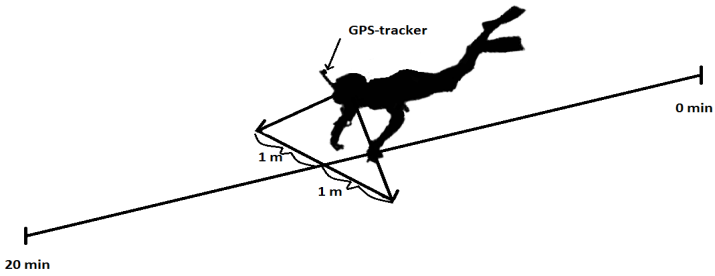

Figure 3.12: *Illustration of the diving approach with indicator of the surveyes area (Rasmus Ebert).*

Snorkeling was, whenever possible, performed in the hours of darkness, but if the conditions turned out dangerous in the specific port, snorkeling was done during daytime. The visibility was estimated, so the "swept area" could be estimated and the number of fish could be presented as number/m^2. Temperature and salinity were recorded.

All fish were counted and identified to species. If other non-resident organisms of e.g. algae, mussels and crabs were observed, they were registered. A representative selection of the fauna was photographed/filmed - the focus was on non-resident species. Concurrently, macroalgae and non-indigenous species of invertebrates were registered and collected for ID-verification.

2.4 Molecular methods

Environmental DNA (eDNA) was sampled from 16 Danish harbours and analysed for presence of DNA from 18 target species. Sampling and analysis of eDNA was divided into three steps: Step 1: Sampling of water, step 2: filtration and storage, and step 3: DNA extraction and qPCR. Two similar samples were taken inside the pier (same sample bag was used for both filters). Samples of sea water were collected in 2017 in June-July and in September-October (see **Table 3.1**).

Table 3.1: *Filtered water samples collected during 2017. All volumes are in mL.*

Harbour	**Spring 2017 (May–Jul)**				**Autumn 2017 (Sep–Oct)**			
	Date sample 1	Volume sample 1	Date sample 2	Volume sample 2	Date sample 1	Volume sample 1	Date sample 2	Volume sample 2
Aarhus	Jul.05	2400	Jul.05	3100	Sep.19	1000	Sep.19	1000
Esbjerg	Jun.27	1000	Jun.27	1000	Oct.17	200	Oct.17	200
Aalborg Harbour	Jun.29	1000	Jun.29	1000	Oct.11	1000	Oct.11	1000
Aalborg Portland	Jun.30	1000	Jun.30	1000	Oct.11	2500	Oct.11	2500
Fredericia	Jul.05	1600	Jul.05	2000	Oct.11	1000	Oct.11	1000
Frederikshavn	Jun.28	1000	Jun.28	1000	Oct.06	3000	Oct.06	450
Gedser	Jul.04	700	Jul.04	700	Sep.23	1000	Sep.23	1000
Grenå	Jul.05	1100	Jul.05	1500	Sep.19	1000	Sep.19	1000
Helsingør	Jul.11	1000	Jul.11	1000	Sep.13	1000	Sep.13	1000
Hirtshals	Jun.28	1000	Jun.28	1000	Nov.08	600	Nov.08	600
Kalundborg	Jul.03	1500	Jul.03	1500	Sep.22	1000	Sep.22	1000
København	Jun.09	1800	Jun.09	1800	Sep.12	1650	Sep.12	1000
Køge	Jul.04	850	Jul.04	850	Sep.12	450	Sep.12	600
Odense	Jul.19	800	Jul.19	800	Sep.15	800	Sep.15	800
Rødby	Jul.04	500	Jul.04	500	Sep.23	200	Sep.23	200
Statiol (Kalundborg)	Jul.02	1500	Jul.02	1500	Sep.22	1000	Sep.22	1000

The amount of eDNA is roughly proportional to the amount of water sampled. Therefore, the amount of filtered water (>1,5 L) was maximised and larger particles in the collected water (i.e. macroalgae and insects) were avoided. Water was sampled using a water sampler with a single-use plastic canister or bag to fill the sample bag through a funnel (**Figure 3.13**).

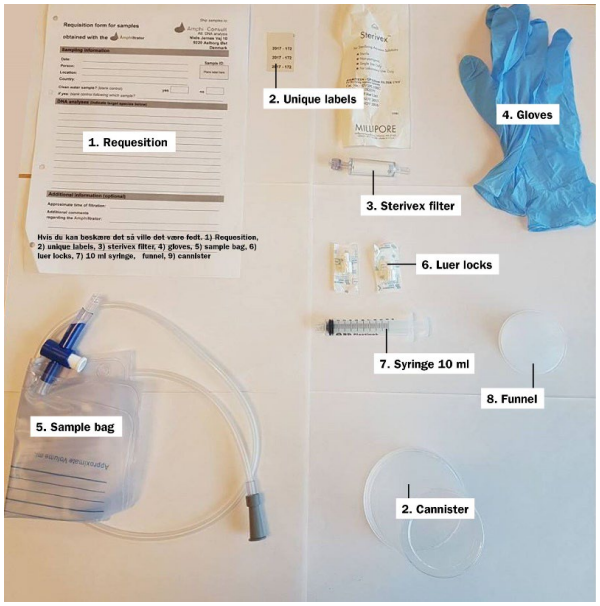

Figure 3.13: *Amphiltrator and sample Kit B.*

If conditions were not suitable for sampling, e.g. due to suspended material, sampling was done outside of the harbour pier. Some algae are expected to be more numerous in spring compared to autumn, while some fish species are found at shallow water during the warm months in early autumn. To increase the chance of detecting all species, sampling was done both in summer and autumn.

Filtration of water with the Amphiltrator was done by attaching a 22 µm Millipore 'Sterivex'-filter (Spens *et al.* (2017)), to the Amphiltrator 2.0 and thereafter to the sample bag (**Figure 3.14**). The sample bag was then mounted and the Amphiltrator was safely closed by placing the O-ring, attaching the lid and clamp and tightening the screw according to the guidance. Then pressure was added to the chamber using a bicycle pump (**Figure 3.15**).

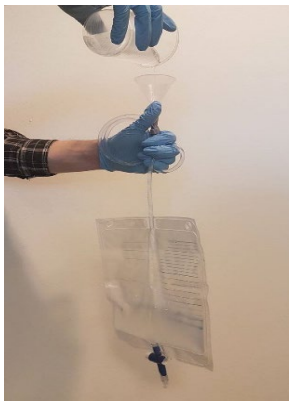

Figure 3.14: *Filling the sample bag.*

Figure 3.15: *Pressure added to the Amphiltrator.*

Figure 3.16: *After carefully emptying all water the Sterivex filter is labelled with unique label, luer-lock stoppers attached and stored on dry ice.*

Filtration ran until the sample bag was empty or to a suggested maximum of 30 minutes. The water temperature was measured along with the amount of filtrated water. After filtration was completed, the Amphiltrator was opened and detached from the Sterivex filter. After the Sterivex filter had been detached, the provided syringe from sample KitB was used to remove the remaining water. This step is important to properly preserve DNA and to avoid filter damage potentially caused by freezing water. When the Sterivex filter was emptied, and luer-lock stoppers attached, a unique label was added to the filter (**Figure 3.16**), and immediately stored on dry ice.

The filters were transferred to a -20 °C freezing facility as soon as possible. For long-term storage (months), the filters were transferred to a -80 °C freezing facility as soon as possible. For each of the harbours, two filters were made, one for analysis in this study and one for archiving. For a more detailed description of eDNA sampling and analysis, see Knudsen *et al.* (2015), Amphi Consult (2017) or this video about the sampling procedure applied: https://youtu.be/2BniniPEpTc.

Extraction of DNA from filters was done by using DNeasy Blood & Tissue kit (Qiagen) and storage of filters follow the protocol described by Agersnap *et al.* (2017) and Knudsen *et al.* (2015, 2018, 2019). Extracted eDNA is best preserved below -15 °C and for long-term storage the concentration from the extracted eDNA is measured (i.e. Nanodrop or Qubit).

Design, test and validation of each species-specific assay follows the protocol described by Agersnap *et al.* (2017) and Knudsen *et al.* (2019) and was performed in a qPCR set up on DNA extracted from both target and non-target species as listed by Andersen *et al.* (2018), ensuring that only DNA from the target-species could return positive amplification in a qPCR set up.

The protocol for inferring optimal concentration of each primer and probe follows the set up described by Agersnap *et al.* (2017) and Knudsen *et al.* (2019). For each specific primer-pair and matching probe (**Table 3.2**), a qPCR was performed on DNA extracted from tissue from the target species. Each sample was performed in two replicates and each reaction well set to have a total volume of 25 µL, comprising 10 µL of TaqMan Environmental Master Mix 2.0 (Life Technologies), 2 µL ddH_2O, 5 µL of each primer (forward and reverse) (in concentrations from 1 to 6 µM each), 1 µL of probe (2.5 µM) and 2 µL of template DNA extracted from tissue. The qPCR settings were set to have an initial preheat at 50 °C for 5 minutes, 10 min at 95 °C, followed by 50 cycles at 95 °C for 30 s and 60 °C for 1 min, with fluorescence collected at the endpoint in the final 1 min 60°C step. The qPCRs were all set up on a Stratagene Mx3000P machine using the qPCR MxPro software. The optimal primer concentration for both forward and reverse primer was found by identifying the concentration that returned the lowest Cq-value (i.e. the earliest amplification). This qPCR was carried out using varying concentrations of forward and reverse primer (0.2 µM, 0.4 µM, 0.6 µM, 0.8 µM, 1.0 µM and 1.2 µM) as calculated per final reaction volume (25 µL) for the qPCR well. Once the optimal concentration for primers was inferred, this concentration of the primers was applied in a qPCR set up with settings as described above, but with the probe varying in final concentrations from 0.10 µM, 0.15 µM, 0.20 µM, 0.25 µM, and 0.30 µM. The optimal concentration was again found by identifying the concentration that returned the lowest Cq-value (**Table 3.2**).

Table 3.2: *List of the 18 species-specific assays and oligos applied and optimal concentrations.*

Assay ID	Common name (Danish)	Species	Primer (F and R) and probe name (P)	Sequence (5'-> 3'), primer and probe	Probe Modification 5'-end:	Probe Modification 3'-end:	Optimal primer/probe concentration (nM)
01	Rødtot alge	*Bonnemaisonia hamifera*	Bonham_rbcL_F02	CAATTACTAGATTACCTGGGCAAT			1200
			Bonham_rbcL_R02	CTTCTTTTACAAAGTCCCGACCT			200
			Bonham_rbcL_P01	TCGTGCCATAACCATAGACTCTAAAGCC	FAM	BHQ-1	300
02	Dinoflagelat	*Prorocentrum cordatum*	Promin_28S_F03	CTTGGCAAGATTGTCGGGT			1200
			Promin_28S_R03	TATTCACTCACCCATAGACGA			1200
			Promin_28S_P03	ACACACAAGGCAAGAGACGATCAAGC	FAM	BHQ-1	300
03	Heterokont flagelat	*Pseudochattonella farcimen*	PsFa28SF	GGGAGAAATTCTTTGGAACAAGG			200
			PsFa28SR	GCAACTCGACTCCACTAGG			800
			PsVeFa28SP1	TCAGAGAGGGTGACAATCCCGTCT	FAM	BHQ-1	300
04	Heterokont flagelat	*Pseudochattonella verruculosa*	PsVe28SF	GGGAGAAGTCCTTTGGAACAAGG			200
			PsVe28SR	GCAACTCGACTCCATTAGC			600
			PsVeFa28SP1	TCAGAGAGGGTGACAATCCCGTCT	FAM	BHQ-1	300
05	Dinoflagelat	*Karenia mikimotoi*	KarmikF3	CCGAGTGACTGAATGTCCTC			200
			KarmikR3	GATCGCAGGCAAGCACATGA			200
			KarmikP3	GCAGTGCTACCAGACACACAGAG	FAM	BHQ-1	300
06	Sølvkarusse	*Carassius auratus*	Caraur_COI_F01	TTCTTCCCCCATCATTCCTGT			200
			Caraur_COI_R01	GTATACTGTCCATCCGGAGG			600
			Caraur_COI_P02	TAGCTTCCTCTGGTGTTGAAGCCGGAG	FAM	BHQ-1	100
07	Karpe	*Cyprinus carpio*	CCcytbF	CTAGCACTATTCTCCCCTAACTTAC			200
			CCcytbR	ACACCTCCGAGTTTGTTTGGA			400
			CCcytbP	CCCTCTAGTTACACCACC	FAM	TAMRA	200
08	Østerstyv	*Colpomenia peregrina*	Colper_COX_3_F01	GCAAGCTTTTGAATATGCTAATG			400
			Colper_COX_3_R01	CAGCTAAAAATATTGTACCGATT			600
			Colper_COX_3_P01	TTCAGTTTTTTACATGGCTACAGGCTTC	FAM	TAMRA	100
09A	Sortmundet kutling	*Neogobius melanostomus*	Neomel_COI_F01	CTTCTRGCCTCCTCTGGWGTTG			200
			Neomel_COI_R01	CCCWAGAATTGASGARATKCCGG			600
			Neomel_COI_P01	CAGGCAACTTRGCACATGCAG	FAM	BHQ-1	100
10	Regnbueørred	*Oncorhynchus mykiss*	Oncmyk_CytB_F01	ACCTCCAGCCATCTCTCAGT			400
			Oncmyk_CytB_R01	AGGACGGGGAGGGAAAGTAA			600
			Oncmyk_CytB_P01	TGAGCCGTGCTAGTTACTGCTGTCCTT	FAM	BHQ-1	100
13	Pukkellaks	*Oncorhyncus gorbuscha*	Oncgor_CO1_F09	TCCTTCCTCCTCCTCCTTTC			400
			Oncgor_CO1_R06	TGGCCCCTAAAATTGATGAG			1000
			Oncgor_CO1_P06	CAGGGGCATCCGTCGACTTAACTAT	FAM	BHQ-1	300

Assay ID	Common name (Danish)	Species	Primer (F and R) and probe name (P)	Sequence (5'-> 3'), primer and probe	Probe Modification 5'-end:	Probe Modification 3'-end:	Optimal primer/probe concentration (nM)
14	Stillehavsøsters	*Magallana gigas*	Cragig_CO1_F07	TTGAGTTTTGCCAGGGTCTC			200
			Cragig_CO1_R09	ACCAGCAAGGTGAAGGCTTA			1200
			Cragig_CO1_P06	AACATTGTAGAAAACGGAGTTGGGGC	FAM	BHQ-1	200
15	Sandmusling	*Mya arenaria*	Myaare_CO1_F01	CCCTCCGTTGTCGAGAAATA			200
			Myaare_CO1_R02	ACGCATGTTACCCCAAGTTC			1200
			Myaare_CO1_P06	TATCCCTTCATATTGGAGGGGCTTCAT	FAM	BHQ-1	200
16	Mudderkrabbe	*Rhithropanopeus harrisii*	Rhihar_co1_F03	GTCAACCTGGTACTCTCATTGGT			200
			Rhihar_co1_R03	ACGAGGAAATGCTATATCAGGGG			1200
			Rhihar_co1_P03	TGTTGTAGTAACAGCTCACGCCTTTGT	FAM	BHQ-1	150
18	Kinesisk uldhåndskrabbe	*Eriocheir sinensis*	Erisin_cytb_F02	ACCCCTCCTCATATCCAACCA			200
			Erisin_cytb_R02	AAGAATGGCCACTGAAGCGG			1200
			Erisin_cytb_P02	TTTGCTTACGCTATTTTACGATCAATTCCT	FAM	BHQ-1	200
21	Brakvandskrølle	*Cordylophora caspia*	Corcas_COI_F01	TCATCTGTACAAGCACATTCTGG			200
			Corcas_COI_R01	TTGAAGAAGCTCCTGCACAGT			200
			Corcas_COI_P01	CCTTCTGTAGACATGGCTATATTTAGTC	FAM	BHQ-1	100
22	Amerikansk ribbegoble	*Mnemiopsis leidyi*	Mnelei_its2_F04	ACGGTCCCTTGAAGTAGAGC			400
			Mnelei_its2_R06	TCTGAGAAGGCTTCGGACAT			1000
			Mnelei_its2_P06	GTGCCTCTCGGTGTGGTAGCAATATCT	FAM	BHQ-1	300
23	Siberisk stør	*Acipenser baerii*	Acibae_CR_F02	CAGTTGTATCCCCATAATCAGCC			800
			Acibae_CR_R03	TTATTCATTATCTCTGAGCAGTCGTGA			1200
			Acibae_CR_P01	ATGCCGAGAACCCCATCAACATTTGGT	FAM	BHQ-1	250

Detection of species-specific eDNA using qPCR on each filter was done by analysing a minimum of three replicates for each target species. These replicates were analysed for presence/absence of eDNA from the target species. Hence, the expected number of analyses was minimum: 64 filters, 18 species, and 3 replicates; in total 3.456 analyses.

The results from the qPCR can be quantified from the included standards with known concentration of target DNA. This is only possible if eDNA concentration in the samples is sufficiently high. From the volume of filtrated water and the volume of elution buffer added, the amount of target species-specific eDNA in the original water sample can be calculated. This may give an indication of quantitative differences between the investigated sites. It is currently not possible to estimate the population size of a given species using this method.

The DNA extracted was tested in three technical qPCR replicates per filtered water sample (**Table 3.1**) using the species-specific primers and probes in the optimal final reaction concentrations. The qPCR was set up similar to the protocol described by Agersnap *et al.* (2017) and Knudsen *et al.* (2019), where each sample was prepared in three replicates and each reaction well set to have a total volume of 25 µL. A qPCR well with a total volume of 25 µL comprised 10 µL of TaqMan Environmental Master Mix 2.0 (Life Technologies), 7 µL ddH_2O, 1µL of each primer (forward and reverse) in the optimal concentration inferred earlier, 1 µL of probe in the optimal concentration inferred earlier and 5 µL of template extracted from the filtered water sample. A purified dsPCR amplicon obtained in an initial PCR performed with an AccuPol DNA proofreading polymerase (AccuPOL DNA polymerase, Ampliqon, VWR # 733-1324) and the specific primers, served as both positive control and template for a standard dilution series. The molecular weight of the purified amplicon was calculated with OligoCalc (Kibbe 2007) and diluted to a concentration that equals 1 x 10^6 copies per µL. This amplicon was then diluted in steps to equal 1 x 10^5 copies/µL, 1 x 10^4 copies/µL, 1 x 10^3 copies/µL, 1 x 10^2 copies/µL, 1 x 10^1 copies/µL and 1 x 10^0 copies/µL. For each of these dilution steps three technical qPCR repliactes were included, using 5 µL of the diluted template from each step. The qPCR settings were set to have an initial preheat at 50 °C for 5 min, 10 min at 95 °C, followed by 50 cycles at 95 °C for 30 s and 60 °C for 1 minute, with fluorescence collected at the endpoint in the final 1 minute 60 °C step. Four wells were tested without any template and served as Non-Target-Controls (NTC). A qPCR was performed for each assay for each season. The qPCRs were all set up on a Stratagene Mx3000P machine using qPCR MxPro software.

All qPCR assays included a standard dilution series diluted until extinction of amplification signal (i.e. 'No Ct'). Limit of detection (LOD) and Limit of Quantification (LOQ) was defined for each assay and each specific qPCR run. Limit of detection (LOD) was defined at the lowest dilution of the standards when at least one of the three replicate standard dilutions gave a positive result in the qPCR assay. Limit of quantifications (LOQ) was defined as the lowest dilution of standards which gave a reproducible result in the qPCR assay – i.e. all three technical qPCR replicate dilutions where positive. This follows the definition and the eDNA quantification provided by Ellison *et al.* (2006). Plots of standard dilution series for each species-specific primer-probe assay for each season (i.e. spring and fall) were prepared using R v3.2.4 (R Core Team 2016). The level of specificity will vary between the different species-specific assays, and low detection levels are not comparable across species. Although the eDNA amplification signals below LOD are regarded as being below the limit of detection, there is a stochastic probability of obtaining a few template eDNA molecules (below 1–10 copies) in the qPCR well that can give rise to a late qPCR amplification.

For preparation of plots, maps and tables the resulting data was exported from the MxPro software as text reports and analysed using R v.3.2.4 (R Core Team 2016), and the packages: 'fields' (Nychka *et al.* 2015), 'gplots' (Warnes *et al.* 2016), 'plyr' (Wickham 2011), 'ReporteRs' (Gohel 2018), 'scales' (Wickham 2017), and 'stringr' (Wickham 2018).

3 Results

The results of the monitoring in the 16 Danish harbours are presented in two groups as follows:

1. Conventional sampling methods
    1.1. Plankton (section 4.1)
        1.1.1. Phytoplankton (section 4.1.1)
        1.1.2. Zooplankton (section 4.1.2)
    1.2. Fauna (section 4.2)
        1.2.1. Mobile epifauna (section 4.2.1)
        1.2.2. Benthic infauna (section 4.2.2)
            - Sediment infauna
            - Sediment epifauna
    1.3. Fouling organisms (section 4.4)
    1.4. Fish (section 4.4)
        1.4.1. Fish, standard methods (section 4.5)
        1.4.2. Fish, snorkelling (section 4.4.2)
2. Molecular methods (section 4.5)

3.1 Plankton

3.1.1 Phytoplankton

In June, the three localities in Aarhus Harbour were dominated by diatoms and in particular *Dactyliosolen fragilissimus* was abundant. *Coscinodiscus radiatus* was present at all three stations together with the dinoflagellates *Tripos muelleri* and *T. longipes* and the dictyochophyte *Dictyocha speculum*. At the station 'Aarhus 5' two species of the diatom *Aulacosira* were numerous in addition to *D. fragilissimus*. Aarhus 5 had the highest species diversity with quite high numbers of cryptophytes (only a few species) and chlorophytes (ca. 20 freshwater species). At Aarhus 1 some haptophytes (*Chrysochromulina* and *Haptolina*) and chrysophytes (*Dinobryon*) were also recorded. At Aarhus eight of the diatoms *Gyrosigma/Pleurosigma* and *Thalassionema* were quite abundant as well as the small dinoflagellate *Heterocapsa rotundata.*

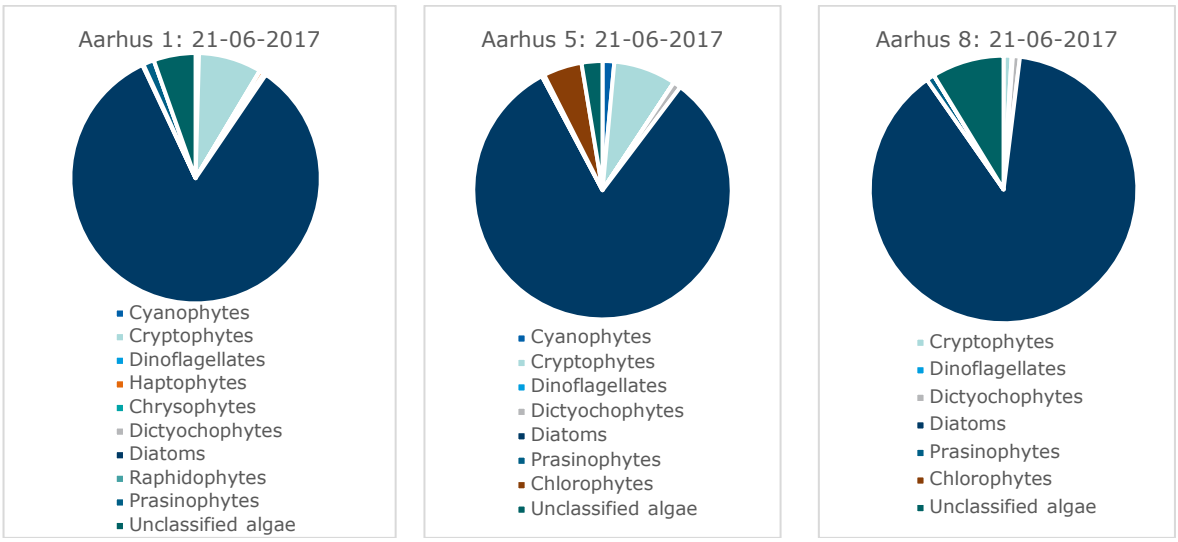

Figure 4.1: *Aarhus Harbour, cell count, summer (June).*

In October, the three localities were dominated by diatoms. At Aarhus 5, the diatom *Aulacosira* (common in freshwater) was by far the most abundant and small unidentified flagellates (5–10 µm) were also numerous. Very few dinoflagellates were observed. At Aarhus AB5 diatoms such as *Cerataulina pelagica, Phaeodactylum tricornutum* and *Chaetoceros* were recorded together with several *Tripos*-species (*T. fusus, T. longipes, T. lineatus* and *T. muelleri*). Aarhus AB7 was almost completely dominated by diatoms and *Aulacosira* was the most abundant. A few dinoflagellates such as *Tripos fusus, T muelleri* and *T. macroceros* were observed.

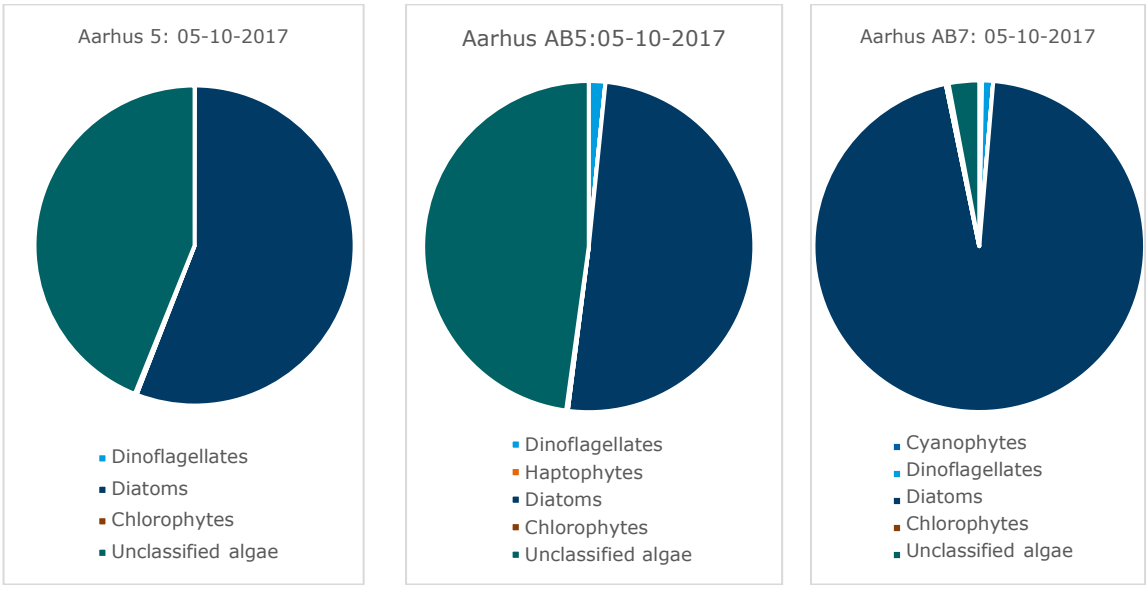

Figure 4.2: *Aarhus Harbour, cell count, autumn (October).*

Some less abundant species such as the diatoms *Rhabdonema* and *Coscinodiscus concinnus* and the dinoflagellates *Dinophysis norvegica* and *Oxytoxum criophilum* were only encountered in the net-samples (see **Annex 3**, list of taxa**).**

Diatoms dominated in abundance at all locations. *Cylindrotheca closterium* and *Rhizosolenia pungens* were abundant at Esbjerg 1 and Esbjerg 7 as were the chrysophyte *Dinobryon,* cryptophytes and the euglenoid *Eutreptiella. Scrippsiella* and unidentified thecate species (20–60 µm) were common dinoflagellates. *Actinoptychus* was present at all locations. At E3 small centric diatoms and *Entomoneis*-like cells were most abundant. Some small *Pyramimonas* species were observed as well as unidentified monads (5-10 µm) and flagellates (< 5 µm).

The Esbjerg 7 sample was the most diverse. The cryptophytes were the most abundant, but the larger diatoms and dinoflagellats most likely accounted for most of the biomass. Common diatoms were *Tabellaria flocculosa*, *Rhizosolenia*, *Pseudo-nitzschia, Dactilysolen fragilissimus* and *Guinardia flaccida*. Abundant dinoflagellates were *Heterocapsa rotundata, Prorocentrum* and *Tripos.* Esbjerg 3 had the lowest total abundance. *Meringosphaera tenerima* was the most abundant species, but these are small cells and do not contribute much to the total phytoplankton biomass which is also true for the haptophytes present. Diatoms such as *Pseudo-nitzschia* and *Guinardia flaccida* were common. Dinoflagellates such as *Scrippsiella* and *Gyrodinium* were also recorded. Esbjerg 1 was dominated by diatoms such as *Entomoneis, Chaetoceros, Leptocylindrus minimus* and small unidentified pennate diatoms (< 20 µm). *Lithodesmium* and *Pseudo-nitzschia* were also recorded. The dictyochophyte *Dictyocha speculum* was observed and some small prasinophytes (5–10 µm). Unidentified monads (5-15 µm) were quite abundant.

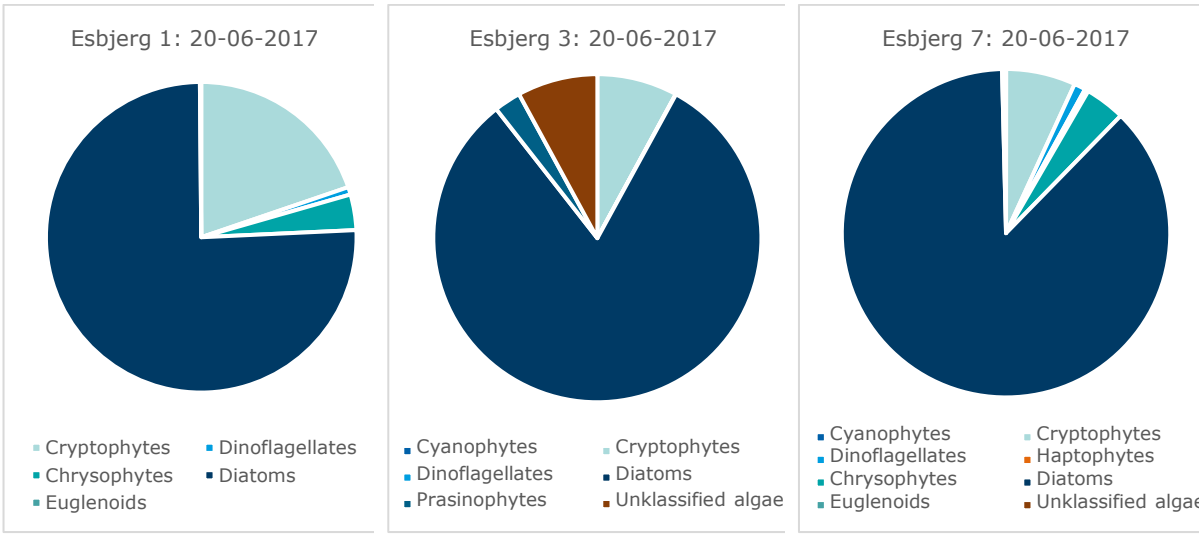

Figure 4.3: *Esbjerg Harbour, cell count summer (June).*

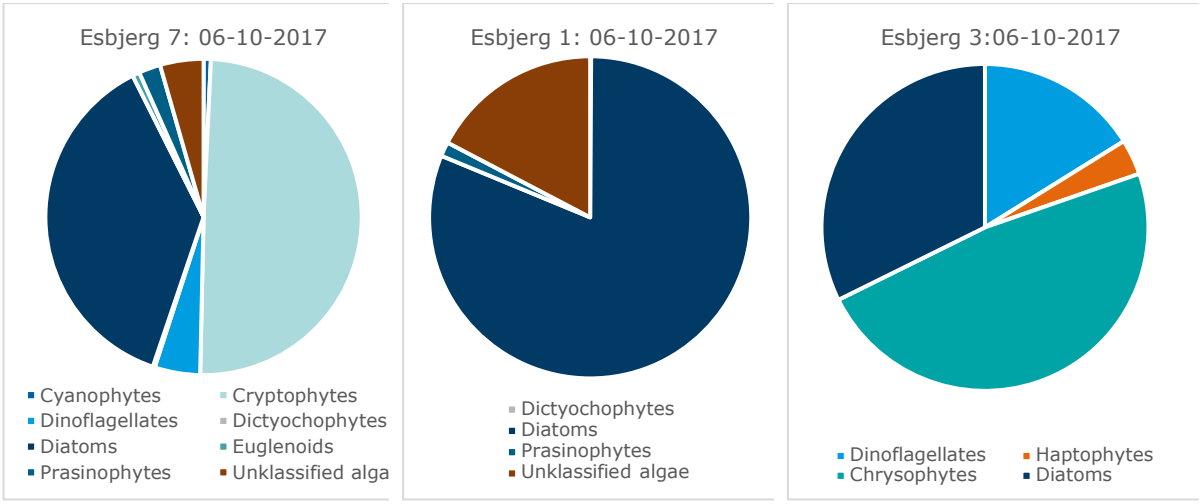

Figure 4.4: *Esbjerg Harbour, cell count, autumn (October).*

Some less abundant species such as the diatom *Lauderia annulata* and the dinoflagellates *Alexandrium pseudogonyaulax* and *Protoperidinium oblongum* were only encountered in the net-samples (see the list of taxa in **Annex 3**).

3.1.2 Zooplankton

Catches differed conspicuously between net hauls made with 200 and 500 µm mesh sizes with very few animals in the coarse net samples. The results presented, thus derive mainly from the 200 µm net hauls. In addition, a few observations of jellyfish were recorded in the field by photography. Since sampling was done within the harbours in proximity of hard substrates, several organisms that are not pelagic were present in the samples. In some cases, freshwater species occurred in the samples, indicating recent runoff. Three species of non-indigenious taxa were recorded. In Esbjerg, the calanoid copepod ***Acartia (Acanthacartia) tonsa*** occurred in all three basins in the autumn samples but were absent in the June samples. The species was also found in Aarhus, but only in one of three autumn samples. An alien cladoceran, ***Penilia avirostris***, was present only at Aarhus, and only in the autumn samples. Finally, two individuals of the ctenophore ***Mnemiopsis leydyi*** were recorded in Esbjerg in September. All three alien species are previously known from the North Sea and Baltic Sea. (see **Annex 4**, list of taxa).

3.2 Mobile epifauna

In Aarhus, 13 species were caught in the traps (**Table 4.1**). The most important species groups were snails, crabs and fish. The most abundant species was the scavenger snail *Hinia reticulata* that was caught in huge quantities in the fine-mesh netting traps. The most common fish species were the 'common wrasse' *Ctenolabrus rupestris* and gobies (*Gobius niger*), whereas other common species were the green shore crab *Carcinus maenas* and the red sea star *Asterias rubens*. With regard to biomass, the snail *Hinia* dominated altogether, whereas the green shore crab and sea stars also contributed substantially to the total biomass (**Table 4.2**). All species are native to Danish waters, with the possible exception that a few small specimens of the black goby might belong to the alien species ***Gobius melanostomus***. For these specimens, morphological characters usually used to distinguish the species (position of black fin dots, number of stiff fin rays), fell between the typical character states for the two species.

Table 4.1: *Catch in crab traps and mesh net traps at the stations in Aarhus (AT1–AT9). Details of the catches (size of collected specimens, biomass) are given in* ***Annex 5****.*

	AT1	AT2	AT3	AT4	AT5	AT6	AT7	AT8	AT9
Crab trap									
Crustacea									
Carcinus maenas	6	6	-	2	5	3	2	3	1
Mollusca									
Hinia reticulata	28	-	8	-	-	-	5	9	-
Echinodermata									
Asterias rubens	2	1	6	-	-	2	-	4	3
Fish									
Taurulus bubalis	-	-	1	-	-	-	-	-	
Mesh netting trap									
Crustacea									
Carcinus maenas	1	8	5	3	-	6	7	4	-
Macropodia rostrate	-	-	-	-	-	-	1	-	-
Crangon crangon	-	-	-	-	-	-	-	2	-
Mollusca									
Hinia reticulata	800	>1000	900	202		209	48	350	>1000
Echinodermata									
Asterias rubens	2	1	-	-	-	-	-	-	1
Psammechinus miliaris	-	-	5	-	-	1	-	-	-
Fish									
Ctenolabrus rupestris	1	1	-	-	1	1	12	3	5
Zoarches viviparous	1	-	-	-	1	-	1	-	-
Gobius niger	2	-	-	3	-	-	8	7	-

Table 4.2: *Summary data for biomass (g wet weight) of catches in crab traps and mesh net traps at the stations in Aarhus. Complete data are given in* ***Annex 5****.*

	AT1	AT2	AT3	AT4	AT5	AT6	AT7	AT8	AT9
Crab trap									
Crustacea	160	140	-	24	80	83	45	60	29
Mollusca	31	-	8	-	-	-	6	9	-
Echinodermata	10	10	290	-	-	8	-	40	7
Fish	-	-	23	-	-	-	-	-	-
Mesh netting trap									
Crustacea	6	15	7	14	-	18	12	6	-
Mollusca	910	2018	1050	230	-	247	52	360	1140
Echinodermata	4	0.8	12	-	-	4	-	-	0.9
Fish	71	2	-	15	42	2	217	24	46

In Esbjerg, 18 species were caught in the traps (**Table 4.3**). The most important species groups were crustaceans and fish. The most abundant species was the green shore crab *Carcinus maenas* that was found at all stations. Prawns (*Crangon* and *Palaemon*) were regularly caught in the fine-mesh traps. With regard to biomass, the shore crab was strongly dominant (**Table 4.4**). The two other crab species, *Hyas* and *Cancer*, were also of moderate to large size. Two sampling stations from Esbjerg harbour is shown in **Figure 4.5**.

Figure 4.5: *Trap stations in Esbjerg harbour. Left: launching of traps in the Dock/Ferry Harbour (Station ET4). Right: position of traps in the North Harbour (Station ET8). White spot at the ladder is the label on the rope for retrieval of traps. Note the very turbid water.*

Three non-indigenous species were recorded. Two species of barnacles, ***Austrominius modestus*** and ***Amphibalanus improvisus*** were established on the carapax (dorsal surface) of several specimens of the green shore crab from about half of the sampling stations (**Figure 4.6**). These species are presumably well established in the area. At one station in the North Harbour, an incidental catch of the pelagic comb medusa ***Mnemiopsis leidyi*** was done. There were quite a few specimens in the fine mesh traps that presumable had been caught from water currents flowing through the trap.

Table 4.3: *Catch in crab traps and mesh net traps at the stations in Esbjerg (ET1–ET9). Details of the catches (size of collected specimens, biomass) are given in **Annex 5**. (+) = recorded, not counted.*

	ET1	ET2	ET3	ET4	ET5	ET6	ET7	ET8	ET9
Crab trap									
Crustacea									
Carcinus maenas	16	43	14	17	20	4	9	6	2
Hyas araneus	-	-	-	1	-	1	-	-	-
Cancer pagurus	-	-	-	-	1	-	-	-	1
Austrominius modestus (on *Carcinus*)	10	(+)	(+)	5	-	-	-	(+)	-
Amphibalanus improvisus (on *Carcinus*)	13	(+)	(+)	24	-	-	-	(+)	-
Echinodermata									
Asterias rubens	-	-	-	-	-	-	-	-	6
Fish									
Zoarches viviparous	-	-	-	-	-	-	-	1	
Mesh netting trap									
Ctenophora									
Mnemiopsis leidyi	-	-	-	-	-	-	-	++	-
Crustacea									
Carcinus maenas	2	-	-	1	-	1	-	-	-
Crangon crangon	-	-	-	1	-	1	-	1	2
Palaemon serratus	2	-	1	-	2	-	-	-	1
Praunus flexuosus	-	-	-	-	-	-	-	1	1
Mollusca									
Hinia reticulata	-	-	-	-	-	1	-	-	-
Echinodermata									
Asterias rubens	-	-	-	-	-	-	-	-	2
Fish									
Zoarches viviparous	-	-	1	-	-	-	2	-	-
Taurulus bubalis	-	-	-	-	-	-	1	-	-
Pomatoschistus minutus	-	-	-	-	-	1	-	-	-
Ciliata mustela	-	1	-	-	1	-	-	2	-

Table 4.4: *Summary data for biomass (g wet weight) of catches in crab traps and mesh net traps at the stations in Esbjerg. Complete data are given in **Annex 5**.*

	ET1	ET2	ET3	ET4	ET5	ET6	ET7	ET8	ET9
Crab trap									
Crustacea	600	1530	570	840	878	169	480	120	262
Echinodermata	-	-	-	-	-	-	-	-	30
Fish	-	-	-	-	-	-	-	134	-
Mesh netting trap									
Crustacea	7	-	0.8	0.5	2.4	0.7	-	0.7	2
Mollusca	-	-	-	-	-	0.7	-	-	-
Echinodermata	-	-	-	-	-	-	-	-	18
Fish	-	14	9	-	30	0,8	50	31	-

Figure 4.6: *The green shore crab* Carcinus maenas *with non-indigenous barnacles* **Austrominius modestus** *and* **Amphibalanus improvisus** *on the carapax.*

3.3 Benthic infauna

3.3.1 Sediment infauna

In Aarhus, a total of 35 species taxa were recorded in the grab samples (**Annex 1**). In the innermost harbour basin (Basin 1) and outermost basin (Basin 9), the fauna was quite impoverished, probably due to high organic load and insufficient water exchange. In Basin 1 (Stns AB1–3), nematods dominated (not sampled quantitatively), whereas the spionid polychaete *Polydora* cf *aggregata* totally dominated at one station (AB4) in Basin 9. In the central basin (Basin 4), fauna species-richness was about normal, but several of the species (oligochaetes, *Mediomastus*, *Chaetozone*) are known to increase in disturbed or organically enriched environments.

One of the collected species is considered non-indigenous in Denmark, viz. the polychaetes ***Alitta succinea***. The species is presumable of West Atlantic origin but was transferred to the Mediterranean Sea a long time ago. The spionid polychaete *Polydora* cf *aggregata* is probably not native, but it belongs in a species complex with quite unclear species affinities. The form in Aarhus agrees closely with ***Polydora aggregata*** from north-east US waters as described by Blake (1971), but also resembles ***P. limicola*** from the North Pacific, that has been reported from California and Germany as well. There is presently much uncertainty as to the origin of the various species in the complex. In the present case ***P. aggregata*** is probably an American species that has been transferred to Europe, but the opposite is quite feasible. Modern taxonomy that incorporates genetic analyses as a new tool may help solving problems with translocated species of uncertain origin.

In Esbjerg, a total of 55 species taxa were recorded in the grab samples (**Annex 1**). Most species and the highest abundances were found in the Traffic Harbour and the Ferry Harbour whereas the Eastern Harbour under construction had both lower species numbers and abundances. Polychaetes, bivalves and crustaceans were the most important groups.

Among the polychaetes there are several non-indigenous species or species of uncertain origin. The most distinctive of these is the spionid ***Streblospio benedicti***, that is native for the North American

east coast (Mahon et al. 2009). This species has been reported from the Netherlands and Great Britain (Radashevsky 2012). Also of North American origin is the phyllodocid ***Eteone heteropoda***. To our knowledge, this species has not been reported from Europe before. Among species of uncertain origin is ***Polydora cornuta***. It is generally believed that this is of North American origin, but it seems to have been transferred early to Europe where it is now widely distributed. It is considered one of the worst invasive species in the Mediterranean Sea (Radashevsky & Selifonova 2013). ***Polydora cornuta*** has previously been reported (as ***Polydora ligni***) from Denmark (Rasmussen 1973) and the Oslofjord (Ramberg & Schram 1983). Also, ***Alitta succinea*** was recorded in Esbjerg.

The high abundance of ***Tharyx* cf *robusta*** is remarkable. The form is close to the newly described ***T. robusta*** from the Swedish west coast (Blake & Göransson 2015) but differ in several characteristic features. If further studies document the two species to be different, the form in Esbjerg is an undescribed species (*J. Blake, pers. comm.*). It then remains an open question if it is introduced in the Esbjerg harbour.

Species in other groups appears to be native to Danish waters, except for a single record of the barnacle ***Austrominius modestus***.

3.3.2 Sediment epifauna

In total, 42 and 36 species were recorded in the dredge samples from Aarhus and Esbjerg harbours, respectively (**Table 4.5**). In Aarhus there were very few species in the sample from the inner Basin 1 and no species in the sample from the outer Basin 9. In the middle Basin 4, 39 species were found. In Esbjerg, the samples from the Traffic Harbour and the Ferry/South Harbour were species-rich, whereas in the East Harbour only some few species and specimens were found. Generally, the same species were found as in the grab samples.

Several non-indigenous species and species of uncertain origin were recorded. Most of these were also found in the grab samples, viz. the polychaetes ***Alitta succinea***, ***Polydora cf aggregata*** in Aarhus and ***Eteone heteropoda***, ***Polydora cornuta*** and ***Streblospio benedicti*** in Esbjerg. In addition, the American razor clam ***Ensis directus*** were found in both harbours and the ascidian ***Styela clava*** in Esbjerg. The two collected specimens of ***S. clava*** were of large size and were partly overgrown by specimens of *Ascidiella obliqua*. Both ***E. directus*** and ***S. clava*** have a wide distribution in the North Sea area and has been reported from several places in Denmark.

Table 4.5: *Number of species in major groups in dredge samples from Aarhus and Esbjerg harbour areas. There were no living animals in the sample from Aarhus Basin 9. Complete results are presented in* ***Annex 2****.*

Area	**Aarhus**			**Esbjerg**		
	Basin 1	**Basin 4**	**Basin 9**	**Traffic Harbour**	**South Harbour**	**East Harbour**
Date	5 Oct	5 Oct	5 Oct	21 Sept	6 Oct	21 Sept
Porifera	-	-	-	-	++	-
Anthozoa	-	-	-	1	1	-
Nematoda	1	-	-	1	1	-
Oligochaeta	-	2	-	-	-	-
Polychaeta	-	14	-	9	4	1
Gastropoda	1	5	-	1	2	1
Bivalvia	-	11	-	5	6	1
Crustacea	-	5	-	3	3	-
Pycnogonida	-	-	-	-	1	-
Echinodermata	-	2	-	1	2	-
Ascidiacea	1	-	-	1	3	-

3.4 Fouling organisms

The list of all non-indigenous and invasive species in Denmark (revised January 10th, 2018) provided by The Danish Environmental Protection Agencies was used as source for assessing the status of the recorded species (Miljøstyrelsen 2018). The list includes all non-indigenous species recorded in Denmark and provides an assessment score of how harmful, "invasive", each of the species is considered according to six categories (e.g. “distribution potential”, “impact on natural habitat” and “economic consequences”). The categories cover the potential overall environmental impact of the presence of the non-indigenous species in Denmark and each species is rated in respect to these categories. The species invasion potential is finally evaluated based on the overall score.

The non-indigenous species is considered invasive if it scores more than two in the categories "Impact on indigenous species" and "Ecosystem Impact Effects" and if the overall score is seven or higher. Ten non-indigenous species were identified from assessing fouling organisms in Esbjerg and Aarhus harbour. Seven of these species are considered invasive according to the list of all non-indigenous and invasive species in Denmark (revised January 10th, 2018) and will be presented below. The presence of algae was low both in Esbjerg and Aarhus and all fouled substrates (PVC plates, RAS substrates and scraped samples) were dominated by colonizing animals. In Esbjerg, the bottom substrate consisted of fine grain silt which contributed to a high sedimentation rate on the substrates and to turbid water with low visibility. Light is crucial for algal growth, hence high levels of particles in the water is probably the major reason for the low presence of sessile algae, in Esbjerg in particular.

Scraped substrate

The abundance and distribution of non-indigenous species were higher in Esbjerg Harbour than in Aarhus harbour (**Table 4.10)**. A total of 23 species were identified from scraped substrates in Esbjerg harbour. Six non-indigenous species were identified among the total number of species, four of them considered invasive according to the list of all non-indigenous and invasive species in Denmark (revised January 10th, 2018).

In Aarhus harbour a total of 27 species were identified from the scraped substrates, three of them non-indigenous and considered invasive in Denmark (**Table 4.6**). The barnacle ***Amphibalanus improvisus*** was the most widely distributed non-indigenous species within the survey areas, found in five of six sampled areas and with high prevalence relative to other non-indigenous species found. A complete list of species recorded in the scrapings is presented in **Annex 6**.

Table 4.6: *Non-indigenous species observed from scrapings of hard substrates in three areas in Esbjerg harbour and three areas in Aarhus harbour in September 2017. The numbers indicate average coverage of the species in each of the three basins assessed in the two harbours; Esbjerg and Aarhus. 1=individual finding, 2=0–25 % coverage, 3=25–75 % coverage, 4=75–100 % coverage).*

Group	Species / Area	Esbjerg			Aarhus		
		North harbour	Dock harbour	East harbour	Basin 9	Basin 3+4	Basin 1
Barnacle	***Amphibalanus improvisus***	3-4	2-3	4	-	2	2
	Austrominius modestus*	2	-	2	-	-	-
Crustacean	***Caprella mutica***	-	-	-	-	-	2
	Hemigrapsus sanguineus	1	2	-	-	-	1
Bivalve	***Crassostrea gigas***	1	2	1	-	-	-
Red algae	***Neosiphonia harveyi****	-	2	-	-	-	-
Ascidian	***Styela clava***	-	-	2	-	-	-

** Not considered invasive.*

Rapid assessment survey (RAS)
Submerged substrates (floating buoys, fender constructions, ropes, etc.) were examined at two sites within the three survey areas of Esbjerg and Aarhus harbours, respectively. Underwater camera was tested but was not considered useful due to the poor visibility in the watermasses. No non-indigenous species was identified from rapid assessment surveys.

A complete species list from the survey is shown in **Annex 6**.

Settlement plates
The settlement plates were densely colonized with fouling organisms when they were collected. The plates deployed in Esbjerg harbour were also heavily laden with sediment, which reduced the biological quality of the sampled organisms and made proper identification of some organisms difficult (**Figure 4.7**). Most settlement plates deployed at shallow water (1 and 3 m) were densely covered with indigenous species of ascidians. In Esbjerg harbour, the solitary ascidian *Ascidiella aspersa* dominated while *Ciona intestinalis* dominated on settlement plates from Aarhus harbour (**Figure 4.7**).

A total of seven non-indigenous species were recorded on the settlement plates, five of them considered invasive according to the list of all non-indigenous and invasive species in Denmark (revised January 10th, 2018; **Table 4.7**). No non-indigenous species were observed on the plates in Basin 9 in Aarhus Harbour. The barnacle ***Amphibalanus improvisus*** was the most common invasive species, found on 13 of the 19 plate units.

Figure 4.7: *Left: Settlement plate from Esbjerg harbour (Dock harbour, station 3, at 3 m depth, upward facing side), dense sediment loading covering the ascidian* Ascidiella aspersa*. Right: Settlement plate from Aarhus harbour (Basin 4, station 8, at 1 m depth, downward facing side) covered by the ascidian* Ciona intestinalis*.*

Table 4.7: *Non-indigenous species observed on 9 settlement plate units in Esbjerg harbour (1–9) and 9 units in Aarhus harbour (1–9) in September 2017. The numbers indicate average coverage of the species: 1=individual finding, 2=0–25 % coverage, 3=25–75 % coverage, 4=75–100 % coverage).*

Group	Species/Sampling site	Esbjerg									Aarhus								
		1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
Anemone	***Diadumene lineata****	-	1	-	2	2	2	1	-	-	-	-	-	-	1	-	-	2	1
Ascidian	***Molgula manhattensis***	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	1
Barnacle	***Amphibalanus improvisus***	3	3-4	2	2	2	2	2	2	2	-	-	-	-	2	-	2	2	1
	Austrominius modestus	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
Crustacean	***Caprella mutica***	-	-	2	-	2	2	2	2	-	-	-	-	-	-	-	2	2	-
Red algae	***Heterosiphonia japonica***	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2
	Neosiphonia harveyi*	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

** Not considered invasive*.

A complete list for indigenous and non-indigenous species identified from settling plates is shown in **Annex 6**. In addition, brief descriptions of the non-indigenous species identified from examining fouling organisms are presented below. Photos of non-indigenous species that were sampled during the survey are shown in **Figure 4.8**.

Amphibalanus improvisus – it is assumed that this barnacle (formerly *Balanus improvisus*) originates from the southern Pacific, but it was introduced to European waters already in the 1800s. The first record of ***A. improvisus*** in Danish waters dates to 1880. Today ***A. improvisus*** is commonly found everywhere along the Danish coast. The pathway of introduction was likely through ballast water or hull fouling. ***A. improvisus*** has a large distribution potential and is transported with the coastal flow during its pelagic larval phase. ***A. improvisus*** is a tolerant species adapted to low salinity and low oxygen level. It colonizes hard substrate from the supralittoral zone (spray zone) down to a depth of approximately two meters. ***A. improvisus*** is considered invasive in accordance to the list of all non-indigenous and invasive species in Denmark.

Caprella mutica (**Figure 4.8:, panel e**) is commonly known as the Japanese skeleton shrimp and is indigenous to the north-western Pacific. It is believed to have been accidentally introduced by ship traffic or aquaculture and was firstly observed in Europe in the early 1990s. The first record of ***C. mutica*** in Denmark is from 2005. The species has direct development and hence little ability to propagate by itself. Hence, the spread to new areas probably occurs when the organism is attached to floating material or on a boat hull. It is mainly found on artificial substrate such as buoys, pontoons, and moorings, where it can occur in extreme high densities and pose a threat to indigenous skeleton shrimps. ***C. mutica*** is considered invasive according to the list of all non-indigenous and invasive species in Denmark.

Crassostrea gigas (**Figure 4.8:, panel b**), commonly known as the Pacific oyster, is indigenous to the Pacific coast of Asia. The oyster was intentionally imported to Europe in the 1960s and introduced to Limfjorden and Lillebelt in the 1970s for aquaculture purpose. Today the oyster is widely distributed and can occur in high densities (> 1000 in/m^2) and form reef formations that completely overlay the bottom substrate. This is particularly known from the Wadden Sea. ***C. gigas*** has a high distribution potential due to a high fecundity and long-lasting larval phase. ***C. gigas*** is considered invasive according to the list of all non-indigenous and invasive species in Denmark.

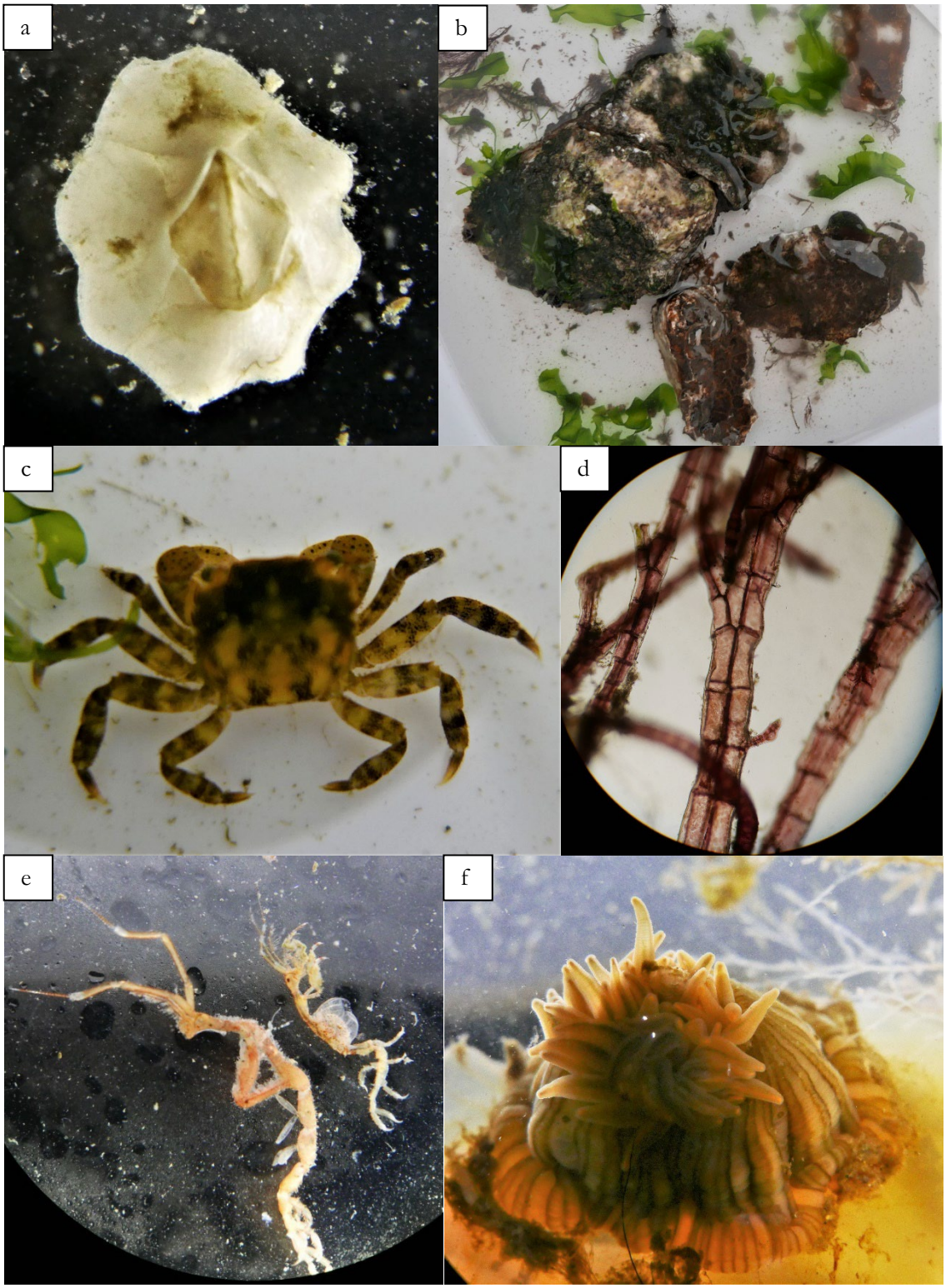

Figure 4.8: *Photos of non-indigenous species sampled from Esbjerg and Aarhus harbour. a:* **E. modestus**. *b:* **C. gigas**. *c:* **H. sanguineous**. *d:* **N. harveyi**. *e:* **C. mutica**. *f.* **D. lineata**.

Diadumene lineata (**Figure 4.8, panel f**) is a small sea anemone with indigenous origin from the north-western Pacific. It was probably unintentionally introduced to Europe in the late 19th century and is now found throughout Western Europe. It propagates by division and it is estimated to have low

distribution potential. ***D. lineata*** is not considered invasive according to the list of all non-indigenous and invasive species in Denmark.

Austrominius modestus: The Australien barnacle ***E. modestus*** (**Figure 4.8, panel a**) is indigenous to the coast of South Australia and New Zealand. It was unintentionally introduced to Europen, probably by ballast water or attached to ship hulls. In Denmark, ***E. modestus*** has been recorded since the 1960s, but it has probably died during cold winters and has not been considered permanently established. It has been found every year between 2004 and 2008 in the Wadden Sea, and in 2007 it was found near the eastern entrance of the Limfjord. ***E. modestus*** is not considered invasive according to the list of all non-indigenous and invasive species in Denmark.

Hemigrapsus sanguineus: The Japanese shore crab ***H. sanguineus*** (**Figure 4.8:, panel c**) originates from the northern Pacific. The species was probably introduced to the Le Havre area in France in the period around 1998. It was first recorded in Denmark (Esbjerg harbour) in 2011. ***H. sanguineus*** is considered invasive according to the list of all non-indigenous and invasive species in Denmark.

Heterosiphonia japonica: The red algae ***H. japonica*** originates from the North Pacific and was recorded in Europe in the 1990s. The species has spread rapidly since its establishment and was discovered several sites in Denmark in 2006, including Limfjorden. ***H. japonica*** grows rapidly and spreads over large areas and may displace indigenous algal species. It also attaches to other algae and to benthic animals. ***H. japonica*** is considered invasive according to the list of all non-indigenous and invasive species in Denmark.

Neosiphonia harveyi: The red algae ***N. harveyi*** (**Figure 4.8:, panel d**) was observed first time in Europe in the early 1900s. The algae usually grow epithetically in larger algae on shallow water but also on eelgrass, shells and rocks, and in different types of artificial substrates such as rope and wood. It appears that the species has become significantly more common along European coastlines over the course of the last 30 years. It was firstly recorded in Denmark in 1986 but is not considered invasive according to the list of all non-indigenous and invasive species in Denmark.

Molgula manhattensis: In Western Europe, this solitary ascidian has been reported back to 1760, and today the species is found along the coasts of Western Europe from Portugal to the UK and Scandinavia. The species is taxonomically difficult to identify, and several synonyms are described. It has long been uncertain whether the species originally is a Western European species that has spread with planktonic larvae to East America or if the spread has occurred by ship traffic from America to Europe. Recent genetic studies indicate that the species is naturally occurring in North America, but it is still unclear whether it is naturally occurring in Europe or introduced here. ***M. manhattensis*** is considered invasive according to the list of all non-indigenous and invasive species in Denmark.

Styela clava is a solitary ascidian indigenous to the northwest Pacific. It is likely that it spread to Europe with British warships returning after the end of the Korean War in 1951. The first record in Denmark was around 1980 when it was found in Limfjorden. ***S. clava*** occurs on hard bottom and all kinds of artificial substrate from the low littoral zone to about 40 m depth. It is an active competitor to ingenious mussels and sedentary filtering organisms for space and nutrition. In recent years, the species has spread very quickly and is now commonly distributed throughout the western European coast from Portugal to Denmark and Norway. ***S. clava*** is considered invasive according to the list of all non-indigenous and invasive species in Denmark.

3.5 Fish, standard methods

In the standard fishing method campaign, a total of 37 sites were sampled. All are closely connected with the 16 harbours chosen by the project (**Table 4.8**).

We classified sampling sites into three categories, according to harbour activity. Industrial harbours include activity ranging from shipping and ferrying to oil refinery plants. Fishing harbours ranged from local marinas with few recreational fishing vessels to industrial scale fishing vessels. Leisure harbours housed sailing boats, yachts and other recreational boats.

Fifteen sites were classified as industrial (such as Aarhus industrihavn, Rødby industrihavn and Kalundborg Statoil-havn, which harboured an oil refinery plant). Six sites were classified as fishery activity, with Hirtshals being the largest fishing harbour, and sixteen sites were classified as leisure harbours (**Table 4.8**).

