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# Monitoring of non-indigenous species in Danish marine waters

Background and proposals for a monitoring  
strategy and a monitoring network

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**Title: Monitoring of non-indigenous species in Danish marine waters**

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# Preface

This report is a product of the MONIS project, or in full "Monitoring of Non-Indigenous Species in Danish Marine Waters", which has been funded by the Danish Ministry for the Environment, 2014.

The objectives of MONIS have been to:

1. Define the optimal monitoring strategy and design a cost-effective Marine Strategy Framework Directive (MSFD) monitoring programme targeting non-indigenous species in Danish waters (MSFD Descriptor 2; abbreviated to D2),
2. scrutinise how to make best use of existing data and make linkages to other monitoring activities (understood as other MSFD descriptors than D2) in order to fulfil obligations related to both the MSFD and the Regulation of the European Parliament and the Commission on the prevention and management of the introduction and spread of invasive alien species, and
3. ensure that the Danish proposal for a D2-targeted monitoring programme focusing on non-indigenous species is consistent with the MSFD cycle, e.g. production of MSFD Initial Assessment, design and implementation of monitoring programmes, design of Programmes of Measures, and implementation of Programmes of Measures, and ultimately also fulfilment of the overarching MSFD target of 'good environmental status'.

The report has been compiled and edited by Jesper H. Andersen, Søren Anker Pedersen and Jens Thaulow, all from NIVA Denmark; Frank Stuer-Lauridsen and Ditte Kristensen from Litehauz Aps, and Sabine Cochrane, Akvaplan-niva.

The work leading to this report has been coordinated by Jesper H. Andersen (NIVA Denmark), Frank Stuer-Lauridsen (Litehauz Aps), Ulrik Chr. Berggreen and Benny L. Bruhn (Danish Nature Agency).

# Sammenfatning

Med EU's havstrategidirektiv skal Danmark sikre en god miljøtilstand i de danske farvande og overvåge tilstand og udvikling, også i forhold til ikke-hjemmehørende arter (descriptor 2 i Havstrategidirektivet). Til dato har der ikke været gennemført en landsdækkende og systematisk overvågning af ikke-hjemmehørende arter i de danske farvande. Derfor har Naturstyrelsen finansieret udarbejdelsen af den foreliggende undersøgelse og rapport, med det formål at beskrive et omkostnings-effektivt overvågningsprogram med fokus på: (1) størst mulig synergi i forhold til eksisterende og planlagte overvågningsaktiviteter, (2) vurderinger af eksisterende metoder og tidssvarende biomolekylære teknologi (barcoding/eDNA), og (3) udarbejdelse af et forslag til overvågningsprogram.

Den eksisterende overvågning af miljø- og naturforholdene i de danske farvande (NOVANA-programmet) og fisk (ved DTU Aqua og Københavns Universitet) dækker forholdsvis bredt i forhold til vurderinger af forekomster af ikke-hjemmehørende arter i de kystnære farvande, men er imidlertid ikke landsdækkende, da visse farvandsområder og potentielle hotspots ikke er omfattet. De anvendte metoder (Tekniske Anvisninger mv.) er gennemlæst, og er overordnet set vurderet som værende hensigtsmæssige, selvom der mangler registrering af vigtige støtteparametre i nogle af anvisningerne. Endelig er andre og nyere teknikker, blandt andet automatisk prøvetagning og barcoding/eDNA, også vurderet med henblik på eventuelt brug i den fremtidige overvågning.

Med udgangspunkt i de gennemførte faglige vurderinger, specielt det forhold at eDNA-metoden er vurderet robust og velegnet i forbindelse med rutinemæssig overvågning, er der foreslået 6 strategiske målsætninger for den fremtidige overvågning af ikke-hjemmehørende arter i de danske farvande: (1) Overvågningsprogrammet skal være landsdækkende. (2) Programmet skal i størst mulige omfang gøre brug af den eksisterende eller planlagte biologiske overvågning, bl.a. a plante- og dyreplankton, bundplanter, bunddyr, fisk, og havpattedyr. (3) Programmet skal bestå af to komponenter, en komponent baseret på konventionel prøvetagning i andre delprogrammer og en komponent baseret på vandprøver og eDNA-analyser. (4) En særlig fokus skal rettes mod hotspots som større havne og områder med kølevandsudledning. (5) Prøver til eDNA-analyser skal dels oparbejdes til den årlige rapportering og dels gemmes i et filterarkiv, så man efterfølgende har mulighed for at genanalysere og vurdere eventuelle introduktion af nye arter. (6) Data fra andre kilder (fx fiskeovervågning) bør inddrages i analyserne og rapporteringen.

Forslagene til suppleringer af den eksisterende overvågning, som er baseret på konventionel prøvetagning, ligger alle inden for rammen for Havstrategiovervågningen til ikke-hjemmehørende arter. Der er således ikke tale om merudgifter i forhold til, hvad der allerede er planlagt. Overvågning af i alt 13 hotspots er foreslået på en måde, hvor arbejdet kan klares uden brug af skibe, men fra bil. Endelig er forslagene til et eDNA-baseret overvågningsprogram i 48 områder tilrettelagt, så det vil kunne integreres 100% i det eksisterende eller planlagte vandkemiprogram. Det vurderes, at den foreslåede strategi, og det afledte programforslag, i videst muligt omfang udnytter eksisterende og/eller planlagte aktiviteter og ressourcer. Med forslaget om et eDNA-baseret program har det, som følge af kost-effektiviteten og efter aftale med Naturstyrelsen, også været muligt at inkludere et forslag til rapportering m.v. En gennemførelse af strategien og de foreslåede overvågningsaktiviteter vil føre til et landsdækkende og kost-effektivt program for overvågning af ikke-hjemmehørende arter i de danske farvande.

# Summary

The Marine Strategy Framework Directive (MSFD) requires Member States to establish good environmental status and to monitor it, also regarding the descriptor on non-indigenous species (D2). However, so far, no organised monitoring of invasive or non-indigenous species has been carried out in Denmark. The Danish Nature Agency initiated the present study with the aim to outline a cost effective monitoring programme for D2. The scope of the study (Monitoring of Non-Indigenous Species in Danish Marine Waters; MONIS) was to focus on: (1) taking the greatest possible advantage of existing monitoring activities; (2) assessing the applicability of existing monitoring guidelines and of contemporary biomolecular technologies (i.e. barcoding/eDNA); and (3) developing a proposal for a national D2 monitoring programme.

This report presents the results of the MONIS project including a proposal for a monitoring programme. The existing monitoring activities of biological and chemical status in Danish waters under the national NOVANA monitoring programme and the monitoring of fish (by DTU Aqua and the University of Copenhagen) are comprehensive but insufficient with regard to monitoring of non-indigenous species and their geographical coverage. The NOVANA Technical Guidance Manuals are by MONIS considered as being appropriate also for non-indigenous species, but in some case important supporting parameters (i.e. salinity and temperature) are not registered. More recent methodologies e.g. automated sampling or biomolecular techniques (barcoding/eDNA) are not in use and they should be included in the monitoring of non-indigenous species.

The technical assessments of potential useful methods point to the eDNA method as a key technique in establishing a comprehensive and cost effective routine monitoring programme. Six strategic objectives for a future monitoring of non-indigenous species in Danish waters are listed: (1) The programme must provide appropriate coverage of Danish waters; (2) the existing NOVANA and planned MSFD monitoring of biological indicators shall be included to the fullest possible extent, i.e. phyto- and zooplankton, macroalgae, benthic organisms, fish and marine mammals; (3) the programme should be two pronged with one based on conventional sampling via other existing programmes and one based on water sampling and eDNA testing partly based on existing sampling; (4) the programme should include suspected hotspots, i.e. selected ports and cooling water outlets; (5) eDNA filter samples should be analysed annually and replicate samples kept in a sample bank ensuring availability for confirmatory analyses and examination for new species; and (6) data from other sources, e.g. fish surveillance, should be included in analysis and reporting.

The strategic objectives proposed by MONIS are incorporated into a proposed monitoring programme including: (1) 13 complimentary hot spot stations for non-indigenous species situated in ports or coastal environments that may be reached with smaller vessels/dinghies or directly from the quays; and (2) a part based on conventional sampling (44 stations) entirely contained within the existing NOVANA and the planned MSFD monitoring programme, thus adding no additional costs to monitoring stations onto the monitoring programme. Finally, the proposal by MONIS for an eDNA-based monitoring programme targeting 48 sites is 100% compatible with the planned water chemistry programme employing not only the same stations but also very similar sampling technologies. The proposed strategy and monitoring programme makes full use of the existing and planned activities and resources. It ensures compatibility with existing conventional sampling and allows considerable synergy to be harvested through the use of biomolecular methods, i.e. eDNA, which emerges as a cheap and versatile tool in the case of monitoring of non-indigenous species. Implementing this proposal will lead to cost effective and geographically comprehensive monitoring of non-indigenous species in Danish waters.

# 1. Introduction

The EU Marine Strategy Framework Directive (MSFD), or in full “Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive)”, entered into force on the 15th of July 2008 (Anon., 2008).

The MSFD focuses on implementing an ecosystem-based approach to the management of human activities and the collective pressures affecting the marine environment. The MSFD itself does not provide a definition of the ecosystems approach, although many different organisations (e.g. UN Convention on Biological Diversity, HELCOM, OSPAR) provide some definitions. In principle, the MSFD covers all European marine waters including coastal waters (the latter only in regard to issues not dealt with by the Water Framework Directive (WFD)) and has as an overarching aim of achieving or maintaining “good environmental status” in all European marine waters by 2020.

In order to reach this goal a timeline with a set of milestones is defined by the MSFD. These are:

- MS 1. Define criteria and methodological standards for identifying “Good Environmental Status” by 15th July 2010.
- MS 2. Prepare an initial assessment by 15th July 2012.
- MS 3. Determine Good Environmental Status by 15th July 2012.
- MS 4. Establish environmental targets and associated indicators by 15th July 2012.
- MS 5. Establish and implement a monitoring programme by 15th July 2014.
- MS 6. Develop a programme of measures by 2015 and operationalize the programme by 2016.
- MS 7. Member States shall by 2020 at the latest take the necessary measures to achieve “Good Environmental Status”.

In a short term perspective, an important part of this work has been the development of Initial Assessments of Member States marine water to be published for public consultation in the beginning of 2012 and agreed and published within 2012. The Initial Assessments should as a minimum include the following three elements:

1. An analysis of the essential qualities and characteristics and current environmental status of marine waters based on the indicative lists in Table 1 in the Directives Annex III and the physical chemical properties, habitat types, biological properties and hydromorphology.
2. An analysis of the predominant pressures and impacts, including human activities on environmental conditions: (i) based on the indicative lists in Table 2 in the Directives Annex III and the various pressures, their qualitative and quantitative composition as well as temporal trends, (ii) include the most important cumulative and synergistic effects, and (iii) take in consideration relevant assessments prepared pursuant to applicable Community legislation.
3. An economic and social analysis of the state of water use and of the cost of degradation of the marine environment.

This report builds upon the MSFD requirements in regard to monitoring of non-indigenous species (Anon 2008, Anon. 2010), the Danish Initial Assessment (Naturstyrelsen 2013a), the established Danish targets for non-indigenous species (Naturstyrelsen 2013b), and the outline monitoring programme for Danish marine waters (Naturstyrelsen 2014). Further, the objectives of the MONIS project have been to:

1. Define the optimal monitoring strategy and design a cost-effective Marine Strategy Framework Directive (MSFD) monitoring programme targeting non-indigenous species (NIS) in Danish waters (MSFD Descriptor 2; or simply D2).
2. Scrutinise how to make best use of existing data and make linkages to other monitoring activities (understood as other MSFD descriptors than D2) in order to fulfil obligations related to both the MSFD and the Regulation of the European Parliament and the Commission on the prevention and management of the introduction and spread of invasive alien species.
3. Ensure that the Danish proposal for a D2 targeted monitoring programme focusing on NIS is consistent with the MSFD cycle, e.g. production of MSFD Initial Assessment, design and implementation of monitoring programmes, design of Programmes of Measures, and implementation of Programmes of Measures, and ultimately also fulfilment of the overarching MSFD target of 'good environmental status'.

In the context of the present MONIS project, it would be appropriate to define what we are focusing on. The MSFD does not include a definition of non-indigenous species (NIS), but a useful definition can be found in the report from the MSFD D2 Task Group on non-indigenous species (Olenin et al. 2010):

*Non-indigenous species (NIS; synonyms: alien, exotic, non-native, allochthonous) are species, subspecies or lower taxa introduced outside of their natural range (past or present) and outside of their natural dispersal potential. This includes any part, gamete or propagule of such species that might survive and subsequently reproduce. Their presence in the given region is due to intentional or unintentional introduction resulting from human activities. Natural shifts in distribution ranges (e.g. due to climate change or dispersal by ocean currents) do not qualify a species as a NIS. However, secondary introductions of NIS from the area(s) of their first arrival could occur without human involvement due to spread by natural means.*

Non-indigenous species can enter into an area through a variety of pathways and routes (Olenin et al. 2010). A pathway is the route an alien species takes to enter or spread through a non-native ecosystem, one example of which is shipping. Each pathway may have a number of vectors that are involved in a species transmission. A vector is a transfer mechanism and is the physical means by which organisms are transported from one geographic region to another, such as in ballast water or attached to the ship's hull. The monitoring of pathways and vectors is not considered in MONIS, although these in principle should be reported as parts of Initial Assessments.

MONIS does not include a paradigm for analysis of monitoring data as well as annual reporting. These activities are in principle a task for M-FDC (Miljøministeriets Marine Fagdatacenter) or a skilled expert. Although NIVA Denmark and the partnership possess the experiences and competences required, we refrain from developing a D2-specific paradigm. Further, MONIS will not set up a Target Species List to consider as non-indigenous species (in principle a 'negative list'), because this is closely related to the reporting. Instead, we suggest a provisional list which will have to be revised once reporting processes begin. Then it would be a task for the Nature Agency, M-FDC or a skilled expert to revise it if needed.

# 2. Where are we now?

## 2.1 The MSFD and descriptor 2

With the adoption and implementation of the MSFD, Denmark is required to characterise Danish marine waters and to continuously monitor these with the aim of updating the characterisations, so-called Initial Assessments, every sixth year. The characterisation shall include an analysis of the essential qualities and characteristics and current environmental status of marine waters based on the indicative lists in MSFD Annex III Table 1 and the physical chemical features, habitat types, biological features and other ecologically relevant features (see Table 1 on the next page). It is also worth noting that the characterisation and Initial Assessment has its own structure, which differs slightly for the list of MSFD descriptors, which in principle is a structure too.

A first characterisation of non-indigenous species in Danish marine water was carried out in 2012-2013 as a two-step process. Firstly, a technical background report was compiled by the Danish Centre for Environment and Energy (DCE) at Aarhus University (Stæhr & Thomsen 2012). Subsequently, the Danish MSFD Initial Assessment was compiled by the Danish Nature Agency based on technical background reports from DCE (n = 18) and DTU Aqua (n = 2) and a single one produced by the Agency itself.

The Initial Assessment is a comprehensive document (100 pages), and addresses all required characteristics and descriptors, including descriptor 2 on non-indigenous species (Naturstyrelsen 2013a). Although variations in the level of details and also some criticism arising from a pan-European evaluation of all available MSFD Initial Assessments, the Danish one comes out relatively good and is, based on a screening for the scoring of assessment reports, probably among the top five Initial Assessments produced by the Member States.

In parallel to the Initial Assessment, national definitions and operational target values for good environmental status were specified by the Danish Nature Agency (Naturstyrelsen 2013b). The Danish description of good environmental status for non-native species (criterion D2.1.1, D2.2.1 and D2.2.2) 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 development 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).*

Further, an outline of the planned MSFD monitoring activities has been compiled by the Danish Nature Agency (Naturstyrelsen 2014).

## 2.2 Existing monitoring activities in Denmark

Two long-term monitoring activities are of particular interest to the monitoring of non-indigenous species in Danish marine waters. The NOVANA programme, which is coordinated and to a large extent carried out by the Danish Nature Agency, focuses on eutrophication, contaminants, biodiversity, and fish monitoring carried out by DTU Aqua.

