



Annual changes in abundance of non-indigenous marine benthos on a very large spatial scale

Mads S. Thomsen^{1,2*}, Thomas Wernberg², Peter A. Stæhr³, Brian R. Silliman⁴, Alf B. Josefson¹, Dorte Krause-Jensen¹ and Nils Risgaard-Petersen¹

¹National Environmental Research Institute, University of Aarhus, Denmark

²Centre for Ecosystem Management, Edith Cowan University, Joondalup WA 6027, Australia

³Freshwater Biological Laboratory, University of Copenhagen, 3400 Hilleroed, Denmark

⁴Department of Zoology, University of Florida, Gainesville, FL 32609, USA

*Corresponding author

E-mail: m.thomsen@ecu.edu.au

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Abstract

Non-indigenous marine species (NIMS) have only recently caught general interest in Denmark, and baseline studies are needed to identify what species are of particular importance in order to prioritize management and research efforts. We used large data sets compiled in monitoring databases to quantify annual nation-wide changes in abundance of non-indigenous soft-bottom invertebrates (from grab samples) and hard-bottom macroalgae (from diver based percent cover values) in Denmark. Based on criteria of being either abundant (constituting >1% of the entire Danish assemblages) or increasing in abundance, NIMS of particular interest were found to be *Mya arenaria* and *Bonemaissonia hamifera* (abundant), *Crepidula fornicata*, *Ensis americanus*, *Neanthes succinea* (a cryptogenic species), *Marenzelleria* spp. (increasing), and *Sargassum muticum* (abundant and increasing). In addition, new and/or warm-water euohaline NIMS such as *Gracilaria vermiculophylla* and *Crassostrea gigas*, should be given attention as these species are expected to increase in the future. Finally, species not included in existing monitoring programs (hard-bottom estuarine invertebrates, fish, parasites, highly mobile species) should also be targeted in future sampling programs.

Key words: Denmark, non-indigenous marine macrobenthos, large-scale patterns, long-term trends

Introduction

Non-indigenous marine species (NIMS) can become invasive and potentially out-compete or eat native species, alter habitats, homogenize biota, and may cause irreversible effects on native communities and ecosystems (Leppakoski et al. 2002b; Reise et al. 2002) and, consequently, research into, and management of,

NIMS has accelerated in the last few decades. In recent years, Danish researchers, managers and politicians have shown an increasing interest in the subject, and in 2007 the Danish Minister for the Environment commissioned a report to review the status of NIS in Denmark with focus on invasive species, black-lists, and management recommendations. To support guidelines of which species should be monitored, researched

and managed, quantitative overview studies are needed. A first approach to prioritize research and management efforts is to analyze how abundant NIS are on the broadest scales possible (here national level) and whether they are increasing in abundance (suggesting increasing impacts in the future) (Parker et al. 1999). In Denmark, while efforts related to NIMS have been small, the commitments to tracking environmental threats from pollution and reduced water quality have been great. Comprehensive nation-wide monitoring programs have been in place for many years and among the data compiled are extensive species-abundance lists from standardized and quantitative samples.

The objective of this study was to quantify large-scale annual changes in the abundance of NIMS in Denmark, ultimately to prioritize NIMS for future research and management on a nation-wide level. We used 'increasing' and/or 'high' abundances of NIMS (the latter defined as NIMS constituting >1% of the entire assemblages) as simple *a priori* chosen criteria of importance. The focus was on soft-bottom invertebrates and hard-bottom macroalgae, two groups of benthic NIMS that have received considerable attention (e.g. Schaffelke et al. 2006). These groups provide an excellent analytical starting point because they represent dominant biota in two distinct habitats that are ubiquitous and have been impacted by 'super invasions' around the world (e.g. *Caulerpa taxifolia* (M. Vahl) C. Agardh, *Undaria pinnatifida* (Harvey) Suringar, *Carcinus maenas* L., Lowe et al. 2001).

Methods

Study region

Denmark is located at the entrance to the Baltic Sea (Figure 1). The primary environmental factor that varies horizontally is salinity, which decreases markedly from the North Sea in the west (34 psu) to the Baltic Proper around Bornholm in the southeast (8 psu). Bottoms along the coasts of Denmark are, except for the rocky coast of northern Bornholm, sedimentary where hard substratum mainly is composed of stones and boulders deposited by glacial activity. Most marine areas are micro-tidal and only the Wadden Sea in south-western Denmark has any significant tidal influence (1-2 m vs. < 0.5 m in the rest of Denmark).