Overall, the standard fishing method campaign caught 32 different fish species, five different crab and prawn species plus a whelk and a sea urchin species (see **Annex 7**). 4.908 individuals were registered. 3.456 of these were common shore crab. This species was thus, by far, the most abundant species overall, and it was present in all harbours and sample sites.

The round goby (***Neogobius melanostomus***) was the only invasive species caught with standard fishing methods (**Table 4.9**). It was caught at four locations in two harbours, Gedser Harbour and Køge Harbour. All ***N. melanostomus*** specimens were closely examined, and species identified on site if possible, following the guidelines of Carl *et al.* (2016). If there was any doubt in connection to the species determination, later identification was performed either based on photographs of the individuals, or by expert physical inspection.

The following section decribes how to identify the species, translated to English from Carl *et al.* (2016): Like the resident goby species, round goby has fused pelvic fins forming a suction cup. The two dorsal fins are separate but closely positioned together. The anterior dorsal fin consists of 5–7 fin rays, while the posterior dorsal fin consists of one spine and 15–17 fin rays (sometimes one spine and 14–16 fin rays). In comparison, the black goby (*Gobius niger*), which is often mistaken for round goby, has only 13–14 fin rays in the posterior dorsal fin (Sand goby (*Pomatoschistus minutus*), common goby (*Pomatoschistus microps*) and painted goby (*Pomatoschistus pictus*) has 8–13 fin rays). The body of Round goby is strong and slightly compact laterally, especially in the posterior part of the body. The caudal peduncle is tall and powerful. The head is large and very wide. The mouth is large, and the lips are large and thick. The colour can vary a lot according to gender, age and surroundings. The base colour is yellowish, brownish or greyish. The fish usually have a darker speckled/checkerboard pattern. On the dorsal part of the body, sometimes there are areas of brighter colour. Dominant males become significantly darker during breeding season, and they can become completely black with yellowish or bright edges on especially the posterior dorsal fin and the caudal fin. One of the best characteristics is that there is a distinct black spot in the posterior part of the anterior dorsal fin, which can however be hard to see in the darkest individuals. In the younger individuals, the black spot is often surrounded by a slightly brighter ring. By comparison, black goby usually has a black area in the anterior part of the anterior dorsal fin. The round goby is the biggest goby species in Danish waters and can in many cases be identified just on its size. In foreign literature, the maximum length is usually 25 cm. In Denmark there are documented catches of Round goby up to 23 cm in length and undocumented catches of fish up to 30 cm in length. By comparison, Black goby only extremely rarely grow longer than 15 cm in length.

Tabel 4.8: *Standard fishing methods - sample overview and locations.*

Harbours and sample locations	Activity					
	INDUS	**FISH**	**YACHT**	**Lat**	**Long**	**Date**
1 Aarhus Harbour						
Aarhus industrihavn	x			56.14873	10.23107	7.9.2017
Aarhus lystbådehavn			x	56.16947	10.22627	7.9.2017
Aarhus Marselisborg			x	56.13702	10.21390	7.9.2017
2 Esbjerg Harbour						
Esbjerg industrihavn	x			55.46018	8.44030	6.9.2017
Esbjerg lystbådehavn			x	55.47287	8.42318	6.9.2017
3 Aalborg Portland Harbour						
Aalborg Portland Harbour	x			57.06562	9.97245	11.9.2017
4 Aalborg Harbour						
Aalborg industrihavn	x			57.05075	10.05793	12.9.2017
Aalborg lystbådehavn			x	57.05818	9.90418	11.9.2017
Aalborg fjordparken			x	57.05682	9.87392	12.9.2017
5 Fredericia Harbour						
Fredericia industrihavn	x			55.55885	9.73555	5.9.2017
Fredericia lystbådehavn			x	55.55353	9.73018	5.9.2017
6 Frederikshavn Harbour						
Frederikshavn industrihavn	x			57.43703	10.55470	14.9.2017
Frederikshavn marina			x	57.42528	10.53300	14.9.2017
7 Gedser Harbour						
Gedser fiskerihavn		x		54.57167	11.92862	28.8.2017
Gedser lystbådehavn			x	54.58133	11.91962	28.8.2017
8 Grenå Harbour						
Grenå industrihavn	x			56.41457	10.92633	10.9.2017
Grenå fiskerihavn		x		56.41015	10.92520	10.9.2017
Grenå marina			x	56.40277	10.92400	10.9.2017
9 Helsingør Harbour						
Helsingør færgehavn	x			56.03365	12.61697	23.8.2017
Helsingør fiskerihavn		x		56.03710	12.61770	23.8.2017
Helsingør nordhavn			x	56.04430	12.61837	23.8.2017
10 Hirtshals Harbour						
Hirtshals industrihavn	x			57.59562	9.97643	13.9.2017
Hirtshals fiskerihavn*		x		57.59343	9.95843	13.9.2017
Hirtshals lystbådehavn			x	57.59720	9.96387	13.9.2017
11 Kalundborg Harbour						
Kalundborg industrihavn	x			55.67640	11.09283	30.8.2017
Kalundborg Gisseløre			x	55.67732	11.07560	30.8.2017
Kalundborg Vesthavnen			x	55.67777	11.08160	30.8.2017
12 Københavns Harbour						
København industrihavn	x			55.70963	12.60375	21.8.2017
København fiskerihavn		x		55.72483	12.60182	21.8.2017
København lystbådehavn			x	55.71892	12.59075	21.8.2017
13 Køge Harbour						
Køge industrihavn	x			55.45327	12.19878	22.8.2017
Køge marina			x	55.47138	12.20095	22.8.2017
14 Odense havn						
Odense Fynsværket	x			55.42942	10.40238	4.9.2017
Odense Centrum			x	55.41053	10.37913	4.9.2017
15 Rødby Harbour						
Rødby industrihavn	x			54.65080	11.35217	29.8.2017
Rødby fiskerihavn		x		54.65388	11.34560	29.8.2017
16 Statoil (Kalundborg)						
Statoil	x			55.66408	11.09002	30.8.2017

*BOLD font indicates harbour locations closest to the sampling points of eDNA and night diving. * Due to insufficient space, it was only possible to deploy the fykenet in Hirtshals Fiskerihavn.*

Table 4.9: *Data on **Neogobius melanostomus** caught using standard fishing methods.*

Harbour	Gear	Numbers	Total length, cm	Total weight, g	Photo in Annex
Køge industrihavn	Gill	5	7,8,9,10,11	54	IMG_3200
Køge industrihavn	Fyke	1	8	10	IMG_3200
Køge marina	Gill	1	17	76	IMG_3198
Køge marina	Fyke	2	11,17	110	IMG_3198
Gedser fiskerihavn	Fyke	1	13	40	IMG_3266
Gedser lystbådehavn	Gill	6	10,10,10,11,11,15	158	IMG_3271
Gedser lystbådehavn	Fyke	1	12	30*	IMG_3270

**estimate.*

In total, 17 ***N. melanostomus*** were caught with an average total length of 11 cm (range: 7–17 cm) and an average weight of 28 g. (range: 10–76 g.). Nine individuals were caught in Køge Harbour and eight in Gedser Harbour. In the two harbours respectively, 67 % and 75 % were caught in gillnets, the rest in fykenets (**Figure 4.9, Table 4.13**).

Of the non-invasive fish species caught, it is worth mentioning some of the rarer species. The thinlip mullet (*Liza ramada*) caught in Hirtshals Harbour is the largest specimen ever caught on record in Denmark (length: 62 cm, weight: 1966 g). The species is considered very rare in Danish waters. The twaite shad (*Alosa fallax*) caught in Aarhus Harbour (length: 26 cm, weight: 150 g) is also considered rare. Its population is believed to have been reduced significantly over the years due to pollution and stream regulation. The two whiting-pout (*Trisopterus luscus*) caught in Esbjerg Harbour (one fish, length 16 cm, weight 52 g) and the one caught in Hirtshals Harbour (length: 22 cm, weight: 128 g) are also considered rare. The two small-mouthed wrasse (*Centrolabrus exoletus*) caught in Aarhus Harbour (length: 9 and 10 cm, total weight: 22 gr) represent the only species of the four that breed in Danish waters, but is at the same time considered to be a very rare species (Carl *et al.* 2004, Muss *et al.* 2006).

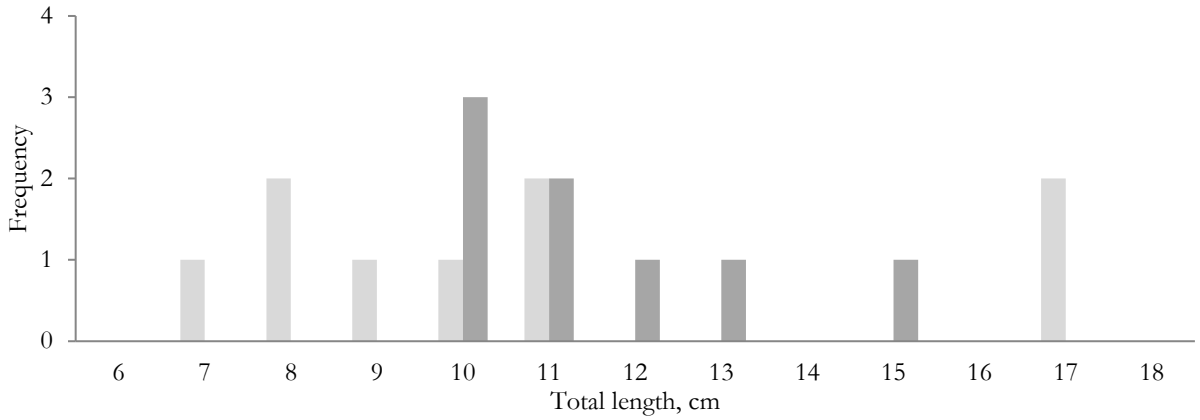

Figure 4.9: *Length distribution of round goby caught using standard fishing methods. Fish caught in Køge Harbour (light grey), fish caught in Gedser Harbour (dark grey).*

3.6 Fish, snorkeling

A total of 41 fish species were recorded, hereof a single non-indigenous species (***Neogobius melanostomus***). Data on observed non-indigenous species, i.e. five invertebrates and a single macroalge were also collected. The following section gives a brief summary of the observed fish species and non-indigenous species in each harbour. Abundance data are provided for non-indigenous fish (***N. melanostomus***). A short note on observed non-indigenous invertebrates is also given.

1. Aarhus: Field work was carried out twice. The 5th of July 2017, nine fish species (all native) were registered during snorkeling (day): Black goby (*Gobius niger*), Cod (*Gadus morhua*), Common goby (*Pomatoschistus microps*), Corkwing wrasse (*Symphodus melops*), Flounder (*Platichthys flesus*), Goldsinny wrasse (*Ctenolabrus rupestris*), Greater sand-eel (*Hyperoplus lanceolatus*), Sand goby (*Pomatoschistus minutus*) and Two-spotted goby (*Gobiusculus flavescens*). Of non-indigenous invertebrates, Atlantic razor clam (***Ensis directus***) was seen. Sargassum seaweed ***(Sargassum muticum***) was also common. 19th of September 2017, nine fish species (all native) were registered during snorkeling (day): Black goby (*Gobius niger*), Cod (*Gadus morhua*), Corkwing wrasse (*Symphodus melops*), Eel (*Anguilla anguilla*), Goldsinny wrasse (*Ctenolabrus rupestris*), Nilsson's pipefish (*Syngnathus rostellatus*), Sea stickleback (*Spinachia spinachia*), Three-spined stickleback (*Gasterosteus aculeatus*) and Two-spotted goby (*Gobiusculus flavescens*). Of non-indigenous invertebrates a single Warty comp jelly (***Mnemiopsis leidyi***) was seen. Sargassum seaweed (***S. muticum***) was common.

2. Esbjerg: Field work was carried out 8th of November 2017 (night). Due to extremely low visibility, snorkeling was impossible, and a fine-meshed beach seine and shrimp push net was used instead. 11 fish species were caught (all native): Cod (*Gadus morhua*), Common goby (*Pomatoschistus microps*), Dab (*Limanda limand*a), Flounder (*Platichthys flesus*), Plaice (*Pleuronectes platessa*), Pogge (*Agonus cataphractus*), Sand goby (*Pomatoschistus minutus*), Short-horn sculpin (*Myoxocephalus scorpius*), Sprat (*Sprattus sprattus*), Turbot (*Scophthalmus maximus*) and Whiting (*Merlangius merlangus*). Of non-indigenous invertebrates, Common slipper shell (***Crepidula fornicata***), Pacific oyster (***Crassostrea gigas***) and Warty comp jelly (***Mnemiopsis leidyi***) were caught.

3. Aalborg Portland: Field work was carried out 11th of October 2017 (day). Besides snorkeling a shrimp push net was used in the seaweed. Seven fish species were caught (all native): Broadnosed pipefisk (*Syngnathus typhle*) (only caught in the shrimp net), Common goby (*Pomatoschistus microps*), Flounder (*Platichthys flesus*), Lumpsucker (*Cyclopterus lumpus*), Sand goby (*Pomatoschistus minutus*), Three-spined stickleback (*Gasterosteus aculeatus*) and Two-spotted goby (*Gobiusculus flavescens*). Of non-indigenous invertebrates, Common slipper shell (***Crepidula fornicata***), Pacific oyster (***Crassostrea gigas***), Stalked sea squirt (***Styela clava***) and Warty comp jelly (***Mnemiopsis leidyi***) were caught.

4. Aalborg: Field work was carried out 11th of October 2017 (day). Besides snorkeling a shrimp push net was used in the seaweed. Snorkeling revealed seven fish species (all native): Black goby (*Gobius niger*), Broadnosed pipefisk (*Syngnathus typhle*), Common goby (*Pomatoschistus microps*), Flounder (*Platichthys flesus*), Sand goby (*Pomatoschistus minutus*), Three-spined stickleback (*Gasterosteus aculeatus*), Two-spotted goby (*Gobiusculus flavescens*). In addition, Sea stickleback (*Spinachia spinachia*) and Straight-nosed pipefish (*Nerophis ophidion*) were caught in the shrimp push net. Of non-indigenous invertebrates, Common slipper shell (***Crepidula fornicata***), Stalked sea squirt (***Styela clava***) and Warty comp jelly (***Mnemiopsis leidyi***) were recorded.

5. Fredericia: Field work was carried out twice. The 5th of July 2017, ten fish species (all native) were registered during night snorkeling: Black goby (*Gobius niger*), Cod (*Gadus morhua*), Dab (*Limanda limanda*), Flounder (*Platichthys flesus*), Goldsinny wrasse (*Ctenolabrus rupestris*), Pogge (*Agonus cataphractus*), Sand goby (*Pomatoschistus minutus*), Three-spined stickleback (*Gasterosteus aculeatus*), Transparent goby (*Aphia minuta*) and Two-spotted goby (*Gobiusculus flavescens*). The 11th of October 2017, 13 fish species (all native) were registered during snorkeling (dusk): Black goby (*Gobius niger*), Broadnosed pipefisk (*Syngnathus typhle*), Cod (*Gadus morhua*), Common dragonet (*Callionymus lyra*), Common goby (*Pomatoschistus microps*), Corkwing wrasse (*Symphodus melops*), Goldsinny wrasse (*Ctenolabrus rupestris*), Nilsson's pipefish (Syngnathus rostellatus), Painted goby (*Pomatoschistus pictus*), Sand goby (*Pomatoschistus minutus*), Sea stickleback (*Spinachia spinachia*), Sea trout (*Salmo trutta*) and Two-spotted goby (*Gobiusculus flavescens*). Of non-indigenous invertebrates, Warty comp jelly (***Mnemiopsis leidyi***) was seen. Besides snorkeling, a shrimp push net was used in the seaweed, and beside some of the species seen during snorkeling, a Sea scorpion (*Taurulus bubalis;* in Danish: Langtornet ulk) was caught.

6. Frederikshavn: Field work was carried out 6th of October 2017 (day). Nine fish species (all native) were registered: Broadnosed pipefisk (*Syngnathus typhle*), Common goby (*Pomatoschistus microps*), Flounder (*Platichthys flesus*), Lesser sand-eel (*Ammodytes tobianus*), Nilsson's pipefish (*Syngnathus rostellatus*), Sand goby (*Pomatoschistus minutus*), Sea stickleback (*Spinachia spinachia*), Three-spined stickleback (*Gasterosteus aculeatus*) and Viviparous eelpout (*Zoarces viviparus*). Of non-indigenous invertebrates, Warty comp jelly (***Mnemiopsis leidyi***) was seen.

7. Gedser: Field work was carried out 23th of September 2017 (day). 13 fish species were registered during snorkeling. 12 of these were native: Black goby (*Gobius niger*), Broadnosed pipefisk (*Syngnathus typhle*), Common goby (*Pomatoschistus microps*), Eel (*Anguilla anguilla*), Flounder (*Platichthys flesus*), Nine-spined stickleback (*Pungitius pungitius*), Painted goby (*Pomatoschistus pictus*), Sand goby (*Pomatoschistus minutus*), Sea stickleback (*Spinachia spinachia*), Straight-nosed pipefish (*Nerophis ophidion*), Three-spined stickleback (*Gasterosteus aculeatus*) and Two-spotted goby (*Gobiusculus flavescens*). The invasive Round goby (***Neogobius melanostomus***) was found in relatively low numbers. Of non-indigenous invertebrates, Warty comp jelly (***Mnemiopsis leidyi***) was present in large numbers.

8. Grenå: Field work was carried out twice. The 5th of July 2017 (day), 14 fish species (all native) were registered during snorkeling: Black goby (*Gobius niger*), Brill (*Scophthalmus rhombus*), Cod (*Gadus morhua*), Common sole (*Solea solea*), Corkwing wrasse (*Symphodus melops*), Eel (*Anguilla anguilla*), Flounder (Platichthys flesus), Goldsinny wrasse (*Ctenolabrus rupestris*), Great pipefish (*Syngnathus acus*), Greater weever (*Trachinus draco*), Sea scorpion (*Taurulus bubalis*), Sea stickleback (*Spinachia spinachia*), Short-horn sculpin (*Myoxocephalus scorpius*) and Two-spotted goby (*Gobiusculus flavescens*). Sargassum seaweed (***Sargassum muticum***) was present in large quantities. The 19th of September 2017 (day), 16 fish species (all native) was seen during snorkeling: Black goby (*Gobius niger*), Broadnosed pipefisk (*Syngnathus typhle*), Butterfish (*Pholis gunnellus*), Common goby (*Pomatoschistus microps*), Corkwing wrasse (*Symphodus melops*), Eel (*Anguilla anguilla*), Flounder (*Platichthys flesus*), Goldsinny wrasse (*Ctenolabrus rupestris*), Sand goby (*Pomatoschistus minutus*), Sea stickleback (*Spinachia spinachia*), Sea trout (*Salmo trutta*), Short-horn sculpin (*Myoxocephalus scorpius*), Straight-nosed pipefish (*Nerophis ophidion*), Three-spined stickleback (*Gasterosteus aculeatus*), Transparent goby (*Aphia minuta*) and Two-spotted goby (*Gobiusculus flavescens*). Of

non-indigenous invertebrates, Warty comp jelly (***Mnemiopsis leidyi***) was present in large numbers. Sargassum seaweed (***Sargassum muticum***) was again present in large quantities.

9. Helsingør: This harbour was subject to thorough investigations and snorkeling was carried out a total of 5 times. The 11th of July 2017, nine fish species (all native) were registered: Black goby (*Gobius niger*), Broadnosed pipefisk (*Syngnathus typhle*), Common goby (*Pomatoschistus microps*), Corkwing wrasse (*Symphodus melops*), Flounder (*Platichthys flesus*), Sand goby (*Pomatoschistus minutus*), Straight-nosed pipefish (*Nerophis ophidion*), Three-spined stickleback (*Gasterosteus aculeatus*) and Two-spotted goby (*Gobiusculus flavescens*). The 31st of July 2017, 20 fish species (all native) were registered during daytime: Black goby (*Gobius niger*), Brill (*Scophthalmus rhombus*), Broadnosed pipefisk (*Syngnathus typhle*), Cod (*Gadus morhua*), Corkwing wrasse (*Symphodus melops*), Eel (*Anguilla anguilla*), Goldsinny wrasse (*Ctenolabrus rupestris*), Greater sand-eel (*Hyperoplus lanceolatus*), Greater weever (*Trachinus draco*), Herring (*Clupea harengus*), Lesser sand-eel (*Ammodytes tobianus*), Nilsson's pipefish (*Syngnathus rostellatus*), Painted goby (*Pomatoschistus pictus*), Saithe (*Pollachius virens*), Sand goby (*Pomatoschistus minutus*), Sea scorpion (*Taurulus bubalis*), Sea stickleback (*Spinachia spinachia*), Sea trout (*Salmo trutta*), Short-horn sculpin (*Myoxocephalus scorpius*) and Two-spotted goby (*Gobiusculus flavescens*). During night snorkeling the same night, 16 fish species (all native) were seen: Black goby (*Gobius niger*), Cod (*Gadus morhua*), Corkwing wrasse (*Symphodus melops*), Eel (*Anguilla anguilla*), Flounder (*Platichthys flesus*), Great pipefish (*Syngnathus acus*), Greater weever (*Trachinus draco*), Herring (*Clupea harengus*), Plaice (*Pleuronectes platessa*), Saithe (*Pollachius virens*), Sand goby (*Pomatoschistus minutus*), Sea scorpion (*Taurulus bubalis*), Sea stickleback (*Spinachia spinachia*), Sea trout (*Salmo trutta*), Short-horn sculpin (*Myoxocephalus scorpius*) and Viviparous eelpout (*Zoarces viviparus*). Of non-indigenous invertebrates, Warty comp jelly (***Mnemiopsis leidyi***) was present. Snorkeling was also continued the 1st of September 2017 (day). This time 12 fish species (all native) were registered: Black goby (*Gobius niger*), Cod (*Gadus morhua*), Common sole (*Solea solea*), Corkwing wrasse (*Symphodus melops*), Goldsinny wrasse (*Ctenolabrus rupestris*), Great pipefish (*Syngnathus acus*), Greater sand-eel (*Hyperoplus lanceolatus*), Plaice (*Pleuronectes platessa*), Saithe (Pollachius virens), Transparent goby (*Aphia minuta*), Two-spotted goby (*Gobiusculus flavescens*) and Viviparous eelpout (*Zoarces viviparus*). The last snorkeling (day) was done on the 13th of September 2017, and ten fish species (all native) were seen: Black goby (*Gobius niger*), Broadnosed pipefish (*Syngnathus typhle*), Common goby (*Pomatoschistus microps*), Goldsinny wrasse (*Ctenolabrus rupestris*), Greater weever (*Trachinus draco*), Saithe (*Pollachius virens*), Sand goby (*Pomatoschistus minutus*), Sea stickleback (*Spinachia spinachia*), Three-spined stickleback (*Gasterosteus aculeatus*) and Two-spotted goby (*Gobiusculus flavescens*). Of non-indigenous invertebrates, a single Pacific oyster (***Crassostrea gigas***) was registered.

10. Hirtshals: Field work was carried out the 8th of November 2018. Despite calm water the visibility was low and not a single fish was seen during snorkeling. During angling from the pier, three fish species (all native) were caught: Cod (*Gadus morhua*), Dab (*Limanda limanda*) and Saithe (*Pollachius virens*). A shrimp push net was used in the tidal pools just east of the harbour, and five native fish species were caught: Common goby (*Pomatoschistus microps*), Nilsson's pipefish (*Syngnathus rostellatus*), Plaice (*Pleuronectes platessa*), Sand goby (*Pomatoschistus minutus*) and Three-spined stickleback (*Gasterosteus aculeatus*).

11. Kalundborg: Field work was carried out 22th of September 2017 (day). During snorkeling, 14 fish species were registered. 13 of these were native: Black goby (*Gobius niger*), Broadnosed pipefisk (*Syngnathus typhle*), Common goby (*Pomatoschistus microps*), Corkwing wrasse

(*Symphodus melops*), Flounder (*Platichthys flesus*), Goldsinny wrasse (*Ctenolabrus rupestris*), Sand goby (*Pomatoschistus minutus*), Sea stickleback (*Spinachia spinachia*), Sea trout (*Salmo trutta*), Straight-nosed pipefish (*Nerophis ophidion*), Three-spined stickleback (*Gasterosteus aculeatus*), Two-spotted goby (*Gobiusculus flavescens*) and Viviparous eelpout (*Zoarces viviparus*). The invasive Round goby (***Neogobius melanostomus***) was found in large numbers and mostly young of the year – an indication of a newly established breeding population at the locality. Of non-indigenous invertebrates, Pacific oyster (***Crassostrea gigas***) and Warty comp jelly (***Mnemiopsis leidyi***) were seen.

12. Københavns havn: Field work was carried out 12th of September 2017 (day). During snorkeling, 16 fish species were registered. 15 of these were native: Black goby (*Gobius niger*), Broadnosed pipefisk (Syngnathus typhle), Common goby (*Pomatoschistus microps*), Corkwing wrasse (*Symphodus melops*), Eel (*Anguilla anguilla*), Flounder (*Platichthys flesus*), Goldsinny wrasse (*Ctenolabrus rupestris*), Nilsson's pipefish (*Syngnathus rostellatus*), Nine-spined stickleback (*Pungitius pungitius*), Saithe (*Pollachius virens*), Sand goby (*Pomatoschistus minutus*), Sea stickleback (*Spinachia spinachia*), Straight-nosed pipefish (*Nerophis ophidion*), Three-spined stickleback (*Gasterosteus aculeatus*) and Two-spotted goby (*Gobiusculus flavescens*). A single invasive Round goby (***Neogobius melanostomus***) was seen – the most northern registration in Øresund so far. Of non-indigenous invertebrates, Pacific oyster (***Crassostrea gigas***) and Warty comp jelly (***Mnemiopsis leidyi***) were registered.

13. Køge: Field work was carried out 12th of September 2017 (day). During snorkeling, 11 fish species were registered. Ten of these were native: Black goby (*Gobius niger*), Broadnosed pipefisk (Syngnathus typhle), Common goby (*Pomatoschistus microps*), Eel (*Anguilla anguilla*), Goldsinny wrasse (*Ctenolabrus rupestris*), Nine-spined stickleback (*Pungitius pungitius*), Perch (*Perca fluviatilis*), Sea stickleback (*Spinachia spinachia*), Three-spined stickleback (*Gasterosteus aculeatus*) and Two-spotted goby (*Gobiusculus flavescens*). The invasive Round goby (***Neogobius melanostomus***) was found in relatively high numbers. Of non-indigenous invertebrates a single Warty comp jelly (***Mnemiopsis leidyi***) was registered.

14. Odense: Field work was carried out 15th of September 2017 (night). During snorkeling, ten fish species (all native) were registered: Black goby (*Gobius niger*), Broadnosed pipefisk (*Syngnathus typhle*), Common goby (*Pomatoschistus microps*), Eel (*Anguilla anguilla*), Flounder (*Platichthys flesus*), Herring (*Clupea harengus*), Nilsson's pipefish (*Syngnathus rostellatus*), Sand goby (*Pomatoschistus minutus*), Sea trout (*Salmo trutta*) and Three-spined stickleback (*Gasterosteus aculeatus*).

15. Rødby: Field work was carried out 23rd of September 2017 (day). During snorkeling, 15 fish species were registered. 14 of these were native: Black goby (*Gobius niger*), Broadnosed pipefisk (*Syngnathus typhle*), Common goby (*Pomatoschistus microps*), Flounder (*Platichthys flesus*), Garfish (*Belone belone*), Greater sand-eel (*Hyperoplus lanceolatus*), Herring (*Clupea harengus*), Sand goby (*Pomatoschistus minutus*), Sea stickleback (*Spinachia spinachia*), Straight-nosed pipefish (*Nerophis ophidion*), Three-spined stickleback (*Gasterosteus aculeatus*) and Two-spotted goby (*Gobiusculus flavescens*). Two specimens of the invasive Round goby (***Neogobius melanostomus***) were seen. Many more could have been present, but the big ferries at the harbour caused a lot of sediment from the bottom to rise up in the water column and made it difficult to see bottom dwelling fish. Of non-indigenous invertebrates a few Warty comp jelly (***Mnemiopsis leidyi***) were seen.

16. Statoil (Kalundborg): Field work was carried out 22th of September 2017 (day). During snorkeling, 15 fish species were registered. 14 of these were native: Black goby (*Gobius niger*), Broadnosed pipefisk (*Syngnathus typhle*), Common goby (*Pomatoschistus microps*), Corkwing wrasse (*Symphodus melops*), Eel (*Anguilla anguilla*), Flounder (*Platichthys flesus*), Goldsinny wrasse (*Ctenolabrus rupestris*), Painted goby (*Pomatoschistus pictus*), Sand goby (*Pomatoschistus minutus*), Sea stickleback (*Spinachia spinachia*), Sea trout (*Salmo trutta*), Three-spined stickleback (*Gasterosteus aculeatus*), Two-spotted goby (*Gobiusculus flavescens*) and Viviparous eelpout (*Zoarces viviparus*). The invasive Round goby (***Neogobius melanostomus***) was found in relatively large numbers and mostly young of the year – an indication of a newly established breeding population at the locality. Of non-indigenous invertebrates a few Warty comp jelly (***Mnemiopsis leidyi***) were seen.

Species list for the fish and other organisms recored via snorkeling is presented in **Annex 8**.

3.7 Molecular methods

The eDNA levels in the filtered water samples, all qPCR results were evaluated from the Cycle threshold of quantification (Cq) and in relation to limit of detection (LOD) and limit of quantification (LOQ) (Klymus et al. 2020).

The result of each qPCR assay was evaluated on the observed Cycle threshold of quantification (Cq) values and were for each replicate of each tested water sample categorized as: (1) No Cq – i.e. no amplification at all, (2) amplification detected but below LOD, (3) amplification detected above LOD but below LOQ, (4) amplification detected with levels above LOQ (**Table 4.10** and **4.11**).

Each sample (comprising triplicate qPCR assays) was evaluated according to the lowest Cq score of each set of technical triplicates, and categorized as follows: NEGATIVE (WHITE): No Cq observed in any of the triplicates; WEAK POSSIBLE POSITIVE (YELLOW): amplification observed in at least one triplicate, but amplification below LOD; POSSIBLE POSITIVE (ORANGE): Cq observed in at least one triplicate, and amplification detected above LOD but below LOQ; POSITIVE (RED): Ct observed in at least one triplicate with levels above LOQ; POSITIVE AND POSSIBLE TO QUANTIFY (BLACK): All triplicates with Cq amplification levels above LOQ (**Table 4.10 and 4.11** and **Annex**).

“Yellow” and “Orange” categories ("weak possible positive" and "possible positive") contains inadequate amount of target DNA to obtain convincing detection within the range of the standard curve. These samples represent detections that could be caused by either stochastic sampling of the targeted eDNA fragment or caused by unspecific amplification in the qPCR. It is recommended that "Yellow" and "Orange" samples are further analysed to achieve a fully conclusive result. A more conclusive result may be obtained by increasing the level of eDNA template in the sample by increasing the filtered sample volume and/or the number of biological filter replicates. To further confirm the "Yellow" and "Orange" samples it is also possible to sequence the obtained PCR products.

A summary of invasive species recorded by the conventional monitoring carried out as a part of this project was supplemented by previously known distributions and records and combined with assumed presence in Danish marine waters (**Table 4.12)**. This table was prepared from both literature and by consulting different experts as described (**Table 4.12)**.

Table 4.10: *Monitoring by eDNA in May–July 2017. The numbers divided by slashes indicates the number of technical qPCR replicates that resulted in: No Cq / Below LOD / Above LOD below LOQ / Above LOQ. The color coding reflects the highest amplifcation level, and thereby reflects the level of eDNA detected. White equals 'NoCq' - i.e. no amplification in any of the replicates, which reflects no target eDNA present in the water sample. Yellow equals at least one replicate below LOD. Orange equals at least one replicate above LOD but below LOQ. The yellow and orange coloring reflects there is an inadequate amount of target DNA to obtain conclusive detection. Black equals all three replicates above LOQ. The black colorings reflect a sufficient level of target DNA to confirm the detection of the invasive species by eDNA.*

Species	Assay IDNo	AalborgHavn	Aalborgportland	Aarhus	Esbjerg	Fredericia	Frederikshavn	Gedser	Grenå	Helsingør	Hirtshals	Kalundborg	KalundborgStatiol	København	Køge	Odense	Rødby
Bonnemaisonia hamifera	1	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Prorocentrum cordatum	2	'0/0/0/3'	'0/0/0/3'	'0/3/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'0/3/0/0'	'1/2/0/0'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'0/3/0/0'	'0/3/0/0'	'0/0/0/3'	'1/2/0/0'	'3/0/0/0'
Pseudochattonella farcimen	3	'3/0/0/0'	'0/3/0/0'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'0/3/0/0'	'0/0/0/3'
P. verruculosa	4	'0/1/2/0'	'2/1/0/0'	'3/0/0/0'	'0/2/1/0'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Karenia mikimotoi	5	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Carassius auratus	6	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Cyprinus carpio	7	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Colpomenia peregrina	8	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Neogobius melanostomus	09	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'0/3/0/0'
Oncorhynchus mykiss	10	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Oncorhyncus gorbuscha	13	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/0/0/0'	'3/0/0/0'	'3/0/0/0'
Crassostrea gigas	14	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Mya arenaria	15	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'
Rhithropanopeus harrisii	16	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/0/1/0'	'2/0/1/0'	'3/0/0/0'
Eriocheir sinensis	18	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Cordylophora caspia	21	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Mnemiopsis leidyi	22	'0/0/0/3'	'0/0/0/3'	'2/1/0/0'	'0/3/0/0'	'3/0/0/0'	'2/1/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Acipenser baerii	23	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'

Table 4.11: *Monitoring by eDNA in Sep-Oct 2017. The numbers divided by slashes indicates the number of technical qPCR replicates that resulted in: No Cq / Below LOD / Above LOD below LOQ / Above LOQ. The color coding reflects the highest amplifcation level, and thereby reflects the level of eDNA detected. White equals 'NoCq' - i.e. no amplification in any of the replicates, which reflects no target eDNA present in the water sample. Yellow equals at least one replicate below LOD. Orange equals at least one replicate above LOD but below LOQ. The yellow and orange coloring reflects there is an inadequate amount of target DNA to obtain conclusive detection. Red equals at least one replicate above LOQ. Black equals all three replicates above LOQ. The red and black colorings reflect a sufficient level of target DNA to confirm the detection of the invasive species by eDNA.*

Species	Assay IDNo	AalborgHavn	Aalborgportland	Aarhus	Esbjerg	Fredericia	Frederikshavn	Gedser	Grenå	Helsingør	Hirtshals	Kalundborg	KalundborgStatiol	København	Køge	Odense	Rødby
Bonnemaisonia hamifera	1	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'0/2/0/1'
Prorocentrum cordatum	2	'0/3/0/0'	'0/3/0/0'	'3/0/0/0'	'2/1/0/0'	'0/3/0/0'	'0/3/0/0'	'0/0/0/3'	'0/3/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'0/0/0/3'	'0/0/0/3'	'0/2/0/1'	'0/0/0/3'
Pseudochattonella farcimen	3	'0/1/0/2'	'0/0/0/3'	'0/3/0/0'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'0/3/0/0'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'2/1/0/0'
P. verruculosa	4	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/2/0/1'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/1/0/2'	'3/0/0/0'
Karenia mikimotoi	5	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'
Carassius auratus	6	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Cyprinus carpio	7	'3/0/0/0'	'2/1/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Colpomenia peregrina	8	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'2/1/0/0'	'0/0/0/3'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Neogobius melanostomus	09	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'0/3/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'0/3/0/0'
Oncorhynchus mykiss	10	'1/2/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Oncorhyncus gorbuscha	13	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Crassostrea gigas	14	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/2/1/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Mya arenaria	15	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'0/0/0/3'	'3/0/0/0'	'3/0/0/0'	'0/3/0/0'	'1/2/0/0'
Rhithropanopeus harrisii	16	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'1/2/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'2/1/0/0'	'2/1/0/0'	'3/0/0/0'	'3/0/0/0'
Eriocheir sinensis	18	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Cordylophora caspia	21	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'
Mnemiopsis leidyi	22	'0/0/0/3'	'0/0/0/3'	'2/1/0/0'	'0/0/0/3'	'0/0/0/3'	'3/0/0/0'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'0/1/0/2'	'0/0/0/3'	'0/0/0/3'	'0/0/0/3'	'0/3/0/0'	'0/3/0/0'	'0/0/0/3'
Acipenser baerii	23	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'	'3/0/0/0'

Table 4.12: *Conventional monitoring. Not found previously or unknown occurence = 0, found before MONIS4 = 1 and found during MONIS4 in 2017 = 2. The letters refer whether the NIS was (A) found in Frederikshavn in 1981, (B) mentioned as* Prorocentrum *on page 24, (C) Mentioned as* Pseudochattonella *on page 52, (D) mentioned as* Karenia *on page 52, (E) caught in 2010, (F) Peter Rask Møller, Zool. Mus. Univ. Copenhagen: pers. obs., 2010, (G) NIRAS A/S, (H) FiskeAtlas, (I) Tom Schiøte, Zool. Mus. Univ. Copenhagen: pers. com., 2018.*

Species name	Ass Id No	Aalborg Havn	Aalborg Portland	Aarhus	Esbjerg	Fredericia	Frederikshavn	Gedser	Grenå	Helsingør	Hirtshals	Kalundborg	Statiol (Kalundborg)	København	Køge	Odense	Rødby
Bonnemaisonia hamifera	01	1	1	1	1	1	1A	1	1	1	1	1	1	1	1	1	1
Prorocentrum cordatum	02	1G	1G	1G	2B	1G	1G	1G	1G	1G	1G	1G	1G	1G	1G	1G	1G
Pseudochattonella farcimen	03	0	0	1G	2C	1G	1G	0	1G	0	1G	1G	1G	0	0	1G	0
P. verruculosa	04	0	0	1G	2C	1G	1G	0	1G	0	1G	1G	1G	0	0	1G	0
Karenia mikimotoi	05	1G	1G	1G	2D	1G	1G	0	1G	1G	1G	1G	1G	1G	0	1G	0
Carassius auratus	06	0	0	0	0	0	0	0	0	0	0	0	0	1H	0	0	0
Cyprinus carpio	07	0	0	0	0	0	0	0	0	0	0	1E	1E	0	0	0	0
Colpomenia peregrina	08	1F	1F	0	0	0	1F	0	0	0	0	0	0	0	0	0	0
Neogobius melanostomus	09	0	0	0	0	0	0	2	0	0	0	2	2	2	2	0	2
Oncorhynchus mykiss	10	1H	1H	1H	0	0	0	0	1H	1H	0	1H	1H	1H	1H	1H	0
Oncorhyncus gorbuscha	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Crassostrea gigas	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mya arenaria	13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhithropanopeus harrisii	14	2	2	0	2	0	0	0	0	2	0	1F	0	2	0	0	0
Paralithodes camtschaticus	15	1I	1I	1I	1I	1I	1I	1I	1I	1I	1I	1I	1I	1I	1I	1I	1I
Eriocheir sinensis	16	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	1
Homarus americanus	17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cordylophora caspia	18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mnemiopsis leidyi	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acipenser baerii	20	2	2	2	2	2	2	2	2	2	1	2	2	2	2	1	2
Bonnemaisonia hamifera	21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prorocentrum cordatum	22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

In the following, the obtained eDNA results for each harbour are interpreted from the standard dilution series plots (**Annex 9, graphs A1-A20**) and the tables with eDNA evaluation categories (**Tables 4.15 and 4.16**) are presented for each season of sampling (i.e. for spring and autumn).

1. In Aalborg harbour, eDNA was detected in the spring from ***Mnemiopsis leidyi*** and ***Prorocentrum cordatum***. The detection was supported by all three technical qPCR replicates for each species, and above the limit of quantification (LOQ) for all three replicates. ***Pseudochattonella verruculosa*** was detected above LOD (**Table 4.15**. The autumn sample detected eDNA from ***Karenia mikimotoi*** and ***Mnemiopsis leidyi***, ***Pseudochattonella verruculosa*** above LOQ for all three qPCR replicates, and above LOQ for two replicates for ***Pseudochattonella farcimen*** and low traces of eDNA from ***Mya arenaria***, ***Oncorhynchus mykiss*** and ***Prorocentrum cordatum*** below the limit of detection (LOD) **(Table 4.16)**.

2. In Aalborg Portland harbour, eDNA was detected in the spring from ***Mnemiopsis leidyi***, ***Prorocentrum cordatum*** and ***Mya arenaria***. The detection was supported by all three technical qPCR replicates for each species, and above the limit of quantification (LOQ) for all three replicates (**Table 4.15**). ***Pseudochattonella farcimen***, ***Pseudochattonella verruculosa*** was detected at levels above LOD (**Table 4.15**) For the autumn sample eDNA was detected in levels above LOQ for ***Karenia mikimotoi***, ***Pseudochattonella farcimen***, ***Pseudochattonella verruculosa*** and ***Mnemiopsis leidyi***, and in levels below LOD for ***Cyprinus carpio***, ***Oncorhynchus mykiss*** and ***Prorocentrum cordatum*** (**Table 4.16**).

3. In Aarhus, ***Bonnemaisonia hamifera***, ***Colpomenia peregrina***, ***Mnemiopsis leidyi***, ***Prorocentrum cordatum*** were detected in the spring by low traces of eDNA, indicated by traces of eDNA below LOD (**Table 4.15**). ***Pseudochattonella farcimen*** was detected above LOQ (**Table 4.15**). In the autumn sample ***Bonnemaisonia hamifera*** was detected in all three qPCR replicates above LOQ, while ***Cyprinus carpio***, ***Mnemiopsis leidyi*** and ***Pseudochattonella farcimen*** was traced in eDNA levels below LOD (**Table 4.16**).

4. In Esbjerg harbour, the sample collected in the spring only had traces of eDNA from **Crassostrea gigas** and ***Mnemiopsis leidyi*** at levels below LOD (**Table 4.15**). ***Pseudochattonella verruculosa*** was detected above LOD (**Table 4.15**). While the autumn sample had eDNA levels above LOQ for all three qPCR replicates for ***Mnemiopsis leidyi***, and low traces of eDNA, below LOD, for ***Crassostrea gigas***, ***Karenia mikimotoi*** and ***Prorocentrum cordatum***, and a single replicate above LOQ for ***Pseudochattonella verruculosa*** (**Table 4.16**).

5. In Fredericia harbour, eDNA was traced in the spring from ***Prorocentrum cordatum*** at levels below LOD and from ***Pseudochattonella farcimen*** above LOQ (**Table 4.15**). In the autumn, sample eDNA was found for ***Mnemiopsis leidyi*** and ***Pseudochattonella farcimen*** in levels above LOQ and in levels below LOD for ***Colpomenia peregrina*** and ***Prorocentrum cordatum*** (**Table 4.16**).

6. In Frederikshavn harbour, the water sample collected in the spring held eDNA traces from ***Karenia mikimotoi***, ***Mnemiopsis leidyi***, ***Pseudochattonella verruculosa*** and ***Oncorhynchus mykiss*** in levels below LOD and above LOQ for ***Pseudochattonella farcimen*** (**Table 4.15**). The autumns sample contained eDNA traces from ***Mya arenaria*** and ***Pseudochattonella farcimen*** in levels above LOQ, and eDNA from ***Colpomenia peregrina***, ***Prorocentrum cordatum*** and ***Rhithropanopeus harrisii*** in levels below LOD (**Table 4.16**).

7. In Gedser harbour, the spring sample held traces of eDNA from ***Mnemiopsis leidyi***, ***Neogobius melanostomus*** and ***Prorocentrum cordatum*** at levels below LOD, and eDNA from ***Mya arenaria*** in levels above LOQ (**Table 4.15**). The autumn sample held eDNA in levels above LOQ for ***M. leidyi***, ***M. arenaria*** and ***Prorocentrum cordatum***, and low traces of eDNA from ***N. melanostomus*** below LOD (**Table 4.16**).

8. In Grenå harbour, the spring sample had low traces of eDNA from ***Bonnemaisonia hamifera***, ***Mya arenaria*** and ***Prorocentrum cordatum***. All species with eDNA levels below LOD (**Table 4.15**). Higher levels of eDNA, above LOQ, was detected for ***Pseudochattonella farcimen***. The autumn sample indicated the presence of ***Bonnemaisonia hamifera***, ***Mnemiopsis leidyi***, ***Mya arenaria*** and ***Pseudochattonella farcimen*** at eDNA levels above LOQ for all three qPCR replicates, and low traces of eDNA from ***Crassostrea gigas, Karenia mikimotoi*** and ***Prorocentrum cordatum*** with eDNA levels below LOD (**Table 4.16**).

9. In Helsingør harbour, the water sample collected in the spring showed traces of eDNA from ***Prorocentrum cordatum*** and ***Pseudochattonella farcimen*** above the LOQ for all three qPCR replicates (**Table 4.15**). In the autumn sample the presence of ***Mnemiopsis leidyi***, ***Prorocentrum cordatum*** and ***Pseudochattonella farcimen*** was detected with eDNA levels above the LOQ for all three qPCR replicates, and for ***Colpomenia peregrina*** by eDNA levels below LOD (**Table 4.16**).

10. In Hirtshals harbour, the samples collected in the spring supported the presence of ***Mnemiopsis leidyi*** with eDNA levels above the LOQ for all three qPCR replicates, and ***Pseudochattonella verruculosa*** in levels below LOD (**Table 4.15**). The autumn samples supported the presence of ***M. leidyi*** for two out of three qPCR replicates in levels above LOQ and ***P. verruculosa*** for all three replicates in levels above LOQ, and for ***Oncorhynchus mykiss*** and ***Pseudochattonella farcimen*** in levels below LOD (**Table 4.16**).

11. In Kalundborg harbour, eDNA was found in the spring sample from ***Mya arenaria, Pseudochattonella farcimen, Pseudochattonella verruculosa*** and ***Prorocentrum cordatum*** in levels above LOQ, and for ***Colpomenia peregrina***, ***Neogobius melanostomus*** and ***Oncorhynchus mykiss*** in levels below LOD (**Table 4.15**). The autumn sample supported the presence of ***Mnemiopsis leidyi*** and ***P. farcimen*** in levels of eDNA above LOQ, and hinted the presence of ***C. peregrina***, ***Cyprinus carpio*** and ***N. melanostomus*** with levels of eDNA below LOD (**Table 4.16**).

12. In Kalundborg Statoil Harbour, the water sample collected in the spring had eDNA from ***Mya arenaria*** and ***Pseudochattonella farcimen*** in levels above LOQ, and from ***Colpomenia peregrina***, ***Mnemiopsis leidyi*** and ***Prorocentrum cordatum*** in levels below LOD (**Table 4.15**). The water sample collected in the autumn supported the presence of ***C. peregrina***, ***M. leidyi***, ***M. arenaria*** and ***P. farcimen*** by levels of eDNA above LOQ, and the presence of ***Cyprinus carpio***, ***Neogobius melanostomus***, ***Oncorhynchus mykiss*** and ***P. cordatum*** by levels of eDNA below LOD (**Table 4.16**).

13. In København harbour, the water sample collected in the spring indicated the presence of ***Mya arenaria*** and ***Pseudochattonella farcimen*** with eDNA levels above LOQ, and the presence of ***Prorocentrum cordatum*** with eDNA levels below LOD (**Table 4.15**). The water sample collected in the autumn supported the presence of ***Mnemiopsis leidyi***, ***P. farcimen*** and ***P. cordatum*** by eDNA levels above LOQ and hinted the presence of ***Colpomenia peregrina***, ***Crassostrea gigas*** and ***Rhithropanopeus harrisii*** by eDNA levels below LOD (**Table 4.16**).

14. In Køge harbour, for the water sample collected in the spring, the presence of ***Prorocentrum cordatum*** and ***Pseudochattonella farcimen*** was supported by eDNA levels above LOQ for all three qPCR replicates and the presence of ***Neogobius melanostomus*** indicated by eDNA levels below LOD, and the presence of ***Rhithropanopeus harrisii*** indicated by eDNA levels above LOD but below LOQ (**Table 4.15**). The sample collected in the autumn supported the presence of ***Prorocentrum cordatum*** by levels of eDNA above LOQ, and the presence of ***Mnemiopsis leidyi***, ***N. melanostomus*** and ***R. harrisii*** was indicated by levels of eDNA below LOD (**Table 4.16**).

15. In Odense harbour, eDNA was detected in the spring from ***Mnemiopsis leidyi*** in levels above LOQ, from ***Rhithropanopeus harrisii*** in levels above LOD but below LOQ, and from ***Prorocentrum cordatum*** and ***Pseudochattonella farcimen*** in levels below LOD (**Table 4.15**). The autumn sample supported the presence of ***Bonnemaisonia hamifera***, ***Karenia mikimotoi***, ***P. cordatum***, ***Pseudochattonella farcimen*** and ***Pseudochattonella verruculosa*** with eDNA levels above LOQ and indicated the presence of ***Mnemiopsis leidyi*** and ***Mya arenaria*** by levels of eDNA below LOD (**Table 4.16**).

16. In Rødby, the presence of ***Mnemiopsis leidyi*** and ***Pseudochattonella farcimen*** was supported by eDNA levels above LOQ, and the presence of ***Mya arenaria*** indicated be levels of eDNA below LOD (**Table 4.15**). The autumn sample supported the presence of ***Bonnemaisonia hamifera***, ***Mnemiopsis leidyi*** and ***Prorocentrum cordatum*** by levels of eDNA above LOQ, and indicated the presence of ***Mya arenaria***, ***Neogobius melanostomus*** and ***P. farcimen*** by traces of eDNA below LOD (**Table 4.16**).

4 Discussion

The results are discussed according to the structure of Chapter 4 and with a specific focus on the records on non-indigenous species found in the 16 harbours.

4.1 Plankton

Phytoplankton
Three phytoplankton species regarded as non-indigenous in Danish waters (cf. Andersen *et al.* 2014) were identified in light microscopy; ***Prorocentrum cordatum*** (syn. *Prorocentrum minimum*), ***Karenia mikimotoi***, and ***Pseudochattonella verruculosa***. All of them are challenging to identify in light microscopy alone and for a precise identification electron microscopy and/or genetic tools should be used in addition. For all June samples and the Esbjerg September sample 25 mL were analysed. The October samples were full of detritus and it was only practically possible to analyse 5 mL. Some of the samples, in particular station 5 in Aarhus from June contained a lot of freshwater green algae. A few icthyotoxic species such as ***K. mikimotoi***, *Alexandrium pseudogonyaulax*, *Heterosigma akashiwo*, *Vicicitus globosus* and *Pseudochattonella* were observed in low numbers. *Pseudo-nitzschia* spp. was common in most of the samples. Several of the species are known demoic acid (neurotoxin) producers. *Dinophysis acuminata* and *D. norvegica* known to produce shellfish toxins were recorded.

Zooplankton
Identification of *Acartia* species is time consuming and difficult, partly because some characters are very variable (e.g., spinulation on various body segments, occurrence of rostral filaments), and partly because the diagnostic fifth pair of legs is very small and hard to observe. Dissection and microscopic slide preparation are usually required to obtain proper resolution. This was not possible to do for the many *Acartia* individuals in the samples. Thus, we dissected a low number (4-19) of individuals from each sample and reported them as '***Acartia tonsa***' or '*Acartia* spp. (*non tonsa*)'. The latter category comprises mainly *Acartia (Acanthacartia) bifilosa* (Giesbrecht 1881) and some *Acartia (Acartiura) clausi*. All three species mentioned are often found in estuaries.

Acartia tonsa is considered native to the western Atlantic and the eastern Pacific but has been known to occur in the eastern Atlantic already from first half of the 20th century. It has invaded the Gironde estuary in France, to become a dominant member of the plankton community (David *et al.* 2007). The species is well-known in the Baltic and has recently been observed in brackish lakes in southern Norway.

Penilia avirostris is a cosmopolitan species, found mainly in neritic waters in the tropics and subtropics, but the distribution also extends into warm temperate waters. Recent years have seen an expansion into the North Sea since 1990 (Johns *et al.* 2005). It was firstly observed in Danish waters in 2001, and now occurs regularly in Kattegat. ***P. avirostris*** also appears regularly on the Swedish west coast and the Norwegian south coast and may occasionally build dense populations in the autumn. It is likely that the current range expansion of ***P. avirostris*** is linked to increasing summer temperatures, which allows rapid parthenogenetic reproduction. However, anthropogenic dispersal cannot be ruled out. Unlike other marine cladocerans, ***P. avirostris*** feeds on nanoplankton. Thus, competition with native phytoplankton feeders is possible, but it is yet unclear whether the species may disturb coastal food webs.

Mnemiopsis leidyi has its native range along North America's east coast (Costello *et al.* 2012). From this area it has spread over the Atlantic, most likely with ballast water as a primary invasion vector. In the beginning of the millennium, it was observed in the North Sea and the Baltic Sea for the first time and during the 10 years that have passed since the first observations, ***M. leidyi*** has apparently come to stay in Danish waters (Riisgård 2017). Recent studies indicate that after ***M. leidyi*** firstly was established in the North Sea area, the ocean currents were important in driving the secondary spread (Jasper *et al.* 2018). Hydrodynamic modelling shows strong connectivity via ocean currents in the North Sea area with rapid recolonization from high-abundance hubs after local extinction (Jasper *et al.* 2018). The possible harmful effects of ***M. leidyi*** have so far not been thoroughly studied in Danish waters, but recent studies from Gulmar Fjord on the Swedish west coast shows profound changes in the pelagic ecosystem (Tiselius & Møller 2017). The authors described a trophic cascade where a strong reduction in the abundance of the targeted prey of ***M. leidyi*** released the primary producers from grazing pressure leading to a significant increase in phytoplankton biomass.

4.2 Mobile epifauna

No non-indigenous species were caught in the traps in Aarhus Harbour. In both traps mainly native and common species were caught. In the coarse-mesh crab trap, larger specimens of the green shore crab *Carcinus* and sea star *Asterias* were the dominant organisms. In the fine mesh netting traps, small specimens of *Carcinus*, snails and several species of shallow water fish species were caught. The large abundances of the snail *Hinia*, which is a scavenger, may be related to rather poor environmental conditions at several sites in the harbour with organically enriched black sediments. It appeared that both trap types were efficient for sampling the resident species at the sampling stations.

In Esbjerg, the catches in the crab trap were dominated by large specimens of the the green shore crab *Carcinus*. Two non-indigenous barnacle species, ***Austrominius modestus*** and ***Amphibalanus improvisus,*** were found attached to the dorsal side of the carapax of several crab specimens. Both species are well-known to Danish waters. The fine mesh netting traps caught small specimens of *Carcinus*, several species of prawns and shallow water fish. There was a presumably accidental catch of the non-indigenous comb jelly ***Mnemiopsis leidyi*** at one station, but no other alien species were found. It appeared that both trap types also in Esbjerg were efficient for sampling the resident species at the sampling stations.

4.3 Benthic infauna

Several non-indigenous species and species of uncertain origin were found in the grab samples, particularly of marine bristle worms. In Aarhus, the nereid ***Alitta succinea*** was present in parts of the harbour area. This species is generally common in shallow soft-bottom areas in Denmark. ***A. succinea*** is of uncertain origin but are considered to be alien in Denmark. In several other countries, e.g. Norway, it is considered native. At one station in Aarhus there was a mass occurrence of ***Polydora*** cf ***aggregata***. This species belongs in a group of species with insufficiently developed taxonomy. It is probable that the species in Aarhus has been translocated from the eastern coast of USA, but further studies of the taxonomy are needed to clarify the identitiy of the species and its native distribution. In Aarhus no non-indigenous species from other taxonomic groups were recorded in grab samples.

In Esbjerg, two non-indigenous bristle worms that appear to be new to Denmark were found. The spionid ***Streblospio benedicti*** was among the dominant species and was found at all sampling localities. ***S. benedicti*** has previously been reported from scattered localities in the Netherlands and UK (Radashevsky 2012) and seems presently to be spreading in Dutch waters (*A. Gittenberger, pers. comm.*). The other species, the phyllodocid ***Eteone heteropoda***, is presumably recorded in Europe for

the first time. Both species are native to the US east coast. Also, in Esbjerg a species of the *Polydora*-group was found, viz. ***Polydora cornuta***. Like other species of *Polydora,* it is of uncertain origin, but is believed to be of North American origin and is now considered invasive in Mediterranean waters (Radashevsky & Selifonova 2013). The occurrence and abundances of these species in Esbjerg give the impression of a fauna that is similar to the fauna of North-East American harbours. The status of the most abundant species in Esbjerg, the cirratulid ***Tharyx*** sp., is unclear both with regard to species identity and native distribution. No similar species are known from northeast US waters *(J. Blake, pers. comm.*).

In the dredge samples, mainly the same species were found as in the grab samples. Two additional non-indigenous species were recorded: the American razor clam ***Ensis directus*** in both harbours and the ascidian ***Styela clava*** in Esbjerg. Both these species are considered invasive and are well-known from Danish waters.

4.4 Fouling organisms

Ten non-indigenous species were identified from assessing fouling organisms in Esbjerg and Aarhus harbour. Seven of these species are considered invasive according to the list of all non-indigenous and invasive species in Denmark (revised January 10th, 2018). A higher abundance and distribution of non-indigenous species were found in Esbjerg harbour compared to Aarhus harbour, both from PVC plates and scraped material. No non-indigenous species were identified from the rapid assessment surveys (RAS). All non-indigenous species observed among the fouling organisms have previously been recorded in Denmark and many of these species were recorded more than fifty years ago.

The following non-indegenous species that were recorded; ***M. manhattensis***, ***A. improvisus***, ***E. modestus***, ***H. japonica***, ***C. gigas*** and ***S. clava***, are today all well established and commonly observed among native Danish fauna. The red algae ***N. harveyi***, the small sea anemone ***D. lineata***, the Japanese shore crab ***H. sanguineus*** and the Japanese skeleton shrimp ***C. mutica*** are however among the more recent species introduced to Denmark. ***N. harveyi*** was only sampled in Esbjerg harbour while the remaining three species were found both in Esbjerg Harbour and Aarhus Harbour during this survey. Both the red algae ***N. harveyi*** and the sea anemone ***D. lineata*** are believed to have low impact on native species and are not considered to have an invasive potential while the two latter species, ***H. hemigraspus*** and ***C. mutica***, both are considered invasive in Danish waters according to the list of all non-indigenous and invasive species in Denmark (revised January 10th, 2018). The Japanese skeleton shrimp ***C. mutica*** pose a significant threat to native caprellides due to their large size and aggressive behavior. ***C. mutica*** also tend to reach extremely high densities, particular on artifical substrates which are usually easiliy accessible in harbour areas. The Japanese shore crab ***H. sanguineus*** are also considered invasive, mainly due to their large distribution potential and their negative impact on native crab species. ***H. sanguineus*** is oppurtunistic and an effective predator of juvenile bivalves, hence they may potentially restructure prey communities in habitats into which they have been introduced (Brousseau *et al.* 2001). ***H. memigraspus*** and ***C. mutica*** were found both in Esbjerg Harbour and Aarhus Harbour during this survey.

4.5 Fish, standard methods

With a maximum size of 23 cm reported for round goby caught in Danish waters, the specimens caught in the present campaign, using standard fishing methods, were of a small size (Carl *et al.* 2016). However, as some of the larger individuals were showing dark coloration and no ring around the spot on the dorsal fin, it is likely that our catches represent a mixture of juvenile and adult individuals.

The invasive round goby ***Neogobius melanostomus*** was caught in Gedser Fiskerihavn, Gedser Lystbådehavn, Køge Industrihavn and Køge Marina, using standard fishing methods. This was to some extent expected as the species has been recorded near to these locations before in 2016 (Carl *et al.* 2016). However, no invasive fish species were caught in Rødby industrihavn or Rødby fiskerihavn using standard fishing methods, even though these locations are also known as previous sites of ***N. melanostomus*** (Carl *et al.* 2016). According to local fishermen interviewed on site, during the present campaign, ***N. melanostomus*** is however still present. We therefor expect that this result, from the standard fishing methods, is a false negative and that ***N. melanostomus*** presumably still is present at Rødby Færgehavn. Possibly the presence of potential predators of ***N. melanostomus***, such as cod and eel, could be part of the reason we did not catch the species at this location. According to Almqvist *et al.* (2010), in the Gulf of Gdańsk during summer, cod with a total length of 26-35 cm can have a diet comprised of on average 79 % ***N. melanostomus***. The average total length of cod caught in this campaign was 26 cm (N=139, range: 15–43 cm). In Rødby færgehavn the average total length of cod was 24 cm (N=6, range: 21–27 cm). This indicates that some of the cod present in the habitats of these harbours have a size that makes them potential predators on ***N. melanostomus***. Similarly, the European eel is expected to prey on ***N. melanostomus*** (*Mads Christoffersen, DTU Aqua, unpublished data*). The average total length of eel caught in this campaign was 45 cm (N=31, range: 19–77 cm). In Rødby færgehavn the average total length of eel was 42 cm (N=4, range: 30–53 cm).

The standard fishing methods did not catch any round goby in København Harbour. However, ***Neogobius melanostomus*** has previously been observed in the southern parts of the harbour, whereas the effort of the present campaign was focused on the northern parts. In 2016, ***N. melanostomus*** was observed about 5–10 km south from our sampling locations (Carl *et al.* 2016). Given the fact that ***N. melanostomus*** is believed to increase its distribution with 30 km pr year, it is expected that we would catch it at the northern sampling sites in 2017 (Azour *et al.* 2015). However, we did not. Based on our effort in this harbour we do not expect that these results are false negatives.

Behrens *et al.* (2017) found no physiological differences in ***Neogobius melanostomus***, when exposed to salinity of a PSU similar to what is found in the northern part of København Harbour. The study did however conclude that salinity may play a role in the distribution of this species. Perhaps the seemingly absence of the invasive species in the northern part of København Harbour, indicates that ***N. melanostomus*** is struggling with an increased salinity in Øresund, or in other ways have been obstructed in its further distribution northward in the harbour.

Kalundborg Harbour and Helsingør Harbour represent harbours that are located closest to the known distribution of ***Neogobius melanostomus*** in the inner Danish marine waters. With an estimated increase in distribution of 30 km pr year, it's expected that we would catch ***N. melanostomus*** at these locations in 2017. However, we did not. Based on the extent of our effort at these harbours, we do not expect that round goby is present at these locations.

The rest of the harbours and sample sites are located mainly on the Jutland peninsular. They therefore have a large distance to the known distribution of round goby. The absence of the invasive species at these locations indicates that there has been no subsequent introduction or translocation of ***Neogobius melanostomus*** following its invasion to the Danish marine waters.