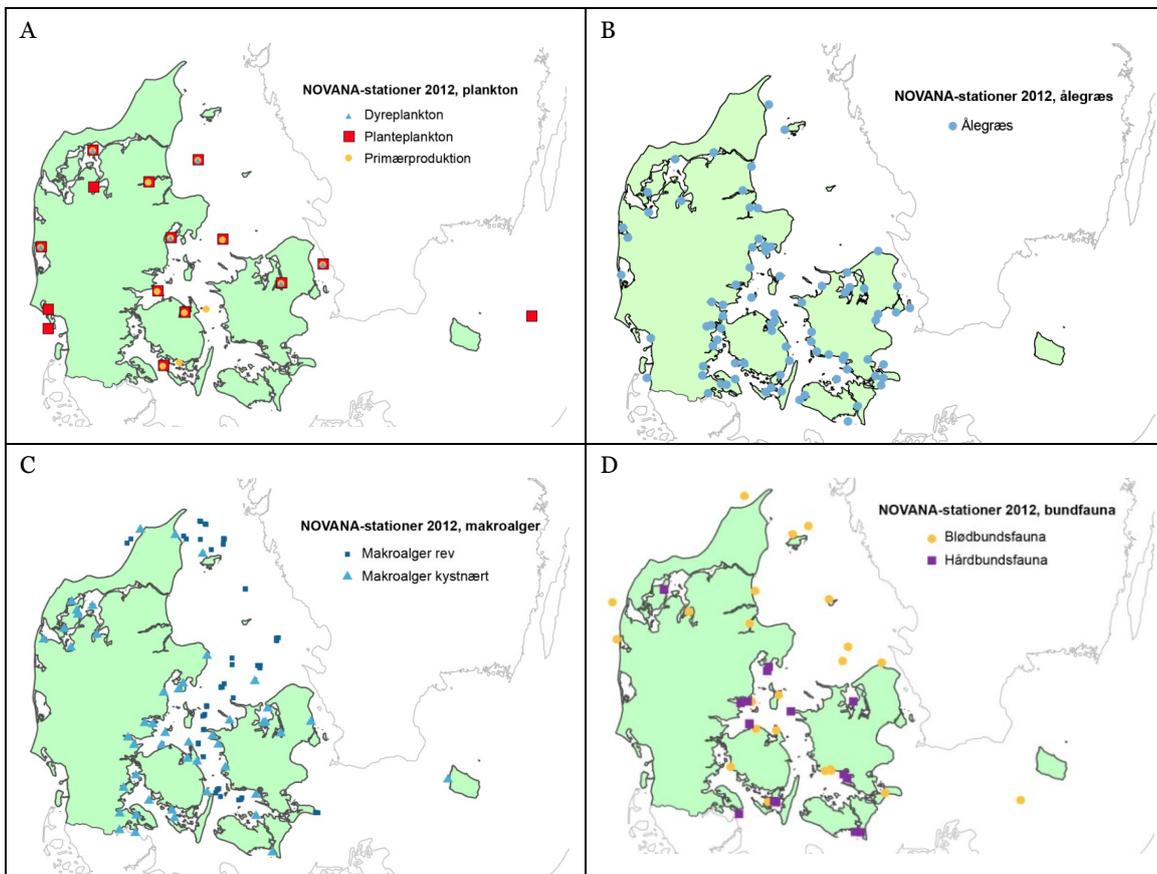
**Table 1.** Characteristics to be addressed in MSFD Initial Assessments (MSFD Annex III, Table 1). Those relevant to non-indigenous species in Danish marine waters, directly or indirectly, have been underlined.

| Features                       | Characteristics  |
|--------------------------------|--|
| Physical and chemical features | <ul style="list-style-type: none"> <li>• Topography and bathymetry of the seabed,</li> <li>• annual and seasonal temperature regime and ice cover, current velocity, upwelling, wave</li> <li>• exposure, mixing characteristics, turbidity, residence time,</li> <li>• spatial and temporal distribution of salinity,</li> <li>• spatial and temporal distribution of nutrients (DIN, TN, DIP, TP, TOC) and oxygen,</li> <li>• pH, pCO<sub>2</sub> profiles or equivalent information used to measure marine acidification.</li> </ul>  |
| Habitat types                  | <ul style="list-style-type: none"> <li>• The predominant seabed and water column <u>habitat type(s)</u> with a description of the characteristic physical and chemical features, such as depth, water temperature regime, currents and other water movements, salinity, structure and substrata composition of the seabed,</li> <li>• identification and mapping of <u>special habitat types</u>, especially those recognised or identified under Community legislation (the Habitats Directive and the Birds Directive) or international conventions as being of special scientific or biodiversity interest,</li> <li>• <u>habitats</u> in areas which by virtue of their characteristics, location or strategic importance merit a particular reference. This may include areas subject to <u>intense or specific pressures or areas which merit a specific protection regime</u>.</li> </ul>   |
| Biological features            | <ul style="list-style-type: none"> <li>• A description of the biological communities associated with the predominant seabed and water column habitats. This would include information on the <u>phytoplankton</u> and <u>zooplankton</u> communities, including the species and seasonal and geographical variability,</li> <li>• information on <u>angiosperms</u>, <u>macro-algae</u> and <u>invertebrate bottom fauna</u>, including species composition, biomass and annual/seasonal variability,</li> <li>• information on the structure of <u>fish</u> populations, including the abundance, distribution and age/size structure of the populations,</li> <li>• a description of the population dynamics, natural and actual range and status of species of <u>marine mammals</u> and reptiles occurring in the marine region or subregion,</li> <li>• a description of the population dynamics, natural and actual range and status of species of <u>seabirds</u> occurring in the marine region or subregion,</li> <li>• a description of the population dynamics, natural and actual range and status of <u>other species</u> occurring in the marine region or subregion which are the subject of Community legislation or international agreements,</li> <li>• an inventory of the temporal occurrence, abundance and spatial distribution of <u>nonindigenous, exotic species</u> or, where relevant, <u>genetically distinct forms of native species</u>, which are present in the marine region or subregion.</li> </ul> |
| Other features                 | <ul style="list-style-type: none"> <li>• A description of the situation with regard to chemicals, including chemicals giving rise to concern, sediment contamination, hotspots, health issues and contamination of biota (especially biota meant for human consumption),</li> <li>• a description of any other features or characteristics typical of or specific to the marine region or subregion.</li> </ul>  |

### 2.2.1 Monitoring of the aquatic environment and nature (NOVANA)

The national monitoring of Danish marine waters in relation to key policy drivers, i.e. the EC Habitats Directive (Anon. 1992) and the EU Water Framework Directive (Anon. 2000), takes place under the umbrella of NOVANA programme. HELCOM (COMBINE) and OSPAR (CEMP) requirements are also included in the monitoring programme and data are reported to several fora, i.e. WISE-Marine and ICES. The marine sub-programme of NOVANA focuses primarily on: (1) nutrient enrichment and eutrophication, including hydrography, (2) hazardous substances, and (3) marine nature types in Natura 2000 areas. The monitoring in regard to nutrient enrichment and eutrophication includes sampling of a wide range of indicators ranging from inputs, nutrient concentrations to direct and indirect effects of eutrophication, e.g. chlorophyll-a, phytoplankton species composition, zooplankton species composition, cover and species composition of angiosperms, macro-algae, and species composition of benthic invertebrates. The monitoring of marine nature types is focused on species composition and coverage of macro-algae on stone reefs and bubble reefs. NOVANA also includes a sub-programme for biodiversity. The primary focus is on terrestrial ecosystems, but seabirds and marine mammals are also included and monitored regularly.

The existing monitoring of pelagic biological indicators, i.e. phytoplankton and zooplankton covers most coastal waters but only few offshore parts (Figure 1A). The monitoring of benthic communities, i.e. submerged aquatic vegetation and benthic invertebrates, also covers most coastal waters, and again with a limited coverage in offshore parts such as the North Sea, Skagerrak and Baltic Sea (Figure 1B-D).



**Figure 1.** NOVANA monitoring stations in Danish marine waters. Panel A: Phyto- and zooplankton. Panel B: Eelgrass. Panel C: Macro-algae. Panel C: Benthic macro-invertebrates. Source: Hansen (2013).

A screening of the ongoing Danish monitoring activities under the NOVANA programme has revealed that the programme is not nation-wide as it was originally meant to be.

Monitoring of phytoplankton (Figure 1A) is focused on selected estuaries, some coastal waters and a few offshore stations. No monitoring stations are located in the North Sea, Skagerrak, the Great Belt, Smålandsfarvandet, and the sea area west of Bornholm (Figure 1A). Monitoring of zooplankton is limited to very few stations meaning that a nation-wide picture cannot be made. No zooplankton monitoring is being carried out in the open parts of the North Sea, Skagerrak, Great Belt, Little Belt, or the Baltic Sea.

Monitoring of benthic communities focuses on angiosperms (seagrasses), macro-algae and benthic invertebrates. The monitoring of angiosperms is providing knowledge about the present distribution of seagrasses almost nation-wide. The only missing area seems to be the coastal waters at Bornholm (Figure 1B).

Monitoring of macro-algae is concentrated in estuaries, coastal waters and the Inner Danish Water. No offshore monitoring activities are being carried out in the North Sea or around Bornholm. Only one station is located in Øresund (Figure 1C). Regarding benthic invertebrates, no soft-bottom monitoring is carried out in the open parts of the North Sea, Øresund, or east of Bornholm. Concerning hard bottom benthic invertebrates, there is no monitoring in the North Sea, Skagerrak, Kattegat, Øresund, or in the Baltic Sea including coastal waters around Bornholm (Figure 1D).

**Table 2.** Estimated spatial coverage and representativeness of existing NOVANA monitoring activities. 0 = Low, - = Adequate, and O = High.

| Area           |          | PHY-PLA | Zoo-PLA | SAV | Benthic invertebrates |             |
|----------------|----------|---------|---------|-----|-----------------------|-------------|
|                |          |         |         |     | Soft bottom           | Hard bottom |
| North Sea      | Coastal  | 0       | 0       | 0   | -                     | 0           |
|                | Offshore | 0       | 0       | 0   | 0                     | 0           |
| Skagerrak      | Coastal  | 0       | 0       | -   | -                     | 0           |
|                | Offshore | 0       | 0       | -   | -                     | 0           |
| Kattegat       | Coastal  | 0       | 0       | -   | -                     | 0           |
|                | Offshore | -       | -       | -   | -                     | 0/-         |
| Danish Straits | Coastal  | -       | -       | O   | 0                     | 0           |
|                | Offshore | -       | 0       | -   | 0                     | 0           |
| Baltic Sea     | Coastal  | 0       | 0       | 0   | 0                     | 0           |
|                | Offshore | 0       | 0       | 0   | 0                     | 0           |

### 2.2.2 Monitoring of fish by DTU Aqua

DTU Aqua, formerly known as the Danish Institute for Fisheries Research (DIFRES), is a national institute responsible for a wide range of specific monitoring activities in Danish marine waters, including commercially important fish and shellfish species. The institute is monitoring most of the open marine waters in Denmark through several standard monitoring surveys. The number of sampling days and stations are similar between years, such that species distributions can be compared over time. According to Storr-Paulsen (2014), fish monitoring activities by DTU Aqua includes the following programmes:

1. Limfjord survey
2. Sole survey
3. Cod survey
4. Kattegat survey (KASU)
5. Baltic International Trawl Survey (BITS)
6. International Bottom Trawl Survey (IBTS)
7. North Sea Herring Acoustic Survey (NHAS)
8. *Nephrops* UTV survey in in Skagerrak and Kattegat
9. North Sea sandeel survey
10. Recreational fish catch registration
11. Coastal juvenile fish survey
12. Commercial vessel sampling

The surveys and sampling listed above are funded by various programmes and/or activities, and it has been estimated that approximately 80% of the costs are covered via the EU Data Collection Framework (DCF) (Storr-Paulsen, *pers. comm.*).

#### Limfjord survey

The Limfjord fish survey 1984–2012 was carried out to monitor abundance, distribution and composition of the fish species in the fjord over space and time (Hofmann, 2005). In addition to fish also larger invertebrates such as crustaceans, shellfish, squids, and jellyfish have been recorded. All data are stored in the DTU Aqua database and survey results are subsequently published by DTU Aqua (in Danish). A standard DTU Aqua TV3-trawl with 20 mm cod-end has been used since 1996. See survey facts in Table 3.

The survey covers 30-35 trawl stations (fishing lanes) revisited from year to year. Areas not covered by the survey are near shore areas and Hjarbæk Fjord (see Figure A2.1a in Annex 2).

#### Sole survey

The sole survey monitors flatfish species in Kattegat and part of the Skagerrak with a commercial trawl. No survey was carried out in 2012 and 2013 but the survey continued in 2014. The fishery is performed during night time and all fish caught are registered. The data collected are species, length and hydrography (CTD). See survey facts in Table 3.

The spatial coverage of the survey is Kattegat and a small part of the Skagerrak area. Areas not covered by the survey are the coastal areas and Fjords, eastern Kattegat and Aalborg Bugt (0-15 m) (see Figure A2.1b in Annex 2).

### **Cod survey**

The cod survey monitors the large demersal fish species in the Kattegat deeper than the 20 m depth contour line using a commercial trawl with a 70 mm cod-end. Two coastal embayments, Laholmsbugten and Skældervigen, are also included in the survey area despite the depth being shallower than 20 metres. The survey is conducted by four commercial chartered trawlers - two Swedish and two Danish. No survey was conducted in 2012. All fish caught are registered. The data collected are species, length and hydrography (CTD). Survey facts in Table 3.

Areas not covered by the survey are coastal areas and fjords, eastern Kattegat and Aalborg Bugt (0-20 m).

### **Kattegat survey (KASU)**

The Kattegat bottom trawl survey monitors the abundance of commercial (mainly cod, flounder and plaice) and non-commercial fish species using a TV3-trawl with a 20 mm cod-end. KASU is part of the Baltic International Trawl Survey (BITS) survey coordinated by the ICES International Bottom Trawl Survey Working Group (see below). The data collected are species, length and hydrography (CTD). Otoliths of commercial species are also collected to assess abundance by age, and the recruiting year classes. See survey facts in Table 3.

The survey covers Kattegat and the Belt seas. Areas not covered by the survey are the eastern part of the Great Belt, shallow areas between the islands, southeast Inner Danish Waters (western Baltic Sea; see Figures A2c and d in Annex 2).

### **Baltic International Trawl Survey (BITS)**

The BITS bottom trawl survey monitors the abundance of commercial (mainly cod, flounder and plaice) and non-commercial fish species using a TV3-trawl with a 20 mm cod-end. The data collected are species, length and hydrography (CTD). Otoliths of commercial species are also collected to assess abundance by age, and the recruiting year classes. Furthermore, a plankton sampler with 3 different mesh sizes (150, 335 and 500 µm) is used to monitor planktonic species. See survey facts in Table 3.

The Danish survey part of BITS covers the deep areas around and east of Bornholm. Areas not covered by the survey are coastal areas and shallow waters around Bornholm (Figure A2.1j).

### **International Bottom Trawl Survey (IBTS)**

The IBTS bottom trawl survey monitors the abundance of commercial and non-commercial fish species using a GOV-trawl with a 20 mm cod-end. The IBTS survey is coordinated by the ICES International Bottom Trawl Survey Working Group and covers the North Sea, Skagerrak and Kattegat. The data collected are species, length and hydrography (CTD). A sampling programme for fish larvae is conducted during the 1st quarter of IBTS and covers around 80 plankton sampling stations with a MIK plankton sampler (2 m diameter ringnet). See survey facts in Table 3.

The Danish survey covers part of the North Sea. Areas not covered by the survey includes coastal areas of Jutland and the Wadden Sea (Figure A2.1g,h).

### **North Sea Herring Acoustic Survey (NHAS)**

The NHAS monitors abundance estimates of herring and sprat in the North Sea (eastern part), Skagerrak and Kattegat. The data collected are acoustic data, species, length and hydrography (CTD). See survey facts in Table 3.

Areas not covered by the survey are coastal areas and shallow waters (0-15 m) (Figure A2.1e).

### ***Nephrops* UTV survey in Skagerrak and Kattegat**

The survey monitors the abundance of *Nephrops* in the Skagerrak and Kattegat using underwater video recording. The video recordings are later analysed and the *Nephrops* abundance is estimated in selected survey areas. The 2012 survey was conducted with R/V Havfisken in April/May (10 days) and completed in August (5 days). The survey covers the main *Nephrops* fishing grounds in the Skagerrak (Subarea 1) and Kattegat (Subarea 2), and station positioning follows a stratified random design. A station is a 10 min video recording on a predefined position. See survey facts in Table 3.

Only *Nephrops* habitats are covered by the survey (Figure A2.1f).

### **North Sea sand-eel survey**

The survey catches sand-eels (*Ammodytes marinus*) buried in the seabed to assess the year class strength. The gear used is a modified commercial mussel dredged with bottom contact sensors. The survey is conducted with a commercial fishing vessel. See survey facts in Table 3.

The spatial coverage of the survey is sand-eel sand banks of the North Sea. Sand banks along the west and southwest coast of Jutland are not covered by the survey (Figure A2.1i).

**Table 3.** Overview of DTU Aqua surveys. From Storr-Paulsen (2014).

| Survey   | Vessel                  | Planned days at sea | Planned fish hauls | Planned plankton hauls | First year of survey | Last year of survey |
|--|-------------------------|---------------------|--------------------|------------------------|----------------------|---------------------|
| Limfjord survey Aug-Sep  | Havkatten/<br>Havfisken | 10-12               | 30-35              | 0                      | 1984                 | 2012                |
| Sole survey 4 <sup>th</sup> quarter                                  | Commercial vessel       | 12                  | 80                 | 0                      | 2004                 | 2011                |
| Cod survey 4 <sup>th</sup> quarter                                   | Commercial vessel       | 24                  | 80                 | 0                      | 2008                 | Present time        |
| BITS 1 <sup>st</sup> / 4 <sup>th</sup> quarter (KASU I/II)           | Havfisken               | 20/20               | 49/49              | 0/0                    | 1995/1994            | Present time        |
| BITS 1 <sup>st</sup> / 4 <sup>th</sup> quarter                       | DANA                    | 18/18               | 50/50              | 80/80                  | 1991                 | Present time        |
| IBTS 1 <sup>st</sup> / 3 <sup>rd</sup> quarter                       | DANA                    | 18/18               | 39/50              | 80/0                   | 1991                 | Present time        |
| NHAS 2 <sup>nd</sup> + 3 <sup>rd</sup> quarter                       | DANA                    | 14                  | 37                 | 20                     | 2006                 | Present time        |
| <i>Nephrops</i> UTV survey 2 <sup>nd</sup> + 3 <sup>rd</sup> quarter | Havfisken               | 15                  | 120                | 0                      | 2006                 | Present time        |
| Sandeel survey   | Commercial vessel       | 15                  | 50                 | 0                      | 2004                 | Present time        |

### **Coastal juvenile fish survey**

The survey monitored distribution and abundance of age 0 and age 1 juveniles in coastal soft bottom areas at depth between 1.2 and 3 m during July-August from 1957 to 2004. However, no surveys have taken place between 1972 and 1984.

The spatial coverage of the survey was limited to selected coastal areas on the east coast of Jutland, northern Funen, northwest Zealand and Øresund. The number of stations sampled varied between years. Many coastal areas were not covered.

