

# Ranking of Danish ports according to shipping activities and to the potential of natural dispersal of nonindigenous species

By Flemming Thorbjørn Hansen, Aurelia Pereira Gabellini and Asbjørn Christensen

DTU Aqua Report no. 369-2020





# Ranking of Danish ports according to shipping activities and to the potential of natural dispersal of non-indigenous species

DTU Aqua Report no. 369-2020

By Flemming Thorbjørn Hansen, Aurelia Pereira Gabellini and Asbjørn Christensen

### Colophon

Title:	Ranking of Danish ports according to shipping activities and to the potential of natural dispersal of non-indigenous species
Authors:	Flemming Thorbjørn Hansen, Aurelia Pereira Gabellini and Asbjørn Christensen
DTU Aqua Report no.:	369-2020
Year:	The scientific work was completed in June 2020 and the report was published in June 2020.
Reference:	Hansen, F.T., Gabellini, A.P. & Christensen, A. (2020). Ranking of Danish ports according to shipping activities and to the potential of natural dispersal of non- indigenous species. DTU Aqua-rapport nr. 369-2020. National Institute of Aquatic Resources, Technical University of Denmark. 62 pp. + appendices
Cover:	Example of map from the report showing cumulated dispersal probability of 23 non-indigenous marine species for each of the 28 major Danish ports, in this case Rødby Port.
Published by:	National Institute of Aquatic Resources, Kemitorvet, 2800 Kgs. Lyngby, Denmark
Download:	www.aqua.dtu.dk/publikationer
ISSN:	1395-8216
ISBN:	978-87-7481-291-3

**DTU Aqua Reports** contain results from research projects, reviews of specific topics, expositions for authorities etc. Unless stated in the colophon, the reports are not peer reviewed, which means that the content has not been reviewed by researchers outside the project group.

## Preface

This study was funded by the Danish Maritime Fund, "Den Danske Maritime Fond. We want to express our gratitude to representatives from the Danish Environmental Agency including Kim Larsen, Kim Lundgreen, Ulrich Christian Berggreen and Anette Maria Christensen, and Frank Stuer-Lauridsen from the maritime consultancy company Litehauz Aps., for participating in the project advisory group.

Kgs. Lyngby, Denmark, June 2020

Flemming Thorbjørn Hansen Senior Consultant DTU Aqua Karen Edelvang Head of Section DTU Aqua

## Contents

1.	Extended abstract								
2.	Introductio	on	8						
3.	Ranking o	f ports based on shipping activity	9						
3.1	Introductior	۱	9						
3.2	Methodolog	Jy	10						
3.2.1	Ris	k Factors	10						
3.2.2	Ma	jor Danish ports	12						
3.2.3	Ris	k factor criteria and data basis	13						
3.3	Results		24						
3.4	Discussion		25						
3.5	Conclusion	S	26						
4.	Ranking p	orts based on natural dispersal of non-indigenous species	28						
4.1	Introduction	۱	28						
4.2	Methodolog	<u>gy</u>	29						
4.2.1	Ove	erview	29						
4.2.2	Tar	get species selection	29						
4.2.3	Lar	val dispersal modelling	31						
4.2.4	Co	nnectivity analyses	34						
4.2.5	Rai	nking of ports according to the connectivity between ports	37						
4.2.6	Rai	nking of ports according to the natural dispersal potential	40						
4.3	Results		41						
4.3.1	Co	nnectivity analysis results	41						
4.3.2	Rai	nking of ports according to the connectivity between ports	41						
4.3.3	Rai	nking of ports according to the natural dispersal potential	47						
4.4	Discussion		51						
4.4.1	Co	nnectivity between ports	51						
4.4.2	Dov	wnstream dispersal potential	51						
4.4.3	Rei	marks	52						
4.5	Conclusion	S	53						
5.	Compariso	on of the different rankings	55						
6.	Reference	9S	59						
Арре	ndix 1 H	Hydrographic regions and habitat maps	63						
Appe	ndix 2 [	Dispersal Probability Maps	87						
Appe	ndix 3 (	Cumulated downstream dispersal probability maps	88						

## 1. Extended abstract

The Ballast Water Management Convention (BWMC) requires that ballast water (BW) meet certain standards before being discharged into the marine environment to reduce the risk of transferring non-indigenous species (NIS) between locations. The discharge of BW typically (but not exclusively) takes place during port visits following the load and un-load of cargo. To meet these standards ship owners need to treat the BW prior to discharge by the use of on-board treatment technology. The BWMC provides an option for national authorities to grant exemptions to ships that operate exclusively between a fixed number of ports or exclusively within a limited geographical area. An exemption can be granted if a risk assessment can conclude that, the discharge of untreated BW only impose a low and acceptable risk of transfer of NIS between locations.

As a basis for future risk assessment there is a need for a better understanding on how NIS may disperse along Danish coastal areas and between ports, including the identification of the most likely hotspots for introduction and spread of NIS. Thus, the main objective of the present study is to contribute to the decision basis for granting exemptions to the BWMC by Danish authorities, by mapping the potential of introduction and spread of NIS to and from 28 major Danish cargo and ferry ports and identifying major hotspot for primary and secondary introduction of NIS in Denmark.

The 28 major ports are ranked according to 1) the shipping activity, 2) the potential natural dispersal of pelagic life stages of NIS between ports, and 3) the potential natural dispersal of pelagic life stages of NIS to neighbouring habitats. While shipping activity is included as a proxy for both primary and secondary introduction, natural dispersal represents the secondary introduction following an eventual introduction by shipping activity.

Shipping activities were analysed by applying a previously developed preliminary risk assessment (PRA) adapted for Danish ports including five shipping categories: cargo ships, passenger ships, ferries, fishing boats, and leisure boats. Ranking of ports according to shipping activity was done based on scores for each port from the PRA.

Natural dispersal was analysed for 23 selected sessile NIS with limited pelagic larvae duration (PLD) of days to weeks, using larval dispersal modelling including species specific traits such as PLD, habitat preference, vertical position in the water column, salinity tolerance and spawning period. Larval dispersal was simulated using a 3D hydrodynamic dataset from 2005, 2010 and 2012 providing data on currents and salinity.

The natural dispersal between ports was analysed by dividing the study area into a regular grid and translating the results from the larval dispersal model simulations into connectivity adjacency matrices with values representing numbers of all pairwise connections in the grid. The ranking of each port according to natural dispersal between ports was then done using three different methods:

- 1. Cluster membership analysis: The extent to which individual ports belong to groups of neighbouring ports in areas with high larvae dispersal connectivity identified using cluster analysis techniques.
- 2. Dispersal probability analysis: The probability of natural dispersal between individual ports.

3. Dispersal duration analysis: The dispersal duration between ports without considering species specific traits.

The ranking of ports according to the natural dispersal potential to neighbouring marine habitats was done by analysing the results from the larval dispersal model simulations and calculating the mean and sum of the number of downstream connections to neighbouring habitats for the 23 NIS.

#### Ranking of ports according to shipping activities

The major Danish ports with the highest PRA scores (between 20 and 35) included in the study and thus with the highest risk to introduce and spread invasive species via shipping activities are (in descending order) the 11 ports of Copenhagen, Esbjerg, Hirtshals, Kalundborg, Aarhus, Frederikshavn, Fredericia, Thyborøn, Grenaa, Rønne and Køge. Ferry ports in general received the lowest scores except for the port of Helsingør.

#### Ranking of ports according to dispersal probability between ports

The connectivity between ports depends on which analytical method is used. Of the three methods applied the "cluster membership analyse" and the "dispersal probability analysis" showed comparable result, while ranking based on the "dispersal duration analysis" deviated considerably from the other two. The "Dispersal duration analyses" doesn't include species specific traits such as salinity tolerance or habitat preference, and these traits are essential when evaluating connectivity in Danish waters where salinity conditions are highly variable in space and time. Thus, dispersal duration was not included in the final ranking.

