TRENDS IN RECORDS AND CONTRIBUTION OF NON-INDIGENOUS SPECIES (NIS) TO BIOTIC COMMUNITIES IN DANISH MARINE WATERS

Scientific Report from DCE – Danish Centre for Environment and Energy No. 179 2016

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Data sheet

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Abstract:	The report investigates trends in the temporal and spatial changes of non-indigenous marine species in the Danish part of the OSPAR and HELCOM regions. The assessment is based on a quantitative analysis of data available in national monitoring databases and covers the period 1989 to 2014 and other documented records of non-indigenous marine species in the Danish waters.
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Preface

The report is a baseline report on non-indigenous marine species in the Danish part of the OSPAR and HELCOM regions. Also the report is the first Danish national assessment of marine NIS (non-indigenous species) and will be used as input to the European Marine Strategy Framework Directive (MSFD) reporting. The analysis serves as a baseline for future annual reporting of the ecological status of Danish marine waters.

The report has been made by DCE in collaboration with DTU Aqua and Andersen Aqua-consult.

The assessment is based on a descriptive analysis of data available in national monitoring databases and covers the period 1989 to 2014 and other documented records of non-indigenous marine species in the Danish waters.

Summary

The objective of this report is threefold: 1) to contribute to the Danish official contribution to the Marine Strategy Framework Directive (MSFD) reporting on non-indigenous species (NIS), 2) to be an independent report on NIS in the OSPAR and HELCOM regions and 3) to act as a baseline in future assessments of NIS in Danish waters. The report is the first national assessment of the occurrence and decadal trends of NIS and how NIS contributes to changes in marine community structures. We also investigate possible relationships between NIS abundance and key water quality conditions characterizing the marine environment. Marine species were grouped into five categories: Phytoplankton, zooplankton, macroalgae, benthic invertebrates and fish. The analyses cover the period 1989 to 2014 for five marine regions in the North Sea (OSPAR) and the Baltic Sea-North Sea transition zone (HELCOM and OSPAR): 1) the Norths Sea including Skagerrak, 2) the Kattegat, 3) Limfjorden, 4) the Belt Sea and 5) the western Baltic Sea.

Since the initiation of the Danish marine monitoring programmes in the 1990s, the number of marine and coastal NIS recorded has increased from 21 to 85 in 2014. Of the 85 marine NIS recorded in total, 63 species appear in the monitoring programmes, while the remaining 22 are documented in various reports (see table 6.2). Several NIS were not detected in the monitoring programme because of limited spatio-temporal sampling. Although monitoring programmes have recorded several NIS, inadequacies in the current sampling technique and strategy are responsible for lack of information on some groups of NIS including gelatinous zooplankton, shallow water fish, parasitic invertebrates, and flowering plants from the littoral zone. We calculated older introduction rates to be approx. two new NIS per decade from 1900 to 1980 followed by a dramatic increase of 16 new NIS per decade from 1980 to 2014 when combining information from monitoring and other documented records. Similar exponential increases in NIS numbers has been observed in other countries and related to a combination of intensified ship traffic, breakdown of dispersal barriers and climatic changes. Parallel to the introduction of NIS, there has been a gradual increase in the total species number sampled following the initiation of the monitoring surveys in the 1980s. However, as the relative number of NIS was not related to sampling effort, changes in this over time are not likely to bias our interpretations of NIS impact. Most of the recent increase (from 1989 to 2014) has occurred within the phytoplankton (45 %), followed by benthic invertebrates (26 %), macroalgae (14 %), fish (5 %), parasites (5 %), zooplankton (4 %) and flowering plants (1 %). Today, most NIS are established in the large sound Limfjorden (30 NIS), and fewest are found in the brackish western Baltic Sea region (11 NIS).

Significant and exponential increases in records of NIS relative to the total species records in the database (hereafter $\%NIS_{record}$) were observed for phytoplankton and benthic invertebrates in all five regions. Only two records of fish NIS existed in the database indicating that trawl surveys primarily conducted in deeper waters over sandy sediments are not suited to monitor NIS of fish, which typically are adapted to shallow vegetated waters. For macroalgae and zooplankton, there was no clear temporal trend in $\%NIS_{record}$. We also analysed changes in relative abundances of NIS compared to the native community (hereafter $\%NIS_{abundance}$) standardised by biomass for phytoplankton, number of individuals for benthic invertebrates and percent

cover for macroalgae (no abundance data were available for zooplankton). This analysis showed a decrease in $\%NIS_{abundance}$ for phytoplankton, but an increase for benthic invertebrates and macroalgae (only in Limfjorden for macroalgae).

Multivariate statistics was used to quantify the impact of NIS on community structures. This analysis showed that in regions where NIS were recorded, their contribution to total community similarity increased over time. This indicates that NIS have, over time, become a more characteristic component of Danish marine communities. The largest recent (i.e. from 2006 to 2014) contribution of NIS to community similarity was observed for phytoplankton where NIS contributed with ~ 10 percent of the similarity (compared to 5, 4, and 2 percent contribution from benthic invertebrate, macroalgal and zooplankton NIS, respectively). Furthermore, the relative importance of NIS to community similarity generally declined from the open saline waters toward the brackish Baltic Sea suggesting that many NIS have been introduced from neighbouring waters rather than long dispersal via ballast water. The stronger influence of NIS in the north-western regions is likely driven by a combination of factors, for example (1) high hydrodynamic connectivity between the Baltic Sea – North Sea transition zone, (2) introduction of NIS generally has to travel through this north-western North Sea Kattegat opening, (3) this region includes large estuarine systems (e.g. Limfjorden), and (4) this region has subsystems characterized by both euhaline waters (for stenohaline NIS) and estuarine brackish water (for euryhaline NIS). While NIS generally explained less than 10 percent of the year-to-year changes in the community similarity for these large-scale regions, NIS often dominated locally and seasonally. Examples of particular dominant NIS include *Pseudochattonella* during early spring in the Kattegat (phytoplankton), *Sargassum muticum* in the Limfjord (macroalgae), *Ensis directus* in all regions (benthic invertebrates), *Mnemiopsis leidyi* in the Belt Sea (zooplankton) and *Neogobius melanostomus* in the Belt Sea (fish).

We performed a univariate correlation analysis to evaluate if changes in $\%NIS_{record}$ over time were related to changes in environmental conditions (water clarity, salinity, temperature, primary production, concentration of chlorophyll and total nitrogen) in the five studied regions. Overall low correlations were found, suggesting that changes in environmental conditions such as those related to climate change were of small importance for changes in NIS number. A multivariate correlation analysis furthermore indicated that changes in species composition within groups (phyto-, zooplankton, macroalgae, benthic fauna) were partly related to $\%NIS_{record}$ but strongly related to salinity, highlighting a general positive relationship between salinity and marine species richness in Danish waters. To further resolve and disentangle the underlying causes for the increasing contribution to, and effect of NIS to Danish marine communities, analyses are needed at a more local scale where the occurrence of NIS (either as records or abundance) can be linked to environmental variables with greater certainty.

Ensis directus/Atlantic jackknife clam.
Photo: Steffen Lundsteen©

Sammenfatning

Rapporten har tre formål: 1) at levere et fagligt udgangspunkt for det danske bidrag til havstrategidirektiv (MSFD)-afrapporteringen om ikke-hjemmehørende marine arter (NIS), 2) at levere en baseline rapport om udbredelse og effekter af marine NIS i den danske del af OSPAR og HELCOM regionerne og 3) at levere en analyse, som danner udgangspunkt for fremtidige vurderinger af udbredelse og effekter af NIS i danske farvande. Rapporten er den første kvantitative danske vurdering af ændringer i udbredelse og forekomst af NIS og deres effekter på strukturen i de marine bentiske og pelagiske samfund. Rapporten indeholder også en undersøgelse af mulige sammenhænge mellem forekomst af NIS og en række vandkvalitetsparametre, som karakteriserer det marine miljø. I den kvantitative analyse har vi opdelt de marine arter in fem kategorier: fytoplankton, zooplankton, makroalger, blødbundsfauna og fisk. Analysen dækker perioden 1989 til 2014, hvor de danske farvande er opdelt i 5 marine regioner, som omfatter Nordsøen inkl. Skagerrak (OSPAR) og overgangen til Østersøen (HELCOM og OSPAR). Følgende 5 regioner blev anvendt i analysen: 1) Nordsøen inkl. Skagerrak, 2) Kattegat, 3) Limfjorden, 4) Bælthavet og 5) den vestlige Østersø.

Siden begyndelsen af moniteringsprogrammerne i 1980erne er antallet af observerede NIS steget fra 21 til 85 i 2014. Af de 85 NIS er 63 arter observeret i forbindelse med overvågningsprogrammerne og registreret i moniteringsdatabaserne, mens de resterende 22 NIS er observeret og dokumenteret i andre sammenhænge. En del af årsagen til at disse NIS ikke er fanget i moniteringsprogrammerne, skyldes formentlig begrænsninger i programmernes rumlige og tidslige dækning. Hertil kommer begrænsninger i indsamlingsstrategien og -metoden som gør, at specifikke NIS (goplerne, fisk på lavt vand, parasitter og blomsterplanter i kystzonen) ikke observeres med den nuværende overvågning.

Ved inddragelse af historiske kilder, finder vi, at der i perioden fra ca. 1900 frem til 1980 er introduceret ca. 2 arter per årti, hvorefter ca. 16 NIS er observeret per årti i de danske farvande frem til 2014. Tilsvarende eksponentielle stigninger i NIS antallet er observeret i andre lande og relateres generelt til en kombination af ballastvand fra øget skibstrafik, nedbrydning af spredningsbarrierer, og miljø/klimaforandringer. Samtidigt med indførelsen af de nye ikke-hjemmehørende marine arter, er der sket en gradvis stigning i det samlede artsantal i takt med at overvågningsindsatsen er forøget siden 1980erne. Dette gælder i øvrigt også det samlede artsantal, hvilket indikerer, at jo mere man leder, desto større er sandsynligheden for at finde en ny art. Den procentvise andel af NIS var dog ikke afhængig af antallet af observationer, hvormed udvikling i NIS andelen og bidraget fra NIS til samfundsstrukturen, antages at være upåvirket af prøvetagningsintensiteten.

Den samlede forøgelse siden 1989 er hovedsageligt sket inden for fytoplankton (45 %), fulgt af blødbundsfaunaen (26 %), makroalger (14 %), fisk (5 %), parasitter (5 %), zooplankton (4 %) og blomsterplanter (1 %). Limfjorden er den region, hvor flest NIS har etableret sig (30 arter), mens færrest NIS er observeret i den artsfattigere brakke Østersø (11 arter).

For nogle organismegrupper er der sket en signifikant og nærmest eksponentiel stigning i den relative andel af NIS-arter (%$NIS_{records}$) igennem perioden 1989 til 2014. Dette gælder fytoplanktonet og blødbundsfaunaen i alle 5 regioner, men ikke makroalger, zooplankton og fisk. Der var i alt kun to observationer af NIS blandt fiskene, hvilket indikerer, at trawlundersøgelser målrettet konsumfisk, som hovedsageligt fanges på dybere vand med blødbund, ikke er velegnet til at registrere NIS, da de arter, som står på NIS-listen, udgøres af fisk, som primært lever på lavt vand i områder med vegetation. Vi analyserede også for tidslige ændringer i NIS opgjort som deres relative abundans ($\%NIS_{abundance}$) målt på biomasse for fytoplankton, antal individer for blødbundsfaunaen og procent dækningsgrad for makroalger (biomassedata var ikke tilgængelig for zooplankton). Denne analyse viste et fald i NIS-andelen for fytoplanktonet, en general stigning for blødbundsfaunaen samt en stigning for makroalgerne i Limfjorden.

Betydningen af NIS for ændringer i samfundsstrukturen blev undersøgt ved anvendelse af multivariat statistik. En similaritetsanalyse viste, at der i de fleste regioner er sket en gradvis forøgelse siden 1989 i bidraget fra de ikke-hjemmehørende arter til den samlede artssammensætning. Dette indikerer, at NIS med tid er blevet en mere fremtrædende del af de bentiske og pelagiske samfund og derved bidrager mere til at karakterisere den marine flora og fauna i de danske farvande. Hvis man ser bort fra regionale forskelle, er det største recente (årene 2006-2014) bidrag sket inden for fytoplanktonet, hvor NIS bidrager med ~ 10 procent af similariteten (ligheden) i samfundet. Til sammenligning er det landsdækkende bidrag 5, 4 og 2 procent for blødbundsfaunaen, makroalgerne og zooplanktonet. Disse tal dækker over store regionale samt sæsonmæssige variationer, hvor NIS lokalt kan blive altdominerende. Eksempler herpå er forårsopblomstring af *Pseudochattonella* i Kattegat, dominans af makroalgen *Sargassum muticum* i det meste af Limfjorden, tætte bestande af den amerikanske knivmusling *Ensis directus* i lokale områder af samtlige regioner, masseforekomster af "dræbergoblen" *Mnemiopsis leidyi* primært i Bælthavet samt Limfjorden og tætte bestande af den sortmundede kutling *Neogobius melanostomus* i det sydlige Bælthav.

Ved hjælp af en multivariat korrelationsanalyse fandt vi, at en mindre del af ændringerne i samfundsstrukturen kan tillægges stigningen i $\%NIS_{records}$, mens forskelle i salinitet fra Østersøen ud mod Nordsøen bidrager væsentligt mere til at forklare regionale forskelle i samfundsstrukturen inden for plante- og dyregrupperne. Udover at ændringer i samfundsstruktur falder sammen med den generelle stigning i artsdiversitet med stigende saltholdighed, så antages den større betydning af NIS i de nordvestlige mere salte regioner at være et resultat af (1) større udveksling af vand og dermed NIS-individer pga. høj hydrodynamisk udveksling, (2) tilførsel af NIS gennem Nordsø-Kattegat åbningen, (3) de salte regioner omfatter en række store forstyrrede fjordsystemer, herunder Limfjorden, og (4) Limfjorden er endvidere et optimalt sted for NIS, da regionen udgøres af en række delsystemer karakteriseret ved både højsalint vand (optimalt for stenohaline arter) samt brakvand velegnet til euryhaline arter. Denne tolkning indikerer, at en væsentlig del af NIS i danske farvande er kommet til som sekundære introduktioner fra tilstødende farvande, og i mindre grad er kommet til via ballastvand langt borte fra.