4.6 Fish, snorkeling

Approximately 20 non-indigenous fish species have previously been found in Danish marine waters, but most of them very rarely (Carl *et al.* 2016). Only ***Oncorhynchus mykiss*** and ***Neogobius melanostomus*** have been reported regularly and are the most likely to find when searching. For ***N.***

melanostomus, harbours are excellent habitats with plenty of hiding places. For the ***Oncorhynchus mykiss***, harbours often act like a big fish trap, where they have trouble finding their way out, once that they have entered the harbour. On top of that, many harbours are known to host a huge amount of food items – such as shrimps, mussels and small fish, which potentially make them a great home for several non-indigenous fish species. In the following, we will discuss the findings of non-indigenous fish species in the current project and compare the results with existing knowledge from the National Fish Atlas database and literature. For a detailed description of ***O. mykiss*** in Denmark, please confer with Rasmussen (2012). A description of the invasion history of ***N. melanostomus*** in Danish waters can be found in the study by Azour *et al.* (2015).

1. Aarhus: The harbour is a popular place for angling, and rainbow trout (***Oncorhynchus mykiss***) has been caught there many times. The presence is linked to escapes from fish farms along the eastern Jutland coast, and large fluctuations are seen. It is therefore not a surprise, that none were seen during snorkeling. Also, large pelagic fish are often underestimated during snorkeling as they tend to get spooked in the presence of a diver. The round goby (***Neogobius melanostomus***) will probably arrive in the Aarhus area within the next 10–15 years and there is no reason to believe that it would not thrive there.

2. Esbjerg: At most other places along the Danish west coast, the visibility is often very poor in the Esbjerg area. It was therefore impossibly to snorkel, and instead other active methods were employed. No non-indigenous fish species were found. As the native black goby (*Gobius niger*) is very uncommon along the west coast, it is not expected that the invasive round goby (***Neogobius melanostomus***) will have any luck settling in the area, should it ever spread so far. The National Fish Atlas Database does not have any records of rainbow trout (***Oncorhynchus mykiss***) from the harbour, but the species has been caught not far from Esbjerg a couple of times and will probably guest the harbour now and then.

3. Aalborg Portland: Snorkeling (and shrimp push net) did not result in any non-indigenous fish species being registered. This was not surprising as only rainbow trout (***Oncorhynchus mykiss***) has been registered in the Aalborg area in the past – and only in very low numbers. In contrast to many other harbours this harbour is missing a semi-closed basin, and therefore rainbow trout will not congregate as they often do in harbour basins. Round goby (***Neogobius melanostomus***) has been reported from the Aalborg area a couple of times, but these records have turned out to be misidentified black gobies (*Gobius niger*). Round goby will, however, probably find its way to Limfjorden within the next decades (sooner if transported with e.g. ballast water), and in Limfjorden one would expect it to become numerus as there are many sheltered areas with low salinity.

4. Aalborg: Snorkeling (and shrimp push net) did not result in any non-indigenous fish species being registered. A local fisherman present at the time of the investigation told that round goby (***Neogobius melanostomus***) had often been caught in the area. When digging deeper into his story it became clear, that he was talking about black goby (*Gobius niger*). Also earlier records of round goby from the area have turned out to be misidentified black gobies. Round goby will, however, probably find its way to Limfjorden within the next decades (sooner if transported with e.g. ballast water), and the brackish water with many sheltered areas is a preferred habitat. Rainbow trout (***Oncorhynchus mykiss***) has previously been registered in the Aalborg area in low numbers and the species will be present from time to time. Escaped rainbow trout does not seem to congregate in the Aalborg area, so they do not play any role in the harbour ecosystem.

5. Fredericia: No non-indigenous fish species were seen during the investigations. Rainbow trout (***Oncorhynchus mykiss***) escaped from fish farms in e.g. Horsens Fjord are, however, rather common in Lillebælt and must be present in the harbour area from time to time. Round goby (***Neogobius melanostomus***) will probably arrive within the next 5–10 year, and it will find perfect conditions in the many sheltered areas near Fredericia (e.g. Kolding Fjord and Vejle Fjord).

6. Frederikshavn: No non-indigenous fish species have ever been registered in the harbour, so it does not come as a surprise that they were not present during the investigation. Rainbow trout (***Oncorhynchus mykiss***) are probably present from time to time, but presumably in low numbers as the nearest fish farms (in saltwater) are situated quite far from the harbour. It is believed that round goby (***Neogobius melanostomus***) could possibly spread to the harbour in the future, but it is too early to guess, as the goby has still to be found anywhere at the Jutland coast.

7. Gedser: The invasive round goby (***Neogobius melanostomus***) was first found in the Gedser area in 2011, and it has been found there quite a few times since and often in relatively high numbers. It was therefore expected that it would be present in the snorkel survey. The harbour area is a suitable habitat for this species, as there are many places to hide. The Fish Atlas Database does not contain any information about rainbow trout (***Oncorhynchus mykiss***) in the harbour, but the species is without a doubt present from time to time as they regularly escape from fish farms in the region and have been registered a couple of times only a few kilometers from the harbour.

8. Grenå: No non-indigenous fish species were found during snorkeling, but it is well known that rainbow trout (***Oncorhynchus mykiss***) is sometime present in the harbour. For example, 5,000–10,000 rainbow trouts escaped from a holding net in the harbour in April 2013 and for some time after that they were caught there by anglers. The harbour provides a suitable habitat for many species, and eventually the round goby (***Neogobius melanostomus***) will probably also spread this far north. Transport with e.g. ballast water could easily speed up this process.

9. Helsingør: Even though a thorough investigation was carried out, no non-indigenous fish species were found. Within a few years it is expected that the round goby (***Neogobius melanostomus***) will spread from the Copenhagen area to the harbour, where it will find many good hiding places – especially in the marina. In the 1970's and 1980's rainbow trout (***Oncorhynchus mykiss***) were stocked in the Helsingør area to enhance local sport fishing. This practice has stopped now, but the species is still sometimes registered. As it is probably escaped fish from Musholm Lax in Storebælt, numbers are generally low.

10. Hirtshals: No non-indigenous fish species were found during the investigations, and there are no records in the National Fish Atlas Database, that any have been found there before. Rainbow trout (***Oncorhynchus mykiss***) have, however, been registered at Kjul Beach just east of the harbour, so they probably also guest the harbour from time to time. Visibility was very poor at the day of the investigation even though there was almost no wind, and as mentioned the result was that not a single fish was seen. Low visibility is a limiting factor for snorkeling most days of the year – as it is most places along the Danish west coast. The Danish west coast is probably not a suitable place for the round goby (***Neogobius melanostomus***) as it is not suitable for the native black goby (*Gobius niger*). It is therefore not expected that the round goby will come to play any role in the harbour, if it should arrive.

11. Kalundborg: As mentioned a large breeding population of round goby (***Neogobius melanostomus***) was found. The species never had been seen north of Reersø before, but as its range is known to expand around 30 km a year (Azour *et al.* 2015), it was expected that the species would show up in Kalundborg. As mostly young-of-the-year and a few adults were present, it seems that the species had only been around the area for about one season. The Kalundborg area is a perfect habitat for the round goby, and this area can aid the expansion further north around Zealand. The whole Kalundborg area is well known for its rainbow trout (***Oncorhynchus mykiss***). The fish regularly escape from the nearby fish farm, Musholm Lax, in Storebælt and when they follow the coast they often end up in the harbour where they become more or less resident (until they are caught – often quite quickly). Only a few months after the field work was carried out, a huge number of rainbow trout escaped from the fish farm and ended up in the harbour. Common carp (***Cyprinus carpio***) have been caught and observed a few times in Kalundborg Havn and Fjord. In 2010 more than 20 was caught in the Kalundborg area and it was concluded that fish came from Saltbæk Vig, where a large population exist. That summer, carp (***Cyprinus carpio***) was found all the way from Kalundborg to Rørvig.

12. København: Round goby (***Neogobius melanostomus***) was first seen in the Copenhagen area in 2016 and already in 2017 it was found many places in the harbour. Numbers are generally low (as seen during the snorkeling), but as the harbour already is home to a large population of the native black goby (*Gobius niger*), it is thought to be the perfect habitat for round goby. It's to be expected that numbers will rise dramatically within the next few years and that the round goby will become the dominant goby species and perhaps the most common of all fish species. Rainbow trout (***Oncorhynchus mykiss***) was earlier on stocked in the harbour area to help local anglers catch more fish. This is not done anymore (now native seatrout are stocked), but rainbow trout are still caught from time to time – fish that have escaped from fish farms in other parts of the country. Also feral goldfish (***Carassius auratus***) has been caught in the harbour area a couple of times in the past. Generally, the salinity is, however, thought to be too high for this freshwater fish, and presence is undoubtable rather sporadic.

13. Køge: Round goby (***Neogobius melanostomus***) was first seen in the Køge area in 2012. In 2015 it had become numerous, and it was no surprise that relative high numbers were seen during snorkeling in 2017. The harbour area provides a suitable habitat for the species and the fish also enter Køge Å where they have been caught in relative high numbers the last couple of years. Rainbow trout (***Oncorhynchus mykiss***) has been caught in the harbour area a couple of times in the past, but as most fish farms are placed rather far from Køge, it is never present in great numbers. It's therefore not surprising that it was not registered during snorkeling. Also carp (***Cyprinus carpio***) that are present in the nearby Køge Å could from time to time be expected to visit the harbour, as freshwater fish – mostly perch (*Perca fluviatilis*), roach (*Rutilus rutilus*) and ide (*Leuciscus idus*) often make op the majority of the fish fauna in the brackish water of the harbour.

14. Odense Havn: No non-indigenous fish species were found during our snorkeling. Earlier rainbow trout (***Oncorhynchus mykiss***) has been caught in the harbour a number of times, and this species is probably present almost every time they escape in huge numbers from fish farms in this part of the country. Round goby (***Neogobius melanostomus***) will probably arrive within the next rather few years, as it has recently been found at the eastern shores of Fyn a couple of times. Odense Fjord and Odense Havn are suitable habitats for this species, and it will almost certainly become numerus in the area. As snorkeling is a very effective method for finding round goby it is, however, highly unlikely that the species is already present in any significant numbers at the time.

15. Rødby: Round goby (***Neogobius melanostomus***) was first confirmed from the Rødby Harbour area in 2009, and in 2011 a couple of local fishermen reported that it was common. The species has been found many places around Lolland since then, and the presence in the area is well documented. Harbours are normally considered to be a good habitat for round goby, and it was surprising, that only two specimens were seen. As mentioned, the ferries at place caused a lot of sediment from the bottom to rise up in the water column and made is difficult to see bottom dwelling fish, so the fish can have been present in larger numbers. The National Fish Atlas Database does not contain any information about rainbow trout (***Oncorhynchus mykiss***) in the harbour, but without doubt this species is present from time to time as it has been caught on many locations around Lolland and several times only a few kilometers from Rødby Harbour.

16. Statoil-havnen (Kalundborg): The presence of a breeding population of round goby (***Neogobius melanostomus***) came as a surprise, since the species never had been seen north of Reersø before. It was, however, expected that the species would show up within a couple of years, as it has colonized most coastal areas in the southeastern part of Denmark since 2008, where it was first found near Bornholm. The Kalundborg area seems to be a perfect habitat for the Round goby, and it is expected, that the population will grow over the next couple of years. This area can be a stronghold for the range expansion further north around Zealand. Snorkeling is behind most of the records of round goby in Denmark, and it has already proven itself as a very cost-effective way of mapping the distribution of the species. This harbour is well known for its rainbow trout (***Oncorhynchus mykiss***) as fish from the nearby fish farm, Musholm Lax, in Storebælt often gather here, when they escape from the fish farm. The presence is however often quite short, as anglers target the trout in the harbour and quickly catch most of them. Only a few months after the snorkeling was done, a huge number of rainbow trout escaped from the fish farm and ended up in the harbour.

4.7 Molecular methods

For the 18 species monitored by eDNA there was an overall congruence with conventional monitoring (**Tables 5.1 and 5.2**). The conventional monitoring was performed throughout May – November 2017, but each harbour was visited two times only, representing spring and autumn. However, the conventional monitoring results (**Table 4.12** also includes past recordings, assumed occurrence and suspected distribution of the non-indigenous target species. The different sources of conventional data on distribution of the target species are explained in **Table 4.12**. Sampling of eDNA represents two sampling-events in the spring (May – July) and autumn (September – November) 2017. eDNA deteriorates fast in the water; probably within less than a couple of days (Thomsen 2012a, 2012b, and Sigsgaard *et al.* 2017). Hence, eDNA analysis is expected to reflect the species present at the time of sampling¸ i.e. within days or weeks.

The agreement and/or disagreements between conventional monitoring can therefore just as well be regarded as a reflection of the difference in life cycle events and annual distribution of the non-indigenous species. Some species (e.g. ***Mnemiopsis leidyi***) will be more widely distributed in the inner Danish marine waters later in the fall (Risgård 2017) and will be rare in the spring. This may potentially result in disagreements between eDNA monitoring and conventional monitoring if the sampling is not done on the same dates.

Another important factor to consider when examining agreements/disagreements between eDNA monitoring and conventional monitoring, is the volume of water filtered prior to the eDNA analysis. A larger volume of filtered water will ensure a higher possibility of capturing eDNA from the target

organisms. In some of the harbours (e.g. Esbjerg Harbour) the autumn sampling was impaired by low volumes of water filtered (200 mL), because of high levels of suspended solids and/or algae in the water, which were leading to fast clocking of the filter (see **Table 3.1**). This will subsequently hamper the ability to detect low concentrations of eDNA in the water, since the analysis only include eDNA from a reduced water volume. If at all possible, it is advised to avoid sampling, when the water is murky and full of suspended solids and/or algae.

The plots with standard dilution series (**Annex 9**) show the inferred levels of eDNA detected for the filtered water samples. For the majority of the plots the Limit of Detection (LOD) and Limit of Quantification (LOQ) are identical, but there are minor disagreements per season – e.g. there is a difference between LOD and LOQ for the species-specific assay against ***Crassostrea gigas*** for spring and autumn. This disagreement is due to the variability in the number of target-copies transferred by pipetting these low concentrations, and it is expected that the LOD and LOQ will vary from assay to assay at the lowest level of concentration. Unfortunately, by an error an incorrect initial high concentration was prepared for standard dilution series for the assay detecting eDNA from ***Carrasius auratus*** and for ***Acipenser baerii***, causing the LOQ and LOD to be set at a very high level. However, this mistake has no consequence for the data, since all analyzed samples proved to be negative anyway for both ***C. auratus*** and ***A. baerii***.

Overall, the study has shown the expected agreement between the results obtained from eDNA and the conventional monitoring in combination with previous knowledge on the distribution of the 20 non-indigenous target-species (**Tables 5.1 and 5.2**). The red colours in **Tables 5.1 and 5.2** highlights the sites where eDNA analysis indicated presence of species, which were neither found by conventional monitoring nor known from previous studies. These eDNA observations must be confirmed by further investigations, to eliminate any potentially false positive results. This is in particular important because this study presents the first full-scale applications of several new eDNA-detection systems.

The rare target species, such as ***Cordylophora caspia***, ***Eriocheir sinensis***, were neither found with the eDNA method nor with the conventional monitoring. The freshwater target-species will rarely occur in the brackish to saline waters found in the investigated harbours. Hence, the freshwater species such as ***Acipenser baerii***, ***Carassius auratus***, ***Cyprinus carpio*** and ***Oncorhynchus gorbuscha*** were rarely detected with the eDNA method, and equivalently rarely recorded by conventional monitoring method. The non-indigenous target-species which are common throughout the inner Danish Seas, such as ***Bonnemaisonia hamifera***, ***Prorocentrum cordatum***, ***Karenia mikimotoi***, ***Mya arenaria***, and ***Mnemiopsis leidyi***, were also detected all over the inner Danish seas from several harbours by the eDNA method as well as conventional monitoring. The non-indigenous target species such as ***Colpomenia peregrina*** and ***Crassostrea gigas*** that previously have been recorded by conventional monitoring in the northwestern part of the inner Danish marine waters and the North Sea is mainly recorded by eDNA in the North Sea, Limfjorden, Skagerak and Kattegat. Whereas the non-indigenous species such as ***Neogobius melanostomus*** and ***Rhithropanopeus harrisii*** that previously have been recorded by conventional monitoring in the southeastern part of the inner Danish Seas, are mainly detected by eDNA in Øresund and south off Falster and Lolland, albeit at very low eDNA levels. The results emphasize the future perspectives of eDNA as a monitoring tool for aquatic biodiversity in general, and non-indigenous species in particular.

In the following, eDNA analysis from each the 18 non-indigenous target species (se data in **Table 4.10** and **4.11**) are compared to the conventional monitoring and the known occurrence in Danish seas for the two seasons sampled (**Table 5.2 and 5.3).**

To validate the eDNA analysis, all DNA results were compared to results from the conventional monitoring and previous knowledge on the distribution of the target organisms (see **Table 5.2 and 5.3**). Green colours (**Table 5.1**) were used to indicate similar results obtained from eDNA analysis and conventional imonitoring. Blue colours indicate positive conventional monitoring and negative eDNA detection. Red colours indicate positive eDNA detection but negative conventional monitoring. Each harbour with "red" and "blue" observations will be treated in section 5.7.

Table 5.1: *Table for interpretation of colors in validation **Table 5.2 and 5.3**. Abbreviations are based on eDNA results and conventional monitoring. Green colors indicate the eDNA monitoring and conventional monitoring are in agreement, whether this means that the species is found present or the species is recorded absent. The blue colors indicate that the conventional monitoring detected the species as being present when the eDNA method was unable to detect the same species. The red colors indicate that the conventional monitoring was unable to detect the presence of the species when the eDNA method supported the species as being present. The abbreviations used refer to the conventional monitoring result on the left side of the underscore ('_') and the eDNA results on the right side. The conventional monitoring was recorded as 'never found' (NF), 'found before' (FB) and 'found during MONIS4' (FM4). The eDNA levels were recorded as: 'not detected at any Cq' (NoCq), 'detected but below limit of detection' (BeLOD), 'detected above LOD but below limit of quantification' (AbLOD), 'one technical replicate above limit of quantification' (1AbLOQ) and 'all three replicates above limit of quantification' (3AbLOQ).*

	CONVENTIONAL RESULT		
eDNA RESULT:	**Not found ever (NF)**	**Found before, but not during MONIS 4 (FB)**	**Found during MONIS 4 (FM4)**
No Cq	NF_NoCq	FB_NoCq	FM4_NoCq
Below LOD	NF_BeLOD	FB_BeLOD	FM4_BeLOD
Above LOD below LOQ	NF_AbLOD	FB_AbLOD	FM4_AbLOD
1Above LOQ	NF_1AbLOQ	FB_1AbLOQ	FM4_1AbLOQ
3Above LOQ	NF_3AbLOQ	FB_3AbLOQ	FM4_3AbLOQ

The eDNA levels detected were plotted on maps for both spring and autumn for each species, together with the results from conventional monitoring (see **Annex** , **Appendix B1-B20**).

Table 5.2: *Monitoring by eDNA compared with conventional monitoring in the spring (May – July 2017). The comparison between eDNA monitoring and conventional monitoring is made for each species for each harbour. The results from both methods are separated by an underscore, with eDNA results on the left, and conventional monitoring on the right. Evaluation of technical qPCR replicates for the presence of eDNA is scored for five different categories: No Ct (NoCt). Below LOD (BeLOD). Above LOD below LOQ (AbLOD). One replicate Above LOQ (1AbLOQ). Three replicates above LOQ (3AbLOQ). The conventional monitoring was scored for three different categories: Not found previously or unknown (NF). Found before, but not during MONIS 4 field work (FB). Found during MONIS 4 field work (FM4). Agreements between the two methods are colored green. Major disagreements between the two methods are colored red (detected by eDNA but never before by conventional monitoring) and blue (not detected by eDNA but detected by conventional monitoring). Minor incongruence between the two methods is colored light green (i.e. not detected by eDNA but recorded in the past). Agreements between eDNA and conventional monitoring are colored dark green (i.e. found by both eDNA and conventional monitoring, or not found by either methods).*

Species	Assay IDNo	AalborgHavn	Aalborg Portland	Aarhus	Esbjerg	Fredericia	Frederik-shavn	Gedser	Grenå	Helsingør	Hirtshals	Kalundborg	Statiol	København	Køge	Odense	Rødby
Bonnemaisonia hamifera	1	NoCt_FB	NoCt_FB	BeLOD_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	BeLOD_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB
Prorocentrum cordatum	2	3AbLOQ_FB	3AbLOQ_FB	BeLOD_FB	NoCt_FM4	BeLOD_FB	NoCt_FB	BeLOD_FB	BeLOD_FB	3AbLOQ_FB	NoCt_FB	3AbLOQ_FB	BeLOD_FB	BeLOD_FB	3AbLOQ_FB	BeLOD_FB	NoCt_FB
Pseudochattonella farcimen	3	NoCt_NF	BeLOD_NF	3AbLOQ_FB	NoCt_FM4	3AbLOQ_FB	3AbLOQ_FB	3AbLOQ_NF	3AbLOQ_FB	3AbLOQ_NF	NoCt_FB	3AbLOQ_FB	3AbLOQ_FB	3AbLOQ_NF	3AbLOQ_NF	BeLOD_FB	3AbLOQ_NF
P. verruculosa	4	AbLOD_NF	BeLOD_NF	NoCt_FB	AbLOD_FM4	NoCt_FB	BeLOD_FB	NoCt_NF	NoCt_FB	NoCt_NF	BeLOD_FB	NoCt_FB	NoCt_FB	NoCt_NF	NoCt_NF	NoCt_FB	NoCt_NF
Karenia mikimotoi	5	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FM4	NoCt_FB	BeLOD_FB	NoCt_NF	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_NF	NoCt_FB	NoCt_NF
Carassius auratus	6	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_FB	NoCt_NF	NoCt_NF	NoCt_NF
Cyprinus carpio	7	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_FB	NoCt_FB	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF
Colpomenia peregrine	8	NoCt_FB	NoCt_FB	BeLOD_NF	NoCt_NF	NoCt_NF	NoCt_FB	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	BeLOD_NF	BeLOD_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF
Neogobius melanostomus	09	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	BeLOD_FM4	NoCt_NF	NoCt_NF	NoCt_NF	BeLOD_FM4	NoCt_FM4	NoCt_FM4	BeLOD_FM4	NoCt_NF	BeLOD_FM4
Oncorhynchus mykiss	10	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_NF	NoCt_NF	BeLOD_NF	NoCt_NF	NoCt_FB	NoCt_FB	NoCt_NF	BeLOD_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_NF
Oncorhyncus gorbuscha	13	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF
Crassostrea gigas	14	NoCt_FM4	NoCt_FM4	NoCt_NF	BeLOD_FM4	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_FM4	NoCt_NF	NoCt_FB	NoCt_NF	NoCt_FM4	NoCt_NF	NoCt_NF	NoCt_NF
Mya arenaria	15	NoCt_FB	3AbLOQ_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	3AbLOQ_FB	BeLOD_FB	NoCt_FB	NoCt_FB	3AbLOQ_FB	3AbLOQ_FB	3AbLOQ_FB	NoCt_FB	3AbLOQ_FB	3AbLOQ_FB
Rhithropanopeus harrisii	16	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_FB	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_FB	AbLOD_FB	AbLOD_NF	NoCt_FB
Eriocheir sinensis	18	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF
Cordylophora caspia	21	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF
Mnemiopsis leidyi	22	3AbLOQ_FM4	3AbLOQ_FM4	BeLOD_FM4	BeLOD_FM4	NoCt_FM4	BeLOD_FM4	BeLOD_FM4	NoCt_FM4	NoCt_FM4	3AbLOQ_FB	NoCt_FM4	BeLOD_FM4	NoCt_FM4	NoCt_FM4	NoCt_FB	NoCt_FM4
Acipenser baerii	23	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF

Table 5.3: *Monitoring by eDNA compared with conventional monitoring in the fall (September – October 2017). The comparison between eDNA monitoring and conventional monitoring is made for each species for each harbour. The results from both methods are separated by an underscore, with eDNA results on the left, and conventional monitoring on the right. Evaluation of technical qPCR replicates for the presence of eDNA is scored for five different categories: No Ct (NoCt). Below LOD (BeLOD). Above LOD below LOQ (AbLOD). One replicate Above LOQ (1AbLOQ). Three replicats above LOQ (3AbLOQ). The conventional monitoring was scored for three different categories: Not found previously or unknown (NF). Found before, but not during Monis4 field work (FB). Found during MONIS 4 field work (FM4). Agreements between the two methods are colored green. Major disagreements between the two methods are colored red (detected by eDNA but never before by conventional monitoring) and blue (not detected by eDNA but detected by conventional monitoring). Minor incongruence between the two methods is colored light green (i.e. not detected by eDNA but recorded in the past). Agreements between eDNA and conventional monitoring are colored dark green (i.e. found by both eDNA and conventional monitoring, or not found by either methods).*

Species	Assay IDNo	Aalborg Havn	Aalborg portland	Aarhus	Esbjerg	Fredericia	Frederikshavn	Gedser	Grenå	Helsingør	Hirtshals	Kalundborg	StatiolHavn	København	Køge	Odense	Rødby
Bonnemaisonia hamifera	1	NoCt_FB	NoCt_FB	3AbLOQ FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	3AbLOQ FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	3AbLOQ FB	1AbLOQ FB
Prorocentrum cordatum	2	BeLOD FB	BeLOD FB	NoCt_FB	BeLOD FM4	BeLOD FB	BeLOD FB	3AbLOQ FB	BeLOD FB	3AbLOQ FB	NoCt_FB	NoCt_FB	BeLOD FB	3AbLOQ FB	3AbLOQ FB	1AbLOQ FB	3AbLOQ FB
Pseudochattonella farcimen	3	1AbLOQ_NF	3AbLOQ_NF	BeLOD_FB	NoCt_FM4	3AbLOQ_FB	3AbLOQ_FB	NoCt_NF	3AbLOQ_FB	3AbLOQ_NF	BeLOD_FB	3AbLOQ_FB	3AbLOQ_FB	3AbLOQ_NF	NoCt_NF	3AbLOQ_FB	BeLOD_NF
P. verruculosa	4	3AbLOQ_NF	3AbLOQ_NF	NoCt_FB	1AbLOQ_FM4	NoCt_FB	NoCt_FB	NoCt_NF	NoCt_FB	NoCt_NF	3AbLOQ_FB	NoCt_FB	NoCt_FB	NoCt_NF	NoCt_NF	1AbLOQ_FB	NoCt_NF
Karenia mikimotoi	5	3AbLOQ_FB	3AbLOQ_FB	NoCt_FB	BeLOD_FM4	NoCt_FB	NoCt_FB	NoCt_NF	BeLOD_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_NF	3AbLOQ_FB	NoCt_NF
Carassius auratus	6	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_FB	NoCt_NF	NoCt_NF	NoCt_NF
Cyprinus carpio	7	NoCt_NF	BeLOD_NF	BeLOD_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	BeLOD_FB	BeLOD_FB	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF
Colpomenia peregrine	8	NoCt_FB	NoCt_FB	NoCt_NF	NoCt_NF	BeLOD NF	BeLOD FB	NoCt_NF	NoCt_NF	BeLOD NF	NoCt_NF	BeLOD NF	3AbLOQ NF	BeLOD NF	NoCt_NF	NoCt_NF	NoCt_NF
Neogobius melanostomus	09	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	BeLOD_FM4	NoCt_NF	NoCt_NF	NoCt_NF	BeLOD_FM4	BeLOD_FM4	NoCt_FM4	BeLOD_FM4	NoCt_NF	BeLOD_FM4
Oncorhynchus mykiss	10	BeLOD_FB	BeLOD_FB	NoCt_FB	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_FB	NoCt_FB	BeLOD_NF	NoCt_FB	BeLOD_FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_NF
Oncorhyncus gorbuscha	13	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF
Crassostrea gigas	14	NoCt FM4	NoCt FM4	NoCt_NF	BeLOD FM4	NoCt_NF	NoCt_NF	NoCt_NF	BeLOD NF	NoCt FM4	NoCt_NF	NoCt_FB	NoCt_NF	AbLOD FM4	NoCt_NF	NoCt_NF	NoCt_NF
Mya arenaria	15	BeLOD FB	NoCt_FB	NoCt_FB	NoCt_FB	NoCt_FB	3AbLOQ FB	3AbLOQ FB	3AbLOQ FB	NoCt_FB	NoCt_FB	NoCt_FB	3AbLOQ FB	NoCt_FB	NoCt_FB	BeLOD FB	BeLOD FB
Rhithropanopeus harrisii	16	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	BeLOD_NF	NoCt_FB	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	BeLOD_FB	BeLOD_FB	NoCt_NF	NoCt_FB
Eriocheir sinensis	18	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF
Cordylophora caspia	21	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF
Mnemiopsis leidyi	22	3AbLOQ_FM4	3AbLOQ_FM4	BeLOD_FM4	3AbLOQ_FM4	3AbLOQ_FM4	NoCt_FM4	3AbLOQ_FM4	3AbLOQ_FM4	3AbLOQ_FM4	1AbLOQ_FB	3AbLOQ_FM4	3AbLOQ_FM4	3AbLOQ_FM4	BeLOD_FM4	BeLOD_FB	3AbLOQ_FM4
Acipenser baerii	23	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF	NoCt_NF

1. AssayIDNo 01: ***Bonnemaisonia hamifera*** is native to the northwestern Pacific Ocean (Hariot 1891) and is known from around the European coast (Guiry 2001). The high eDNA levels detected from ***B. hamifera*** in Aarhus, Grenå, Odense and Rødby harbour matches the wide distribution of ***B. hamifera*** known from the North Sea to Storebælt in the inner Danish seas (Køie & Kristiansen 2000) (**Annex 10, Figure B1**). Tufts of this red algae can easily be swept away with currents, and conventional monitoring might overlook ***B. hamifera*** drifting in the vicinity of the harbour. Since this species easily is dispersed by currents in the inner Danish marine waters the eDNA levels and the knowledge on the distribution of ***B. hamifera*** can be considered congruent.

2. AssayIDNo 02: ***Prorocentrum cordatum*** is known from European seas (Brandt 2001). Originally ***P. cordatum*** was identified from the Caspian Sea (Velikova & Larsen 1999) and algae blooms of have been reported from Japan, France, Netherlands, Norway, and the eastern coast of the United States of America (Heil *et al.* 2005). Blooms of ***P. cordatum*** generally occur under relative high temperatures and low salinities (Heil *et al.* 2005) which match the higher levels of eDNA detected in the autumn in the south-eastern part of the inner Danish seas (**Annex 10, Figure B2**).

3. AssayIDNo 03: ***Pseudochattonella farcimen*** and ***Pseudochattonella verruculosa*** are morphologically very similar and it is hard to differentiate between the two species (Edvardsen *et al.* 2007, Eikrem *et al.* 2009). The species-specific assay developed in this project (**Table 3.2**) for detection of eDNA from ***P. farcimen*** can potentially return positive detection based on eDNA from ***P. verruculosa***, and vice versa for the species-specific assay developed for detecting ***P. verruculosa*** (Annex B3). ***Pseudochattonella farcimen*** was detected in Aalborg Havn, Aalborg Portland, Aarhus, Fredericia, Frederikshavn, Grenå, Helsingør, Hirtshals, Kalundborg Kalundborg Statiol Harbour, København, Odense and Rødby. Based on the eDNA detection, this broadens the known distribution of ***P. farcimen*** to also cover the eastern and southeastern part of the inner Danish marine waters (**Annex 10, Figure B3**). It is possible that ***P. farcimen*** have been overlooked by conventional monitoring in Helsingør, København, Køge, Rødby and Gedser. However, the eDNA analysis in this case indicated the potential presence of the target species, which was neither found by conventional monitoring nor known from previous studies. These eDNA observations must be confirmed by further investigations, to assure that they are not false positive results. This is in particular important because this study presents the first full-scale applications of several new eDNA detection systems.

4. AssayIDNo 04: ***Pseudochattonella verruculosa*** was detected in the southwestern and northwestern part of the Danish marine waters (North Sea and Skagerak, respectively) (**Annex 10, Figure B4**). This occurrence of eDNA matches the previous known distribution of ***P. verruculosa***. The eDNA method did not find any traces of ***P. verruculosa*** in Kattegat, Lillebælt and Storebælt, where this species has been recorded previously. This can be because the water sampling was performed outside the time period where ***P. verruculosa*** is most abundant in the inner Danish seas, or it could be because ***P. verruculosa*** and ***P. farcimen*** have been confused in the past, and the eDNA method in fact is providing a more precise reflection of the distribution of these two species. However, eDNA analysis indicated in this case potential presence of the target species, which was neither found by conventional monitoring nor known from previous studies. In this case the eDNA observations must be confirmed by further investigations, to assure they are not false positive results. This is in particular important because this study presents the first full-scale applications of several new eDNA-detection systems.

5. AssayIDNo 05: ***Karenia mikimotoi*** – This dinoflagellate was originally described from the Northwestern Pacific Ocean and has since been reported from the east coast of the United States of America and the west coast of Europe (Guiry 2018). The high eDNA levels in Aalborg and Odense and low eDNA levels in Esbjerg, Frederikshavn and Grenå (**Annex 10, Figure B5**), is not surprising as ***K. mikimotoi*** is known to be widespread.

6. AssayIDNo 06: Although ***Carassius auratus*** is quite common in all freshwater systems in Denmark (Carl 2012c), and eDNA is bound to reach the shoreline and the harbours around Denmark, no eDNA was detected in any of the 16 harbours (**Annex 10, Figure B6**). However, as ***C. auratus*** rarely visits saline and brackish waters, the absence of eDNA from ***C. auratus*** in the Danish harbours is expected.

7. AssayIDNo 07: ***Cyprinus carpio*** is mainly a freshwater species, but occasional occurrence in brackish water is known from the inner Danish marine waters (Carl 2012b). As ***C. carpio*** is common in many freshwater lakes and streams in Denmark, it is very likely that any eDNA will reach the harbours around the Danish coastline. The faint detections of eDNA in Aarhus and Aalborg are congruent with the broad distribution of ***C. carpio*** in Danish freshwater systems (**Annex 10, Figure B7**).

8. AssayIDNo 08: ***Colpomenia peregrina*** is native in the Eastern Pacific Ocean (Abbott & Hollenberg 1976, Min *et al.* 2014) and is known from European seas (Guiry 2001), and has been recorded at Hirsholmene in Kattegat, and is encountered in the North Sea and Limfjorden (Køie & Kristiansen 2000, Nielsen *et al.* 1995) and along the western coast off Europe (Min *et al.* 2014) and Ireland (Minchin 1991). The low eDNA levels found in Aarhus, Fredericia, Helsingør and København, and the high eDNA levels in Kalundborg Harbour, supports that ***C. peregrina*** is widespread in the inner Danish seas. Although not previously recorded by conventional monitoring in Kalundborg (Table 4.17), the high eDNA levels in Kalundborg Harbour in September - November 2017 supports the presence of ***C. peregrina*** in the northwestern part of Sjælland (**Annex 10, Figure B8**), which matches the ability of ***C. peregrina*** to disperse widely (Green *et al.* 2012, Min *et al.* 2014, Minchin 1991). Nielsen *et al.* (1995) lists ***C. peregrina*** as recorded in 1970 or later from the Kattegat, but not from the southeastern part of the inner Danish marine waters. Given the ability of ***C. peregrina*** to spread, more southern occurrences are not unlikely today. More intensive sampling with multiple biological filter replicates, and additional technical qPCR replicates to analyse is recommended from these 'yellow' eDNA recordings. The eDNA analysis indicated potential presence of this target species in Kalundborg, which was neither found by conventional monitoring nor known from previous studies.

9. AssayIDNo 09A: ***Neogobius melanostomus*** arrived in Danish marine waters in 2008 and is now widespread in southeastern parts (Azour *et al.* 2015). The eDNA levels for ***N. melanostomus*** are low but match the currently known distribution of this species. ***Neogobius melanostomus*** is quite abundant near Køge and Gedser, and higher eDNA levels were anticipated in these two harbours. However, ***N. melanostomus*** is a benthic associated species (Carl 2012a), and the water collected in the surface might hold insufficient levels of eDNA from ***N. melanostomus*** to ensure a strong eDNA signal. The 'yellow' categories assigned to the eDNA levels for ***N. melanostomus*** calls for more intensive sampling, and reanalysis of more biological filter replicates, and additional technical qPCR replicates (**Annex 10, Figure B9**).

10. AssayIDNo 10: Low eDNA levels were detected from ***Oncorhyncus mykiss*** in Aalborg, Frederikshavn, Hirtshals, Kalundborg (**Annex 10, Figure B10**), which supports the introduction of

this species in various aquaculture facilities in Denmark (Rasmussen 2012). The low eDNA levels can potentially also stem from inland freshwater aquaculture facilities, or from ***O. mykiss*** escaped from aquaculture facilities to streams and lakes.

11. AssayIDNo 13: No eDNA was detected from ***Oncorhyncus gorbuscha*** (**Annex 10, Figure B11**), which confirms the rare occurrence of this non-indigenous species.

12. AssayIDNo 14: ***Crassostrea gigas*** - This species (also known as *Magallana gigas;* see section 4.4 and **Figure 4.8, panel b**) is introduced from the Pacific coast of Asia and has been recorded in along the Western coast of Jylland, in Limfjorden and in Øresund. The eDNA detected in this study supports the distribution of Pacific oyster in Øresund, but the eDNA signal is too low (Esbjerg) or absent (Aalborg) areas where this species has been recorded previously (see **Annex 10, Figure B12**).

13. AssayIDNo 15: The mussel ***Mya arenaria*** is known throughout Europe's seas (Gofas *et al.* 2001) and is common around the Danish coastline (*T. Schiøte, Natural History Museum of Denmark, pers. comm.*), which is supported by the eDNA levels detected (**Annex 10, Figure B13**). ***Mya arenaria*** is common but not considered native in Danish waters (Strasser 1999).

14. AssayIDNo 16: The eDNA detected for ***Rhithropanopeus harrisii*** in Øresund confirms the currently known distribution of ***R. harrisii*** from the Baltic Sea and in Øresund (Forstrom & Vasemagi 2016). Although mainly found in brackish waters, the low eDNA levels in Odense are not unlikely, and could represent and overlooked distribution. The low eDNA levels detected in Frederikshavn, however, are quite unlikely, but not impossible, and could be an artefact stemming from unspecific qPCR amplification or a very rare occurrence (see **Annex 10, Figure B14**). The eDNA levels in Odense and Frederikshavn are categorized as 'yellow' and 'orange' and it is therefore recommended that additional samples are collected and analysed from these two harbours. A re-run of the sample from Frederikshavn collected in the autumn confirmed the eDNA detection below LOD for one replicate for ***R. harissii***.

15. AssayIDNo 18: The Chinese mitten crab (***Eriocheir sinensis***) is commonly encountered as a non-indigenous species in southern England and Northeastern coast of Germany (Herborg *et al.* 2005) but has only been occasionally recorded in inner Danish seas (*Jørgen Olsen, Nat. Hist. Mus. Denmark, pers. comm.*). The complete absence of eDNA from all samples is in congruence with the expected distribution and occurrence (see **Annex 10, Figure B16**).

16. AssayIDNo 21: ***Cordylophora caspia*** - This brackish water species has in the past been recorded from the Baltic Sea (van der Land 2001). However, the absence of eDNA from the inner Danish marine waters supports the conventional monitoring (**Annex 10, Figure B18**), suggesting that this species is a rare non-indigenous species to that has yet to be encountered the North Sea, Kattegat, Skagerak and Øresund.

17. AssayIDNo 22: The eDNA detected for ***Mnemiopsis leidyi*** in the northwestern part of Jutland in the spring, and the absence of eDNA from ***M. leidyi*** in the rest of the Inner Danish Waters in the spring matches the recolonization of the Inner Danish Waters performed every year by ***M. leidyi*** (Riisgård 2017) (see **Annex 10, Figure B19**). In the autumn samples ***M. leidyi*** is detected with eDNA from Hirtshals and Aalborg and in the southeastern part of the Inner Danish Waters in Rødby and Gedser Harbour, which agrees with the annual reoccurrence and broad distribution of this invasive comb jelly. During the winter period ***M. leidyi*** disappears again from the Inner Danish Waters, and the following year the Inner Danish Waters are

recolonized again from the North Sea and through Limfjorden, matching the eDNA detections found in Hirtshals and Aalborg during the spring (Risgård 2017). Only low levels of eDNA were found for ***M. leidyi*** in the spring in Esbjerg Harbour, and the North Sea is expected to be inhabited all year round by ***M. leidyi*** (Risgård 2017), but this might as well reflect the low volume of water filtered in this harbour (**Table 3.1**).

18. AssayIDNo 23: ***Acipenser baerii*** - No eDNA was detected from ***A. baerii*** (**Annex 10, Figure B20**), which confirms the rare occurrence of this non-indigenous species (Carl 2012a, Møller 2012).

In the following, the correspondence between eDNA monitoring and conventional monitoring is presented for each harbour:

1. Aarhus: The eDNA in the water sample collected in the spring matched the conventional monitoring for all 18 species, although the eDNA levels were only indicative for ***Mnemiopsis leidyi*** (below LOD) (**Table 5.2**), the snorkel monitoring confirmed the presence of this species later in the year. The conventional monitoring matched the eDNA sampling in the autumn apart for ***Bonnemaisonia hamifera***, which was found present by high levels of eDNA (above LOQ) and not recorded before.

2. Esbjerg: The conventional monitoring and the water sample collected in the spring disagreed on the presence of ***Cyprinus carpio***, ***Karenia mikimotoi***, ***Mnemiopsis leidyi***, ***Prorocentrum cordatum*** and ***Pseudochattonella farcimen***. The conventional monitoring applied in the MONIS 4 project recorded all seven species from Esbjerg harbour, but the eDNA method failed to detect these species and only found indicative traces of eDNA (below LOD) from ***C. carpio*** and ***M. leidyi*** (**Table 5.2**). The water sample collected in the autumn in Esbjerg found low levelks of eDNA from ***Crassostrea gigas***, ***K. mikimotoi*** and ***P. cordatum***, although they had been recorded by conventional monitoring in this study. No eDNA was found for ***Pseudochattonella farcimen*** although this species has been recorded by conventional monitoring during the MONIS 4 project period.

3. Aalborg: The eDNA tracked in the spring matched the conventional monitoring for nearly all 20 species monitored. High levels of eDNA was found for ***M. leidyi*** confirming their presence in this harbour as observed by snorkelling (**Table 5.2**). ***Prorocentrum cordatum*** was not recorded during the MONIS 4 project period but have been recorded from Aalborg harbour in the past and is thereby in agreement with the high eDNA levels found for ***Prorocentrum cordatum*** (**Table 5.2**). The match between the conventional and eDNA monitoring in the autumn matched for all species apart from ***Crassostrea gigas*** which was recorded during the snorkelling in the MONIS4 survey, but not found with the eDNA method (**Table 5.3**). For ***Karenia mikimotoi*** and ***M. leidyi*** high levels of eDNA (above LOQ) matched the presence of these species previously being recorded by conventional monitoring and snorkelling (**Table 5.3**). This agrees with previous records of ***M. leidyi*** from the North Sea (Riisgård 2017). High eDNA levels for ***Pseudochattonella farcimen*** and ***Pseudochattonella verruculosa*** did not match the conventional monitoring for Aalborg harbour, and they have to our knowledge not been recorded from this harbour before (**Table 5.2 and 5.3**). These results must be investigated further to confirm the findings.

4. Aalborg Portland: In the spring, high levels of eDNA (above LOQ) from ***Mnemiopsis leidyi*** and ***Prorocentrum cordatum*** matched the record of these species through snorkelling and conventional monitoring in this project and in the past, respectively (**Table 5.2**). ***Mya arenaria***

was detected by eDNA matching previous findings with conventional monitoring. Snorkelling in Aalborg Portland harbour found ***Crassostrea gigas***, but the presence of this species was not supported in the autumn by the eDNA method (**Table 5.3**). The high levels of eDNA (above LOQ) found for ***Karenia mikimotoi*** and ***M. leidyi*** matched the previous recording of these species by conventional monitoring (**Table 5.3**). ***Oncorhynchus mykiss*** and ***P. cordatum*** have been recorded in the past from Aalborg Portland harbour, and this was matched by low levels of eDNA (below LOD) found for these two species (**Table 5.3**). High eDNA levels for ***Pseudochattonella farcimen*** and ***Pseudochattonella verruculosa*** did not match the conventional monitoring for Aalborg Portland harbour, and they have to our knowledge not been recorded from this harbour before (**Table 5.3**). These results must be investigated further to confirm the findings.

5. Fredericia: There was similarity between the conventional sampling and the eDNA method for all species in the spring, except for ***Mnemiopsis leidyi*** which was recorded by snorkel monitoring, but not found with the eDNA method in the water sample (**Table 5.2**). For the autumn there was a higher level of congruence between the molecular methods and conventional monitoring in Fredericia harbour. High levels of eDNA (above LOQ) were found for ***M. leidyi*** and for ***Pseudochattonella farcimen***, and the snorkel monitoring also recorded these two species in this harbour. Among the other species detected by eDNA in Fredericia, only low traces of eDNA (***Prorocentrum cordatum*** and ***Colpomenia peregrina***) was recorded for species that either had not previously been recorded or have been recorded in the past (**Table 5.3**). No eDNA was found for ***Pseudochattonella verruculosa*** although this species has been recorded from Fredericia in the past.

6. Frederikshavn: The eDNA detected in the water sample collected in the spring matched the conventional monitoring for all species apart from ***Mnemiopsis leidyi***, which was only indicated by eDNA (below LOD) and found in the autumn by the snorkel monitoring in the MONIS 4 project. ***Pseudochattonella farcimen*** and ***Pseudochattonella verruculosa*** were found with eDNA levels above LOQ and below LOD, and both species have been recorded from Frederikshavn in the past (**Table 5.2**). The eDNA detected in the water sample collected in the autumn disagreed with the snorkel monitoring for ***M. leidyi*** which was not detected by eDNA but found using snorkel monitoring. ***Mya arenaria*** and ***P. farcimen*** have been found by conventional monitoring in the past and was detected with high eDNA levels (above LOQ) in the water sample from the autumn (**Table 5.3**). The other invasive species (***P. cordatum*** and ***R. harrissii***) that had their presence indicated by low levels of eDNA (below LOD) in the autumn had not been recorded previously or had never before been recorded by conventional monitoring in Frederikshavn Harbour.

7. Gedser: The conventional monitoring and the eDNA monitoring agreed for all species apart from ***Mnemiopsis leidyi***, ***Mya arenaria*** and ***Neogobius melanostomus*** (**Table 5.2**). ***M. leidyi*** and ***N. melanostomus*** were only indicated by eDNA (below LOD) but found by snorkel and conventional monitoring, and ***M. arenaria*** is known from this area from past sampling and was detected by high eDNA levels (above LOQ) (**Table 5.2**). ***Pseudochattonella farcimen*** was detected with eDNA levels above LOQ although this species not have been recorded by conventional monitoring from Gedser harbour before in the spring. But this could be dependent on the seasonality of sampling as the autumn water samples had no trace of ***P. farcimen*** or ***P. verruculosa***. For the autumn there was a higher level of congruence between conventional monitoring and eDNA monitoring. The presence of ***M. leidyi*** and ***Prorocentrum cordatum*** was supported by high levels of eDNA (above LOQ) and also recorded by snorkel and conventional monitoring. High levels of eDNA (above LOQ) from ***M. arenaria*** matched past known

distribution in this area. Although ***N. melanostomus*** was recorded by the conventional monitoring, only low traces of eDNA supported ***N. melanostomus*** being present in Gedser Harbour in the fall (**Table 5.3**).

8. Grenå: In the spring, the conventional monitoring and the eDNA were congruent for all but one species. Low trace levels of eDNA (below LOD) were matched by conventional monitoring that either had not recorded the species before or only recorded the species in the past (**Table 5.2**). However, ***Mnemiopsis leidyi*** was recorded during the snorkel monitoring, but not found with eDNA in the spring (**Table 5.2**). Agreement between eDNA and conventional monitoring was found for ***Pseudochattonella farcimen***, as this species was detected with eDNA levels above LOQ and have been recorded before with conventional monitoring. Comparison between eDNA traced in the water sample in the autumn matched the conventional monitoring for all but two species. The presence of ***M. leidyi*** was confirmed by findings with snorkel monitoring and was supported by high levels of eDNA (above LOQ). The presence of ***Mya arenaria***, ***Bonnemaisonia hamifera*** and ***P. farcimen*** in the autumn were also supported high levels of eDNA (above LOQ), and all three species are assumed present in this area.

9. Helsingør: eDNA levels from ***Prorocentrum cordatum*** were above LOQ in the spring, which agrees with this species being recorded from Helsingør in the past. The other invasive species traced in this project by eDNA were not detected, and had not been recorded in the past, or found occasionally in the past. ***Mnemiopsis leidyi*** was not recorded by the eDNA method in the spring but was found during the present project by snorkel monitoring . ***Pseudochattonella farcimen*** was detected with high eDNA levels in both the spring and the autumn but have not been found before with conventional monitoring (**Table 5.2-5.3**). The eDNA method and conventional monitoring showed better congruence in the autumn for all species monitored by both methods. ***M. leidyi*** and ***P. cordatum*** were detected in the autumn water sample in levels above LOQ, confirming the presence as found by conventional monitoring. Other species traced were not found using eDNA or only indicated by eDNA levels below LOD and not recorded by conventional monitoring, and if recorded, then in the past and not during the present project. However, ***Crassostrea gigas*** was recorded with snorkel monitoring, but not found with the eDNA method in the autumn (**Table 5.3**).

10. Hirtshals: Conventional monitoring and eDNA tracking were in overall agreement for both spring and autumn samples. ***Mnemiopsis leidyi*** was detected in the spring by high levels of eDNA (above LOQ) and had also been recorded from Hirtshals in the past by conventional monitoring. All other species were not detected by eDNA and had either been recorded in the past or never recorded (**Table 5.2**). ***Pseudochattonella verruculosa*** was detected with low eDNA levels, and have been recorded with conventional monitoring before, but no eDNA was found in the spring from ***Pseudochattonella farcimen***. This could be a because of sampling in the spring and summer differs from the time point where the conventional monitoring is performed, as ***P. farcimen*** and ***P. verruculosa*** was detected with eDNA levels, below LOD and above LOQ, respectively, in the autumn, in agreement with the conventional monitoring that in the past have found these two species in Hirtshals Harbour. For the autumn ***M. leidyi*** was detected by eDNA levels above LOQ, confirming previous conventional monitoring records of this species in Hirtshals harbour. Low traces of eDNA from ***Oncorhynchus mykiss*** (below LOD) was found in the autumn, although ***O. mykiss*** had not been recorded before by conventional monitoring in Hirtshals harbour.

11. Kalundborg: eDNA levels for the 18 invasive species monitored in the spring agreed with conventional monitoring for all species apart from ***Mnemiopsis leidyi*** and ***Neogobius***

melanostomus. ***M. leidyi*** was not found in the spring with eDNA, and ***N. melanostomus*** only indicated by low levels of eDNA (below LOD), whereas snorkel monitoring in this project recorded the presence of both species. The eDNA levels of ***Mya arenaria*** and ***Pseudochattonella farcimen*** in the spring were quite high (above LOQ), which matches the assumed distribution and suspicion of these species being present in Kalundborg harbour. In the autumn the snorkel monitoring and eDNA traces were more congruent, with high levels of eDNA detected for ***M. leidyi*** and ***Pseudochattonella farcimen*** (both above LOQ), which agrees with the finding of these species using conventional monitoring. The other species monitored by eDNA in the autumn had low traces of eDNA and have been recorded occasionally in the past or never been recorded by conventional monitoring. The eDNA level for ***N. melanostomus*** was low (below LOD) in the autumn, even though this species was observed in high numbers by snorkel monitoring during this project. ***P. verruculosa*** was not found with eDNA but have been recorded in the past with conventional monitoring.

12. København: The water sample collected in the spring matched the findings inferred from the conventional monitoring for all species apart from, ***Mnemiopsis leidyi*** and ***Neogobius melanostomus*** and ***Pseudochattonella farcimen***. ***M. leidyi*** and ***N. melanostomus*** were found with snorkel monitoring during this project, but not by eDNA. ***Mya arenaria*** and ***P. farcimen*** were detected by eDNA (above LOQ) but ***M. arenaria*** is common in Øresund and København harbour (**Table 5.2**), and ***P. farcimen*** have not been recorded from Øresund before. The water sample collected in the autumn agreed with the conventional monitoring on all species surveyed, except for ***N. melanostomus***, which was found during the conventional monitoring in this project, but not found with the eDNA method. ***Crassostrea gigas***, ***M. leidyi*** and ***Prorocentrum cordatum*** were detected by eDNA in the autumn and have also in the past and in this project been found during conventional monitoring (**Table 5.2-5.3**). High levels of eDNA in the autumn from ***P. farcimen*** disagrees with conventional monitoring, as this species not have been recorded from Øresund before. No eDNA in the autumn from ***P. verruculosa*** supports that this species has not been recorded from this harbour before and snorkel monitoring (**Table 5.3**).

13. Køge: The water sample in the spring did not detect eDNA from the majority of the 18 invasive species this project screened for. This matched the absence of these species in the conventional monitoring. However, eDNA (above LOQ and above LOD) were found for ***Prorocentrum cordatum*** and ***Rhithropanopeus harrisii*** (**Table 5.2**), and this matched the finding of these two species by conventional monitoring in the past. ***Mnemiopsis leidyi*** was not detected by eDNA in the spring sample but was found with conventional monitoring later in 2017. The presence of ***Neogobius melanostomus*** in Køge harbour was only indicated by relative low levels of eDNA (below LOD), where the conventional monitoring performed later in 2017 supported the presence of ***N. melanostomus*** in Køge harbour. High levels of eDNA in the spring from ***Pseudochattonella farcimen*** disagrees with conventional monitoring, as this species have not been recorded from Øresund before. The autumn water sample returned the same findings as found in the spring. This time the presence of ***M. leidyi*** in Køge harbour was supported by a high level of eDNA (ablove LOQ), confirming the record of this species as found by snorkel monitoring (**Table 5.3**). No eDNA in the autumn from ***Pseudochattonella verruculosa*** and no eDNA from ***P. farcimen*** supports that these species have not been recorded from this harbour before.

14. Odense: In the water sample from the spring, eDNA was detected from ***Mya arenaria***, ***Prorocentrum cordatum*** and ***Rhithropanopeus harrisii*** (**Table 5.2** and **5.3**). ***R. harrisii*** has not been recorded previously from Odense harbour, but ***P. cordatum*** has been recorded by conventio-

nal monitoring in the past. Low levels of eDNA or no eDNA at all in the spring for ***Pseudochattonella farcimen*** and ***P. verruculosa*** is congruent with these species having been recorded with conventional monitoring in the past, this congruence is reflected better by the autumn sample where both species are found with eDNA levels above LOQ (**Table 5.3**). The water sample from the autumn agreed better with the conventional monitoring as both approaches found ***Karenia mikimotoi***, ***Mnemiopsis leidyi*** and ***P. cordatum*** (**Table 5.3**). The only major disagreement between the two methods was the high level of eDNA from ***Bonnemaisonia hamifera*** (above LOQ) that was unsupported by conventional monitoring.

15. Rødby: The eDNA method supported the presence of ***Mya arenaria*** and indicated the presence of ***Neogobius melanostomus*** (eDNA levels above LOQ and below LOD, respectively). But no eDNA was detected for ***Mnemiopsis leidyi*** in the spring, where the conventional monitoring performed later in the year found ***M. leidyi*** (**Table 5.2**). High eDNA levels for ***Pseudochattonella farcimen*** in the spring disagrees with conventional monitoring, as this species is not known from this harbour before. But this could reflect the time point for when the conventional monitoring is performed. The autumn water samples where in better agreement with conventional monitoring, as the eDNA levels for ***Pseudochattonella farcimen*** were low in the autumn (**Table 5.3**). In the water sample collected in the autumn eDNA was detected from ***Bonnemaisonia hamifera***, ***M. leidyi***, ***M. arenaria***, ***N. melanostomus*** and ***Prorocentrum cordatum***. ***M. leidyi***, ***M. arenaria***, ***N. melanostomus*** and ***P. cordatum*** have also been found using conventional monitoring in the past and in 2017 during this project ***B. hamifera*** has not been recorded from Rødby harbour before (**Table 5.3**) but can have been dispersed by currents and be free floating in the water column and have overlooked by conventional monitoring. No eDNA in the spring or in the autumn from ***P. verruculosa*** supports that this species has not been recorded from this harbour before.

16. Statoil, Kalundborg: ***Mnemiopsis leidyi*** and ***Mya arenaria*** were detected in the spring with eDNA, and both species are also recorded by conventional monitoring. ***Neogobius melanostomus*** was also recorded during the snorkel monitoring, but not found in the spring using the eDNA method (**Table 5.2**). The high eDNA levels (above LOQ) for ***Pseudochattonella farcimen*** match the assumed occurrence of this species being present in Kalundborg Statiol harbour. No eDNA was found for ***Pseudochattonella verruculosa*** although this species is assumed present in Kalundborg Statiol harbour. For the water sample collected in the autumn high levels of eDNA was found for ***Colpomenia peregrina***, ***M. leidyi*** and ***M. arenaria*** (above LOQ). ***M. leidyi*** was also found with snorkel monitoring. ***Colpomenia peregrina*** have not been recorded in Kalundborg Statiol harbour in the past or during this survey (**Table 5.3**), but ***Mya arenaria*** is known from this area. High eDNA levels for ***Pseudochattonella farcimen*** in the autumn match that this species has been found before in Kalundborg harbour. No eDNA in the autumn from ***P. verruculosa*** supports that this species has not been recorded from Kalundborg Statiol harbour before. The high eDNA levels for ***C. peregrina*** disagree with the conventional monitoring (**Table 5.2-5.3, Annex 10, Figure B8**), but this might as well be a result stemming from ***C. peregrina*** having been overlooked in the southern parts of the inner Danish Seas. ***Colpomenia peregrina*** have been recorded from the Kattegat Sea (Nielsen *et al.* 1995) back in 1970 and is a species that is capable of dispersing widely (Green *et al.* 2012, Min *et al.* 2014, Minchin 1991) and could have spread to the southern parts of the inner Danish waters.

5 Synthesis and conclusions

We summarized the finding of the baseline study of the occurrence of non-indigenous species in 16 selected Danish harbours.

In Aarhus and Esbjerg harbours, intensive sampling was carried out focusing on a broad range of biological features (phytoplankton, zooplankton, mobile epifauna, benthic infauna, fouling organisms and fish) as well as different sampling approaches (conventional sampling vs. molecular methods).

Based on the monitoring carried out, we conclude:

1. **Aarhus**

- Plankton: Two of four phytoplankton species listed as non-indiginous in Danish waters were observed, ***Pseudochattonella*** cf. ***verruculosa*** and cf. ***Heterosigma akashiwo***, both in June and in low numbers. Two non-indigenous species of zooplankton were recorded: the calanoid copepod ***Acartia tonsa*** and the cladoceran ***Penilia avirostris***.
- Mobile epifauna: No non-indigenous species were recorded in the traps in the investigated basins of Aarhus industrihavn.
- Benthic infauna: Two marine bristle worms of unknown, but presumably west Atlantic origin were found. The nereid ***Alitta succinea*** is well established in Danish waters and has been reported from several localities. The spionid ***Polydora*** cf. ***aggregata*** was abundant at one locality in the harbour and may have been introduced by shipping activities.
- Fouling organisms: Six non-indigenous species were sampled from PVC plates and scraping of hard substrate (***Diadumene lineata***, ***Molgula manhattensis***, ***Amphibalanus improvisus***, ***Caprella mutica***, ***Heterosiphonia japonica*** and ***Hemigrapsus sanguineus***). However, no non-indigenous species were identified from the rapid assessment surveys. However, no non-indigenous species were identified from the rapid assessment surveys.
- Fish, standard methods: No non-indigenous fish species were caught in Aarhus industrihavn, Aarhus lystbådehavn or Aarhus Marselisborg lystbådehavn using standard fishing methods.
- Snorkelling: A total of 13 fish species were observed during two snorkel events. No invasive fish species were seen. No indication of permanent presence of any non-indigenous fish species. ***Sargassum muticum*** was very common, whereas ***Ensis directus*** and ***Mnemiopsis leidyi*** were infrequent.
- eDNA: In the spring, ***Bonnemaisonia hamifera***, ***Colpomenia peregrina***, ***Mnemiopsis leidyi,*** and ***Prorocentrum cordatum*** were detected by low levels of eDNA, and ***Pseudochattonella farcimen*** detected at relative high levels. In the autumn eDNA from ***B. hamifera*** was detected at relative high levels, and ***Cyprinus carpio***, ***P. farcimen*** and ***M. leidyi*** at low levels.

2. **Esbjerg**

- Plankton: Two of four phytoplankton species listed as non-indiginous in Danish waters were observed: ***Karenia mikimotoi*** and ***Prorocentrum*** cf. ***cordatum*** (syn. *Prorocentrum minimum*), both in June and in low numbers. A single non-indinenous species of zooplankton was recorded: ***Acartia tonsa***. In addition, two individuals of ***Mnemiopsis leidyi*** were observed.
- Mobile epifauna: Two non-indigenous barnacle species, ***Austrominius modestus*** and ***Amphibalanus improvisus*** were caught attached to the carapax of the green shore crab *Carcinus*. There was also an accidental catch of the comb jelly ***Mnemiopsis leidyi*** in the basin of the North port (Nordhavnen).

- Benthic infauna: Two non-indigenous bristle worms that have not previously been reported from Denmark were found. The spionid ***Streblospio benedicti*** is of western Atlantic origin and is currently spreading in harbours around the southern North Sea. The phyllodocid ***Eteone heteropoda*** is also of west Atlantic origin and seems to be a new introduction in Europe. In addition, ***Alitta succinea*** and a spionid of uncertain origin, viz. ***Polydora cornuta***, that both are established in Denmark were found. In dredge samples, the razor clam ***Ensis directus*** and the ascidian ***Styela clava*** were caught.
- Fouling organisms: Nine non-indigenous species were sampled from PVC plates and scraping of hard substrate (***Diadumene lineata***, ***Molgula manhattensis***, ***Amphibalanus improvisus***, ***Caprella mutica***, ***Styela clava***, ***Austrominius modestus***, ***Hemigrapsus sanguineus***, ***Crassostrea gigas*** and ***Neosiphonia harveyi***). However, no non-indigenous species were identified from the rapid assessment surveys.
- Fish, standard methods: No non-indigenous fish species were caught in Esbjerg Industrihavn or Esbjerg lystbådehavn. In conclusion, based on the extent of this effort, there are no non-indigenous fish species present in Esbjerg Harbour.
- Snorkelling: Snorkling is generally not possible in Esbjerg Harbour, due to low visibility. An alternative beach seine caught 11 species. No indication of permanent presence of non-indigenous fish species. Three species of non-indigenous invertebrates were observed: ***Crassostrea gigas, Crepidula fornicata*** and ***Mnemiopsis leidyi***.
- eDNA: In the spring, eDNA from ***Crassostrea gigas***, ***Pseudochattonella verruculosa*** and ***Mnemiopsis leidyi*** were traced at low indicative levels. In the autumn eDNA from ***M. leidyi*** were detected at relative high levels, and eDNA from ***C. gigas***, ***Karenia mikimotoi*** and ***Prorocentrum cordatum*** at low indicative levels.