Ports with the highest ranking according to dispersal probability between ports were ports located in the western and southern part of Kattegat, and in the Danish straits of Lillebælt, Storebælt and Øresund, while the ports with the lowest rankings are located on Bornholm, along the west coast of Jutland and in the northern parts of Kattegat. The highest rankings were determined by a combination of good hydrographic conditions, salinity conditions supporting a majority of the selected NIS, and a close geographical proximity between ports.

#### Ranking ports according to dispersal potential to neighbouring habitats

The natural dispersal potential to neighbouring habitats was highest for ports located towards the open water of Kattegat, Skagerrak and the North Sea, except the ports of Esbjerg, Havneby and Studstrup because of local hydrodynamic conditions. The highest rankings were determined by the dynamic hydrographic conditions and the salinity conditions within the tolerance range of the majority of the 23 selected NIS. While the overall dispersal potential of the ports located in the Baltic Sea (e.g. Rønne and Nexø) are limited due to a limited number of NIS with a salinity tolerance to low salinity levels, the dispersal potential of the species with a potential occurrence are still of a magnitude similar to the mean of all 28 ports.

The salinity tolerance distribution among the 23 NIS selected for the current study is considered to reflect the general salinity tolerance gradient of estuarine systems.

#### Major hotspots

The ports of Aarhus, Kalundborg, Copenhagen and Fredericia are identified as the major hotspots among the major Danish ports with a combination of both relative high chance of primary

introduction of NIS and a subsequent potentially efficient secondary dispersal to other marine habitats and both major and minor ports.

Some of the major Danish ports located in fjords and straits was not included in the natural dispersal analysis due to limitations of in the spatial coverage of the hydrodynamic dataset.

## 2. Introduction

This project was funded by the Danish maritime fund "Den Danske Maritime Fond" on request from the Danish Environmental Protection Agency, "Miljøstyrelsen" (MST). The project inspects how non-indigenous species (NIS) may spread along Denmark's shores, partly by primary and secondary introduction by ships arriving at, and operating between, Danish ports, and partly by natural dispersal of pelagic life stages of NIS by ocean currents.

The primary introduction of NIS typically occurs at so-called "hot spots" (e.g. ports) caused by shipping activities either by the uptake and discharge of ballast water or from the ship hulls as fouling agents. If a NIS is introduced in a specific port, the specific location of the port would be important for potential secondary spread of the NIS to other sites. This secondary spread may take place by other shipping activities connecting ports or by natural dispersal.

There is a need for better understanding on how NIS may disperse along Danish coastal areas and between ports, including the identification of the most likely hotspots for introduction and spread of NIS. Such information will provide a basis for future risk assessment and decision-making, specifically in relation to the Ballast Water Management Convention (BWMC). Within the BWMC the national authorities are responsible for any granting of exemptions to the *de facto* requirements of the BWMC to ship-owners that operate between Danish and foreign ports, to treat the ballast water before discharging this into the ambient environment (as outlined in the A4 and Guideline 7 of the BWMC).

Thus, the main objective of the present project is to provide a decision basis for granting exemptions to the BWMC by Danish authorities, by mapping the potential of introduction and spread of NIS to and from major Danish ports.

In this report we will rank the major Danish ports based on;

- 1. shipping activities
- 2. natural dispersal connectivity between major ports, and
- 3. natural dispersal potential to the surrounding marine habitats.

In addition, the projects identification of hotspots as well as the most likely secondary dispersal from major ports to surrounding marine habitats can potentially lead to improvement of the national monitoring of NIS and clarify the importance of vectors such as ballast water and hull fouling as required by the EU marine strategy directive.

While the ranking of ports according to shipping activities is based on existing statistical data and a modification of a previously published risk assessment methodology, the ranking of ports according to the potential natural dispersal of pelagic life stages of NIS, is based on a novel methodology developed within this project.

## 3. Ranking of ports based on shipping activity

### 3.1 Introduction

Introduction of Non-Indigenous Species (NIS) can occur by many pathways including commercial shipping (Fofonoff et al., 2003), recreational watercraft (Ashton et al., 2012), aquaculture (Grosholz et al., 2012), aquarium trade (Williams et al., 2012), live bait trade (Fowler et al., 2015), live seafood trade (Chapman et al., 2003) and marine debris (Barnes, 2002). However, shipping is considered the most important vector of introduction of NIS (Geburzi & McCarthy, 2018). Thus, Gollasch and Leppakoski (1999) developed a preliminary risk assessment (PRA) as a tool to evaluate the risk of future introductions of NIS into German ports focussing on shipping activity as the primary vector (table 1).

Risk factor	Low		Medium	High	
	1*	2*	3*	4*	5*
Shipping					
Volume of released ballast water					
[in million tons]	<1	<10	<25	<50	>50
Number of ship arrivals in the area					
[number of ships]	<100	<1.000	<5.000	<10.000	>10.000
Major shipping routes to areas of matching climate (per region)					
[%]	<20	<40	<60	<80	>80
Major shipping route to areas of matching salinities (per region)					
[%]	<20	<40	<60	<80	>80
Duration of ship voyages					
[days ballast water lasted in tanks before released, in average]	>100	<100	<50	<10	<5
Habitat					
Number of estuaries in port areas per region					
[number]	0	<2	<4	<6	>6
Salinity gradient within port area					
[salinity range in ppt]	0	<5	<10	<15	>20
Number of aquaculture and fish processing sites					
[number of sites in the area]	0	<2	<10	<25	>25
Number of ship yards					
[number of sites in the area]	0	<2	<4	<8	>16
Degree of water pollution					
[eutrophication, chemical, urban waste, power plants]	Low		medium		High
Community					

Table 1. Preliminary risk assessment of future introduction of species. Extracted from (Gollasch &Leppakoski, 1999).

#### Table 1 (continued)

Number of macrozoobenthos species per region					
[species]	>1000	<1000	<500	<250	<50
Number of previously known invasions per region					
[species]	<5	<10	<50	<100	>100
Secondary introductions					
Number of nonnative species in nearby areas					
[number of established and non- established NIS]	<5	<25	<50	<100	>100

In order to assess the risk of introducing NIS to a port the PRA consider four major groups of risk factors:

- Shipping traffic
- Habitat characteristics
- Community structure
- Potential for secondary introductions

Each group represents a number of risk factors of which each factor has to be given a score from 1 to 5 indicating low to high risk respectively. Each risk factor score is associated with given criteria, i.e. a range of values for translating statistical data into a given score, and subsequently the sum of scores of all risk factors will then represent the overall risk score for each port. The risk factors originally proposed by Gollasch and Leppakoski (1999) and associated criteria are shown in table 1.

In the current study, we develop a preliminary risk assessment (PRA) specifically to identify the Danish ports with the highest risks of introduction of NIS based on the principles of the PRA proposed by Gollasch & Leppakoski (1999).