En univariat korrelationsanalyse blev anvendt til at vurdere mulige sammenhænge mellem tidslige ændringer i forekomsten af NIS ($\%NIS_{record}$) og ændringer i forskellige miljøforhold (temperatur, salinitet, sigtdybde, primærproduktion, koncentration af klorofyl *a* og total kvælstof). Analysen viste en meget svag sammenhæng, hvilket tyder på, at det ikke er ændringer i det modtagende miljø, som har været udslagsgivende for stigningen i antallet af NIS. Mere detaljerede analyser af tidslige og rumlige forskydninger i forholdet mellem hjemmehørende og ikke-hjemmehørende arter er dog nødvendig for reelt at kunne vurdere betydningen af lokale miljøforhold for ændringer i udbredelse og dominans af NIS.

1 Introduction

Human activities have over centuries broken down natural dispersal barriers, that in the past separated biota from different oceans, seas, rivers and terrestrial environments (Thomsen et al. 2015). Growth in global transport and deliberate introductions of new species have furthermore facilitated the spread of non-indigenous species (NIS = alien, exotic, non-native, introduced) including species with invasive potential to travel vast distances and colonise novel habitats and environments. NIS are defined as species which due to human activity (direct or indirect introduction by humans) are found outside of their natural range and dispersal potential (IUCN 2000; Thomsen et al. 2008a). It should be acknowledged that some NIS can be cryptogenic species, of unknown origin, which may not have been introduced, but have arrived because of changes in the environment such as warming. Regardless of the definition, NIS may cause severe ecological, economic and cultural impacts on the receiving environments. Today, introduction of NIS is therefore viewed as a potential threat to natural habitats and NIS have been included in legislation at national and international levels in order to counteract current and future negative impacts.

Invasive NIS have caused ecological, economic and public health impacts globally (Ruiz et al.1997; Ojaveer & Kotta 2015; Thomsen et al. 2016). Some invasive NIS can induce considerable changes in the structure and dynamics of marine ecosystems and may also hamper the economic use of the sea or even represent a risk for human health. Ecological impacts include changes in habitats and biological communities as well as alterations in food web functioning, in extreme cases even losses of native species can occur (Galil 2007). Economic impacts range from financial losses in fisheries to expenses for industries for cleaning intake or outflow pipes and structures from fouling (Black 2001; Williams et al. 2010). Public health impacts may arise from the introduction of pathogens or toxic algae (Thomsen et al. 2015).

Only few of all NIS become invasive with the potential to cause negative impacts. Those NIS which cause the most harm on the environment and/or humans are the most important to assess. At the European scale, impact assessment from NIS is required as part of assessing the 'current and changing status of the ecosystems' (the Water Framework Directive and the Marine Strategy Framework Directive) and for the 'the marine management perspective' in order to facilitate a strong move towards implementation of the ecosystem-based approach where the target is to achieve good ecological status.

Studies of the ecological impacts of NIS are scarce. In the Baltic Sea, impact assessment only exists for approx. 43 NIS (Zaiko et al. 2011), corresponding to 25 % of the 174 species currently identified as potential NIS for Danish marine waters alone (MONIS 2 2015 target list). Also it is difficult to compare the impact assessment of those 43 species as sample effort and methods are very different. There is therefore an urgent need to improve our knowledge of the number, distribution and impact of NIS in Danish waters. The single most important attribute that can function as a proxy for both an invasive species success and its impact is its local abundance (Catford et al. 2009; Parker et al. 1999, Thomsen et al. 2011). A first approach to evaluate NIS success and impact is therefore to determine and document the contribution of NIS to the native communities, for example in terms of relative abundances, and to investigate temporal changes in relative abundances in different regions of the Danish waters.

This report provides updated data on the historical changes in the number and abundance of marine NIS in Danish marine waters (Stæhr & Thomsen 2012; Thomsen et al. 2007, 2008b, 2009). As part of the analysis, we make the first national assessment of the quantitative importance of NIS for trends in marine species abundance and community structure based on the Danish marine monitoring programmes.

2 Methodology and data material

2.1 Study area

The Danish waters were divided into 5 broad regions: Region 1 = the North Sea and the Skagerrak, Region 2 = the Kattegat, Region 3 = the Limfjorden, Region 4 = the Belt Sea, and Region 5 = the western Baltic Sea – see *figure 2.1*).

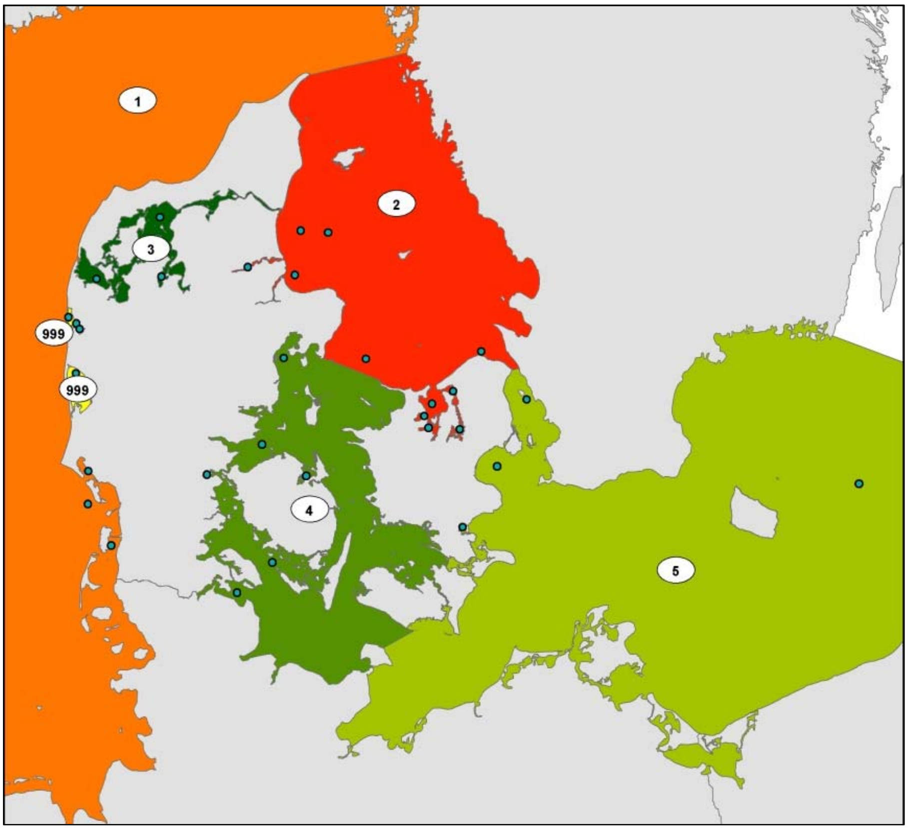


Figure 2.1. Map of phytoplankton sampling stations (dots) in Danish waters utilized in the NIS analysis covering data from 1989 to 2014. Region 1 = the North Sea and the Skagerrak, Region 2 = the Kattegat, Region 3 = Limfjorden, Region 4 = the Belt Sea, and Region 5 = the western Baltic Sea. Regions 999 were not included in the data analysis.

These regions roughly correspond to the HELCOM (2-5)/OSPAR (1-3) boarders except for the distinction between the Kattegat and the western Baltic Sea, where we applied the Kattegat-Sound basin line in order to best separate the hydrographical regions as characterized by differences in several key environmental variables (*table 2.1*).

Table 2.1. The five regions characterized by annual average conditions in selected physical and chemical parameters. All means are average of annual average of water column integrated values from 1989 to 2014. Data in (brackets) are standard error of the mean. Variables with a large standard error have undergone significant changes during the monitored period. TN is total nitrogen and PP is primary production. Sampling stations are shown in *figure 2.6.*

Region	Temperature (°C)	Salinity (psu)	TN (mg m^{-3})	PP (g C m^{-2} y^{-1})	Chlorophyll *a* (mg m^{-3})	Secchi depth (m)
North Sea and Skagerrak	9.5 (0.1)	30.5 (0.1)	343 (12)	195 (29)	2.2 (0.1)	4.1 (0.1)
Kattegat	9.6 (0.1)	23.3 (0.1)	360 (11)	267 (11)	2.5 (0.1)	5.7 (0.1)
Limfjorden	9.9 (0.1)	24.4 (0.2)	776 (51)	280 (25)	5.3 (0.3)	2.6 (0.1)
Belt Sea	9.5 (0.1)	18.1 (0.1)	358 (13)	229 (13)	2.5 (0.1)	5.2 (0.1)
Western Baltic Sea	9.4 (0.1)	12.2 (0.1)	304 (6)	130 (18)	1.6 (0.0)	6.8 (0.1)

2.2 Data sources

We analysed data from fjords, estuaries and coastal and open water sites scattered across Denmark that have been regularly monitored as part of the Danish National Aquatic Monitoring and Assessment Programme (NOVANA, but also referred to as DNAMAP – see Riemann et al. 2016) - and the annual fish survey programme (ICES 2012, 2014). Our investigation included long-term monitoring data series. However, to reduce effects of significant changes in sampling protocols and sampling effort (number of stations and samples per year in each region), we restricted the quantitative part of our analysis to data collected since the nationwide monitoring programmes were established in 1989. Quantitative species data were analysed for macroalgae, phytoplankton, zooplankton, benthic invertebrates, and fish. We also extracted environmental data to correlate changes in NIS abundance to changes in water quality conditions. For all groups except phytoplankton, our search for NIS in the Danish databases relied on the 174 species currently identified as potential NIS for Danish marine waters (MONIS 2 2015 target list). For phytoplankton, the MONIS list was expanded with a few species identified by Per Andersen.

2.2.1 Phytoplankton data

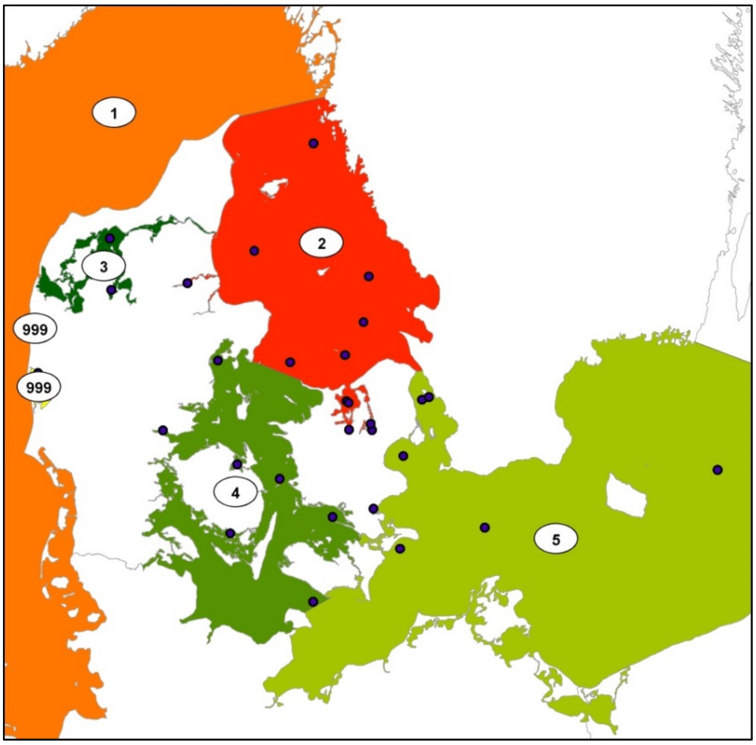
Phytoplankton were identified and quantified using inverted microscope according to a standard protocol (Fossing & Jakobsen 2015). Phytoplankton counts were converted into cells L^{-1} or carbon biomass (µg C L^{-1}). Carbon biomass was estimated by grouping species into taxonomical groups using the cell volume to carbon relationship by Menden-Deuer & Lessard (2000) as outlined in Jakobsen et al. (2015). *Figure 2.1* shows the total distribution of stations sampled within each of the investigated five regions. A total of 23 stations was analysed with 3, 8, 3, 5 and 4 stations, respectively, in regions 1 to 5. A total of 561 “species records” was recorded in the entire data set. A phytoplankton or zooplankton ”species record” included either a full identified species or in some cases only a genus name such as “*Gymnodinium* sp.” or “*Acartia* sp.” which may include multiple unidentified species. For phytoplankton a total of 162, 232, 218, 239 and 195 species was recorded in regions 1 to 5. 33 phytoplankton NIS were observed in the entire data set (*table 6.1*) and an additional six NIS documented outside the monitoring programme were found (*table 6.2*).

2.2.2 Zooplankton data

Mnemiopsis leidyi/ warty comb jelly.
Photo: Hans Ulrik Riisgaard©

Zooplankton are identified and counted using a dissection microscope following a standard protocol (Nielsen & Møhlenberg 2004). Counts were quantified into number of individuals per litre. *Figure 2.*2 shows the total distribution of stations sampled within each of the investigated five regions. A total of 35 stations was analysed with 13, 2, 7 and 13 stations, respectively, in regions 2 to 5. A total of 250 “species” was recorded in the entire data set, with 212, 103, 150 and 155 species from regions 2 to 5. Some of the stations were only visited a few times, and some were only “viable” for a few years. Typically, the stations were visited bimonthly. Only two zooplankton NIS were observed in the entire data set (*table 6.1*) while one other species (*Mnemiopsis leidyi*) was recorded outside the monitoring programme (*table 6.2*).

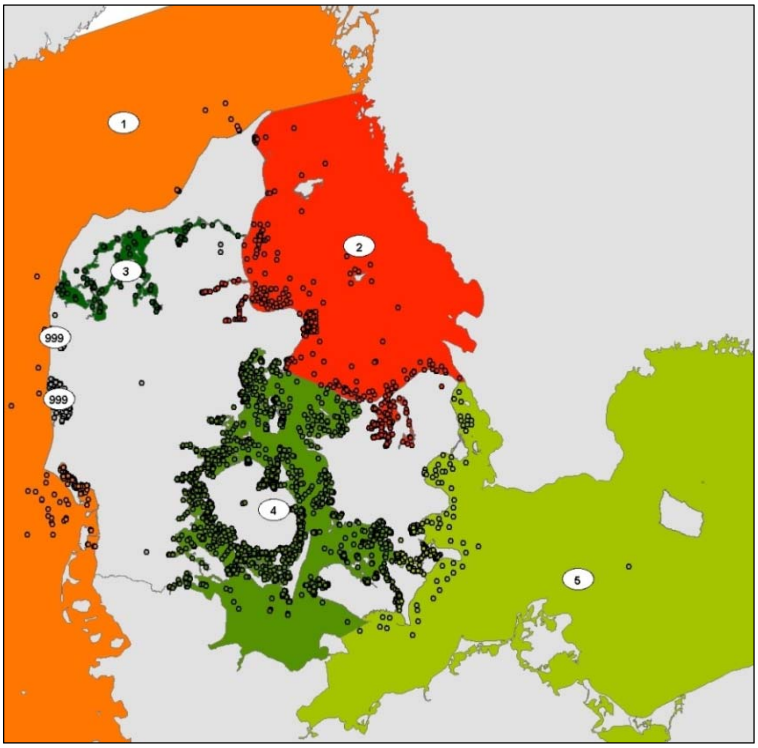
Figure 2.2. Map of zooplankton stations (dots) sampled in Danish waters from 1989 to 2014. Region 1 = the North Sea and Skagerrak, Region 2 = the Kattegat, Region 3 = Limfjorden, Region 4 = the Belt Sea, and Region 5 = the western Baltic Sea. Regions 999 were not included in the data analysis.