### **Commercial vessel sampling**

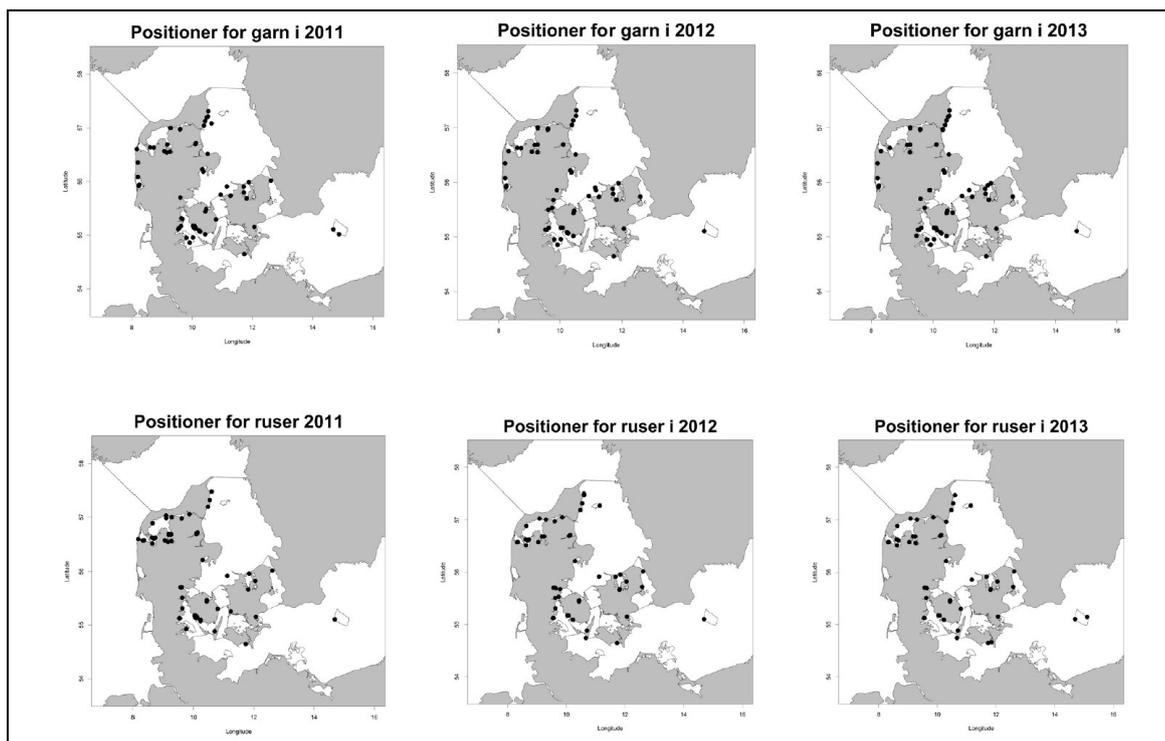
Sampling of catches and discards on board commercial fishing vessels has been ongoing since 1995 by DTU Aqua. In 2012 samples were taken from the catches of 229 commercial fishing trips in five sea areas: North Sea (32 trips), Skagerrak (52 trips), Kattegat (44 trips), Western Baltic (32 trips), Eastern Baltic (30 trips). The data collected are species, length and hydrography (CTD) and for commercial species also age and weight.

The spatial coverage of the sampling programme is the North Sea, Skagerrak, Kattegat, Western Baltic, and Eastern Baltic. Areas not sampled are the coastal waters.

### Recreational fish catch registration

DTU Aqua has since 2002, in collaboration with the Danish Organisation for Amateur Fishermen and the Danish Union of Recreational Fishermen, recorded fish catches in Danish estuaries and coastal waters using standard sampling methods. The objective of the “Key-fishermen” project is to register fish catches and document fish assemblages in Danish coastal waters over a consecutive number of years. The project represents the largest and longest time series on catches in gillnets and fyke-nets along the Danish coasts. This represents a great feat based on voluntary work and reflects the profound interest of recreational fishermen to monitor and preserve fish populations in fjords, bays and coastal areas. Not all species are caught in the nets used. Pelagic fish (fish that live in the water column) are rarely caught, e.g. garfish. Using the same fishing gear and fishing position year after year, makes it possible to compare developments in fish stocks between areas and years. Flounder, eel and eelpout are caught in all areas. This reflects an adaptation of these species to the variable environment that is typical of coastal areas with fluctuations in temperature and salinity. The project will continue until 2016. Fishing positions in 2011, 2012 and 2013 are shown in Figure 2. More information is available in Kristensen et al. (2014) and via the following link: <http://www.fiskepleje.dk/kyst/fangstregistrering.aspx>.

Most of Danish coastal waters are covered by the project. Areas not covered are eastern part of the Great Belt, Smålandsfarvandet, south-eastern parts of the Inner Danish Waters (south-western Baltic Sea) (Figure 2).



**Figure 2.** Monitoring of coastal fish populations by the “Key-fishermen” project in 2011, 2012, and 2013. Gill-net positions (top) and traps positions (bottom). From Kristensen et al. (2014).

### 2.2.3 Other relevant activities

#### Mapping of Danish salt water fish species 2009-2016

University of Copenhagen (Zoological Museum, KU) is in collaboration with DTU Aqua and Krog Consult currently mapping the occurrence of salt water fish species in Denmark (2009-2016). The project is funded

by Aage V. Jensens Fonde for nature conservation and wildlife protection. The objective of the mapping project is to create detailed knowledge about the distributions of the Danish marine fish species. The future increasing temperatures make it important to map the present fish distributions in order to track future changes. The mapping is important in order to keep track of new incoming and potentially invasive fish species. The survey includes all species caught in Danish territorial waters, both the ordinary as herring and cod, the rare visitors such as swordfish and freshwater fish, which often migrate out into the sea from rivers and lakes. The project will collect an amount of fish for various studies. Many fish will be preserved and stored in the Zoological Museum's scientific fish collection, where they together with the associated tissue bank will be available to researchers worldwide in the next hundreds of years. The project will result in a comprehensive book on our saltwater fish similar to the one on the way for freshwater fish. The book is scheduled for publication 2016. More information can be found at: <http://fiskeatlas.ku.dk/>.

### **Ballast water**

There have been no monitoring activities related to ballast water in Denmark. As part of their commercial activity, DHI performs large scale sampling exercises of ballast water treatment systems at their facility in Hundested and of ballast water during tests on-board ships. At present, non-indigenous species have not been identified.

## **2.3 Methods currently in use**

We will review existing and D2 relevant national Technical Guidance Manuals. As a starting point, we will focus on the Technical Guidance Manuals from the national NOVANA monitoring programme, but also include other available types of guidance and/or method descriptions. Any international guidance will be included on the basis of a relevance and maturity criteria.

### **2.3.1 NOVANA**

Priority has been given to the following monitoring methods described in the following NOVANA Technical Guidance Manuals <sup>(1,2)</sup>:

|                               |   |
|-------------------------------|---|
| Plankton:                     | Microzooplankton (TA 2.6), og Mesozooplankton (TA 2.7).   |
| Submerged aquatic vegetation: | Macro-algae in coastal hard bottom (TA M12), Mapping of vegetation on tidal flats (TA M13), and Eelgrass and other vegetation on coastal soft bottom (M18). |
| Benthic invertebrates:        | Soft bottom fauna (TA M19), and Hard bottom fauna (TA 4.2).   |
| Marine mammals:               | Monitoring of harbour porpoise (TA M15), Monitoring of seals (TA M16)   |
| Natura 2000:                  | Monitoring and assessment of coastal lagoon habitats in Natura 2000 areas (TA M 20).  |

The Technical Guidance Manual TA 2.5 on phytoplankton (Phytoplankton species composition, number, biovolume and carbon biomass) has not been considered. A revised draft is in consultation and has not been made available to MONIS – and we found it irrelevant to evaluate the existing and soon outdated version.

Zooplankton: The main groups of protozooplankton in mid-water are flagellates and ciliates. These two groups play an important role as grazers of both phytoplankton and bacteria. In contrast to the multicellular zooplankton (mesozooplankton) protozooplankton have growth rates comparable to the phytoplankton. Mesozooplankton consists primarily of organisms that feed on phytoplankton. Mesozooplankton is dominated by copepods, however, *Daphnia* contribute significantly to the biomass during certain periods of the year.

Angiosperms: Marine macro-vegetation is monitored on tidal flats of e.g. the Wadden Sea. Eelgrass (*Zostera marina*), dwarf eelgrass (*Zostera noltii*) and drifting, opportunistic macro-algae dominate the

<sup>1</sup> <http://bios.au.dk/videnudveksling/til-myndigheder-og-saerligt-interesserede/fagdatacentre/fdcmarintny/tekniskeanvisningernovana20042010/>

<sup>2</sup> <http://bios.au.dk/videnudveksling/til-myndigheder-og-saerligt-interesserede/fagdatacentre/fdcmarintny/ta2011-2015/>

macro vegetation. The angiosperms are also monitored on soft ground along the coasts of the inner fjords, outer fjords and open areas along depth gradients from the coast and out to the greatest depths where vegetation occurs. The monitoring programme has a special focus on eelgrass but also includes other flowering plants, stonewort and drifting macro-algae (seaweed).

Macro-algae: The monitoring of macro-algae (seaweed) on hard bottoms along the coasts of the inner fjords, outer fjords and open areas describes macro-algal diversity, species distribution and dominance along depth gradients. The macro-algae are divided in three main groups: Sessile macro-algae on stable substrate, drifting opportunistic macro-algae and finally other drifting macro-algae. There is a particular focus on the sessile macro-algae on large, stable stones.

Benthic invertebrates: The monitoring of the soft bottom fauna includes studies of species diversity, species composition, individual density and biomass of soft bottom fauna in the coastal and open sea areas. Species diversity and species composition are included in the calculation of the Danish Quality Index, DKI, which describes the environmental status of the fauna communities. Monitoring of the hard bottom fauna is related to the EC Habitats Directive's requirement to describe the favourable conservation status of two of the directive habitats, reefs and bubble reefs. Another fact that makes registration of animals on the hard bottom relevant is the interaction between animals and algae. The animals' presence can be an explanatory factor for algal occurrence. Finally, registration of hard bottom fauna helps to provide better basic knowledge about the different species and the communities they are part of.

Marine mammals: The two seal species in Denmark, harbour seal (*Phoca vitulina*) and grey seal (*Halichoerus grypus*) are monitored at their resting places on land in order to estimate the number of seals and pup production, respectively. Monitoring is performed by aerial surveys on their resting places in relevant periods with high incidence of seals. The harbour porpoise (*Phocoena phocoena*) are monitored at a frequency which makes it possible to assess the population dynamics within each reporting period. Because the harbour porpoises migrate beyond the boundaries of the designated Natura 2000 habitat areas, monitoring include not only the SACs but the whole porpoise distribution range. However, the current coverage of the existing monitoring activities has not been assessed.

Natura 2000: Monitoring and assessment of coastal lagoon habitats in Natura 2000 areas includes observations and recording of characteristic structures, flora and fauna elements, invasive species and individual measurements of physical conditions such as water depth and salinity. The current coverage of the monitoring activities has not been assessed.

Table 4 summaries the reviews of the NOVANA Technical Guidance Manuals. All in all, the Technical Guidance Manuals considered are found fit for purpose although the structure with both thematic Technical Guidance Manuals and a single focusing on a specific nature type is not logical. Only flaw would be that temperature and salinity apparently is not measured consistently when biological parameters are sampled.

### **2.3.2 The Common Fisheries Policy (CFP) and Data Collection Framework (DCF)**

A new Common Fisheries Policy (CFP) aims to get stocks back to sustainable levels, put an end to fishing methods that involve waste of resources. As part of the reformed a ban on fish discards has been agreed upon (3). Instead, all of the catch must be landed and counted against quota. The discard ban creates opportunities to monitor new and invasive species. Therefore, the species-monitoring programme under the new CFP may be useful for monitoring non-indigenous species in Danish marine waters.

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<sup>3</sup> [http://ec.europa.eu/fisheries/cfp/fishing\\_rules/discards/index\\_en.htm](http://ec.europa.eu/fisheries/cfp/fishing_rules/discards/index_en.htm)

**Table 4.** Summary of the review of the NOVANA Technical Guidance Manuals. The evaluation relates to the general methodology and its suitability in regard to identification of non-indigenous species.

| Theme                              | TA  | Status | Comments  |
|------------------------------------|-----|--------|---|
| Phytoplankton                      | 2.5 | ?      | Not considered  |
| Microzooplankton                   | 2.6 | OK     | Pelagic measurements of temperature, salinity, chlorophyll etc. described in TA number 2.1.   |
| Mesozooplankton                    | 2.7 | OK     | Pelagic measurements of temperature, salinity, chlorophyll etc. described in TA number 2.1.   |
| Macro-algae                        | 12  | OK     | Temperature and salinity measurements should be included.   |
| Angiosperms                        | 13  | OK     | Temperature and salinity measurements should be included.   |
| Eelgrass                           | 18  | OK     | Temperature and salinity measurements should be included.   |
| Benthic invertebrates, soft bottom | 19  | OK     | At the sampling stations temperature and salinity measurements at the bottom are missing. Sieve with mesh size 1 mm is used and only fauna in the sieve are determined. Fauna below 1 mm not considered e.g. invertebrate larvae. |
| Benthic invertebrates, hard bottom | 4.2 | OK     | Temperature and salinity measurements should be included.   |
| Harbour porpoise                   | 15  | OK     |   |
| Seals                              | 16  | OK     |   |
| N2000                              | 20  | OK     | Salinity included, temperature not included. Sieve with mesh size 2 mm is used and only fauna in the sieve are determined. Fauna below 2 mm not considered e.g. invertebrate larvae.  |

The EU Data Collection Framework (DCF) was established in 2008 in order to make fisheries data available for scientific advice and management. The data is collected by the EU Member States based on National Programmes evaluated by the EU Scientific, Technical and Economic Committee for Fisheries (STECF). The European Commission (DG MARE) issues data calls and the data collected by the MS is uploaded to databases managed by the JRC. JRC makes the data available for analysis by experts of the STECF and the resulting scientific advice is used to inform the CFP decision making process. The Data Collection Framework (DCF) website<sup>4</sup> gives information and data related to the above described process.

In ICES, fisheries data collected under the DCF is stored by ICES in the regional database Fishframe<sup>(5)</sup> and used in ICES stock assessments working groups and other related working groups such as the Planning Group on Commercial Catches; Discards and Biological Sampling; Study Group on Practical Implementation of Discard Sampling Plans. The European Commission is currently working to develop a new data collection regulation, the Data Collection Multiannual Program (DC-MAP).

### 2.3.3 Port survey guidelines

The HELCOM/OSPAR Joint Harmonised Procedure (JHP; 2013) was developed to assist applicants as well as national authorities regarding biological surveys of non-indigenous species in ports. The surveys are part of a risk assessment undertaken in the exemption application process under the Ballast Water Management Convention as implemented in Danish law. The JHP is voluntary and include a port survey protocol describing the methodology for comprehensive sampling in each port on the route for which an exemption is applied. The approach to surveys of the JHP is to use proven technologies and skills. A port is considered “a contiguous unit, separated by for example a land mass, as peninsula or distance more than 1 km from other ports or port areas”. The minimum of sampling sites is three, but more sites may be required for an adequate analysis, depending on the size and type of port. According to the JHP, two surveys should be conducted at each port, one in spring and one in late summer in order to identify aquatic species throughout all life-cycles. All main substrate types are to be sampled for hard substrate organisms, soft bottom benthos, plankton, and mobile epifauna, and special attention be given to high priority areas, which include active berths, inactive/disused wharves, channel markers, tug and pilot vessel berths, and

<sup>4</sup> <http://datacollection.jrc.ec.europa.eu/index.html>

<sup>5</sup> <http://www.ices.dk/marine-data/data-portals/Pages/RDB-FishFrame.aspx>

slipways. It is highly recommended to also map the underwater habitats either by diving, if possible, or by using underwater cameras (HELCOM/OSPAR, 2013; LITEHAUZ, 2013).

The JHP is based on the survey methods used in the HELCOM Maritime ALIENS-2 project, which were in part based on the CRIMP methods developed in Australia. There was no Danish participation in the ALIENS-2 project.

The IMO<sup>6</sup> risk assessment guideline (G7) entails awarding exemptions for a five year period with a review at midterm. However, options exist to require reviews or surveys at shorter intervals. Thus, the prescribed timing of surveys for maintaining an exemption is as a minimum every five years.

### **2.3.4 Sampling of ballast water, hulls and aquaculture equipment**

There are currently no prescribed methodologies for the sampling of ballast water, hulls or other equipment placed in the marine environment. The IMO sampling guideline for ballast water (G2) does point to a number of methods for sampling, but up to late 2014, it still is not decided which of these are universally appropriate for e.g. port state control or as basis for national monitoring.

## **2.4 Emerging methods**

In regard to new and emerging methods, focus has been put on the potential use of so-called eDNA (section 2.5.1.), automatic sampling by FerryBox (section 2.5.2), and other potentially interesting methods (section 2.5.3).

### **2.4.1 Environmental DNA (eDNA)**

Traditional registration of species is a time consuming task due to the necessity of visual verification that the organism/species of interest actually is present in a given location. Sampling of invertebrates in streams by kick samples and later laboratory species identification or detection of the endangered freshwater pearl mussel (*Margaritifera margaritifera*) by wading or extensive snorkelling, are examples of time consuming species registration methods. In addition to the time aspect, many of these methods are destructive, and thus may affect the habitat and wellbeing of the target species. More abundant species are easier to detect than species that are less abundant. Red listed species often occur in very low densities and may therefore be especially time consuming to register. This also holds true for non-indigenous species where early detection and rapid response may be very critical in preventing invasion and establishment in new habitats.

A new method has recently come forth where visual or destructive species detection methods are not required. This method builds on the underlying fundamental principle that all living organisms have species specific DNA which will be shed in various forms into the environment and that this can be collected from the environment and identified. DNA enters the aquatic ecosystem 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. The detection of this environmental DNA is based on whole DNA extraction found in a water sample and polymerase chain reaction (PCR) assays using species-specific DNA sequences.

The method has been proven effective for registration of fish, amphibians, reptiles and toads (Ficetola et al. 2008; Goldberg et al. 2011; Piaggio et al. 2014; Thomsen et al. 2012a,b). Thomsen et al. (2012a) actually showed, that eDNA was more reliable for detection of rare and endangered species than more conventional methods in locations where an amphibian (*Pelobates fuscus*) was believed to be extinct. In another publication Thomsen et al. (2012b) showed that eDNA isolated from saltwater samples also was a good alternative to numerous conventional detection methods. The eDNA method is therefore not restricted to the freshwater environment despite the majority of the research focus thus far have been focused here. It has been used for detecting eDNA of non-indigenous zebra mussel in the US (Lance & Car 2012), using

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<sup>6</sup> International Maritime Organisation.

commercially and publically available primers and microsatellites. Further, it has recently been reported that the monitoring of benthic macroinvertebrates in coastal waters targeted for classification of Good Ecological Status can move from conventional sampling to eDNA-based methods (Aylagas et al. 2014).

eDNA in the aquatic environment has been shown to have a persistency restricted to some weeks (Dejean et al. 2011; Pilliod et al. 2014). Therefore, positive detection of a target species via eDNA indicate a relative recent occupation or presence in the sampled area, when sampling lakes or ponds, and up to nine km down-stream from sampling position in running water (Deiner & Altermatt 2014; Jane et al. 2014).