In the remaining 14 harbours, sampling focused on conventional fish monitoring, snorkelling and biomolecular methods. Based on these activities, we conclude:

3. Aalborg Portland

- Fish, standard methods: No non-indigenous fish species were caught in Aalborg Portland Harbour. In conclusion, based on the extent of this effort, there are no non-indigenous fish species present in this harbour. Neither did the campaign catch any non-indigenous species at Aalborg fjordparken (distance 6 km) or Aalborg lystbådehavn (distance 4 km) emphasising the absence of non-indigenous fish species.
- Snorkelling: A single snorkelling effort resulted in observation of six indigenous fish species and one additional with push net. A permanent presence of non-indigenous fish species was not indicated. Four species of non-indigenous invertebrates (***Crassostrea gigas***, ***Crepidula fornicata***, ***Mnemiopsis leidyi*** and ***Styela clava***) were observed and photographed.
- eDNA: In the spring, ***Mnemiopsis leidyi, Prorocentrum cordatum*** and ***Mya arenaria*** were detected by eDNA at relative high levels, and ***Pseudochattonella farcimen***, ***Pseudochattonella verruculosa*** were detected at low levels. In the autumn eDNA from ***Karenia mikimotoi***, ***P. farcimen***, ***P. verruculosa*** and ***M. leidyi*** were detected at relative high levels, and ***Cyprinus carpio, Oncorhyncus mykiss*** and ***P. cordatum*** at low indicative levels.

4. Aalborg

- Fish, standard methods: No non-indigenous fish species were caught in Alborg Harbour using standard fishing methods. In conclusion, based on the extent of this effort, there are no inon-indigenous fish species present.
- Snorkelling: Seven fish species were oberserved by snorkelling and two additional species were caught with push net. No indication of permanent presence of non-indigenous fish

species. Four species of non-indigenous invertebrates (***Crassostrea gigas***, ***Crepidula fornicata***, ***Mnemiopsis leidyi*** and ***Styela clava***) were observed and photographed.

- eDNA: In the spring, eDNA from ***Mnemiopsis leidyi*** and ***Prorocentrum cordatum*** were detected at relative high levels, and ***Pseudochattonella verruculosa*** were detected at low indicative levels. In the autumn, eDNA from ***M. leidyi*** were detected at relative high levels, and eDNA from ***Mya arenaria***, ***Oncorhyncus mykiss***, and ***Prorocentrum cordatum*** at low levels.

5. Fredericia

- Fish, standard methods: No non-indigenous fish species were caught in Fredericia industrihavn or Fredericia lystbådehavn. In conclusion, based on the extent of this effort, there are no non-indigenous fish species present in Fredericia.
- Snorkelling: Nineteen fish species were observed or caught during two snokel events. No non-indigenous fish species were observed, so a permanent presence was not indicated. ***Mnemiopsis leidyi*** was observed in the autumn.
- eDNA: In the spring, eDNA from ***Mnemiopsis leidyi***, ***Crassostrea gigas*** and ***Pseudochattonella verruculosa*** were traced at low indicative levels. In the autumn eDNA from ***M. leidyi*** were detected at relative high levels, and eDNA from ***C. gigas***, ***Karenia mikimotoi***, ***Pseudochattonella verruculosa*** and ***Prorocentrum cordatum*** at low indicative levels.

6. Frederikshavn

- Fish, standard methods: No non-indigenous fish species were caught in Frederikshavn industrihavn or Frederikshavn marina. In conclusion, based on the extent of this effort, there are no indigenous fish species present.
- Snorkelling: Nine fish species were observed or caught during one snokel event. No non-indigenous fish species were observed, so a permanent presence was not indicated. ***Mnemiopsis leidyi*** was observed in the autumn.
- eDNA: In the spring, eDNA from ***Pseudochattonella farcimen*** were traced at relatively high indicative levels, and for ***Karenia mikimotoi***, ***Pseudochattonella verruculosa*** and ***Mnemiopsis leidyi*** at relative low levels. In the autumn eDNA from ***Mya arenaria*** and ***P. farcimen*** were detected at relative high levels, and eDNA from ***Colpomenia peregrina***, ***Rhithropanopeus harrisii*** and ***Prorocentrum cordatum*** at low indicative levels.

7. Gedser

- Fish, standard methods: The invasive ***Neogobius melanostomus*** was caught in Gedser fiskerihavn and Gedser lystbådehavn.
- Snorkelling: Thirteen fish species were observed during one snokel event. No non-indigenous fish species were observed, so a permanent presence was not indicated. ***Mnemiopsis leidyi*** was observed in the autumn.
- eDNA: In the spring, eDNA from ***Mnemiopsis leidyi***, ***Neogobius melanostomus*** and ***Prorocentrum cordatum*** were detected at low indicative levels, and from ***Mya arenaria*** at relative high levels. In the autumn eDNA from ***M. leidyi***, ***M. arenaria*** and ***Prorocentrum cordatum*** were found at relative high levels, and eDNA from ***N. melanostomus*** at low indicative levels. The eDNA for ***N. melanostomus*** thus correlate to observations made during snorkelling.

8. Grenå

- Fish, standard methods: No non-indigenous fish species were caught in Grenå industrihavn, Grenå fiskerihavn or Grenå marina.
- Snorkelling: Twentytwo species of fish were observed during two snokel events. No non-indigenous fish species were observed. ***Mnemiopsis leidyi*** was observed in the autumn, and ***Sargassum muticum*** is common in the harbour.

- eDNA: In the spring, eDNA from ***Bonnemaisonia hamifera***, ***Mya arenaria*** and ***Prorocentrum cordatum*** were detected at low indicative levels, and from ***Pseudochattonella farcimen*** at relative high levels. In the autumn eDNA from ***B. hamifera***, ***M. arenaria***, ***P. farcimen*** and ***Mnemiopsis leidyi*** were detected at relative high levels, and eDNA from ***Crassostrea gigas***, ***Karenia mikimotoi*** and ***Prorocentrum cordatum*** at low indicative levels.

9. Helsingør

- Fish, standard methods: No non-indigenous fish species were caught in Helsingør industry-havn, Helsingør fiskerihavn or Helsingør lystbådehavn. In conclusion, based on the extent of this effort, there are no non-indigenous fish species present in Helsingør Harbour.
- Snorkelling: A total of 28 fish were observed during five snorkel events. No non-indigenous fish species were observed. Three species of non-indenous invertebrate were observed (***Ensis directus***, ***Mnemiopsis leidyi*** and ***Crassostrea gigas***).
- eDNA: In the spring, eDNA from ***Prorocentrum cordatum*** and ***Pseudochattonella farcimen*** were detected at relative high levels. In the autumn eDNA from ***Pseudochattonella farcimen*** ***Prorocentrum cordatum*** and ***Mnemiopsis leidyi*** were detected at relative high levels, and eDNA from ***Colpomenia peregrina*** at low indicative levels. The eDNA for ***M. leidyi*** thus correlate to observations made during snorkelling.

10. Hirtshals

- Fish, standard methods: No invasive fish species were caught in Hirtshals industrihavn, Hirtshals fiskerihavn or Hirtshals lystbådehavn. In conclusion, based on the extent of this effort, there are no non-indigenous fish species present in Hirtshals Harbour.
- Snorkelling: Eight species of fish were caught with push net (snorkelling not possible). No permanent presence of non-indigenous fish species and invertebrates was indicated.
- eDNA: In the spring, eDNA from ***Mnemiopsis leidyi*** was detected at relative high levels, and ***Pseudochattonella verruculosa*** at relative low indicative levels. In the autumn, eDNA from ***P. verruculosa*** and ***M. leidyi*** were detected at relative high levels, and eDNA from ***Pseudochattonella farcimen*** and ***Oncorhynchus mykiss*** at low indicative levels.

11. Kalundborg

- Fish, standard methods: No non-indigenous fish species were caught in Kalundborg industrihavn, Kalundborg marina Vesthavnen or Kalundborg Gisseløre lystbådehavn.
- Snorkelling: Fourteen species of fish were observed during snorkelling. The ***Neogobius melanostomus*** was abundant indicating a permanent presense in the harbour and a new front zone for the dispersal of the species along the Zealand coastline. ***Crassostrea gigas*** and ***Mnemiopsis leidyi*** were also observed.
- eDNA: In the spring, eDNA from ***Mya arenaria, Pseudochattonella verruculosa***, ***Pseudochattonella farcimen*** and ***Prorocentrum cordatum*** was detected at relative high levels, and ***Neogobius melanostomus***, ***Colpomenia peregrina*** and ***Oncorhynchus mykiss*** at relative low indicative levels. In the autumn, eDNA from ***Pseudochattonella farcimen*** and ***Mnemiopsis leidyi*** were detected at relative high levels, and eDNA from ***Neogobius melanostomus***, ***Colpomenia peregrina*** and ***Cyprinus carpio*** at low indicative levels. The eDNA for ***M. leidyi*** thus correlate to observations made during snorkelling.

12. København

- Fish, standard methods: No non-indigenous fish species were caught in Københavns industrihavn, Københavns fiskerihavn or Københavns lystbådehavn.
- Snorkelling: Sixteen species of fish were observed during snorkelling. ***Neogobius melanostomus*** was found in low abundance, but the observation indicates a permanent presense in the

harbour and a new front zone for the dispersal of the species along the Zealand coastline. ***Crassostrea gigas*** and ***Mnemiopsis leidyi*** were also observed.

- eDNA: In the spring, eDNA from ***Mya arenaria*** and ***Pseudochattonella farcimen*** were detected at relative high levels, and ***Prorocentrum cordatum*** at relative low indicative levels. In the autumn, eDNA from ***Pseudochattonella farcimen***, ***Prorocentrum cordatum*** and ***Mnemiopsis leidyi*** were detected at relative high levels, and eDNA from ***Crassostrea gigas***, ***Colpomenia peregrina*** and ***Rhithropanopeus harrisii*** at low indicative levels. Detection of eDNA from ***C. gigas*** and ***M. leidyi*** correlates with the observations made during snorkelling.

13. Køge

- Fish, standard methods: The invasive ***Neogobius melanostomus*** was caught in Køge industrihavn and Køge marina.
- Snorkelling: 11 species of fish were observed during snorkelling. ***Neogobius melanostomus*** was abundant confirming a permanent presense in the harbour. ***Mnemiopsis leidyi*** was also observed.
- eDNA: In the spring, eDNA from ***Prorocentrum cordatum*** and ***Pseudochattonella farcimen*** were detected at relative high levels, and ***Neogobius melanostomus*** and ***Rhithropanopeus harrisii*** at relative low indicative levels. In the autumn, eDNA from ***Prorocentrum cordatum*** was detected at relative high levels, and eDNA from *Mnemiopsis leidya*, ***Neogobius melanostomus*** and ***Rhithropanopeus harrisii*** at low indicative levels. Detection of eDNA from ***N. melanostomus*** and ***M. leidyi*** supports the observations made during snorkelling.

14. Odense

- Fish, standard methods: No non-indigenous fish species were caught in Odense industrihavn or Odense lystbådehavn.
- Snorkelling: 10 species of fish were observed during snorkelling. No non-indigenous fish species were observed.
- eDNA: In the spring, eDNA from ***Mnemiopsis leidyi*** were detected at relative high levels, and ***Prorocentrum cordatum***, ***Pseudochattonella farcimen*** and ***Rhithropanopeus harrisii*** at relative low indicative levels. In the autumn, eDNA from ***Bonnemaisonia hamifera***, ***Karenia mikimotoi***, ***Prorocentrum cordatum***, and ***Pseudochattonella farcimen*** and ***Pseudochattonella verrucolosa*** was detected at relative high levels, and eDNA from ***Mnemiopsis leidyi*** and ***Mya arenaria*** at low indicative levels.

15. Rødby

- Fish, standard methods: No non-indigenous fish species were caught in Rødby Industrihavn or Rødby Fiskerihavn. However, this result is a false negative (see eDNA results below).
- Snorkelling: Thirteen species of fish were observed during snorkelling. ***Neogobius melanostomus*** was abundant confirming a permanent presense in the harbour. ***Mnemiopsis leidyi*** was also observed.
- eDNA: In the spring, eDNA from ***Mnemiopsis leidyi*** and ***Pseudochattonella farcimen*** were detected at relative high levels, and ***Mya arenaria*** at relative low indicative levels. In the autumn, eDNA from ***Bonnemaisonia hamifera***, ***Mnemiopsis leidy, Prorocentrum cordatum*** was detected at relative high levels, and eDNA from ***Neogobius melanostomus, Pseudochattonella farcimen*** and ***Mya arenaria*** at low indicative levels. The eDNA from ***M. leidyi*** and ***N. melanostomus*** supports the observations made during snorkelling.

16. Statoil (Kalundborg)

- Fish, standard methods: No non-indigenous fish species were caught in Kalundborg Statoil using standard fishing methods.

- Snorkelling: 15 species of fish were observed during snorkelling. ***Neogobius melanostomus*** was found in high abundance, indicating a permanent presense in the harbour and a new front zone for the dispersal of the species along the Zealand coastline. ***Mnemiopsis leidyi*** was also observed.
- eDNA: In the spring, eDNA from ***Mya arenaria*** and ***Pseudochattonella farcimen*** were detected at relative high levels, and ***Colpomenia peregrina, Prorocentrum cordatum*** and ***Mnemiopsis leidyi*** at relative low indicative levels. In the autumn, eDNA from ***Colpomenia peregrina***, ***M. leidyi, P. farcimen*** were detected at relative high levels, and eDNA from ***Cyprinus carpio***, ***Neogobius melanostomus***, ***P. cordatum*** and ***Oncorhynchus mykiss*** at low indicative levels.

With this baseline study, we have documented the occurence of non-indigenous marine species in 16 selected Danish harbours. The results presented harbour by harbour above, are synthesised and presented in **Table 6.1** (conventional sampling) and **Table 6.2** (eDNA-based) on the following pages.

26 non-indigenous species were recorded using conventional sampling and 13 species were recorded using eDNA-based methods. Excluding overlapping records, we have recorded a total of 34 non-indigenous species in the 16 Danish harbours studied.

The study represents a leap forward. Not only is the occurrence of non-indigenopus species in Danish harbous systematically documented for the first time ever, we also provided a proof of concept with regard to eDNA-based monitoring of non-indigenous marine species in Danish marine water. Hence, we suggest the following next steps:

1. As a first step, the eDNA-based monitoring of non-indigenous species cf. the EU Marine Strategy Framework Directive in Danish marine water can now be executed and operationalized as planned.
2. As a second sted, relevant international conventions such as HELCOM and OSPAR should be informed about the progress made and also invited to collaborate on development of relevant species-specific operational test systems.
3. Finally, as a third step, a critical evaluation of the internationally coordinated and agreed joint monitoring protocols should be carried out in collaboration with neighbouring countries in order to focus future activities and to increase cost-effectiveness of monitoring protocols.

Further, we suggest that similar studies could be considered in other Danish harbours as well as in relevant hot spot areas such as the Wadden Sea and Limfjorden.

And on a final note, we would like to highlight that the results presented in this report have been reported in various ways, e.g. in the Symposium Proceedings from the 1st GEF-UNDP-IMO GloFouling R&D Forum, in the NISAR project report and in a scientific journal:

- Andersen, J.H., E. Kallenbach, M.B. Kjeldgaard, S.W. Knudsen, W. Eikrem, C. Fagerli, E. Oug, T. Dahle, J. Thaulow, J. Gitmark, A. Hobæk, N. Green, M. Hesselsøe, J. Støttrup, J. Kuhn, D. Bekkevold, L.M.W. Jacobsen, P.R. Møller, C.Aa. Olesen, H. Carl & F. Stuer-Lauridsen (2020): Occurrence of non-indigenous species in Danish harbours. GEF-UNDP-IMO GloFouling Proceedings: 31-35.
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Table 6.1: *Synthesis of findings in the 16 Danish harbours studied based on conventional sampling methods. A total of 26 species were identified based on conventional monitoring and observations. Positive recordings are marked with an 'X'.*

Harbour	Planktonic organisms						Benthic communities (vegetation and invertebrates)																			Fish
	Pseudochattonella cf. *verruculosa*	Cf. *Heterosigma akashiwo*	Cf. *Karenia mikimotoi*	*Prorocentrum* cf. *cordatum*	*Acartia tonsa*	*Penilia avirostris*	*Mnemiopsis leidyi*	*Sargassum muticum*	*Alitta succinea*	*Polydora aggregata*	*Diadumene lineata*	*Molgula manhattensis*	*Amphibalanus improvisus*	*Austrominius modestus*	*Caprella mutica*	*Neosiphonia harveyi*	*Hemigrapsus sanguineus*	*Streblospio benedicti*	*Eteone heteropoda*	*Polydora cornuta*	*Ensis directus*	*Styela clava*	*Heterosiphonia japonica*	*Crepidula fornicata*	*Crassostrea gigas*	*Neogobius melanostomus*
1. Aarhus	X	X	-	-	X	X	X	X	X	X	X	X	X	X	X	-	X	-	-	-	X	-	X	-	-	-
2. Esbjerg	-	-	X	X	X	-	X	-	X	-	X	X	X	X	X	X	-	X	X	X	X	X	-	X	X	-
3. Aalborg Havn	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	X	X	-
4. Aalborg Portland	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	X	X	-
5. Fredericia	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6. Frederikshavn	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7. Gedser	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
8. Grenå	-	-	-	-	-	-	X	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9. Helsingør	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	X	-	-	-	X	-
10. Hirtshals	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11. Kalundborg	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		X	X
12. København	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X	X
13. Køge	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
14. Odense	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15. Rødby	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X
16. Statoil (Kalundborg)	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	X

Table 6.2: *Synthesis of findings in the 16 Danish harbours studied based on biomolecular methods (eDNA). The values scored represent the eDNA findings over both spring and fall 2017 together. The values scored are the highest eDNA signal reported over both spring and fall. No eDNA signal (No Ct) (0), a relatively low eDNA signal (below LOQ) (1), and a relatively high eDNA signal (at least one technical replicate above LOQ) (2).*

Harbour	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**09A**	**10**	**13**	**14**	**15**	**16**	**18**	**21**	**22**	**23**
	Bonnemaisonia hamifera	***Prorocentrum cordatum***	***Pseudochattonella farcimen***	***Pseudochattonella verruculosa***	***Karenia mikimotoi***	***Carassius auratus***	***Cyprinus carpio***	***Colpomenia peregrina***	***Neogobius melanostomus***	***Oncorhynchus mykiss***	***Oncorhyncus gorbuscha***	***Crassostrea gigas***	***Mya arenaria***	***Rhithropanopeus harrisii***	***Eriocheir sinensis***	***Cordylophora caspia***	***Mnemiopsis leidyi***	***Acipenser baerii***
1. Aarhus	2	1	2	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0
2. Esbjerg	0	1	0	2	1	0	0	0	0	0	0	1	0	0	0	0	2	0
3. Aalborg Havn	0	2	2	2	2	0	0	0	0	1	0	0	1	0	0	0	2	0
4. Aalborg Portland	0	2	2	2	2	0	1	0	0	1	0	0	2	0	0	0	2	0
5. Fredericia	0	1	2	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0
6. Frederikshavn	0	1	2	1	1	0	0	1	0	1	0	0	2	1	0	0	1	0
7. Gedser	0	2	2	0	0	0	0	0	1	0	0	0	2	0	0	0	2	0
8. Grenå	2	1	2	0	1	0	0	0	0	0	0	1	2	0	0	0	2	0
9. Helsingør	0	2	2	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0
10. Hirtshals	0	0	1	2	0	0	0	0	0	1	0	0	0	0	0	0	2	0
11. Kalundborg	0	2	2	0	0	0	1	1	1	1	0	0	2	0	0	0	2	0
12. København	0	2	2	0	0	0	0	1	0	0	0	1	2	1	0	0	2	0
13. Køge	0	2	2	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0
14. Odense	2	2	2	2	2	0	0	0	0	0	0	0	2	1	0	0	1	0
15. Rødby	2	2	2	0	0	0	0	0	1	0	0	0	2	0	0	0	2	0
16. Statoil (Kalundborg)	0	1	2	0	0	0	1	2	1	1	0	0	2	0	0	0	2	0

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Annex 1: Grab samples

A1.1: *Catch in grab samples at the stations in Aarhus. Table entries show number of specimens. (+) = recorded, not counted.*

van Veen grab	**AB1**	**AB2**	**AB3**	**AB4**	**AB5**	**AB6**	**AB7**	**AB8**	**AB9**
Foraminifera	-	-	-	-	-	-	+	-	-
Anthozoa	-	-	-	-	-	-	2	1	-
Nematoda	412	5	23	-	-	-	-	37	1
Oligochaeta	-	-	-	-	-	-	163	251	64
Tubificoides benedi	-	-	-	-	-	1	-	-	-
Tubificoides sp	-	-	-	1	-	-	-	-	-
Polychaeta									
Eteone longa/flava	-	-	-	-	-	-	1	1	-
Nephtys pente	-	-	-	-	-	-	1	-	-
Hediste diversicolor	-	-	-	-	-	-	-	1	-
Alitta virens	-	-	-	-	-	-	7	2	1
Alitta succinea	1	-	-	-	-	-	6	-	-
Scoloplos armiger	-	-	-	-	-	-	1	5	5
Aricidea suecica	-	-	-	-	-	-	1	2	8
Prionospio fallax	-	-	-	-	-	-	-	4	4
Prionospio cirrifera	-	-	-	-	-	-	1	-	-
Polydora cf aggregata	-	-	-	835	1	1	4	-	-
Malacoceros fuliginosus	1	-	-	-	-	-	-	1	-
Capitella sp	-	-	-	-	-	-	-	6	-
Mediomastus fragilis	-	-	-	-	-	-	46	47	7
Heteromastus filiformis	-	-	-	-	-	-	-	-	1
Chaetozone sp	-	-	-	-	-	-	11	5	26
Pectinaria koreni	-	-	-	-	-	-	-	-	1
Gastropoda									
Hinia reticulata	-	3	2	-	-	-	-	1	-
Philine sp	-	-	-	-	-	-	-	-	3
Egg capsules	-	-	6	-	78	1	-	-	-
Eggs, in sticky clumps	-	-	-	-	300	-	-	-	-
Bivalvia									
Cerastoderma glaucum	-	-	-	-	-	-	1	1	-
Spisula subtruncata	-	-	-	-	-	-	-	1	-
Kurtiella bidentate	-	-	-	-	-	-	-	-	1
Mytilus edulis juv	-	-	-	-	-	-	1	-	-
Barnea candida	-	-	-	-	-	-	1	-	-
Abra prismatica	-	-	-	-	-	-	3	-	-
Phaxas pellucidus	-	-	-	-	-	-	-	1	5
Bivalvia indet juv	-	-	-	-	-	-	-	1	-
Crustacea									
Balanus cf crenatus	-	-	-	-	3	-	-	-	-
Carcinus maenas	-	-	-	-	1	-	5	-	1
Monocorophium insiduosum	1	-	-	-	-	-	-	-	-
Phoronida									
Phoronis muelleri	-	-	-	-	-	-	-	-	1

A1.2: *Catch in grab samples at the stations in Esbjerg. Table entries show number of specimens. (+) = recorded, not counted.*

van Veen grab	TR1	TR2	TR3	E3	EB4	EB5	ØS1	ØS2	ØS3
Foraminifera	-	-	-	-	+	-	-	-	-
Porifera	-	-	-	-	-	+	+++	-	-
Anthozoa	-	-	-	5	1	1	-	-	-
Edwardsia sp	-	-	9	-	-	-	-	-	-
Nemertea	-	-	-	-	2	-	-	-	-
Nematoda	5	-	-	-	26	-	-	-	-
Oligochaeta	2	5	27	16	-	-	-	-	-
Tubificoides benedi	-	1	-	6	2	1	1	-	-
Tubificoides sp	-	-	-	-	-	31	7	1	-
Polychaeta									
Phyllodoce mucosa	-	-	-	-	6	-	-	-	-
Eteone heteropoda	-	1	-	20	11	1	2	-	-
Eumida sp	-	-	-	-	2	-	-	-	-
Alitta succinea	-	-	-	-	5	-	-	-	-
Nephtys hombergi	3	2	10	3	-	2	1	-	1
Polydora cornuta	11	5	19	9	318	2	7	-	-
Streblospio benedictii	110	50	49	524	241	75	61	41	8
Capitella capitata	-	1	-	-	-	-	-	-	-
Tharyx sp	75	623	392	1087	66	195	19	3	2
Gastropoda	-	-	-	-	-	-	-	-	-
Hydrobia ulvae	550	127	1343	15	-	-	9	22	66
Philine sp	-	-	-	1	-	-	-	-	-
Bivalvia									
Astarte sp juv	-	-	-	2	-	-	2	-	-
Cardiidae indet juv	-	-	266	1	-	-	-	-	-
Cerastoderma edule	10	-	-	-	-	-	-	-	-
Cerastoderma glaucum	-	13	116	-	-	-	-	-	-
Laevicardium crassum	1	-	-	-	-	-	-	-	-
Lasaeidae indet juv	-	-	-	-	-	1	-	-	1
Tellimya ferruginosa	-	-	-	-	1	-	-	-	-
Spisula subtruncata	-	-	7	2	-	-	4	-	-
Kurtiella bidentata	-	-	-	2	2	-	-	-	-
Mya truncata	-	-	-	4	-	-	6	-	-
Mytilidae indet juv	-	-	3	-	-	-	-	-	-
Modiolus modiolus juv	-	-	-	-	-	-	4	4	3
Mytilus edulis juv	-	3	-	1	-	-	-	-	-
Gari tellinella	-	-	1	-	-	-	-	-	-
Abra sp juv	-	-	-	-	-	-	-	1	-
Abra alba	-	-	-	-	-	1	1	-	-
Abra prismatica	1	4	33	5	-	-	-	-	-
Solenidae indet juv	1	-	2	14	3	-	8	-	-
Phaxas pellucidus	-	1	-	-	2	-	-	-	-
Ensis sp juv	-	1	-	-	-	-	-	-	-
Tellina tenuis	-	-	3	-	-	-	-	-	-
Bivalvia indet juv	9	1	43	9	-	-	2	-	-
Crustacea									
Austrominius modestus	1	-	-	-	-	-	-	-	-
Carcinus maenas	-	-	-	-	1	-	-	-	-
Parambius typicus	-	-	3	1	1	-	1	1	-
Monocorophium acherusicum	-	-	-	2	6	1	5	-	-
Monocorophium insidiosum	-	-	-	-	2	-	31	-	-
Corophium volutator	-	-	-	-	17	-	-	-	-
Dulichia monacantha	-	-	-	-	6	-	-	-	-
Gitana sarsi	-	-	-	-	-	-	-	-	3
Amphipoda indet	-	-	-	2	5	-	2	-	-
Leucon acutirostris	-	-	-	5	-	1	2	2	3
Bodotria scorpioides	-	-	-	-	14	2	-	-	-
Echinodermata									
Ophiura sp	1	-	-	-	-	-	-	-	-
Ascidiacea									
Ascidiella aspersa	6	-	-	-	-	-	-	-	-

Annex 2: Sediment infauna (grab) and epifauna (dredge) – background data

Data for collection of sediment infauna by hand-operated van Veen grab in Aarhus and Esbjerg harbours; GPS positions (WGS 84), date and time (hours), water depth, and visual observations.

Stn	Coordinates	Time (hours)	Depth m	Grab catch/ Corer	Visual observations
Aarhus		5 Oct 2017			
AB1	56,1546 10,2165	12:40 - 12:50	8.5	G: Full C: 25 cm	Dark grey/black soft mud with thin light brown top layer. Smell of H_2S. Patches of oil.
AB2	56,1666 10,2177	12:20 - 12:35	8.7 – 10.1	G: Full C: 30 cm	Dark grey/black soft mud with thin light brown top layer. Smell of H_2S. Patches of oil.
AB3	56,1560 10,2186	12:00 – 12:15	9.6	G: Full C: 25 cm	Dark grey/black soft mud with thin light brown top layer. Smell of H_2S. Patches of oil.
AB4	56,1437 10,2199	16:05 – 16:15	15	G: 1 /4	Sand and stones with some mud, dark grey. Corer unsuccessful
AB5	56,1485 10,2243	15:35 – 15:45	11.8	G: Full C: 30 cm	Dark grey mud. Faint smell of H_2S
AB6	56,1463 10,2231	15:55 – 16:00	14.3	G: Full C: 25 cm	Dark grey mud. Faint smell of H_2S
AB7	56,1527 10,2206	18:25 – 18:40	9.6 – 10.1	G. 1 /4	Grey sand and mud with gravel. Corer unsuccessful
AB8	56,1536 10,2202	18:44 – 18:55	10.3 – 11.4	G: 3 /4 C: 13 cm	Grey sandmixed mud
AB9	56,1551 10,2225	19:00 – 19:10	11	G: Full C: 18 cm	Grey mud with thin brown surface layer
Esbjerg		21 Sept / 6 Oct (E3, EB4, EB5) 2017			
TR1	55,4725 08,4261	12:20	7	G: 3 /4 C: 33 cm	Dark brown mud with thin light brown surface layer. Faint smell of H_2S.
TR2	55,4702 08,4270	14:00	10.5 – 12.8	G: 3 /4 C: 41 cm	Dark brown mud with 0.5-1 cm light brown surface layer. Faint smell of H_2S.
TR3	55,4694 08,4311	14:10	11.2	G: 3 /4 C: 23 cm	Dark brown mud with 0.5 cm light brown surface layer.
E3	55,4603 08,4365	11:05 – 11:20	9	G: Full C: 40 cm	Grey silt with 2-4 cm greenish-brown surface layer. Corer grey to 18 cm, below darker with black stripes
EB4	55,4605 08,4386	11:35 – 11:50	11	G: 1 /2 C: 11 cm	Dark grey sticky clay with 2-4 cm brown surface layer. Tubes of bristle worms. Corer with black vertical stripes
EB5	55,4604 08,4471		10	G: Full C: 13 cm	Dark grey mud with 2-4 cm greenish-brown surface layer. Faint smell of H_2S. Corer with black vertical stripes.
ØS1	55,4490 08,4767	17:30	6.6	3 /4	Light brown mud. Corer unsuccessful
ØS2	55,4505 08,4768		8.9 – 9.4	G: 3 /4 - full	Brown mud with light brown surface. Corer unsuccessful
ØS3	55,4526 08,4776		11.2	G: Full	Dark brown consolidated mud and clay with clumps. Light brown surface layer. Corer unsuccessful

Sediment catch and data for workup of sediment samples with hand-operated van Veen grab in Aarhus and Esbjerg harbours; components in sieve residue and amount processed for samples that were subsampled.

Stn	Sieve residue	Sub-sampling	Comments
Aarhus			
AB1	Sand, shell fragments from molluscs and crustaceans. Debris of terrestrial plants and algae		
AB2	Sand and shell fragments. Debris of terrestrial plants and algae. Slag fragments.		
AB3	Sand and shell fragments. Empty shells of bivalves and snails. Debris of terrestrial plants and algae		
AB4	Shell fragments from molluscs and crustaceans. Some stiff clay. Debris of terrestrial plants		
AB5	Sand, shell fragments from crustaceans. A large amount of dead juvenile mytilids. Debris of terrestrial plants and algae		
AB6	Sand, shell fragments from Mytilus, sea urchins and crustaceans. Sawdust and terrestrial seeds (?) Debris of terrestrial plants and algae.		
AB7	Sand and gravel, shell fragments. Stiff clay and slag fragments. Debris of terrestrial plants	1 /2	Sieve residue > 2 liter. Subsampled to reduce time of processing.
AB8	Sand, gravel and pebbles, shell fragments. Stiff clay and slag fragments. Debris of terrestrial plants	1 /2	Sieve residue > 2 liter. Subsampled to reduce time of processing.
AB9	Sand, gravel and pebbles, shell fragments. Empty polychaete tubes. Some slag fragments. Debris of terrestrial plants.		
Esbjerg			
TR1	Sand and shell fragments. Debris of terrestrial plants and algae. Fragments of plastics.		
TR2	Sand and shell fragments. Debris of terrestrial plants and algae. Fragments of plastics.		
TR3	Sand and shell fragments. Debris of terrestrial plants. Red algae.		
E3	Sand, shell fragments from bivalves, snails and barnacles. Empty muddy polychaete tubes. Debris of terrestrial plants and algae.		
EB4	Sand and pebbles. Shell fragments from bivalves, snails and barnacles. Empty muddy tubes. Some slag fragments. Debris of terrestrial plants and algae.		Sieve residue > 2 liter
EB5	Sand and gravel. Shell fragments from bivalves, snails and barnacles. Empty muddy tubes. Debris of terrestrial plants and algae.		
ØS1	Sand and shell fragments. Debris of terrestrial plants and algae.		
ØS2	Sand and shell fragments. Debris of terrestrial plants and algae.		
ØS3	Sand and shell fragments. Debris of terrestrial plants and algae.		

Sediment catch and data for workup of sediment samples with light-weight dredge in Aarhus and Esbjerg harbours; components in sieve residue and amount processed for samples that were subsampled.

Stn	Sieve residue	Sub-sam-pling	Comments
Aarhus			
Basin 1	Shell fragments from barnacles and other crustaceans. Empty shells of blue mussels. Debris of terrestrial plants and algae		Very little material
Basin 4	Much sand and gravel, some pebbles. Shell fragments from blue mussels, other molluscs and barnacles. Slag fragments. Sticky oily clods. Debris of terrestrial plants.	1 /2	Sieve residue > 3 liter. All larger specimens sorted out, rest subsampled.
Basin 9	Shell fragments from bivalves and barnacles. Wood chippings and sawdust, much terrestrial seeds (?) Debris of terrestrial plants and algae. Some Zostera.		No animals.
Esbjerg			
Traffic port	Shell fragments from bivalves, snails and barnacles. Debris of terrestrial plants and algae.		
South port	Shell fragments from bivalves, snails, barnacles and other crustaceans. Seaweeds with encrusting bryozoans. Debris of terrestrial plants and algae.		
East port	Debris of terrestrial plants. Some green algae		Very little material

Total catch of animals in dredge samples in Aarhus and Esbjerg harbour areas. There were no living animals in the sample from Esbjerg Basin 9.

City	**Aarhus**			**Esbjerg**		
Area	**Basin 1**	**Basin 4**	**Basin 9**	**Traffic Port**	**Ferry/South Port**	**East Port**
Date	**5. Oct**	**5. Oct**	**5. Oct**	**21. Sept**	**6.Oct**	**21. Sept**
Porifera					++	
Anthozoa				2	27	
Nematoda	+			2	3	
Oligochaeta		24				
Tubificoides benedi		180				
Polychaeta						
Phyllodoce mucosa		2				
Eteone heteropoda				1	1	
Eteone longa/flava		4				
Autolytinae indet				1		
Nephtys hombergi		1		10	13	
Nephtys ciliata		1				
Nephtys pente		2				
Scoloplos armiger		52				
Aricidea suecica		10				
Aricidea cf *catherinae*		18				
Polydora cornuta				11		
Prionospio fallax		20				
Pseudopolydora pulchra				1		
Pygospio elegans				1		
Streblospio benedictii		10		68	43	5
Capitella/Capitomastus		18				
Mediomastus fragilis		130				
Notomastus latericeus				1		
Tharyx sp				904	97	
Chaetozone sp		152				
Pectinaria koreni		16				
Gastropoda						
Helcion pellucidum		8				
Hydrobia ulvae				10	4	8
Aporrhais pespelecani		1				
Hinia reticulata	27	41			1	
Philine sp		14				
Nudibranchia indet		2				
Egg capsules (species indet)	1					4
Bivalvia						
Bivalvia indet juv		2		3		
Cardiidae indet juv						1
Cerastoderma edule		4			5	
Cerastoderma glaucum				8		
Spisula subtruncata		64				
Mya arenaria		10				
Mytilus edulis juv		4		5	5	
Abra prismatica				2	1	
Solenidae indet juv		2				
Phaxas pellucidus		51				
Ensis directus		7			1	
Ensis sp juv				1		
Macoma balthica		2			1	
Corbula gibba		4				
Thracia sp *(villosiuscula/phaseolina)*		58			2	

Crustacea				
Parambius typicus				13
Melita sp		1		
Monocorophium acherusicum			2	
Amphipoda indet		4	2	
Diastylis bradyi		1		
Crangon crangon		42		1
Carcinus maenas		3	4	4
Pycnogonida				
Anoplodactylus cf *exiguus*				1
Echinodermata				
Asterias rubens		4		3
Psammechinus miliaris		1		
Ophiura ophiura			1	1
Ascidiacea				
Ascidia obliqua				13
Ciona intestinalis	5			
Eugyra arenosa			2	5
Styela clava				2

Annex 3: Phytoplankton

A2.1: Esbjerg Harbour, species per station

Eier_takson	Navn_cf	Esbjerg1 06_10_2017	Esbjerg1 20_06_2017	Esbjerg3 20_06_2017	Esbjerg 3 06_10_2017	Esbjerg Trafikkhavn w6 21_09_2017	Esbjerg7 20_06_2017
Diatoms	*Actinoptychus* spp.	X	X	X	X	X	X
Diatoms	*Asterionella* spp.	X			X		
Diatoms	*Bacteriastrum delicatulum*					X	
Diatoms	*Cerataulina pelagica*					X	
Diatoms	*Cerataulina* spp.	X			X		
Diatoms	cf. *Entomoneis* spp.	X			X	X	
Diatoms	cf. *Navicula vanhoeffenii*	X				X	
Diatoms	*Chaetoceos-Phaeceros* spp.		X				
Diatoms	*Chaetoceros affinis*					X	
Diatoms	*Chaetoceros* cf. *danicus*	X		X	X		X
Diatoms	*Chaetoceros* cf. *debilis*	X			X		
Diatoms	*Chaetoceros decipiens*					X	
Diatoms	*Chaetoceros* spp. <10 µm	X			X	X	X
Diatoms	*Chaetoceros* spp. 10-20 µm		X	X	X	X	
Diatoms	*Chaetoceros* spp. 20-40 µm	X					
Diatoms	*Chaetoceros tenuissimus*		X				
Diatoms	*Coscinodiscus* spp. 60-100 µm	X	X	X	X	X	
Diatoms	*Cylindrotheca closterium*	X	X		X	X	X
Diatoms	*Dactyliosolen fragilissimus*					X	
Diatoms	*Ditylum brightwellii*	X			X	X	X
Diatoms	*Eucampia zodiacus*	X			X		
Diatoms	*Fragilaria ulna*					X	
Diatoms	*Guinardia delicatula*	X			X	X	
Diatoms	*Guinardia flaccida*		X	X		X	X
Diatoms	*Gyrosigma/Pleurosigma*		X		X	X	X
Diatoms	*Lauderia annulata*						X
Diatoms	*Leptocylindrus minimus*	X			X		
Diatoms	*Licmophora* spp.						X
Diatoms	*Lithodesmium undulatum*	X	X	X			X
Diatoms	*Navicula* cf. *vanhoeffenii*						X
Diatoms	*Navicula* spp.				X		
Diatoms	*Navicula transitans*	X	X		X	X	
Diatoms	Odontella aurita			X			X
Diatoms	*Odontella* cf. *sinensis*		X				

Eier_takson	Navn_cf	Esbjerg1 06_10_2017	Esbjerg1 20_06_2017	Esbjerg3 20_06_2017	Esbjerg 3 06_10_2017	Esbjerg Trafikkhavn w6 21_09_2017	Esbjerg7 20_06_2017
Diatoms	*Odontella* spp.					X	
Diatoms	*Plagiogrammopsis* spp.	X				X	
Diatoms	*Pseudo-nitzschia seriata*-group		X	X		X	
Diatoms	*Pseudo-nitzschia* spp.	X			X		
Diatoms	*Rhizosolenia hebetata* f. *semispina*					X	
Diatoms	*Rhizosolenia pungens*		X	X			
Diatoms	*Rhizosolenia setigera*	X			X	X	
Diatoms	*Rhizosolenia* spp.	X		X	X		
Diatoms	*Rhizosolenia styliformis*		X	X		X	
Diatoms	*Skeletonema* spp.		X	X	X	X	
Diatoms	*Tabellaria flocculosa*					X	
Diatoms	*Thalassionema nitzschioides*	X			X	X	
Diatoms	*Thalassiosira* cf. *rotula*			X	X		
Diatoms	*Thalassiosira* spp.		X				
Diatoms	Unidentified diatoms	X			X		
Diatoms	Unidentified pennate diatoms <20 µm	X		X	X	X	
Diatoms	Unidentified pennate diatoms >150 µm			X			
Diatoms	Unidentified pennate diatoms 20-50 µm	X	X	X	X		
Diatoms	Unidentified pennate diatoms 50-100 µm		X	X			
Diatoms	Unidentified sentriske diatoms >300 µm				X		
Diatoms	Unidentifiedsentriske diatoms 100-150 µm					X	
Diatoms	Unidentified sentriske diatoms 20-40 µm	X		X	X	X	
Diatoms	Unidentified sentriske diatoms 5-10 µm	X			X		
Diatoms	Unidentified sentriske diatoms 60-80 µm	X				X	
DICTYOCHOPHYTES	*Dictyocha speculum*	X		X	X	X	
DICTYOCHOPHYTES	cf. *Vicicitus globosus*	X					
DINOFLAGELLATES	cf. *Alexandrium pseudogoniaulax*		X	X			
DINOFLAGELLATES	cf. *Diplopelta bomba*		X	X			
DINOFLAGELLATES	*Cochlodinium* spp.					X	
DINOFLAGELLATES	Dinoflagellate cysts			X		X	
DINOFLAGELLATES	*Dinophysis* spp.					X	
DINOFLAGELLATES	cf. *Diplopsalis* spp.		X	X			
DINOFLAGELLATES	*Gyrodinium fusiforme*		X	X			
DINOFLAGELLATES	*Gyrodinium/Gymnodinium* 20-40 µm		X	X			
DINOFLAGELLATES	*Gyrodinium/Gymnodinium* 60-80 µm					X	
DINOFLAGELLATES	*Heterocapsa rotundata*					X	
DINOFLAGELLATES	*Noctiluca scintillans*		X	X			
DINOFLAGELLATES	*Oblea* spp.		X				
DINOFLAGELLATES	*Prorocentrum* cf. *cordatum*					X	
DINOFLAGELLATES	*Prorocentrum micans*	X				X	

Eier_takson	Navn_cf	Esbjerg1 06_10_2017	Esbjerg1 20_06_2017	Esbjerg3 20_06_2017	Esbjerg 3 06_10_2017	Esbjerg Trafikkhavn w6 21_09_2017	Esbjerg7 20_06_2017
DINOFLAGELLATES	*Prorocentrum* spp.					X	
DINOFLAGELLATES	*Prorocentrum triestinum*					X	
DINOFLAGELLATES	*Protoperidinium bipes*		X	X			
DINOFLAGELLATES	*Protoperidinium brevipes*		X			X	
DINOFLAGELLATES	*Protoperidinium* cf. *marielebourae*			X			
DINOFLAGELLATES	*Protoperidinium oblongum*					X	
DINOFLAGELLATES	*Protoperidinium steinii*			X	X		
DINOFLAGELLATES	*Scrippsiella* cf. *trochoidea*		X	X			
DINOFLAGELLATES	*Scrippsiella*-group	X	X	X	X		
DINOFLAGELLATES	*Tripos furca*				X	X	
DINOFLAGELLATES	*Tripos fusus*			X			
DINOFLAGELLATES	*Tripos lineatus*				X	X	
DINOFLAGELLATES	*Tripos macroceros*			X			
DINOFLAGELLATES	*Tripos muelleri*			X		X	
DINOFLAGELLATES	*Tripos* spp.	X				X	
DINOFLAGELLATES	Athecate dinoflaellates < 20 µm				X	X	
DINOFLAGELLATES	Athecate dinoflagellates 20-40 µm		X			X	
DINOFLAGELLATES	Thecate dinoflagellates 20-40 µm		X	X			
DINOFLAGELLATES	Thecate dinoflagellates 40-60 µm			X		X	
PRASINOPHYTES	*Pterosperma* spp.			X			
PRASINOPHYTES	*Pyramimonas* spp. 10-15 µm					X	
PRASINOPHYTES	*Pyramimonas* spp. 5-10 µm	X			X		
CHRYSOPHYTES	*Dinobryon* cf. *balticum*		X				
CHRYSOPHYTES	*Dinobryon* cf. *faculiferum*		X				
CRYPTOPHYTES	cf. *Plagioselmis* spp.		X				
CRYPTOPHYTES	cf. *Teleaulax* spp.		X			X	
CRYPTOPHYTES	Cryptophytes 10-15 µm				X		
CRYPTOPHYTES	Cryptophytes 5-10 µm					X	
CRYPTOPHYTES	Cryptophytes >15 µm					X	
EUGLENOIDS	*Eutreptiella* spp.		X				
EUGLENOIDS	*Eutreptiella* 30-60 µm					X	
UNCLASSIFIED	Flagellates 5-10 µm	X				X	
UNCLASSIFIED	Monads 5-10 µm	X			X		
UNCLASSIFIED	Monads 10-15 µm	X					
UNCLASSIFIED	Flagellates <5 µm				X	X	
CYANOPHYTES	cf. *Pseudanabaena* spp.					X	
CYANOPHYTES	*Oscillatoria/Phormidium*						X

A1.2: Esbjerg Harbour, cells per liter

		Esbjerg1 20_06_2017	**Esbjerg3 06_10_2017**	**Esbjerg3 20_06_2017**	**Esbjerg Trafikkhavn w6 21_09_2017**	**Esbjerg1 06_10_2017**	**Esbjerg7 20_06_2017**
		20-06-2017	**06-10-2017**	**20-06-2017**	**21-09-2017**	**06-10-2017**	**20-06-2017**
Cyanophyta							
	cf. Pseudanabaena sp.	.	.	.	3 204	.	.
	Oscillatoria/Phormidium						160
	Sum - Cyanophyta	0	0	0	3 204	0	0
Cryptophyta							
	cf. Plagioselmis spp.	54 475	.	.	.	.	12 818
	cf. Teleaulax spp.	48 066	.	.	102 542	.	48 066
	Unidentified cryptophytes 5-10 µm	.	.	.	105 746	.	.
	Unidentified cryptophytes 10-15 µm	.	.	17 700	.	.	.
	Unidentified cryptophytes >15 µm	.	.	.	6 409	.	.
	Sum - Cryptophyta	102 542	0	17 700	214 696	0	60 884
Dinoflagellates							
	Cochlodinium spp.	.	.	.	801	.	.
	Dinophysis acuminata	.	.	.	.	.	40
	Dinophysis spp.	.	.	.	80	.	.
	cf. Diplopsalis spp.	160	.	.	.	.	320
	cf. Diplopelta bomba	80	.	.	.	.	6 409
	Gyrodinium fusiforme	160	240	.	.	.	400
	Gyrodinium/Gymnodinium 20-40 µm	160	800	.	.	.	.
	Gyrodinium/Gymnodinium 40-60 µm	.	.	.	.	.	480
	Gyrodinium/Gymnodinium 60-80 µm	.	.	.	80	.	.
	Heterocapsa rotundata	.	.	.	3 204	.	.
	cf. Karenia mikimotoi	.	.	.	.	.	80
	Noctiluca scintillans	.	160	.	.	.	40

		Esbjerg1 20_06_2017	Esbjerg3 06_10_2017	Esbjerg3 20_06_2017	Esbjerg Trafikkhavn w6 21_09_2017	Esbjerg1 06_10_2017	Esbjerg7 20_06_2017
	Oblea spp.	160	.	.	.	.	.
	Prorocentrum micans	.	.	.	320	.	.
	Prorocentrum cf. cordatum	.	.	.	5 608	.	.
	Prorocentrum triestinum	.	.	.	3 204	.	.
	Prorocentrum spp.	.	.	.	801	.	.
	Protoperidinium bipes	160	80	.	.	.	.
	Protoperidinium brevipes	80	.	.	.	.	80
	Protoperidinium cf. marielebourae	.	.	.	.	.	40
	Pyrocystis noctiluca		.	80	.	.	240
	Scrippsiella cf. trochoidea	640	1 520	.	.	.	480
	Scrippsiella-gruppen	480	960	.	.	.	240
	Tripos furca	.	.	.	320	.	.
	Tripos lineatus	.	.	.	80	.	.
	Tripos macroceros	.	.	.	.	.	40
	Tripos spp.	.	.	.	40	.	.
	Unidentified dinoflagellates <20 µm	.	.	.	801	.	.
	Unidentified athecate dinoflagellates 20-40 µm	80	.	.	801	.	80
	Unidentified thecate dinoflagellates 20-40 µm	1 920	640	.	.	.	960
	Unidentified thecate dinoflagellates 40-60 µm	.	.	.	801	.	.
	Dinoflagellate cystes	.	.	.	3 204	.	80
	Sum - Dinoflagellates	4 080	4 400	80	20 147	0	10 009
Haptophytes							
	cf. Corymbellus aureus		960				
	cf. Ophiaster hydroideus	.	.	.	.	.	3 204
	Sum - Haptophytes	0	960	0	0	0	3 204
Chrysophytes							
	Dinobryon cf. balticum	12 818	.	.	.	.	35 249
	Dinobryon cf. faculiferum	6 409	.	.	.	.	.
	cf. Meringosphaera tenerima		13 080				3 270
	Sum - Chrysophytes	19 227	13 080	0	0	0	35 249

		Esbjerg1 20_06_2017	Esbjerg3 06_10_2017	Esbjerg3 20_06_2017	Esbjerg Trafikkhavn w6 21_09_2017	Esbjerg1 06_10_2017	Esbjerg7 20_06_2017
Dictyochophytes							
	Dictyocha speculum	.	.	.	880	400	.
	Sum - Dictyochophytes	0	0	0	880	400	0
Diatomes							
	Actinoptychus sp	480	320	4 200	.	14 400	1 200
	diatoms chain forming		800	1040			
	Asterionellopsis spp.	.	.	1 000	.	6 800	.
	Bacteriastrum delicatulum	.	.	.	40	.	.
	Cerataulina pelagica	.	.	.	160	.	.
	Cerataulina spp.	.	.	1 600	.	800	.
	Chaetoceros affinis	.	.	.	80	.	.
	Chaetoceros danicus	.	400	400	.	1 000	240
	Chaetoceros debilis	.	.	2 400	.	3 200	.
	Chaetoceros decipiens	.	.	.	80	.	.
	Chaetoceros tenuissimus	9 613	.	.	.	.	.
	Chaetoceos-Phaeceros spp.	200	.	.	.	.	.
	Chaetoceros spp. <10 µm	.	.	11 800	6 409	11 800	19 227
	Chaetoceros spp. 10-20 µm	480	.	1 400	8 011	.	.
	Chaetoceros spp. 20-40 µm	.	.	.	.	400	.
	Coscinodiscus spp. 60-100 µm	.	.	200	120	800	.
	Cylindrotheca closterium	320 442	.	2 000	4 807	5 600	704 973
	Dactyliosolen fragilissimus	.	.	.	200	.	.
	Ditylum brightwellii	.	.	400	40	800	80
	cf. Entomoneis spp.	.	.	42 000	40	86 400	.
	Eucampia zodiacus	.	.	.	.	1 600	.
	Fragilaria ulna	.	.	.	80	.	.
	Guinardia delicatula	.	.	4 600	1 602	8 000	.
	Guinardia flaccida	1 920	1 360	.	160	.	560
	Gyrosigma/Pleurosigma	160	.	600	80	.	400
	Heliotheca tameris		880				

		Esbjerg1 20_06_2017	Esbjerg3 06_10_2017	Esbjerg3 20_06_2017	Esbjerg Trafikkhavn w6 21_09_2017	Esbjerg1 06_10_2017	Esbjerg7 20_06_2017
	Leptocylindrus minimus	.	.	2 600	.	18 800	.
	Lithodesmium cf. undulatum	120	.	.	.	1 600	160
	Navicula transitans	.	.	400	801	1 200	.
	cf. Navicula vanhoeffenii	.	.	.	1 602	7 200	.
	Navicula spp.	.	.	400	.	.	160
	Odontella cf. sinensis	80	.	.	.	.	.
	Plagiogrammopsis spp.	.	.	.	.	2 200	.
	Pseudo-nitzschia seriata-gruppen	2 640	3 440	.	1 280	.	4 480
	Pseudo-nitzschia spp.	.	.	1 800	.	11 600	.
	Rhizosolenia hebetata f. semispina	.	.	.	2 403	.	.
	Rhizosolenia pungens	52 873	.	.	.	.	43 260
	Rhizosolenia cf. setigera	.	.	1 000	1 000	600	.
	Rhizosolenia styliformis	80	.	.	801	.	.
	Rhizosolenia spp.	.	160	3 200	.	12 000	.
	Skeletonema spp.	2 080	240	2 400	121 768	.	.
	Tabellaria flocculosa	.	.	.	160	.	.
	Thalassionema nitzschioides	.	.	.	120	.	.
	Thalassiosira cf. rotula	960	720	600	.	.	1 520
	Unidentified centric diatoms 5-10 µm	.	.	53 100	.	11 800	.
	Unidentified centric diatoms 20-40 µm	.	240	400	5 608	4 000	80
	Unidentified centric diatoms 60-80 µm	.	.	.	3 204	.	80
	Unidentified centric diatoms 100-150 µm	.	.	.	360	.	.
	Unidentified centric diatoms >300 µm	.	.	200	.	.	.
	Unidentified pennate diatoms <20 µm	.	.	11 800	801	94 400	.
	Unidentified pennate diatoms 20-50 µm	.	240	800	.	2 400	.
	Unidentified diatoms	.	.	29 500	.	22 400	.
	Sum - Diatoms	392 129	8 800	181 840	161 818	331 800	776 420
Euglenophyta							
	Eutreptiella 30-60 µm	.	.	.	3 204	.	3 204
	Eutreptiella spp.	720	.	.	.	.	.
	Sum - Euglenophyta	720	0	0	3 204	0	3 204

		Esbjerg1 20_06_2017	**Esbjerg3 06_10_2017**	**Esbjerg3 20_06_2017**	**Esbjerg Trafikkhavn w6 21_09_2017**	**Esbjerg1 06_10_2017**	**Esbjerg7 20_06_2017**
Prasinophytes							
	Pyramimonas spp. 5-10 µm	.	.	5 900	.	5 900	.
	Pyramimonas spp. 10-15 µm	.	.	.	9 613	.	.
	Sum - Prasinophytes	0	0	5 900	9 613	5 900	0
Unclassified							
	Unidentified flagellates <5 µm	.	.	5 900	6 409	.	.
	Unidentified flagellates 5-10 µm	.	.	.	12 818	5 900	.
	Unidentified cyster	.	.	.	.	.	240
	Unidentified monads 5-10 µm	.	.	11 800	.	41 300	.
	Unidentified monads 10-15 µm	.	.	.	.	23 600	.
	Sum - Unclassified	0	0	17 700	19 227	70 800	240
	Sum total	518 697	27 240	223 220	432 790	408 900	890 925

A1.3: Aarhus Harbour, species per station

Eier_takson	Navn_cf	Århus1 21_06_2017	Århus5 21_06_2017	Århus8 21_06_2017	ÅrhusAB5 05_10_2017	ÅrhusAB7 05_10_2017	ÅrhusÅ5 05_10_2017
DIATOMS	cf. Actinoptychus				X		
DIATOMS	Asterionella formosa						X
DIATOMS	Aulacoseira italica	X	X		X	X	X
DIATOMS	Aulacoseira granulata	X	X		X	X	X
DIATOMS	Cerataulina pelagica				X	X	X
DIATOMS	Chaetoceros affinis				X	X	X
DIATOMS	Chaetoceros debilis				X	X	X
DIATOMS	Chaetoceros decipiens	X			X		
DIATOMS	cf. Chaetoceros socialis				X		
DIATOMS	Chaetoceros spp. <10 µm				X		
DIATOMS	Chaetoceros spp. 10-20 µm	X				X	X
DIATOMS	Coscinodiscus centralis	X		X			X
DIATOMS	Coscinodiscus concinnus	X					
DIATOMS	Coscinodiscus radiatus	X	X	X	X		X
DIATOMS	Coscinodiscus spp. >200 µm			X			
DIATOMS	Coscinodiscus spp. 60-100 µm		X	X			
DIATOMS	Cylindrotheca closterium	X		X	X	X	X
DIATOMS	Dactyliosolen fragilissimus	X	X	X			
DIATOMS	Ditylum brightwellii	X	X	X	X	X	X
DIATOMS	Fragilaria ulna	X	X	X	X		X
DIATOMS	Guinardia delicatula	X	X	X	X	X	X
DIATOMS	Guinardia flaccida				X	X	
DIATOMS	Gyrosigma/Pleurosigma		X				
DIATOMS	Leptocylindrus mediterraneus				X	X	X
DIATOMS	Leptocylindrus spp.	X			X	X	X
DIATOMS	Licmophora spp.	X		X			
DIATOMS	Melosira sp.		X	X			
DIATOMS	cf. Navicula septentrionalis		X				
DIATOMS	Navicula spp.	X	X				
DIATOMS	Nitzschia sp.						
DIATOMS	cf. Phaeodactylum tricornutum	X					
DIATOMS	Proboscia alata	X	X	X	X	X	X
DIATOMS	Pseudo-nitzschia spp.				X	X	X
DIATOMS	Rhabdonema spp.		X	X		X	
DIATOMS	Rhizosolenia pungens				X		
DIATOMS	Rhizosolenia setigera	X	X	X			
DIATOMS	Rhizosolenia spp.				X	X	
DIATOMS	Skeletonema spp.				X	X	X

Eier_takson	Navn_cf	Århus1 21_06_2017	Århus5 21_06_2017	Århus8 21_06_2017	ÅrhusAB5 05_10_2017	ÅrhusAB7 05_10_2017	ÅrhusÅ5 05_10_2017
DIATOMS	Striatella unipuctata.		X				
DIATOMS	Tabellaria sp.		X				
DIATOMS	Thalassionema nitzschioides	X	X	X	X	X	X
DIATOMS	Ubestemte diatoméer					X	X
DIATOMS	Pennate diatoms <20 µm				X		
DIATOMS	Pennate diatoms 20-50 µm					X	
DIATOMS	Pennate diatoms 50-100 µm		X	X		X	
DIATOMS	Centric diatoms 100-150 µm					X	
DIATOMS	Centric diatoms 40-60 µm		X			X	
DIATOMS	Centric diatoms 5-10 µm				X		
DIATOMS	Centric diatoms 60-80 µm				X	X	X
DIATOMS	Centric diatoms 80-100 µm				X	X	X
CHRYSOPHYTES	Dinobryon spp.	X					
CILIATES	Myrionecta rubra	X		X			
HAPTOPHYTES	Chrysochromulina spp. 5-10 µm	X					
HAPTOPHYTES	cf. Haptolina ericina/hirta	X					
CRYPTOPHYTES	Cryptophytes 5-10 µm	X	X				
CRYPTOPHYTES	Cryptophytes 10-15 µm		X	X			
DICTYOCHOPHYTES	Dictyocha speculum	X	X	X	X		
DICTYOCHOPHYTES	Pseudochattonella cf. verruculosa			X			
DINOFLAGELLATES	cf. Diplopelta bomba	X	X				
DINOFLAGELLATES	cf. Diplopsalis lenticula	X					
DINOFLAGELLATES	Dinoflagellate cysts				X	X	
DINOFLAGELLATES	Dinophysis acuminata			X			
DINOFLAGELLATES	Dinophysis norvegica	X				X	
DINOFLAGELLATES	Diplopsalis spp.	X		X			
DINOFLAGELLATES	Heterocapsa rotundata			X			
DINOFLAGELLATES	cf. Oblea spp.			X			
DINOFLAGELLATES	cf. Diplopelta bomba	X	X	X			
DINOFLAGELLATES	Oxytoxum criophilum				X		
DINOFLAGELLATES	Peridinium sp.		X				
DINOFLAGELLATES	Prorocentrum micans	X			X	X	
DINOFLAGELLATES	Protoperidinium conicum		X				
DINOFLAGELLATES	Protoperidinium crassipes				X		
DINOFLAGELLATES	Protoperidinium cf. curtipes			X			
DINOFLAGELLATES	Protoperidinium depressum			X			
DINOFLAGELLATES	Protoperidinium divergens				X		
DINOFLAGELLATES	Protoperidinium pellucidum	X		X			
DINOFLAGELLATES	Protoperidinium spp. 20-40 µm		X				
DINOFLAGELLATES	Protoperidinium spp. 40-60 µm			X			

Eier_takson	Navn_cf	Århus1 21_06_2017	Århus5 21_06_2017	Århus8 21_06_2017	ÅrhusAB5 05_10_2017	ÅrhusAB7 05_10_2017	ÅrhusÅ5 05_10_2017
DINOFLAGELLATES	Protoperidinium steinii					X	X
DINOFLAGELLATES	Scrippsiella cf. trochoidea					X	X
DINOFLAGELLATES	Scrippsiella-group		X	X			
DINOFLAGELLATES	Tripos furca				X		
DINOFLAGELLATES	Tripos fusus	X			X	X	X
DINOFLAGELLATES	Tripos horridus						
DINOFLAGELLATES	Tripos lineatus				X	X	
DINOFLAGELLATES	Tripos longipes	X	X	X	X	X	
DINOFLAGELLATES	Tripos macroceros	X					X
DINOFLAGELLATES	Tripos spp.				x		
DINOFLAGELLATES	Tripos muelleri	X	X	X	X	X	
DINOFLAGELLATES	Athecate dinoflagellates < 20 µm				X		
DINOFLAGELLATES	Athecate dinoflagellates 20-40 µm				X	X	X
DINOFLAGELLATES	Thecate dinoflagellates 20-40 µm	X		X		X	
DINOFLAGELLATES	Thecate dinoflagellates 40-60 µm	X					
PRASINOPHYTES	Pyramimonas spp. 5-10 µm	X		X			
PRASINOPHYTES	Pyramimonas spp. 10-15 µm		X				
PRASINOPHYTES	Pyramimonas spp.		X				
RAPHIDOPHYTES	cf. Heterosigma akashiwo	X					
UNCLASSIFIED	Flagellates <5 µm	X					
UNCLASSIFIED	Flagellates 5-10 µm	X	X	X		X	
UNCLASSIFIED	Flagellates 10-15 µm		X				
UNCLASSIFIED	Flagellates20-30 µm			X			
UNCLASSIFIED	Monads 5-10 µm			X	X	X	X
UNCLASSIFIED	Monads 10-15 µm					X	
UNCLASSIFIED	Cysts						X
CHLOROPHYTA	cf. Ankistrodesmus falcatus						X
CHLOROPHYTA	cf. Aktinastrum hantzschii		X				
CHLOROPHYTA	Closteriopsis sp		x				
CHLOROPHYTA	Coelastrum astroideum		x				
CHLOROPHYTA	Desmodesmus opoliensis		X				
CHLOROPHYTA	Desmodesmus acuminatus		X				X
CHLOROPHYTA	Desmodesmus spp.				X	X	X
CHLOROPHYTA	Dichtyosphaerium cf. elegans		X				
CHLOROPHYTA	Elakothrix geneviensis		X				
CHLOROPHYTA	Koliella sp.		X				
CHLOROPHYTA	Monoraphidium contortum		X	X			
CHLOROPHYTA	Monoraphidium minimum		X				
CHLOROPHYTA	Micractinium cf. pusillum		X				
CHLOROPHYTA	Pediastrum spp.						X

Eier_takson	Navn_cf	Århus1 21_06_2017	Århus5 21_06_2017	Århus8 21_06_2017	ÅrhusAB5 05_10_2017	ÅrhusAB7 05_10_2017	ÅrhusÅ5 05_10_2017
CHLOROPHYTA	Pediastrum boryanum		X				
CHLOROPHYTA	Pediastrum duplex		X		X		
CHLOROPHYTA	Pediastrum duplex var. gracillimum		X				
CHLOROPHYTA	Pediastrum tetras		X				
CHLOROPHYTA	Pandorina morum		X				
CHLOROPHYTA	cf. Pandorina sp.		X				
CHLOROPHYTA	Romeria sp.		X				
CHLOROPHYTA	Tetrahëdon caudatum		X				
CHLOROPHYTA	Coccoid chlorophyte in gelatinous colony		X		x		
CYANOPHYTA	Anabaena spp.		X				
CYANOPHYTA	cf. Pseudanabaena sp.		X				
CYANOPHYTA	Filamentous cyanophyte					X	
CYANOPHYTA	Coccoid cyanophytes in gelatinous colony		X				X

A1.4: Aarhus Harbour, cells per liter

		Århus 1	**Århus 5**	**Århus 8**	**Århus Å5**	**Århus AB5**	**Århus AB7**
		21-06-2017	**21-06-2017**	**21-06-2017**	**05-10-2017**	**05-10-2017**	**05-10-2017**
Cyanophytes							
	cf. Pseudanabaena sp.	19 620	36 771	.	.	.	.
	Unidentified cyanophytes	.	.	.	.	.	200
	Sum - Cyanophytes	19 620	36 771	0	0	0	200
Cryptophytes							
	Unidentified cryptophytes 5-10 µm	365 304	130 741	.	.	.	.
	Unidentified cryptophytes 10-15 µm	.	65 370	32 685	.	.	.
	Sum - Cryptophytes	365 304	196 111	32 685	0	0	0
Dinoflagellates							
	Dinophysis acuminata	.	.	40	.	.	.
	cf. Diplopsalis lenticula	320	200	.	.	.	.
	Diplopsalis spp.	560	.	40	.	.	.
	Heterocapsa rotundata	.	.	4 086	.	.	.
	Oblea spp.	.	.	40	.	.	.
	Prorocentrum micans	160	.	.	.	.	.
	Protoperidinium depressum	.	.	40	.	.	.
	Protoperidinium spp. 20-40 µm	.	80	.	.	.	.
	Protoperidinium spp. 40-60 µm	.	.	40	.	.	.
	Scrippsiella-group	.	.	80	.	.	.
	Tripos fusus	.	.	.	.	200	400
	Tripos lineatus	.	.	.	.	600	.
	Tripos longipes	240	40	80	.	400	.
	Tripos macroceros	.	.	.	.	.	200
	Tripos muelleri	80	40	80	.	400	200
	Unidentified athecate dinoflagellates <20 µm	.	.	.	.	200	.