2.2.3 Benthic invertebrate data

Benthic invertebrate data cover 258 soft bottom fauna sampling locations. Soft bottom fauna was recorded as number of individuals per species. *Figure 2.3* shows the total distribution of stations sampled within each of the investigated five regions. For the period 1989 to 2014, a total of 258 fauna stations was analysed with 23, 38, 27, 149 and 21 stations, respectively, in regions 1 to 5. A total of 1304 benthic invertebrate species was recorded in the 1989 to 2014 period, with 525, 853, 465, 939 and 452 species from regions 1 to 5. A total of 16 benthic invertebrate NIS was observed in the entire data set (*table 6.1*) and an additional 6 species were recorded outside the monitoring programme (*table 6.2*).

Figure 2.3. Map of benthic soft bottom invertebrate sampling areas (dots) in the Danish waters from 1989 to 2014. Region 1 = the North Sea and Skagerrak, Region 2 = the Kattegat, Region 3 = Limfjorden, Region 4 = the Belt Sea, and Region 5 = the western Baltic Sea. Regions 999 were not included in the data analysis.



2.2.4 Macroalgal data

Macroalgal data were recorded by divers for each species as percent cover of suitable substrate in different depth intervals. *Figure 2.4* shows the location of stations sampled within each of the investigated five regions. A total of 898 sampling stations was monitored from 1989 to 2014 with a total of 29, 151, 68, 563 and 99 stations, respectively, in regions 1 to 5. A total of 327 species was recorded in the entire data set, with 57, 299, 141, 253 and 140 species from regions 1 to 5. Most stations in region 1 were located in The Wadden Sea area. Ten macroalgal NIS were observed in the entire data set (*table 6.1*) with an additional two species recorded outside the monitoring programme (*table 6.2*).

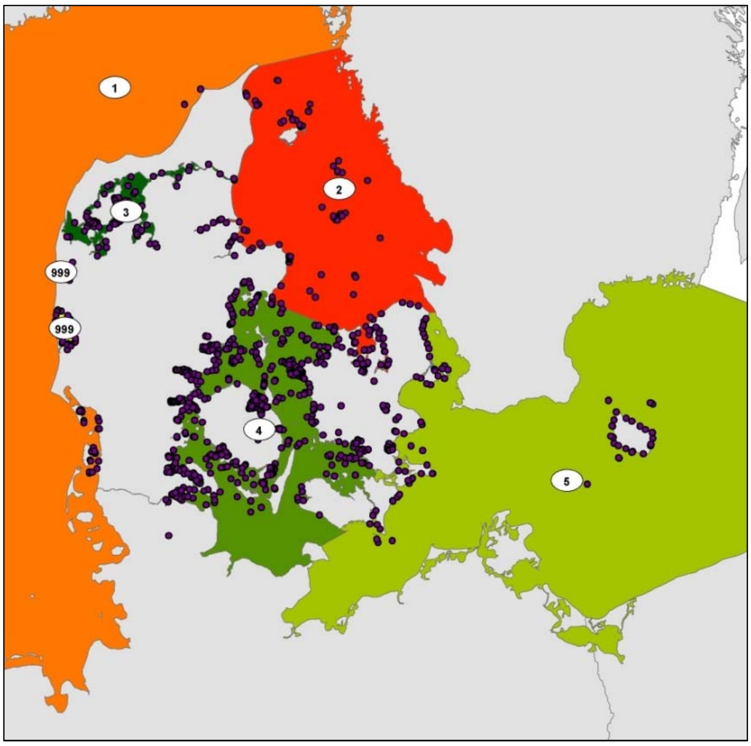


Figure 2.4. Map of macroalgal sampling stations (dots) utilized in the NIS analysis covering data from 1989 to 2014. Region 1 = the North Sea and Skagerrak, Region 2 =the Kattegat, Region 3 = Limfjorden, Region 4 = the Belt Sea, and Region 5 = the western Baltic Sea. Regions 999 were not included in the data analysis.

2.2.5 Fish data

DTU Aqua provided data from 6 annual surveys conducted in the waters around Denmark. Two surveys a year are conducted in the North Sea in the first and third quarter with the larger vessel DANA. Another two surveys a year are conducted in the Kattegat in the first and fourth quarter with "Havfisken" and finally two surveys per year are conducted in the eastern Baltic Sea during the first and fourth quarter with DANA. The main purpose of the surveys is to estimate abundance of commercial and non-commercial fish species by means of bottom trawling. The surveys are using slightly different trawls, but all of the trawls are designed to target juvenile fish at the bottom. The Kattegat and Baltic surveys are both using a TV3-trawl with a 20 mm cod-end, however, scaled in two different sizes to fit the different vessels. The survey conducted in the North Sea is using a different bottom trawl (a GOV), however, the mesh size is similar with 20 mm in the cod-end. Fish abundance for each species is recorded as number of fish per trawled hour. Data span the period from 1991 to 2014. *Figure 2.5* shows the total distribution of trawls conducted within each of the investigated five regions. A total of 3661 trawl stations was analysed with 542, 1036, 615 and 1468 stations, respectively, in regions 1, 2, 4 and 5. These surveys do not cover region 3 = Limfjorden.

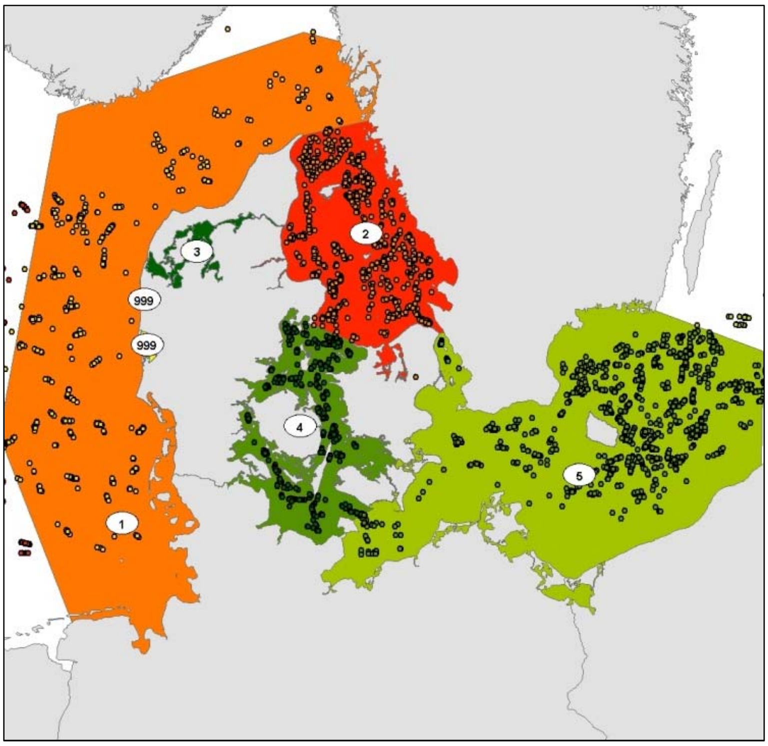


Figure 2.5. Map of fish survey trawls (dots) used in the analysis covering data from 1991 to 2014. Region 1 = the North Sea and Skagerrak, Region 2 = the Kattegat, Region 3 = Limfjorden, Region 4 = the Belt Sea, and Region 5 = the western Baltic Sea. Regions 999 were not included in the data analysis.

2.2.6 Environmental data

We extracted data from DNAMAP for total nitrogen (TN), chlorophyll *a* (Chl), primary production (PP), Secchi depth, and water column integrated values of temperature and salinity. A total of 355 stations was analysed with 60, 54, 35, 134 and 50 stations, respectively, in regions 1 to 5 (*figure 2.6*). The number of sampling stations has decreased over time, especially in region 1 following reductions in the monitoring programme in 2007.

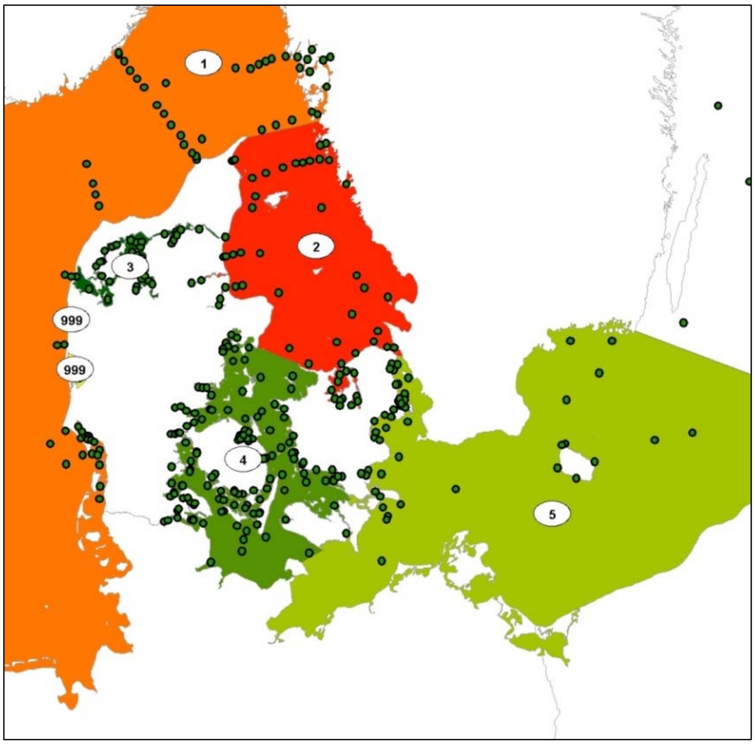


Figure 2.6. Map of sampling stations for environmental variables (dots) used in the analysis covering data from 1989 to 2014. Region 1 = the North Sea and Skagerrak, Region 2 = the Kattegat, Region 3 = Limfjorden, Region 4 = the Belt Sea, and Region 5 = the western Baltic Sea. Regions 999 were not included in the data analysis.

2.3 Data analysis

2.3.1 Time series data analysis

We used the monitoring data to produce yearly and regionally weighted estimates of species presence and abundance and environmental (nutrients, temperature, salinity, Chl, primary production, Secchi depth) data. For species data, the data analysis was carried out in a 3 step procedure: 1) selection of species data from databases, 2) quality control – names, coordinates, station numbers, obvious outlines, and 3) estimation of spatially aggregated data across regions for each year.

Due to unbalanced sampling in time and space, we used a general linear model (GLM) to estimate the marginal means of each variable in each basin on a seasonal and annual scale. The GLM approach was used on both species composition data and water quality monitoring data to produce annual time series within each region.

2.3.2 Analysis of fish data

All six surveys were carried out according to international standards, indicating that similar equipment and materials were used, same trawl speed (3 knots), same haul duration and same procedure to work up the samples. Our analysis focused on the relative abundances of NIS vs. native communities, and all sampled species from this survey were therefore required to compile a total species-abundance list. All species in the surveys were compared to the MONIS target species list. Data on weight and number were aggregated with respect to survey year, month, station, species, longitude and latitude and haul duration. Information in databases about non-living objects (e.g., litter and garbage) was deleted from the data set. All hauls were conducted as approx. 30 min. tows, and the abundance was recalculated as numbers per 1 hour tow. In a few cases, data on haul duration were missing from the database but was assumed to be 30 min.

2.3.3 Community analysis of NIS

The species composition of communities of phytoplankton, zooplankton, macroalgae and benthic invertebrates was analysed using the software package PRIMER (Clarke & Gorley 2015). Data on fish communities were not analysed due to absence of NIS. Analysis of the community composition across the global data set was based on Bray-Curtis similarity of presence/absence of data grouped by region and sampling year. Presence/absence transformation was chosen to reduce problems associated with changes in sampling technique over time. The similarity among species communities was also quantified in terms of Bray-Curtis similarity using the routine "RESEMBLANCE" and the contribution of NIS to total community similarity within regions and time periods was analysed using the routine "SIMPER".

2.3.4 Analysis of environmental data

We used Spearman Rank correlation analysis to investigate relationships between %NIS contribution to species numbers (presence/absence data) and trends in key environmental variables expected to influence the distribution of marine species. To enable comparison among regions, the time trends in each variable were first normalized to the mean value in each region over the period 1989 to 2014. To reduce the probability of analysing irrelevant spurious correlations, e.g. caused by unmeasured environmental drivers or co-varying drivers, we performed the analysis on detrended time data. The time detrending was done by performing a simple linear regression model of each variable as a function of time (years). The regression coefficients (slope and intercept) were then used to estimate each environmental variable as a function of time (years) and these estimates were then subtracted from the original values. As a result of the time detrending, none of the time detrended variables (%NIS, TN, Chl, primary production, Secchi depth, water temperature and salinity) had any correlation with time (years). This should allow us to exclude the time effect from relations between NIS abundance and environmental variables.

The PRIMER program BEST (Clarke & Gorley 2015) was used to calculate which set of environmental factors was best correlated with the structure of the phytoplankton, zooplankton, macroalgae and benthic invertebrate communities. For each possible combination of environmental factors, a dissimilarity matrix based on normalized Euclidean distances was calculated. The correlation between the biotic and environmental matrices was calculated using Spearman rank coefficient.

3 Results and discussion

3.1 Overall changes in NIS numbers in Danish waters

Since 1989, the number of NIS recorded in the marine national monitoring has almost doubled from 37 in 1989 to 61 in 2014 (*figure 3.1* and supplementary material, *table 6.1*). A linear regression of accumulated NIS gives a slope of 1.1, i.e. 11 new marine NIS have been recorded per decade in Danish waters. Most of this increase has occurred within the phytoplankton (slope = 0.55), followed by benthic invertebrates (slope = 0.23), macroalgae (slope = 0.14), fish (slope = 0.05) and zooplankton (slope = 0.03).