Due to the high sensitivity and the time restricted persistence of eDNA in water, the method has proven a strong tool for future implementation into registration of biodiversity within a wide range of habitats.

#### **2.4.2 FerryBox and CPR**

The on-going FerryBox monitoring potentially enables sampling of surface water and subsequent counting of phytoplankton species, zooplankton, and fish larvae at ships between i) Oslo-Kiel, ii) Hirtshals-Færøerne/Island, and iii) Bergen-Esbjerg-Beverwijk and could potentially provide a platform for monitoring of non-indigenous species.

NIVA's Ferrybox system<sup>7</sup> measures temperature, salinity, oxygen, chlorophyll and particle content at a depth of four metres along the fixed route of a vessel. This amounts to about one measurement every 500 metres. Some of the ships with Ferrybox equipment also carry advanced instruments for measuring solar radiation and reflection from the ocean surface. The data is transmitted to NIVA in real time. The system can activate sampling of water from predetermined sites. Sampling can also be triggered by NIVA during a voyage as necessary. NIVA downloads data from the environmental satellite ENVISAT. This data is processed and presented together with data from the ships. The sampling equipment can be customised for sampling of water, which subsequent could be screened for presence/absence of non-indigenous pelagic species, i.e. phytoplankton, zooplankton and specific fish species.

Another system to be considered for monitoring of non-indigenous pelagic species is that of SAFHOS<sup>8</sup>. SAFHOS operates the Continuous Plankton Recorder (CPR) survey. The CPR Survey's marine monitoring programme has been collecting data from the North Atlantic and the North Sea on the ecology and biogeography of plankton since 1931.

#### **2.4.3 Ballast water indicative methods**

A number of technologies are available for the determination of species in ballast water, but most are species unspecific relating to chlorophyll, ATP and other measures of occurrence and viability (BWM.2-Circ.42). Automated species recognition for a range of zooplankton and microalgae is available through the use of flow-through cameras and image matching in extensive databases, but considered of limited use due to an inadequate sample size and relatively low speed of analysis.

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<sup>7</sup> <http://www.niva.no/en/miljoedata-paa-nett/ferrybox-og-satellitdata>

<sup>8</sup> <http://www.sahfos.ac.uk/sahfos-home.aspx>

# 3. Where are we going?

We are designing and implementing a cost-effective MSFD D2 targeted monitoring programme, taking relevant authorised requirements into consideration. The design of a monitoring network subsequently builds on the strategy and the available resources. The proposed monitoring strategy builds upon requirements from the MSFD, the Commission Decision on criteria and other relevant requirement, e.g. the Danish GES definitions, the EU regulation on invasive species, the proposed outline of a Danish MSFD monitoring programme, and agreements in Regional Marine Conventions (HELCOM and OSPAR).

## 3.1 Proposed monitoring strategy

The overall objective is to carry out monitoring activities targeting non-indigenous species in Danish marine water in a way that enables answering the following questions: (1) In which parts of the Danish marine waters can non-indigenous species be found?, (2) are non-indigenous species more abundant or common in some areas than others?, (3) can potential hotspots be identified?, (4) can critical combinations of environmental factors and potential vectors be identified?, and (5) can the year and place of introduction be identified?

Bearing in mind that the environmental target for the Danish implementation of MSFD regarding non-indigenous species is to prevent and/or reduce the introduction of these species via ship-borne vectors, fisheries and/or aquaculture, we suggest a monitoring strategy anchored in the following six strategic objectives.

Strategic objective 1: The Danish D2 monitoring programme should be nation-wide covering coastal and offshore parts of relevant regions and sub-regions.

To answer the raised questions, a national MSFD D2 monitoring programme has to cover the ecological variations in Danish marine water from coast to offshore in the North Sea, including the Kattegat and northern parts of the Sound, and the Baltic Sea including the Danish Straits (the Great Belt and Little Belt).

Strategic objective 2: The Danish D2 monitoring programme should to the extent possible make use of existing and already planned sampling of ecologically relevant information, i.e. the NOVANA and MSFD monitoring of eutrophication and biodiversity (phytoplankton, zooplankton, angiosperms, macro-algae, benthic invertebrates) and DTU Aqua's monitoring of fish (commercial fish species and coastal fish species).

The existing and planned monitoring of other relevant MSFD descriptors, such as biodiversity, seafloor integrity, fish, food webs and eutrophication include sampling of biological parameters/indicators, which are of value when assessing non-indigenous species. This wealth of information should be re-analysed with aim of answering the above questions. Where relevant, the existing and planned programmes should be supplemented in order to support D2 specific analyses and assessments, for example in the open parts of the North Sea, Skagerrak, Kattegat and Baltic Sea. Further, the data generated from the sampling should be cross-checked with a 'Target Species List' of non-indigenous species in Danish marine waters (to be developed, see example in appendix 1).

Strategic objective 3: The Danish D2 monitoring programme should be stratified with: (1) a component based on conventional methods (see strategic objective 2) and frequencies used in the NOVANA programme, and (2) a component based on annual eDNA sampling.

The majority of benthic communities, i.e. submerged aquatic vegetation and benthic invertebrates, are in NOVANA sampled with a low frequency, i.e. 1/6 (one year out of 6), 2/6 (two years out of 6) or 3/6 (3 years out of 6). Fish species are generally sampled once a year, with the “Key fishermen” project as an exception with a significantly higher frequency. In contrast, pelagic communities are sampled with high frequency, normally > 20/year for phytoplankton and less for zooplankton.

Hence, the aim of the suggested eDNA sampling-based component is to supplement the conventional methods and frequencies and especially to guarantee a nation-wide MSFD D2 programme with reasonable chance of detection newly introduced non-indigenous species. This component should therefore include coastal and offshore parts of all regions and sub-regions as well as potential hotspots.

**Strategic objective 4:** Potential hotspots should be included in the monitoring programme, in particular coastal waters to which cooling water is discharged and ports. To the extent possible both primary introductions via ports with inter-biogeographic transport and secondary transfers e.g. with international ferry traffic should be covered.

Some areas are considered more susceptible to introduction or even invasion of non-indigenous species. For example, ports could potentially be hotspots because of discharge of ballast water. Another example of special interest is water bodies to which cooling water is discharged and potentially creating a niche where non-indigenous species could survive during cold periods.

For ports, we suggest the following selection criteria:

- *Hotspots for primary introductions*  
The major port hubs receiving vessels from transoceanic voyages are key locations for primary introduction of invasive species, particularly when exporting goods, i.e. discharging ballast water. These hotspots are ports with high volumes of cargo being loaded.
- *International ferry route between only two ports (secondary transfers)*  
Secondary transfers may occur between ports of lower traffic/cargo volumes or frequent line traffic. It is expected that international ferry lines may be exempted from the Ballast Water Management Convention and such ports may be included in the mapping of non-indigenous species.
- *Proximity to protected areas*  
Ports located in or close to protected areas may be more critical if invasive species should appear and proximity criteria may be applied to monitor non-indigenous species.
- *Environmental conditions*  
A spatial distribution of the key ports in the monitoring programme is emphasised to ensure coverage of the main water bodies of the Danish jurisdiction.

In regard to discharges of cooling water, we suggest three areas of concern: (1) Copenhagen Port at H.C. Ørstedværket, (2) Odense Port ved Odense Kraftvarmeværk and (3) the coastal waters outside of Studstrupværkets kølevandskanal (north-western part of Kalø Vig). These areas are designated based on Hansen et al. (2012).

**Strategic objective 5:** All eDNA samples should be preserved and stored in order to enable backtracking of recently introduced species.

Once a new species is identified either by the programme based on conventional sampling methods or via activities outside the MSFD D2 programme, the species in question should be added to the suggested check list (see strategic objective 2) and old samples should be reanalysed. This would potentially enable reconstruction of the invasion history of recently introduced species.

**Strategic objective 6:** Information from other relevant data sources should be included in the MSFD-specific analyses and reporting.

Information from other activities than monitoring, such as research projects and mapping studies should be included in the MSFD D2 analyses and reporting. Examples of such activities are: (1) the Danish “Fiskeatlas”, (2) monitoring carried out by neighbouring countries (Germany, Norway, Poland, and Sweden), and (3) EU projects such as DEVOTES and other international projects – see for example ICES (2014).

Fulfilling the six strategic objectives above should in principle result in a monitoring programme compliant with MSFD and RMC requirement and also fit for answering the raised questions.

### 3.2 Proposed monitoring network

Given the proposed monitoring strategy (section 3.1) and the budget available for monitoring in 2015-2017, we suggest carrying out a D2-targeted monitoring programme consisting of three elements/activities:

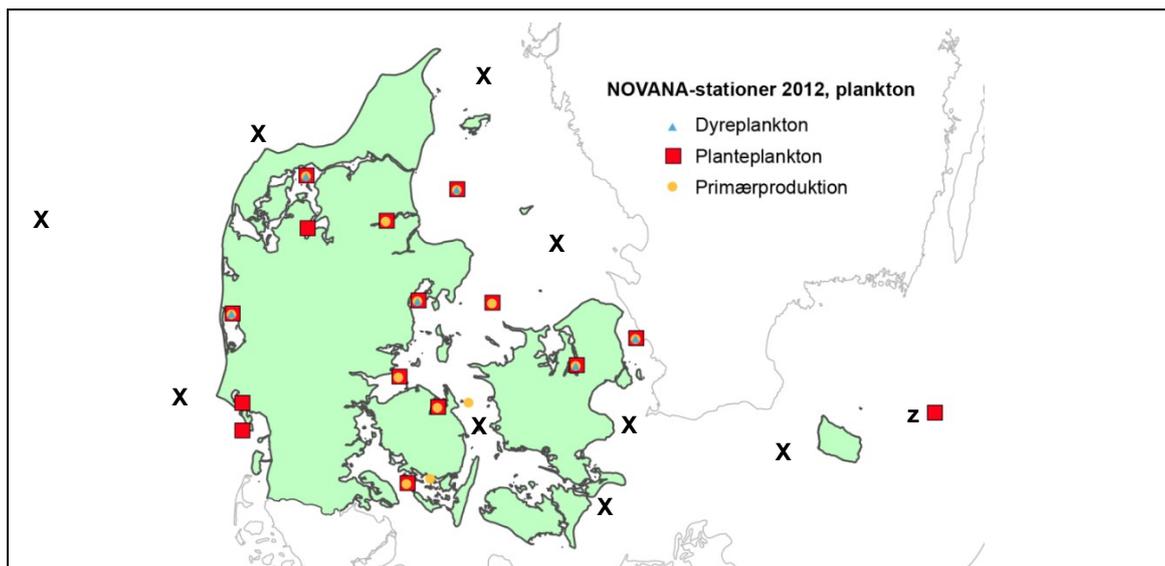
1. Activity D2-1: A general non-specific programme package based on existing activities and conventional monitoring techniques, supposedly with an appropriate spatial coverage.
2. Activity D2-2: A specific programme package targeting currently identified problematic indigenous species applying eDNA techniques, which we now consider operational and fit for purpose.
3. Activity D2-3: Annual reporting including data flow.

#### 3.2.1 Activity D2-1: supplementary monitoring activities

In regard to sampling based on conventional methods, we propose a number of additions to the existing NOVANA activities as well as the outlined MSFD-specific monitoring activities. The costs of these suggested supplementary monitoring activities are assumed to be covered via already planned extensions related to the MSFD-specific activities (Naturstyrelsen 2014). The proposed supplementary activities are presented in the following sections.

#### Phyto- and zooplankton

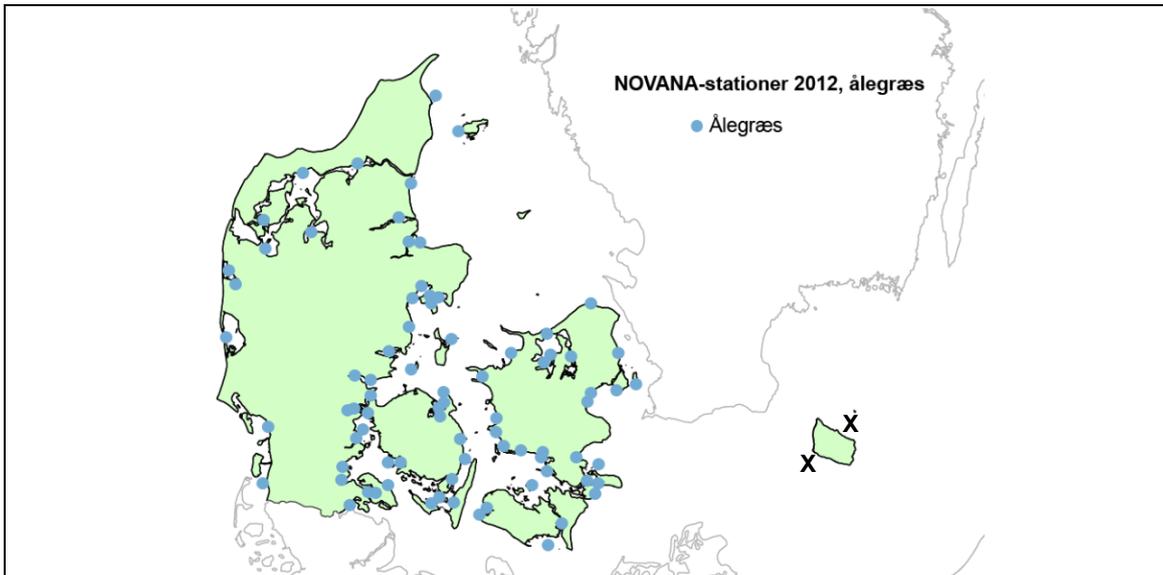
In the NOVANA programme there are no phytoplankton monitoring stations in the North Sea, Skagerrak, the Great Belt, Smålandsfarvandet, western Baltic Sea and the sea west of Bornholm. Zooplankton is monitored at only three monitoring stations: Aarhus Bay, north-western Kattegat and Øresund. Therefore we propose 10 additional phyto- and zooplankton monitoring stations to supplement the existing NOVANA programme (Figure 3).



**Figure 3.** Proposed network of stations for monitoring of phyto- and zooplankton in Danish marine waters. Suggested areas supplementing the existing NOVANA programme are indicated by a bold cross (X) (both phyto- and zooplankton). z denotes zooplankton only. Based on Fig. 1A.

### Angiosperms

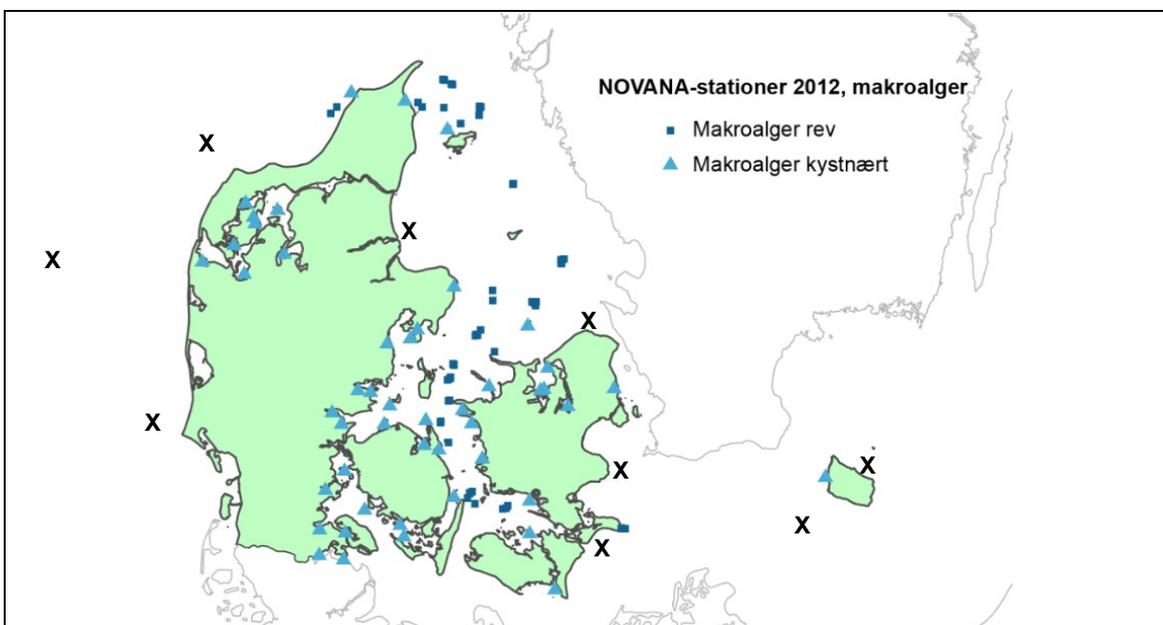
We propose to add monitoring of angiosperms at two stations at Bornholm to supplement the existing NOVANA programme (Figure 4).



**Figure 4.** Proposed network of stations for monitoring of Eelgrass (submerged aquatic vegetation) in Danish marine waters. Suggested areas to include are indicated by a bold cross (X). Based on Fig. 1B.

### Macro-algae

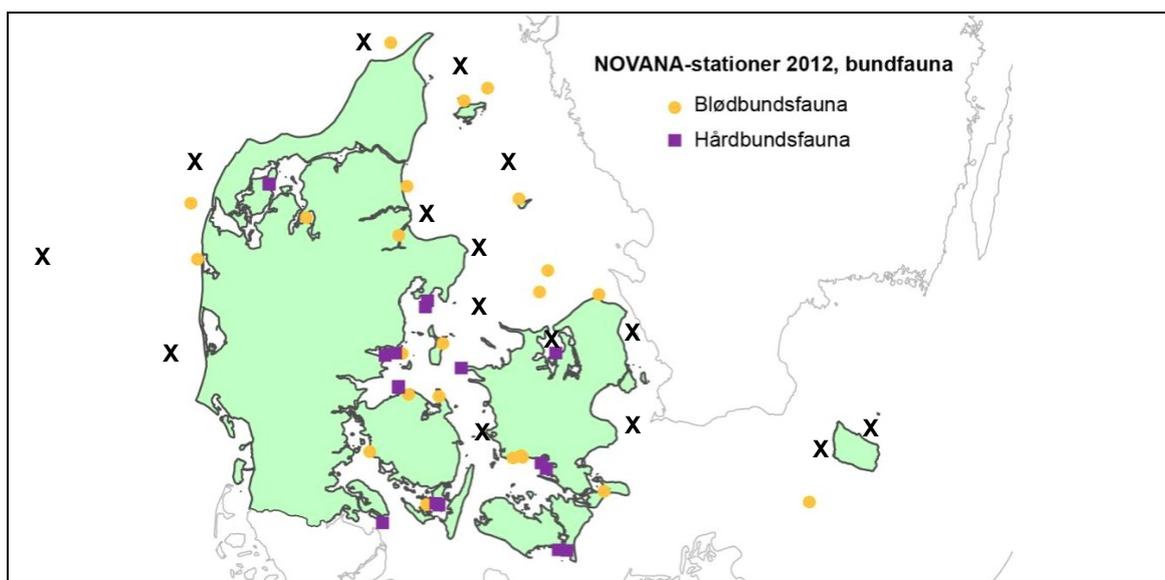
There are only two macro-algae monitoring stations on stone reefs in the North Sea and none around Bornholm and no coastal macro-algae monitoring along the coasts of northeast Jutland and eastern Zealand. Therefore we propose nine additional monitoring stations (Figure 5).