		Århus 1	Århus 5	Århus 8	Århus Å5	Århus AB5	Århus AB7
	Unidentified athecate dinoflagellates 20-40 µm	.	.	.	200	200	.
	Unidentified thecate dinoflagellates 20-40 µm	80	.	320	.	.	.
	Unidentified thecate dinoflagellates 40-60 µm	1 200	.	.	.	.	.
	Sum - Dinoflagellates	2 640	360	4 846	200	2 000	800
Haptophytes							
	Chrysochromulina spp. 5-10 µm	12 818	.	.	.	.	.
	Haptolina ericina/hirta	12 818	.	.	.	.	.
	Sum - Haptophytes	25 635	0	0	0	0	0
Chrysophytes							
	Dinobryon spp.	6 409	.	.	.	.	.
	Sum - Chrysophytes	6 409	0	0	0	0	0
Dictyochophytes							
	Dictyocha speculum	6 880	24 514	28 599	.	.	.
	Pseudochattonella spp.	.	.	160	.	.	.
	Sum - Dictyochophytes	6 880	24 514	28 759	0	0	0
Diatoms							
	Actinoptychus cf. senarius	.	.	.	.	200	.
	Asterionella formosa	.	.	.	1 600	.	.
	Aulacoseira spp.	208 368		.	207 200	2 600	37 000
	Aulacoseira cf granulata		216 954				
	Aulacoseira cf. italica		119 112				
	Cerataulina pelagica	.	.	.	600	4 600	2 400
	Chaetoceros affinis	.	.	.	1 800	.	1 000
	Chaetoceros debilis	.	.	.	.	200	200
	Chaetoceros decipiens	160	.	.	.	.	.
	Chaetoceros spp. <10 µm	.	.	.	.	23 600	.
	Chaetoceros spp. 10-20 µm	240	.	.	200	.	400
	Coscinodiscus centralis	80	.	.	.	.	.
	Coscinodiscus radiatus	1 040	120	640	.	.	.

		Århus 1	Århus 5	Århus 8	Århus Å5	Århus AB5	Århus AB7
	Coscinodiscus spp. 60-100 µm	.	80	240	.	.	.
	Coscinodiscus spp. >200 µm	.	.	80	.	.	.
	Cylindrotheca closterium	6 409	.	8 171	400	.	1 000
	Dactyliosolen fragilissimus	3 284 856	1 581 143	2 622 982	.	.	.
	Ditylum brightwellii	80	80	.	400	400	400
	Fragilaria ulna	9 613	8 171	.	.	.	.
	Guinardia delicatula	76 906	20 428	106 227	1 000	800	400
	Gyrosigma/Pleurosigma	.	40	.	.	.	.
	Leptocylindrus mediterraneus	.	.	.	.	.	1 400
	Leptocylindrus spp.	12 818	.	.	1 800	5 000	4 200
	Licmophora spp.	160	.	240	.	.	.
	Melosira arctica	.	12 000	240	.	.	.
	Navicula septentrionalis	.	2 400	.	.	.	.
	Navicula spp.	80	32 685	.	.	.	.
	Phaeodactylum tricornutum	6 540	12 762	34 033		11 800	
	Proboscia alata	320	40	.	.	.	200
	Pseudosolenia calca-avis					200	
	Pseudo-nitzschia spp.	.	.	.	2 600	5 400	3 800
	Rhabdonema spp.	.	.	.	.	.	200
	Rhizosolenia pungens	.	.	.	.	200	.
	Rhizosolenia setigera	320	80	360	.	.	.
	Rhizosolenia spp.	.	.	.	.	1 200	200
	Skeletonema spp.	.	.	.	4 200	.	15 000
	Thalassionema nitzschioides	144 199	44 942	208 368	.	.	.
	Unidentified centric diatoms 5-10 µm	.	.	.	.	5 900	.
	Unidentified centric diatoms 40-60 µm	.	40	.	.	.	200
	Unidentified centric diatoms 60-80 µm	.	.	.	200	100	.
	Unidentified centric diatoms 80-100 µm	.	.	.	.	.	200
	Unidentified centric diatoms 100-150 µm	.	.	.	.	.	200
	Unidentified pennate diatoms 20-50 µm	.	.	.	.	.	200
	Unidentified pennate diatoms 50-100 µm	.	80	80	.	.	200
	Unidentified diatoms	.	.	.	70 800	.	1 800
	Sum - Diatoms	3 752 188	2 051 158	2 981 660	292 800	62 200	70 600

		Århus 1	Århus 5	Århus 8	Århus Å5	Århus AB5	Århus AB7
Raphidophytes							
	cf. Heterosigma akashiwo	6 409	.	.	.	.	.
	Sum - Raphidophytes	6 409	0	0	0	0	0
Prasinophytes							
	Pyramimonas spp. 5-10 µm	64 088	.	32 685	.	.	.
	Pyramimonas spp. 10-15 µm	.	8 171	.	.	.	.
	Pyramimonas spp.	.	40	.	.	.	.
	Sum - Prasinophytes	64 088	8 211	32 685	0	0	0
Chlorophytes							
	Monoraphidium contortum	.	57 199	.	.	.	.
	Aktinastrum hantzschii		59 556				
	cf. Tetrastrum komareki		4 254				
	cf. Closteriopsis		46 794	4 254			
	Crusigenia sp.		4 254				
	Desmodesmus accuminatus		51 048	4 254			
	Desmodesmus cf. armatus	.	72 318	200	800	200	200
	Elakotothrix geneviensis		34 033				
	Lagerheimia geneviensis cf		8 508				
	Micractinium sp.		4 254				
	Monoraphidium cf dubrowskii		34 033				
	Monoraphidium contortum		59 556	4 254			
	Koliella spp						1 200
	Pediastrum cf. angulosum			160			
	Pediastrum cf. boryatum			40			
	Pediastrum duplex		4 254				
	Pediastrum spp.	.	.	.	200	.	.
	Pediastrum tetras		4 254				
	cf. Romeria sp.		68 064				
	Tertrahëdron spp.		4 254				
	Sum - Chlorophytes	0	121 009	400	1 000	200	200

		Århus 1	Århus 5	Århus 8	Århus Å5	Århus AB5	Århus AB7
Unclassified							
	Unidentified flagellates <5 µm	51 271	.	.	.	.	.
	Unidentified flagellates 5-10 µm	192 265	32 685	196 111	.	.	800
	Unidentified flagellates 10-15 µm	.	32 685	.	.	.	.
	Unidentified flagellates 20-30 µm	.	.	200	.	.	.
	Unidentified cysts	.	.	.	59 000	.	.
	Unidentified monads 5-10 µm	.	.	98 055	171 100	59 000	1 000
	Unidentified monads 10-15 µm	.	.	.	.	.	400
	Sum - Unclassified	243 536	65 370	294 366	230 100	59 000	2 200
Ciliates							
	Myrionecta rubra	3 204	.	40	.	.	.
	Sum - Ciliophora :	3 204	0	40	0	0	0
	Sum totalt :	4 495 915	2 701 316	3 381 823	524 100	123 400	74 600

Annex 4: Zooplankton

A3.1: Aarhus Harbour.

	AA1		AA5		AA8	
	21.06 2017	**05.10 2017**	**21.06 2017**	**05.10 2017**	**21.06 2017**	**05.10 2017**
Copepoda Calanoida						
Acartia tonsa	-	28	-	-	-	-
Acartia sp. (non tonsa)	34189	60	16605	31	27145	-
Temora longicornis	236	2	212	2	177	+
Centropages hamatus	147	1	+	-	2	-
Calanus helgolandicus	-	1	-	2	-	-
Pseudocalanus cf. elongatus	236	65	371	23	3183	17
Paracalanus parvus	-	88	-	55	-	157
Copepoda Cyclopoida						
Oithona similis	589	634	955	1377	1636	821
Copepoda Harpacticoida						
Harpacticoida indet	-	1	17	3	9	+
Copepoda Monstrilloida						
Monstrilloida indet.	-	1	-	2	4	+
Copepod larvae						
Unidentified copepodite larvae	3497	50	3554	26	2657	43
Calanoid nauplius larvae	-	-	+	-	-	-
Unidentified nauplius larvae	-	-	-	-	-	+
Cladocera						
Evadne nordmanni	88	44	265	25	619	44
Podon leuckarti	88		424	-	227	-
Podon intermedius	-	9	-	2	23	11
Penilia avirostris	-	1	-	3	-	-
Amphipoda						
Amphipod (juvenile)	-	-	-	-	-	+
Unidentified Amphipod exuviae	+	-	+	-	-	-
Other crustacean larvae						
Cirripedia nauplius larvae	29	-	-	57	2	43
Cirripedia cypris larvae	+	9	-	25	-	-
Brachyura megalopa larvae	-	-	-	1	-	-
Caridea zoea larvae	-	-	-	2	2	-
Appendicularia						
Appendicularia indet	-	24	3	-	-	27
Cnidaria						
Clytia hemisphaera	-	1	-	-	-	+
Aglantha digitale	-	-	-	-	-	+
Corymorpha nutans	-	-	-	-	-	+
Siphonophora nectophores	-	+	-	+	-	85
Unidentified small Hydrozoa	147	+	53	+	265	-
Other larvae						
Juvenile musssels	648	24	371	129	2432	72
Juvenile snails	5069	124	159	480	442	332
Bryozoan cyphonautes larvae	147	4	371	38	619	17
Polychate larvae	-	85	-	88	2	19
Fish fry	-	-	11	-	-	-
Miscellanea						
Unidentified eggs	-	-	+	-	5	-
Freshwater organisms						
Alona affinis	-	-	1	-	-	-
Alona spp.	-	-	-	2	-	-
Daphnia cucullata	-	-	1	-	-	-
Daphnia cf longispina	-	-	-	1	-	-
Megacyclops sp	-	-	3	-	-	-
Polyphemus pediculus	-	-	-	1	-	-
Sida crystallina	-	-	-	1	-	-
Ceriodaphnia sp.	-	-	-	3	-	-
Bryozoan statoblasts	-	-	+	-	-	1

A4.2: Esbjerg Harbour.

	E1		E7		E3	
	20.06.2017	**06.10.2017**	**20.06.2017**	**21.09.2017**	**20.06.2017**	**21.09.2017**
Copepoda Calanoida						
Acartia tonsa	-	165	-	557	-	1096
Acartia spp. (non tonsa)	884	-	4	-	60	-
Temora longicornis	+	2	-	-	7	+
Centropages hamatus	248	-	11	9	48	+
Centropages typicus	-	-	-	-	2	-
Pseudocalanus cf. elongatus	283	-	-	-	2	-
Paracalanus parvus	-	6	2	-	7	-
Copepoda Cyclopoida						
Oithona similis	-	6	3	9	2	9
Copepoda Harpacticoida						
Euterpina acutifrons	-	83	-	62	-	62
Harpacticoida indet	-	-	1	-	2	+
Copepod larvae						
Unidentified copepodite larvae	354	21	14	88	12	88
Calanoid nauplius larvae	-	-	-	-	-	-
Cladocera						
Evadne nordmanni	283	-	113	-	157	-
Podon intermedius	-	-	-	-	-	-
Podon leuckarti	-	-	10	-	19	-
Pleopis polyphemoides	-	118	-	18	-	+
Amphipoda						
Amphipoda indet.	-	3	-	-	2	-
Unidentified Amphipod exuviae		-	-	-	-	-
Cumacea						
Cumacea indet	-	-	-	-	-	+
Other crustacean larvae						
Cirripede nauplius larvae	6260	100	417	442	1028	133
Cirripede cypris larvae	424	6	4	-	9	-
Brachyura zoea-larvae	-	-	7	-	48	-
Caridea zoea-larvae	-	9	-	35	11	-
Unidentified crustacean larvae	-	-	1	-	-	-
Appendicularia						
Oikopleura sp.	35	12		+	7	9
Cnidaria			4			
Clythia hemisphaerica	-	-	-	-	-	+
Unidentified Ctenophore	-	-	-	-	-	+
Unidentified small Hydrozoa	-	+	-	+	-	-
Beroe spp.	1	-	-	-	1	-
Mnemiopsis leidyii	-	-	-	2	-	-
Other larvae						
Bryozoan cyphonautes larvae	-	-	-	-	-	-
Polychaete larvae	+	+	-	27	2	-
Juvenile musssels	+	+	-	+	-	+
Juvenile snails	5977	+	2	27	99	+
Fish fry	-	-	-	-	2	-
Miscellanea						
Insect remains	-	-	-	-	-	-
Unidentified egg (Ø 200 µm)	-	-	-	-	-	-
Foraminifera	-	-	-	-	4	-

Annex 5: Mobile epifauna (traps)

Data for collection of mobile epifauna with traps in Aarhus and Esbjerg harbours; GPS positions WGS 84, date and time (hh:mm) of trap deployment (D) and retrieval (R), water depth, and a short station description.

Stn	Coordinates	Deployment	Depth (m)	Description of station
Aarhus		D: 18 Oct 2017 R: 20 Oct 2017		
AT1	56,1549 10,2186	D 12:15 / R 10:15	7	Vertical stone quay
AT2	56,1559 10,2193	D 12:50 / R 11:05	5.5	Vertical stone quay, black sediment
AT3	56,1571 10,2206	D 13:30 / R 11:50	8	Vertical quay front with iron plates
AT4	56,1510 10,2162	D 14:15 / R 12:40	8	Vertical stone quay
AT5	56,1501 10,2195	D 14:55 / R 13:10	8.5	Vertical stone quay
AT6	56,1528 10,2206	D 15:25 / R 13:40	9	Vertical stone quay
AT7	56,1464 10,2281	D 16:15 / R 14:15	10	Vertical stone quay
AT8	56,1482 10,2240	D 16:50 / R 14:40	8	Channel, beneath small bridge
AT9	56,1490 10,2310	D 17:30 / R 15:15	7	Breakwater with iron plates
Esbjerg		D: 21 Oct 2017 R: 23 Oct 2017		
ET1	55,4506 08,4674	D 11:50 / R 10:05	10	Recently constructed quay with fenders
ET2	55,4508 08,4667	D 11:55 / R 10:50	10	Recently constructed quay with fenders
ET3	55,4621 08,4508	D 13:00 / R 11:40	8	Vertical stone quay
ET4	55,4625 08,4447	D 13:30 / R 12:20	7	Vertical stone quay
ET5	55,4646 08,4420	D 14:00 / R 13:35	7	Vertical stone quay
ET6	55,4649 08,4331	D 14:40 / R 13:00	14	Vertical stone quay
ET7	55,4700 08,4245	D 15:10 / R 14:15	2	Inside breakwater, at wooden quay
ET8	55,4758 08,4234	D 15:40 / R 14:40	5	Vertical stone quay
ET9	55,4728 08,4233	D 16:10 / R 15:30	5	Vertical stone quay

Biomass (g wet weight) of species caught in crab traps and mesh neting traps at the stations in Aarhus. nd = not determined. Sizees of collected specimens are given below table.

	AT1	AT2	AT3	AT4	AT5	AT6	AT7	AT8	AT9
Crab trap (C)									
Crustacea									
Carcinus maenas	160	140		24	80	83	45	60	29
Mollusca									
Hinia reticulata	31		8				6	9	
Echinodermata									
Asterias rubens	10	10	290			8		40	7
Fish									
Taurulus bubalis			23						
Mesh netting trap (M)									
Crustacea									
Carcinus maenas	6	15	7	14		18	12	3	
Macropodia rostrata							nd		
Crangon crangon								3	
Mollusca									
Hinia reticulata	910	2018	1050	230		247	52	360	1140
Echinodermata									
Asterias rubens	4	0,8							0,9
Psammechinus miliaris			12			4			
Fish									
Ctenolabrus rupestris	13	2			8	2	112	15	46
Zoarches viviparous	27				34		23		
Gobius niger	31			15			82	9	

Size of specimens:

Carcinus maenas (m=male, f=female; width of carapax mm). **AT1C**: m 85, m 86, f 40, m 46, m 38, m 42, **AT1M**: f 30; **AT2C**: m 58, m 52, m 43, m 44, f 38, f 34; **AT2M**: m 14, m 13, m 13, m 12, f 12, f 11; **AT3M**: m 27, m 15, m 13, m 12, m 10; **AT4C**: m 40, f 24; **AT4M**: m 30, f 30, f 28; **AT5C**: f 40, f 48, f 34, f 36, f 35; **AT6C**: f 38, m 55, m 50; **AT6M**: m 25, f 28, m 29, m 20, m 13, m 13; **AT7C**: m 54, f 40; **AT7M**: f 25; **AT8C**: f 35, m 54, m 37; **AT8M**: m 14, m 14, m 14, m 11; **AT9C**: m 48.

Asterias rubens (arm length mm). **AT1C**: 35, 10; **AT2C**: 30, **AT2M**: 20; **AT3C**: 75, 80, 85, 40, 28, 22; **AT6C**: 32, 30; **AT8C**: 45, 37, 34, 47; **AT9C**: 16, 18, 17; **AT9M**: 22.

Psammechinus miliaris (diameter mm). **AT3M**: 20, 20, 18, 14, 15; **AT6M**: 22.

Ctenolabrus rupestris (length cm). **AT1M**: 9.5; **AT2M**: 6.0; **AT5M**: 9; **AT6M**: 6; **AT7M**: 10, 9.5, 10, 9, 9, 8.5, 9.5, 8, 8, 9, 7; **AT8M**: 9.5, 5, 4.5; **AT9M**: 9, 8.5, 9, 9, 7.5.

Zoarches viviparous (length cm). **AT1M**: 19.5; **AT5M**: 18; **AT7M**: 18.

Gobius niger (length cm). **AT1M**: 10.5, 9.5; **AT4M**: 9, 7, 5; **AT7M**: 9.5, 10.5, 10, 8.5, 9.5, 7, 9.5, 6; **AT8M**: 5, 5, 4.5, 5, 4.5, 4.5, 4.5.

Taurulus bubalis (length cm). **AT3C**: 12.

Biomass (g wet weight) of species caught in crab traps and mesh netting traps at the stations in Esbjerg. nd = not determined. Sizees of collected specimens are given below table.

	ET1	ET2	ET3	ET4	ET5	ET6	ET7	ET8	ET9
Crab trap									
Crustacea									
Carcinus maenas	600	1530	570	760	816	115	480	120	55
Hyas araneus				80		54			
Cancer pagurus					62				207
Austrominius modestus (on *Carcinus*)	nd	nd	nd	nd				nd	
Amphibalanus improvisus (on *Carcinus*)	nd	nd	nd	nd				nd	
Echinodermata									
Asterias rubens									30
Fish									
Zoarches viviparous								134	
Mesh netting trap									
Ctenophora									
Mnemiopsis leidyi								nd	
Crustacea									
Carcinus maenas	5			0.3		0,5			
Crangon crangon				0,2		0,2		0,6	1
Palaemon serratus	2		0,8		2,4				0,9
Praunus flexuosus								0,1	0,1
Mollusca									
Hinia reticulata						0,7			
Echinodermata									
Asterias rubens									18
Fish									
Zoarches viviparous			9				42		
Taurulus bubalis							8		
Pomatoschistus minutus						0,8			
Ciliata mustela		14			30			31	

Sizes of specimens:

Carcinus maenas (m=male, f=female; width of carapax mm). **ET1C**: m 55, m 60, m 53, m 48, m 45, m 55, m 52, m 60, m 65, f 60, m 46, m 36, m 55, m 42, m 46, f 36; **ET2C**: m 60, m 56, m 58, m 64, m 60, m 52, m 48, m 55, m 55, m 58, m 65, m 46, m 56, m 70, m 55, m 43, m 42, m 41, m 41, f 40, f 34, m 60, m 40, m 62, m 47, m 52, f 35, f 35, m 38, m 55, m 56, f 35, m 45, m 58, f 32, m 46, m 50, m 45, m 51, f 35, m 38, m 36, m 35; **ET3C**: m 60, m 50, m 35, m 51, m 50, m 56, m 58, m 47, m 51, m 53, m 55, m 50, m 60, m 46; **ET4C**: m 55, m 60, m 56, m 57, m 52, m 63, m 56, m 60, m 53, m 60, m 41, m 45, m 44, m 57, m 54, m 50, m 53; **ET4M**: 10; **ET5C**: m 45, m 55, f 43, m 55, m 55, m 65, m 52, m 55, m 55, m 50, m 67, m 58, m 56, f 42, m 44, m 43, f 40, m 60, m 52, m 54; **ET6C**: m 57, m 50, m 41, f 41; **ET6M**: m 12; **ET7C**: m 75, m 67, m 50, m 57, f 46, m 60, m 54, m 50, m 57; **ET8C**: m 38, f 42, m 45, m 48, m 40, m 42; **ET9C**: m 55, f 36.

Hyas araneus (width/length of carapax mm). **ET4C**: 55/74; **ET6C**: 45/60.

Cancer pagurus (width of carapax mm). **ET5C**: 75; **ET9C**: 110.

Asterias rubens (arm length mm). **ET9C**: 37, 21, 25, 20, 20, 24; **ET9M**: 35, 28.

Zoarches viviparous (length cm). **ET3M**: 13; **ET7M**: 17.5, 13.5; **ET8C**: 26.

Gobius niger (length cm). **AT1M**: 10.5, 9.5; **AT4M**: 9, 7, 5; **AT7M**: 9.5, 10.5, 10, 8.5, 9.5, 7, 9.5, 6; **AT8M**: 5, 5, 4.5, 5, 4.5, 4.5, 4.5.

Taurulus bubalis (length cm). **ET7M**: 8.

Ciliata mustela (length cm). **ET2M**: 14; **ET5M**: 15; **ET8M**: 15.5, 11.

Annex 6: Fouling organisms – background data

A6.1: Sampling sites and water depths.

Esbjerg harbour					Aarhus harbour				
Area	**Method**	**Lat**	**Long**	**Depth (m)**	**Area**	**Method**	**Lat**	**Long**	**Depth (m)**
Østhavn East port	Scraping	55,44843	8,47700	1	Basin 1	Scraping	56,15503	10,21871	1
	Scraping	55,44870	8,47627	0		Scraping	56,15581	10,21933	0
	Scraping	55,45176	8,47401	> 0		Scraping	56,15716	10,21784	0
	RAS	55,45032	8,47914	0		RAS	56,15783	10,21929	0
	Video	55,45040	8,47863	7		Video	56,15581	10,21933	5,5
	Video	55,45090	8,47884	7		Video	56,15600	10,21913	7,1
	Plate (E1)	55,44844	8,47677	1, 3		Plate (Å5)	56,15583	10,21931	1, 3
	Plate (E2)	55,44844	8,47677	1, 3		Plate (Å6)	56,15500	10,21864	1, 3
Fergekai Dock port	Scraping	55,45734	8,43904	> 0	Basin 3 & 4	Scraping	56,14993	10,21962	0
	Scraping	55,45888	8,43552	0		Scraping	56,15279	10,22047	0,5
	Scraping	55,46035	8,43652	0		Scraping	56,15490	10,22394	0,3
	Plate (E3)	55,46051	8,43657	1, 3, 7		Video	56,14980	10,21937	7,6
	Plate (E4)	55,46051	8,43657	1, 3, 7		Video	56,14986	10,21922	9,9
	RAS	55,45895	8,43556	0		Plate (Å7)	56,15495	10,22403	1, 3, 7
	RAS	55,46036	8,43646	0		Plate (Å8)	56,15268	10,22052	1, 3, 7
1. Bassin + Trafikkhavn North port + Traffic port	Scraping	55,46798	8,42569	0		Plate (Å9)	56,14986	10,21944	1, 3
	Scraping	55,46653	8,43143	0	Basin 9	Scraping	56,14644	10,22317	0
	Scraping	55,47243	8,42405	0		Scraping	56,14895	10,23102	0
	RAS	55,46718	8,43121	0		Scraping	56,14855	10,22450	0
	Video	55,46658	8,43078	2,1		RAS	56,14923	10,22852	0
	Video	55,46654	8,43065	1,9		Plate (Å1)	56,14635	10,22320	1, 3, 7
	Plate (E5)	55,47008	8,42632	1, 3, 7		Plate (Å2)	56,14812	10,22405	1, 3, 7
	Plate (E6)	55,47083	8,42794	1, 3, 7		Plate (Å3)	56,14812	10,22405	1, 3, 7
	Plate (E7)	55,47024	8,42691	1, 3, 7		Plate (Å4)	56,14906	10,23102	1, 3, 7
	Plate (E8)	55,47282	8,42333	1, 3					
	Plate (E9)	55,47287	8,42337	1, 3					

A6.2: List of species identified from scraping fouling organisms.

ESBJERG			
Area	**Species**	**Cover**	**Non-indige-nous**
TRAFIKKHAVN			
SCRAPE1	*Austrominius mod-estus*	2	x
SCRAPE1	*Balanus im-provisus*	3	x
SCRAPE1	*Crassostrea gigas*	1	x
SCRAPE1	*Mytilus sp.*	1	
SCRAPE1	*Ulva lactuca*	2	
SCRAPE1	*Ceramium rubrum*	2	
SCRAPE1	*Littorina littorea*	1	
SCRAPE2	*Hemigrapsus san-guineus*	1	x
SCRAPE2	*Fucus vesiculosus*	4	
SCRAPE2	*Balanus im-provisus*	4	x
SCRAPE2	*Ulva lactuca*	3	
SCRAPE2	*Ceramium rubrum*	2	
SCRAPE2	*Ulva intestinalis*	2	
SCRAPE2	*Elachista fucicola*	2	
SCRAPE3	*Crassostrea gigas*	1	x
SCRAPE3	*Fucus vesiculosus*	4	
SCRAPE3	*Mytilus sp.*	2	
SCRAPE3	*Austrominius mod-estus*	2	x
SCRAPE3	*Ulva intestinalis*	2	
SCRAPE3	*Ulva lactuca*	2	
SCRAPE3	*Elachista fucicola*	2	
FERGEKAI			
SCRAPE1	*Crassostrea gigas*	2	x
SCRAPE1	*Ulva lactuca*	3	
SCRAPE1	*Hemigrapsus san-guineus*	1	x
SCRAPE1	*Audouinella sp.*	1	
SCRAPE1	*Balanus im-provisus*	3	x
SCRAPE1	*Mytilus sp.*	2	
SCRAPE1	*Fucus vesiculosus*	2	
SCRAPE1	*Littorina littorea*	1	
SCRAPE1	*Littorina saxatilis*	1	

AARHUS			
Area	**Species**	**Cover**	**Non-indige-nous**
BASSIN 9			
SCRAPE1	*Mytilus* sp.	4	
SCRAPE1	*Metridium senile pallidus*	2	
SCRAPE1	*Ulva lactuca*	2	
SCRAPE1	*Asterias rubens juvenile*	2	
SCRAPE1	*Electra pilosa*	2	
SCRAPE1	*Actiniaria indet.*	1	
SCRAPE1	*Hiatella arctica*	2	
SCRAPE1	*Hydroidea indet.*	2	
SCRAPE1	*Modiolus modiolus*	1	
SCRAPE1	*Pycnogonida indet.*	1	
SCRAPE2	*Metridium senile pallidus*	2	
SCRAPE2	*Ulva lactuca*	2	
SCRAPE2	*Ceramium rubrum & C. cimbricum*	4	
SCRAPE2	*Polysiphonia fucoides*	3	
SCRAPE2	*Asterias rubens juvenile*	1	
SCRAPE3	*Metridium senile pallidus*	2	
SCRAPE3	*Mytilus sp.*	4	
SCRAPE3	*Balanus sp.*	2	
SCRAPE3	*Ceramium cimbricum*	2	
SCRAPE3	*Ulva lactuca*	2	
SCRAPE3	*Carcinus maenas* juvenile	1	
2KRAP3	*Electra pilosa*	S	
BASSIN 3/4			
SCRAPE1	*Ulva lactuca*	4	
SCRAPE1	*Ulva compressa*	2	
SCRAPE1	*Carcinus maenas juvenile*	1	
SCRAPE1	*Mytilus sp.*	1	
SCRAPE1	*Callithamnion corymbo-sum*	1	
SCRAPE1	*Balanus improvisus*	2	x
SCRAPE2	*Mytilus sp.*	4	
SCRAPE2	*Balanus improvisus*	2	x

SCRAPE1	*Laomedea geniculata*	2	
SCRAPE1	*Elachista fucicola*	2	
SCRAPE2	*Crassostrea gigas*	2	x
SCRAPE2	*Mytilus sp.*	2	
SCRAPE2	*Ulva lactuca*	2	
SCRAPE2	*Ceramium rubrum*	2	
SCRAPE2	*Dynamena pumila*	2	
SCRAPE2	*Hemigrapsus sanguineus*	2	x
SCRAPE2	*Botryllus schlosseri*	1	
SCRAPE2	*Balanus improvisus*	2	x
SCRAPE3	*Mytilus sp.*	2	
SCRAPE3	*Balanus improvisus*	2	x
SCRAPE3	*Ceramium virgatum*	3	
SCRAPE3	*Ulva lactuca*	2	
SCRAPE3	*Neosiphonia harveyi*	2	x
SCRAPE3	*Diadumene lineata*	1	
ØSTHAVN			
SCRAPE1	*Ulva lactuca*	4	
SCRAPE1	*Balanus improvisus*	4	x
SCRAPE2	*Crassostrea gigas*	1	X
SCRAPE2	*Styela clava*	2	X
SCRAPE2	*Ulva lactuca*	2	
SCRAPE2	*Balanus improvisus*	2	x
SCRAPE3	*Balanus improvisus*	4	x
SCRAPE3	*Austrominius modestus*	2	x
SCRAPE3	*Crassostrea gigas*	1	x
SCRAPE3	*Porphyra sp*	1	
SCRAPE3	*Carcinus maenas juvenile*	1	
SCRAPE3	*Mytilus sp. juvenile*	2	
SCRAPE3	*Harmothoe sp.*	1	
SCRAPE3	*Ceramium virgatum*	1	

SCRAPE2	*Littorina saxatilis*	1	
SCRAPE2	*Gammarus sp.*	2	
SCRAPE3	*Mytilus sp.*	4	
SCRAPE3	*Carcinus maenas juvenile*	2	
SCRAPE3	*Fucus vesiculosus*	1	
SCRAPE3	*Ulva intestinalis*	2	
SCRAPE3	*Balanus improvisus*	2	x
SCRAPE3	*Laomedea geniculata*	2	
SCRAPE3	*Clava multicornis*	2	
SCRAPE3	*Asterias rubens juvenile*	2	
SCRAPE3	*Ulva lactuca*	2	
SCRAPE3	*Ceramium cimbricum*	2	
BASSIN1/2			
SCRAPE1	*Mytilus sp.*	4	
SCRAPE1	*Ceramium cimbricum*	2	
SCRAPE1	*Balanus improvisus*	2	x
SKRAP1	*Asterias rubens juvenile*	S	
SCRAPE1	*Hydroidea indet.*	2	
SCRAPE1	*Hemigrapsus sanguineus*	1	x
SCRAPE2	*Mytilus sp. juvenile*	4	
SCRAPE2	*Balanus improvisus*	2	x
SCRAPE2	*Laomedea geniculata*	2	
SCRAPE2	*Hydroidea indet.*	2	
SCRAPE2	*Ceramium cimbricum*	2	
SCRAPE2	*Laminaria sp. seedling*	1	
SCRAPE2	*Hemigrapsus sanguineus*	1	x
SCRAPE2	*Asterias rubens juvenile*	2	
SCRAPE3	*Mytilus sp.*	3	
SCRAPE3	*Saccharina latissima*	2	
SCRAPE3	*Caprella mutica*	2	x
SCRAPE3	*Ulva intestinalis*	2	
SCRAPE3	*Polycera quadrilineata*	1	
SCRAPE3	*Gammarus locusta*	2	
SCRAPE3	*Asterias rubens juvenile*	2	
SCRAPE3	*Ciona intestinalis*	2	
SCRAPE3	*Harmothoe spirifera*	1	

A6.3: List of species identified from RAS.

ESBJERG				
Area	**Species**	**Coverage**	**Non-indige-nous**	**Comments**
TRAFFIC PORT				
Bouy	*Ulva lactuca*			
	Ceramium rubrum			
Drop-camera	Soft bottom			2 m, zero visibility
DOCK PORT				
Chain	*Fucus vesiculosus*	2		
	Balanus sp. juvenile	4		
	Ulva lactuca	2		
Rope	*Mytilus cf. edulis*	4		
	Ceramium rubrum	2		
	Ulva lactuca	2		
EAST PORT				
Bouy	*Ulva lactuca*	2		
	Porphyra sp.	2		
	Polysiphonia sp.	2		
	Semibalanus balanoides	2		
Drop-camera	-			Soft bottom. Low visibility, 7 m
AARHUS				
Area	**Species**	**Coverage**	**Non-indige-nous**	
BASSIN 9				
Bouy	Filamentous algae	2		
BASSIN 4				
UV camera	*Mytilus cf. edulis*	2		Soft bottom. Low visibility, 7.6-9 m depth
	Empty bivalve shells	2		
Floating fender	Sediment	4		
	Mytilus cf. edulis	2		
	Ulva lactuca	2		
BASSIN1/2				
UV camera	-			Soft bottom. No visible life. 5.5-7.1 m depth
Tire	*Balanus sp.*	2		
	Ulva sp.	2		

A6.4: Non-indigenous species identified from settlement units.

Harbour	Area	Unit	Depth	Plate side	Species	Cover
Esbjerg harbour	Østhavn East port	E1	1	Down	*Amphibalanus improvisus*	4
			3	Up	*Amphibalanus improvisus*	3
			3	Down	*Amphibalanus improvisus*	2
		E2	1	Up	*Amphibalanus improvisus*	3
			1	Up	*Diadumene lineata*	1
			1	Down	*Amphibalanus improvisus*	4
			3	Up	*Amphibalanus improvisus*	4
			3	Down	*Amphibalanus improvisus*	3
	Fergekai Dock port	E3	1	Up	*Amphibalanus improvisus*	3
			1	Down	*Neosiphonia harveyi*	2
			1	Down	*Amphibalanus improvisus*	2
			1	Down	*Caprella mutica*	2
			3	Up	*Amphibalanus improvisus*	2
			3	Up	*Neosiphonia harveyi*	1
			3	Down	*Amphibalanus improvisus*	2
			7	Up	*Amphibalanus improvisus*	2
			7	Down	*Amphibalanus improvisus*	2
		E4	1	Up	*Amphibalanus improvisus*	2
			1	Down	*Diadumene lineata*	2
			3	Up	*Amphibalanus improvisus*	2
			7	Up	*Amphibalanus improvisus*	2
			7	Down	*Amphibalanus improvisus*	2
	1. Bassin + Trafikkhavn North port + Traffic port	E5	1	Up	*Amphibalanus improvisus*	2
			1	Down	*Amphibalanus improvisus*	2
			1	Down	*Caprella mutica*	2
			3	Up	*Diadumene lineata*	2
			3	Down	*Amphibalanus improvisus*	2
			3	Down	*Molgula manhattensis*	1
			7	Up	*Amphibalanus improvisus*	2
			7	Down	*Amphibalanus improvisus*	2
		E6	1	Up	*Diadumene lineata*	2
			1	Down	*Diadumene lineata*	2
			1	Down	*Amphibalanus improvisus*	2
			3	Up	*Amphibalanus improvisus*	2
			3	Down	*Caprella mutica*	2
			7	Up	*Amphibalanus improvisus*	2
			7	Down	*Caprella mutica*	2
			7	Down	*Amphibalanus improvisus*	2
		E7	1	Up	*Amphibalanus improvisus*	2
			1	Down	*Amphibalanus improvisus*	2
			1	Down	cf. *Austrominius modestus*	2
			3	Up	*Amphibalanus improvisus*	2
			3	Down	*Amphibalanus improvisus*	2
			3	Down	*Austrominius modestus* juvenile	2
			3	Down	*Diadumene lineata*	1
			7	Up	*Amphibalanus improvisus*	2
			7	Down	*Caprella mutica*	2
		E8	1	Up	*Amphibalanus improvisus*	2
			3	Up	*Amphibalanus improvisus*	2
			3	Up	*Caprella mutica*	2
			3	Down	*Amphibalanus improvisus*	2
		E9	1	Up	*Amphibalanus improvisus*	2
			1	Down	*Amphibalanus improvisus*	2
			3	Down	*Amphibalanus improvisus*	2

A6.5: All species identified from settlement plates.

Site	Depth	Plate	Species	Cover	Non-indigenous
E1	1	UP	*cf. Metridium senile pallidus*	2	
			Acmaea sp.	1	
			Molgula occulta	2	
			Mytilus edulis juvenile	2	
			Tubularia larynx	2	
			Balanus sp. juvenile	2	
		DOWN	*Mytilus edulis*	2	
			Botryllus schlosseri	2	
			Balanus improvisus	4	x
			Pomatoceros triqueter	2	
			Ascidiacea indet.	1	
			Tubularia larynx	2	
			Molgula occulta	3	
			Botrylloides leachi	2	
	3	UP	*Molgula occulta*	2	
			Tubularia larynx	2	
			Pomatoceros triqueter	2	
			Balanus improvisus	3	x
		DOWN	*Molgula occulta*	4	
			Balanus improvisus	2	x
			Pomatoceros triqueter	2	
			Tubularia larynx	3	
			Botrylloides leachi	2	
			Botryllus schlosseri	2	
			Ciona intestinalis juvenile	1	
E2	1	UP	*Balanus improvisus*	3	x
			Sediment	4	
			Ascidiacea indet.	2	
			Diadumene lineata	1	x
			Ascidiella aspersa	2	
		DOWN	*Molgula occulta*	4	
			Tubularia larynx	3	
			Mytilus edulis juvenile	2	
			Pomatoceros triqueter	2	
			Balanus improvisus	4	x
	3	UP	*Balanus improvisus*	4	x
			Balanus sp.	2	
			Molgula occulta	2	
			Pomatoceros triqueter	2	
			Metridium senile pallidus	2	
			Laomedea geniculata	2	
			Tubularia larynx	2	
		DOWN	*Balanus improvisus*	3	x
			Pomatoceros triqueter	3	
			Tubularia larynx	2	
			Molgula occulta	4	
			Liocarcinus arcuatus	1	
			Ascidiella aspersa	2	
E3	1	UP	*Ascidiella aspersa*	2	
			Balanus improvisus	3	x
			Electra pilosa	2	
			Pomatoceros triqueter	2	
		DOWN	*Polysiphonia fucoides*	3	
			Neosiphonia harveyi	2	x
			Molgula sp.	4	
			Balanus improvisus	2	x
			Ulva lactuca	1	

Site	Depth	Plate	Species	Cover	Non-indigenous
			Caprella mutica	2	x
	3	UP	*Sediment*	4	
			Actiniaria indet.	2	
			Balanus improvisus	2	x
			Pomatoceros triqueter	2	
			Botrylloides leachi	2	
			Laomedea longissima	2	
			Ceramium virgatum	2	
			Neosiphonia harveyi	1	x
			Mytilus edulis juvenile	2	
		DOWN	*Ascidiella aspersa*	4	
			Balanus improvisus	2	x
			Pomatoceros triqueter	2	
			Botrylloides leachi	2	
			Laomedea geniculata	2	
			Mytilus edulis juvenile	2	
	7	UP	*Balanus improvisus*	2	x
			Pomatoceros triqueter	2	
			Ulva lactuca	1	
			Metridium senile pallidus	2	
		DOWN	*Ascidia virginea*	4	
			Pomatoceros triqueter	3	
			Balanus improvisus	2	x
			Metridium senile pallidus	2	
			Botrylloides leachi	2	
			Hydroida indet. død	1	
E4	1	UP	*Pomatoceros triqueter*	2	
			Ascidiella aspersa	2	
			Mytilus sp	2	
			Botrylloides leachi	2	
			Balanus improvisus	2	x
			Semibalanus balanoides juvenile	2	
			Tubularia indivisa død	2	
			Polysiphonia fucoides	1	
		DOWN	*Diadumene lineata*	2	X
			Ascidiella aspersa	4	
	3	UP	*Botrylloides leachi*	2	
			Botryllus schlosseri	2	
			Pomatoceros triqueter	2	
			Ascidiella aspersa	2	
			Harmothoe spinifera	1	
			cf. Laomedea geniculata	2	
			Semibalanus balanoides juvenile	2	
			Balanus improvisus	2	x
			Mytilus edulis juvenile	2	
			Tubularia indivisa	2	
			Carcinus maenas	1	
		DOWN	*Ascidiella aspersa*	4	
			Metridium senile pallidus	2	
			Mytilus edulis juvenile	2	
	7	UP	*Sediment*	4	
			Actiniaria indet.	2	
			Laomedea geniculata	2	
			Actiniaria indet.	1	
			Pomatoceros triqueter	2	
			Balanus improvisus	2	x
			Electra pilosa	2	
		DOWN	*Ascidiella aspersa*	4	

Site	Depth	Plate	Species	Cover	Non-indigenous
			Pomatoceros triqueter	2	
			Balanus improvisus	2	x
			Caprella sp.	1	
E5	1	UP	*Carcinus maenas*	1	
			Liocarcinus arcuatus	1	
			Metridium senile pallidus	2	
			Balanus improvisus	2	x
			Laomedea geniculata	2	
			Pomatoceros triqueter	2	
		DOWN	*Ceramium cf. cimbricum*	2	
			Ascidia virginea	4	
			Balanus improvisus	2	x
			Laomedea longissima	2	
			Caprella mutica	2	x
			Lanice conhilega	1	
			Amphipoda/Isopoda sp.	2	
	3	UP	*Pomatoceros triqueter*	2	
			Sediment	4	
			Carcinus maenas	1	
			Diadumene lineata	2	x
			Carcinus maenas		
		DOWN	*Ascidiella aspersa*	3	
			Laomedea geniculata	2	
			Botrylloides leachi	2	
			Bugula purpurotincta	2	
			Balanus improvisus	2	x
			Ciona intestinalis	2	
			Molgula manhattensis	1	x
	7	UP	*Sediment*	4	
			Pomatoceros triqueter	2	
			Balanus improvisus	2	x
			Actiniaria indet.	2	
		DOWN	*Ascidiella aspersa*	4	
			Balanus improvisus	2	x
			Pomatoceros triqueter	2	
			Ciona intestinalis juvenile	2	
			Botrylloides leachi	2	
			Botryllus schlosseri	2	
			Laomedea geniculata	2	
E6	1	UP	*Laomedea geniculata*	2	
			Diadumene lineata	2	x
			Electra pilosa	2	
			Ascidiella aspersa	3	
			Laomedea longissima	2	
		DOWN	*Ascidiella aspersa*	4	
			Electra pilosa	2	
			Pomatoceros triqueter	2	
			Diadumene lineata	2	x
			Balanus improvisus	2	x
			Botryllus schlosseri	2	
			Botrylloides leachi	2	
			Laomedea geniculata	2	
	3	UP	*Pomatoceros triqueter*	2	
			Balanus improvisus	2	x
			Botrylloides leachi	2	
			Botryllus schlosseri	2	
			Ascidiella aspersa	2	
			Electra pilosa	2	

Site	Depth	Plate	Species	Cover	Non-indigenous
			Laomedea longissima	2	
			Laomedea geniculata	2	
			Hydroida indet.	2	
			Bugula purpurotincta	2	
		DOWN	*Laomedea geniculata*	2	
			Ascidiella aspersa	2	
			Semibalanus balanoides juvenile	2	
			Pomatoceros triqueter	2	
			Electra pilosa	2	
			Caprella mutica	2	x
			Isopoda indet.	1	
	7	UP	*Balanus improvisus*	2	x
			Pomatoceros triqueter	1	
			Bugula purpurotincta	1	
		DOWN	*Ascidiella aspersa*	2	
			Caprella mutica	2	x
			Balanus improvisus	2	x
			Pomatoceros triqueter	3	
			Botrylloides leachi	2	
			Botryllus schlosseri	2	
			Bugula purpurotincta	2	
E7	1	UP	*Balanus improvisus*	2	x
			Sediment	4	
			Ulva intestinalis	2	
		DOWN	*Ascidiella aspersa*	2	
			Botryllus schlosseri	2	
			Botrylloides leachi	2	
			Balanus improvisus	2	x
			Pomatoceros triqueter	2	
			Molgula sp.	2	
			cf. Austrominius modestus	2	X
	3	UP	*Balanus improvisus*	2	x
			Ascidiella aspersa	2	
			Pomatoceros triqueter	2	
			Acmaea virginea	1	
		DOWN	*Botrylloides leachi*	2	
			Electra pilosa	2	
			Balanus improvisus	2	x
			Austrominius modestus juvenile	2	x
			Ciona intestinalis juvenile	2	
			Diadumene lineata	1	x
	7	UP	*Pomatoceros triqueter*	2	
			Metridium senile pallidus	2	
			cf. Metridium senile pallidus	2	
			Balanus improvisus	2	x
		DOWN	*Ascidiella aspersa*	2	
			Botrylloides leachi	2	
			Botryllus schlosseri	2	
			Asterias rubens juvenile	1	
			Bugula purpurotincta	2	
			Ciona intestinalis	2	
			Caprella linearis	2	
			Caprella mutica	2	x
E8	1	UP	*Polysiphonia fucoides*	2	
			Ascidiella aspersa	2	
			Balanus improvisus	2	x
		DOWN	*Ascidiella aspersa*	4	
			Pomatoceros triqueter	2	

Site	Depth	Plate	Species	Cover	Non-indigenous
			Botrylloides leachi	2	
			Electra pilosa	2	
	3	UP	*Ascidiella aspersa*	4	
			Balanus improvisus	2	x
			Caprella mutica	2	x
		DOWN	*Pomatoceros triqueter*	3	
			Balanus improvisus	2	x
			Laomedea geniculata	2	
			Harmothoe spinifera	1	
			Acmaea sp.	2	
E9	1	UP	*Sediment*	4	
			Ascidiella aspersa	4	
			Balanus improvisus	2	x
		DOWN	*Ascidiella aspersa*	4	
			Botrylloides leachi	2	
			Balanus improvisus	2	x
			Pomatoceros triqueter	2	
	3	UP	*Ascidiella aspersa*	4	
			cf. Metridium senile pallidus	2	
			Botrylloides leachi	2	
			Hydroida indet. død	2	
			Sediment	4	
		DOWN	*Balanus improvisus*	2	x
			Pomatoceros triqueter	2	
			Ascidiella aspersa	2	
			Acmaea sp.	2	
			Botrylloides leachi	2	
			Molgula sp.	1	
Å1	1	UP	*Botryllus schlosseri*	2	
			Carcinus maenas	1	
			Ciona intestinalis	2	
			Polysiphonia fucoides	2	
			Ceramium cimbricum	2	
			Ulva cf. clathrata	2	
		DOWN	*Ciona intestinalis*	4	
			Botryllus schlosseri	2	
	3	UP	*Botryllus schlosseri*	2	
			Polysiphonia fucoides	2	
		DOWN	*Laomedea geniculata*	2	
			Botryllus schlosseri	2	
			Ciona intestinalis	4	
	7	UP	*Laomedea longissima*	2	
			Ciona intestinalis	2	
			Metridium senile pallidus	2	
			Botryllus schlosseri	2	
		DOWN	*Laomedea geniculata*	2	
			Ciona intestinalis	4	
Å2	1	UP	*Sediment*	4	
			Callithamnion corymbosum	2	
			Metridium senile pallidus	1	
			Polysiphonia fucoides	2	
			Ulva cf. clathrata	1	
		DOWN	*Ciona intestinalis*	4	
			Botryllus schlosseri	2	
			Electra pilosa	2	
			Laomedea longissima	2	
	3	UP	*Ciona intestinalis*	3	
		DOWN	*Ciona intestinalis*	4	

Site	Depth	Plate	Species	Cover	Non-indigenous
	7	UP	*Ciona intestinalis*	2	
			Botryllus schlosseri	2	
			Asterias rubens juvenile	1	
		DOWN	*Halecium beanii*	2	
			Ciona intestinalis	4	
Å3	1	UP	*Sediment*	4	
			Botryllus schlosseri	2	
			Polysiphonia fucoides	2	
			cf. Pylaiella littoralis	1	
			Ulva intestinalis	1	
			Ceramium cimbricum	2	
		DOWN	*Ciona intestinalis*	4	
			Laomedea longissima	2	
	3	UP	*Sediment*	4	
			Ciona intestinalis	3	
			Liocarcinus arcuatus	1	
		DOWN	*Ciona intestinalis*	4	
	7	UP	*Ciona intestinalis*	2	
		DOWN	*Ciona intestinalis*	4	
			Bougainvillia ramosa	2	
Å4	1	UP	*Callithamnion corymbosum*	2	
			Polysiphonia fucoides	2	
			Sediment	4	
			Ciona intestinalis	2	
			Ulva prolifera	1	
		DOWN	*Ciona intestinalis*	4	
			Halecium beanii	2	
	3	UP	*Sediment*	4	
			Callithamnion corymbosum	2	
			Callithamnion cf. byssoides	2	
			Ulva flexuosa	2	
			Metridium senile pallidus	1	
			Mytilus edulis juvenile	2	
			Electra pilosa	3	
		DOWN	*Botryllus schlosseri*	3	
			Halecium beanii	2	
			Callithamnion corymbosum	2	
			Callithamnion cf. byssoides	2	
	7	UP	*Sediment*	4	
			Botryllus schlosseri	2	
			Metridium senile pallidus	1	
			Polysiphonia fucoides	1	
			Pterothamnion plumula	2	
		DOWN	*Ciona intestinalis*	4	
			Laomedea geniculata	2	
			Bougainvillia ramosa	2	
Å5	1	UP	*Strongylocentrotus droebachiensis juve-*	1	
			Sediment	4	
			Botryllus schlosseri	2	
			Balanus improvisus	2	x
			Polysiphonia fucoides	2	
			Callithamnion corymbosum	2	
		DOWN	*Ciona intestinalis*	4	
			Ulva lactuca	2	
			Botryllus schlosseri	1	
			Diadumene lineata	1	x
	3	UP	*Sediment*	3	
			Laomedea geniculata	2	

Site	Depth	Plate	Species	Cover	Non-indigenous
			cf. Scagelia sp.	1	
		DOWN	*Ciona intestinalis*	4	
			Metridium senile pallidus	1	
			Botryllus schlosseri	2	
			Metridium senile pallidus	1	
Å6	1	UP	*Sediment*	4	
			Botryllus schlosseri	2	
			Tubularia larynx	2	
			Asterias rubens juvenile	1	
			Callithamnion corymbosum	1	
			Polysiphonia fucoides	2	
			Bougainvillia ramosa	2	
			Caprella sp.	1	
			Pylaiella littoralis	1	
	1	DOWN	*Ciona intestinalis*	4	
			Botryllus schlosseri	2	
			Hydroida indet.	2	
	3	UP	*Botryllus schlosseri*	2	
			Mytilus edulis	1	
			Ciona intestinalis	2	
			Sediment	3	
			Hydroida indet. død	2	
		DOWN	*Ciona intestinalis*	4	
			Botryllus schlosseri	2	
			Hydroida indet. død	2	
			Metridium senile pallidus	1	
Å7	1	UP	*Polysiphonia fucoides*	2	
			Ceramium strictum	1	
			Ulva intestinalis	2	
			Sediment	4	
			Asterias rubens juvenile	1	
			Ciona intestinalis	2	
			Laomedea longissima	2	
			Halecium beanii	2	
			Caprella cf. mutica	2	x
			Caprella mutica	2	x
			Mytilus edulis juvenile	1	
			Botryllus schlosseri	2	
			Electra pilosa	2	
		DOWN	*Ciona intestinalis*	4	
			Caprella sp.	2	
			Mytilus edulis juvenile	1	
			Balanus improvisus	2	x
			Laomedea geniculata	2	
	3	UP	*Balanus improvisus*	2	x
			Sediment	4	
			Ciona intestinalis	2	
			Botryllus schlosseri	2	
			Mytilus edulis juvenile	2	
		DOWN	*Ciona intestinalis*	4	
			Laomedea longissima	2	
			Mytilus edulis juvenile	2	
	7	UP	*Laomedea longissima*	2	
			Tubularia larynx	2	
			Sediment	4	
			Mytilus edulis juvenile	2	
		DOWN	*Laomedea longissima*	2	
			Tubularia larynx	2	

Site	Depth	Plate	Species	Cover	Non-indigenous
			Ciona intestinalis	4	
			Asterias rubens juvenile	1	
			Metridium senile pallidus	1	
Å8	1	UP	*Sediment*	4	
			Polysiphonia fucoides	2	
			Ulva flexuosa	1	
			Diatome-kjeder	3	
			Bougainvillia ramosa	2	
			Laomedea geniculata	2	
			Laomedea longissima	2	
			Diadumene lineata	2	x
			Macropodia rostrata	1	
			Asterias rubens juvenile	1	
			Psammechinus miliaris juvenile	2	
			Laomedea geniculata	2	
		DOWN	*Ciona intestinalis*	4	
			Diadumene lineata	1	x
			Botrylloides leachi	1	
			Carcinus maenas juvenile	1	
			Bougainvillia ramosa	2	
			Laomedea geniculata	2	
			Laomedea longissima	2	
	3	UP	*Ciona intestinalis*	2	
			Strongylocentrotus droebachiensis juve-	1	
			Laomedea longissima	2	
		DOWN	*Asterias rubens juvenile*	2	
			Carcinus maenas juvenile	1	
			Botryllus schlosseri	2	
			Laomedea geniculata	2	
			Balanus improvisus	1	x
			Ciona intestinalis	4	
			Hydroida indet. død	2	
			Caprella sp.	2	
	7	UP	*Sediment*	3	
		DOWN	*Ciona intestinalis*	4	
			Botryllus schlosseri	2	
			Pomatoceros triqueter	2	
			Caprella mutica	2	x
			Asterias rubens juvenile	2	
			Balanus improvisus	2	x
			Strongylocentrotus droebachiensis juve-	1	
			Flabellina verrucosa	1	
			Liocarcinus arcuatus	1	
Å9	1	UP	*Sediment*	4	
			Polysiphonia fucoides	2	
			Ciona intestinalis	2	
			Ulva flexuosa	1	
		DOWN	*Ciona intestinalis*	4	
			Asterias rubens juvenile	2	
			Pomatoceros triqueter	1	
			Balanus improvisus	1	x
			Molgula cf. occulta	1	
			Diadumene lineata	1	x
	3	UP	*Sediment*	3	
			Laomedea geniculata	2	
			Botryllus schlosseri	2	
			Ceramium cf. cimbricum	1	
			Heterosiphonia japonica	2	X

Site	Depth	Plate	Species	Cover	Non-indigenous
			Polysiphonia fucoides	2	
		DOWN	*Molgula manhattensis*	1	x
			Ciona intestinalis	4	
			Balanus improvisus	1	x

Annex 7: Fish – caught species and their numbers

Fish	Harbor nr and name
Atlantic cod *Gadus morhua*	**Århus industrihavn**
Atlantic herring *Clupea harengus*	Århus lystbådehavn
Atlantic horse mackerel *Trachurus trachurus*	Århus Marselisborg
Atlantic mackerel *Scomber scombrus*	1 Århus havn n=3
Black goby *Gobius niger*	Esbjerg industrihavn
Common dab *Limanda limanda*	**Esbjerg lystbådehavn**
Common roach *Rutilus rutilus*	2 Esbjerg havn n=2
Common sole *Solea solea*	**Aalborg Portland**
Cuckoo wrasse *Symphodus melops*	3 Aalborg Portland n=1
European eel *Anguilla anguilla*	Aalborg industrihavn
European flounder *Platichthys flesus*	**Aalborg lystbådehavn**
European perch *Perca fluviatilis*	Aalborg fjordparken
European plaice *Pleuronectes platessa*	4 Aalborg havn n=3
European pollack *Pollachius pollachius*	Fredericia industrihavn
European sprat *Sprattus sprattus*	**Fredericia lystbådehavn**
Goldsinny wrasse *Ctenolabrus rupestris*	5 Fredericia havn n=2
Greater weever *Trachinus draco*	**Frederikshavn industrihavn**
Longspined bullhead *Taurulus bubalis*	Frederikshavn marina
Pipefishes and seahorses *Syngnathidae*	6 Frederikshavn havn n=2
Rock gunnel *Pholis gunnellus*	**Gedser fiskerihavn**
Round goby ***Neogobius melanostomus***	Gedser lystbådehavn
Saithe *Pollachius virens*	7 Gedser havn n=2
Sea stickleback *Spinachia spinachia*	**Grenaa industrihavn**
Sea trout *Salmo trutta trutta*	Grenaa fiskerihavn
Shorthorn sculpin *Myoxocephalus scorpius*	Grenaa marina
Small-mouthed wrasse *Centrolabrus exoletus*	8 Grenaa havn n=3
Thinlip mullet *Liza ramada*	Helsingør færgehavn
Twaite shad *Alosa fallax*	**Helsingør fiskerihavn**
Two-spotted goby *Gobiusculus flavescens*	Helsingør nordhavn
Viviparous eelpout *Zoarces viviparus*	9 Helsingør havn n=3
Whiting *Merlangius merlangus*	Hirtshals industrihavn
Whiting-pout *Trisopterus luscus*	**Hirtshals fiskerihavn***
Crabs and lobsters	Hirtshals lystbådehavn
Baltic prawn *Palaemon elegans*	10 Hirtshals havn n=3
Common shore crab *Carcinus maenas*	
Edible crab *Cancer pagurus*	
European lobster *Homarus gammarus*	
Long-legged spider crab *Maemopodia rostrata*	
Others	
Common whelk *Buccinum undatum*	
Green sea urchin *Strongylocentrotus droebachiensis*	
Total number of species	
Total number of individuals	
Total number of invasive species	
Total number of invasive individuals	

Harbor nr and name	Fish	Atlantic cod *Gadus morhua*	Atlantic herring *Clupea harengus*	Atlantic horse mackerel *Trachurus trachurus*	Atlantic mackerel *Scomber scombrus*	Black goby *Gobius niger*	Common dab *Limanda limanda*	Common roach *Rutilus rutilus*	Common sole *Solea solea*	Cuckoo wrasse *Symphodus melops*	European eel *Anguilla anguilla*	European flounder *Platichthys flesus*	European perch *Perca fluviatilis*	European plaice *Pleuronectes platessa*	European pollack *Pollachius pollachius*	European sprat *Sprattus sprattus*	Goldsinny wrasse *Ctenolabrus rupestris*	Greater weever *Trachinus draco*	Longspined bullhead *Taurulus bubalis*	Pipefishes and seahorses *Syngnathidae*	Rock gunnel *Pholis gunnellus*	Round goby ***Neogobius melanostomus***	Saithe *Pollachius virens*	Sea stickleback *Spinachia spinachia*	Sea trout *Salmo trutta trutta*	Shorthorn sculpin *Myoxocephalus scorpius*	Small-mouthed wrasse *Centrolabrus exoletus*	Thinlip mullet *Liza ramada*	Twaite shad *Alosa fallax*	Two-spotted goby *Gobiusculus flavescens*	Viviparous eelpout *Zoarces viviparus*	Whiting *Merlangius merlangus*	Whiting-pout *Trisopterus luscus*	Crabs and lobsters	Baltic prawn *Palaemon elegans*	Common shore crab *Carcinus maenas*	Edible crab *Cancer pagurus*	European lobster *Homarus gammarus*	Long-legged spider crab *Maemopodia rostrata*	Others	Common whelk *Buccinum undatum*	Green sea urchin *Strongylocentrotus droebachiensis*	Total number of species	Total number of individuals	Total number of invasive species	Total number of invasive individuals
Århus industrihavn		4				2				6		1										38																												
Århus lystbådehavn		27								3							46					46																												
Århus Marselisborg		1														1	25					25																												
1 Århus havn n=3		32				2				9		2				1	109					109					2																						
Esbjerg industrihavn											5																																						
Esbjerg lystbådehavn											2			3																																		
2 Esbjerg havn n=2		0				0				0	7	0		3		0	0					0					0																				
Aalborg Portland						1																1					1																				
3 Aalborg Portland n=1		0				0				0		0				0	0					0					0																				
Aalborg industrihavn																																															
Aalborg lystbådehavn												5																																			
Aalborg fjordparken																																															
4 Aalborg havn n=3		0				0				0		5				0	0					0					0																				
Fredericia industrihavn		8				1						2				1	11					1																										
Fredericia lystbådehavn		30										1					49																														
5 Fredericia havn n=2		38				1						3					60																														
Frederikshavn industrihavn		2										2				1	3			1																												
Frederikshavn marina		2				1						1									1																												
6 Frederikshavn havn n=2		4				1						3					3				1	1																										
Gedser fiskerihavn		2				1						3		8								1																										
Gedser lystbådehavn						1						1		12								7																										
7 Gedser havn n=2		2				0				0		4		20			0					0																										
Grenaa industrihavn		8				3				1		2					30					16																										
Grenaa fiskerihavn		5								3		1																																				
Grenaa marina		6																																													
8 Grenaa havn n=3		19				0				0		4					46					84					7																				
Helsingør færgehavn		7																				1																										
Helsingør fiskerihavn		2				2				3		2					7					1					10																				
Helsingør nordhavn		7				1						1					1					5					20																				
9 Helsingør havn n=3		16				0				1		3					92					0					30																				
Hirtshals industrihavn		4				2						3					2					1																										
Hirtshals fiskerihavn*										5												15					8																				
Hirtshals lystbådehavn		1										1										16					8																				
10 Hirtshals havn n=3		5				2				0		1					2					0					8																				
		1	166	105	149	419	18	4	6	7	7	7	2	5	1	6	9	8	11	11	11	16	7	6	8	2	8	11	7	7	14	6	11	9	15	10	5	9	16
		0	12	8	11	18	4	6	7	7	7	2	5	1	6	9	8	11	11	11	16	7	6	8	2	8	11	7	7	14	6	11	9	15	10	5	9	16
		0	29	18	104	151	290	126	416	45	45	292	193	85	569	53	135	188	66	141	207	184	74	258	42	76	165	283	5	11	21	37	61	51	75	187
		0	1			0			0						0			0	1	1	2			0				0				0					12
		0				0			0						0			0		56	56			0				0				0					0
		0				0			0						0			0						0				0				0					0
		0				0		7	7				1		0			0						0				0				0					0
		1				0			0						0			0						0				0				0					0
		0				0			0						0			0						0				0				0					0
		0				0			0						0			0						0				0				0					0

BOLD font indicates invasive species and harbours locations closest to the sampling points of eDNA/ragndring

* Do to insufficient space, it was only possible to deploy the fykenet in Hirtshals fiskerihavn

	Harbor nr and name	Kalundborg industrihavn	Kalundborg Gisseløre	Kalundborg Vesthavnen	11 Kalundborg havn n=3	Københavns industrihavn	København Nordhavnen	**København lystbådehavn**	12 Københavns havn n=3	**Køge industrihavn**	Køge marina	13 Køge havn n=2	Odense Fynsværket	Odense Centrum	14 Odense havn n=2	Rødby industrihavn	**Rødby fiskerihavn**	15 Rødby havn n=2	Kalundborg Statoil	16 Kalundborg Statiol n=1
Fish																				
Atlantic cod	*Gadus morhua*	5			5		4		4			0	1	6	7	5	2	7	1	1
Atlantic herring	*Clupea harengus*		1	8	9				0	3		3	2		2	9	27	36	1	1
Atlantic horse mackerel	*Trachurus trachurus*	1			1				0			0			0			0		0
Atlantic mackerel	*Scomber scombrus*				0				0			0			0			0		0
Black goby	*Gobius niger*			1	1	3	2	1	6			0	3	1	4			0		0
Common dab	*Limanda limanda*				0	1			1			0			0			0		0
Common roach	*Rutilus rutilus*				0				0	155	52	207			0			0		0
Common sole	*Solea solea*				0				0			0			0			0		0
Corkwing wrasse	*Symphodus melops*				0				0			0			0			0		0
European eel	*Anguilla anguilla*		1	1	2	3	1		4		1	1			0	4		4		0
European flounder	*Platichthys flesus*				0	2			2			0		1	1	1	2	3		0
European perch	*Perca fluviatilis*				0				0	6	6	12			0		1	1		0
European plaice	*Pleuronectes platessa*				0				0			0			0			0		0
European pollack	*Pollachius pollachius*				0				0			0			0			0		0
European sprat	*Sprattus sprattus*				0				0			0	2	2	4			0		0
Goldsinny wrasse	*Ctenolabrus rupestris*				0		7	23	30			0			0			0	1	1
Greater weever	*Trachinus draco*				0				0			0		1	1			0		0
Longspined bullhead	*Taurulus bubalis*				0				0			0			0			0		0
Pipefishes and seahorses	*Syngnathidae*				0				0			0			0			0		0
Rock gunnel	*Pholis gunnellus*				0				0			0			0			0		0
Round goby	***Neogobius melanostomus***				0				0	**6**	**3**	**9**			0			0		0
Saithe	*Pollachius virens*				0	5	17	36	58		1	1			0		1	1		0
Sea stickleback	*Spinachia spinachia*		1	1	2				0			0			0			0		0
Sea trout	*Salmo trutta trutta*	1			1				0			0	2		2	1		1		0
Shorthorn sculpin	*Myoxocephalus scorpius*	2			2			1	1			0			0			0	8	8
Small-mouthed wrasse	*Centrolabrus exoletus*				0				0			0			0			0		0
Thinlip mullet	*Liza ramada*				0				0			0			0			0		0
Twaite shad	*Alosa fallax*				0				0			0			0			0		0
Two-spotted goby	*Gobiusculus flavescens*				0				0			0			0			0		0
Viviparous eelpout	*Zoarces viviparus*	3		2	5		1		1		2	2	1		1	1	3	4		0
Whiting	*Merlangius merlangus*				0				0			0			0			0		0
Whiting-pout	*Trisopterus luscus*				0				0			0			0			0		0
Crabs and lobsters					0				0			0			0			0		0
Baltic prawn	*Palaemon adspersus*				0		3	4	7		7	7	1		1			0		0
Common shore crab	*Carcinus maenas*	93	20	15	128	31	32	19	82	10	26	36	12	6	18	429	397	826	26	26
Edible crab	*Cancer pagurus*				0				0			0			0			0		0
European lobster	*Homarus gammarus*				0				0			0			0			0		0
Long-legged spider crab	*Macropodia rostrata*				0				0			0			0			0		0
Others					0				0			0			0			0		0
Common whelk	*Buccinum undatum*				0				0			0			0			0		0
Green sea urchin	*Strongylocentrotus droebachiensis*				0				0			0			0			0		0
Total number of species		6	4	6	10	6	8	6	11	5	8	9	8	6	10	7	7	9	5	5
Total number of individuals		105	23	28	156	45	67	84	196	180	98	278	24	17	41	450	433	883	37	37
Total number of invasive species					0				0	1	1	2			0			0		0
Total number of invasive individuals					0				0	6	3	9			0			0		0

BOLD font indicates invasive species and harbours locations closest to the sampling points of eDNA/nightdiving

* Do to insufficient space, it was only possible to deploy the fykenet in Hirtshals fiskerihavn

Annex 8: Snorkeling - Abundance, CPUE ('catch per unit effort'), number pr. m² of fish and selected invertebrates and algae in Danish harbours

Annex 7A: Results from Arhus, Esbjerg, Aalborg Portland and Aalborg Harbours.