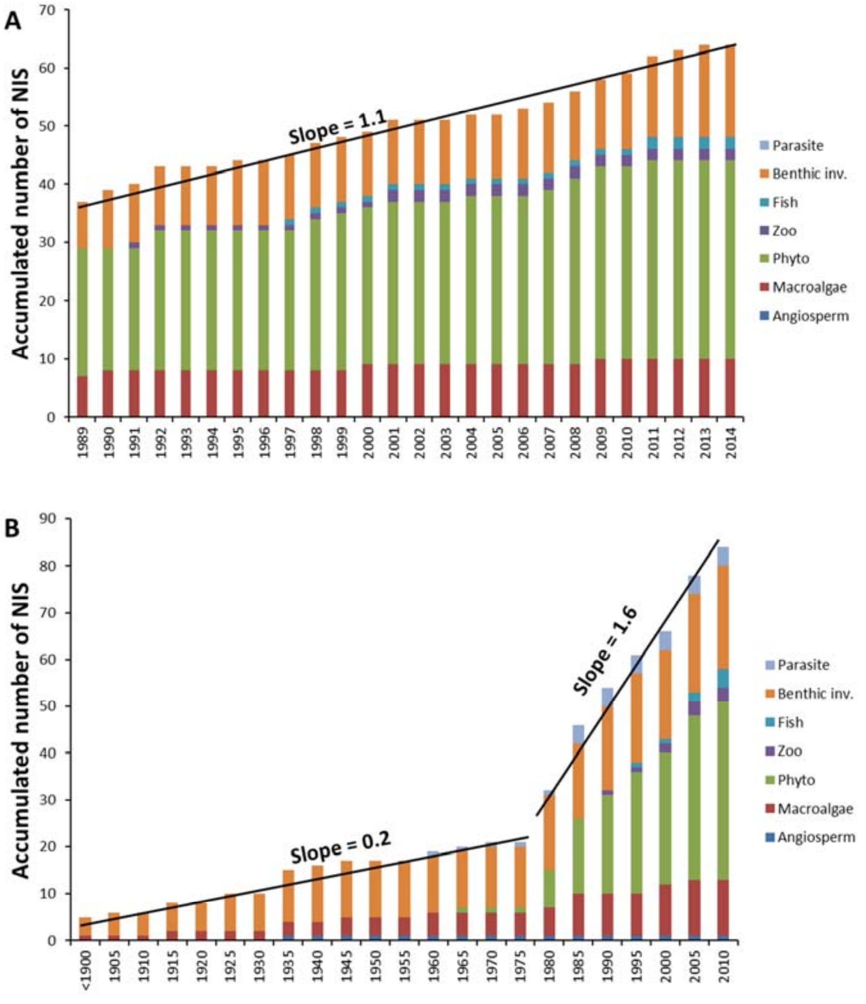


Figure 3.1. Accumulated number of NIS according to A) the Danish marine surveys since 1989 (*table 6.1*) and B) accumulated NIS including documented records outside the monitoring programmes (see *table 6.1* and *6.2*). Once the observation of a species has been confirmed, it is assumed that the species remains part of the Danish biota regardless of lack of observations in some of the following years. For figure 3.1B, the first document year of arrival was used for all species.

In addition to the accumulated number of NIS shown in *figure 3.1A* and *table 6.1*, another 22 marine NIS have been recorded outside the national monitoring programme (*figure 3.1B* and *table 6.2*). These NIS include one angiosperm, two macroalgae, six phytoplankton, one zooplankton, six benthic invertebrates, four invertebrate parasites and two fish species. Six of these species were recorded before 1989. In total there were 40 recorded NIS before 1989 – this number more than doubled to 86 in 2014, mostly as a result of a huge increase in phytoplankton NIS. Note that some of the phytoplankton species may already be included in the monitoring programme by their genus names as for example may be the case for *Pseudochatonella verruculosa* as *Pseudochatonella sp*. Looking at the long-term changes in the accumulated number of marine NIS in Danish waters (*figure 3.1B*) suggests a slow gradual increase until around 1980 of two species per decade. After this, the rate of NIS introductions was around 16 species per decade, when including data on NIS observed outside the national monitoring programmes. While the accumulated number of NIS is 85, the actual number of NIS recorded by the monitoring programme on an annual basis has remained below 30 in all five regions (*figure 3.2*). A given NIS is therefore not necessarily recorded every year after its first observation. The accumulated number of NIS (currently 85) represents NIS which are likely to be found in the Danish waters.

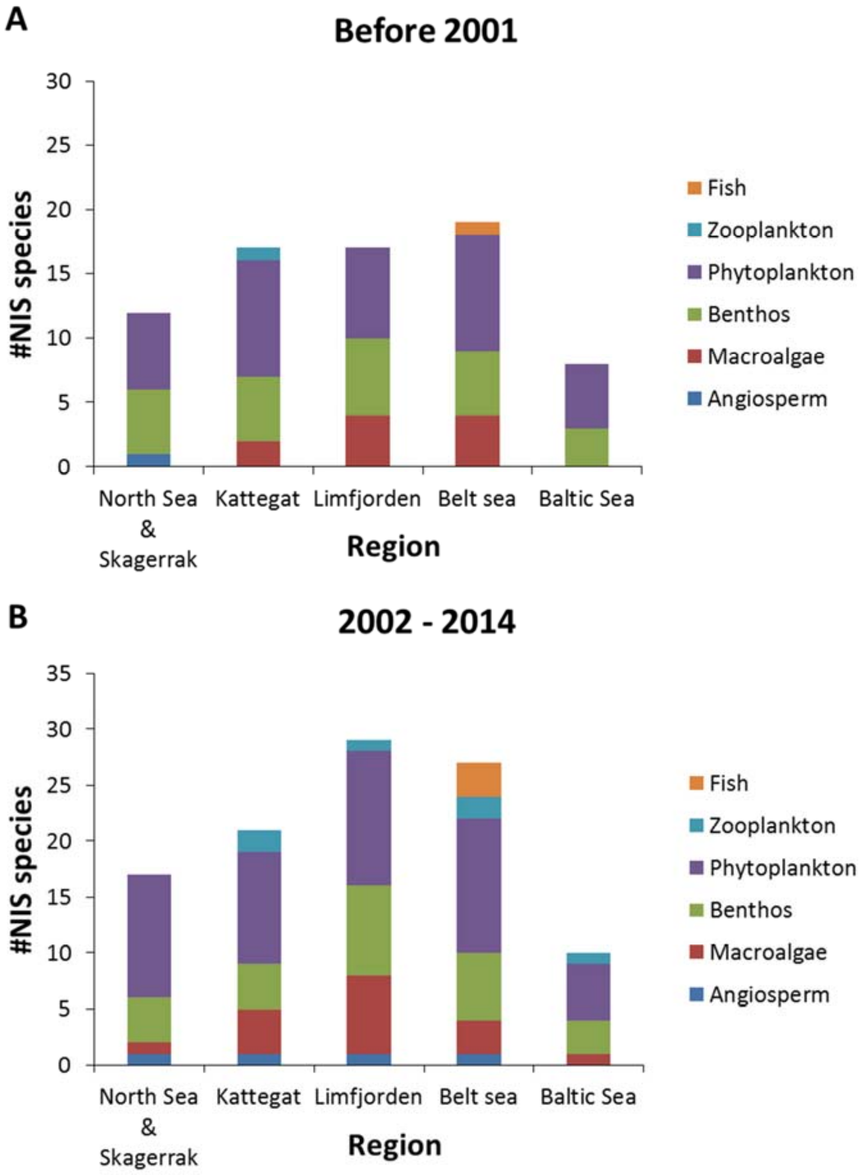


Figure 3.2. Average number of observed marine NIS in five Danish regions for two periods: A) before 2001 and B) 2002-2014. Data include the information on 85 NIS recorded in Danish monitoring databases and other documented records. Parasite NIS are not included due to lack of geographical information.

Of the 85 marine NIS recorded in Danish waters (*figure 3.1B*), most are phytoplankton (45 %), followed by benthic invertebrates (26 %), macroalgae (14 %), fish (5 %), parasites (5 %), zooplankton (4 %) and flowering plants (1 %). Information about the geographical distribution of parasitic NIS was not available and therefore not included in *figure 3.2*. A list of the NIS observed in the monitoring programmes with notes on their origin, time of arrival and relative importance is shown in the supplementary material (*table 6.1*). *Table 6.2* provides information on NIS recorded outside the monitoring programmes. We have no time series data for NIS recorded outside the monitoring programmes, and these species (*table 6.2*) were therefore excluded from our analysis of changes to community structures.

3.2 Temporal trends in number and relative abundance of marine NIS in five Danish regions

3.2.1 Phytoplankton

A total of 33 phytoplankton NIS was listed in the monitoring database in 2014, 14 species were recorded prior to 1989 (*table 6.1* and *6.2*). The ten most frequently observed phytoplankton NIS (Rank P / A) overlap with five of the highest ranking NIS according to average biomasses (Rank abund) (*table 6.1*). Of the ten highest ranking NIS, six are dinoflagellates, two flagellates and two diatoms. Importantly, six out of the ten most abundant species are also classified as HAB species (that can form 'Harmful Algal Blooms'). The fact that the two species which dominate $\%NIS_{abundance}$ (*Phaeocystis pouchetii* and *Lepidodinium chlorophorum*) are not among the ten highest ranking based on $\%NIS_{records}$, indicates that these species are found in extreme biomasses during ephemeral blooms.

The tendency to increased numbers of NIS observed during the monitoring period based upon the total data set covering all monitoring stations is also observed when the phytoplankton communities are analysed on a regional basis (*figure 3.3A*).

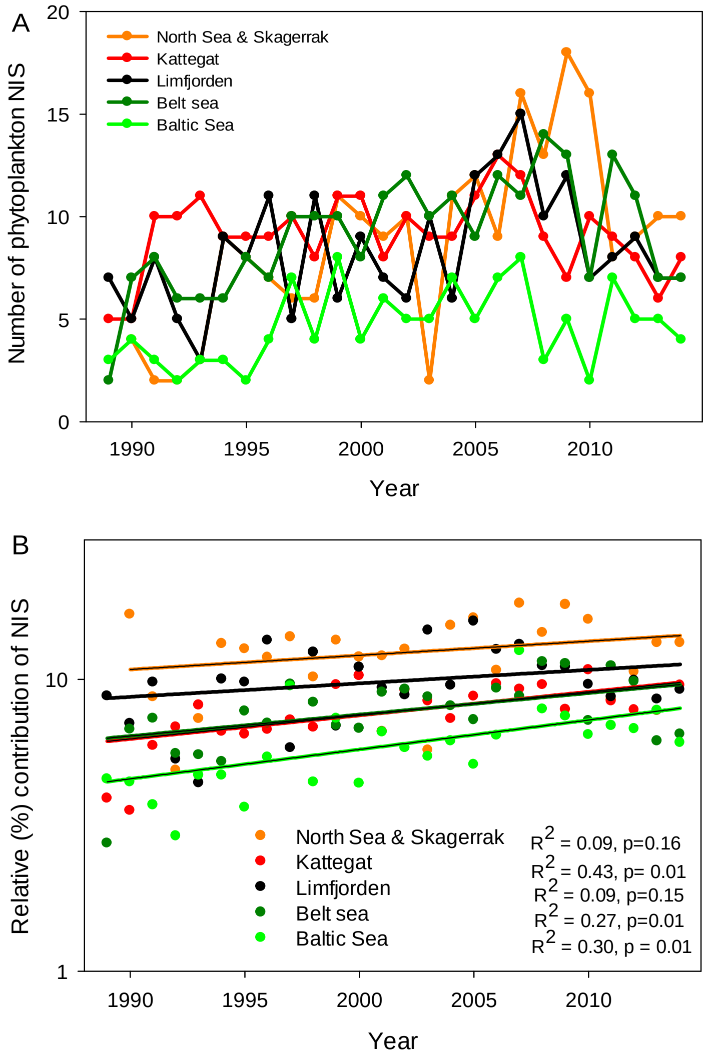


Figure 3.3. A) Number of phytoplankton NIS observed and B) their relative contribution to the total phytoplankton species number based on presence/absence data. Note that the y-axis in fig. 3.3B is log-transformed as increases in %NIS were exponential in most regions.

The highest average number of NIS (eight) as well as the highest relative occurrence of $\%NIS_{records}$ (12.4 %) during the period 1989-2014 was observed in the high saline North Sea / Skagerrak region.

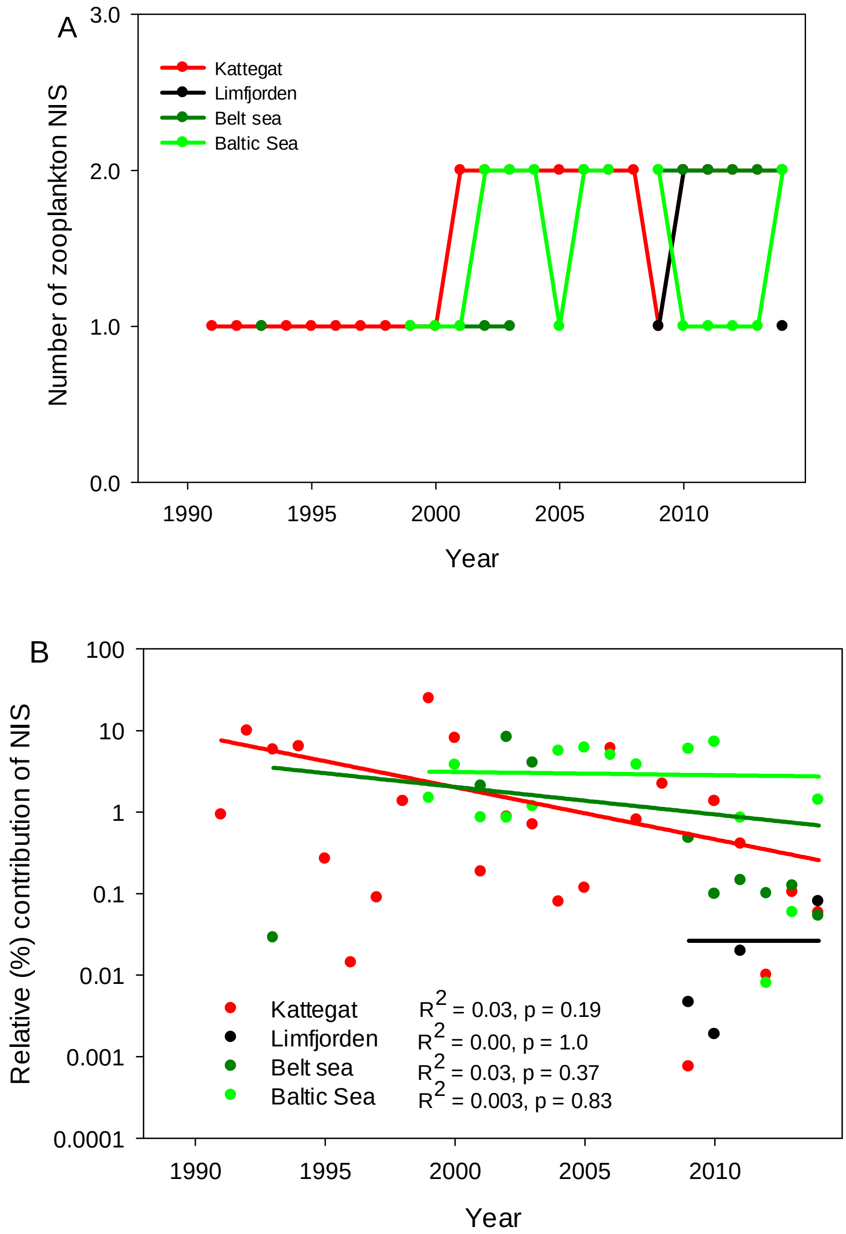
The observed increase in $\%NIS_{records}$ was 0.14-0.21 % per year considering all regions. The highest rates of increase in relative abundance of NIS were observed in the Kattegat and Belt Sea regions with 0.21 and 0.20 % per year, respectively (*figure 3.3B*).