**Figure 5.** Proposed network of stations for monitoring of macro-algae in Danish marine waters. Suggested areas to include are indicated by a bold cross (X). Based on Fig. 1C.

### Benthic invertebrates

There is no hard bottom benthic invertebrate monitoring in the North Sea, Kattegat, western Baltic Sea and around Bornholm. Therefore we propose 15 additional monitoring stations (Figure 6).



**Figure 6.** Proposed network of stations for monitoring of benthic invertebrates in Danish marine waters. Suggested areas to include are indicated by a bold cross (X). Based on Fig. 1D.

The supplements are all justified by the proposed strategic objective no. 1, i.e. a nation-wide monitoring programme. The proposals are summarised below (Table 5). It should be noted that the suggested station/areas are indicative in order to provide some flexibility for the Danish Nature Agency when implementing the proposals. For example, the suggested number of stations/areas in the North Sea should not be reduced – focus should merely be on where to implement the proposals in a cost-effective way.

**Table 5.** Proposed supplement of the existing and planned monitoring of biological indicators in the NOVANA programme. PHYTO = phytoplankton; ZOO = zooplankton; ANGIO = angiosperms (within to Eelgrass activities; MACRO = macro-algae on hard-bottom; BENTHOS= soft-bottom invertebrates and hard-bottom-invertebrates.

| Area                     | PHYTO | ZOO | ANGIO | MACRO | BENTHOS <sup>1</sup> |
|--------------------------|-------|-----|-------|-------|----------------------|
| North Sea, coastal       | 2     | 2   | -     | -     | 2                    |
| North Sea, off shore     | 1     | 1   | -     | 3     | 1                    |
| Skagerrak, coastal       | -     | -   | -     | -     | -                    |
| Skagerrak, off shore     | -     | -   | -     | -     | 1                    |
| Kattegat, coastal        | -     | -   | -     | 2     | 3                    |
| Kattegat, offshore       | 2     | 2   | -     | -     | 3                    |
| Danish Straits, coastal  | 1     | -   | -     | -     | -                    |
| Danish Straits, offshore | -     | -   | -     | -     | 2                    |
| Baltic Sea, coastal      | 1     | 2   | 2     | 2     | 2                    |
| Baltic Sea, off shore    | 2     | 3   | -     | 2     | 1                    |
| Total                    | 9     | 10  | 2     | 9     | 15                   |

1: We have not differentiated between soft and hard bottom monitoring due to lack of information on potential monitoring sites, i.e. stone reefs, bubble reefs, and stony areas. Whether to implement the supplementary activities at soft-bottom or hard-bottom sites should be decided by the Danish Nature Agency when doing the detailed planning of the monitoring.

### 3.2.2 Activity D2-2: eDNA-based monitoring activity

We propose sampling of seawater, filtration and subsequent eDNA analysis in 48 coastal and offshore marine areas and in 16 potential hot spot areas encompassing 3 areas with discharges of cooling water, 6 ports with mainly cargo export, 4 port with international ferry lines, and 3 optional sites. A proposal for monitoring sites is presented on the following pages.

**Table 6.** Suggested distribution of 48 stations in the eDNA-based sub-programme.

| Area           | Coastal   | Offshore  |
|----------------|---|---|
| North Sea      | Wadden Sea, inner parts<br>Ringkøbing Fjord<br>Limfjorden, central<br>Limfjorden, southern<br>Limfjorden, Halkjær Bredning  | Wadden Sea, outer parts<br>North Sea, western Danish parts<br>North Sea, northern Danish parts<br>North Sea, off Ringkøbing Fjord |
| Skagerrak      | Jammer Bugt<br>Tannis Bugt  | Hirtshals<br>North of Skagen  |
| Kattegat       | Ålbæk Bugt<br>Ålborg Bugt<br>Hevring Bugt<br>Grenå<br>Isefjorden, outer parts<br>Isefjorden, inner parts<br>Roskilde Fjord, northern parts<br>Roskilde Fjord, southern parts<br>Sjællands Odde<br>Gilleleje | Læsø Rende<br>Læsø Trindel<br>Kattegat, central<br>Kattegat, southern   |
| Danish Straits | Århus Bugt<br>Horsens Bugt<br>Vejle Fjord<br>Sejerø Bugt<br>Stavns Bugt<br>Lillebælt, southern parts<br>Sydfynske Øhav<br>Smålandsfarvandet<br>Øresund, Nivå Bugt<br>Øresund, Tragten                       | Great Belt, central<br>Køge Bugt<br>Øresund, central  |
| Baltic Sea     | Bornholm, west<br>Bornholm, east<br>Præstø Fjord<br>Fakse Bugt<br>Hjelm Bugt<br>Femern Belt   | Arkona Basin<br>Bornholm Basin  |

The following ports as marked in the tables below are prioritised for eDNA monitoring:

- Three sites in and outside of ports are selected because of the outlet of cooling water (C1-C3);
- six sites are included due to the cargo exports in the ports and the suspected ballast water volume discharged;
- four ports with intense international ferry traffic; and
- three optional sites.

The three optional sites may be included subject to Nature Agency decision. They will serve the monitoring objectives and each provides additional value to the monitoring programme.

### Three sites for cooling water

The cooling water outlets from power plants have historically been refuges for non-indigenous aquatic species. Selected sites in ports of Copenhagen and Odense are listed here to be included to cover impact areas of the cooling water outlets in the ports. In addition, the area outside Studstrupværket near Port of Århus is included for the same reason.

**Table 7.** Sites related to cooling water hotspots of non-indigenous species.

| Port                         | Characteristics   | Sampling site                                      |
|------------------------------|-------------------|--|
| C1. Port of Copenhagen       | Suspected hotspot | H.C. Ørstedsværkets cooling water outlet           |
| C2. Port of Odense           | Suspected hotspot | Odense Kraftvarmeværks cooling water outlet        |
| C3. Outside of Port of Århus | Suspected hotspot | Studstrupsværkets cooling water outlet to Kalø Vig |

### Six sites for cargo export

The six prioritised ports (marked with E) presumably receive ballast water discharge associated with exporting goods. We propose to include Port of Kalundborg (E6) as a second site in the Kalundborg fjord, since it is by its own merit number six on the cargo list with the adjacent Statoil pier as number three. The local energy plant Asnæsværket is a producer of cooling water although it is expected that the industrial symbiosis system in Kalundborg does remove a large part of the surplus heat.

**Table 8.** Top 10 ports without international ferry lines in the order of cargo loaded volume.

| Port                            | Characteristics                                   | Sampling site                                  |
|---------------------------------|---|--|
| E1. Port of Fredericia          | Exposed but several basins                        | Oil harbour                                    |
| E2. Port of Aarhus              | Several basins                                    | Container terminal                             |
| E3. Statoil-havnen (Kalundborg) | Smaller port                                      | Oliepier                                       |
| E4. Port of Esbjerg             | Many basins, exposed, proximity to protected area | Several sites possible; Optional more than one |
| E5. Aalborg Portland            | Exposed quay                                      | Cement loading quay                            |
| E6. Port of Kalundborg          | Deep protected fjord several basins               | Sydhavn  |
| Port of Copenhagen              | Several basins                                    | Prioritised for cooling water                  |
| Port of Odense                  | Several basins                                    | Prioritised for cooling water                  |
| Port of Aalborg                 | Several basins                                    | Optional                                       |
| Port of Køge                    | Outlet from stream in Southern basin              | Northern basin                                 |

The four ferry ports are the prioritised ferry line ports for the local transfer issues. The prioritised ports (marked with F) presumably receive ballast water discharge including trim water associated with frequent loading and offloading of goods and passengers. We propose to include both Frederikshavn and Grenå since these ports are presumably biologically comparable in Denmark but their ferry lines' corresponding ports in Sweden, Gothenburg and Varberg are widely different with respect to other international traffic, and hence presumed risk of non-indigenous species.

**Table 9.** Top 10 ports with international ferry lines based on cargo loaded volume.

| Port                   | Characteristics                 | Sampling site                 |
|------------------------|---------------------------------|-------------------------------|
| F1. Rødby Færgehavn    | Simple basin, brackish          | Near East quay                |
| F2. Helsingør Havn     | Simple basin, traffic intensive | Near East quay                |
| F3. Frederikshavn Havn | Kattegat, pair Gothenburg       | Cargo basin (Fragthavnsbasin) |
| Gedser Havn            | Gedser Havn                     | Optional                      |
| Hirtshals Havn         | Hirtshals Havn                  | Optional                      |
| F4. Grenaa Havn        | Grenaa Havn                     | At ferry berth                |
| Københavns Havn        | Københavns Havn                 | -                             |
| Rønne Havn             | Rønne Havn                      | -                             |

The following ports and sites are relevant for a monitoring programme of non-indigenous species, but may not be selected at the time of planning since due to various issues as mentioned below (three may be chosen, currently not prioritised):

- The Lindø shipyard under the administration of the Port of Odense may be relevant as vessels going for repair in the docks may discharge ballast water;
- the berthing area of Royal Arctic Line in the Port of Aalborg is relevant as the traffic on Greenland may serve as a pathway for Arctic/Subarctic species, if such species are included in final target species list;
- the small ferry port of Gedser would qualify based on cargo volume, but has been excluded as it is part of a pending application for exemption;
- the Port of Hirtshals can be considered although the ferries calling will be fitted with ballast water treatment systems, since it is the only port facing Skagerrak;
- more sites may be included in Esbjerg since it is a port with partitioned basins and a different trading pattern in different basins, and
- finally, Thyborøn Canal bordering the North Sea and the Limfjord may be a relevant site for sampling.

The estimation of the costs related to the proposed eDNA-based activities is based on the existing calculation principles and unit prices. In some cases, new unit prices have been estimated. The estimations are not included in this report, but reported exclusively to the Danish Nature Agency.

### **3.2.3 Activity D2-3: reporting**

Although not originally included in the work, we have in dialogue with the Danish Nature Agency developed an outline for the planned annual reporting in regard to MSFD D2. The activities included under this heading encompass: (1) collation of data from relevant sources, (2) analysis of available data, and (3) reporting as part of the annual report on the state of Danish marine areas (M-FDC's Havrapport).

In addition, some prerequisites in regard to reporting have been included, e.g. (1) the writing of a D2-specific Technical Guidance Manual (Teknisk Anvisning), (2) a process resulting in a national Targets Species List (with 50 species), (3) barcoding of the species on the Target Species List, and (4) costs in regard to establishment and running of a national eDNA filter archive.

The estimation of the costs related to the reporting is based on the existing calculation principles and unit prices. In some cases, new unit prices have been estimated. The estimations are not included in this report, but reported exclusively to the Danish Nature Agency.

# 4. Conclusions and recommendations

The EU Marine Strategy Framework Directive requires Member States to establish good environmental status and to monitor it, also regarding the descriptor on non-indigenous species (D2). To the present day, there is no organised monitoring of invasive or non-indigenous species in Denmark.

The Danish Nature Agency initiated the present study (abbreviated to MONIS) with the aim to outline a cost effective monitoring programme for D2. The scope of the study was to focus on:

1. Taking the greatest possible advantage of existing monitoring activities;
2. applicability of existing monitoring guidelines (Tekniske Anvisninger) and of contemporary biomolecular technologies (i.e. barcoding/eDNA); and
3. development of a proposal for a monitoring programme covering Danish marine waters.

This report presents the results of the MONIS project study including a proposal for a monitoring programme. The existing monitoring activities of biological and chemical status in Danish waters under the NOVANA programme and the monitoring of fish (by DTU Aqua and Copenhagen University) are comprehensive but insufficient as they are not scoped for monitoring of non-indigenous species and their geographical coverage. The applied NOVANA Technical Guidance Manuals are generally assessed as being appropriate also for non-indigenous species, but in some case important supporting parameters are not registered. More recent methodologies e.g. automated sampling or biomolecular techniques (barcoding/eDNA) are not in use and they should be included in the monitoring of non-indigenous species.

The technical assessments point to the eDNA method as a key technique in establishing a comprehensive and cost effective routine monitoring programme. Six strategic objectives for a future monitoring of non-indigenous species in Danish waters are proposed:

1. The programme must provide appropriate coverage of Danish waters;
2. the existing and planned biological monitoring activities shall be included to the fullest possible extend, i.e. phyto- and zooplankton, macro-algae, benthic organisms, fish and marine mammals;
3. the programme should be two pronged with one branch based on conventional sampling via other existing programmes and another branch based on water sampling and eDNA testing largely based on existing sampling;
4. the programme should include suspected hotspots, i.e. selected ports and cooling water outlets;
5. eDNA filter samples should be analysed annually and replicate samples kept in a sample bank ensuring availability for confirmatory analyses and examination for new species; and
6. data from other sources, e.g. fish surveillance, should be included in analysis and reporting.

The strategic objectives are converted to a proposed monitoring programme including:

1. A part based on conventional sampling entirely contained within the existing NOVANA and the planned Marine Strategy Framework monitoring programme, thus not forcing any additional costs the monitoring programme;

2. an eDNA-based sub-programme fully integrated in the existing or planned ship-based sampling of sea-water; and
3. 13 complimentary hot spot stations for non-indigenous species situated in ports or coastal environments that may be reached with smaller vessels/dinghies or directly from the quays.

The proposed total supplements to the existing NOVANA or MSFD-specific sub-programmes based on conventional sampling comprises 44 sampling stations and should be carried out in relation to other descriptors than D2 ((Table 10).

**Table 10.** Summary of proposed supplements to other sub-programmes than D2.

| Area                    | PHYTO    | ZOO       | ANGIO    | MACRO    | BENTHOS   | Total     |
|-------------------------|----------|-----------|----------|----------|-----------|-----------|
| North Sea               | 3        | 3         | -        | 3        | 3         | 12        |
| Skagerrak               | -        | -         | -        | -        | 1         | 1         |
| Kattegat                | 2        | 2         | -        | 2        | 6         | 12        |
| Danish Straits, coastal | 1        | -         | -        | -        | 2         | 2         |
| Baltic Sea, coastal     | 3        | 5         | 2        | 4        | 3         | 17        |
| <b>Total</b>            | <b>9</b> | <b>10</b> | <b>2</b> | <b>9</b> | <b>15</b> | <b>44</b> |

The proposed eDNA-based monitoring programme targets 48 sites and is 100% compatible with the planned NOVANA / MSFD water chemistry programme employing not only the same stations but also very similar sampling technologies (Table 11).

**Table 11.** Proposed distribution of 48 stations in the eDNA-based sub-programme.

| Area           | Coastal   | Offshore  | Total     |
|----------------|-----------|-----------|-----------|
| North Sea      | 5         | 4         | 9         |
| Skagerrak      | 2         | 2         | 4         |
| Kattegat       | 10        | 4         | 14        |
| Danish Straits | 10        | 3         | 13        |
| Baltic Sea     | 6         | 2         | 8         |
| <b>Total</b>   | <b>33</b> | <b>15</b> | <b>48</b> |

Hot spot programme is suggested to include 13 port and 3 areas with discharges of cooling water. The following ports should be included in the proposed hotspot programme:

1. E1 – Port of Fredericia
2. E2 – Port of Aarhus
3. E3 – Statoil-havnen (Kalundborg)
4. E4 – Port of Esbjerg
5. E5 – Aalborg Portland
6. E7 – Port of Kalundborg
7. F1 – Rødby Færgehavn
8. F2 – Helsingør Havn
9. F3 – Frederikshavn Havn
10. F4 – Grenaa Havn
- 11.-13. Any three of the proposals I-VI (Lindø, Aalborg, Gedser, Hirtshals, Esbjerg, or Thyborøn).

Further, the following locations, all with discharges of cooling water, are proposed in the hotspot programme with sampling twice a year:

1. Københavns Havn, close to Ørstedværket;
2. Odense Havn, close to Odense Kraftvarmeværk; and
3. Kalø Vig, close to Studstrupværket.

The costs of the proposed activities, excluding the proposal targeting other sub-programmes than the D2 sub-programme, have been estimated to be 932.218,00DKK per year in the period 2015-2017. These estimates, which are based on estimated unit prices and official NOVANA-rates, are reported exclusively to the Danish Nature Agency.

The proposed strategy and monitoring programme makes full use of the existing and planned activities and resources for relevant environmental monitoring in Denmark. It ensures compatibility with existing conventional sampling and allows considerable synergy to be harvested through the use of biomolecular methods, i.e. eDNA, which emerges as a cost-efficient and versatile tool in the case of monitoring of non-indigenous species.

Implementing this proposal will lead to cost effective and geographically comprehensive monitoring of non-indigenous species in Danish waters.

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# Appendices

Appendix 1: Draft indicative Target Species List of non-indigenous species in Danish marine waters

Appendix 2: Maps of DTU Aqua's fish monitoring activities

Appendix 3: Hot spot monitoring network

Appendix 4: Statement of quality assurance

## Appendix 1: Draft indicative Target Species List of non-indigenous species in Danish marine waters

The following table (in Danish) represents a draft Target Species List, and is taken from Stæhr & Thomsen (2013): Opgørelse over rumlig udbredelse, tidlig udvikling og tæthed af ikke-hjemmehørende arter i danske farvande. Fagligt notat fra DCE - Nationalt Center for Miljø og Energi. 14 pp. (In Danish).

For macro-algae, benthic invertebrates and zooplankton, the frequency of occurrence is presented in % (individual species/total species × 100). References are given to the dispersal history of individual species as well as quantitative estimates when available.