Fish		**Aarhus Havn** 05.07 2017 Day	**Aarhus Havn** 19.09 2017 Day	**Esbjerg Havn** 08.11 2007 Night	**Aalborg Portland** 11.10 2017 Day	**Aalborg Havn** 11.10.2017 Day
Pogge	*Agonus cataphractus*			*		
Lesser sand-eel	*Ammodytes tobianus*					
Eel	*Anguilla anguilla*		0.001			
Transparent goby	*Aphia minuta*					
Garfish	*Belone belone*					
Common dragonet	*Callionymus lyra*					
Herring	*Clupea harengus*					
Goldsinny wrasse	*Ctenolabrus rupestris*	0.250	0.400			
Lumpsucker	*Cyclopterus lumpus*				0.001	
Cod	*Gadus morhua*	0.001	0.001	*		
Three-spined stickleback	*Gasterosteus aculeatus*		0.015		0.004	0.001
Black goby	*Gobius niger*	0.003	0.001			0.001
Two-spotted goby	*Gobiusculus flavescens*	0.100	15.000		0.143	0.01
Greater sand-eel	*Hyperoplus lanceolatus*	0.001				
Dab	*Limanda limanda*			*		
Whiting	*Merlangius merlangus*			*		
Short-horn sculpin	*Myoxocephalus scorpius*			*		
Round goby	***Neogobius melanostomus***					
Straight-nosed pipefish	*Nerophis ophidion*					*
Perch	*Perca fluviatilis*					
Butterfish	*Pholis gunnellus*					
Flounder	*Platichthys flesus*	0.002		*	0.001	0.001

Fish		**Aarhus Havn** 05.07 2017 Day	**Aarhus Havn** 19.09 2017 Day	**Esbjerg Havn** 08.11 2007 Night	**Aalborg Portland** 11.10 2017 Day	**Aalborg Havn** 11.10.2017 Day
Plaice	*Pleuronectes platessa*			*		
Saithe	*Pollachius virens*					
Common goby	*Pomatoschistus microps*	0.020		*	7.143	0.05
Sand goby	*Pomatoschistus minutus*	0.001		*	0.143	0.2
Painted goby	*Pomatoschistus pictus*					
Nine-spined stickleback	*Pungitius pungitius*					
Sea trout	*Salmo trutta*					
Turbot	*Scophthalmus maximus*			*		
Brill	*Scophthalmus rhombus*					
Common sole	*Solea solea*					
Sea stickleback	*Spinachia spinachia*		0.001			*
Sprat	*Sprattus sprattus*			*		
Corkwing wrasse	*Symphodus melops*	0.100	0.600			
Great pipefish	*Syngnathus acus*					
Nilsson's pipefish	*Syngnathus rostellatus*		0.001			
Broadnosed pipefisk	*Syngnathus typhle*				*	0.001
Sea scorpion	*Taurulus bubalis*					
Greater weever	*Trachinus draco*					
Viviparous eelpout	*Zoarces viviparus*					
Non-fish						
Pacific oyster	***Crassostrea gigas***			*	0.143	0.001
Common slipper shell	***Crepidula fornicata***			*	0.007	0.01
Atlantic razor clam	***Ensis directus***	0.001				
Warty comp jelly	***Mnemiopsis leidyi***		0.001	*	1.429	0.001
Sargassum seaweed	***Sargassum muticum***	0.200	0.250			
Stalked sea squirt	***Styela clava***				0.071	0.001

** caught by other methods than snorkelling e.g. push net, angling.*

A7B: Results from Fredericia, Frederikshavn and Grenå Harbours.

Fish		**Fredericia** 05.07 2017 Day	**Fredericia** 11.10 2017 Day	**Frederikshavn** 06.10 2017 Day	**Gedser Havn** 23.09 2017 Day	**Grenå Havn** 05.07 2017 Day	**Grenå Havn** 19.09 2017 Day
Pogge	*Agonus cataphractus*	0.004					
Lesser sand-eel	*Ammodytes tobianus*			0.004			
Eel	*Anguilla anguilla*				0.001	0.001	0.001
Transparent goby	*Aphia minuta*	0.001					0.100
Garfish	*Belone belone*						
Common dragonet	*Callionymus lyra*		0.003				
Herring	*Clupea harengus*						
Goldsinny wrasse	*Ctenolabrus rupestris*	0.038	0.188			0.350	0.075
Lumpsucker	*Cyclopterus lumpus*						
Cod	*Gadus morhua*	0.001	0.001			0.002	
Three-spined stickleback	*Gasterosteus aculeatus*	0.038	*	0.013	0.125		0.250
Black goby	*Gobius niger*	0.003	0.008		0.003	0.003	0.003
Two-spotted goby	*Gobiusculus flavescens*	0.019	6.250		3.125	0.030	15.000
Greater sand-eel	*Hyperoplus lanceolatus*						
Dab	*Limanda limanda*	0.009					
Whiting	*Merlangius merlangus*						
Short-horn sculpin	*Myoxocephalus scorpius*					0.001	0.001
Round goby	***Neogobius melanostomus***				0.031		
Straight-nosed pipefish	*Nerophis ophidion*				0.001		0.001
Perch	*Perca fluviatilis*						
Butterfish	*Pholis gunnellus*						0.001
Flounder	*Platichthys flesus*	0.001		0.001	0.001	0.005	0.001
Plaice	*Pleuronectes platessa*						
Saithe	*Pollachius virens*						
Common goby	*Pomatoschistus microps*		0.188	0.375	0.375		0.300
Sand goby	*Pomatoschistus minutus*	0.006	0.125	0.625	0.313		0.040
Painted goby	*Pomatoschistus pictus*		0.063		0.001		
Nine-spined stickleback	*Pungitius pungitius*				0.001		

Fish		**Fredericia** 05.07 2017 Day	**Fredericia** 11.10 2017 Day	**Frederikshavn** 06.10 2017 Day	**Gedser Havn** 23.09 2017 Day	**Grenå Havn** 05.07 2017 Day	**Grenå Havn** 19.09 2017 Day
Sea trout	*Salmo trutta*		0.001				0.001
Turbot	*Scophthalmus maximus*						
Brill	*Scophthalmus rhombus*					0.001	
Common sole	*Solea solea*					0.002	
Sea stickleback	*Spinachia spinachia*		0.004	0.006	0.005	0.001	0.004
Sprat	*Sprattus sprattus*						
Corkwing wrasse	*Symphodus melops*		0.004			0.150	0.500
Great pipefish	*Syngnathus acus*					0.002	
Nilsson's pipefish	*Syngnathus rostellatus*		0.001	0.001			
Broadnosed pipefisk	*Syngnathus typhle*		0.001	0.013	0.031		0.006
Sea scorpion	*Taurulus bubalis*		*			0.001	
Greater weever	*Trachinus draco*					0.001	
Viviparous eelpout	*Zoarces viviparus*			0.001			
Non-fish							
Pacific oyster	***Crassostrea gigas***						
Common slipper shell	***Crepidula fornicata***						
Atlantic razor clam	***Ensis directus***						
Warty comp jelly	***Mnemiopsis leidyi***		0.063	0.001	0.344		0.100
Sargassum seaweed	***Sargassum muticum***					0.050	0.050
Stalked sea squirt	***Styela clava***						

** caught by other methods than snorkelling e.g. push net, angling.*

A7C: Results from Helsingør Harbour.

Fish		**Helsingør Havn** 11.07 2017 Night	**Helsingør Havn** 31.08 2017 Day	**Helsingør Havn** 31.08 2017 Night	**Helsingør Havn** 01.09 2017 Day	**Helsingør Havn** 13.09 2017 Day
Pogge	*Agonus cataphractus*	1.000				
Lesser sand-eel	*Ammodytes tobianus*		0.002	0.005		
Eel	*Anguilla anguilla*				0.002	
Transparent goby	*Aphia minuta*					
Garfish	*Belone belone*					
Common dragonet	*Callionymus lyra*		0.002	0.003		
Herring	*Clupea harengus*		0.030		0.008	0.070
Goldsinny wrasse	*Ctenolabrus rupestris*					
Lumpsucker	*Cyclopterus lumpus*			0.02	0.005	
Cod	*Gadus morhua*	0.004				0.300
Three-spined stickleback	*Gasterosteus aculeatus*	0.050	0.001	0.001	0.002	0.020
Black goby	*Gobius niger*	0.003	0.050		0.083	5.000
Two-spotted goby	*Gobiusculus flavescens*		0.500		0.017	
Greater sand-eel	*Hyperoplus lanceolatus*					
Dab	*Limanda limanda*					
Whiting	*Merlangius merlangus*		0.001	0.001		
Short-horn sculpin	*Myoxocephalus scorpius*					
Round goby	***Neogobius melanostomus***	0.003				
Straight-nosed pipefish	*Nerophis ophidion*					
Perch	*Perca fluviatilis*					
Butterfish	*Pholis gunnellus*	0.001		0.01		
Flounder	*Platichthys flesus*			0.005	0.002	
Plaice	*Pleuronectes platessa*		0.200	0.1	0.333	0.030
Saithe	*Pollachius virens*	0.009				0.010
Common goby	*Pomatoschistus microps*	0.025	0.010	0.04		0.400
Sand goby	*Pomatoschistus minutus*		0.002			
Painted goby	*Pomatoschistus pictus*					
Nine-spined stickleback	*Pungitius pungitius*		0.001	0.002		

Fish		**Helsingør Havn** 11.07 2017 Night	**Helsingør Havn** 31.08 2017 Day	**Helsingør Havn** 31.08 2017 Night	**Helsingør Havn** 01.09 2017 Day	**Helsingør Havn** 13.09 2017 Day
Sea trout	*Salmo trutta*					
Turbot	*Scophthalmus maximus*		0.001			
Brill	*Scophthalmus rhombus*				0.002	
Common sole	*Solea solea*		0.020	0.01		0.300
Sea stickleback	*Spinachia spinachia*					
Sprat	*Sprattus sprattus*	0.063	0.050	0.003	0.008	
Corkwing wrasse	*Symphodus melops*			0.002	0.002	
Great pipefish	*Syngnathus acus*		0.003			
Nilsson's pipefish	*Syngnathus rostellatus*	0.001	0.005			0.003
Broadnosed pipefisk	*Syngnathus typhle*		0.002	0.002		
Sea scorpion	*Taurulus bubalis*		0.005	0.01		0.003
Greater weever	*Trachinus draco*			0.005	0.002	
Viviparous eelpout	*Zoarces viviparus*					
Non-fish						0.001
Pacific oyster	***Crassostrea gigas***					
Common slipper shell	***Crepidula fornicata***		0.005			
Atlantic razor clam	***Ensis directus***			0.001		
Warty comp jelly	***Mnemiopsis leidyi***					
Sargassum seaweed	***Sargassum muticum***					
Stalked sea squirt	***Styela clava***	1.000				

** caught by other methods than snorkelling e.g. push net, angling.*

A7D: Results from Hirtshals, Kalundborg, København, Køge, Odense, Rådby and Statoil Harbours.

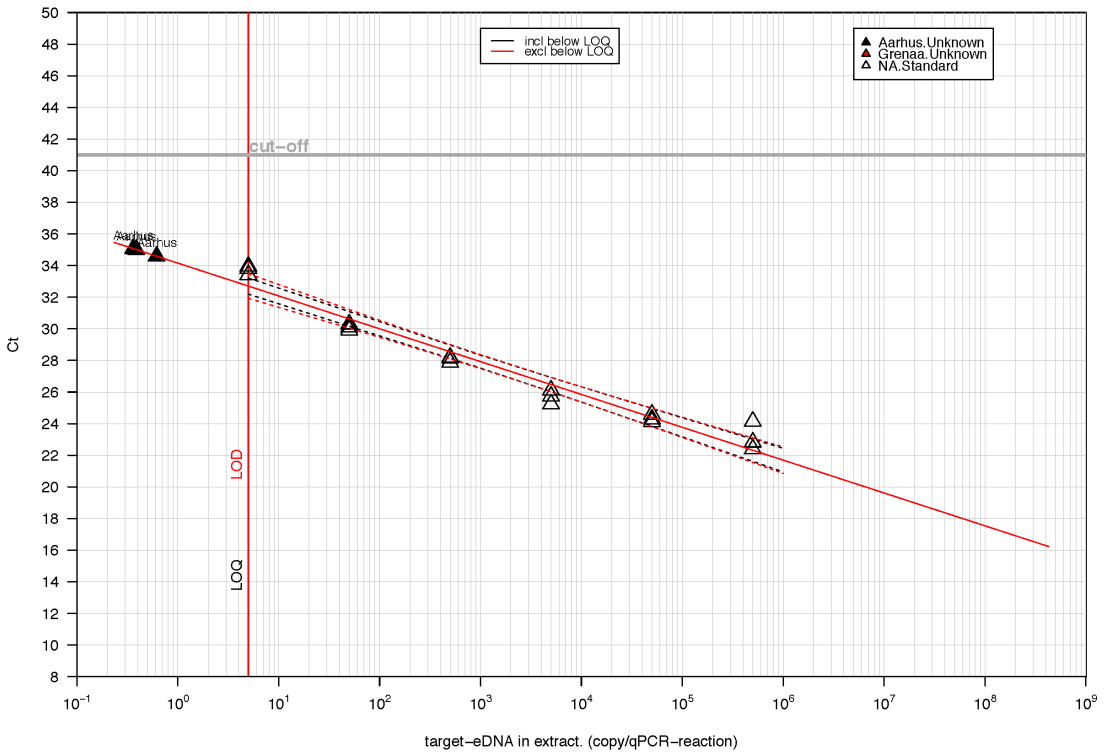
Fish		**Hirtshals** 08.11 2017 Day	**Kalundborg** 22.09 2017 Day	**København** 12.09 2017 Day	**Køge** 12.09 2017 Day	**Odense** 15.09 2017 Night	**Rødby** 23.09 2017 Day	**Statoil** 22.09 2017 Day
Pogge	*Agonus cataphractus*							
Lesser sand-eel	*Ammodytes tobianus*			0.002	0.001	0.008		0.001
Eel	*Anguilla anguilla*							
Transparent goby	*Aphia minuta*						0.003	
Garfish	*Belone belone*							
Common dragonet	*Callionymus lyra*					0.008	16.667	
Herring	*Clupea harengus*		0.050	0.004	0.002			0.100
Goldsinny wrasse	*Ctenolabrus rupestris*							
Lumpsucker	*Cyclopterus lumpus*	*						
Cod	*Gadus morhua*	*	0.001	10.000	0.010	25.000	25.000	0.005
Three-spined stickleback	*Gasterosteus aculeatus*		0.005	0.025	0.010	0.188	0.003	0.030
Black goby	*Gobius niger*		250.000	25.000	25.000		0.833	10.000
Two-spotted goby	*Gobiusculus flavescens*						0.008	
Greater sand-eel	*Hyperoplus lanceolatus*	*						
Dab	*Limanda limanda*							
Whiting	*Merlangius merlangus*							
Short-horn sculpin	*Myoxocephalus scorpius*		0.250	0.001	0.125		0.003	0.070
Round goby	***Neogobius melanostomus***		0.001	0.001			0.002	
Straight-nosed pipefish	*Nerophis ophidion*				0.002			
Perch	*Perca fluviatilis*							
Butterfish	*Pholis gunnellus*		0.002	0.004		0.003	0.002	0.009
Flounder	*Platichthys flesus*	*						
Plaice	*Pleuronectes platessa*	*		0.020				
Saithe	*Pollachius virens*	*	0.025	0.050	0.015	25.000	0.025	0.150
Common goby	*Pomatoschistus microps*	*	0.050	0.750		0.500	0.003	0.002
Sand goby	*Pomatoschistus minutus*							0.010
Painted goby	*Pomatoschistus pictus*			0.010	0.002			
Nine-spined stickleback	*Pungitius pungitius*		0.001			0.025		0.001

Sea trout	*Salmo trutta*							
Turbot	*Scophthalmus maximus*							
Brill	*Scophthalmus rhombus*							
Common sole	*Solea solea*		0.002	0.050	0.001		0.003	0.003
Sea stickleback	*Spinachia spinachia*							
Sprat	*Sprattus sprattus*		0.100	0.010				0.150
Corkwing wrasse	*Symphodus melops*							
Great pipefish	*Syngnathus acus*	*		0.001		0.025		
Nilsson's pipefish	*Syngnathus rostellatus*		0.001	0.006	0.006	0.125	0.003	0.002
Broadnosed pipefisk	*Syngnathus typhle*							
Sea scorpion	*Taurulus bubalis*							
Greater weever	*Trachinus draco*		0.001					0.001
Viviparous eelpout	*Zoarces viviparus*							
Non-fish			0.001	0.001				
Pacific oyster	***Crassostrea gigas***							
Common slipper shell	***Crepidula fornicata***							
Atlantic razor clam	***Ensis directus***		0.005	0.100	0.001		0.008	0.010
Warty comp jelly	***Mnemiopsis leidyi***							
Sargassum seaweed	***Sargassum muticum***							
Stalked sea squirt	***Styela clava***							

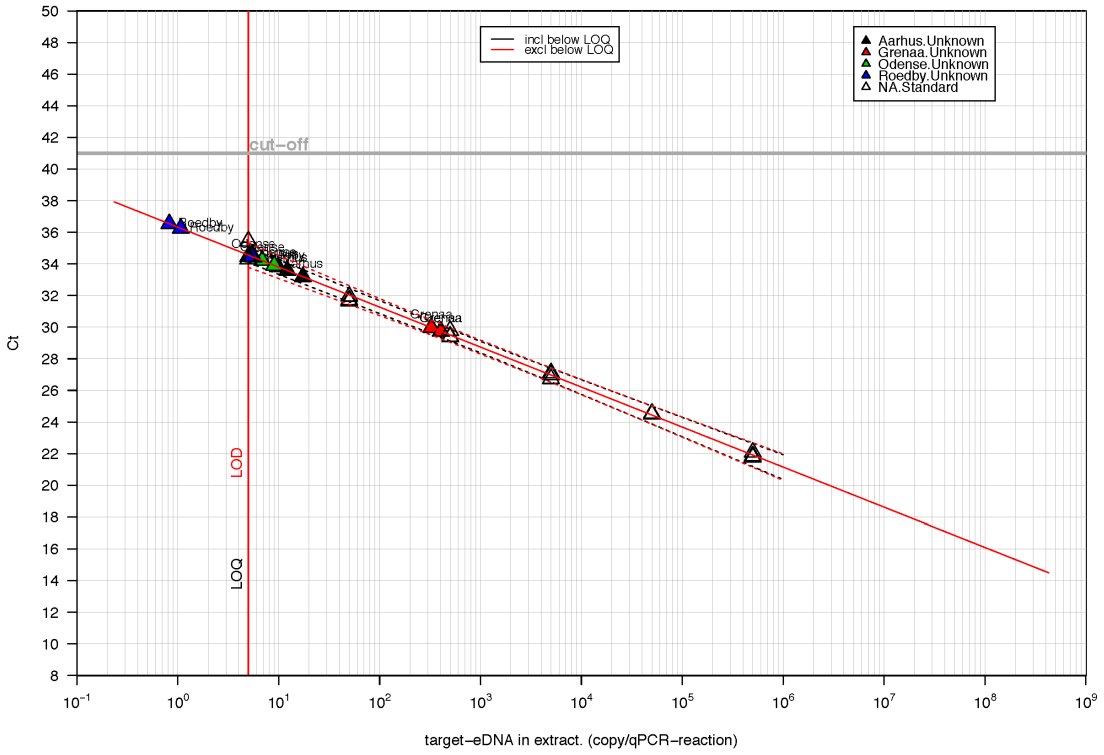
** caught by other methods than snorkelling e.g. push net, angling*

Annex 9: eDNA assay-specific standard curves

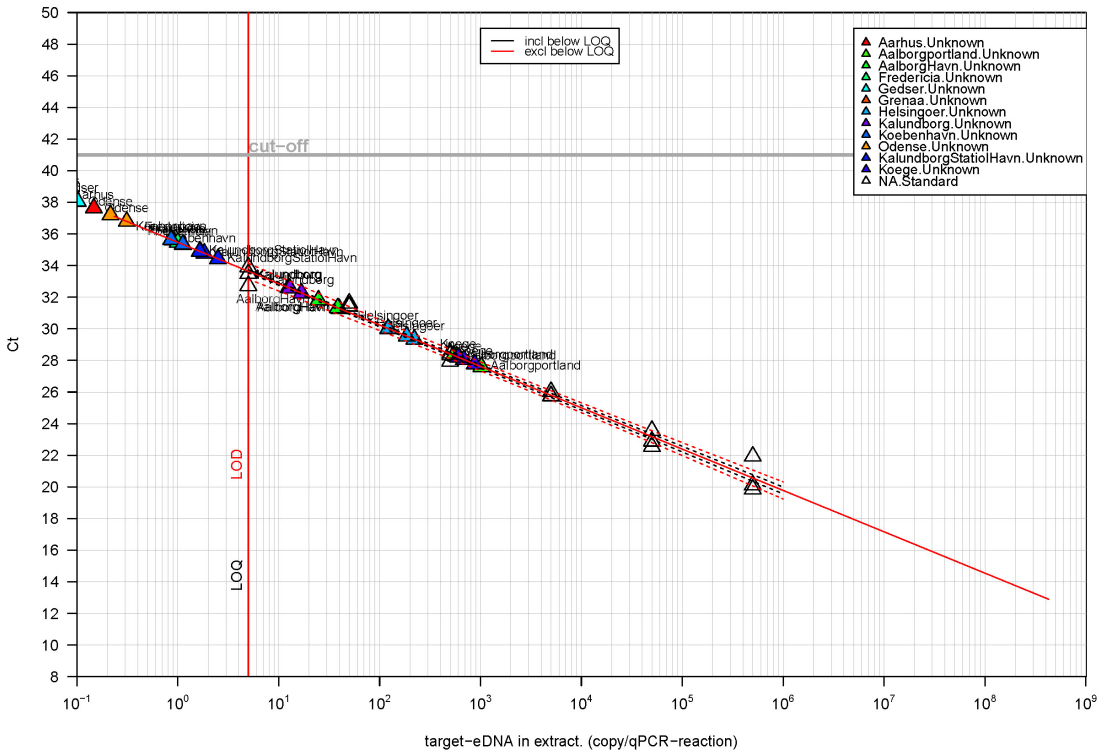
Appendix A 1 . qPCR standard curve for *Bonnemaisonia hamifera* (roedtotalge), AssayNo 1 , spring



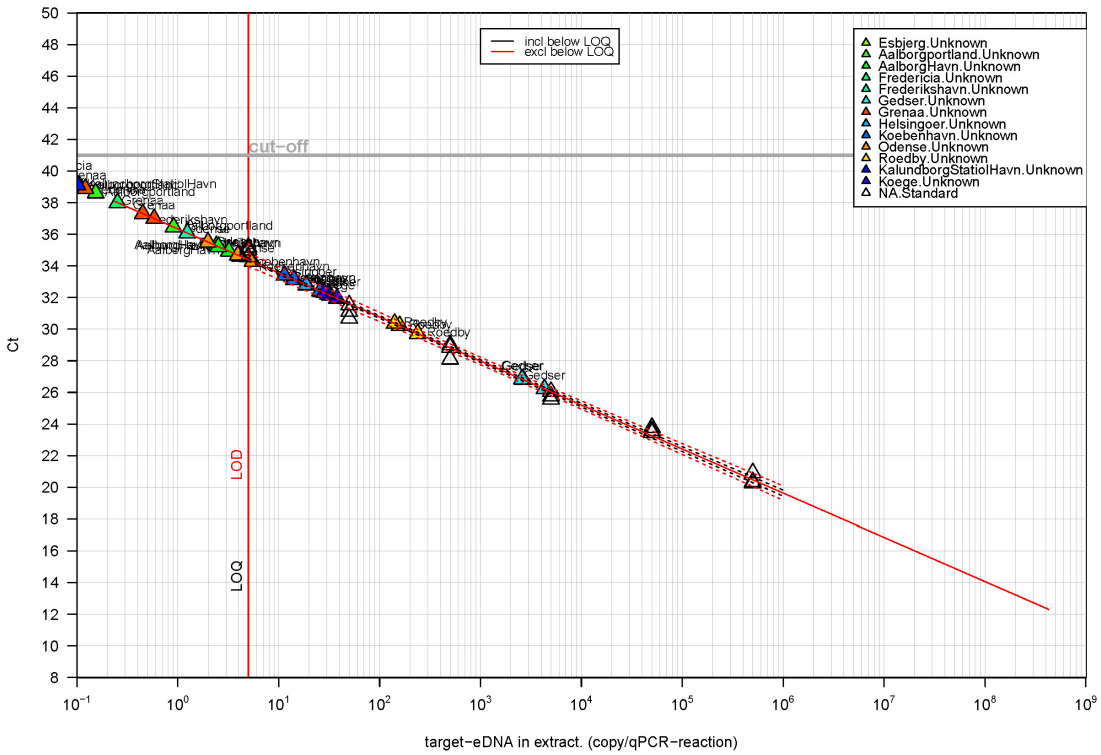
qPCR standard curve for *Bonnemaisonia hamifera* (roedtotalge), AssayNo 1 , autumn



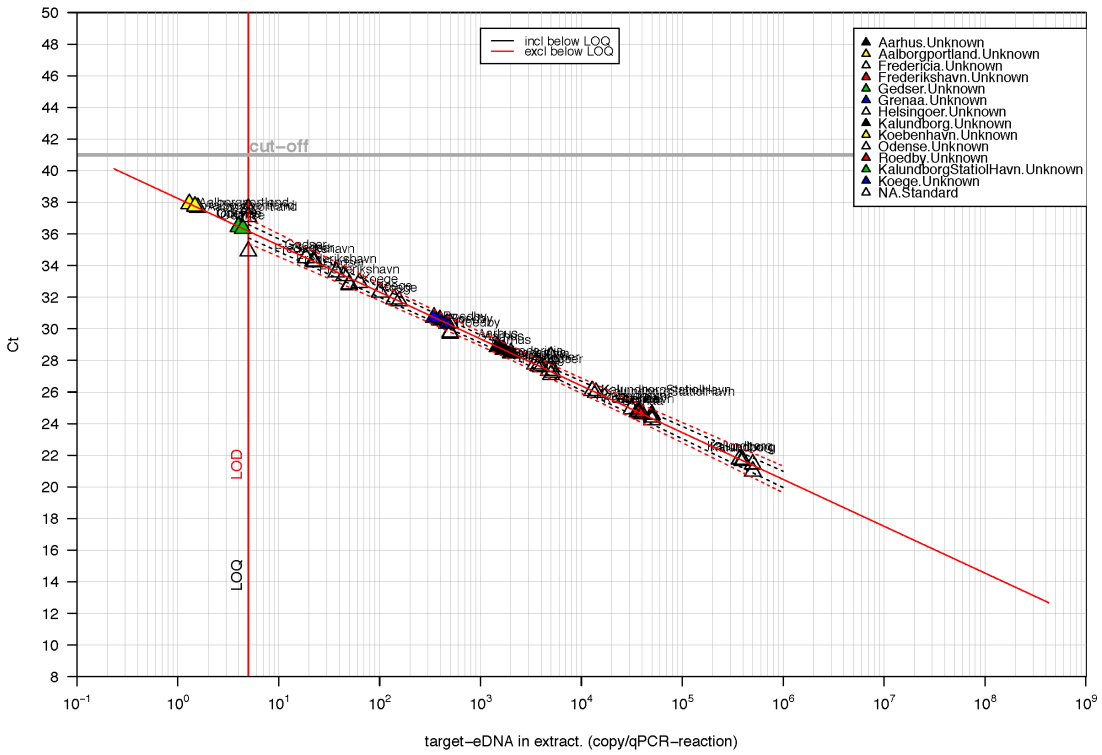
Appendix A 2 . qPCR standard curve for *Prorocentrum cordatum* (dinoflagelat), AssayNo 2 , spring



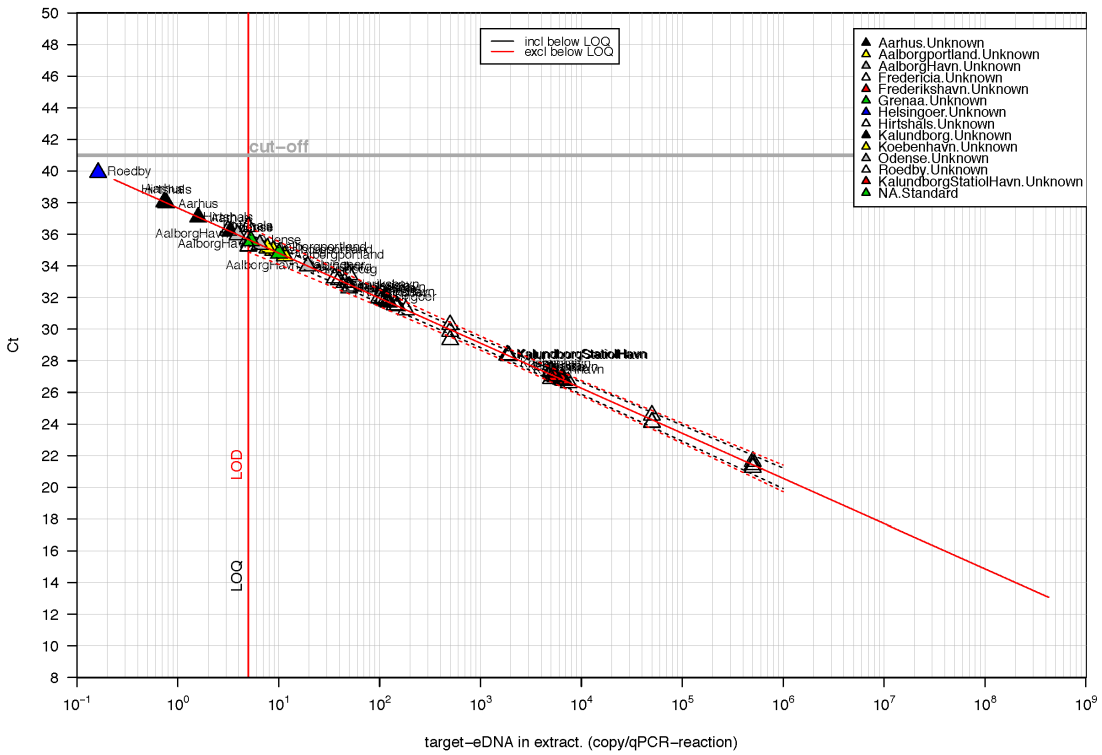
qPCR standard curve for *Prorocentrum cordatum* (dinoflagelat), AssayNo 2 , autumn



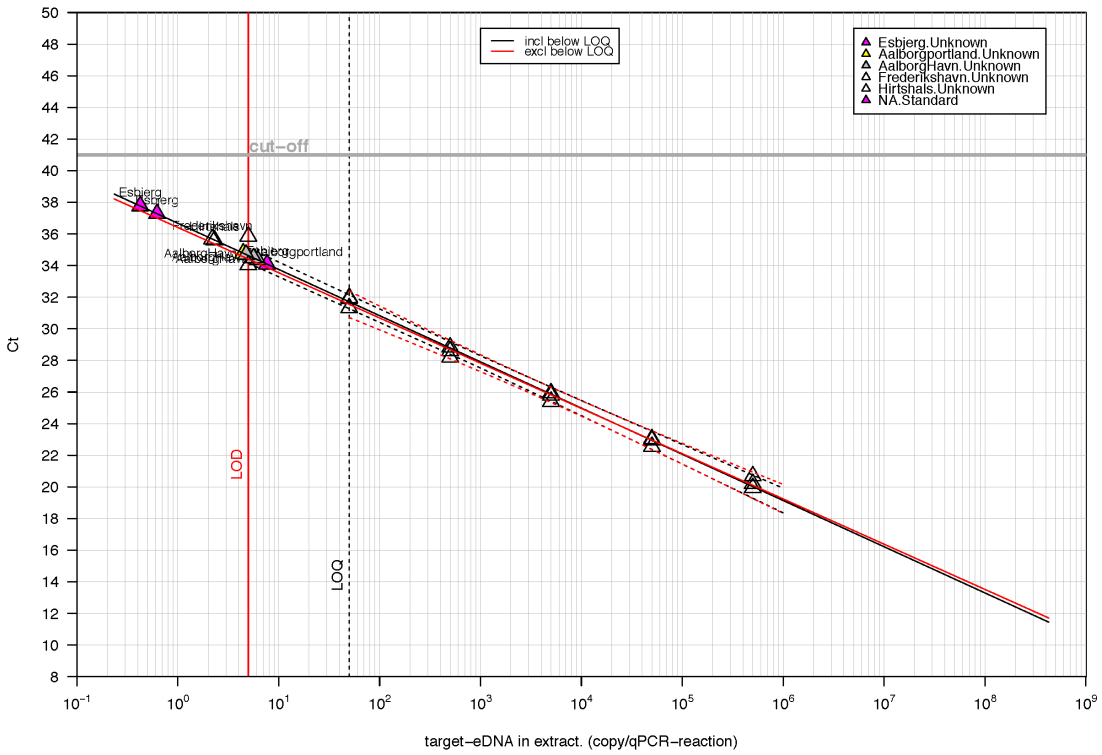
Appendix A 3 . qPCR standard curve for *Pseudochattonella farcimen* (heterokontflagelat), AssayNo 3 , spring



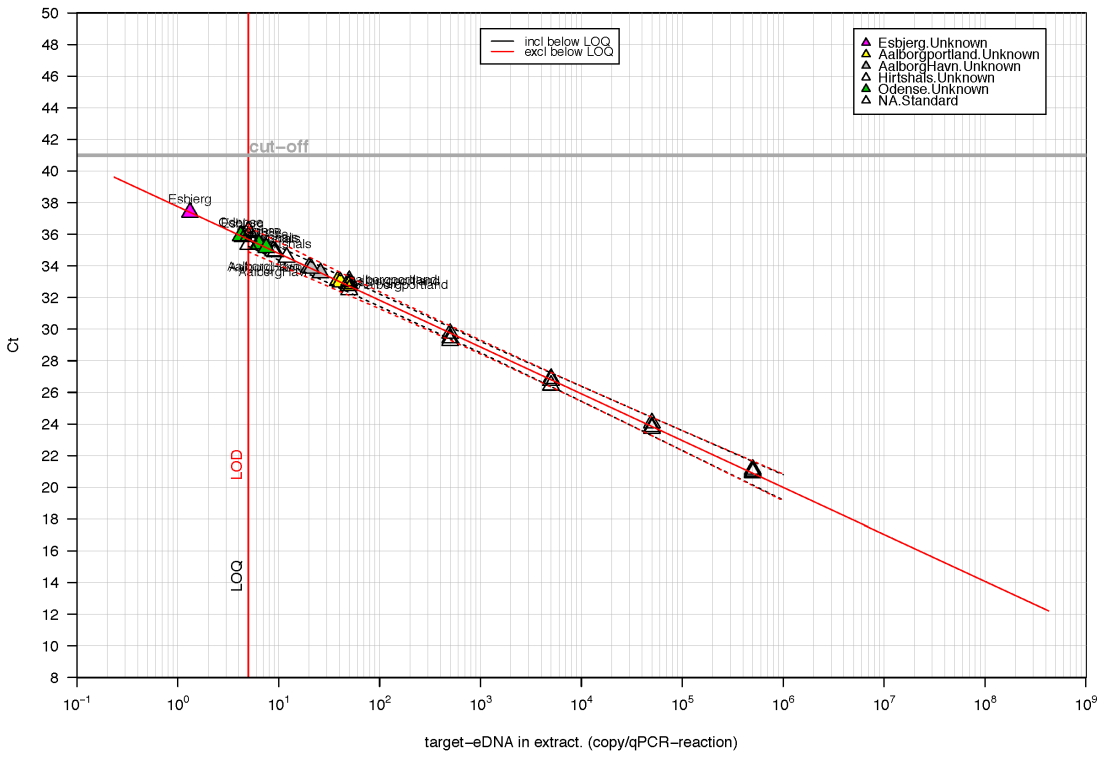
qPCR standard curve for *Pseudochattonella farcimen* (heterokontflagelat), AssayNo 3 , autumn



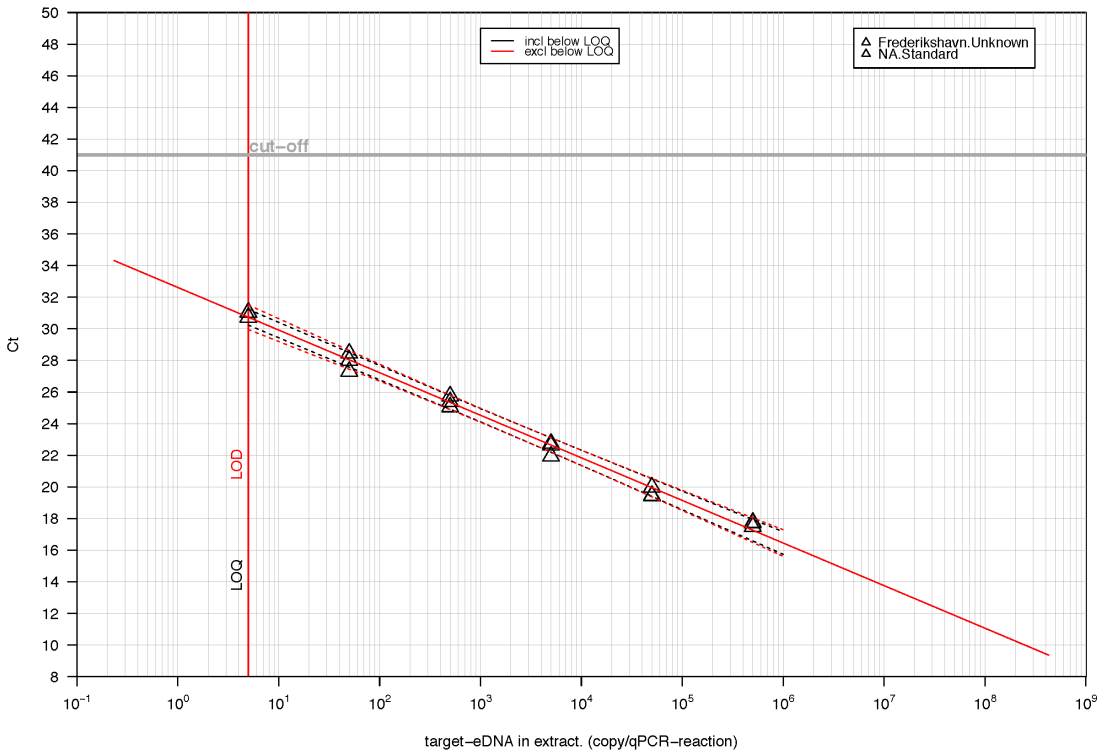
Appendix A 4 qPCR standard curve for *Pseudochattonella verruculosa* (heterokontflagelat), AssayNo 4 , spring



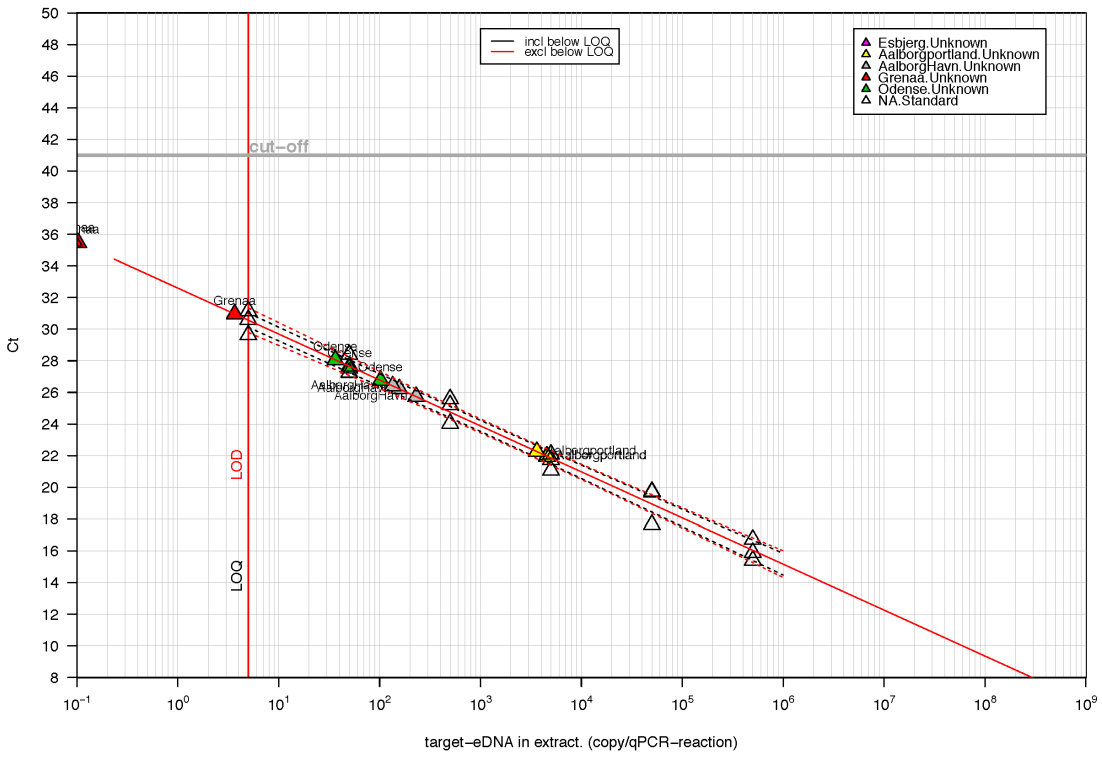
qPCR standard curve for *Pseudochattonella verruculosa* (heterokontflagelat), AssayNo 4 , autumn



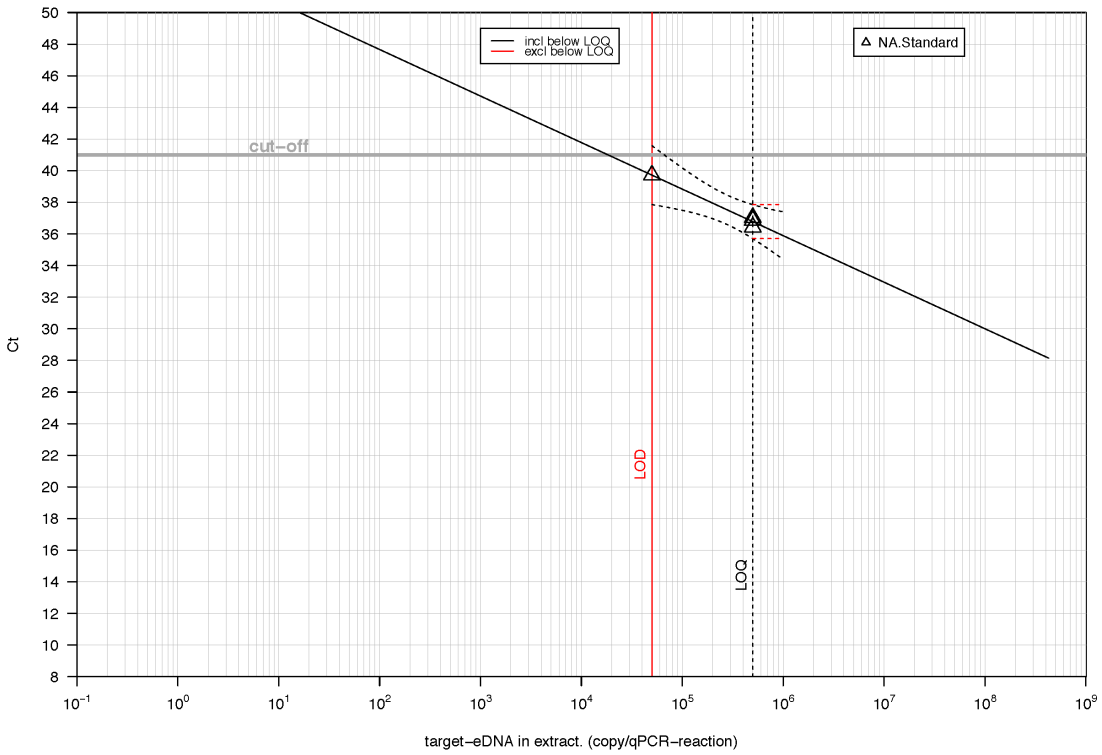
Appendix A 5 . qPCR standard curve for *Karenia mikimotoi* (dinoflagelat), AssayNo 5 , spring



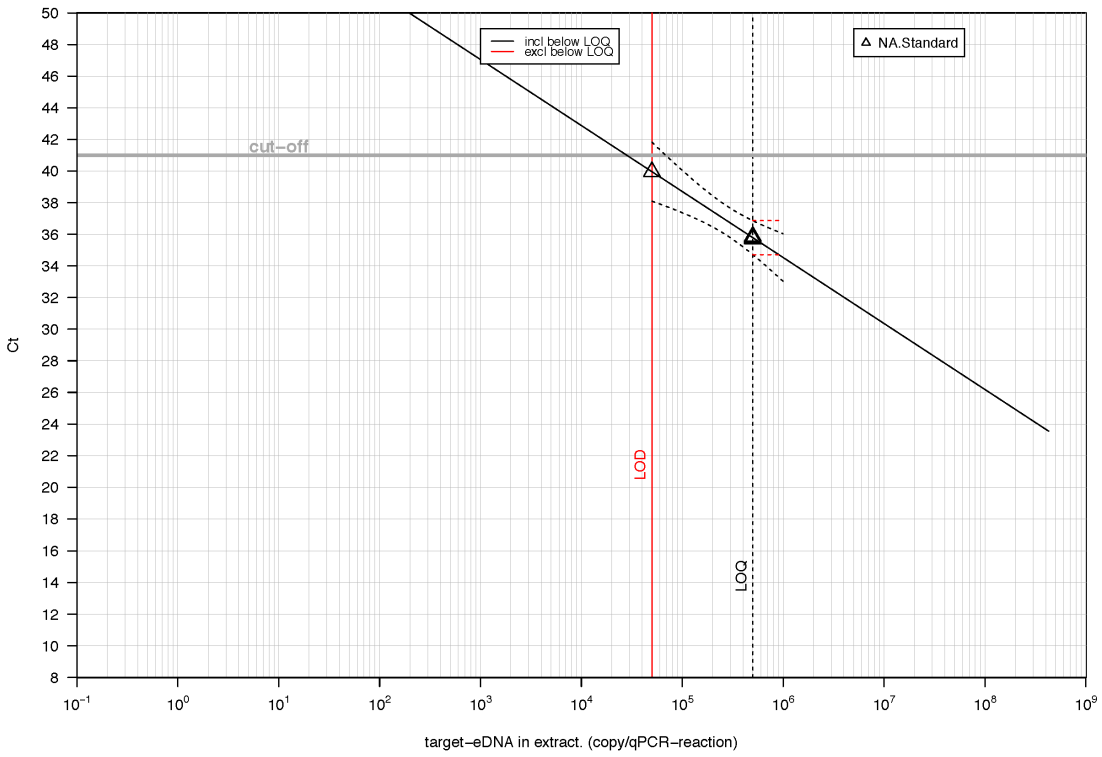
qPCR standard curve for *Karenia mikimotoi* (dinoflagelat), AssayNo 5 , autumn



Appendix A 6 . qPCR standard curve for *Carassius auratus* (soelvkarusse), AssayNo 6 , spring

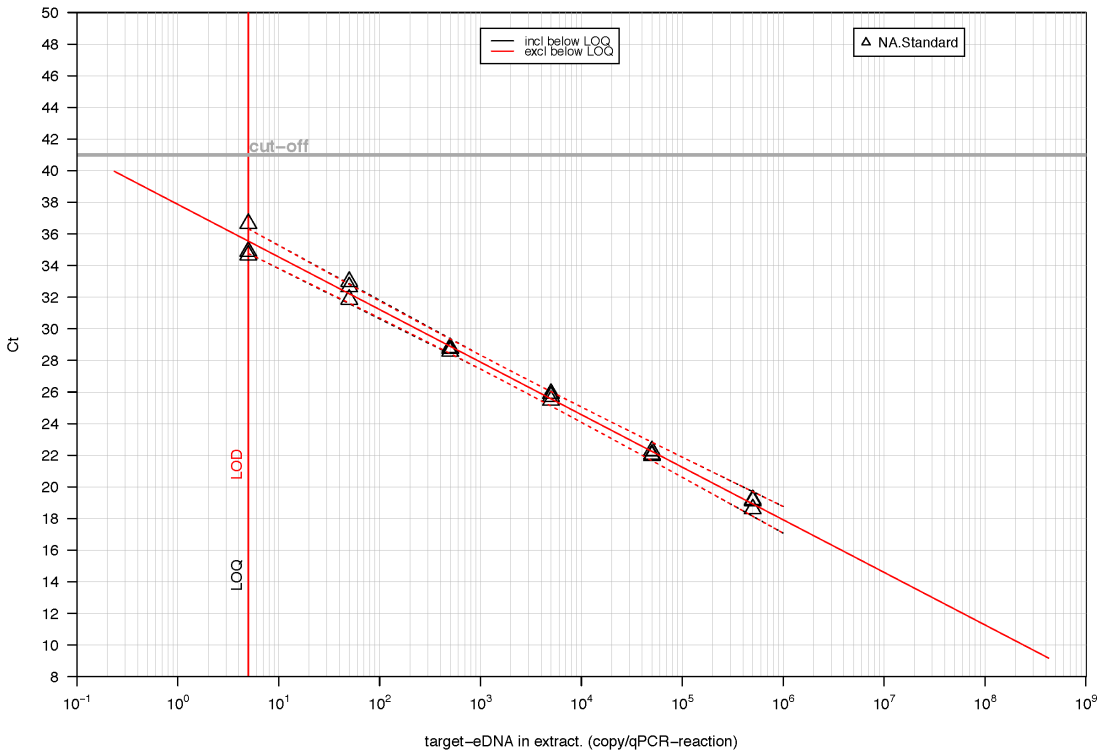


qPCR standard curve for *Carassius auratus* (soelvkarusse), AssayNo 6 , autumn

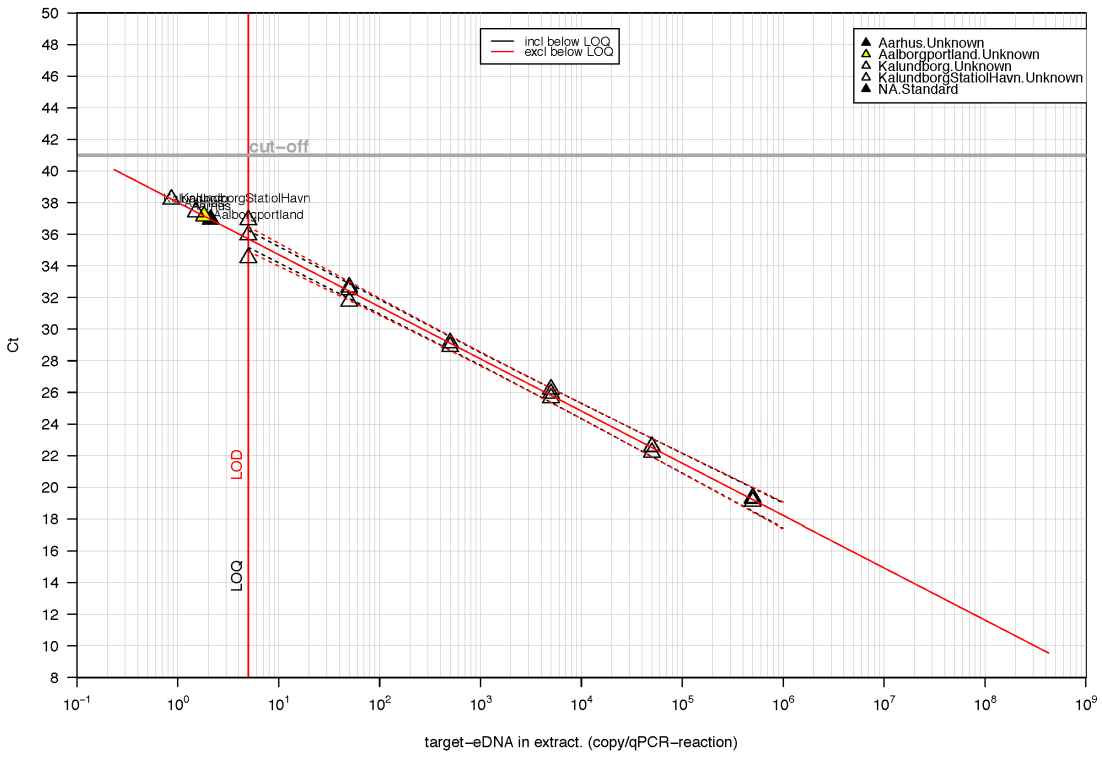


Appendix A 7 .