For all regions except the Belt Sea, the biomass of phytoplankton NIS as well as the relative importance of NIS to the total phytoplankton C-biomass ($\%NIS_{abundance}$) decreased during the monitoring period (data not shown). The species responsible for the increase in C-biomass level in the Belt Sea are dictyophycean flagellates from the genus *Pseudochattonella (*which produces fish toxins), which can bloom during late winter and early spring and the diatom *Rhizosolenia calcar-avis* which mainly occurs during summer. Both species are recorded for the first time in the second "decade" (2002-2014) of the monitoring period. The quantitative occurrences of high biomass bloomers such as the toxic dinophycean *Karenia mikimotoi* (syn: *Gyrodinium aureolum*) are markedly reduced in the second period which suggest a coupling with the observed reduction in nutrient load of the Danish coastal waters (Riemann et al. 2016).

3.2.2 Zooplankton

The temporal changes in zooplankton NIS number and their relative contribution to the total zooplankton species number in the five regions are shown in *figure 3.4*. Only two zooplankton NIS were recorded in the database since 1989 (the calanoid copepod *Acartia tonsa* and the marine daphnia *Penilia avirostris*) with highest frequency in the Kattegat region, where their relative contribution tended to decrease over time. However, none of the trends were significant (see *figure 3.4B*).

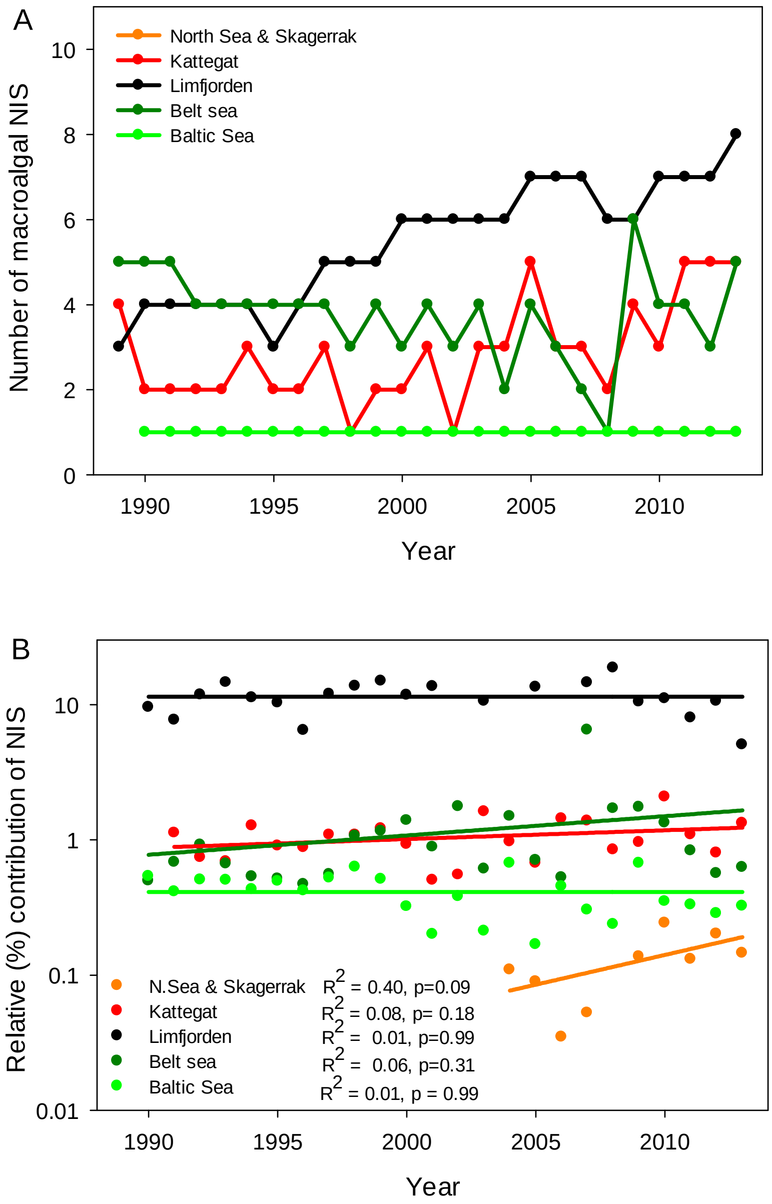
Figure 3.4. A) Number of zooplankton NIS observed and B) their relative contribution to the total zooplankton species number based on presence/absence data.



3.2.3 Macroalgae

The temporal changes in macroalgal NIS number and their relative contribution to the total macroalgal species number in the five regions are shown in *figure 3.5*. Of the ten NIS recorded in the monitoring programme, 8 were observed in the Limfjorden, which is the only region where there has been observed an increase in NIS numbers since 1989. Based on presence/absence data, none of the regions showed significant changes in the relative contribution of NIS to total species number (*figure 3.5B*), most of which were related to increasing cover of the invasive *Sargassum muticum* (Stæhr et al. 2000). Evaluating the relative quantitative contribution based on %cover, however, shows a significant increase in the Limfjorden, where %NIS increased significantly from around 7 % in 1989 to 23 % in 2014. Small but significant increases in %NIS were similarly observed in the North Sea (from 0 to 1 %) and the Kattegat region (from 0.1 to 0.3 %).

Figure 3.5. A) Number of macroalgal NIS observed, B) their relative contribution to the total macroalgal species number based on presence/absence data for monitoring data collected from 1989 to 2014.

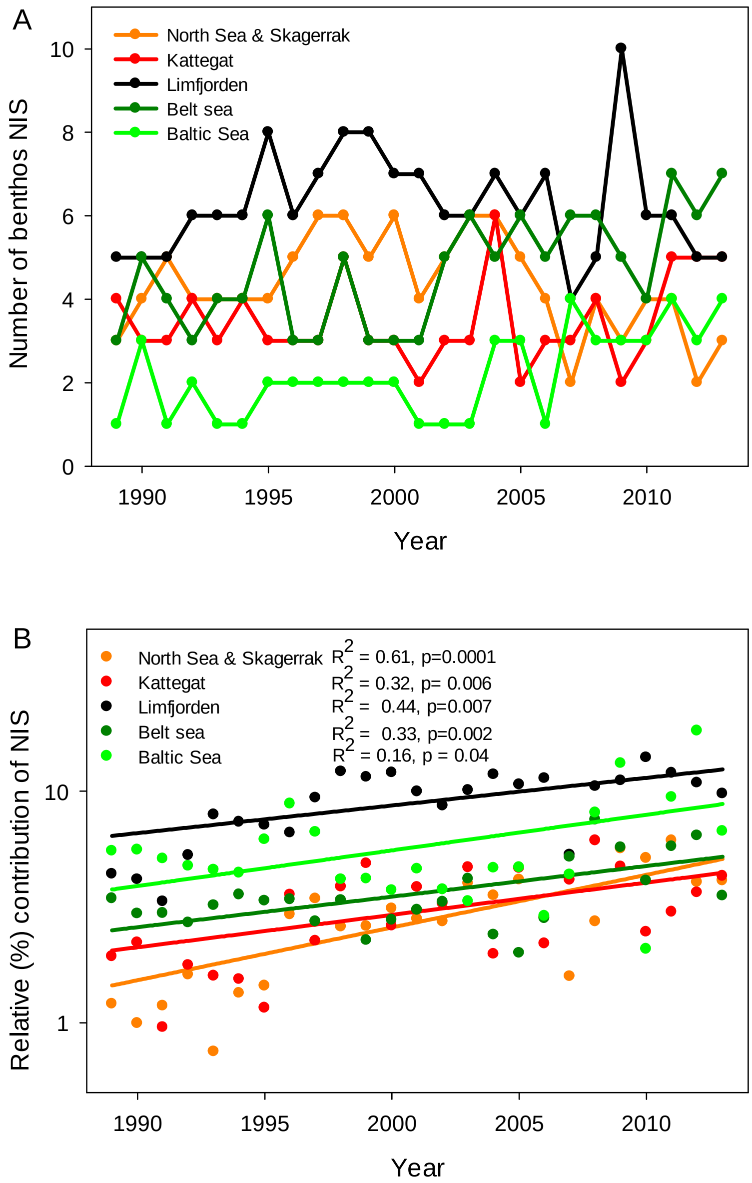


3.2.4 Benthic invertebrates

The occurrence of benthic invertebrate NIS and their relative contribution to the total species number (%NIS$_{records}$) in the five regions are shown in *figure 3.6*. Of the 16 NIS recorded in the monitoring programme, most species were observed in the Limfjorden and the Belt Sea. Although no clear trend was observed in the number of benthic invertebrate NIS in the five regions, a significant increase in %NIS$_{records}$ can be observed in all regions (*figure 3.6B*). The largest current (2014) NIS contribution was recorded in the Limfjorden (10 %), followed by the western Baltic Sea (6 %) with the other three regions having 4-5 %NIS contribution to the total benthic invertebrate species number. An increase in %NIS was similarly found when evaluating the relative NIS contribution from abundance data (%NIS$_{abundance}$ calculated as number of NIS individuals relative to total species individuals). The trend was clearest when the abundant species *Mya arenaria* was excluded from the analysis NIS, indicating that this species which was introduced more than 800 years ago, have habituated to the Danish marine systems long before monitoring commenced. Excluding *Mya arenaria* showed that %NIS contribution in recent years was highest in Limfjorden (5 %), followed by the western Baltic

Sea (4 %), the Belt Sea (2 %) and with less than 1 % in the North Sea / Skagerrak and Kattegat regions. Analysis of data including *Mya arenaria* elevated the NIS contribution by 1 % in Limfjorden and the Belt Sea and 3 % in the western Baltic region.

Figure 3.6. A) Number of benthic invertebrate NIS and B) their relative contribution to the total benthic invertebrate species number based on presence/absence data.



3.2.5 Fish

A total of 140 species was recorded in the entire data set with 112, 104, 79 and 60 species from region 1, 2, 4 and 5, respectively. Only one recording of a non-indigenous species was made in the entire data set. This was of *Neogobius melanostomus* in the Belt Sea during one cruise in 2011 where two fish were caught per hour of trawling. Search for other non-indigenous fish species in the DTU Aqua databases from other fish surveys, revealed the presence of *Oncorhynchus mykiss* (rainbow trout) in the Belt Sea, near Aarhus Bay in 1997 with further recordings until 2009. *Oncorhynchus mykiss* is grown in marine aquaculture in the Belt Sea region and the origin of the fish observed in the DTU surveys is most likely escapes from the aquaculture sites. At present there are no indications of a self-reproducing wild population of *Oncorhynchus mykiss* in Danish marine waters (Mellergaard et al. 2002).

The areas investigated by DTU Aqua in the Baltic, Kattegat and North Sea surveys are mainly covering depth strata from 20-120 metres where the main part of the commercially important species are inhabited. This indicates that the survey was not conducted close to land but at the deeper areas of the Danish waters. As the trawl was very fine meshed (20 mm in cod end), the gear was not well suited for rough grounds as stone reefs, etc. The main trawling hauls were therefore conducted on sand bottom. If the NIS are mainly habituated at shallow waters or on more rough bottom, these species would not be caught at the allocated stations. The species *Neogobius melanostomus* is known to be located in shallow waters and it is therefore not surprising that this species is not caught regularly in the deeper water. However, if other NIS were habituated in the deeper water at the sand bottom, we would expect the trawl to catch the species and that the scientist participating in the survey to be qualified to do the correct species identification.

3.3 Impact of NIS on community structure over the last three decades

3.3.1 Phytoplankton

An ordination plot based on non-parametric multi dimension scaling (nMDS) of data from the sampling period 1989-2014 showed that observations grouped together region by region had different communities in all regions (*figure 3.7*). However, data from the North Sea and from the western Baltic Sea varied more than observed for the Kattegat, the Belt Sea and in the Limfjorden. The similarity between regions followed the overall salinity pattern in such a way that the North Sea phytoplankton community was most similar to the communities in the Kattegat and the Limfjorden which, in turn, were most similar to the Belt Sea communities. The Belt Sea and the Kattegat communities were overall the most similar communities reflecting that these regions more or less share the same body of surface water (Bendtsen et al. 2009; Hansen & Bendtsen 2013). In contrast, the western Baltic Sea and the North Sea shared the lowest community similarity (*figure 3.7)*. Clustering within regions was not strongly related to the relative abundance of non-indigenous species (NIS) which generally accounted for less than 20 percent of the phytoplankton records.