A Target Species List should be regarded as a living document to updated when relevant.

| Taxa                   | Art   | Oprindelse                | Sprednings-vektor      | Ankomst Danmark                                     | Hyppighed & udvikling   | Kommentarer  | Referencer  |
|------------------------|---|---------------------------|------------------------|---|---|--|---|
| Blomster-plante        | <i>Spartina anglica</i><br>(Engelsk vadegræs)                                 | N. Amerika /<br>England   | Udplantning            | 1930-40<br><br>Vadehavet                            | Danner tætte bestande i<br>tide-vandszonen (~ 10%)<br><br>I vækst | Veletableret i<br>Vadehavet. fjorde i<br>Kattegat og Bælthavet                       | Nehring &<br>Adersen 2006<br><br>Randløv 2007                           |
| Makroalge<br>(rødalge) | <i>Aglaothamnion<br/>halliae</i>  | Muligvis V.<br>Atlantehav | Muligvis<br>Skibsskrog | 2003?<br>I Norge i<br>1980erne, i<br>Sverige i 2003 | Ukendt  | Samme som<br><i>Callithamnion halliae</i> ,<br><i>Aglaothamnion<br/>westbrookiae</i> | <a href="http://www.frammandearter.se">http://www.frammandearter.se</a> |
| Makroalge<br>(rødalge) | <i>Bonnemaisonia<br/>hamifera</i> Hariot<br>(Krogalge)                        | V. Stillehav              | Skibsskrog             | 1900  | Avg: 1%<br>Max: 1.8%<br>Stagneret                                 | Kun tetrasporophyter<br><br>Nordsøen, Skagerak,<br>Kattegat                          | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b                       |
| Makroalge<br>(rødalge) | <i>Dasya<br/>bailloiwiana</i> (S. G.<br>Gmelin)<br>(Dusktang)                 | Middelhavet               | Østers?<br>skibsskrog? | 1961<br>Nyborg                                      | Avg: 0.4%<br>Max: 1%<br>Stagneret                                 | Stigende forekomst de<br>seneste år<br>Kattegat, Bælthavet,<br>Østersøen             | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b                       |
| Makroalge<br>(rødalge) | <i>Gracilaria<br/>vermiculophylla</i><br>(Ohmi) Papenfuss<br>(Gracilariatang) | V. Stillehav              | Østers                 | 2003<br>Horsens Fjord                               | Avg: 0.02%<br>Max: 0.10%<br><br>I vækst                           | Stigende forekomst de<br>seneste år<br><br>Nordsøen, Kattegat                        | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b                       |
| Makroalge<br>(rødalge) | <i>"Heterosiphonia<br/>japonica"</i>  | Stillehavet               | Østers?<br>skibsskrog? | 2005<br>Limfjorden                                  | Avg: 0.13%<br>Max: 0.13%<br><br>I vækst                           | Samme som<br><i>Dasysiphonia</i> sp.?<br>Normal i Norge<br>Kattegat                  | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b                       |

|                         |   |                              |                          |                        |   |   |   |
|-------------------------|---|------------------------------|--------------------------|------------------------|---|---|---|
| Makroalge<br>(rødalge)  | <i>Neosiphonia harveyi</i> (J. Bailey)<br>Kim, Choi, Guiry & Saunders                                     | Stillehavet/NV<br>Atlantehav | Epifyt?                  | 1986<br>Ukendt         | Avg: 0.006%<br>Max: 0.10%<br><br>I vækst  | Samme som<br><i>Polysiphonia harveyi</i> .<br>Forveksles nemt med<br>andre <i>Polysiphonia</i> -<br>arter<br>Kattegat   | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b |
| Makroalge<br>(brunalge) | <i>Colpomenia peregrina</i><br>Sauvageau<br>(Østerstyv)   | Vestlige Stillehav           | Østers                   | 1939<br>Limfjorden     | Avg: 0.02%<br>Max: 0.15%<br><br>Stagneret | Forekommer mest om<br>foråret og overses<br>derfor nemt i sommer-<br>kortlægningen.<br>Kattegat   | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b |
| Makroalge<br>(brunalge) | <i>Dictyota dichotoma</i><br>(Hudson) J.V.<br>Lamouroux<br>(Tvedelt bændel-<br>alge)                      | Atlantehavet                 | Østers?<br>Naturlig?     | 1939<br><br>Limfjorden | Avg: 0.6%<br>Max: 1.2%<br><br>Aftagende   | Kan være<br>hjemmehørende<br><br>Skagerrak, Limfjorden  | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b |
| Makroalge<br>(brunalge) | <i>Fucus evanescens</i><br>C. Agardh<br>(Langfrugtet<br>klørtang)   | Nordatlanten                 | Skibsskrog?<br>Naturlig? | 1948<br>Øresund        | Avg: 0.08%<br>Max: 0.24%<br>Aftagende     | Muligvis<br>hjemmehørende. Kan<br>forveksles med andre<br><i>Fucus</i> -arter - især små<br>individer<br>Skagerrak, Kattegat,<br>Limfjorden, Bælthavet  | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b |
| Makroalge<br>(brunalge) | <i>Sargassum muticum</i> (Yendo)<br>Fensholt  | V. Stillehav                 | Østers?                  | 1984<br>Limfjorden     | Avg: 2%<br>Max: 6%<br>Stagneret?          | Meget hyppig i<br>Limfjorden<br>Nordsøen, Skagerrak,<br>Limfjorden, Kattegat  | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b |
| Makroalge<br>(grønalge) | <i>Codium fragile</i> ssp.<br><i>tomentosoides</i> (van<br>Goor) P.C. Silva<br>(Gaffelgrenet<br>plysalge) | V. Stillehav                 | Østers?<br>skibsskrog?   | 1919<br>Hirsholmene?   | Avg: 0.2%<br>Max: 0.6%<br>Aftagende       | <i>Codium</i> -underarter er<br>svære at skelne<br>morfologisk og<br>behandles derfor som<br>en art i overvågnings-<br>programmet<br>Nordsøen, Skagerrak,<br>Limfjorden, Kattegat,<br>Bælthavet | Thomsen et al.<br>2007<br>Thomsen et al.<br>2008b |

|   |   |   |   |                        |  |  |                       |
|---|---|---|---|------------------------|--|--|-----------------------|
| Fytoplankton (dinoflagellat)              | <i>Prorocentrum minimum</i>   | ? | ? | 1981                   | Op til > 50 mio celler/L<br><br>Aftagende? | Dannede specielt i 1980'erne og 1990'erne meget kraftige opblomstringer i mange danske fjorde. Er siden år 2000 forekommet i lavere koncentrationer  | Bjergskov et al. 1990 |
| Fytoplankton (dinoflagellat)              | <i>Karenia mikimotoi</i> (= "Gyrodinium aureolum", = "Gymnodinium mikimotoi") | ? | ? | 1968                   | Op til > 10 mio celler/L<br>Aftagende?     | Er med sikkerhed set i specielt Nordsøen, Skagerrak, Kattegat (inkl. Limfjorden) - og i få tilfælde i Bælthavet og Øresund.  | Bjergskov et al. 1990 |
| Fytoplankton (dinoflagellat)              | <i>Gymnodinium chlorophorum</i>   | ? | ? | 1999?                  | Op til > 7,8 mio celler/L<br>I vækst       | Dannede massiv opblomstring i Skagerrak-Kattegat i det sene efterår/vinter 2009. Arten er i modsætning til de fleste andre dinoflagellater grøn, hvilket ikke kan ses i lugolfikserede prøver. Det kan derfor ikke med sikkerhed siges, at den ikke har været i danske farvande tidligere. | Hansen et al. 2000    |
| Fytoplankton (dictyochophyceae)           | <i>Pseudochattonella verruculosum</i>   | ? | ? | 1998                   | Op til > 19 mio celler/L<br>I vækst        | Første opblomstring forekom i Kattegat-Skagerrak-Nordsøen i vinter-forår 1998. Opblomstringen medførte massiv fiskedød. Siden da er denne art blevet en næsten årligt tilbagevendende del af forårsfytoplankton.   | Markager et al. 1999  |
| Fytoplankton (raphidophyceae = flagellat) | <i>Heterosigma akashiwo</i>   | ? | ? | 1988<br><br>Limfjorden | Op til ca. 2 mio celler/L                  | Observeret i Kattegat  | Bjergskov et al. 1990 |

|  |  |                                    |   |                          |  |  |  |
|--|--|------------------------------------|---|--------------------------|--|--|--|
| Pelagisk makroinvertebrat (ribbegoble) | <i>Mnemiopsis leidyi</i> ("Dræbergoble")             | V. Atlanterhav                     | Ballast vand                            | 2007 Lille- og Storebælt | Op til ca. 600 indiv./m <sup>3</sup><br>Stagneret? | Observeret i samtlige danske farvande i 2005-07<br><br>Næsten ingen observationer i 2011 | Tendal et al. 2007<br>Riisgård et al. 2010 |
| Pelagisk invertebrat (vandloppe)       | <i>Acartia tonsa</i>                                 | Indo-pacific                       | Ballast vand?                           | 1925?                    | Avg: < 4%<br>Max: 19%<br><br>Aftagende             | Kattegat og Østersøen (Mest i Kattegat)  | MADS<br>BSASD                              |
| Pelagisk invertebrat (dafnie)          | <i>Penilia avirostris</i>                            | Subtropiske og tropiske havområder | Ballast vand                            | 2001                     | Avg: < 0.5%<br>Max: 2%                             | Kattegat og Østersøen (Mest i Kattegat)  | MADS<br>Søndergaard et al. 2006            |
| Parasit invertebrat (fladorm)          | <i>Pseudodactylogyru s anguillae</i> Yin & Sproston  | V. Stillehav                       | Import af Japanske ål?                  | 1985                     | ?<br><br>?   | Vært (ål), parasit<br><br>Kattegat, Bælthavet  | Jensen et al. 2005<br>Køie 1988<br>BSASD   |
| Parasit invertebrat (fladorm)          | <i>Pseudodactylogyru s bini</i> Kikuchi              | V. Stillehav                       | Import af Japanske ål?                  | 1985<br>Esrum sø         | ?<br><br>?   | Vært (ål), parasit<br><br>Østersøen?   | Jensen et al. 2005<br>Køie 1988            |
| Parasit invertebrat (nematod)          | <i>Anguillicola crassa</i> Kuwahara, Niimi & Itagaki | V. Stillehav                       | Import af Japanske ål?                  | 1985<br><br>?            | ?<br><br>?   | Vært (ål), parasit<br><br>Kattegat, Bælthavet, Østersøen                                 | Jensen et al. 2005<br>Køie 1988<br>BSASD   |
| Parasit invertebrat (copepod)          | <i>Mytilicola intestinalis</i> Steuer                | Middelhavet                        | Ballast vand?<br>Østers? På skibsskrog? | 1964                     | ?<br><br>?   | Parasit i muslinger<br><br>Limfjorden  | Jensen et al. 2005                         |
| Bentisk makroinvertebrat (børsteorm)   | <i>Ficopomatus enigmaticus</i> Fauvel                | Australia? India?                  | Skibsskrog?<br>Naturlig?                | 1939<br><br>Marstal      | ?<br><br>Stagneret?                                | Fasthæftet, hårdbund, epifauna, kan danne rev på blødbund, filtrator<br>Kattegat         | Jensen et al. 2005                         |
| Bentisk makroinvertebrat (hydroid)     | <i>Cordylophora caspia</i> Pallas                    | Ponto - Caspiske hav               | Skibsskrog                              | 1895<br>Ringkøbing Fjord | ?<br><br>?   | Findes pt. i Ringkøbing Fjord og Nissum Fjord  | (Jensen og Knudsen 2005)                   |

|                                     |   |   |                     |  |   |   |   |
|-------------------------------------|---|---|---------------------|--|---|---|---|
| Bentisk makroivertebrat (børsteorm) | <i>Marenzelleria viridis</i> Verrill            | NV Atlanterhav                            | Ballast vand?       | 1990<br><br>Ringkøbing Fjord             | Avg: 0.5%<br>Max: 1.5%<br><br>I vækst     | Samme art som <i>M. wireni</i> . Muligvis flere arter ( <i>M neglecta</i> ). Fasthæftet, blødbund, infauna<br>På lavt vand (< 5m). Nordsøen, Kattegat, Bælthavet    | Jensen et al. 2005<br>BSASD<br>Thomsen et al. 2008b, 2009<br>Banta 2010 (i Thomsen og Stæhr 2010) |
| Bentisk makroivertebrat (børsteorm) | <i>Neanthes succinea</i> Frey og Leuckart       | Nordamerika, Øslige Sydamerika, V. Afrika | ?                   | 1940<br><br>Kattegat                     | Avg: 0.2%<br>Max: 0.9%<br><br>I vækst     | Samme art som <i>Nereis succinea</i> og <i>Alitta succinea</i> . Lever på blødt (og hårdt) substrat, både som in- og epifauna, mobil predator<br>Isefjord, Limfjord | Jensen et al. 2005<br>Thomsen et al. 2008b  |
| Bentisk makroivertebrat (snegl)     | <i>Crepidula fornicata</i> L.                   | NV Atlanterhavet                          | Østers?             | 1934<br><br>Vadehavet og Nissum Bredning | Avg: 0.02%<br>Max: 0.1%<br><br>I vækst    | Lever på hårdbund, epifauna, filtrator<br><br>Limfjorden, Kattegat, Vadehavet   | Jensen et al. 2005<br>Thomsen et al. 2008b  |
| Bentisk makroivertebrat (snegl)     | <i>Potamopyrgus antipodarum</i> (Gray)          | New Zealand                               | Ballast vand?       | < 1914<br><br>Randers Fjord              | Avg: 1%<br>Max: 7%<br>Aftagende           | Lever på blødbund, epifauna, substratæder.<br>Kattegat, Østersøen   | Jensen et al. 2005<br>Thomsen et al. 2008b  |
| Bentisk makroivertebrat (snegl)     | <i>Ocenebrellus inornatus</i> (Østersboresnegl) | N. Stillehav                              | På Stillehavsøsters | 2006<br>Nissum Bredning                  | ?<br>I vækst                              | Observeret i store dele af Limfjorden   | Lützen et al (i Thomsen og Stæhr 2010)  |
| Bentisk makroivertebrat (musling)   | <i>Petricola pholadiformis</i> Lamarck          | NV Atlanterhavet                          | Østers?             | 1905<br><br>Vadehavet                    | Avg: 0.03%<br>Max: 0.1%<br><br>Stagneret? | Lever i relativt hårdt substrat (kalksten, skiffer), infauna (borer), filtrator<br>Vadehavet, Skagerrak, Limfjorden, Kattegat                                       | Jensen et al. 2005<br>Thomsen et al. 2008b  |
| Bentisk makroivertebrat (musling)   | <i>Ensis americanus</i> Gould                   | NV Atlanterhavet                          | Ballast vand?       | 1981<br>Vadehavet                        | Avg: 0.01%<br>Max: 0.1%<br>I vækst        | Lever på blødbund, infauna, filtrator<br><br>Vadehavet, Skagerrak, Limfjorden, Kattegat, Bælthavet  | Jensen et al. 2005<br>Thomsen et al. 2008b  |

|                                  |   |                          |                               |                                       |   |  |  |
|----------------------------------|---|--------------------------|-------------------------------|---------------------------------------|---|--|--|
| Bentisk makrovertebrat (musling) | <i>Crassostrea gigas</i> Thunberg       | V. Stillehav             | Larvedrift og akvakultur      | 1980 Vadehavet                        | ?                                       | Fasthæftet, lever på hårbund, epifauna, kan danne rev på blødbund, filtrator   | BSASD<br>Wrangle et al 2009                |
| Bentisk makrovertebrat (musling) | <i>Teredo navalis</i> L                 | Kinesiske hav            | I skibsskrog eller driftømmer | 1853 Kiel bugt<br><br>Nissum Bredning | ?                                       | Kattegat, Bælthavet<br><br>Lever på hårdt substrat deposit feeder  | Jensen et al. 2005<br>BSASD                |
| Bentisk makrovertebrat (musling) | <i>Mya arenaria</i> L.                  | NV Atlanterhavet         | Importeret?                   | < 1200?<br><br>?                      | Avg: 3%<br>Max: 9%<br><br>Stagneret     | Sandsynligvis introduceret fra Nordamerika af vikingerne. Diskuteres om arten skal betragtes som introduceret. Nordsøen, Kattegat, Østersøen | Jensen et al. 2005<br>Thomsen et al. 2008b |
| Bentisk makrovertebrat (rur)     | <i>Balanus improvisus</i> Darwin        | V. Atlanterhav           | I skibsskrog eller driftømmer | 1880<br><br>København                 | Avg: 0.1%<br>Max: 0.3%<br><br>Stagneret | Fasthæftet, lever på hårbund, epifauna, filtrator<br><br>Almindelig i det meste af Danmarks farvande   | Jensen et al. 2005<br>Thomsen et al. 2008b |
| Bentisk makrovertebrat (rur)     | <i>Elminius modestus</i> Darwin         | Australien, New Zealand? | Skibsskrog                    | 1978 Vadehavet                        | ?                                       | Fasthæftet, lever på hårbund, epifauna, filtrator<br><br>Vadehavet   | Jensen et al. 2005                         |
| Bentisk makrovertebrat (krabbe)  | <i>Eriocheir sinensis</i> Milne-Edwards | V. Stillehav             | Ballast vand?                 | 1927<br><br>Nordjylland               | ?                                       | Lever på blødbund (i banker), ses hovedsageligt i ferskvand og brakvand<br><br>Kattegat, Bælthavet, Østersøen                                | BSASD                                      |

|                                 |   |                                |   |                        |  |   |   |
|---------------------------------|---|--------------------------------|---|------------------------|--|---|---|
| Bentisk makrovertebrat (krabbe) | <i>Rhithropanopeus harrisi</i><br>(Østamerikansk brakvandskrabbe) | Amerikanske østkyst            | Ballast vand?                           | 1936<br>(i Østersøen)  | ?<br><br>I vækst?                                | Gammel gæst?<br><br>Østersøen   | BSASD<br><br>Tendal (i Thomsen og Stæhr 2010)                                   |
| Bentisk makrovertebrat (Søpung) | <i>Styela clava</i><br>Herdman                                    | NV stillehav                   | Ballast vand?<br>Østers? På skibsskrog? | 1984<br><br>Limfjorden | Avg: < 0.0001%<br>Max: 0.0001%<br><br>Stagneret  | Fasthæftet, lever på hårbund, epifauna, filtrator<br>Vadehavet, Limfjorden  | Jensen et al. 2005<br>Thomsen et al. 2008b<br>BSASD                             |
| Pelagisk vertebrat (Fisk)       | <i>Neogobius melanostomus</i><br>(sortmundet kutling)             | Sortehavet og Det Kaspiske Hav | Ballastvand eller via kunstige kanaler  | 2008<br>Bornholm       | Observeret mindst 10 gange i 2009<br><br>I vækst | Eneste invasive fiskeart vi har i Danmark. Den eneste ikke-hjemmehørende saltvandsfisk, som yngler i dansk farvand.<br><br>Bælthavet og Østersøen | <a href="http://www.fiskeatlas.dk">www.fiskeatlas.dk</a><br>Azour et al. (2011) |
| Pelagisk vertebrat (Fisk)       | <i>Oncorhynchus mykiss</i><br>(Regnbueørred)                      | Nordamerika                    | Dambrugsfisk                            | 1894                   | Hyppig, især nær dambrug                         | Yngler sporadisk. Undslipper fra dambrug<br>Nordsøen, Kattegat, Bælthavet, Østersøen  | <a href="http://www.fiskeatlas.dk">www.fiskeatlas.dk</a>                        |