Figure 1. Study area of Denmark with its three main regions; Jutland, Zealand, and Fyn. Invertebrate and macroalgal samples were collected throughout Danish waters from east of Bornholm (Bh) to west of Jutland and included samples from both open waters and from all major estuaries, e.g. Limfjorden (Lf) and Ringkøbing fjord (Rf).

Taxonomy and sample method

We used the compiled lists provided in recent reviews as a template for the species to include as NIMS, thereby excluding *Mastocarpus stellatus* (Stackhouse) Guiry but including *Mya arenaria* L., *Neanthes succinea* Frey and Leuckart, *Dictyota dichotoma* (Hudson) J.V. Lamourous, and *Fucus evanescens* C. Agardh (Behrends et al. 2005; Jensen and Knudsen 2005; Thomsen et al. 2007a; Thomsen et al. 2007c). It should be noted that *D. dichotoma* and *N. succinea* are generally not considered to be NIMS in Europe (e.g. Leppakoski et al. 2002a; Reise et al. 2002). However, because both species were not observed in Danish waters until the 1930's and 1940's they could potentially be introduced from other European regions. In this paper we adopted a conservative approach (guilty till proven innocent) to what species to analyze and included those to represent 'cryptogenic' species that needs additional historical, biogeographical and molecular analysis before conclusions regarding their origin can be drawn. The relative abundances, i.e. the percentage contribution of each NIMS compared to the summed assemblage abundances, were calculated for individuals of soft-bottom invertebrates collected with grab-samples (1970 to 2004, a total of 44,999 samples distributed throughout Danish waters down to

500 m depth) and for cover of hard substratum of macroalgae estimated by scuba divers (1989-2005, a total of 10,004 samples distributed throughout Danish waters down to 30 m depth, not all 2004 and 2005 samples have yet been incorporated to the national database). Nationwide annual 'relative abundances', were calculated by standardizing abundances to unit sample size and then total sample abundances (i.e. percentage contribution of specific NIMS out of the total abundance per sample) and finally averaging the percent contribution for each year across all samples. Spearman's rank correlations were subsequently applied to test if NIMS increased or decreased over time. Number of samples per time period were for invertebrates: 1975 (70-79) = 648, 1981 (80-82) = 1173, 1984 (83-85) = 2382, 1986 = 1178, 1987 = 1226, 1988 = 1651, 1989 = 2542, 1990 = 2876, 1991 = 2695, 1992 = 2720, 1993 = 3146, 1994 = 3067, 1995 = 2863, 1996 = 2420, 1997 = 3234, 1998 = 1192, 1999 = 1501, 2000 = 1603, 2001 = 1476, 2002 = 1831, 2003 = 1793, 2004 = 1783, and for macroalgae: 1989 = 637, 1990 = 664, 1991 = 781, 1992 = 836, 1993 = 920, 1994 = 858, 1995 = 845, 1996 = 817, 1997 = 777, 1998 = 337, 1999 = 805, 2000 = 447, 2001.5 (01-02) = 82, 2003 = 518, 2004.5 (04-05) = 275. The calculation of relative abundances, instead of absolute abundances (only standardized to sample size) can potentially reduce some methodological biases, for example, if a grab did not collect all the expected sediment (making it difficult to compare absolute values between samples), or if one diver systematically estimated macroalgal cover with higher values compared to other divers. Changes in relative abundances can occur either because NIMS become more abundant and/or because native species become less abundant. We repeated the correlation analyses with absolute abundance data but results were generally similar, and we only report relative abundances here. Detailed descriptions of the sampling methods have been presented numerous times (e.g. see Middelboe et al. 1998; Josefson and Rasmussen 2000; Stæhr et al. 2000; Josefson and Hansen 2004). For the invertebrate samples it should be noted that a grab rarely catches highly mobile species (e.g. many decapods) or species that can retract fast and deep into sediments (e.g. *Ensis americanus* Gould), and also under-represent species associated with other habitats (e.g. *Balanus improvisus* Darwin attached to hard substratum). However, we

choose to present data on such 'underestimated' MNIS because any potential sampling bias is likely to be consistent between years, i.e. it may still be possible to detect if these NIMS increase over time.