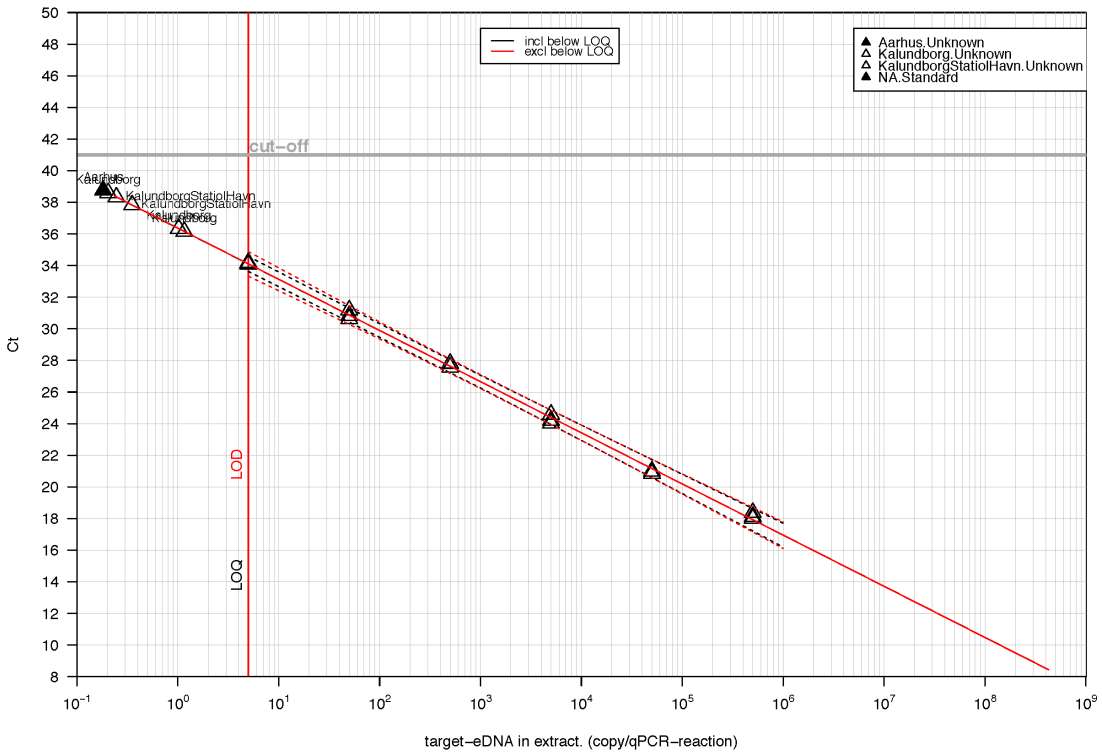
qPCR standard curve for *Cyprinus carpio* (karpe), AssayNo 7 , spring



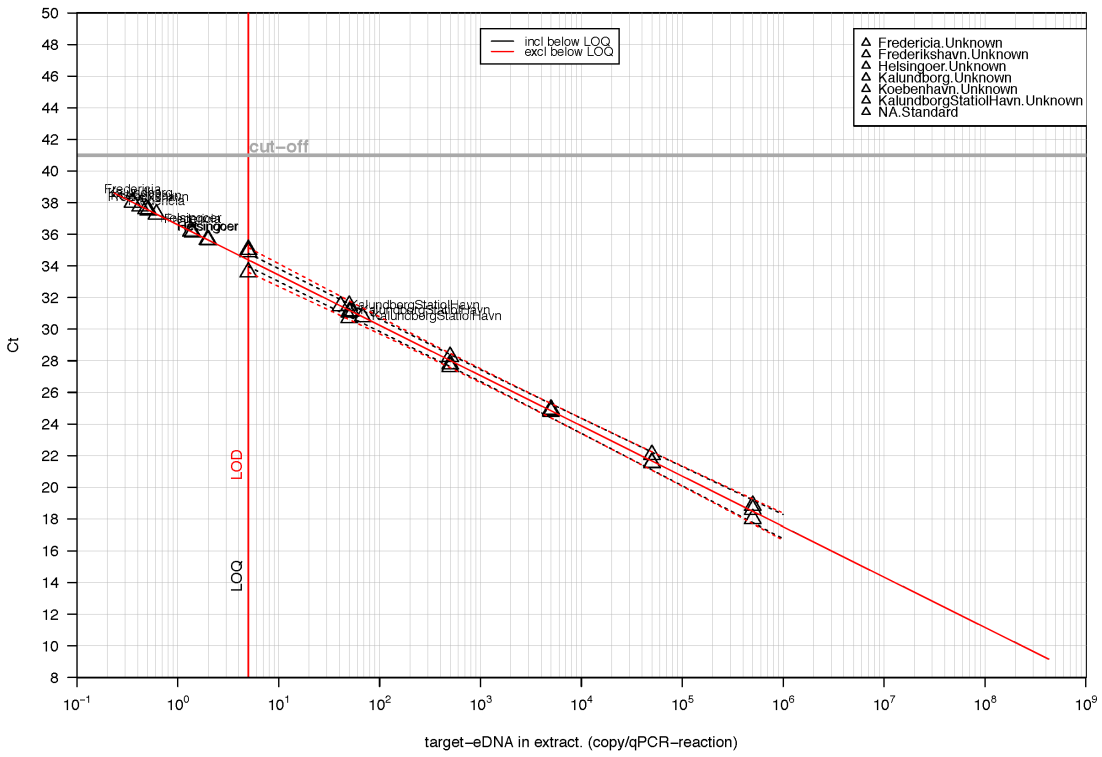
qPCR standard curve for *Cyprinus carpio* (karpe), AssayNo 7 , autumn



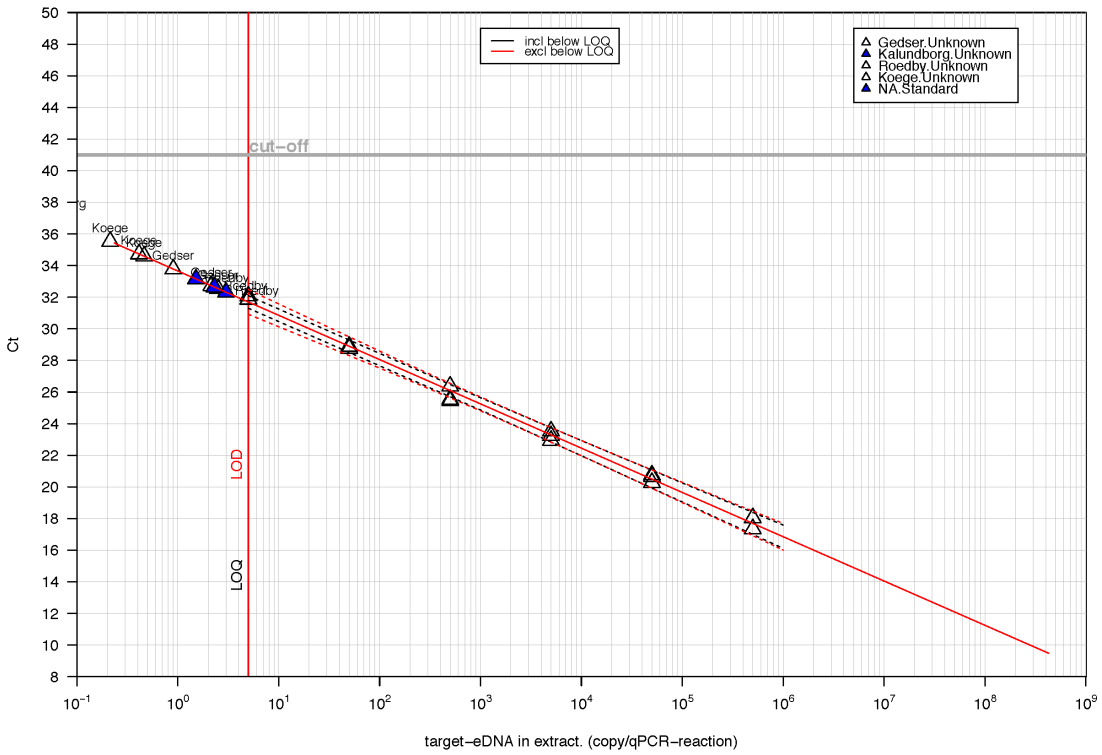
Appendix A 8 . qPCR standard curve for *Colpomenia peregrine* (oesterstyv), AssayNo 8 , spring



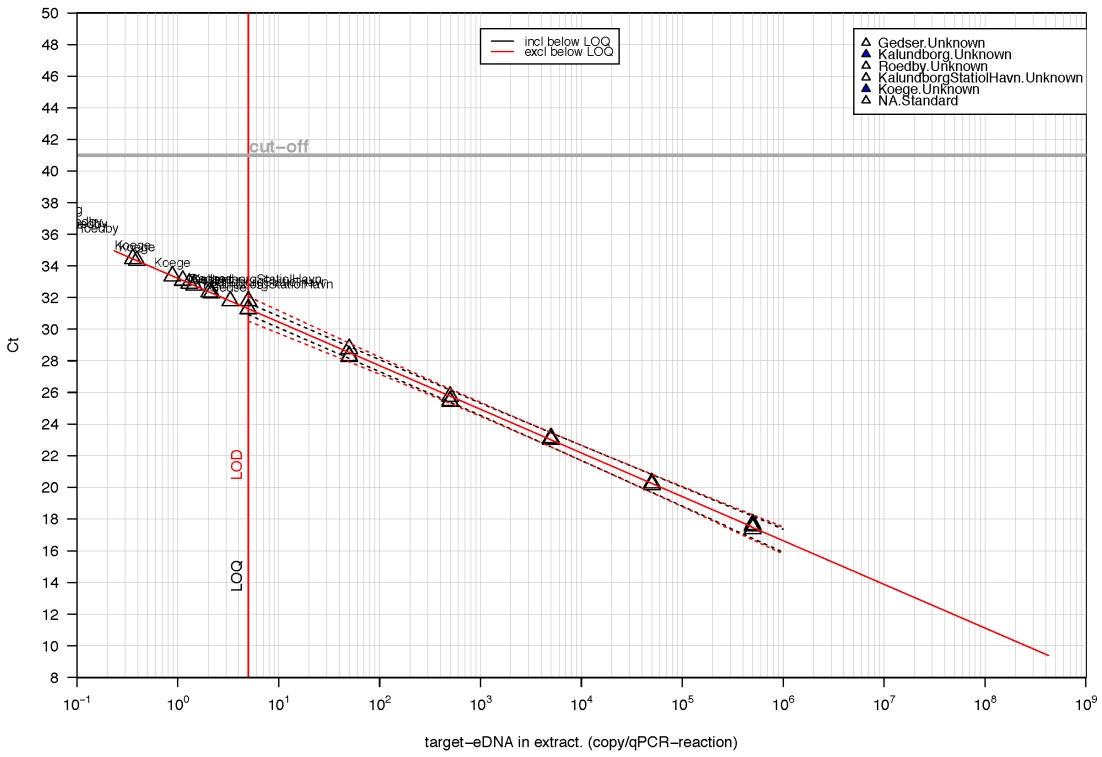
qPCR standard curve for *Colpomenia peregrine* (oesterstyv), AssayNo 8 , autumn



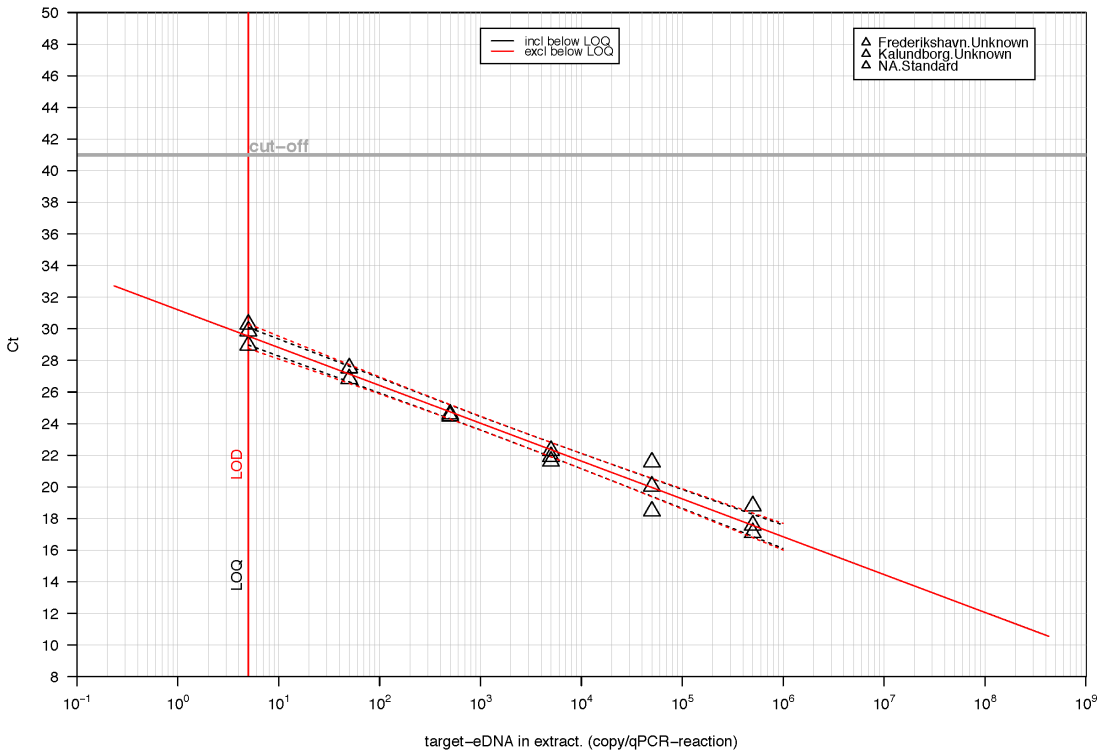
Appendix A 9 ,qPCR standard curve for *Neogobius melanostomus* (sortmundetkutling), AssayNo 09A , spring



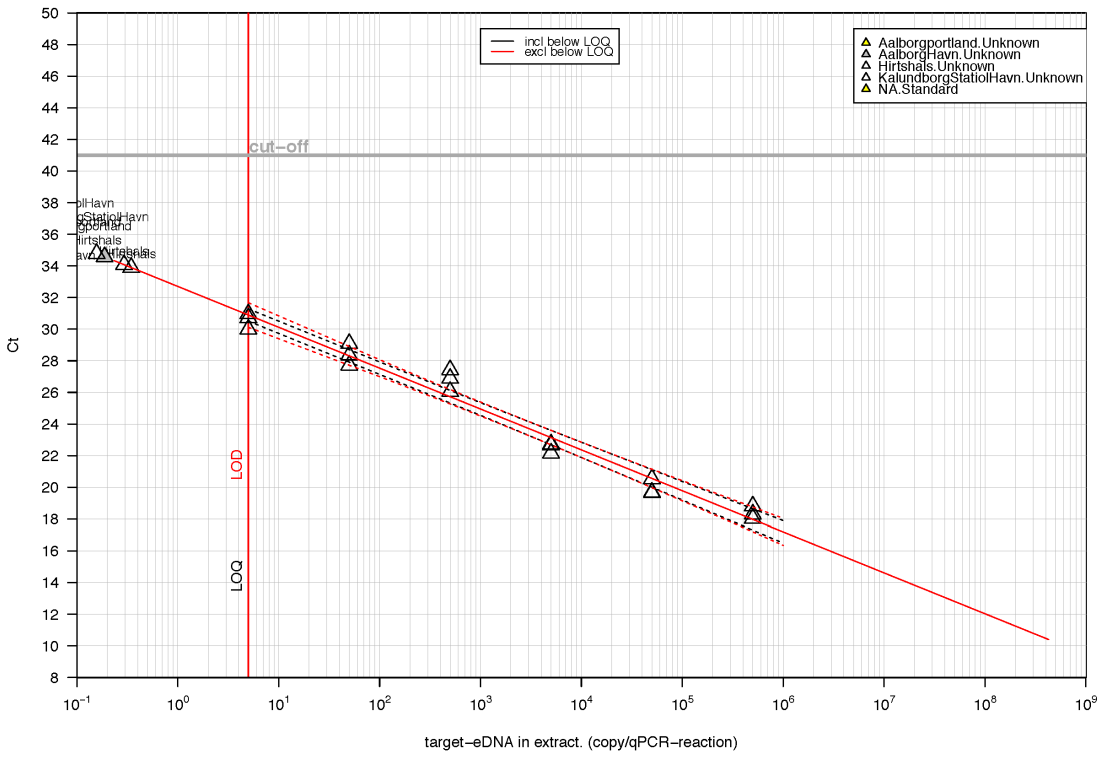
qPCR standard curve for *Neogobius melanostomus* (sortmundetkutling), AssayNo 09A , autumn



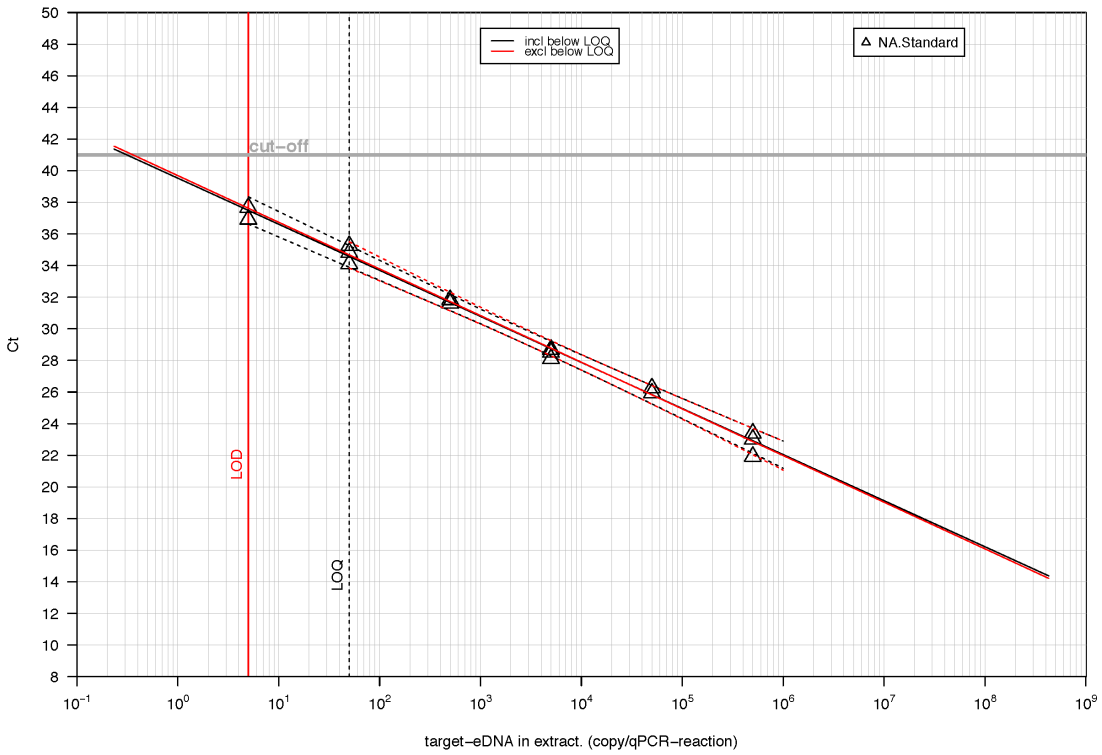
Appendix A 10 . qPCR standard curve for *Oncorhynchus mykiss* (regnbueoerred), AssayNo 10 , spring



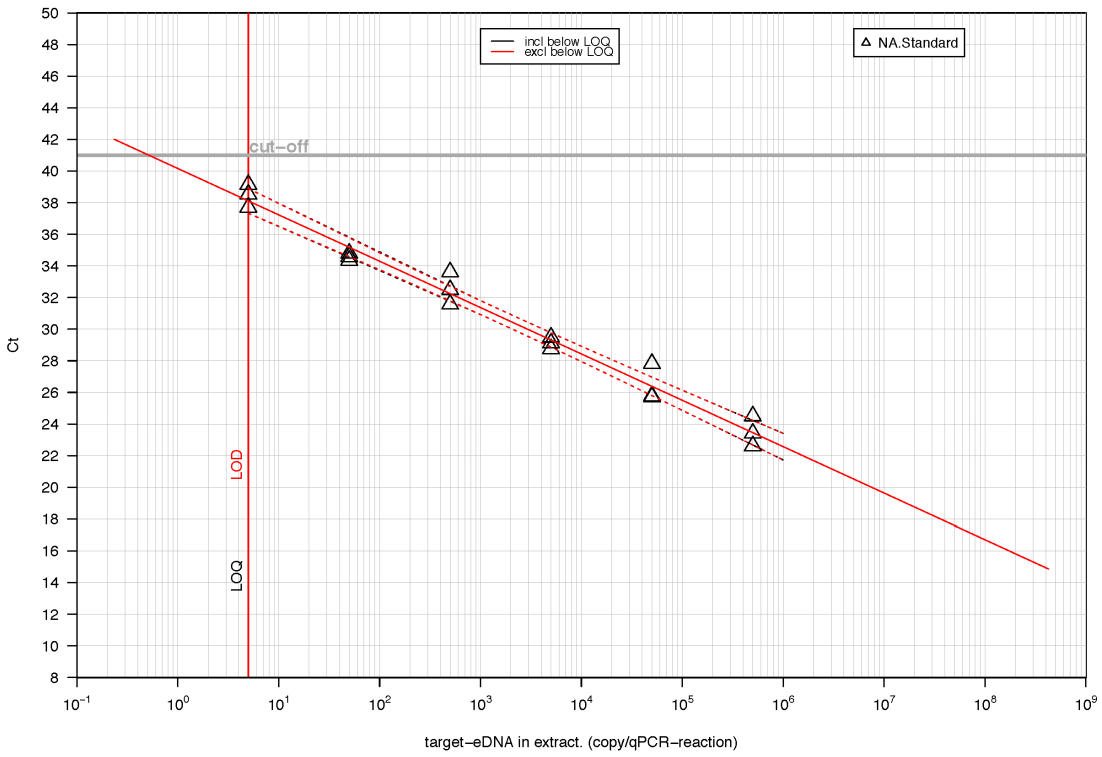
qPCR standard curve for *Oncorhynchus mykiss* (regnbueoerred), AssayNo 10 , autumn



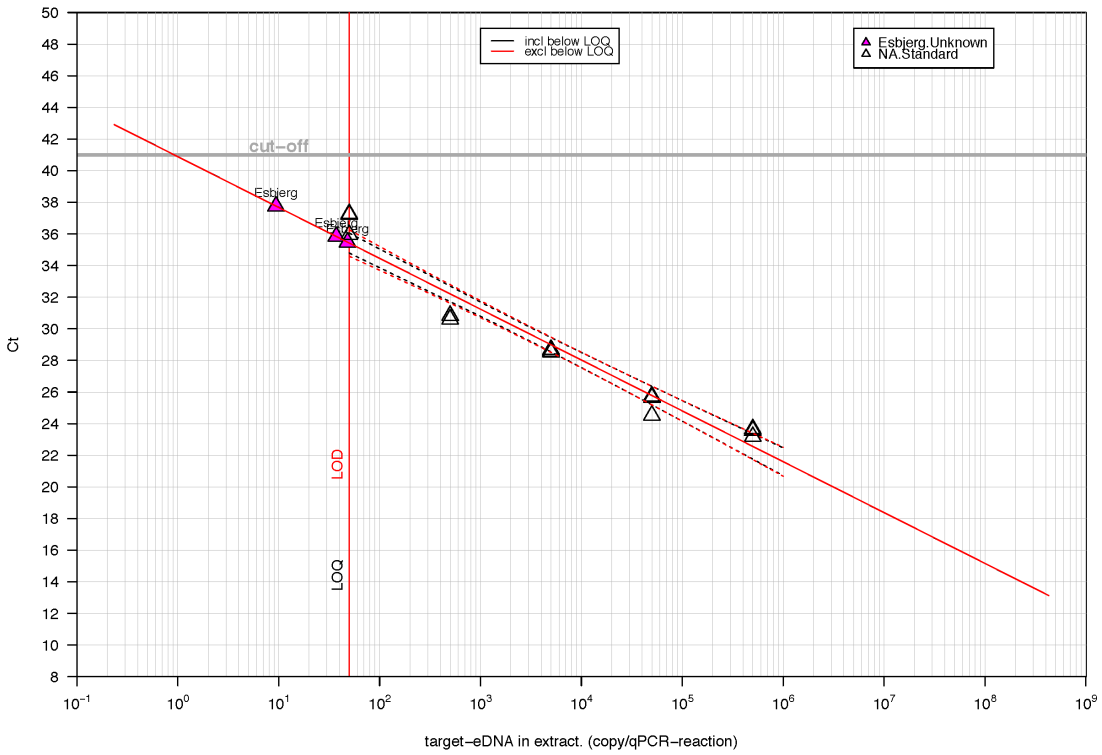
Appendix A 11 . qPCR standard curve for *Oncorhyncus gorbuscha* (pukkellaks), AssayNo 13 , spring



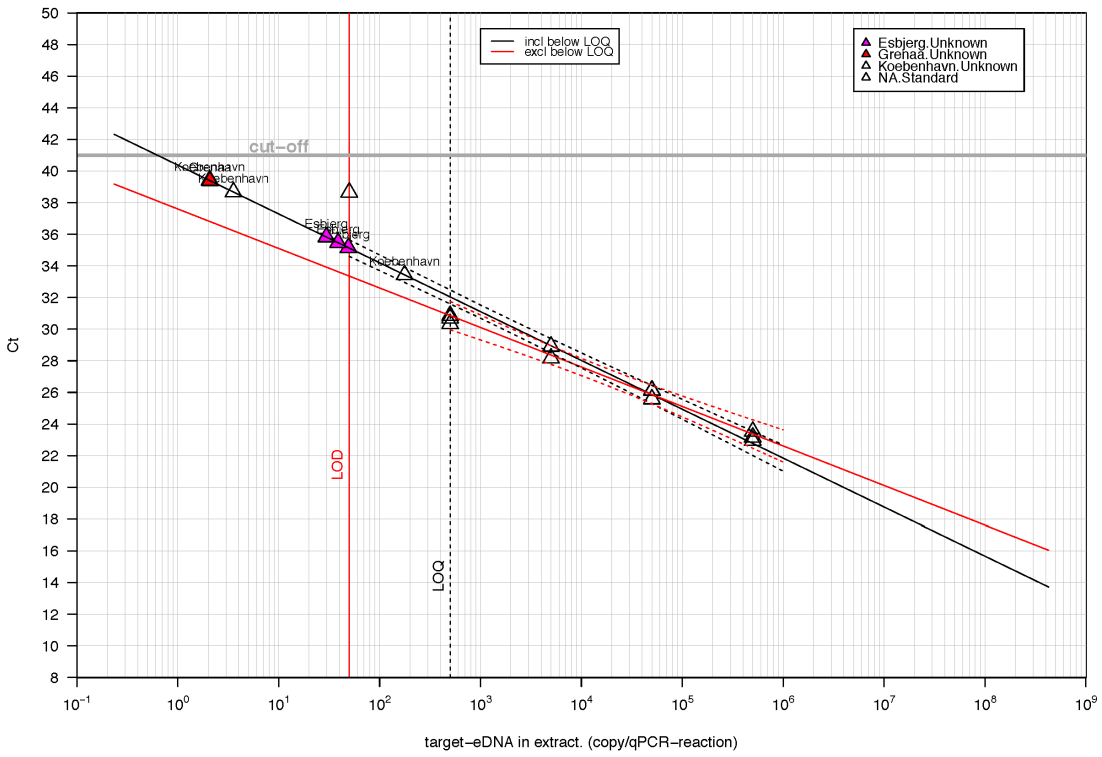
qPCR standard curve for *Oncorhyncus gorbuscha* (pukkellaks), AssayNo 13 , autumn



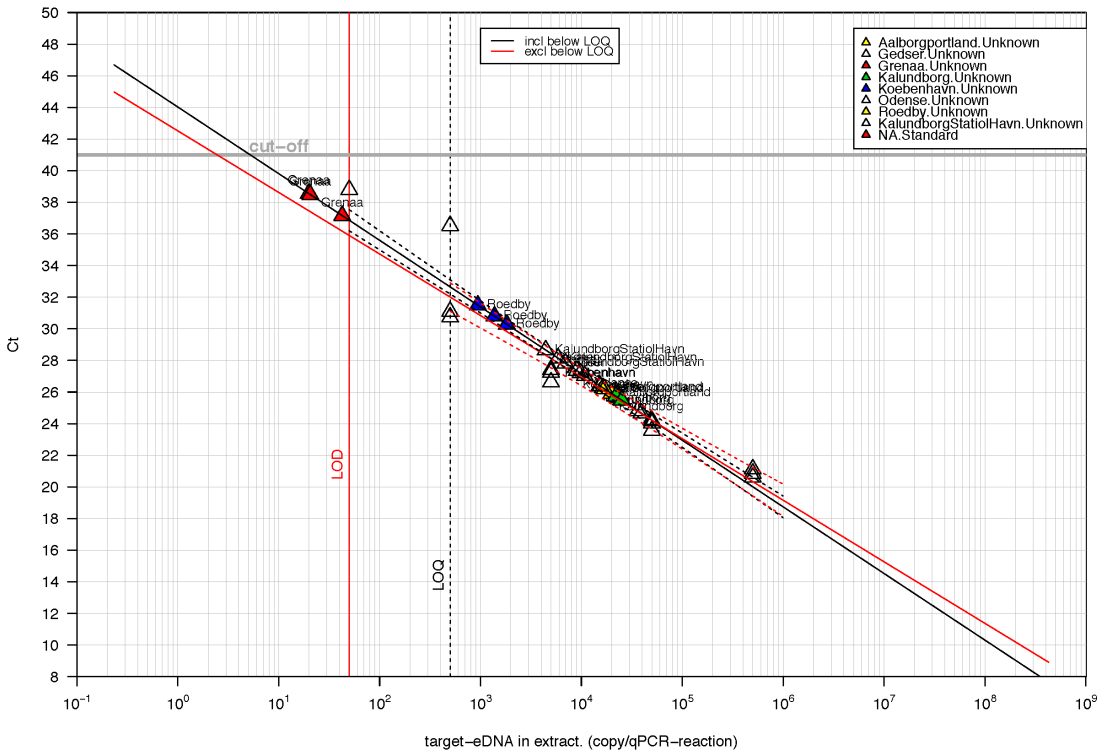
Appendix A 12 . qPCR standard curve for *Crassostrea gigas* (stillehavsoesters), AssayNo 14 , spring



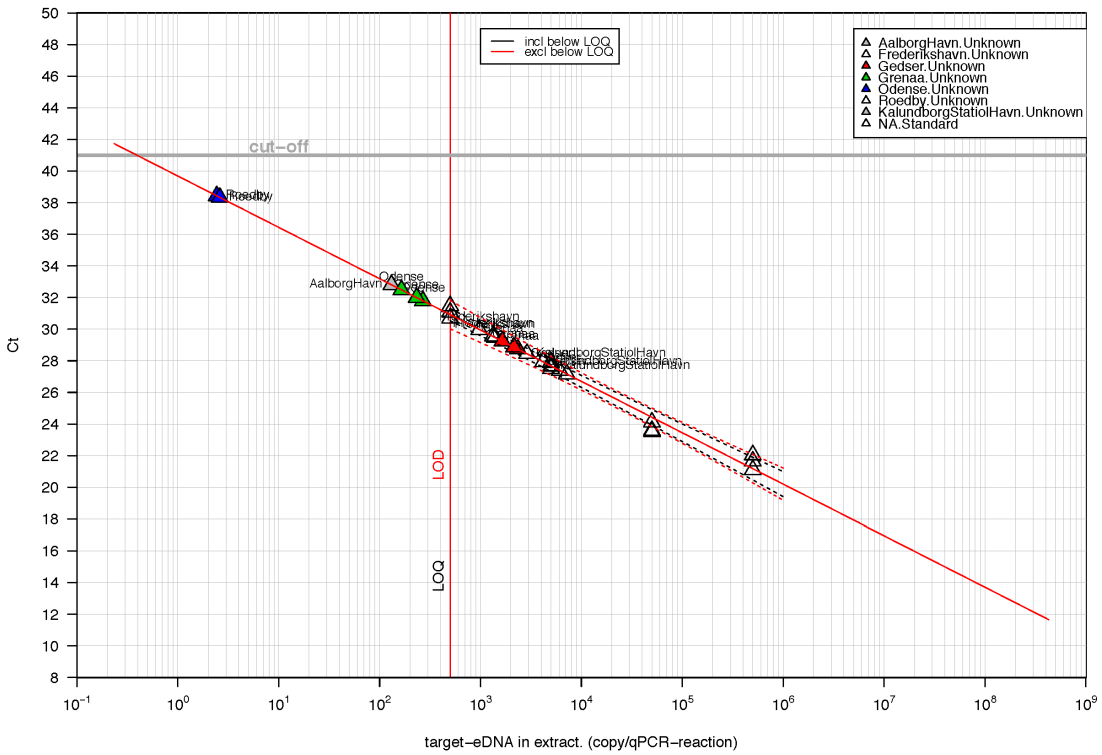
qPCR standard curve for *Crassostrea gigas* (stillehavsoesters), AssayNo 14 , autumn



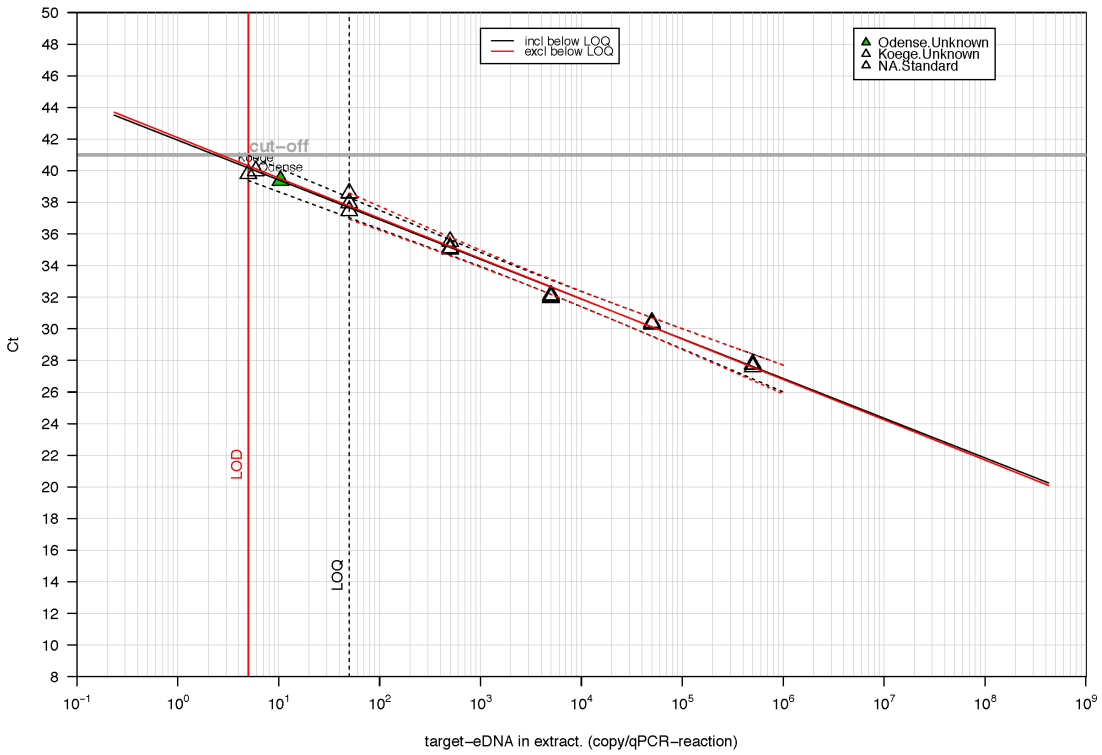
Appendix A 13 . qPCR standard curve for *Mya arenaria* (sandmusling), AssayNo 15 , spring



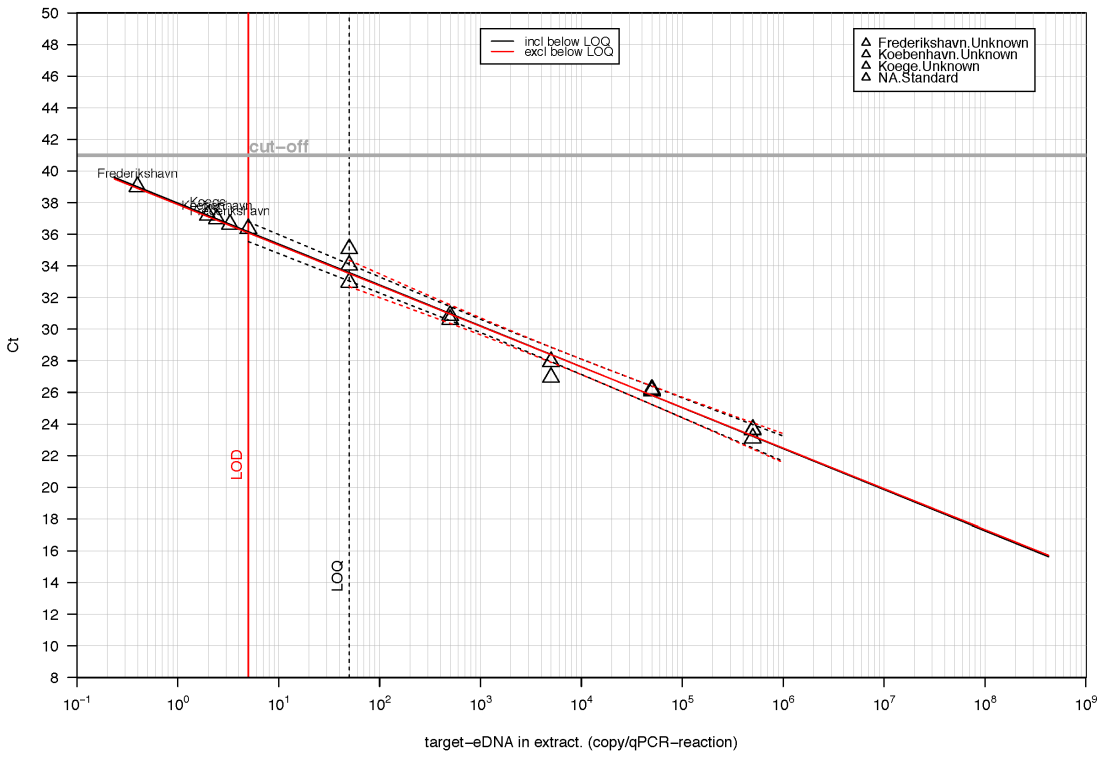
qPCR standard curve for *Mya arenaria* (sandmusling), AssayNo 15 , autumn



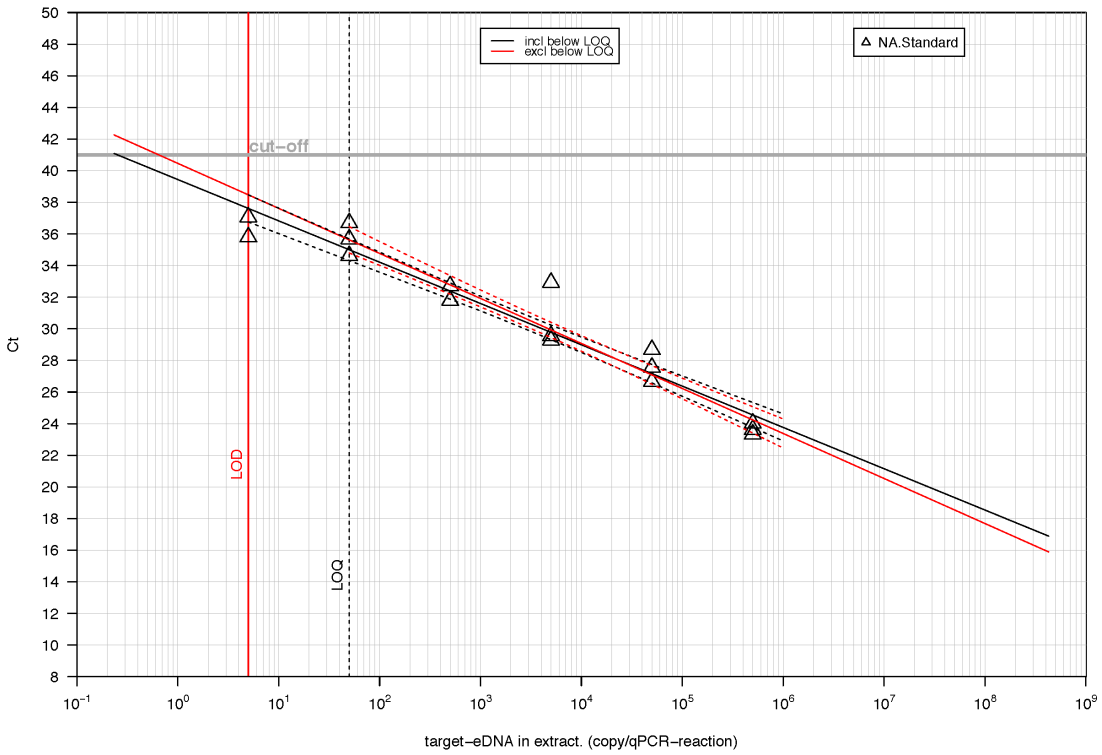
Appendix A 14 . qPCR standard curve for *Rhithropanopeus harrisii* (mudderkrabbe), AssayNo 16 , spring



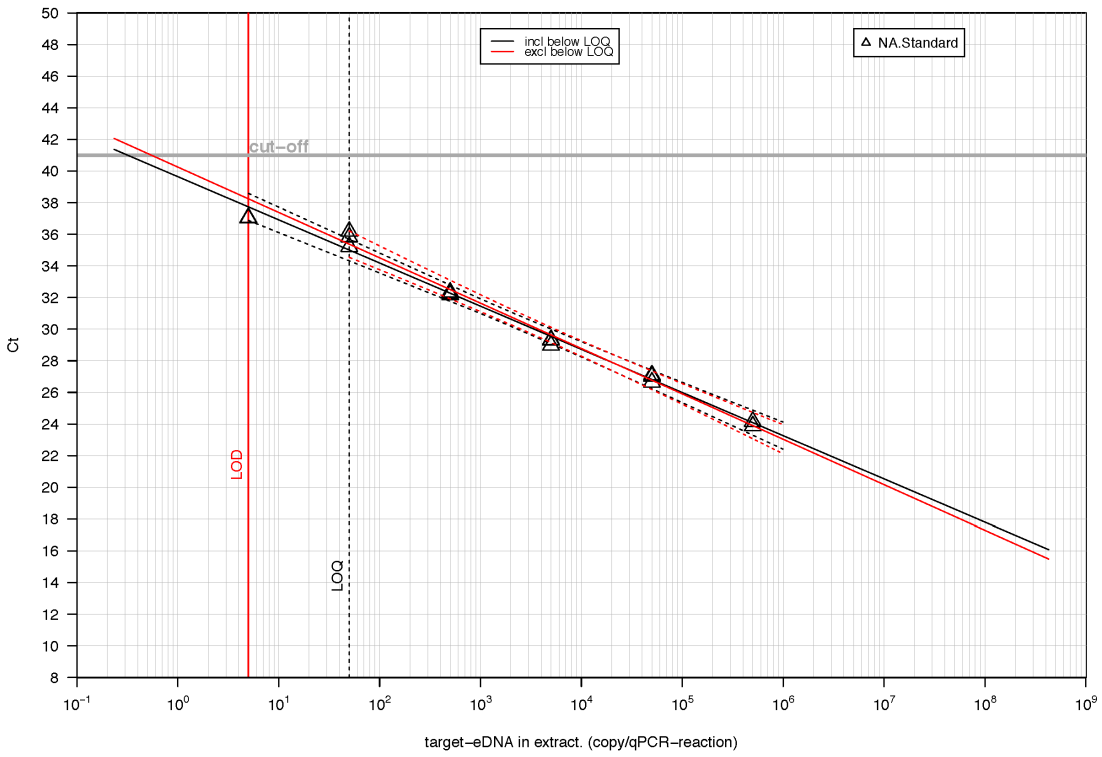
qPCR standard curve for *Rhithropanopeus harrisii* (mudderkrabbe), AssayNo 16 , autumn



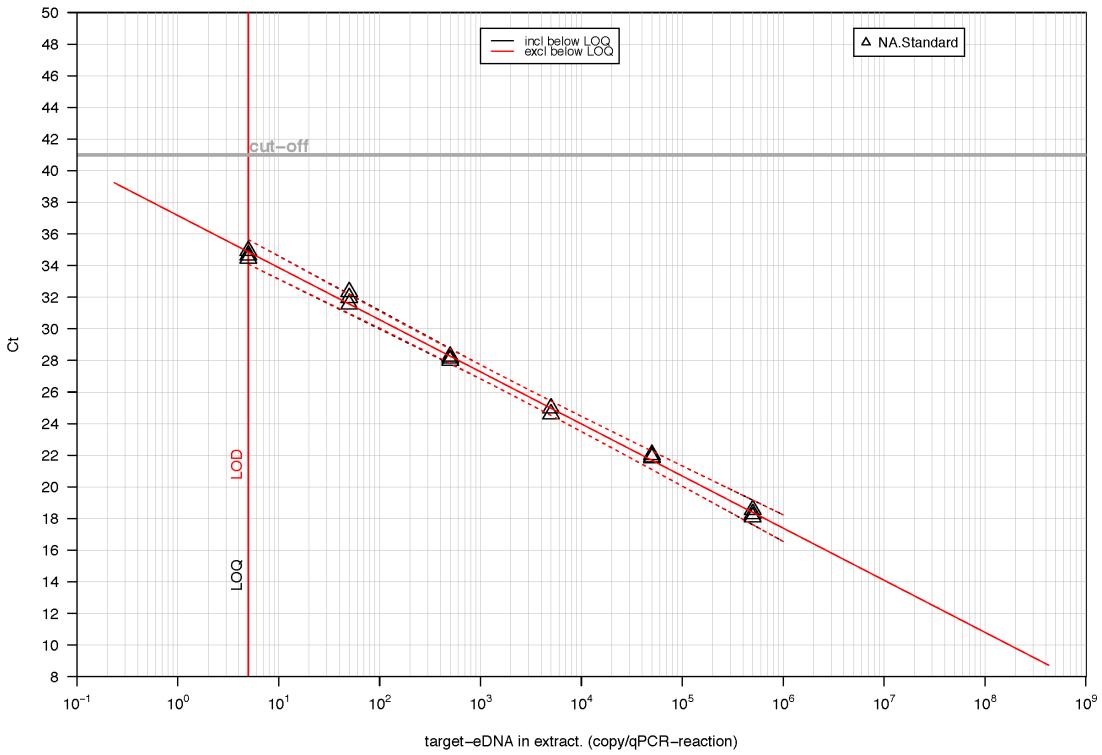
Appendix A 16 . qPCR standard curve for *Eriocheir sinensis* (kinesiskuldhaandskrabbe), AssayNo 18 , spring



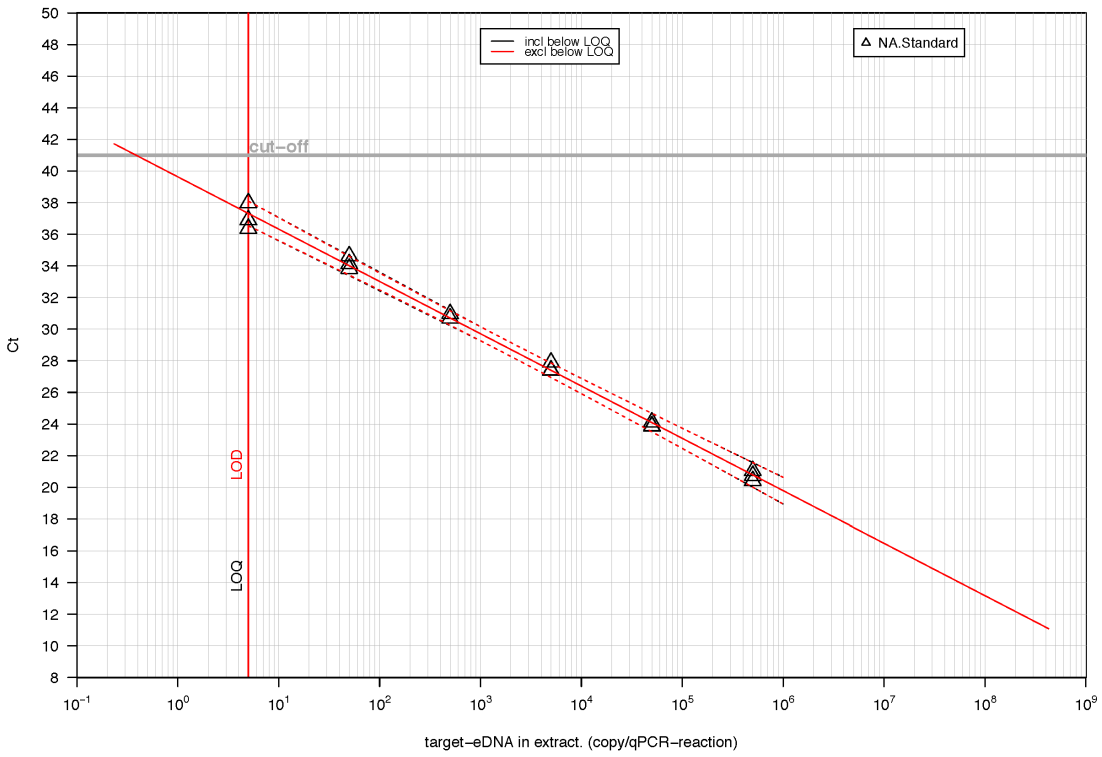
qPCR standard curve for *Eriocheir sinensis* (kinesiskuldhaandskrabbe), AssayNo 18 , autumn



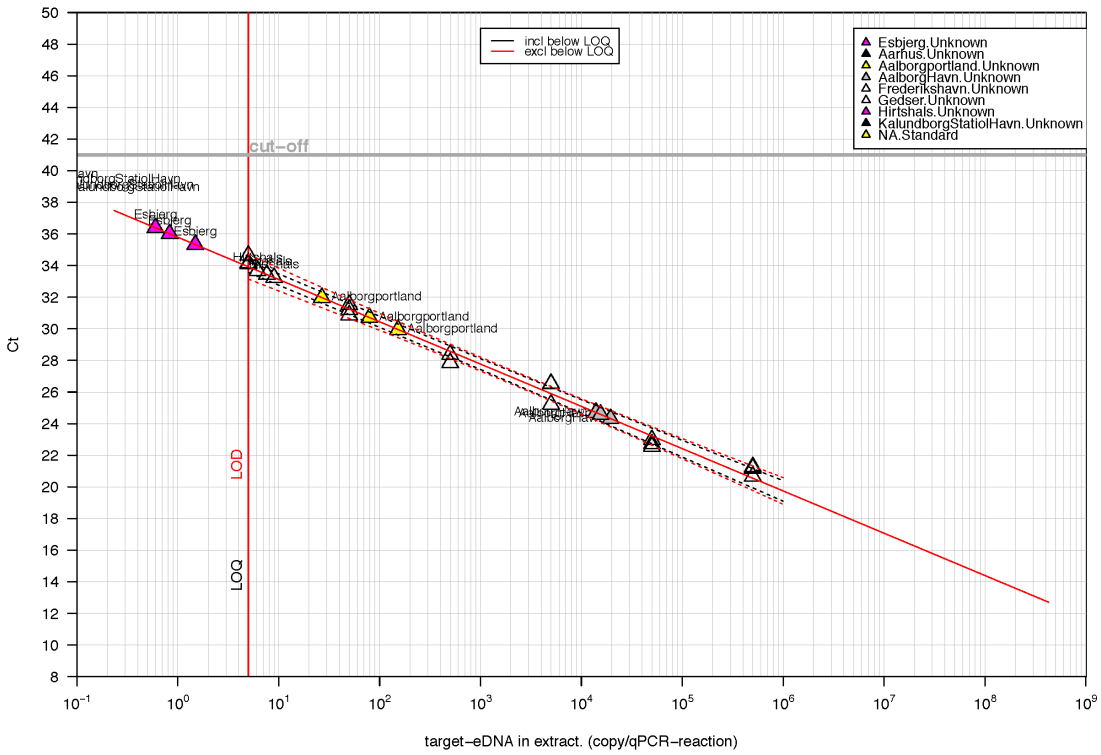
Appendix A 18 . qPCR standard curve for *Cordylophora caspia* (brakvandskroellepolyp), AssayNo 21 , spring



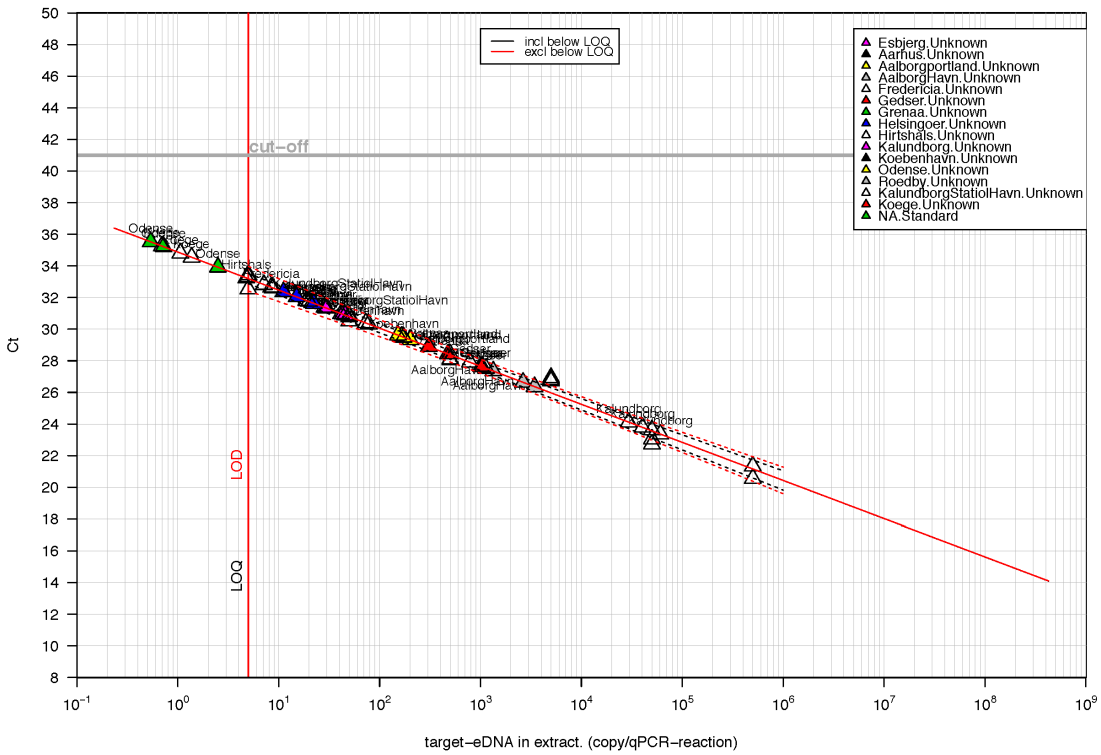
qPCR standard curve for *Cordylophora caspia* (brakvandskroellepolyp), AssayNo 21 , autumn



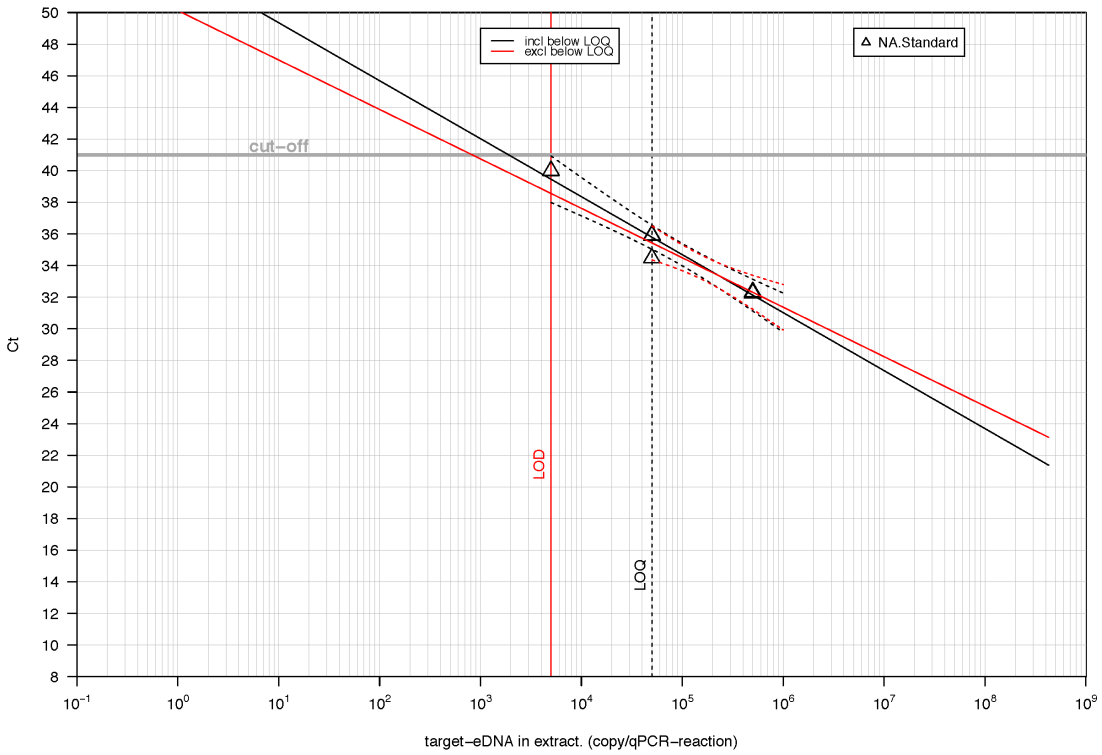
Appendix A 19 . qPCR standard curve for *Mnemiopsis leidyi* (amerikanskribbegoble), AssayNo 22 , spring



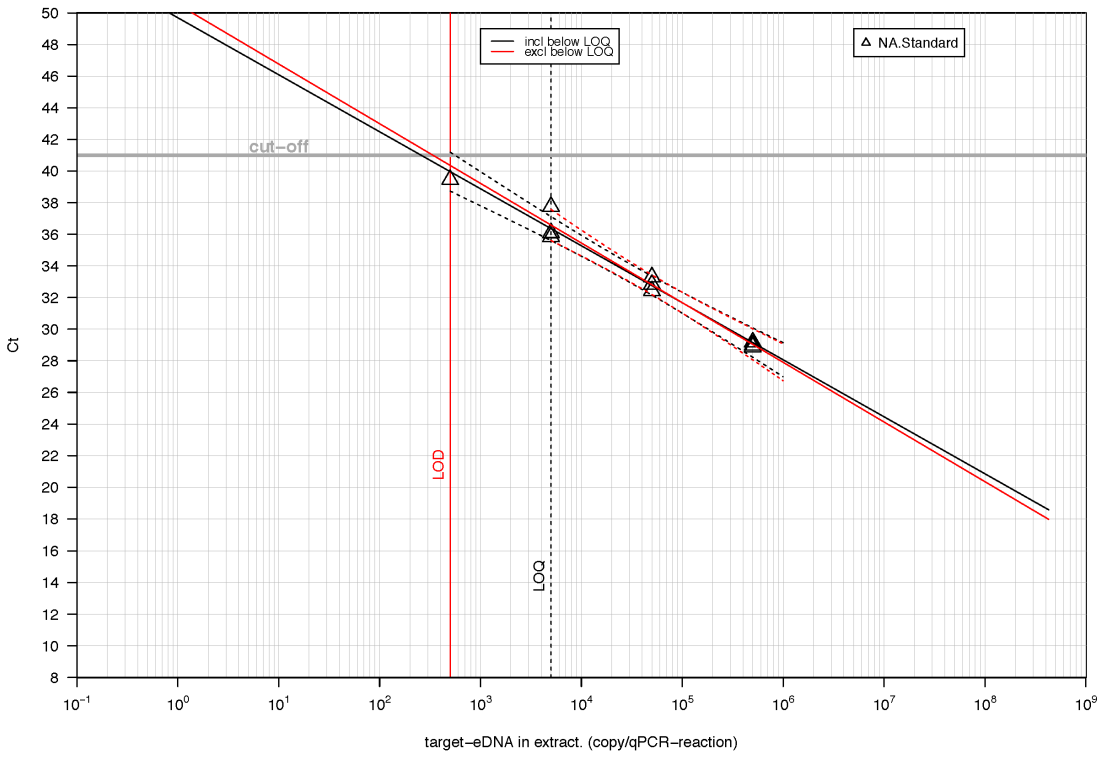
qPCR standard curve for *Mnemiopsis leidyi* (amerikanskribbegoble), AssayNo 22 , autumn



Appendix A 20 . qPCR standard curve for *Acipenser baerii* (siberiskstoer), AssayNo 23 , spring



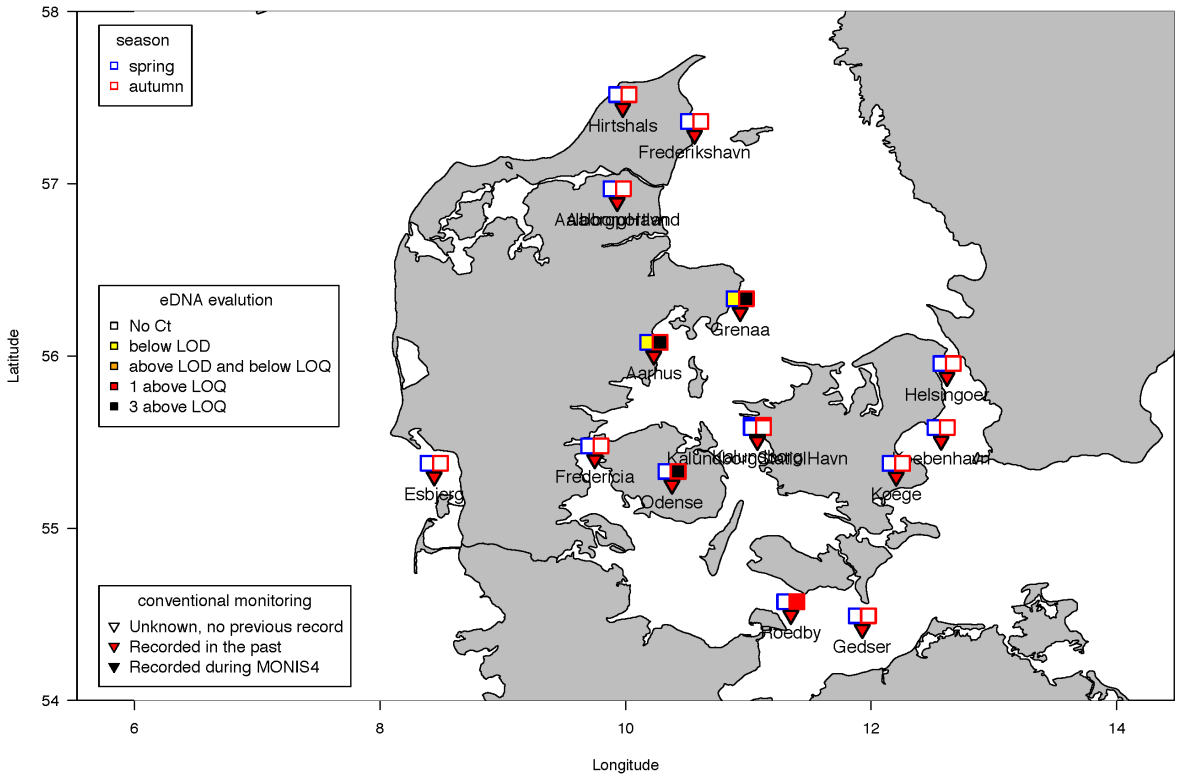
qPCR standard curve for *Acipenser baerii* (siberiskstoer), AssayNo 23 , autumn



Annex 10: eDNA assay-specific maps

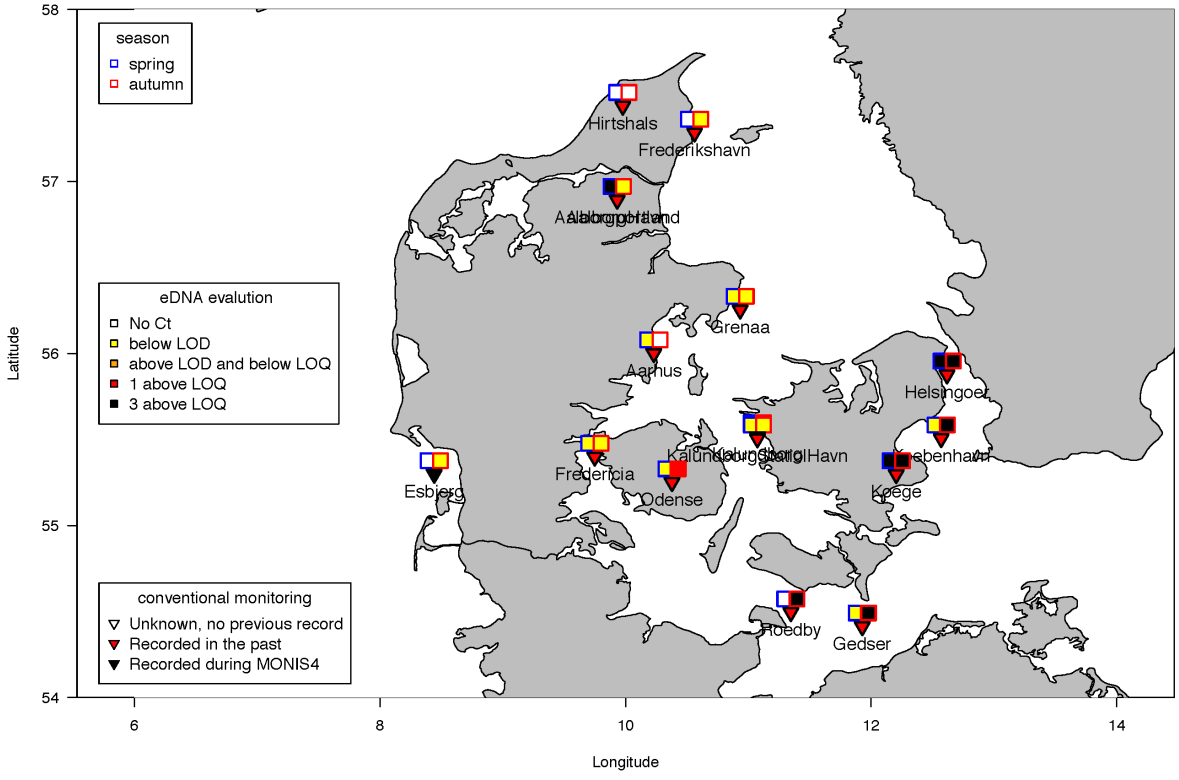
Appendix B 1 .

eDNA detected from *Bonnemaisonia hamifera* (roedtotalge), Assay Id No: 01

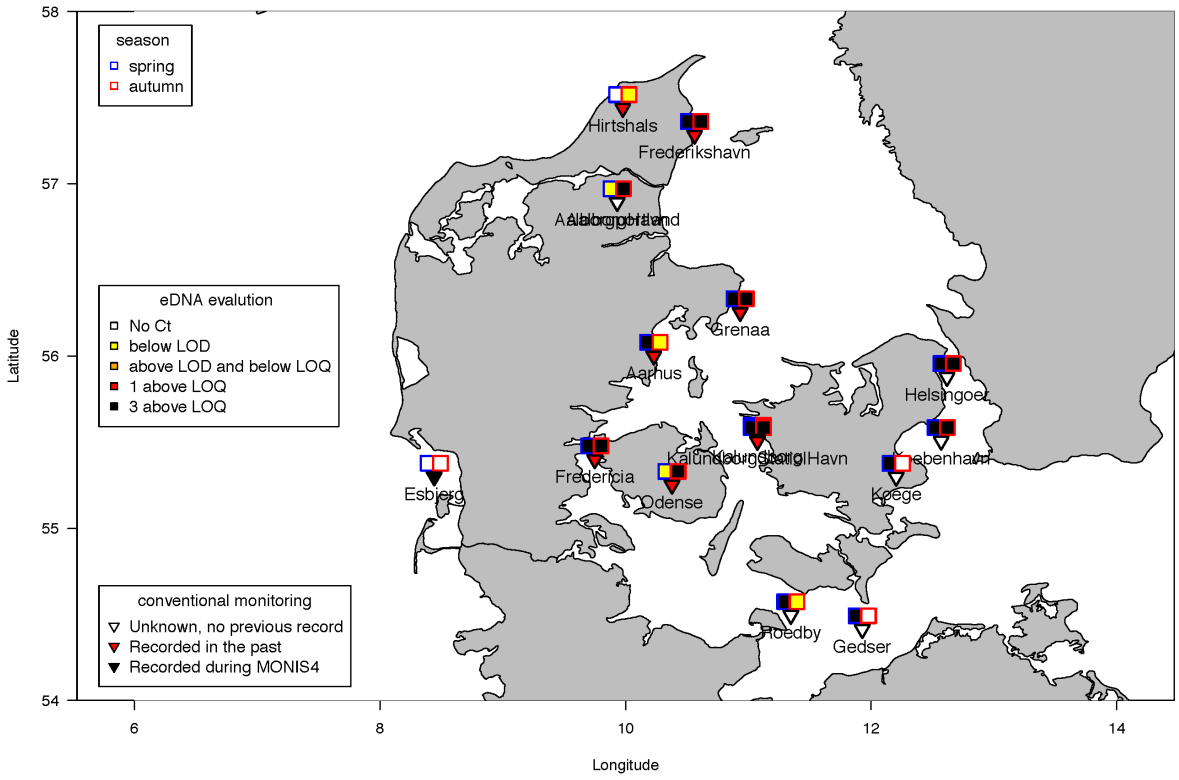


Appendix B 2 .