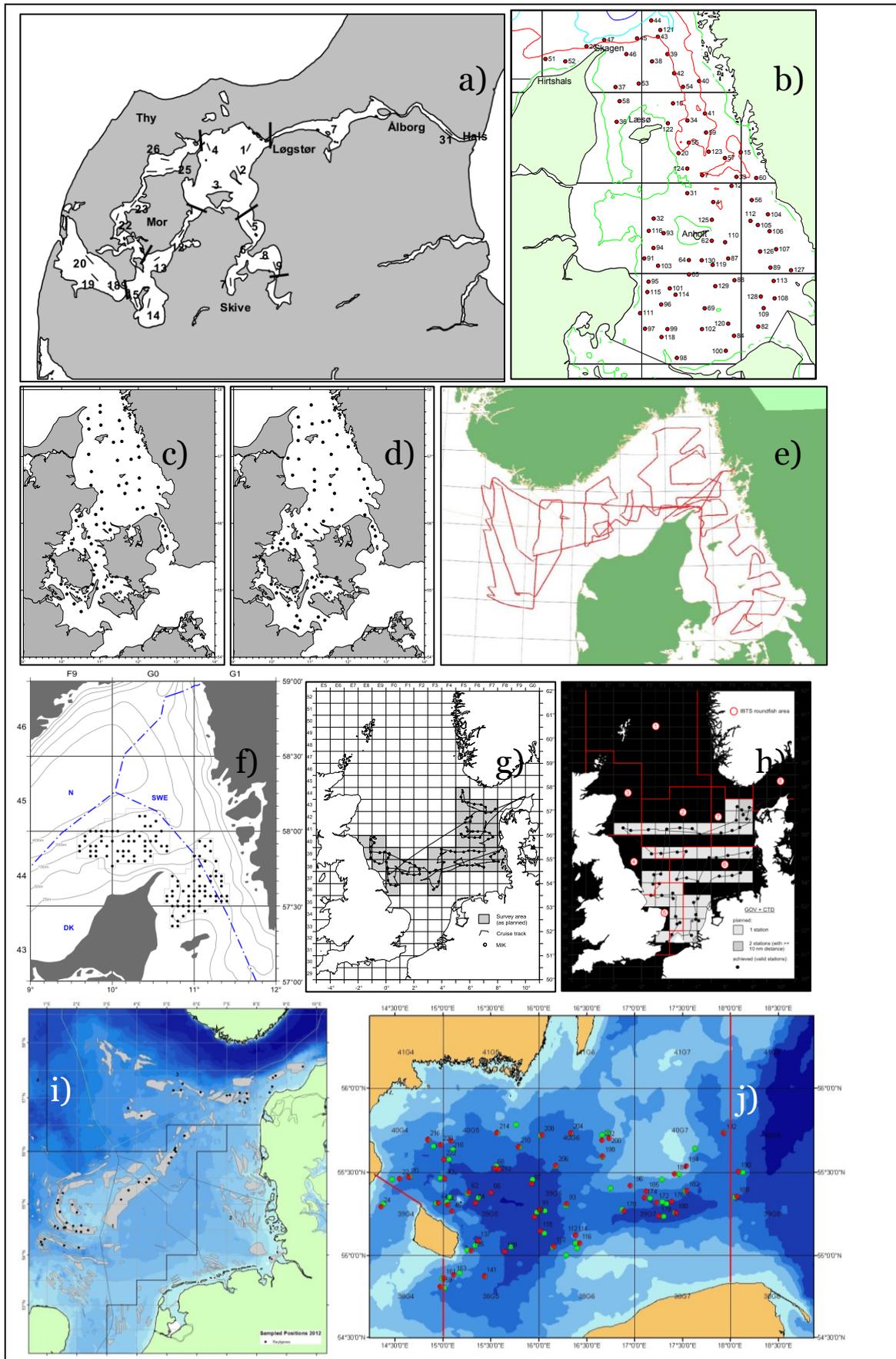
## **Appendix 2: Maps of DTU Aqua's fish monitoring activities**

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**Figure A2.1:** Monitoring surveys in Danish marine waters by DTU Aqua:

- a) Limfjorden surveys, 1980 – 2012;
- b) Sole survey in 2011;
- c) Kattegat survey, BITS 1<sup>st</sup> quarter 2012 (KASU);
- d) Kattegat survey, BITS 4<sup>th</sup> quarter 2012 (KASU);
- e) North Sea Herring Acoustic Survey 2012;
- f) Nephrops UTV survey in Skagerrak and Kattegat, 2012;
- g) International Bottom Trawl Survey, BITS 1<sup>st</sup> quarter 2012;
- h) International Bottom Trawl Survey, BITS 3<sup>rd</sup> quarter 2012;
- i) North Sea sandeel survey 2012;
- j) Baltic International Trawl Survey, BITS 1<sup>st</sup> quarter 2012.

Source: Storr-Paulsen (2014)



**Figure A2.1.**  
See legend on the previous page.

## Appendix 3: Hot spot monitoring network

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### Proposed monitoring strategy

Monitoring of non-indigenous species may be carried out in ports where there is a suspected high frequency of such species and/or in ports where there is a high risk for introduction of non-indigenous species. At the same time it is preferred to establish a representative program, where monitoring is carried out in ports with different environmental conditions, physical characteristics and geographical locations.

### Criteria for port selection

In order to choose where to carry out monitoring of invasive species different criteria are proposed. The first criterion is to identify the 20 ports in Denmark with highest traffic intensity and presumably the highest risk for presence of invasive species. We do acknowledge that the similarity of the conditions at the port of origin may have more explanatory power than the number of port calls or cargo loaded, but the former is difficult to obtain whereas the latter is readily available and should be a reasonable proxy for the ballast water volume in a port.

The ports identified will comprise the study's long-list. To choose among these ports other criteria are also added. The second criteria is in which of these ports there are international ferry connections, since surveys of non-indigenous species may be useful in risk assessments carried out by ferry operators that wish to apply for an exemption from the Ballast Water Management Convention. The third criterion take proximity to protected areas in Denmark into account, since the impact in such areas may be more severe, and the fourth to select ports, which are located geographically different to get the most representative monitoring program. The criteria will be explained into further details in the following.

#### *Traffic intensity (loaded cargo and calls)*

The major international hubs receiving vessels from transoceanic voyages are the key locations for primary introduction of invasive species.<sup>9</sup> It is assumed that no risk of primary invasions can be attached to intra-regional voyages, only secondary transfer. The hotspots are ports with high traffic volumes. Here there may be an increased risk of primary introduction and secondary transfer of invasive species if there is also a great exchange of ballast water. No data is available that directly describes the discharge volumes, and consequently other means of estimation was developed for this assessment. Instead is used cargo exported abroad in ports as proxy for volume of ballast water (Litehauz 2013) under the assumption that vessels transporting cargo abroad comes from abroad - thus having an increased risk of introducing non-indigenous species. The 20 ports of Denmark with most cargo exported are then reviewed with regards to ship calls to avoid representing extreme large tonnage calls (e.g. Triple E class container vessels).

#### *International ferry routes between only two ports*

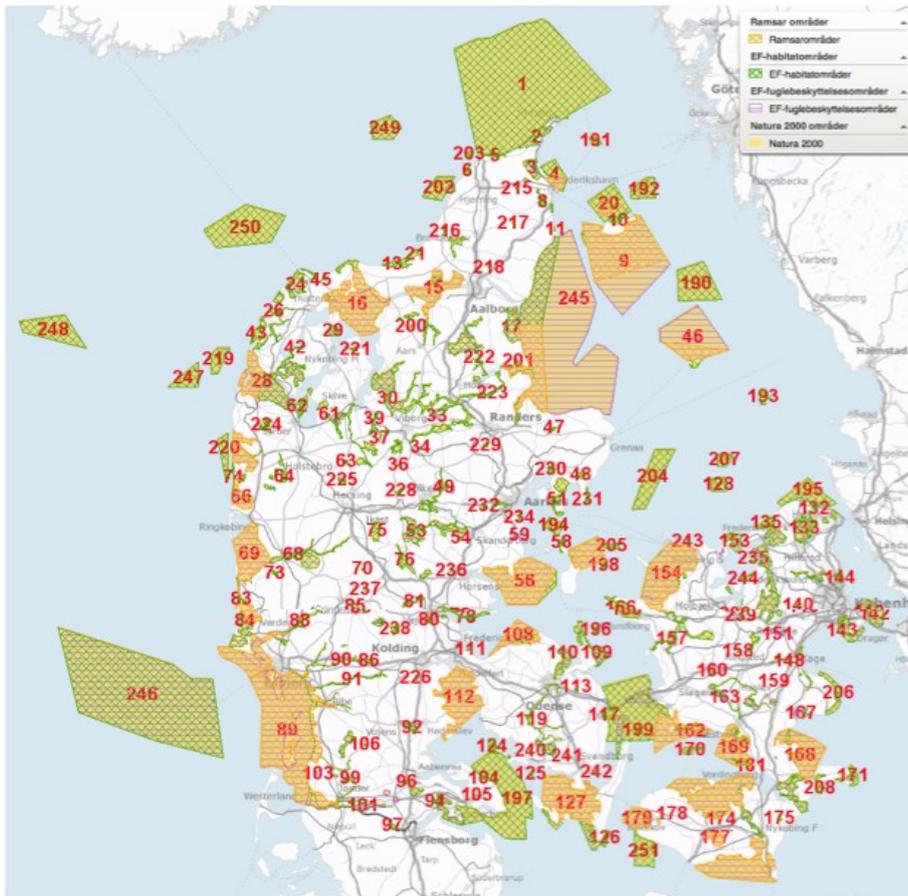
The second selection criterion addresses the long-list port to identify those with international line connections to one other specific port, i.e. we do not include (short sea or ro-ro) lines with multiple port calls. When the Ballast Water Management Convention enters into force every vessel sailing in international waters must manage its ballast water to the discharge criteria, typically by installing a ballast water treatment system. However, it is possible to apply for exemption under Regulation A.4 for ships operating between specific ports where it is shown that there is no risk of transferring non-indigenous species. Because risk assessments for lines with multiple port calls are expected not to shown identical risks, exemption applications for such routes are considered unlikely. The added value of comparing or event using monitoring data in a risk assessment context is expected only for the two port routes included here.

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<sup>9</sup> It is expected that the risk of primary introduction will decrease considerably once the D-2 standard is fully implemented and thereafter, associated primarily with hull fouling. However, in light of the new implementation schedule, where the period in which the D-2 requirements are implemented is longer, it may be expected that the discharge profile in the international hubs may include both treated and untreated water and therefore continue to be a risk of primary introduction of invasive species in a transitional period.

### Proximity to protected areas

There are protected sea and coastal areas in Denmark as shown in **Figure A3.1**. The Natura 2000 areas, which include Ramsar wetlands, Special Areas of Conservation, and Special Protection Areas (Danish Ministry of Environment, 2014) are in some cases close to ports. The port of Esbjerg is located in the Wadden Sea, which is a UNESCO site.



**Figure A3.1.** Natura 2000 areas in Denmark (Danish Ministry of the Environment, 2014).

### Environmental Conditions and Location

The main water bodies around Denmark are the North Sea, Skagerrak, Kattegat and the Baltic Sea. The salinity gradient ranges from largely oceanic in the North Sea to low salinity brackish around Bornholm. It is thus preferred to include ports located in different areas in the short list, in order to represent different circumstances, including different waters, substrate and salinities. Also, different positions of the ports may be considered, e.g. ports located more or less protected and ports exposed to open sea.

### Long-list

The long-list comprises the 20 ports in Denmark with most cargo exported abroad. Furthermore, ship calls, and whether there is an international ferry route is also included in the list (Table A3.1).

The following port's statistics and descriptions are based on the national Danish statistical bureau (Statistics Denmark)<sup>10</sup> and the pilot information provided by the Danish Geodata Agency<sup>11</sup>.

<sup>10</sup> <http://www.statistikbanken.dk/statbank5a/SelectTable/Omradeo.asp?SubjectCode=11&ShowNews=OFF&PLanguage=0>

<sup>11</sup> <http://www.danskehavnelds.dk/#HID=592>

**Table A3.1.** The 20 ports of Denmark with most cargo loaded and exported abroad on freighters and ferries in 2013 and the number and ship calls of freighters and ferries. In the last column international ferry routes is shown. A colour code show decisive data for inclusion in short list.

| Port                  | Loaded cargo to freighters (1000 ton) | Loaded cargo to ferries (1000 ton) | Total loaded cargo (1000 ton) | Freighter ship calls | Ferry ship calls | International ferry route*                                    |
|-----------------------|---------------------------------------|------------------------------------|-------------------------------|----------------------|------------------|---|
| Fredericia Havn       | 7302                                  | 0                                  | 7302                          | 1184                 | 0                |   |
| Rødby Færgehavn       | 0                                     | 3232                               | 3232                          | 0                    | 16966            | - Puttgarden  |
| Helsingør Havn        | 0                                     | 2254                               | 2254                          | 0                    | 28144            | - Helsingborg   |
| Aarhus Havn           | 2098                                  | 0                                  | 2098                          | 2001                 | 3288             |   |
| Statoil-havnen        | 2075                                  | 0                                  | 2075                          | 651                  | 0                |   |
| Esbjerg Havn          | 1324                                  | 0                                  | 1324                          | 2191                 | 14980            | - Harwich<br>- Immingham                                      |
| Frederikshavn Havn    | 30                                    | 1045                               | 1075                          | 570                  | 3724             | - Oslo<br>- Gothenburg  |
| Aalborg Portland Havn | 812                                   | 0                                  | 812                           | 430                  | 0                |   |
| Gedser Havn           | 0                                     | 765                                | 765                           | 0                    | 3169             | - Rostock   |
| Hirtshals Havn        | 7                                     | 683                                | 690                           | 609                  | 2019             | -<br>- Kristiansand<br>- Larvik<br>- Stavanger<br>- Langesund |
| Københavns Havn       | 526                                   | 147                                | 673                           | 2138                 | 362              | - Oslo  |
| Kalundborg Havn       | 626                                   | 0                                  | 626                           | 383                  | 1556             |   |
| Grenaa Havn           | 250                                   | 314                                | 564                           | 434                  | 836              | - Varberg   |
| Odense Havn           | 483                                   | 0                                  | 483                           | 849                  | 0                |   |
| Rønne Havn            | 292                                   | 56                                 | 348                           | 839                  | 1942             | - Sassnitz<br>- Ystad   |
| Aalborg Havn          | 346                                   | 0                                  | 346                           | 847                  | 0                |   |
| Køge Havn             | 304                                   | 0                                  | 304                           | 644                  | 377              |   |
| Aabenraa Havn         | 255                                   | 0                                  | 255                           | 379                  | 0                |   |
| Stålvalseværkets Havn | 243                                   | 0                                  | 243                           | 344                  | 0                |   |
| Nakskov Havn          | 222                                   | 0                                  | 222                           | 157                  | 0                |   |

\*Ferry rout only between two specific ports

Six sites for cargo export: The six prioritised ports (marked with E) presumably receive ballast water discharge associated with exporting goods.

We propose to include Port of Kalundborg (E6) as a second site in the Kalundborg fjord, since it is by its own merit number six on the cargo list with the adjacent Statoil pier as number three. The local energy plant Asnæsværket is a producer of cooling water although it is expected that the industrial symbiosis system in Kalundborg does remove a large part of the surplus heat.

**Table A3.2.** *Top 10 ports without international ferry lines in the order of cargo loaded volume.*

| Port                            | Characteristics                                   | Sampling site                                  |
|---------------------------------|---|--|
| E1. Port of Fredericia          | Exposed but several basins                        | Oil harbour                                    |
| E2. Port of Aarhus              | Several basins                                    | Container terminal                             |
| E3. Statoil-havnen (Kalundborg) | Smaller port,                                     | Oliepier                                       |
| E4. Port of Esbjerg             | Many basins, exposed, proximity to protected area | Several sites possible; Optional more than one |
| E5. Aalborg Portland            | Exposed quay                                      | Cement loading quay                            |
| E6. Port of Kalundborg          | Deep protected fjord several basins               | Sydhavn  |
| Port of Copenhagen              | Several basins                                    | Prioritised for cooling water                  |
| Port of Odense                  | Several basins                                    | Prioritised for cooling water                  |
| Port of Aalborg                 | Several basins                                    | Optional                                       |
| Port of Køge                    | Outlet from stream in Southern basin              | Northern basin                                 |

The four ferry ports are the prioritised ferry line ports for the local transfer issues. The prioritised ports (marked with F) presumably receive ballast water discharge including trim water associated with frequent loading and offloading of goods and passengers. We propose to include both Frederikshavn and Grenå since these ports are presumably biologically comparable in Denmark but their ferry lines' corresponding ports in Sweden, Gothenburg and Varberg are widely different with respect to other international traffic, and hence presumed risk of non-indigenous species.