Results

Invertebrates

We found significant increases in abundance of *Crepidula fornicata* L. ($r = 0.570$, $p < 0.000$), *Ensis americanus* ($r = 0.712$, $p < 0.000$), *Marenzelleria* spp. (possibly several species, Sikorski and Bick 2004, $r = 0.613$, $p = 0.002$), and *N. succinea* ($r = 0.686$, $p = 0.000$), but a significant decrease in *Potamopyrgus antipodarum* (Gray) ($r = -0.509$, $p = 0.016$) (Figure 2). No changes were observed for *Balanus improvisus* (with a total average contribution across all years of the entire Danish invertebrate assemblage of 0.043%), *Mya arenaria* (2.425%), *Petricola pholandiformis* Lamarck (0.013%), *Styela clava* Herdman ($<0.001\%$, a large hard-bottom sessile tunicate, only recorded 4 times, data not included on Figure 2) or all invertebrate NIMS pooled (3.917%). Note that for these 'temporarily stationary' species, only *M. arenaria* was found with a nation-wide abundance above our 1% criteria (calculating total averages covering all years for the 'increasing' MNIS, showed 'means' $<1\%$). All these invertebrates constitute most of the known soft-bottom macrobenthic NIMS in Denmark, although reef-formers such as *Ficopomatus enigmaticus* Fauvel (only found in Copenhagen harbor) and *Crassostrea gigas* Thunberg (common in intertidal habitats in Wadden Sea and some areas of Limfjorden), introduced parasites (e.g. *Mytilicola intestinalis* Steuer common in *Mytilus edulis*) or the sessile barnacle *Elminius modestus* Darwin were obvious absentees.

Macroalgae

We only found significant increases in abundance of *Sargassum muticum* (Yendo) Fensholt ($r = 0.72$, $p = 0.0026$) but a significant decrease in abundance of *Codium fragile* (van Goor) Silva ($r = -0.83$, $p = 0.0001$) and a near significant decrease in abundance of *Fucus evanescens* ($r = -0.48$, $p = 0.0687$) (Figure 3).