## 3.2 Methodology

### 3.2.1 Risk Factors

In table 2, a modification of the PRA developed by Gollasch and Leppakoski (1999) is presented, adapted in order to fit the available data for Danish ports. The PRA originally developed by Gollasch and Leppakoski (table 1) has 13 categories divided into 4 major groups. The PRA presented here specifically addressing Danish ports has 11 categories divided in to 2 major groups of risk factors:

- Shipping traffic
- Habitat characteristics

Although the risk associated with take up and release of ballast water primarily concerns cargo ships and passenger ships (including ferries), we also include risk factors associated with other types of shipping including fishing vessels and leisure boats. The inclusion of leisure boats activities is supported by an increasing evidence that secondary introduction of NIS via recreational boating could be a major vector contributing to the spread of NIS (e.g. Murray et al. 2011,

Anderson et al. 2015, Ferrario et al. 2017, Simard et al. 2017). A study from 2012 (Ashton et al. 2012) in California found significant correlation between the number of NIS in bays and the number of berths of marinas in the area, and this correlation was significant for both NIS with hull fouling as the only vector, and for NIS with both fouling and ballast water as possible vectors. While the BWMC does not consider NIS with biofouling as the only vector, several NIS shortlisted in the HELCOM/OSPAR Joined Harmonised Procedure for the BWMC A-4 exemptions (HELCOM/OSPAR 2014) have both ballast water and hull fouling as possible vectors.

A parallel study to Ashton et al. (2012) investigated the role of fishing vessels as vectors for NIS in California, and concluded that fishing vessels serve as an important potential vector for the spread of NIS (Davidson et al. 2012). Fishing vessel activities potentially create strong connections between ports not necessarily maintained by other vectors.

The habitat risk factors as proposed by Gollasch and Leppakoski (1999) is here represented by one subcategory only, i.e. "degree of water pollution". We merge the presence of shipyards where introductions are likely to occur, and the presence of power plants, where favourable temperature conditions can serve as stepping stones for dispersal of NIS that may have a limited tolerance to low temperature during winter. Water pollution constituents like chemicals or nutrients are not considered. Geburzi and McCarthy 2018 reviewed the existing knowledge on the success of marine invasive species and concluded that there is no clear evidence that water pollution in general favour the introduction of marine invasive species. Observation of increased presence of NIS in disturbed areas may be due to the fact that these areas are more often affected by introduction vectors. The risk factors "Number of estuaries in port areas per region" and "Salinity gradient within the port area" are also not included. Danish marine waters are located within a salinity transition zone between the saline North Sea and the brackish Baltic Sea. At the same time, the freshwater out flow close to port areas constitute relatively small volume of freshwater from small rivers and streams with a limited effect, if any, on the risk of introduction to major Danish ports. The risk factor "Number of aquaculture and fishing processing sites" is not included since the scope of this study is the shipping activity.

The community structure risk factors "Number of macrobenthos species in the region" and "Number of previously invasions in the region" are not included in the PRA for Danish ports since there is no full inventory available on the number of macro-zoobenthos species, nor the number of previous NIS invasions in individual ports.

Similarly, the group of risk factors covering "secondary introductions" is not included since only limited data is currently available on the number of NIS in areas close to the ports, and estimations might consequently be biased.

To adapt the PRA to the Danish conditions, we adjusted the scoring criteria for each risk factor to fit the Danish data. As an example, the volume of ballast water which in Gollasch and Leppakoski (1999) is based on an absolute scale of millions of tonnes, in this PRA for Danish ports it is assessed in thousand tonnes. Similarly, all risk factors were adjusted to the range of values found for Danish ports. We also chose to divide values of each risk factor into scoring intervals using simple linear assumptions, contrary to the approach proposed by Gollasch and Leppakoski (1999) (table 1). We analysed all risk factors for the year 2018, 2018 being the most recent year for which complete information has been published.

## 3.2.2 Major Danish ports

The Danish coastline is long (approximately 8,750 km) and includes a large number of larger and smaller ports, ranging from small marinas with less than 10 berths to large commercial ports with more than 1 mill. tonnes of ships passing through. Some of these ports support two or more of the four shipping categories (i.e. cargo ships, ferries, leisure boats and fishing vessels) considered in this study. To limit the number of ports to be considered in the final ranking we applied the following criteria:

- Cargo ports with an annual throughput of 1 million tons or more
- Ports with international ferry routes
- Ports with national ferry routes connecting major cargo ports.

These criteria were chosen to represent ports with shipping activities primarily associated with ballast water exchange activities.

"Statistics Denmark" publishes data on maritime transport. In this study, ports with an annual throughput of over 1 million tons are considered major ports as more data is available for these ports. The major ports in 2018 then consist of 28 ports: Asnæs Inter Terminals, Asnæsværkets, Kalundborg, Statoil (Kalundborg), Copenhagen, Fredericia, Aarhus, Aalborg, Aalborg Portland, Esbjerg, Grenaa, Thyborøn, Hirtshals, Stigsnæs Inter Terminals, Stigsnæsværket, Gulf-havnen Inter Terminals, Odense, Rønne, Frederikshavn, Kolding, Køge, Randers, Avedøre, Studstrup, Frederiksværk, Aabenraa, Ensted Inter Terminals and Enstedværkets. In addition to the major cargo ports we also included the 5 ports of Helsingør, Rødby, Nexø, Havneby, and Gedser while these have international ferry routes, and we included the 5 ports of Omø, Anholt, Sjællands Odde, Samsø and Læsø because they have ferry routes to major cargo ports.

Due to the proximity between some of the ports, we merged the information of Asnæs Inter Terminals, Asnæsværkets, Kalundborg and Statoil, which together we considered as "Kalundborg"; we merged Aabenraa, Ensted and Enstedværkets, which together are referred to as "Aabenraa"; and lastly, we merged Stigsnæs, Stigsnæsværkets and Gulf Havnen which are referred as "Stignæs". Thus, a total number of 31 of major ports (or port locations) were included, illustrated in Figure 1. The ports of Aalborg, Aalborg Portland and Randers were included in the shipping activity analysis, but these ports were not included in the natural dispersal analyses as outlined in the following section due to their locations outside the hydrodynamic model domain (see later).



Figure 1. Map showing the approximate locations of the 31 Danish ports (or port locations) included in the study.

### 3.2.3 Risk factor criteria and data basis

The criteria applied for translating available statistics to a score for each risk factor are given in Table 2. A short description of each factor and the data applied are subsequently given below the table.

Table 2. Criteria used in the Prelimenary Risk Assessment of Danish Ports into five risk factor categories,
adapted from "Initial Risk Assessment of Alien Species in Nordic Coastal waters" (Gollasch & Leppakoski,
1999).

Risk factor	1	2	3	4	5
Shipping - Cargo Vessels					
A. Volume of ballast water (in thousand tonnes)	< 332	< 662	< 994	< 1325	< 1656
B. Number of ship arrivals in the area (Number of calls)	< 409	< 818	< 1227	< 1636	< 2045
C. Major shipping routes to areas of matching climate [%]	< 20	< 40	< 60	< 80	< 100
D. Major shipping routes to areas of matching salinity [%]	< 20	< 40	< 60	< 80	< 100
E. Loaded goods in international traffic (in thousand tonnes)	< 803	< 1605	< 2407	< 3210	< 4013
Shipping – Ferries					
F. Number of ship arrivals in the area (number of calls)	< 2894	< 5787	< 8681	< 11575	< 14468
G. Average Distance between routes (in km)	< 101	< 202	< 302	< 403	< 504
H. Number of routes	1	2	3	4	5
Shipping - Fishing vessels					
I. Number of ship arrivals in the area (number of calls)	< 1648	< 3295	< 4942	< 6590	< 8237
Shipping - Leisure boats					
J. Number of berths	< 220	< 440	< 660	< 880	< 1100
Habitat					
K. Degree of water pollution (presence of shipyards or power plants (PP))	PP in the bay		Shipyard or PP		Shipyard and PP

#### A. Volume of ballast water

The volume of discharged ballast water is assumed to be correlated to the probability of future species introduction (Hallegraeff and Bolch 1991, 1992). Because no data exists on the total amount of ballast water released, we calculated volume of ballast water discharged in each port based on the method developed by Stephan Gollasch (n. d.) originally developed to estimate ballast water discharged in Germany. Ballast water are typically released and taken up during port visits when cargo are loaded and un-loaded, and thus the calculation is done based on the number of calls to each port and considering a number of additional parameters. The model by Gollasch (n.d.) is available online as a Microsoft Excel application, and the equations in this model can be summarized as:

#### Discharged ballast water of foreign origin (t)

- = Number of calls of an specific type of vessel
- \* average deadweight tonnage (DWT) of ships
- \* maximum ballast tank capacity in percentage of DWT
- $\ast$  volume of ballast water onboard  $\ast$  percentage of discharged ballast water in ports
- \* (1 + percentage discharged in water ways)
- \* percentage of discharged ballast water of foreign origin

#### Where:

The number of calls of each specific type of vessel is extracted from *SKIB23* Call of cargo ships and cruise ships on major Danish ports by time seaport and type of vessel (Statistics Denmark).