**Table A3.3.** *Top 10 ports with international ferry lines based on cargo loaded volume.*

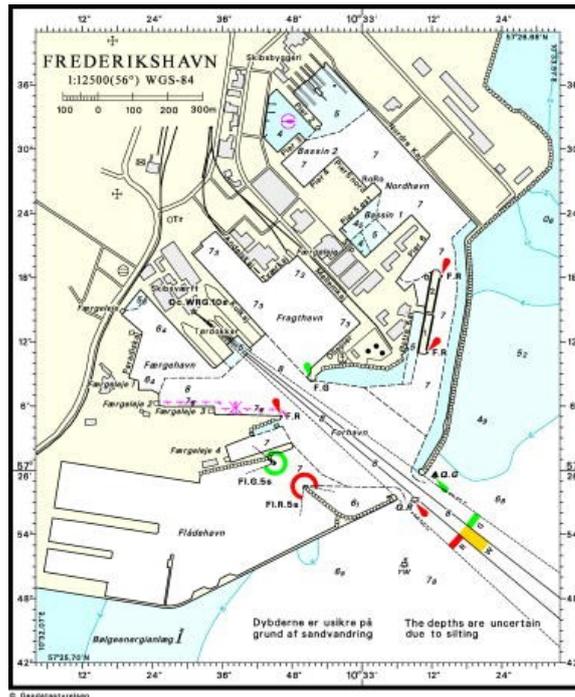
|                        |                                 |                               |
|------------------------|---------------------------------|-------------------------------|
| F1. Rødby Færgehavn    | Simple basin, brackish          | Near East quay                |
| F2. Helsingør Havn     | Simple basin, traffic intensive | Near East quay                |
| F3. Frederikshavn Havn | Kattegat, pair Gothenburg       | Cargo basin (Fragthavnsbasin) |
| Gedser Havn            | Gedser Havn                     | Optional                      |
| Hirtshals Havn         | Hirtshals Havn                  | Optional                      |
| F4. Grenaa Havn        | Grenaa Havn                     | At ferry berth                |
| Københavns Havn        | Københavns Havn                 | -                             |
| Rønne Havn             | Rønne Havn                      | -                             |

### Monitoring program

The longlist is reduced to four ports based on the criteria, i.e. Fredericia, Esbjerg, Rødby and Helsingør. These ports together fulfil the criteria very well, as they represent hotspots (Fredericia), protected area (Esbjerg), ports with international ferry routes (Esbjerg, Rødby and Helsingør) and they are located in different geographical areas.

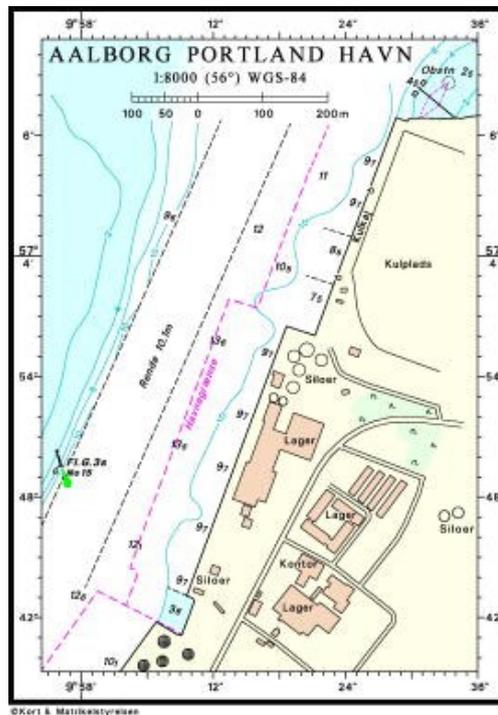
### Frederikshavn

The size of the ships, which can enter the port of Frederikshavn depends on the weather and wave conditions. In fortuitous circumstances the ships can be up to 180 m long. At dock the ships can be up to 215 m long. Plans for deepening and improving access have been published. There are no freshwater outlets from streams or other sources in the port of Frederikshavn (Danish Geodata Agency 2014).



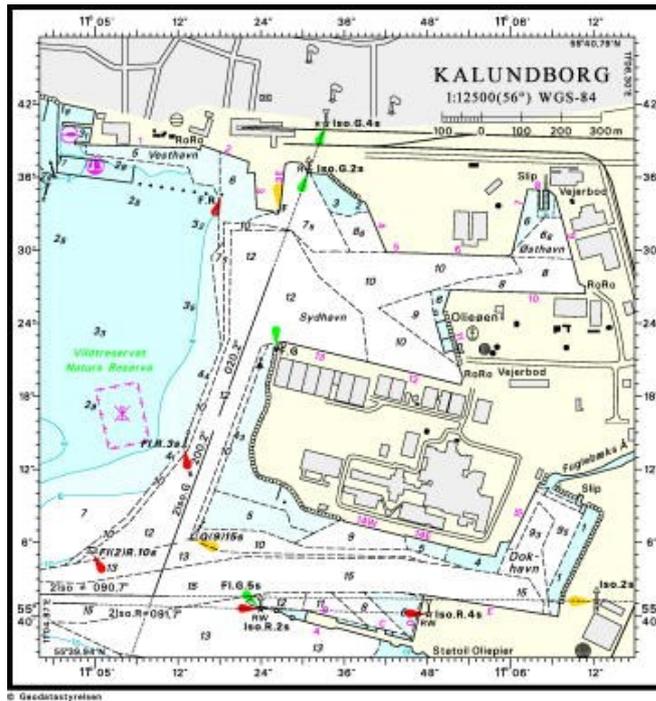
### Aalborg Portland

The port is open and exposed with a relatively shallow draught of 9 m. Many vessels arrive in ballast.



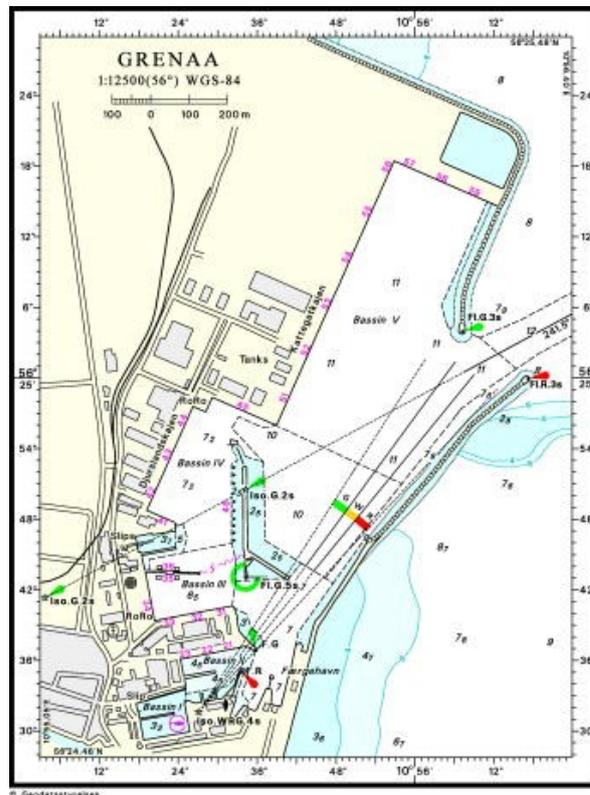
*Kalundborg Sydhavn (top part of map) and Statoil Oil pier (lower part of map)*

Two areas in Kalundborg are proposed as the oil pier in the south and the Sydhavn are potential hotspots. There is a small stream entering the southern basins. Vessels up to 240 m with 11.6 m draught may call Kalundborg and at the oil pier larger vessels up to 264 m with 12.5 m draught.



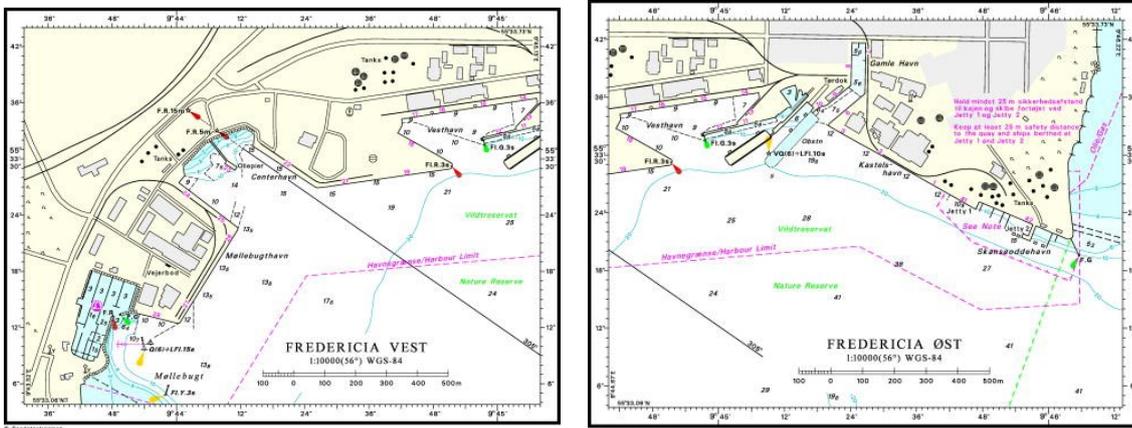
*Grenaa*

The port is partitioned into several basins. Largest vessels to call are 230m and the approach channel is dredged to 11 m. It is proposed to sample off the berths 21 and 31 to the NE of the ferry berth.



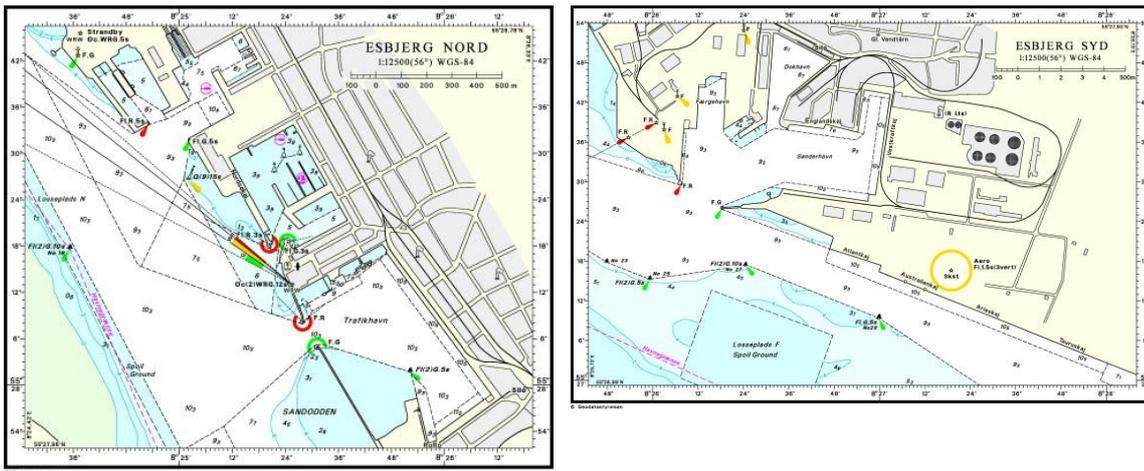
## Fredericia

As seen on the maps the port of Fredericia is more than 1 km wide. The port consists of multiple quays and basins, which from East to West are: "Skanseodde Harbor", "Citadel Harbor", "Old Harbor", "Dockyard Harbor", "Boat Harbor", "West Harbor", "Oil Harbor", "Central Harbor", "Chemistry Harbor", "Møllebugt Harbor" and "Pleasure Craft Harbor". The largest ships, which can use the port can be up to 300 m long and draught of no more than 14 m. There are no freshwater outlets from streams or other sources in the port of Fredericia (Danish Geodata Agency 2014).



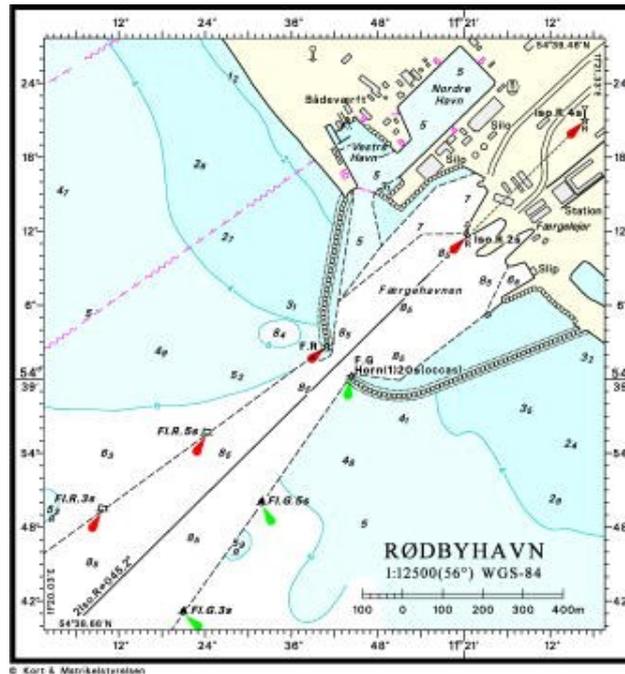
## Esbjerg

The port of Esbjerg consists of different basins and quays as seen on the harbor overview. Sailing into Port of Esbjerg occurs through the North Sea, "Grådyb Barre" and "Grådyb". The depths of the channel in "Grådyb Barre" to the port is by mean low water spring 10.3 m decreasing to 9.8 m at the edges. The width of the channel is at least 200 m. The biggest ships, which can enter the port may have a maximum draught of 10.5 m and a length of 225 m in "Trafikhavn" and "Sønderhavn", while the length can be up to 245 m in "Australienkaj". There are no freshwater outlets from streams or other sources in the port of Esbjerg (Danish Geodata Agency 2014).



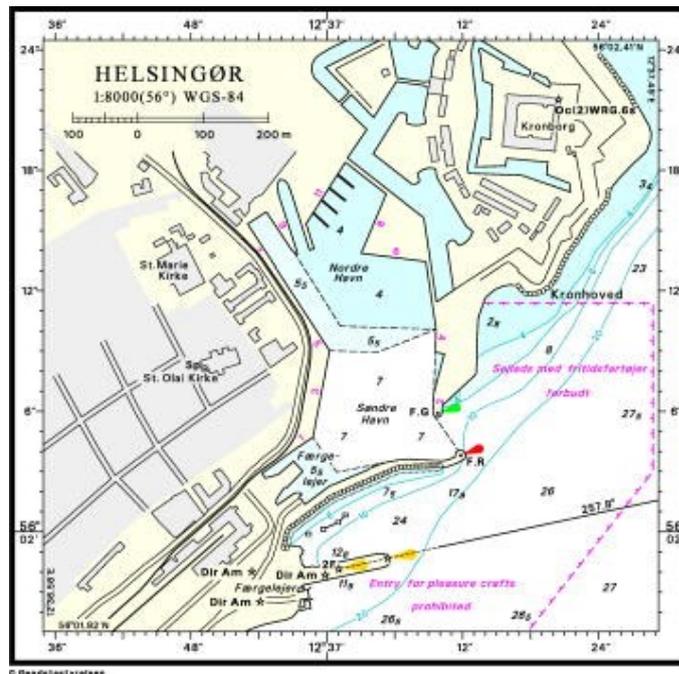
## Rødby

Port of Rødby consists of Rødby Traffic Harbour divided into the West Harbour and North Harbour, in addition to Rødby Ferry Harbour. A channel with a depth of 8.5 m and 1000 m long has been dredged to Rødby Færgehavn. The ships, which can call the port can have a maximum draught of 4.5 m, a width of 20 m and a length of 80 m. The difference between mean low tide and mean high tide is 0.3 m. There are no freshwater outlets from streams or other sources in the port of Rødby (Danish Geodata Agency 2014).



## Helsingør (Elsinore)

Elsinore Harbour is divided into Northern and Southern Harbour. The SW corner is frequented by private ferry companies. The depth in the entry is 7.5 m, however only 7.3 m near the head of the N-pier. The ships, which can safely enter the port of Helsingør depends on the weather and wave conditions. At fortuitous circumstances the ships can be up to 150 m long and 30 m wide. The difference between mean low tide and mean high tide is 0.2 m. There are no freshwater outlets from streams or other sources in the port of Helsingør (Danish Geodata Agency 2014).



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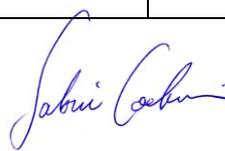
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| <p>Abstract</p> <p>This report presents the results of the MONIS project study including a proposal for a monitoring programme. The existing monitoring activities of biological and chemical status in Danish waters under the NOVANA programme and the monitoring of fish (by DTU Aqua and Copenhagen University) are comprehensive but insufficient with regard to monitoring of non-indigenous species and their geographical coverage. The applied Technical Guidance Manuals are generally assessed as being appropriate also for non-indigenous species, but in some case important supporting parameters are not included. More recent methodologies e.g. automated sampling or biomolecular techniques (barcoding; eDNA) are not currently in use and they should be included in the monitoring of non-indigenous species. The proposed strategic objectives are developed into a proposed monitoring programme including: (1) 13 complimentary hot spot stations for non-indigenous species situated in ports or coastal environments that may be reached with smaller vessels/dinghies or directly from the quays; and (2) a part based on conventional sampling (44 stations/sites) entirely contained within the existing NOVANA programme and the planned Marine Strategy Framework Directive monitoring programme, thus adding no further costs to the monitoring programme. Finally, the proposal for an eDNA-based monitoring programme targeting 48 sites is 100% compatible with the planned water chemistry programme employing not only the same stations but also very similar sampling techniques.</p> |
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|--|---|



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## **Monitoring of non-indigenous species in Danish marine waters**

### **Monitoring of non-indigenous species in Danish marine waters**

This report is a product of the MONIS project, or in full "Monitoring of Non-Indigenous Species in Danish Marine Waters", which has been funded by the Danish Ministry for the Environment, 2014.

MONIS has had three overarching objectives. Firstly, to define the optimal monitoring strategy and to design a cost-effective Marine Strategy Framework Directive (MSFD) monitoring programme targeting non-indigenous species in Danish waters (MSFD Descriptor 2; or simply D2).

Secondly, to scrutinize how to make best use of existing data and to make linkages to other monitoring activities (understood as other MSFD descriptors than D2) in order to fulfil obligations related to both the MSFD and the Regulation of the European Parliament and the Commission on the prevention and management of the introduction and spread of invasive alien species.

Thirdly, to ensure that the Danish proposal for a D2-targeted monitoring programme focusing on non-indigenous species is consistent with the MSFD cycle, e.g. production of MSFD Initial Assessment, design and implementation of monitoring programmes, design of Programmes of Measures, and implementation of Programmes of Measures, and ultimately also fulfilment of the overarching MSFD target of 'good environmental status'.



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