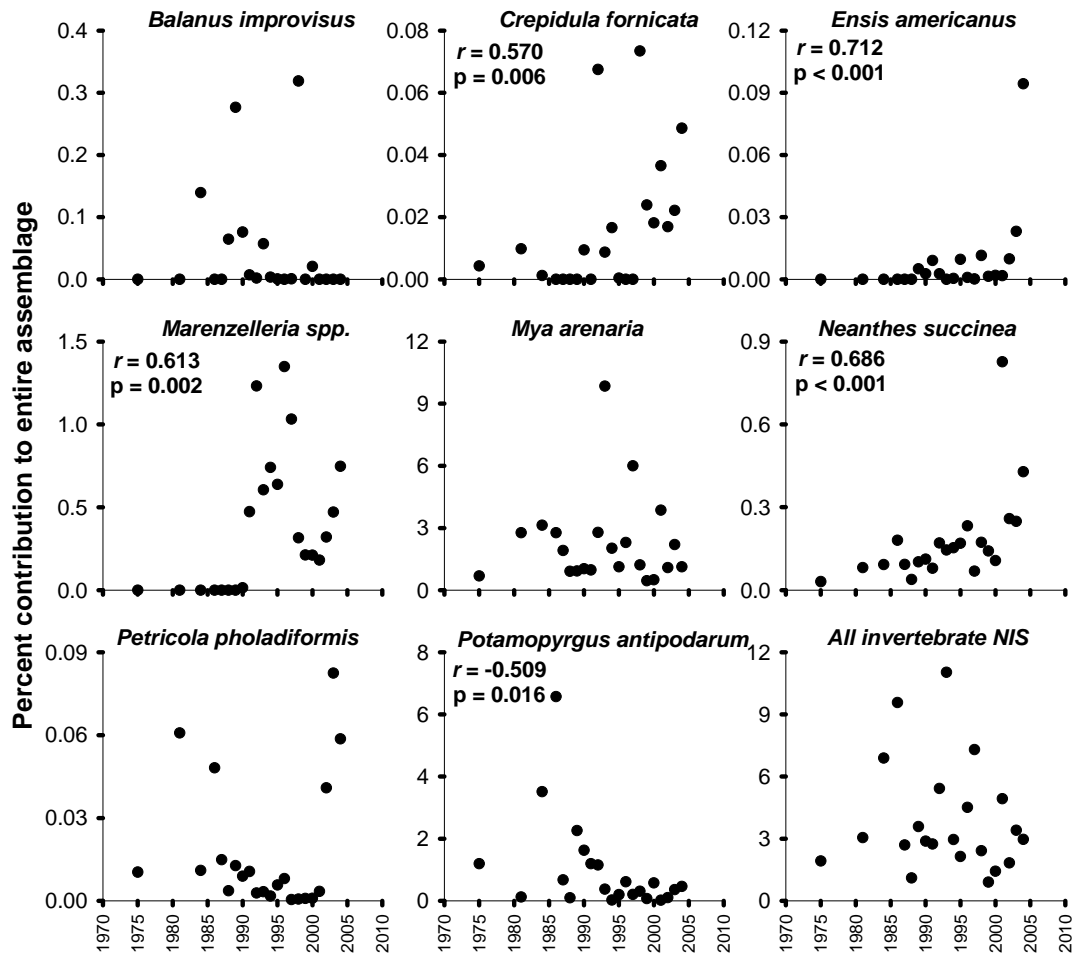


Figure 2. Relative abundance of soft-bottom benthic invertebrate NIMS (divided by all individuals summed from all samples within a specific time period). Only significant correlation results are included on graphs ($p > 0.32$ for rest of tests, $n = 22$)

In contrast no changes were observed for *Bonnemaisonia hamifera* Harriot (with a total average contribution across all years of the entire Danish macroalgal assemblage of 1.072%), *Dictyota dictyota* (0.603%), *Dasya baillouviana* (S.G. Gmelin) (0.290%), *Colpomenia perigrina* Sauvageau (0.016%), or all macroalgal MNIS combined (3.885%). Only *B. hamifera*, of the ‘stationary’, and *S. muticum* of the ‘increasing’

MNIS were found with nation-wide abundances above our 1% criteria. No formal tests were conducted on *Heterosiphonia japonica* Yendo (0.007%), *Gracilaria vermiculophylla* (Ohmi) Papenfuss (0.002%) and *Neosiphonia harveyi* (J. Bailey) Kim, Choi, Guiry & Saunders (0.001%), as these were only found in the last 4, 2 and 1 years. Above species represent all published known macroalgal NIMS from Denmark.