For containerships, bulkers, reefers, tankers, passenger ships and special ships, respectively, the following values are used:

- Average DWT of ships: 29 990, 44 000, 10 700, 125 00, 46 000 and 1350.
- Maximum ballast water capacity: 27.8, 3.8, 14.7, 33.0, 5.9 and 9.7 %.
- Percentage of discharged ballast water in ports: 20, 10, 15, 60, 5 and 5%.
- Volume of ballast water onboard: 30, 1, 10, 33, 10, 10 multiplied by export trade balance which is 36.7% (based in loaded goods divided by the inwards and outwards).
- Discharged ballast water in waterways: 10, 10, 5, 5, 5 and 5%.
- Discharged ballast water of foreign origin: 79.4% (based on the unload in international traffic divided by unloaded goods in total).

Finally, the total ballast water is the sum of discharged ballast for the six types of vessels:

#### Total ballast water (t)

- = Discharged ballast water of foreign origin<sub>container</sub>
- + Discharged ballast water of foreign origin<sub>bulker</sub>
- + Discharged ballast water of foreign origin<sub>reefer</sub>
- + Discharged ballast water of foreign origin<sub>tanker</sub>
- + Discharged ballast water of foreign origin<sub>passenger ship</sub>
- + Discharged ballast water of foreign origin<sub>special ship</sub>

We obtained the data on trade and ship characteristics from the online statistical table *SKIB72 Throughput of goods in major Danish seaport by type of goods, seaport, direction and time summed for all major ports* (Statistics Denmark).

Two types of cargo vessels, "barges" (approximately 4% of the calls) and "other general cargo ships" (approximately 21%), were excluded from the analysis because these had no estimation in Gollasch model. According to Kern and Stuer-Lauridsen (2009), a significant proportion of calls from "other general cargo ships" are from smaller vessels engaged in local or regional trade.

We extracted number of calls for the major ports from the statistical table *SKIB23 Call of cargo ships and cruise ships on major Danish ports by time, seaport and type of vessel* (Statistics Denmark) for the year 2018 (Figure 2).

For each of the major ports the calculated amounts of discharged ballast water from the different types of vessels are shown in figure 3.



Figure 2. Number of calls of cargo ships for Danish ports in 2018. In blue is the total number of calls from containerships, bulkers, reefers, tankers, passenger ships and special ships included in the analysis. In orange and grey are the number of calls from barges and other general cargo ships respectively, both of which are not included in the analysis. The size of each bar represents the total number of calls for each port.



#### Figure 3. Total ballast water volume from cargo ships estimated based in Gollasch (n.d.).

#### B. Number of cargo ship arrivals in the area

The number of calls in the port would be a risk indicator since one single ship is able to introduce a NIS, however multiple introductions, due to high number of ship arrivals increase the chances of successful introductions (Gollasch and Leppakoski, 1999). We extracted the number of calls from cargo ships for the major ports (figure 4) from statistical table *SKIB23 Call of cargo ships and cruise ships on major Danish ports by time seaport and type of vessel* (Statistics Denmark).



Number of calls - Cargo ships

Figure 4. Number of cargo ship calls to major Danish ports.

#### C. Major cargo shipping routes to areas of matching climate

An important risk factor for an introduction of NIS from distant regions is the climate conditions. In the case where the climate conditions are similar in the source and destination areas, it is more likely that a NIS taken in at one place can survive and thrive in the new location. We extracted total throughput of goods in tonnes for each major Danish port with reference to country of origin for major international shipping routes from the statistical table *SKIB44 Throughput of goods in international traffic in major Danish ports by direction, seaport, country and time* (Statistics Denmark). We consider a matching climate (Table 3) when the other country also has a cold temperate climate (Kern and Stuer-Lauridsen, 2009).

Table 3. List of countries with climate matching with Danish ports according to Kern and Stuer-Laurid	dsen
(2009).	

Germany	Estonia	Faroe Islands	Iceland
Lithuania	Finland	Ireland	Netherlands
Norway	United Kingdom	Latvia	Canada
Poland	Belgium	Sweden	

We divided the throughput of goods from countries with matching climate (Table 3) by the total throughput of goods (figure 7) to estimate a percentage of major shipping routes from areas of matching climate (Figure 5).



#### Figure 5. Percentage of throughput of international goods coming from ports of matching climate.

#### D. Major shipping routes to areas of matching salinity

Another important risk factor for introduction of NIS is the water salinity. If the salinity is very different between source and destination ports, the NIS that can survive and establish will be limited to species that have a very wide salinity tolerance range.

To identify major shipping routes to areas of matching salinity, we extracted data for each of the major Danish ports from the statistical table *SKIB44 Throughput of goods in international traffic in major Danish ports by direction, seaport, country and time* (Statistic Denmark), including the total tonnes and the country origin.

We used the salinity criteria developed by Kern and Stuer-Lauridsen (2009), which discriminates between ports located in the Baltic Sea and the North Sea (Table 4), where the line from Skagen to Gothenburg separates the Baltic Sea and North Sea (Kern & Stuer-Lauridsen, 2009).

#### Table 4. Criteria used to determine matching salinity.

	High risk salinity
Baltic Sea	7 – 22.5
North Sea	28 - 35

If there was a match between salinity of port and country of ship origin (Table 5), then we summed the tonnes of goods from countries with matching salinity and calculated the percentage relative to total tonnes of goods (Figure 6).

Baltic Sea	North Se	a
Germany	Germany	Malta
Lithuania	Netherlands	Albania
Netherlands	Norway	Slovenia
Norway	Sweden	Croatia
Poland	Turkey	Serbia and Montenegro
Sweden	Central and South America	Greenland
Romania	Belgium	Africa, North
Russia	Faroe Islands	Africa, West
Bulgaria	Ireland	Australia
Albania	United Kingdom	Cyprus
Turkey	Canada	United States of America
Central and South America	Iceland	Asia
	Portugal	Near and Middle East
	France and Monaco	Italy
	Greece	Spain

Table 5. List of countries with salinity matching based in Kern and Stuer-Lauridsen (2010).



Figure 6. Percentage of throughput of international goods coming from ports of matching salinity.

#### E. Loaded goods in international traffic

We extracted the amount of loaded goods in international traffic from the statistical table *SKIB72 Throughput of goods in major Danish seaport by time, type of goods, seaport and direction* (Statistics Denmark), Figure 7). This risk factor was included because ports exporting goods discharge ballast water (Andersen et al. 2014), and since release of ballast water takes place when the ship arrives at the port following cargo unload. When the cargo is being loaded the water from the ballast tanks are discharged by pumps (Rata et al. 2018) and we choose international traffic to consider primary and secondary introduction of NIS regions outside Denmark.



Figure 7. Loaded goods via international traffic.