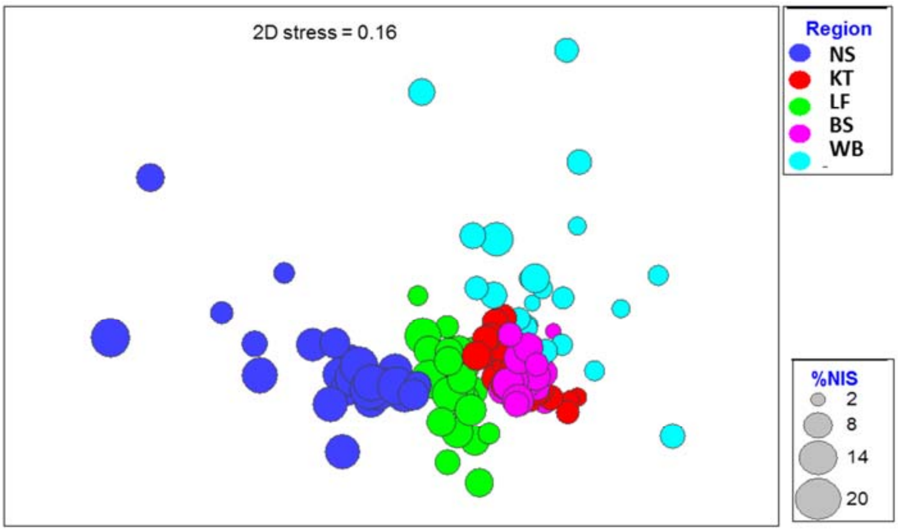
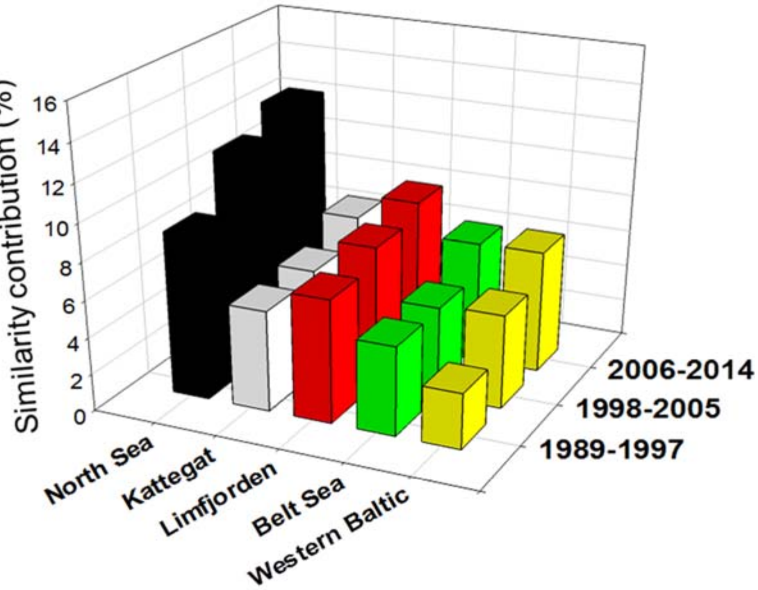


Figure 3.7. nMDS plot of phytoplankton community data (presence/absence) from Danish waters 1989-2014 based on Bray-Curtis similarity of presence/absence data. Points represent data aggregated region by region and year by year: Symbol sizes scaled to % NIS contribution to species number. NS: North Sea, KT: Kattegat, LF: Limfjorden, BS: Belt Sea and WB: Western Baltic.

A SIMPER analysis (Clarke 1993) showed that the group of non-indigenous species (NIS) accounted for less than 13 percent of the within region similarity (*figure 3.8*). When the analysis was applied to each of the periods 1989-1997, 1998-2005 and 2006-2014, a consistent pattern emerged showing that NIS contributed increasingly to the community similarity through the three consecutive periods in all five regions. NIS contributed most to the similarity in the North Sea samples and least in the western Baltic Sea. NIS contribution to the diversity (gamma-diversity) followed the same pattern with a gradual increase during 1989-2014 (data not shown). The community analysis showed that NIS-phytoplankton generally had become more abundant and that NIS were increasingly characterizing the phytoplankton communities during the monitoring programmes going from 1989 to 2014. This indicates that NIS also have become more abundant *in situ*.

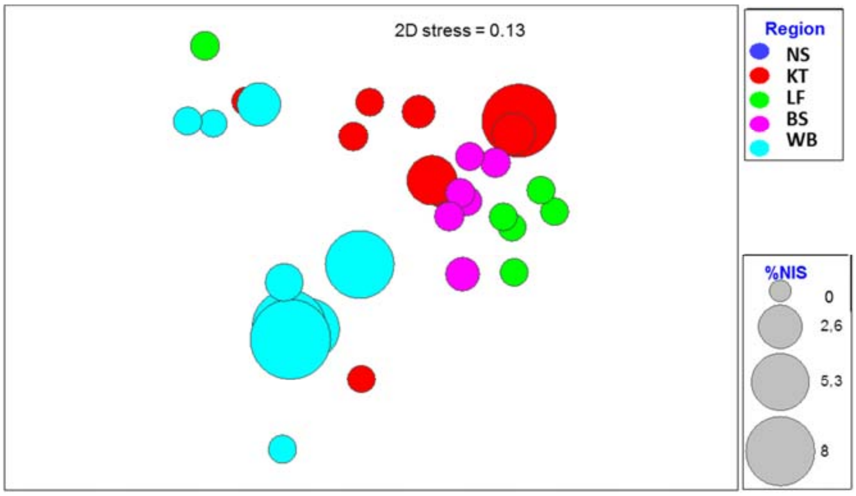
Figure 3.8. Contribution of phytoplankton-NIS to total community similarity within regions for each of the three periods 1989-1997 (period 1), 1998-2005 (period 2) and 2006-2014 (period 3).



3.3.2 Zooplankton

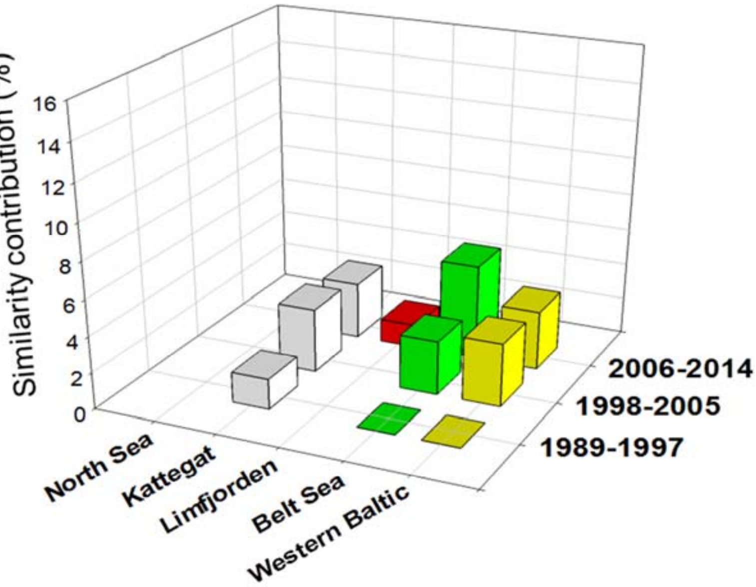
Zooplankton data from the four regions: the Kattegat, the Limfjorden, the Belt Sea and the western Baltic Sea were analysed as described for macroalgal and phytoplankton data (*figure 3.9*). There exist no comparable monitoring data from the North Sea area. nMDS ordination of the similarity data showed a regional grouping. However, the regional communities did not segregate as distinct as the phytoplankton and macroalgal communities did. The regional clustering was furthermore not clearly related to the abundance of NIS-records (%NIS).

Figure 3.9. nMDS plot of zooplankton community data (presence/absence) from Danish waters 1989-2014 based on Bray-Curtis similarity of presence/absence data. Points represent data aggregated region by region and year by year: Symbol sizes scaled to % NIS contribution to species number. NS: North Sea, KT: Kattegat, LF: Limfjorden, BS: Belt Sea and WB: western Baltic. No data were available for the North Sea region.



The SIMPER analysis showed that NIS generally explained less than 6 % of the total within community similarity and only a weak tendency to increase their importance during the observation period (*figure 3.10*).

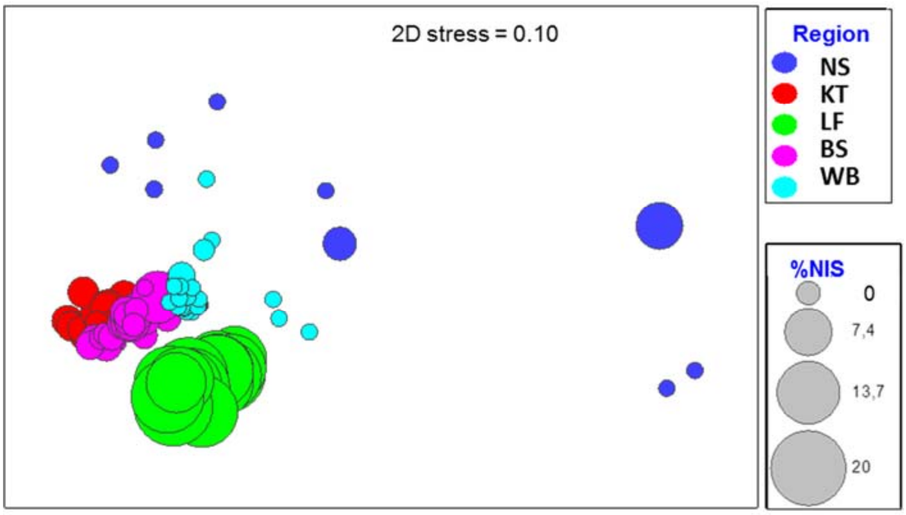
Figure 3.10. Contribution of zooplankton NIS to total community similarity within regions for each of the three periods 1989-1997 (period 1), 1998-2005, (period 2) and 2006-2014 (period 3).



3.3.3 Macroalgae

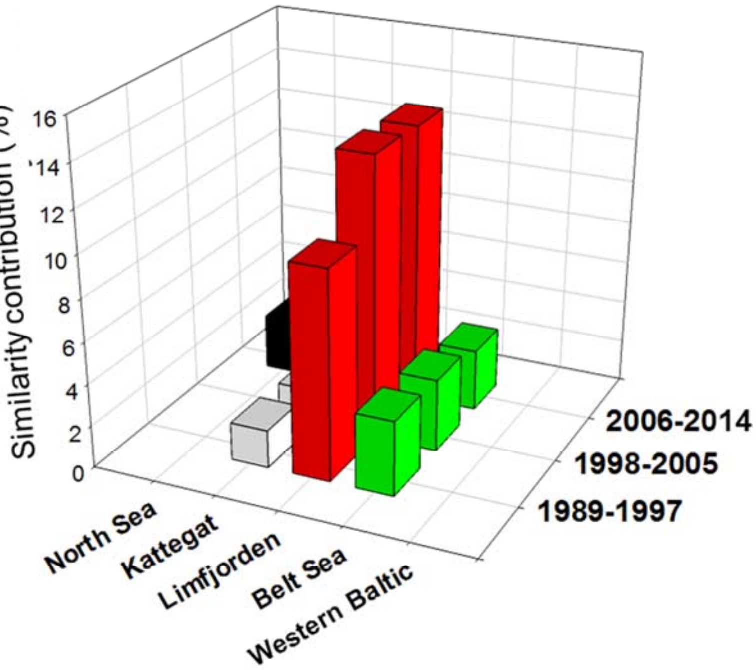
The community composition of the macroalgal communities showed the same overall clustering as was seen for the phyto- and zooplankton communities. Non-indigenous macroalgae (NIS) were reported in all five regions, but with very low contribution in the North Sea & Skagerrak and western Baltic Sea regions (*figure 3.11*). The communities from each of the regions were different from each another. However, a low number of sampling station in the North Sea and from the western Baltic Sea caused these communities to be considerably more scattered than the Kattegat, the Belt Sea and in the Limfjorden. Nevertheless the clustering of community data followed the overall salinity pattern such that the North Sea macroalgal community was most similar to the communities in the Kattegat and Limfjorden.

Figure 3.11. nMDS plot of macroalgal community data (presence/absence) from Danish waters 1989-2014 based on Bray-Curtis similarity of presence/absence data. Points represent data aggregated region by region and year by year: Symbol sizes scaled to % NIS contribution to species number. NS: North Sea, KT: Kattegat, LF: Limfjorden, BS: Belt Sea and WB: western Baltic.



The SIMPER analysis showed that macroalgal NIS were only important for the community composition in Limfjorden where an increase in their importance was observed during 1989-2015 but no overall trends were observed (*figure 3.12*).

Figure 3.12. Contribution of macroalgal NIS to total community similarity within regions for each of the three periods 1989-1997 (period 1), 1998-2005 (period 2) and 2006-2014 (period 3).



3.3.4 Benthic invertebrates

The bottom fauna is covered by two data sets: 1) data set covering 22 stations in the open parts of the inner Danish waters and 2) a larger data set covering Danish fjords and coastal regions. The data from the open waters are balanced with respect to sampling season and spatial coverage and cover a fairly homogeneous habitat. Data from the Danish fjords and coastal areas are highly heterogeneous. However, as the community analysis was based on presence/absence data this enables a direct comparison of community structure across the data sets. In the open water data set, only three species were recorded *Caulleriella killariensis*, *Crepidula fornicata* and *Mya arenaria*. The former two were only recorded twice. Contribution of NIS in this data set to alpha diversity was 0.1 percent across the entire data set from a global average (n = 2627) of 10.15874 to 10.1732 species per sample. Their contribution to the overall Shannon-diversity index showed an even less increasing tendency from 2.593549 to 2.594961. While diversity indices suggest a small impact from NIS, the nMDS analysis of similarities in species composition showed a clear clustering with respect to region (*figure 3.13*).