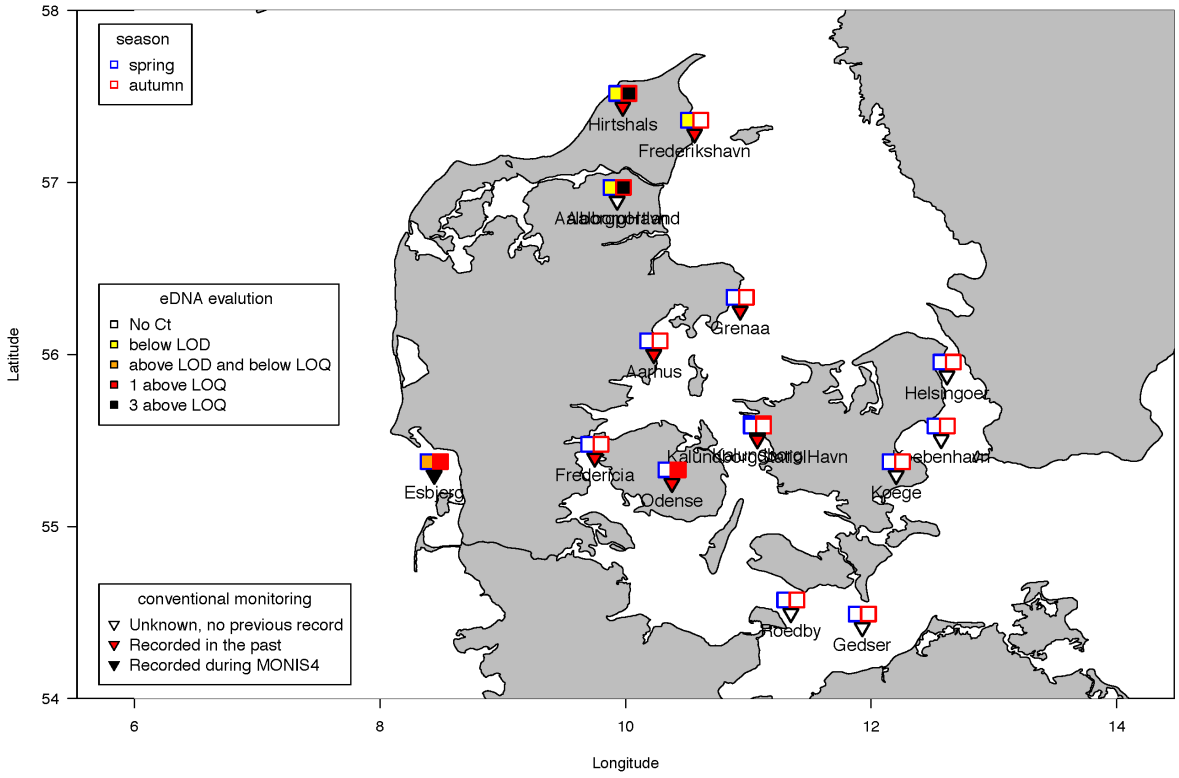
eDNA detected from *Prorocentrum minimum* (dinoflagelat), Assay Id No: 02



Appendix B 3 . eDNA detected from *Pseudochattonella farcimen* (heterokontflagelat), Assay Id No: 03

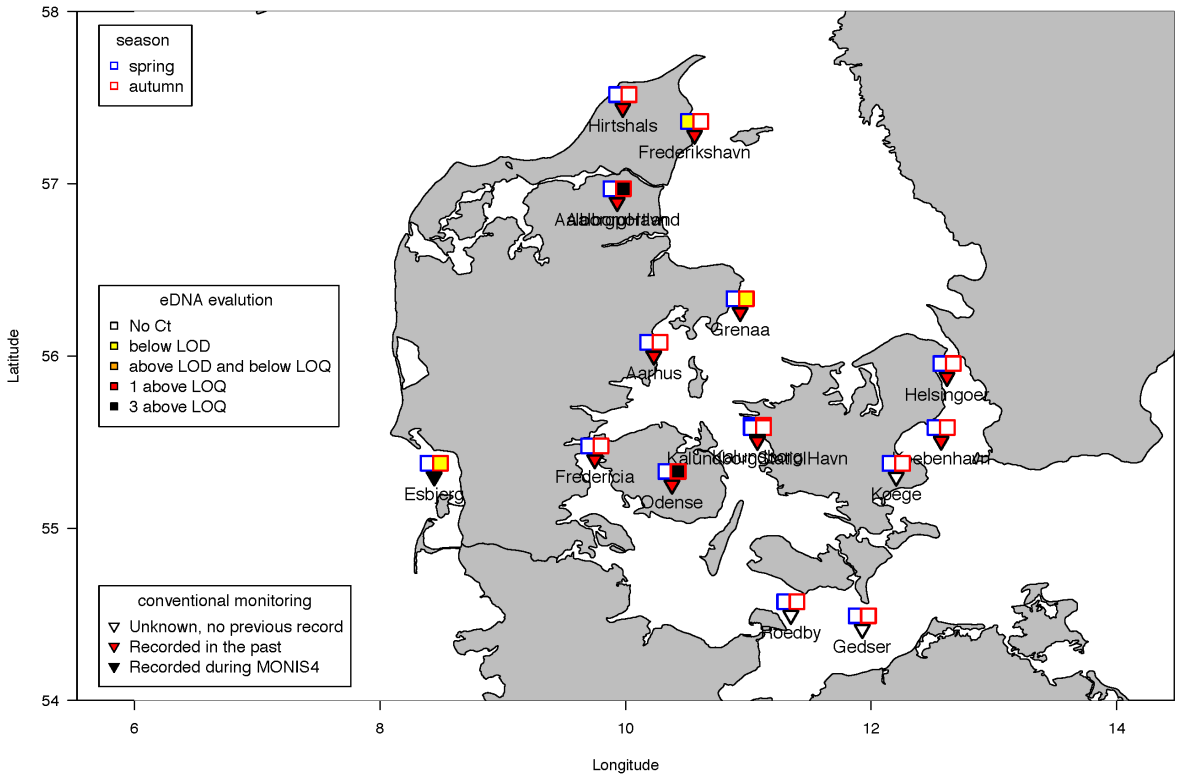


Appendix B 4 . eDNA detected from *Pseudochattonella verruculosa* (heterokontflagelat), Assay Id No: 04



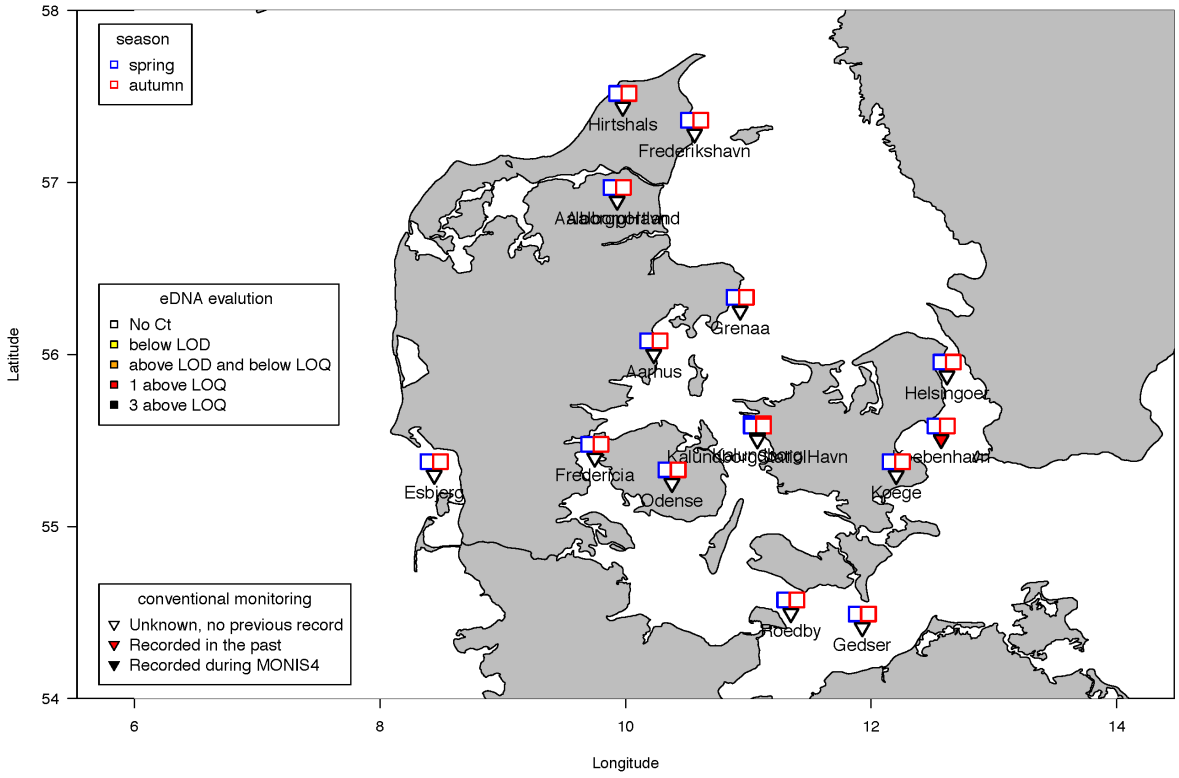
Appendix B 5 .

eDNA detected from *Karenia mikimotoi* (dinoflagelat), Assay Id No: 05



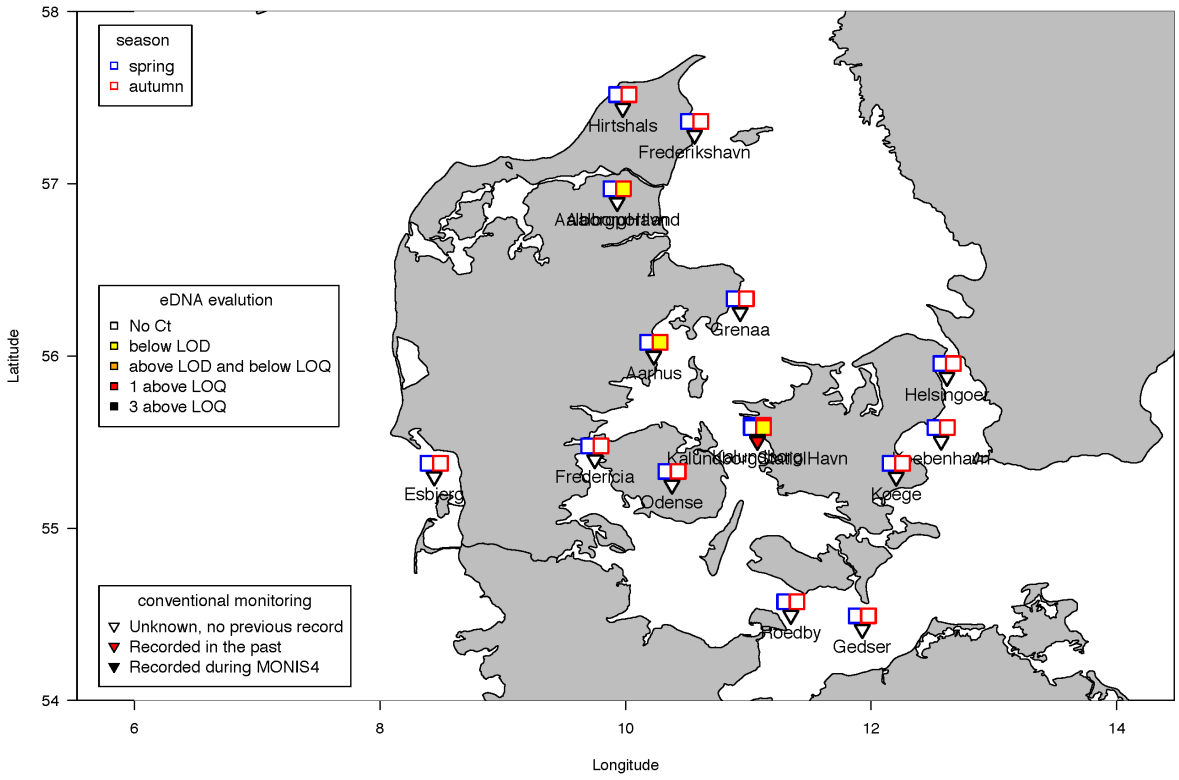
Appendix B 6 .

eDNA detected from *Carassius auratus* (soelvkarusse), Assay Id No: 06



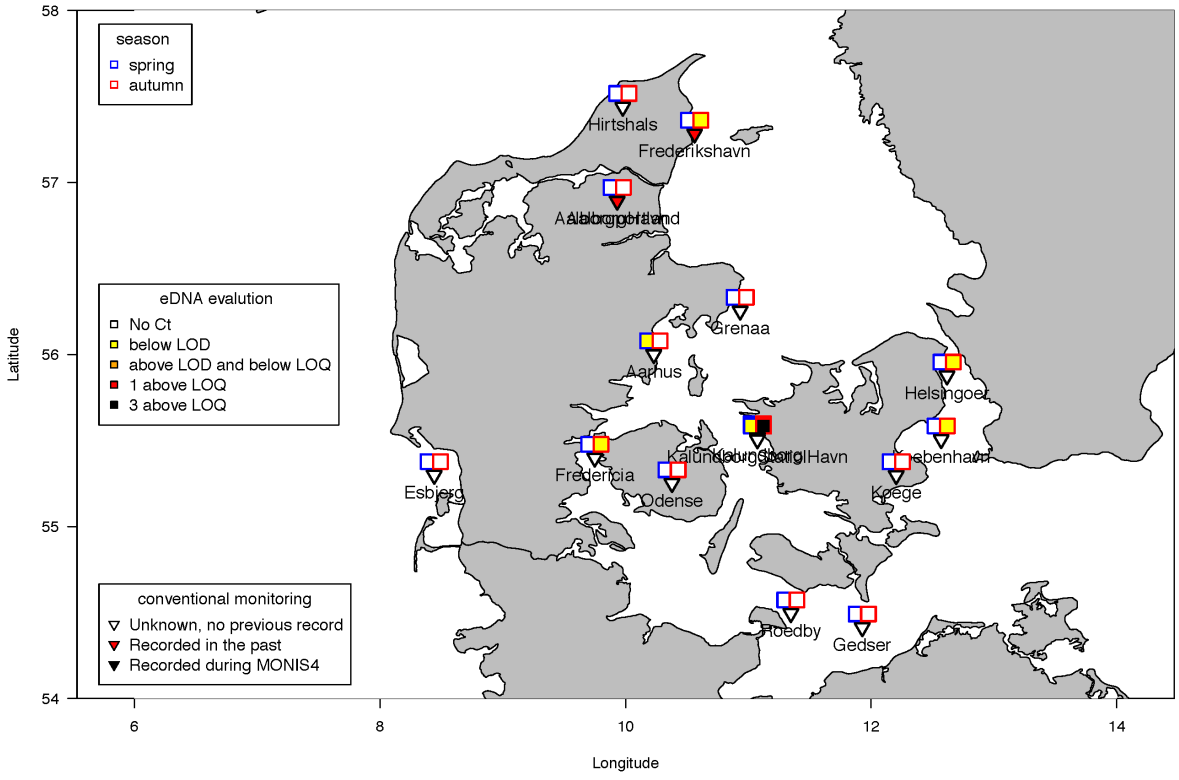
Appendix B 7 .

eDNA detected from *Cyprinus carpio* (karpe), Assay Id No: 07

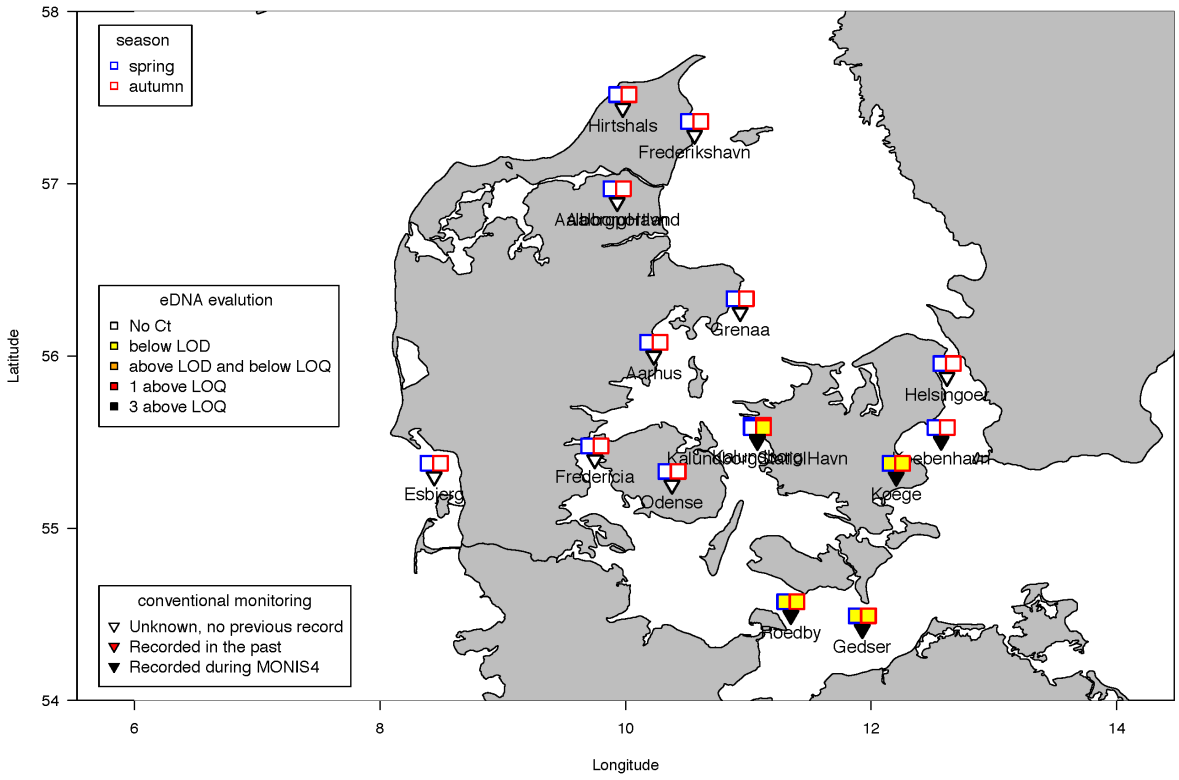


Appendix B 8 .

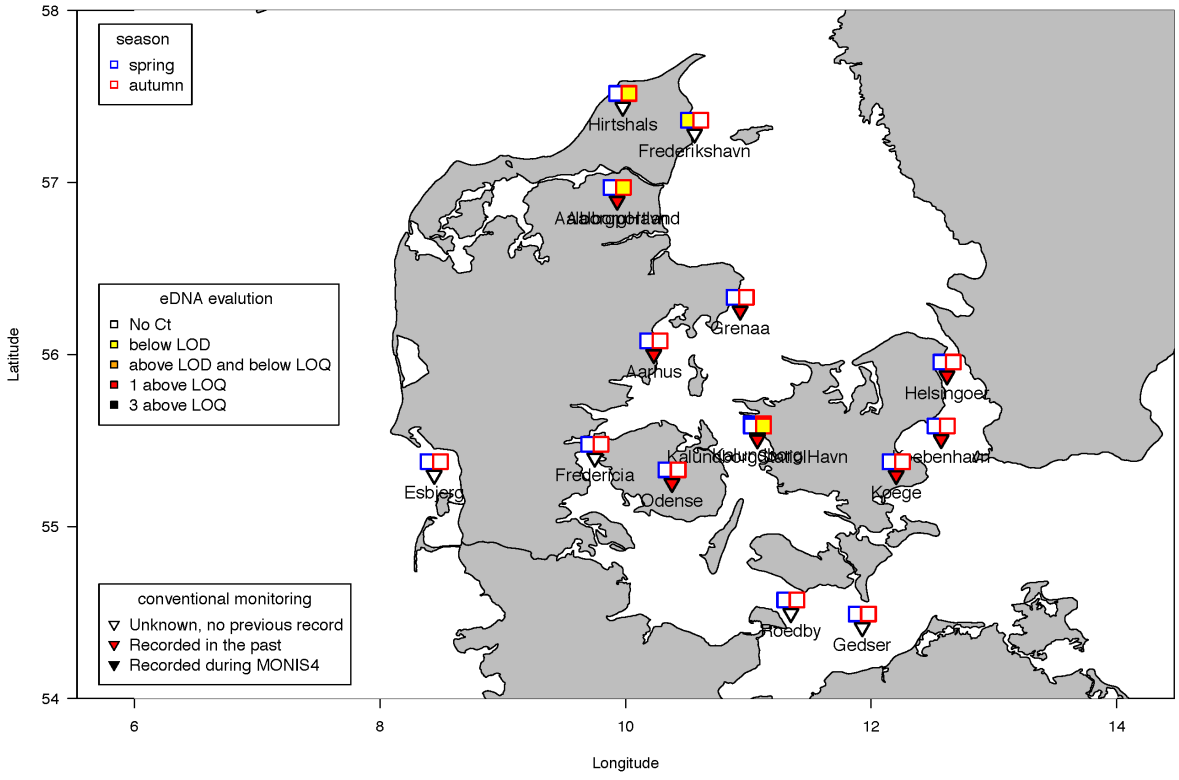
eDNA detected from *Colpomenia peregrine* (oesterstyv), Assay Id No: 08



Appendix B 9 . eDNA detected from *Neogobius melanostomus* (sortmundetkutling), Assay Id No: 09A

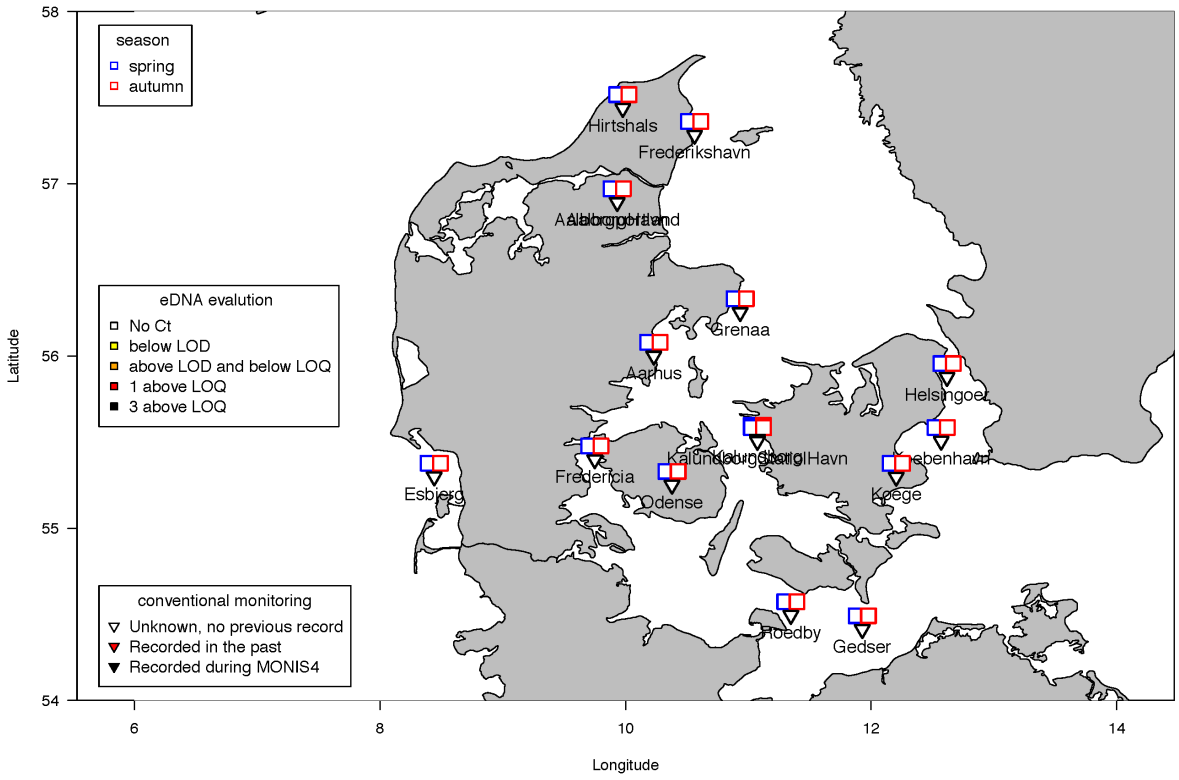


Appendix B 10 . eDNA detected from *Oncorhynchus mykiss* (regnbueoerred), Assay Id No: 10



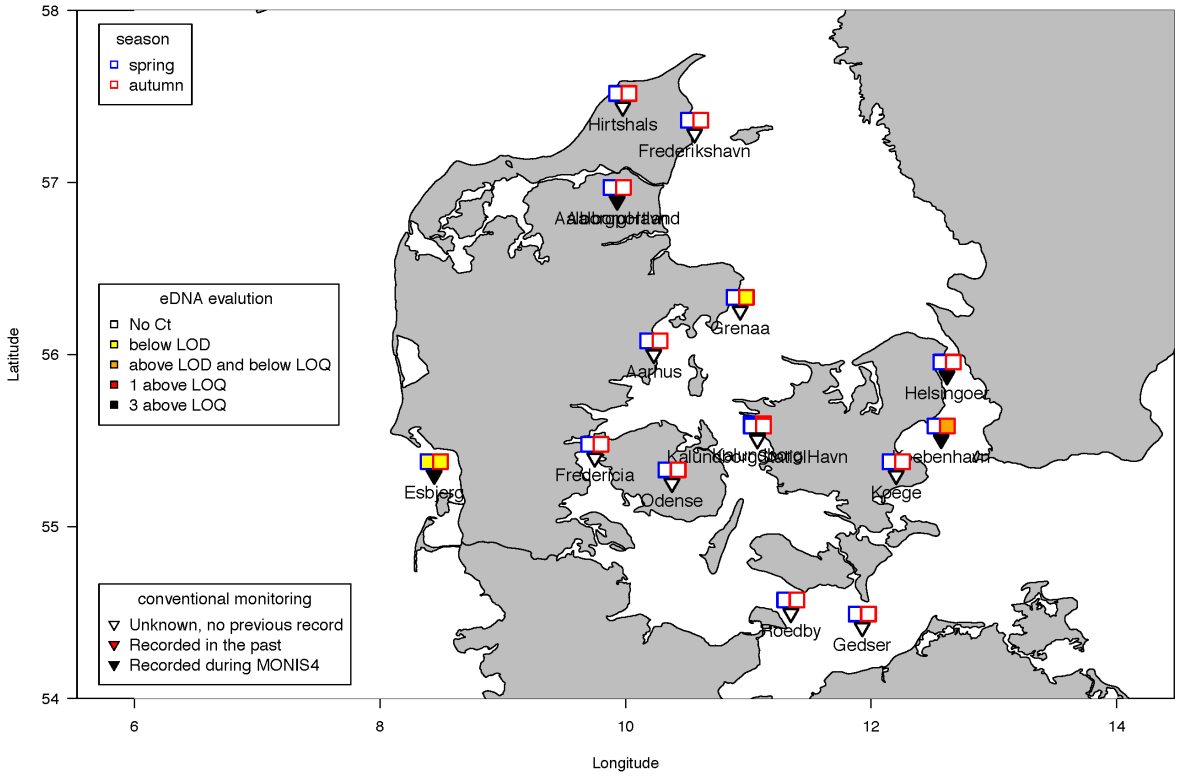
Appendix B 11 .

eDNA detected from *Oncorhyncus gorbuscha* (pukkellaks), Assay Id No: 13



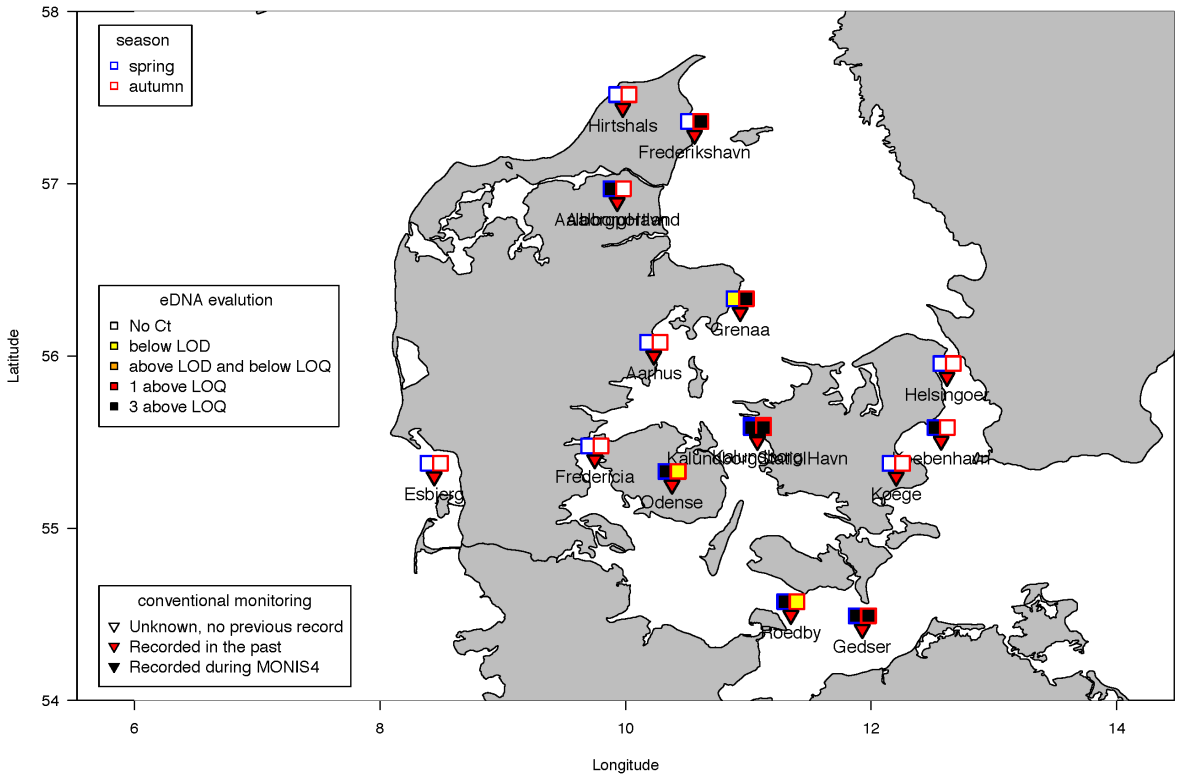
Appendix B 12 .

eDNA detected from *Crassostrea gigas* (stillehavsoesters), Assay Id No: 14



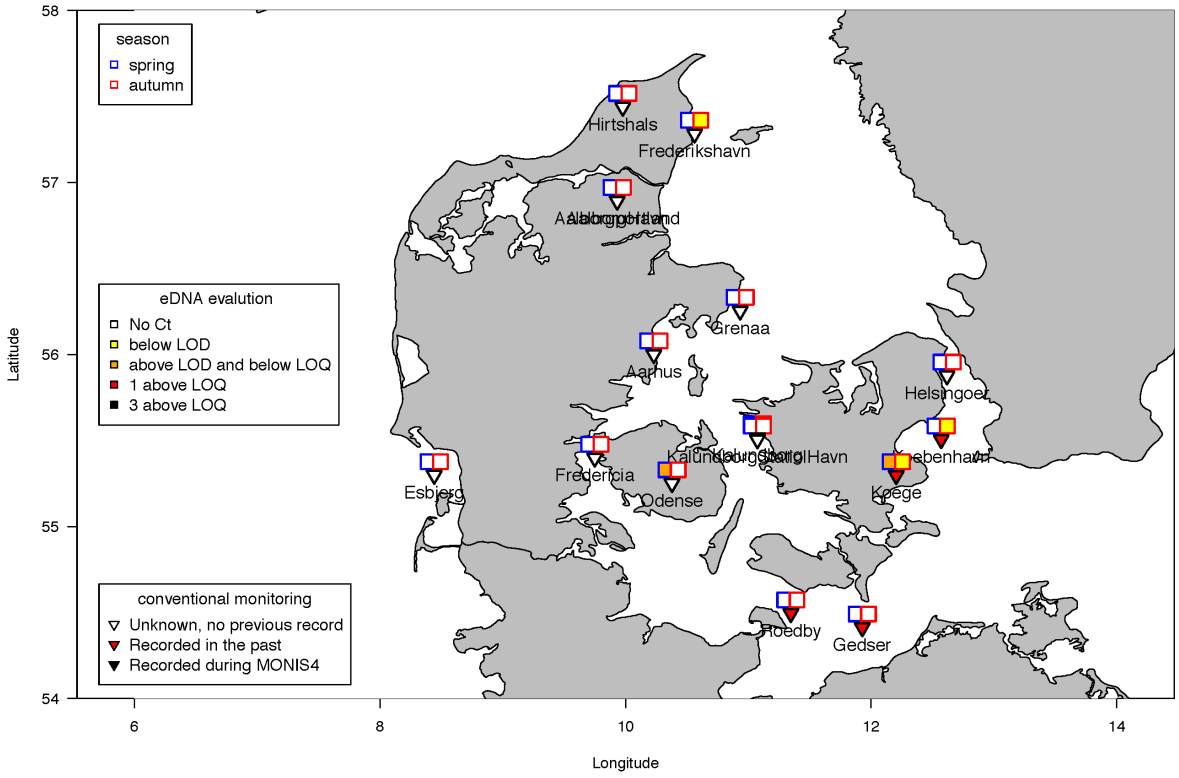
Appendix B 13 .

eDNA detected from *Mya arenaria* (sandmusling), Assay Id No: 15

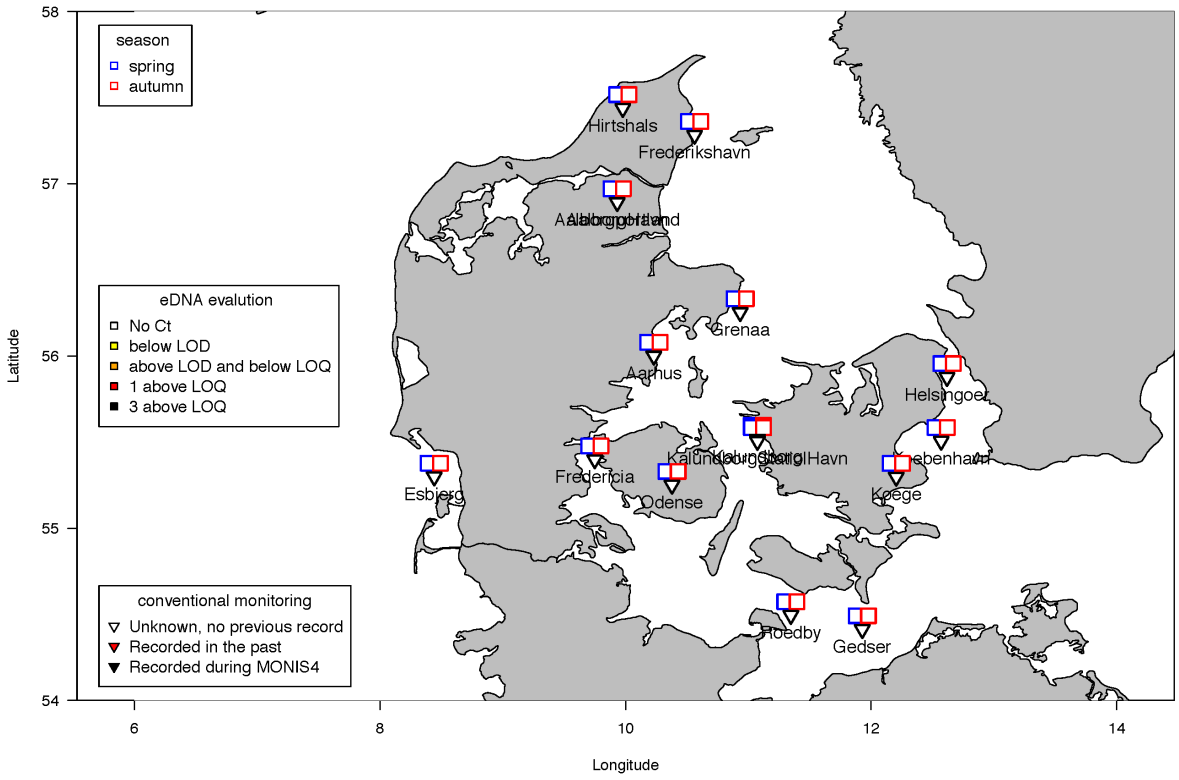


Appendix B 14 .

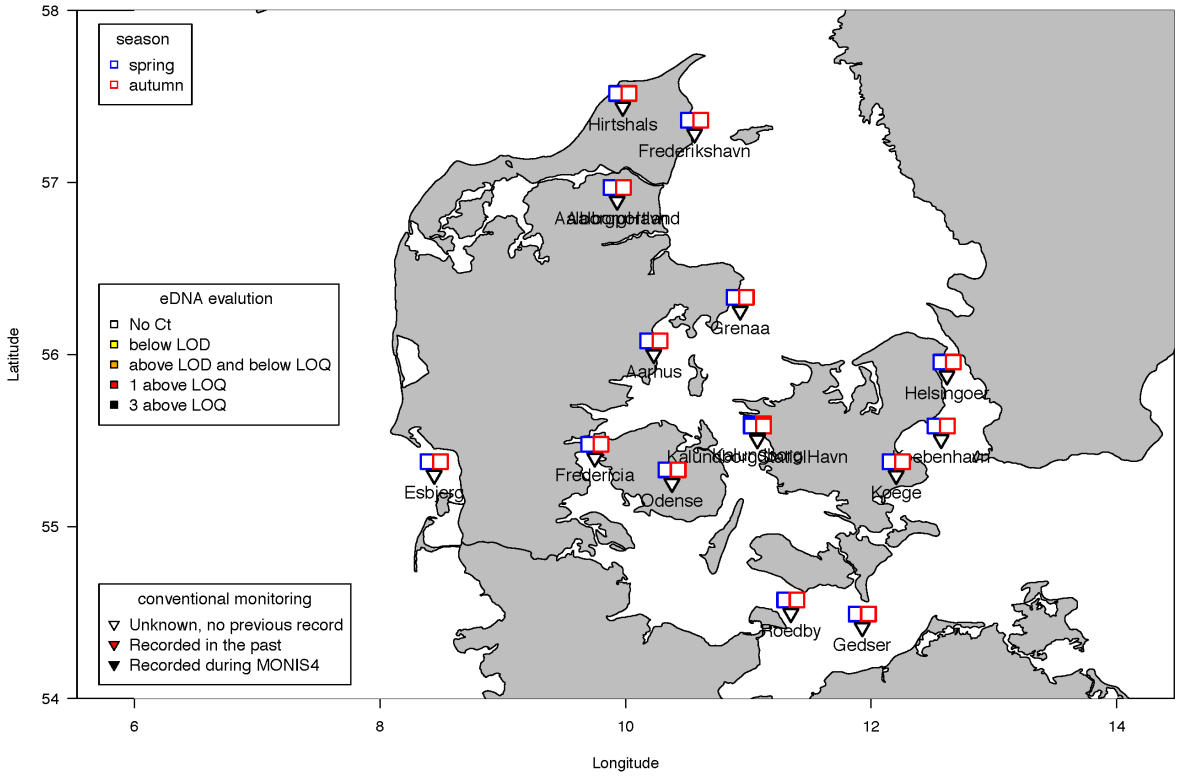
eDNA detected from *Rhithropanopeus harrisii* (mudderkrabbe), Assay Id No: 16



Appendix B 16 . eDNA detected from *Eriocheir sinensis* (kinesiskuldhaandskrabbe), Assay Id No: 18

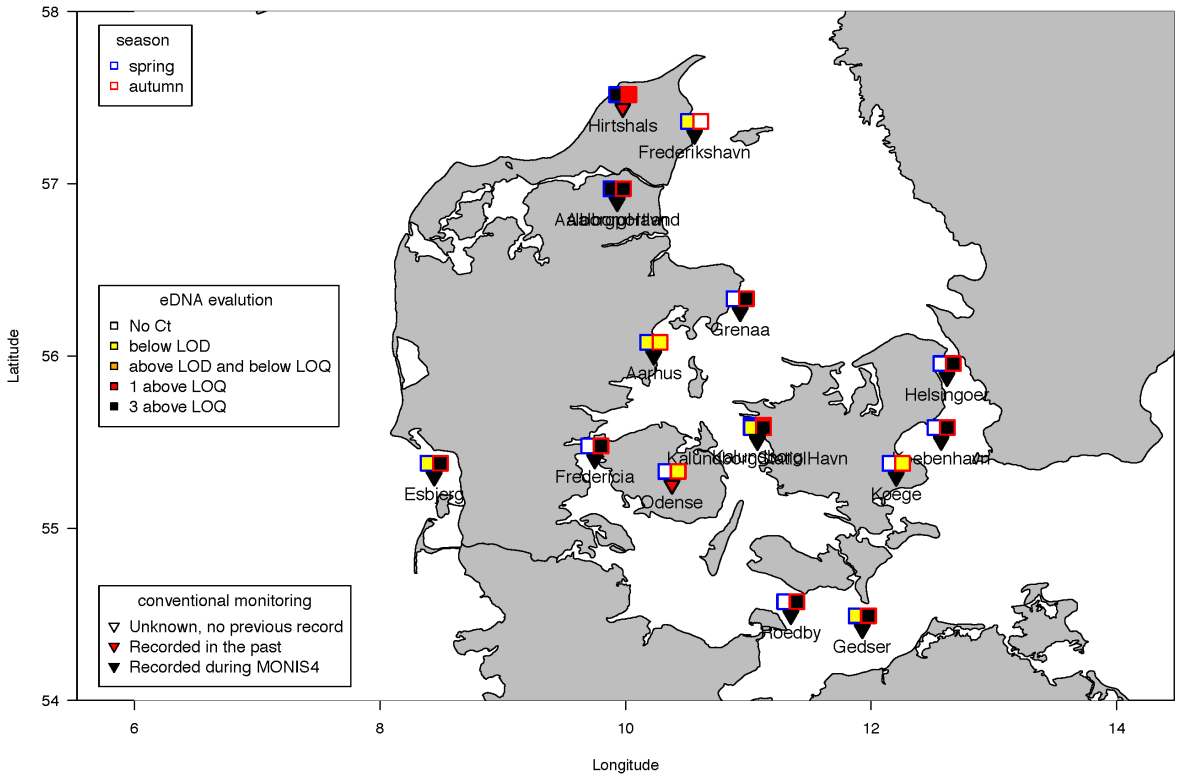


Appendix B 18 . eDNA detected from *Cordylophora caspia* (brakvandskroellepolyp), Assay Id No: 21



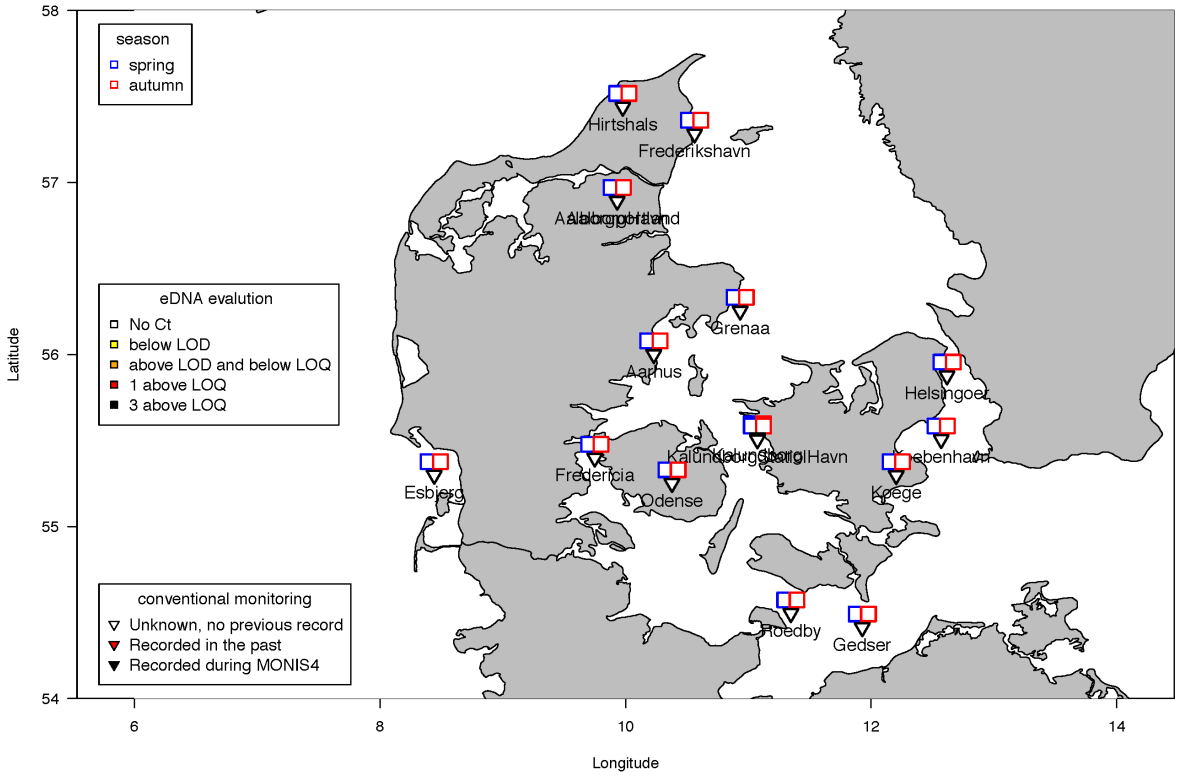
Appendix B 19 .

eDNA detected from *Mnemiopsis leidyi* (amerikanskribbegoble), Assay Id No: 22



Appendix B 20 .

eDNA detected from *Acipenser baerii* (siberiskstoer), Assay Id No: 23



Annex 11: Plots of salinity, temperature and oxygen profiles

Temperature, salinity and oxygen were measured using a SAIV CTD (SD204, see http://saiv.no/elasticslider/ctd-w-optional-sensors-1-2). To estimate the extent of the euphotic zone, a white Secchi disc (**Figure 3.3 section 3.2.1.1**) was released into the water and lowered until it was no longer visible, and the depth was recorded.

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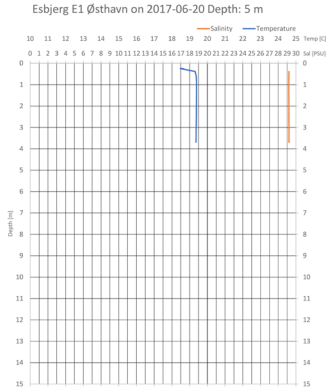
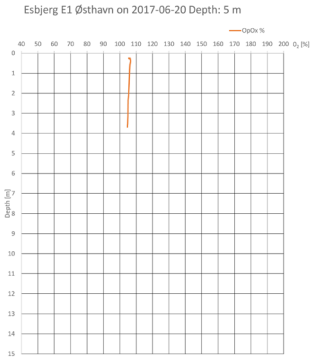
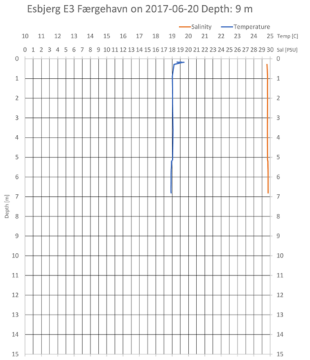
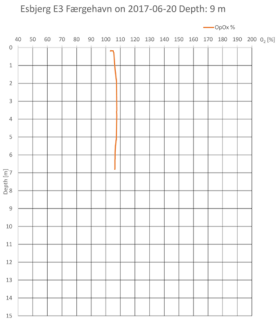


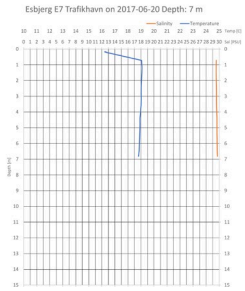


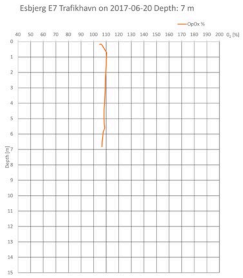
Esbjerg E3 Færgehavn





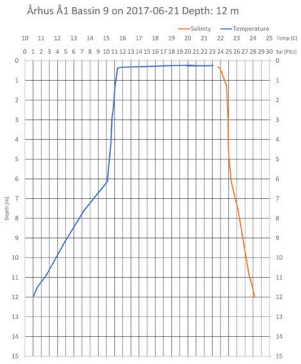
Esbjerg E7 Trafikhavn

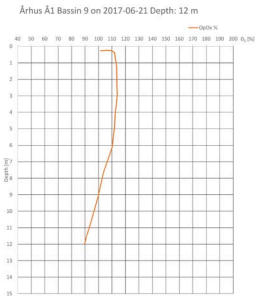




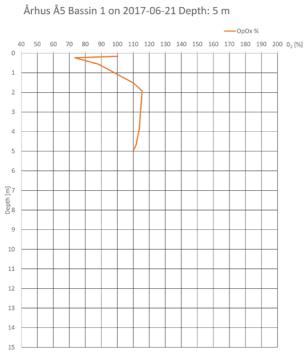
Aarhus – June 2017

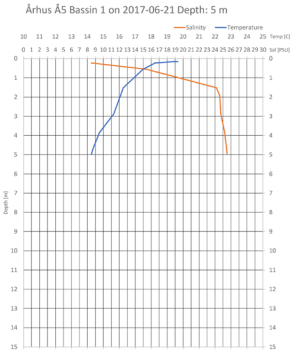
Aarhus Å1 Bassin 9



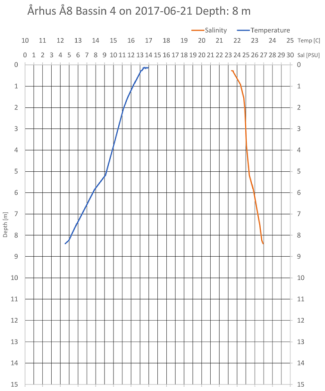


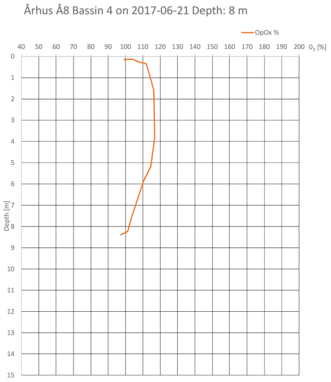
Aarhus A5 Bassin 1





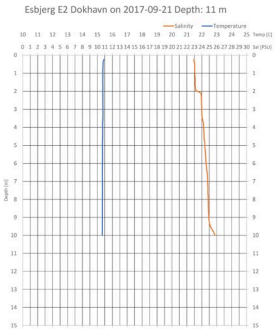
Aarhus Å8 Bassin 4

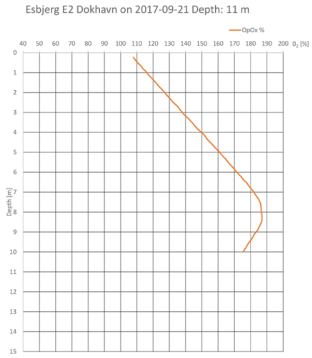




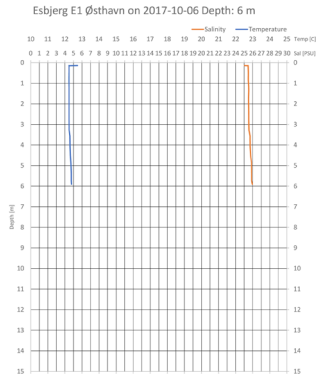
Esbjerg – September/October 2017

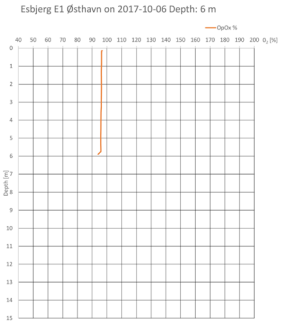
Esbjerg E1 Østhavn



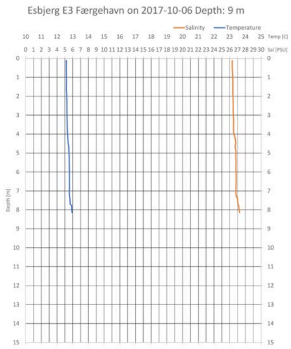


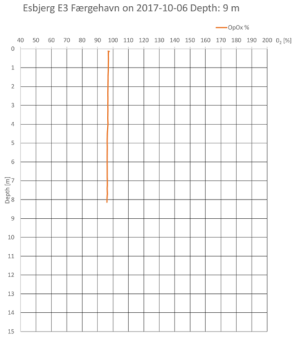
Esbjerg E3 Færgehavn





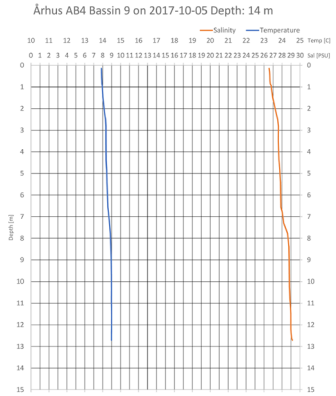
Esbjerg E7 Trafikhavn

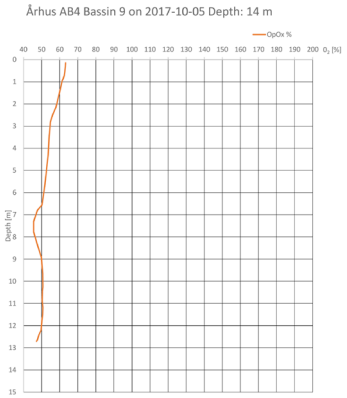




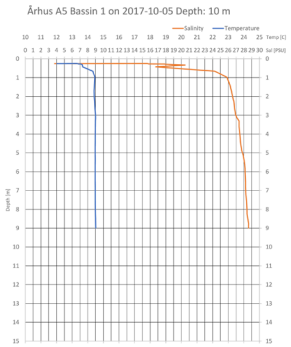
Aarhus - October 2017

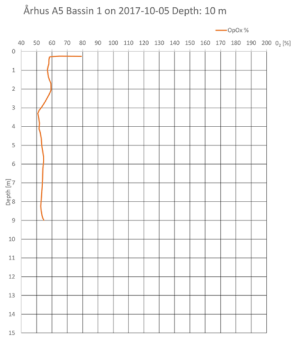
Aarhus Å1 Bassin 9



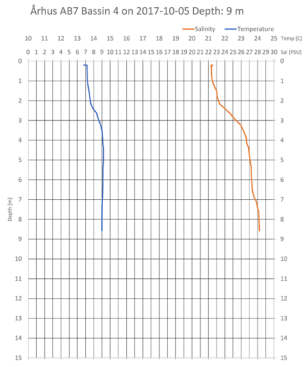


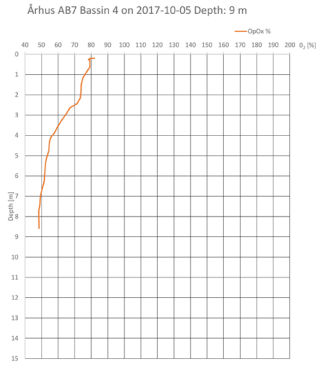
Aarhus A5 Bassin 1





Aarhus Å8 Bassin 4





NIVA Denmark is the name, water is our game

NIVA Denmark Water Research is a regional office of the Norwegian Institute for Water Research (NIVA) established in 2014 to resolve environmental issues concerning the freshwater and marine systems that relate to Denmark.

NIVA Denmark has primary focus on research-based implementation of a number of EU's directives *inter alia* the Water Framework Directive, the Marine Strategy Framework Directive, and the Maritime Spatial Planning Directive together with international conventions (HELCOM, OSPAR, BDC). We occasionally provide consultancy to authorities and small and medium-sized companies.

NIVA Denmark is a place for practice, observation, testing and synthesis. Key research and test areas include eutrophication, hazardous substances, biodiversity, and ecosystem health as well as the implications of multiple human activities in marine waters and in streams, rivers and lakes. We develop indicators, monitoring methods and tools to assess the state of an ecosystem in order to carry out analyses and contribute to evidence based and sustainable solutions to the challenges we and the environment face.

NIVA Denmark, as a regional office to NIVA, has thus the backing of more than 200 dedicated researchers and experts.

NIVA Denmark

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