#### F. Number of ferries arrivals in the area

Ferries typically operate very intensively between a limited numbers of ports, and thus, potentially serve as key transfer of NIS between parts of the country or internationally, especially when a connecting port can be interpreted as a hotspot for NIS introduction for other reasons, such as being a major cargo port. We extracted the number of international ferry calls from the statistical table *SKIB32 International transport by ferry by time, ferry route and unit* (Statistics Denmark). Similarly, we extracted number calls from domestic routes connecting to major Danish cargo ports from the statistical table *SKIB31 Domestic transport by ferry by unit, ferry route and time* (Statistics Denmark). The sum of calls is shown in Figure 8.



Figure 8. Number of calls from domestic and international ferries.

#### G. Average Distance of ferry routes

The reason for including the distance of ferry routes is the assumption that the longer the distance the larger the risk of introducing species not yet introduced in the area. The average distances of ferry routes were based on data on the international and domestic ferry traffic (Statistical tables SKIB32 and SKIB31 from Statistics Denmark). We estimated the weighted average distance between the different routes (Figure 9).



Figure 9. Weighted mean distance of ferry routes.

#### H. Number of ferry routes

The risk of introducing NIS is expected to increase the higher the number of ferry routes, thus ports with multiple ferry routes could be central for transferring NIS between regions. The number of domestic and international ferry routes for each port is based on the statistical tables *SKIB32* and *SKIB31* (Statistics Denmark) (Figure 10).



Figure 10. Number of ferry routes including international routes and national routes with a connection to one of the major cargo ports.

#### I. Fishing vessels – Number of ship arrivals in the area

Fishing vessels have been identified as a potential vector for NIS (Asthon et al. 2012), however no studies on Danish fishing ports or on Danish fishing vessels as a vector have been reported. Here we assume that the number of calls by fishing vessels is correlated to the risk of introduction of NIS. We extracted the number of calls of fishing vessels from the Danish Fisheries Analysis Database (DFAD) for the year 2018, Figure 11 (Logbook data, Danish Fisheries Agency). The number of calls include only fishing vessels registered in Denmark, and only ports with calls from two or more fishing vessels. Data from 2018 was compared with data from 2013-2017 showing only a limited variability between years. Although a number of foreign fishing vessels is known to enter Danish ports, data on the number of calls from these vessels was not available.



Figure 11. Number of fishing calls to the selected 28 Danish ports or port locations.

#### J. Number of berths

The number of calls of leisure boats would be expected to correlate with the risk of introduction of NIS, however such data is not available. Instead, we use the number of berths as a proxy for the leisure boat activity. Number of berths in Danish marinas were obtained from a dataset collected as part of a project mapping marinas in Denmark (Miljøstyrelsen, 2014). This dataset included 365 marinas, however, for 65 of these no data had been found on the number of berths. For these 65 marinas we searched a variety of other data sources on the web<sup>1</sup> including the homepages of individual marinas, and found data available for 40 marinas resulting in a total of 340 marinas included in the analysis. Numbers of berths for the 28 selected ports are shown in Figure 12. The number of berths was considered as a risk factor since local traffic of leisure boats may facilitate secondary spread of NIS towards other regions (Ferrario et al., 2017). Indications are found in a study from California which found strong correlation (Spearmans' r < 0.001) between the number of berths available and the number of fouling NIS recorded in a bay (Ashton et al. 2012).

<sup>&</sup>lt;sup>1</sup> Danskehavnelods.dk; Sailbuddy.com; Kommas havnelods; marine-guide.dk; portsmap.com;



Figure 12. Number of berths of Danish marinas connected to major cargo and ferry ports.

#### K. Degree of water pollution

This factor was adapted from Gollasch and Leppakoski (1999), which used one category for shipyards and another for water pollution (eutrophication, chemical, urban waste and power plants). Shipyards is added as a risk factor since regions with intensive ship building industries receive higher amounts of NIS due to ballast water and sediment discharges during maintenance work in shipyards (Gollasch and Leppakoski, 1999). Likewise, power plants is added as a risk factor because cooling water discharge can offer a favourable environment for the survival and reproduction of NIS and act as a stepping stone towards spread into other environments (Gollasch and Leppakoski, 2007). For example, the NIS species *Mytilopsis leucophaeata* was found in the vicinity of a Finish power plant in 2003 (Laine et al. 2006), later, in 2006, it was found near another Finish power plant (Laine and Urho, 2007), and then in the spring of 2011 it was found near a power plant in Sweden (Florin et al 2013). In the present study we address one point to a port if there is a shipyard or power plant, three points if there is a shipyard or a power plant in the port and five if both are present in the port, Figure 13.



Figure 13. PRA score points due to the presence of power plant and/or shipyard in the port.

## 3.3 Results

We created the preliminary risk assessments (PRA) based on all risk factors described in the previous section, and except for Helsingør, all ferry ports obtained the lowest ranking (figure 14). Helsingør scored 13 points, more than four cargo ports (Avedøre, Odense, Studstrup and Aalborg Portland), due to the high number of berths in the marina, which gave 5 points and high number of ferry calls, which also gave 5 points. Other ferry ports scored a maximum of 8 points.



Figure 14. Major Danish cargo ports and important ferries ports classified according to the eleven risk factors included in the preliminary risk assessment (PRA). Important ferry ports not considered a major Danish cargo port are indicated with a star next to the labels.

Based on the points scored from the preliminary risk assessments we ranked ports (Table 6).

	DP A Pank	PRA Score	
Copenhagen		35	<u> </u>
Echiora	2	32	
	2	31	
Hillshals Kalundhara	3	30	
Aarbus	4	27	
Aarnus	5	26	
Frederiksnavn	6	25	
	7	23	
Inyborøn	8	24	
Grenaa	9	22	
Rønne	9	22	
Køge	10	21	
Kolding	11	17	
Aabenraa	12	15	
Randers	13	14	
Aalborg	13	14	
Frederiksværk	13	14	
Stigsnæs	14	13	
Helsingør	14	13	
Avedøre	15	12	
Odense	15	12	
Studstrup	16	11	
Aalborg Portland	16	11	
Rødby	17	8	
Nexø	18	6	
Anholt	18	6	
Gedser	19	5	
Havneby	19	5	
Omø	19	5	
Læso	19	5	
Siællands Odde	20	4	
Samsø	20	4	

Table 6. Ranking based on the preliminary risk assessment. In bold major cargo ports, and the others are ports included due their ferry activities (either international traffic or connection with major cargo ports).

## 3.4 Discussion

The focus of the preliminary risk assessment (PRA) of Danish ports presented here is the major Danish cargo ports considered as the potential hotspots for introductions of NIS via ballast water. We also included ferry ports with international routes and ferry ports with routes connecting to major Danish cargo ports. All ferry ports, except Helsingør, are located at the end of the ranking with scores more than half of those of the lowest ranging cargo ports.

The method we have applied for a PRA for Danish ports is relatively simple and will be sensitive to changes in the assumptions on which the method is based, e.g. the type and number of risk factors included. For example, the number of risk factors within each group "shipping traffic" and "habitat characteristics" affects the relative contribution of each of the two categories to the overall score. As an example, the risk factor group "shipping traffic" has 10 risk factors while the risk factor group "habitat characteristics" has only 1 risk factor, and thus the overall score will be dominated by risk factors associated with shipping traffic. Another example is risk factor sub-group "leisure boats" which are only represented by one subcategory (number of berths). However, due to a number of studies finding strong links between leisure boat activities and distribution of NIS, it could be argued that the contribution of leisure boats to the overall risk score should be higher.

An option to account the effect for the number of risk factors included could be to apply weights to each risk factor to reflect their relative importance. Justifications for a general application of weights can be difficult since it will be subject to interpretation, and weights may vary according to in which context of the decision making process the port ranking will be applied. Therefore, we present the raw scorings only to ensure maximum transparency of the presented rankings, and instead we suggest that the rankings should be reviewed critically. Depending on the application of the ranking in the decision-making process, the relevance and importance of each risk factor should be considered carefully and any subsequent inclusion/exclusion of risk factors, or weighting, should be considered.