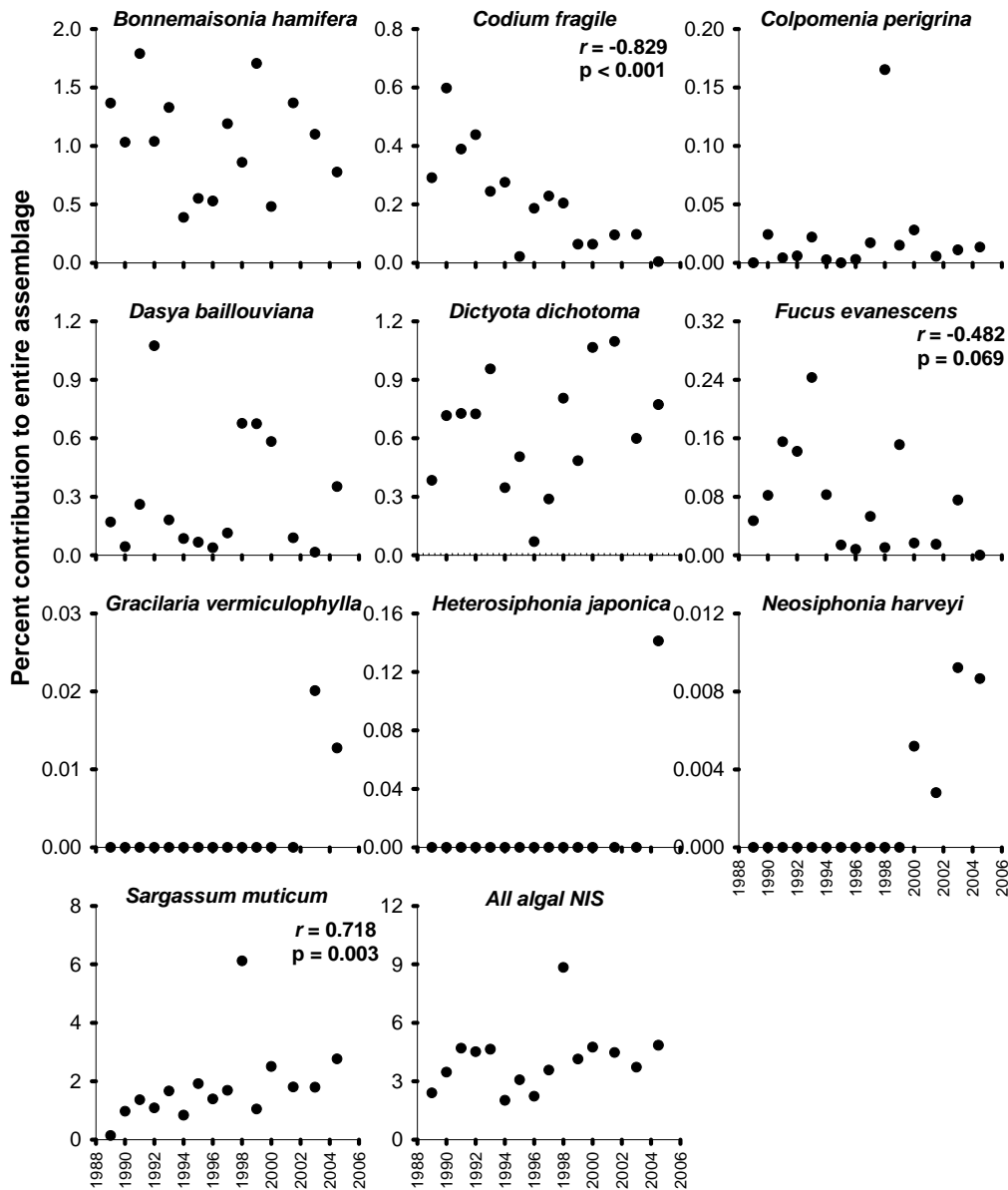


Figure 3. Relative abundance of hard-bottom benthic macroalgal NIMS (divided by all cover values summed from all samples within a specific time period, n = 15). Tests were not performed on the very recent NIMS (*Gracilaria vermiculophylla*, *Heterosiphonia japonica*, *Neosiphonia harveyi*), only significant and near-significant correlation results are included on graphs (p > 0.16 for rest of tests, n = 15)

Discussion

Non-indigenous marine species (NIMS) are today of general importance in Denmark, with both soft-bottom invertebrates and hard-bottom macroalgae each constituting ca. 4% of the entire assemblage abundances, but with no overall increases over the considered time periods (1975-

2004 and 1989-2005 respectively). However, we are not aware of any other quantitative analysis of relative MNIS abundances published on a similar large spatial scale, making it impossible to evaluate the generality of the findings. Still, it is important to emphasize that the large-scale pooling procedures typically will result in relatively 'low' NIMS abundances compared to most small scale surveys. Thus, small scale surveys are often targeted to areas with known

infestations of specific NIMS (e.g. Thomsen et al. 2006a, b; Thomsen et al. 2007b, c) and hence an increased likelihood of detecting high NIMS abundances (i.e. many NIMS surveys are not based on sampling programs randomized with respect to where NIMS are expected to be found). A narrowing of the spatial scale coupled with an increase in the degree of targeted sampling, will likely result in an increase in MNIS abundance, with the extreme case corresponding to only sampling known monocultures of MNIS. Such targeted studies provide efficient information on potential maximum impacts, but they are nevertheless spatially and taxonomically biased in relation to what species should be prioritized for research and administration, as both funding and legislation typically operates on nation-wide scales.