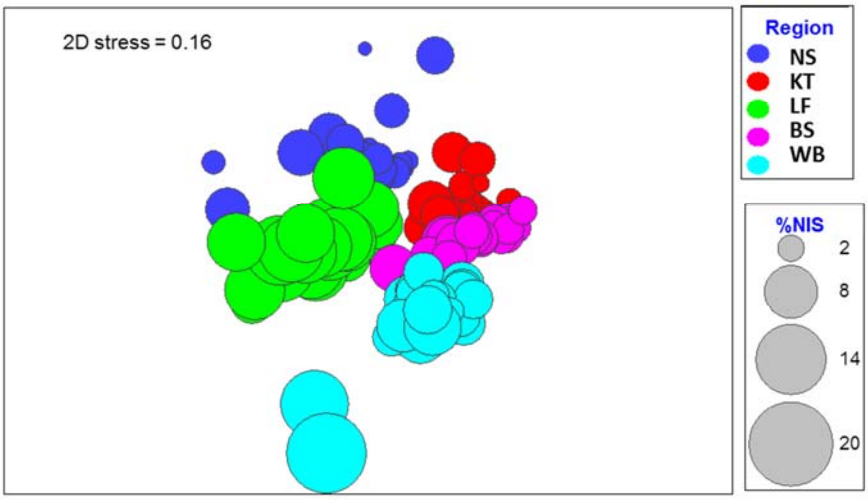
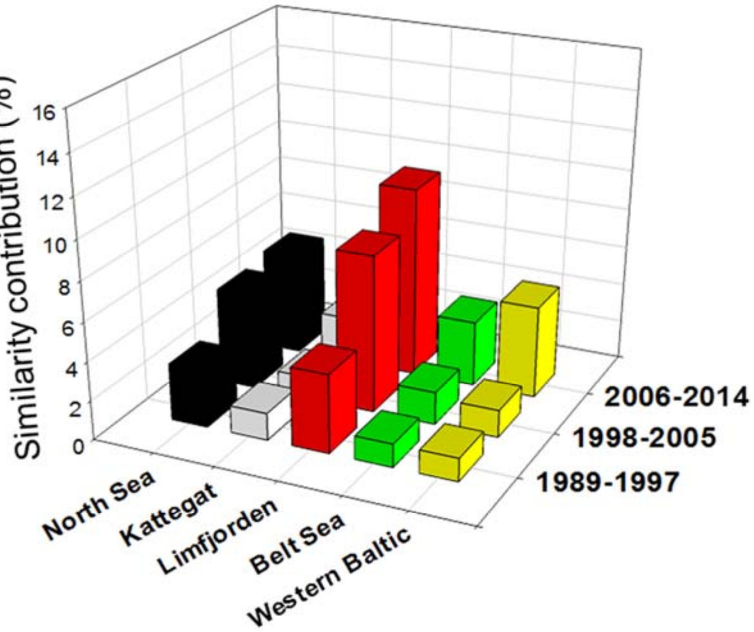


Figure 3.13. nMDS plot of benthic invertebrate community data (presence/absence) from Danish waters 1989-2014 based on Bray-Curtis similarity of presence/absence data. Points represent data aggregated region by region and year by year: Symbol sizes scaled to % NIS contribution to species number. NS: North Sea, KT: Kattegat, LF: Limfjorden, BS: Belt Sea and WB: western Baltic.

The clustering of samples shows the same overall pattern as seen for phytoplankton, zooplankton and macroalgae, which relates to the overall differences in species richness caused by salinity. Also the clustering relates to connectivity of water masses, as the North Sea data are closest to the Kattegat and Limfjorden. The Kattegat and the Belt Sea region are more different than seen in the case of the phytoplankton data which reflects that the stations, due to differences in water depth, are affiliated to two distinct water masses: the bottom water layer and the surface layer. Although the overall clustering was not related to the relative abundance of NIS, the SIMPER analysis showed a consistent pattern with NIS being gradually more and more important for the community composition in all five regions (*figure 3.14*). During the period 1989-1997, NIS could explain about two percent of the community similarity within regions; the same number was about five percent in the latest period 2006-2014.

Figure 3.14. Contribution of benthic invertebrate NIS to total community similarity within regions for each of the three periods 1989-1997 (period 1), 1998-2005 (period 2) and 2006-2014 (period 3).



3.4 Characterization of environmental conditions favouring NIS presence

3.4.1 Correlation analysis

We used correlation analysis of time de-trended data to investigate possible relations between environmental variables and the relative contribution of NIS to total species richness. The use of time de-trended time series allows us to focus on the year-to-year variation rather than a weak trend during the period. This decreases the risk of getting a “false positive” correlation (type I error) due to time trend caused by other factors than those investigated in this study.

The analysis aimed to evaluate if communities strongly influenced by non-indigenous species were characterized by a certain combination of environmental conditions, such as changes in temperature or nutrient status. Performing the analysis separately for each region showed that correlation levels were generally low, non-significant and the importance of the different environmental variables varied more or less randomly from one region to another (data not shown). As an example, the analysis showed that for the soft bottom fauna in the North Sea / Skagerrak, Kattegat and to some extent the Limfjord region, the contribution of NIS (%$NIS_{records}$) tended to be high under conditions with low primary production, TN and water temperature but also high water clarity (SD). The environmental conditions characterizing high NIS presence were, however, the complete opposite in the Belt Sea and western Baltic. Given that there is a shift in the composition of soft bottom NIS with decreasing salinity from the North Sea towards the Baltic Sea, is seems reasonable that the environmental conditions favouring NIS prevalence will vary across the investigated regions.

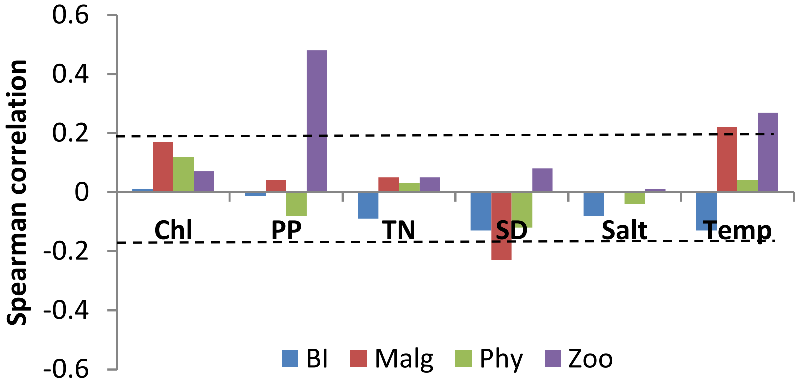


Figure 3.15. Spearman rank correlations between environmental variables and % NIS for all five regions. Correlations outside dashed lines are significant ($p < 0.05$). Environmental variables are: chlorophyll a (Chl), primary production (PP), total nitrogen (TN), Secchi depth (SD), salinity (Salt) and water temperature (Temp). Taxonomic groups are: benthic invertebrates (BI), macroalgae (Malg), phytoplankton (Phy) and zooplankton (Zoo).

Nevertheless, the correlations are very weak and the conditions affecting NIS prevalence cannot easily be interpreted in terms of the selected environmental parameters. Similar unclear and more or less random regional differences appeared for macroalgae, phyto- and zooplankton.

Analysing correlations across all regions (*figure 3.15*) gave similarly weak relationships. There was, however, a tendency for the phytoplankton and macroalgal NIS groups to show an overall increase in prevalence ($\%NIS_{records}$) under high Chl, low water clarity and high temperature conditions, suggesting that there is a higher presence of plant NIS under high productive/eutrophic and warm/summer conditions. A similar pattern was observed for macroalgae and phytoplankton at the sub-regional scale, although some differences appeared among regions. Given that very little zooplankton was available at the regional scale, correlations were all non-significant and tendencies here were difficult to evaluate or non-conclusive. The soft bottom NIS showed no correlation at the large spatial scale.

3.4.2 Multivariate correlation analysis

The degree to which biotic community structure agreed with key environmental parameters and the relative contribution ($\%NIS_{records}$) of non-indigenous species, was further investigated for each taxonomic group using the BEST function in the PRIMER software (*figure 3.16*). The BEST function provides Spearman rank correlation coefficients representing the level of agreement between the biotic matrix for each taxonomic group and corresponding matrices of environmental factors.

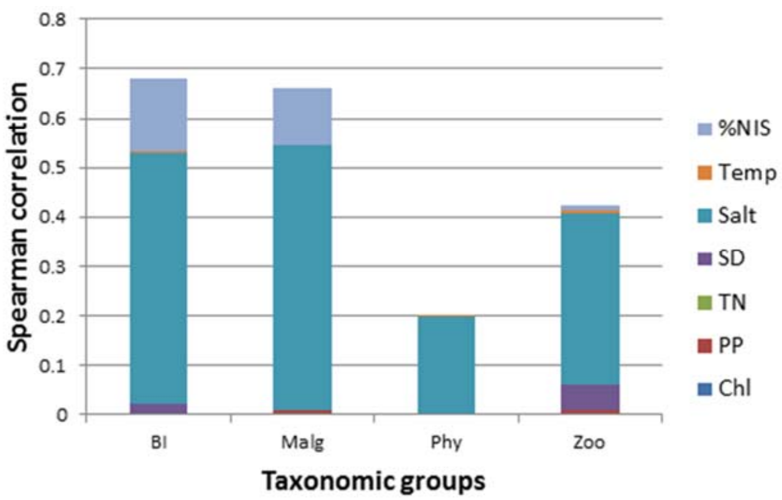


Figure 3.16. The agreement between the biotic matrix for each taxonomic group and corresponding matrices of environmental factors. The agreement is expressed as the Spearman rank correlations. Only variables which contributed significantly ($p < 0.05$) were included. Environmental and taxonomic variables are the same as in *figure 3.15* except that the relative contribution of NIS ($\%NIS_{records}$) was included in the analysis.

Given the significant gradient in salinity of the Danish waters and the fact that our regional aggregation of environmental variables and biotic community data was influenced by this gradient, it was expected that community structure would correlate strongly with salinity. Data on water clarity (SD), primary production (PP) and temperature showed a very small agreement with the community data, whereas total nitrogen showed none. The effect of NIS contribution seemed to be strongest for the benthic groups (benthic invertebrates and macroalgae).

3.5 General trends in the occurrence of NIS

Although the Danish marine monitoring programmes were not established to investigate trends in NIS, the databases enabled us to document significant trends in NIS abundance and contribution to the overall community structure within phyto- and zooplankton, macroalgae and benthic invertebrates.

Our analyses of the monitoring data sets showed that an increase in NIS numbers occurred parallel to an increase in the total number of species and that both increased with the number of samples recorded over time most notably during the first years of sampling. This pattern was most obvious for phytoplankton but also present for benthic invertebrates and macroalgae (*figure 6.1*). However, the relative contribution of NIS ($\%NIS_{records}$) showed no significant dependency on sampling effort when examining the entire period of sampling (data not shown). This is because the majority of the observed new species are not identified as NIS. Accordingly, while sampling effort is likely to have affected the total number of NIS observed, it should not affect the relative NIS abundance (both $\%NIS_{record}$ and $\%NIS_{abundance}$), or the changes in the contribution of NIS to overall community structure.

The quantitative analysis for the 1989 to 2014 period was performed on highly aggregated data (grouped into five large regions). Although GLM modelling was applied to reduce the importance of spatial and temporal heterogeneities in the sampling efforts, it is possible that our results partly are affected by changes to the sampling design (including number of sampled stations and frequency of sampling) from 1989 to 2014. However, since we work on presence / absence data this effect is assumed to be of minor importance for our findings.

Ranking NIS according to number of observations in the total data ($\%NIS_{records}$) gives an indication of how well established the NIS are within the Danish coastal waters. Furthermore, ranking NIS according to percent contribution to the abundance of the total community ($\%NIS_{records}$ based on either C-biomass, number of individuals or percent cover) gives an indication of the potential impact of the NIS (e.g. through competition with other species, effects on other trophic levels, on cycling of carbon and nutrients and oxygen demand).

Based on presence / absence data ($\%NIS_{records}$), we found that NIS generally explained less than ten percent of the annual changes in the community composition, indicating that NIS populations have a small but noticeable impact on the large-scale structure of Danish marine communities. However, NIS can still dominate communities on shorter time scales and on smaller spatial scales, as shown for the phytoplankton species *Pseudochattonella*, the macroalgae *Sargassum muticum* and the benthic invertebrate *Marenzelleria viridis* (however, these dominance patterns are cancelled out when samples are pooled across entire regions and years). To identify the causes for the increasing contribution of NIS to the community structures, further analyses focused on smaller time and spatial scales are needed (e.g. Stæhr et al. 2000; Riisgård et al. 2010; Azour et al. 2015).

NIS typically invade habitats and ecosystems that are strongly modified by human activities (Thomsen et al. 2016). For example, certain ecosystem processes, such as resistance to invasions, may be impeded in stressed low-diversity systems to favour NIS. In the Danish waters, eutrophication, physical disturbance of the seafloor, hazardous substances, and climate change may all function to lower resistance to invasions. An area such as the Limfjord is known to be a very human affected and disturbed system (eg. Christensen et al. 2006). This may partly explain why Limfjorden is the most NIS impacted region in the Danish waters. However other characteristics such as high salinity, warm shallow waters and deliberate introductions of marine organisms most likely also have contributed to the high NIS impact in Limfjorden. Effects of NIS on community structure, ecosystem processes and services can be very difficult to document as NIS have many attributes similar to indigenous species and because of the magnitude of possible interactions (Thomsen et al. 2016). In this perspective it is interesting that our analyses show that the presence (%$NIS_{records}$) and contribution of NIS to community structure has been increasing over time for almost all groups, with NIS characterizing up to twelve percent of the “indigenous” community. This number covers much larger impacts at the local scale in some regions, especially Limfjorden. The overall impact assessment of NIS provided in this report, should therefore be supplemented with investigations of the impact of specific NIS in local areas such as those performed on *Sargassum muticum* (e.g. Stæhr et al. 2000; Pedersen et al. 2005) and *Mnemiopsis leidyi* (Riisgård et al. 2010, 2015).

4 Conclusions and recommendations

We collated large data sets from monitoring of the Danish marine systems, which made it possible to perform the first quantitative analyses of long-term changes to marine NIS covering all taxonomic groups in Danish waters. A total of 63 NIS was recorded in the marine monitoring programmes and documentation exists for an additional 22 introduced species. Most NIS were recorded in Limfjorden (30 NIS) and least in the western Baltic Sea (11 NIS). Aggregating data across five large regions, showed significant temporal increases in NIS numbers and in the relative contribution of NIS to the total species records (%$NIS_{records}$) for phytoplankton, benthic invertebrates and macroalgae. Only two zooplankton NIS and no fish or parasitic NIS were recorded in the survey programmes. As other documentation exists for the introduction of these NIS, the absent monitoring records for zooplankton, fish and parasites suggest that sampling is currently inadequate designed to monitor these NIS groups in Danish waters. Furthermore, as the number of new species and NIS are sensitive to the sampling effort, it is important to maintain a stable monitoring effort. The number of sampling stations and sampling frequencies should adequately cover the different regions in Danish waters and the seasonality of key organisms (planktonic organisms in particular). A strengthened monitoring effort is therefore recommended in in the Danish part of the North Sea and the Skagerrak where monitoring is currently scarce.