To facilitate the decision making process based on the PRA presented here, we recommend a dynamic spreadsheet where factors can be selected or deselected and the ranking subsequently automatically updated. A dynamic spreadsheet can easily be extended to add weights to each of the included risk factors. This way, relevant experts can review the relative importance of individual risk factors, and the weights of individual risk factors can be adjusted accordingly.

## 3.5 Conclusions

The aim of this section was to rank Danish ports according to shipping activities as a proxy for the risk of introduction of non-indigenous species. The ranking is based on a preliminary risk assessment (PRA) originally developed for German conditions and adopted to Danish conditions using 11 critically selected risk factors. Each risk factor was divided into 5 scores (1-5 with 5 indicating the highest risk), and a simple linear assumption was applied for translating the underlying statistical data used for each risk factor into each of the 5 scores.

In the PRA for Danish ports, we considered the major Danish cargo ports as the first priority represented by five risk factors since these ports receive the largest volumes of ballast water from a variety of both national and international destinations, and since ballast water is the focus of the BWMC. A range of different types of vessels (container, bulker, reefer, tanker, special ship and cruiser ship) was considered which differs with respect to their potential for the release of ballast water. These differences were taken into account when ballast water volumes were estimated using a model previously developed by Gollasch (n.d.). Ferries were given second priority in the PRA using three risk factors. Ferries represent strong links for the transfer of NIS between fixed destination and are of particular concern when one of the destination ports are also a major Danish cargo port. Due to the low amount of ballast water in the case of fishing and leisure boats we attributed only one risk factor each of these. In total, 10 risk factors was included representing

shipping traffic. In addition, one risk factor considering the habitat characteristic of the port was included represented by the absence or presence of shipyards and/or power plants. We subsequently calculated the sum of PRA scores for 28 major Danish cargo ports and/or ferry ports.

The overall conclusions of the ranking of ports according to ship activities:

- The major Danish ports with the highest PRA scores (between 20 and 35) included in the study and thus with the highest risk to introduce and spread invasive species are (in descending order) the 11 ports of Copenhagen, Esbjerg, Hirtshals, Kalundborg, Aarhus, Frederikshavn, Fredericia, Thyborøn, Grenaa, Rønne and Køge.
- The major ports with intermediate PRA scores (between 11 and 17) are Kolding, Aabenraa, Randers, Aalborg, Frederiksværk, Stigsnæs, Helsingør, Odense, Avedøre, Aalborg Portland and Studstrup.
- The major ports with the lowest PRA scores (between 4 and 8) are all ferry ports except for Helsingør, and include Rødby, Nexø, Anholt, Gedser, Havneby, Omø, Sjællands Odde, Læso and Samsø.

## 4. Ranking ports based on natural dispersal of nonindigenous species

## 4.1 Introduction

While the "primary introduction" (i.e. of NIS in the marine environment) typically refers to the introduction of species directly from its native region via ballast water, hull fouling or another anthropogenic vectors of introduction, "secondary introduction" typically refers to the subsequent spread of NIS from the site of primary introduction (Minchin et al. 2009) to the surrounding geographic region. Apart from such additional spread via anthropogenic vectors, secondary spread would be driven by natural dispersal. This could be by active movement and migration behaviour of larger organisms, or by a predominantly passive dispersal by ocean currents of pelagic life stages including planktonic larvae, seeds or spores.

The focus of this section is the natural dispersal of pelagic life stages of benthic NIS with limited pelagic larval duration time (e.g. days to weeks). The rationale for this prioritisation is outlined in Hansen and Christensen (2018b). In short, if the natural dispersal of a NIS in an area is likely to be high, and if the NIS could be introduced to one port within the area, there is a high risk that this NIS may spread to the surrounding region and ports by natural dispersal. Thus, in the case of a high natural dispersal the release of untreated ballast water taken up by ships in the first port and released in another neighbouring port may impose only a low additional risk. If this can be considered a low and acceptable risk for a given NIS and area of concern, authorities may grant an exemption to ship owners that operate exclusively within such an area, permitting ship owners to release ballast water without prior treatment. This type of an area based risk assessment, is referred to as a Same-Risk-Area (SRA) (Stuer-Lauridsen et al. 2016, Stuer-Lauridsen et al. 2018) , and an SRA risk assessment is explicitly mentioned in the G7 guideline to the BWMC<sup>2</sup>. Thus, benthic NIS with limited movement and migration behaviour during adult life stages, and with relatively short pelagic larvae durations (days to weeks) are expected to be the primary NIS of concern in an SRA risk assessment addressing a possible exemption to the BWMC.

An SRA case study for Kattegat and Øresund (Hansen and Christensen 2018b) analysed 23 NIS including NIS with a potential for being introduced to, or already introduced but not yet widely distributed within, the Kattegat and Øresund region. The study analysed the dispersal potential of all 23 NIS in the region using larval dispersal modelling based on oceanographic data (currents, salinity and temperature), species biology data (Pelagic Larvae Duration, spawning period, salinity tolerance, vertical positioning in the water column, habitat preferences) and habitat data (seabed substrate, depth, salinity). Connectivity analyses were used, including cluster analyses techniques, to identify areas which are potentially highly connected (referred to at hydrographic regions) and for identifying the potential location of possible dispersal barriers.

In the present project we employ a similar approach as in the SRA Case Study (Hansen and Christensen 2018b) extending the study area to cover all Danish coast lines and adjacent marine areas, and including the same 23 NIS as in the Case study. Based on the larval dispersal modelling results we analyse the dispersal potential *of*, and the connectivity *between*, the 28 major Danish ports (or port locations) described in the previous section. We present three different methods for

<sup>&</sup>lt;sup>2</sup> Resolution mepc.289 (71) adopted on 7 July 2017 in the guidelines for risk assessment under regulation a-4 of the BWM convention, G7.

ranking the 28 ports according to both the connectivity to other ports as well as to the dispersal potential to other parts of the marine system.

## 4.2 Methodology

#### 4.2.1 Overview

The methodology used for ranking ports according to natural dispersal of NIS is divided into the following parts:

- Target species selection
- Larval dispersal modelling
- Connectivity analyses
- Ranking of ports according to connectivity to other ports
- Ranking of ports according to dispersal potential

### 4.2.2 Target species selection

Because natural dispersal is considered a limiting factor in a risk assessment addressing the possibility for granting exemption to the BWMC (specifically an SRA), only sessile NIS with limited dispersal potential are considered. Species NOT considered include:

- Species with the entire life cycle in the water column
- Species that are already fully established in the region
- Species with no or very limited salinity tolerance (<10 PSU)</li>
- Macro algae and macrophytes with typically long dispersal ranges (drifting tallus or drifting reproductive organs)

The 23 species included in the study are shown in table 7 along with collected relevant biological parameters (for details see: Hansen and Christensen 2018b, appendix 1)

Table 7. Short-list of non-indigenous marine species included in the study. From Hansen and Christensen (2018b). Species-specific data include life history traits and environmental tolerances retrieved or estimated from exiting databases and the literature. Values followed by a '\* ' are based on assumptions where no empirical data or descriptions could not be found.