Five NIMS were found to increase significantly in abundance, potentially due to the species being 'new' (e.g. population increases could be a simple consequence of establishment, consolidation and secondary dispersal) and/or because environmental/ecological conditions are becoming increasingly benign (e.g. due to climatic trends, in particular rising temperature or changes to biological interactions such as the appearance of a key NIMS facilitator). We cannot separate these factors here as this requires detailed small-scale analysis of individual NIS (work in progress), as previously demonstrated for *Sargassum muticum* (Stæhr et al. 2000; Thomsen et al. 2006b) and *Gracilaria vermiculophylla* (Thomsen et al. 2006a; Thomsen et al. 2007b). An interesting example is *Neanthes succinea* which was observed for the first time in Denmark in the 1940's. Its relatively recent increase in abundance can therefore both be due to lag-time (Lockwood et al. 2007) and/or improved environmental conditions. As described earlier, *N. succinea* is not considered NIMS in Europe (Leppakoski et al. 2002a; Reise et al. 2002; Streftaris et al. 2005). Despite its commonality in Danish waters today, we are not aware of any ecological studies on this species, and it is not included in several popular keys to marine life, indicating a relatively unknown ecology and low abundances (e.g. Ursing 1971; Hayward et al. 1996; Hayward and Ryland 1996; Køie et al. 2000). Interestingly, *N. succinea* has been suggested to be a NIMS on both the west coast of North America and in Australia (<http://www.issg.org/database/species/>). We therefore reiterate our call for a review of the taxonomy, evolutionary history, biogeography,

biology, ecology, and molecular DNA analysis of this common and apparently cryptogenic species. The remaining 'increasing' NIMS arrived in Denmark during the monitoring program, and these species are therefore potentially suitable for more detailed analysis of invasion pathways and potential impacts. Still, *Crepidula fornicata* is a typical hard-substratum species, and adult individuals of *Ensis americanus* can be too fast and deeply buried to be efficiently caught by grab sampling. For these two species, additional sampling using targeted techniques are needed to avoid underestimating abundances (as for other hard-bottom, reef-forming or fast-moving NIMS). Two NIMS actually decreased in abundance (*Potamopyrgus antipodarum* and *Codium fragile*), and both have been in Denmark for approximately a century. The decrease in abundance of *C. fragile* is particularly interesting, given its invasive status in North and South America, the Mediterranean, Australia and New Zealand (e.g. Mathieson et al. 2003; Bulleri et al. 2006; Neill et al. 2006; Scheibling and Gagnon 2006; Thomsen and McGlathery 2007). It is possible that this decrease is partly attributed to the more recent invasion by *S. muticum*, given that the two species tend to occupy similar habitats in the saltier regions of Denmark (Stæhr et al. 2000). A post-hoc analysis confirmed that the abundances of the two species are negatively correlated ($n = 15$ years, $r_{\text{Spearman}} = -0.696$, $p = 0.004$). Finally, it should be noted that three macroalgal MNIS were very only recorded within the last few years and therefore were not analyzed statistically. Of these species, *G. vermiculophylla* has traits, and a documented early invasion history, that suggest it will become highly abundant throughout Danish shallow and low-energy waters (Thomsen and McGlathery 2007, Thomsen et al 2007b).

We conclude with the first simple nation-wide recommendation of what species should be prioritized in research and management, as outlined in the study objectives, and based on the criteria of targeting NIMS that are 'abundant' and/or 'increasing in abundances' on very large spatial scales: The 'abundant' NIMS were *Mya arenaria* (of special historical interest given its old invasion history, Behrends et al 2005) and *Bonnemaisonia hamifera*, the 'increasing' NIMS were *C. fornicata*, *E. americanus*, *Marenzelleria* spp., and *N. succinea* (and the un-tested *G. vermiculophylla*) and the only 'abundant and increasing' NIMS was *S. muticum*. In addition,

NIMS that are known to respond positively to predicted climatic or environmental trends (e.g. warm-water species) and which do not have limitations to major geographical and environmental barriers (e.g. eurohaline species) should be given extra scrutiny, for example *B. hamifera*, *G. vermiculophylla* as well as *Crassostrea gigas* and *Dasya baillouviana*. To understand how NIMS have transformed Danish marine benthic systems, and will continue to do so, we now need (the more typical) smaller-scale distribution analyses that compare and contrast depth intervals, geographical regions, estuaries, and proximity to stressors, as well as manipulative impact experiments.

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