Analysing temporal trends since 1989 in %NIS based on presence-record revealed significant and exponential increases in phytoplankton and benthic invertebrates (in all five regions), but no trend was observed for macroalgae and zooplankton. However, analysing temporal trends in %NIS based on abundance data, revealed a different pattern with a decrease of phytoplankton NIS, but an increase in benthic invertebrates and macroalgal NIS (for macroalgae - only in Limfjorden).

Using multivariate statistics to investigate potential impacts of NIS on community structure showed that in regions where NIS have been recorded, their contribution to total community similarity increased over time. As this does not occur to result from changes in sampling effort, NIS have over time, become a more characteristic component of Danish marine communities.

Correlation of environmental conditions to changes in %$NIS_{records}$ and community structures, demonstrated a strong influence of salinity for both benthic and pelagic communities, which also changed moderately with changes in number of NIS. Correlations between %$NIS_{records}$ and other environmental variables were, however, weak. To elucidate the causes for the increasing contribution of NIS to Danish marine communities, analyses are needed at more local scales where the occurrence of NIS can be linked to environmental variables with greater certainty. Such analyses will furthermore clarify potential species-specific effects on ecosystem and habitat levels and should be pursued.

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6 Supplementary material

Table 6.1. Non-indigenous marine species recorded in the Danish marine monitoring programme. Region 1 = North Sea and Skagerrak, Region 2 = Kattegat, Region 3 = Limfjorden, Region 4 = the Belt Sea, and Region 5 = western Baltic Sea. HAB = harmful algal blooms. Latitude (Lat) and Longitude (Long) and region, refers to the location of first observation. Rank (P/A) and Rank (abund) are the rank order of the NIS according to presence/absence data and relative abundance. Years in the note coloumn are the year of arrival, and numbers in (brackets) are regions of introduction according to previous reports (see Stæhr & Thomsen 2012). Region numbers in brackets show the first observation according to the HELCOM Sound-Arkona subbasin line.

Tax group	Species	Year	Region	Lat	Long	Rank (P/A)	Rank (abund)	Note
Macroalgae	*Bonnemaisonia hamifera*	1989	4	10.31	56.23	1	2	1900 (?)
	Codium fragile	1989	2	10.96	56.44	2	10	1919 (2)
	Dasya baillouviana	1989	4	9.98	55.87	3	6	1961 (4)
	Sargassum muticum	1989	3	8.29	56.6	4	1	1984 (3)
	Colpomenia peregrina	1990	3	8.74	56.53	5	9	1939 (3)
	Dictyota dichotoma	1989	3	8.29	56.6	6	4	1939 (3)
	Fucus evanescens	1989	2	10.96	56.44	7	3	1948 (5)
	Neosiphonia harveyi	2000	3	8.25	56.6	8	8	1986 (?)
	Gracilaria vermiculophyl	2009	4	9.78	55.37	9	5	2003 (4)
	Porphyra umbilicalis	1989	4	9.94	55.69	10	7	
Phytoplankton	*Prorocentrum cordatum*	1981	5 (2)	12.45	55.52	1	6	1981 HAB
	Pseudochattonella spp.	1998	4	10.192	56.09	2	3	HAB
	Biddulphia rhombus	1983	3	8.245	56.38	3	20	
	Thalassiosira punctigera	1990	4	10.05	55.40	4	13	
	Phaeocystis spp.	1980	5 (2)	12.45	55.52	5	1	HAB
	Prorocentrum triestinum	1983	3	8.25	56.37	6	17	HAB
	Akashiwo sanguinea	1983	5 (2)	12.45	55.52	7	5	HAB
	Alexandrium ostenfeldii	1986	3	9.038	56.57	8	19	
	Karenia mikimotoi	1981	5 (2)	12.45	55.52	9	7	1968 HAB
	Peridiniella danica	1983	3	9.046	56.37	10	9	
	Tripos macroceros	1983	3	8.25	56.37	11	15	
	Odontella sinensis	1983	3	8.25	56.37	12	10	
	Rhizosolenia calcar-avis	2009	3	9.038	56.57	13	8	
	Peridiniella catenata	1987	5	12.25	55.30	14	14	
	Trieres mobiliensis	1983	3	8.25	56.37	15	26	
	Alexandrium pseudogonyaulax	1997	4	10.19	56.09	16	12	
	Alexandrium tamarense	1983	3	8.25	56.37	17	24	
	Lepidodinium chlorophorum	1999	4	10.10	54.60	18	2	
	Bacteriastrum hyalinum	1983	3	8.25	56.37	19	18	
	Trieres regia	1989	3	8.25	56.37	20	21	
	Stephanopyxis turris	1983	3	8.25	56.37	21	23	
	Tripos arietinus	1992	2	10.27	56.37	22	27	
	Chaetoceros peruvianus	2008	2	10.48	56.51	23	28	HAB
	Chaetoceros circinalis	2001	4	10.10	54.60	24	22	
	Coscinodiscus wailesii	1983	3	8.25	56.37	25	11	
	Prorocentrum lima	1989	3	8.25	56.37	26	30	
	Heterosigma akashiwo	1989	2	12.19	56.10	27	4	
	Protoperidinium quinquecorne	2007	999	8.13	56.04	28	29	
	Prorocentrum gracile	2008	2	11.45	55.51	29	16	

Tax group	Species	Year	Region	Lat	Long	Rank (P/A)	Rank (abund)	Note
Phytoplankton	*Emiliania huxleyi*	2004	2	10.48	56.51	30	25	
	Corymbellus aureus	1992	2	12.187	56.0939	31	No quanti-tative data	
	Chaetoceros concavicornis	2011	2	11.09	56.08	32	31	HAB
	Alexandrium minutum	1998	999	8.13	56.04	33	No quanti-tative data	
Zoo	*Acartia tonsa*	1991	2	12.07	55.71	1	1	
	Penilia avirostris	2001	2	11.94	56.95	2	2	
Benthic invertebrates	*Mya arenaria*	1921	4	10.48	55.18	1	1	< 1200 (?)
	Neanthes succinea	1972	4	10.28	55.27	2	5	1940 (2)
	Ensis directus	1981	1	8.36	55.11	3	8	1981 (1)
	Marenzelleria viridis	1990	1	8.31	55.27	4	4	
	Crepidula fornicata	1978	3	8.4	56.31	5	6	1934 (1)
	Potamopyrgus antipodarum	1973	4	11.44	55.11	6	2	1914 (2)
	Molgula manhattensis	1973	4	10.1	55.04	7	9	
	Balanus improvisus	1972	4	10.32	55.31	8	7	1880 (5)
	Styela clava	1981	3	8.25	56.36	9	10	
	Palaemon elegans	1995	4	9.37	54.55	10	14	
	Marenzelleria neglecta	2006	3	8.15	56.18	11	3	
	Caulleriella killariensis	2012	2	11.14	57.25	12	12	
	Gammarus tigrinus	1990	4	10.16	56.35	13	13	
	Rhithropanopeus harrisii	2011	5	12.14	54.6	14	11	1936 (5)
	Petricolaria pholadiformis	2010	4	10.52	57.38	?	?	1905 (1)
	Crassostrea gigas	2013	3	8.8	56.72	?	?	1980 (1)
Fish	*Oncorhynchus mykiss*	1997	4	10.26	56.07	?	?	
	Neogobius melanostomus	2011	4	11.15	54.58	?	?	2008 (4)

Table 6.2. Non-indigenous marine species known to occur in Danish waters, but not recorded in monitoring databases. Three phytoplankton species (*Phaeocystis pouchetii, Pseudochattonella verruculosa* and *Pseudochattonella verruculosa*) were possibly represented in *table 6.1*. However, the genus was not determined to species level in the monitoring programme which therefore only refer to these as spp.

Tax group	Species	Year	Location first observed	References
Angiosperm	*Spartina anglica*	1930-40	the Wadden Sea	Nehring & Adsersen 2006, Randløv 2007
Macroalgae	*Anglaothamnion halliae*	2003	Uncertain	Thomsen et al. 2007, 2008b;
	Heterosiphonia japonica	2005	Limfjorden	Thomsen et al. 2007, 2008b
Phytoplankton	*Phaeocystis pouchetii*	1980	Ven	Bjergskov et al. 1990
	Dinophysis sacculus	2004	Isefjorden	Hansen, J. W. 2008
	Alexandrium margalefii	2006	Limfjorden	Hansen, J. W. 2008
	Alexandrium leei	2006	Nissum Bredning	Hansen, J. W. 2008
	Pseudochattonella verruculosa	2015	Horsens Fjord	Hansen, P. J. pers comm
Zooplankton	*Mnemiopsis leidyi*	2007	The Belt Sea	Tendal et al. 2007; Riisgård et a. 2010
Benthic invertebrates	*Ficopomatus enigmaticus*	1939	Marstal	Jensen & Knudsen 2005
	Cordylopohora caspia	1895	Ringkøbing Fjord	Jensen & Knudsen 2005
	Ocenebra inornata	2006	Nissum Bredning	Lützen et al. (in Thomsen and Stæhr 2010)
	Teredo navalis	1853	Kiel Bright + later in Nissum Bredning	Jensen & Knudsen 2005 + BSASD
	Eriocheir sinensis	1927	Kattegat	BSASD
	Elminius modestus	1927	Kattegat	BSASD
Parasite invertebrates	*Pseudodactylogyrus anguillae*	1985	?	Jensen & Knudsen 2005
	Pseudodactylogyrus bini	1985	Esrum sø	Jensen & Knudsen 2005
	Anguillicola crassa	1985	?	Jensen & Knudsen 2005
	Mytilicola intestinalis	1964	?	Jensen & Knudsen 2005
Fish	*Huso huso*	2010	the Belt Sea, Langeland	Fiskeatlas 2010a
	Acipenser stellatus	2010	the Belt Sea, Hejlsminde	Fiskeatlas 2010a

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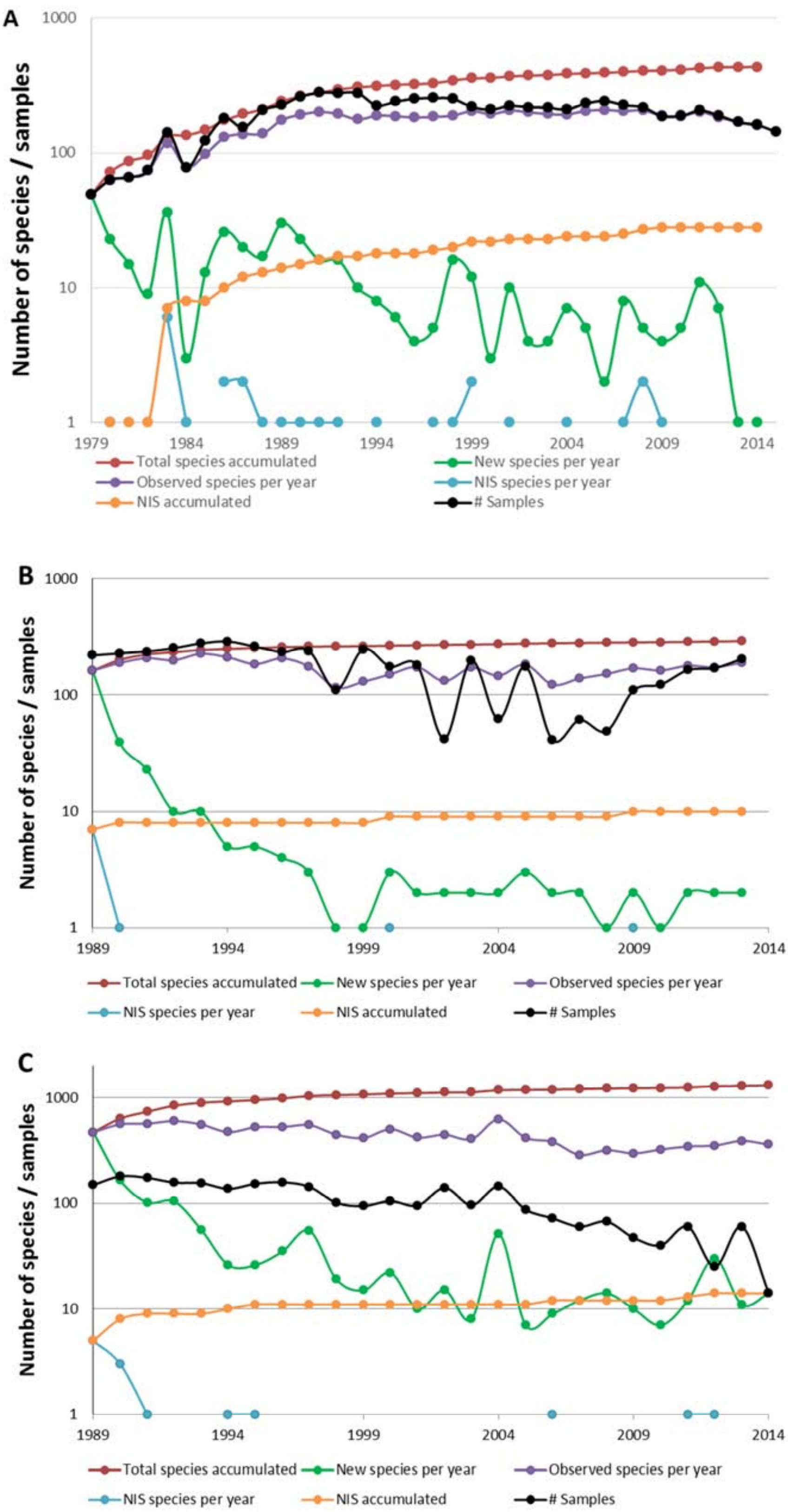
Figure 6.1. Development of the accumulated number of species and samples recorded per year. Species numbers are shown as total and NIS in Danish marine waters for A) phytoplankton, B) macroalgae and C) soft bottom fauna. Also the annually recorded number of total species and NIS is shown as well as number of new species not included in NIS.

The accumulated number of total species and NIS is high to begin with and saturates over time. Also the number of NIS and other new species observed was highest in the early periods.



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TRENDS IN RECORDS AND CONTRIBUTION OF NON-INDIGENOUS SPECIES (NIS) TO BIOTIC COMMUNITIES IN DANISH MARINE WATERS

The report investigates trends in the temporal and spatial changes of non-indigenous marine species in the Danish part of the OSPAR and HELCOM regions. The assessment is based on a quantitative analysis of data available in national monitoring databases and covers the period 1989 to 2014 and other documented records of non-indigenous marine species in the Danish waters.

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