SPECIES	Taxon	PLD (min)	PLD (max)	Generations per year	Spawning start	Spawning end	Habitat Substrate	Habitat Depth	Temp. Min (Adult)	Temp. Max (Adult)	Salinity Min (Adult)	Salinity Max (Adult)	Temp. Min (Larvae)	Temp. Max (Larvae)	Salinity Min (Larvae)	Salinity Max (Larvae)
		days	days	no.s	month	month	type	m	С	С	PSU	PSU	С	С	PSU	PSU
Arcuatula senhousia	Mollusca	14	55	1	7	8	All	20	0	33	17	35	22.5	30	17	30
Asterias amurensis	Echinodermata	41	120	1	6	10	All	220	0	25	18	41	17	20	18	41
Austrominius modestus	Crustacea	10	15	1	5	10	Hard	5	0	26	14	40	6	25	25	32
Bugula neritina	Bryozoan	0.5	2	1	7	9	Hard	10	0	25	18	30	12	26	14	32
Bugulina simplex	Bryozoan	1	1	1	7	9	Hard	20	0	25	18	40	?	25	18	40
Callinectes sapidus	Crustacea	31	49	1	5	8	Mud, Sand	36	5	30	3	40	15	25	20	40
Crassostrea gigas	Mollusca	21	28	1	7	8	All	15	3	35	12	42	18	26	10	42
Didemnum vexillum	Tunicata	0.5	1	1	7	9	Hard	65	2	28	18	40	14	20	18	40
Ensis directus	Mollusca	14	21	1	3	4	Mud, Sand	12	0	26	7	32	15	28	15	32
Eriocheir sinensis	Crustacea	30	60	0.5	3	7	All	10	0	25	0	30	12	35	15	32
Ficopomatus enigmaticus	Annelida	20	25	1	7	9	hard	10	0	30	5	40	18	26	10	30
Hemigrapsus sanguineus	Crustacea	16	55	1	5	9	Sand, Hard	40	5	30	15	33	15	30	20	35
Hemigrapsus takanoi	Crustacea	30	30	1	5	9	All	20	0	20	7	35	15	30	25	35
Hydroides dianthus	Annelida	5	14	2	6	10	Hard	200	5	30	28	50	?	20	25	50
Laonome calida	Annelida	1	1.5	1*	7*	8*	All	40*	0	30	0.1	35	?	25	0.1	35
Marenzelleria viridis	Annelida	28	49	1	9	11	Mud	65	0	25	1	32	15	25	5	30
Mytilopsis leucophaeata	Mollusca	6	14	1	5	10	Hard	40	5	37	0	20	13	27	1	25
Mytilus galloprovincialis	Mollusca	14	28	1	6	9	Sand, Hard	40	0	31	12	38	15	25	10	38
Palaemon macrodactylus	Crustacea	15	20	6	4	10	All	40	2	26	1	36	15	27	1	34
Potamocorbula amurensis	Mollusca	14	21	2	5	10	All	30	0	30	0.1	32	6.4	23	0.1	27.6
Rangia cuneata	Mollusca	7	7	0.5	5	10	Mud, Sand	15	1	29	1	15	8	30	2	20
Rapana venosa	Mollusca	14	80	1	4	11	All	40	4	27	7	32	13	26	15	30
Rhithropanopeus harrisii	Crustacea	7	43	1	6	9	Hard	37	0	35	5	30	14	27	5	30

## 4.2.3 Larval dispersal modelling

#### Hydrodynamic data

Data on ocean current speed and direction, water temperature and salinity were extracted from a hydrographic dataset generated by the hydrodynamic model, HBM, for the North Sea, Skagerrak, Kattegat, Inner Danish Straits and the Baltic Sea (for details: Berg and Poulsen, 2012). The spatial resolution of the model is 5 nm in the North Sea, Skagerrak and central and eastern Baltic Sea, and 0.5 nm in the transitional areas of Kattegat, Inner Danish Straits (IDS) and the western Baltic Sea. The vertical resolution is 50 and 52 layers respectively. As in Hansen and Christensen (2018b) three years were selected based on inter annual variations in the North Atlantic Oscillation index (NAO index), namely 2005, 2010 and 2012 representing a "neutral", a "negative" and a "positive" NAO index respectively to reflect expected range of hydrographic variations between years.

#### Agent Based Model

The simulation of larval dispersal was carried out using the SRAAM tool (Hansen and Christensen, 2018a). The tool consists of an agent-based modelling library (IBMlib) which is a freeware developed by DTU Aqua (Christensen, 2008; Christensen et al., in review). The IBMlib implementation in the SRAAM tool supports a number of larval behaviours and parameters important for predicting larval dispersal. The larval behaviour parameters and inputs used in the larval dispersal modelling for this study include:

- Pelagic larval duration
- Dispersal depth interval
- Spawning start and end
- Spawning and settling habitat
- Vertical dispersion
- Horizontal dispersion

During the larval dispersal simulation, IBMlib keeps track of start and end positions of each simulated larvae, and the environmental conditions experienced during the pelagic phase such as the minimum and maximum values of salinity and temperature. The minimum and maximum salinities are used as input to the connectivity analysis to construct connectivity matrices and to account for environmental tolerances, see later.

The pelagic larval duration (PLD) represents the duration of the life stage (typically a larval stage) where the species drift freely in the water column and hence are subject to passive transport by ocean currents. At the end of the PLD, the larvae will then settle on the seabed. In the current study we use the minimum values of the PLD reported for each species. Data on beginning and end of spawning are typically given by respective months of the year and with a reference to specific locations. We use these start and end months as input to the larvae dispersal simulations interpreting the start month as the first day of the month and the end month as the last day of that month. Dispersal depth during the PLD was set to between 0 - 40 meters to comply with general patterns in vertical distribution of pelagic larvae observed by Corell et al. (2012) in the Baltic Sea. To ensure a random distribution across this depth interval, we applied a constant vertical dispersion of  $0.001 \text{ m}^2/\text{s}$ . Horizontal dispersion is included primarily to reflect the unresolved hydrodynamics of the hydrographic data at scales smaller than the spatial resolution of the model. The horizontal dispersion was set to  $10 \text{ m}^2/\text{s}$ .

#### Habitat

Habitat maps for each species were created based on information on species specific preferences of seabed substrate, depth distribution and adult life-stages salinity tolerances (**Appendix 1**).

Seabed substrate distribution was derived from data available under the European Marine Observation Data Network (EMODnet) Seabed Habitats project (http://www.emodnetseabedhabitats.eu/), funded by the European Commission's Directorate-General for Maritime Affairs and Fisheries (DG MARE). EMODNET substrate data classification was regrouped into three main categories "Mud", "Sand" and "Hard substrate". In both the "hard" substrate and "Sand" categories, we included "Mixed Sediments" and "Coarse Sediments" to reflect transition between the two habitat types. The category "hard substrate" was supplemented by coastline geomorphological classification available from the European Environmental Agency (EEA) representing coastlines with hard substrates (category A "Rocks and Hard Cliffs", and category J "Harbor areas"). For the coastline of southern Norway, we assumed that the entire coastline consisted of hard substrates.



Figure 15. An example of a habitat map for one out of 23 non-indigenious species included in the study(*Arcuatula senhousia*) created based on species preferences of water depth, seabed substrate and salinity tolerance of adult life stages. Green: Optimal salinity conditions. Yellow: Suboptimal salinity conditions, where salinity levels are outside the range of the salinity tolerance of the species in shorter or longer periods.

Data on water depth was based on GEBCO bathymetry data set (IOC, IHO and BODC 2003). Data on salinity was based on the hydrographic data from the HBM model by extracting

minimum and maximum values of bottom salinity for each year 2005, 2010 and 2012, and by interpolation between extraction points (we used inverse distance weighted interpolation, IDW).

Habitat maps were produced as raster grids with a 0.01° grid resolution. See **Appendix 1**. An example of a habitat map for *Arcuatula senhousia* is shown Figure 15.

#### Simulation setup

The full spatial extent within which the larval dispersal simulations for each species were carried out was set to a gross area extending 4° - 16° E, and 53°– 60° N (Figure 16). This was done to include not only the study area of the Danish coastal regions, but also to include the adjacent areas considered to affect the larval dispersal and population connectivity outputs.



Figure 16. The spatial extent of the larval dispersal model setup for a gross area extending 4°-16° E, and 53°- 60° N (red outline). Blue colors show bathymetry (IOC, IHO, BODC 2003).

Three sets of simulations scenarios were carried out:

 Agents distributed in all potential habitats (species specific) By distributing agents randomly throughout all potential habitat it is possible to analyse multiple generation (stepping stone) dispersal for individual species.

The setup for each species and for each year (in total 69 simulations) included 200 000 agents (in total 600 000 agents for all three years per species) distributed randomly in space within the maximum area coverage of the species habitat map, and randomly in time within the spawning period

Agents distributed only in each of the 28 port locations (species specific)
 This scenario was used specifically to analyse the downstream dispersal probability for each of the 28 port locations for a single generation for individual species.
 The setup for each species and each year (in total 69 simulations) included 10 000 agents distributed randomly within, and adjacent to, each of the 28 ports (in total 840

000 agents for all three years per species), and randomly distributed in time within the spawning period.

3. Agents distributed only in each of the 28 ports locations (no species parameters) This scenario was included to analyse the minimum dispersal duration between ports.

The setup considered only passive drifting agents without species-specific traits, habitat preferences or environmental tolerance. Three simulations were set up, one for each year, including 20 000 agents distributed randomly within and adjacent to each of the 28 ports, and randomly over the course of the simulation. The simulation period was set to 1 March to 31 December, and particles could settle after one day until 91 days. Agents were programmed to drift until a neighbouring port location was reached and the duration time was recorded.

For all three scenarios, we used a time step of 1800 seconds.

#### 4.2.4 Connectivity analyses

As a basis for ranking ports according to natural dispersal of marine NIS, we use larval dispersal probability maps, and connectivity analyses of larval dispersal modelling results similar to the methodology outlined in Hansen and Christensen (2018b).

The connectivity analyses were based on a sub-division of the extended area (Figure 17) into a regular grid of 40 x 24 cells corresponding to a spatial resolution of approximately 0.3 degree in both the latitudinal and longitudinal direction, in the following referred to as the connectivity grid. Connectivity adjacency matrices were constructed from the larval dispersal modelling results comprising start and end positions of each agent, and counting the number of all pairwise connections between sub-areas in the connectivity grid. Only agents with end positions within the maximum range of the species specific habitat were included.



Figure 17. The subdivision of the study area into subareas using a regular grid of ca. 0.30x0.30 decimal degrees (~20x30 km) applied for the connectivity analysis.

The connectivity adjacency matrices with absolute numbers of connections were lumped into one matrix for each species representing all years and subsequently translated into connectivity probability matrices for each species. Hydrographic regions were delineated using cluster analysis, each cluster representing assemblies of sub-areas (grid-cells in the 40 x 24 connectivity grid) where the connectivity between sub-areas within the clusters is high, and where the connectivity to neighbouring clusters is low. Here we use the clustering method "Infomap" (Rosvall and Bergstrom, 2008) available in the R package "igraph". The Infomap method is based on information theory principles and has been used previously to delineate hydrographic regions in the Mediterranean (Rossi et al., 2014) and the Kattegat and eastern Baltic Sea (Hansen and Christensen, 2018b). An example of a graphic representation of the connectivity analysis and the clustering of hydrographic regions is shown in Figure 18. All hydrographic regions delineations were calculated based on an assumption of multiple generation stepping stone dispersal (see: Hansen and Christensen 2018b) using the estimated number of generations within a 5 year period for each species and a between generation survival of 10%.



Figure 18. Example of a graph plot representing the outline of hydrographic regions (individual coloured polygons) identified for the species *Arcuatula senhousia* based on larval dispersal simulation results for the year 2005 using an initial number of 50 000 agents per year. The number of agents successfully settled within suitable habitat included in the connectivity analysis is 11 018 (indicated in the top left corner by "n"). The WITHIN region connectivity for each region is represented by node values (within circles) representing the percentage of agents with an initial position in each region that end up in the same region. The BETWEEN regions connectivities are indicated by arrows representing the direction of the connectivity and arrow thicknesses representing the relative magnitude of the connectivity (max thickness set to 17% after which it remains unchanged). Bars next to nodes represent the number of agents supporting the delineation of each individual region relative to the region with the largest number of agents. White areas represent areas outside the larval dispersal extend due to lack of suitable habitat and/or due to unfavourable salinity conditions exceeding the larval salinity tolerance limit during drift. Land areas are displayed in grey.
In addition to the delineation of hydrographic regions, a number of maps for each species were produced visualizing the upstream and downstream dispersal probabilities for each of the 28 major ports considering multiple generation (stepping stone) dispersal. Examples of upstream and downstream connectivity maps are shown in Figure 19.

The above methodology was applied for larval dispersal simulation results from scenario 1 considering multiple generation (stepping stone) dispersal. Results from scenario 2 where agents were only released in, and adjacent to, individual ports, were used for extracting downstream dispersal probability maps for each port for a single generation. Scenario 3 was included specifically to analyse the minimum dispersal duration between ports as a measure of port connectivity (see later). Agents were programmed to drift until a neighbouring port location was reached and the duration time was recorded.

For reference, a number of sensitivity analysis was carried out during the course of the SRA Case Study for Kattegat and Øresund (Hansen and Christensen, 2018b) e.g. the between year variability, the number of agents per simulation and the influence of different drift depth ranges for the vertical distribution of agents in the water column. These sensitivity analyses were conducted to evaluate the robustness of the analyses results, and to evaluate to which degree model assumptions may affect the connectivity analysis results, i.e. the hydrographic region delineation and the downstream/upstream dispersal probabilities of individual ports. Since we apply a similar modelling approach in this study, including the same 23 marine NIS, the same 3 years and the approximately same geographical location, we refer to the findings presented in Hansen and Christensen (2018b – appendices 2 and 3).

All data analyses were carried out using the statistical and data analysis software R (R Core team 2013) using scripts and procedures developed for SRAAM tool (see Hansen and Christensen 2018a).



Figure 19. Examples of downstream (A) and upstream (B) dispersal probability maps based on multiple generation stepping stone dispersal (5 years generations and 10 % survival between generations) for the port of Frederikshavn (location indicated by a large black dot). Only agents successfully settled inside expected species habitats are included. Agents exposed to salinity levels outside the larval salinity tolerance thresholds during the pelagic phase are not included. The colour legend is linear and relative to the largest probability value in each plot. Hatched areas with light blue colours indicate dispersal probability less than 0.1 %. White areas are areas with dispersal probability of "0". Number of agents (n) included in the downstream and upstream probability plot is 354 and 1843 respectively.

#### 4.2.5 Ranking of ports according to the connectivity between ports

The ranking of ports according to the potential natural dispersal of marine NIS between ports will depend on how the potential natural dispersal and/or connectivity is analysed. At present, we are aware of only two SRA studies that have been published (Baetens et al., 2018; Hansen and Christensen, 2018b) and where dispersal modelling and connectivity analysis has been used as part of, or as a basis for, an SRA risk assessment. In the two studies, different methods have been used to address the potential natural dispersal of NIS. Hansen and Christensen (2018b) applied a species specific approach modelling the potential dispersal of individual species based on information on species traits, habitat preferences and environmental tolerances. The dispersal modelling included analysis of the potential dispersal of larvae in the entire study area considering both the direct dispersal between ports, and stepping stone dispersal via potential habitats throughout the region. Baetens et al. (2018) used the minimum dispersal duration time between ports of passive drifters as a measure for port connectivity. It was a generic approach where no species specific trait, habitat preference or environmental tolerance were included. However, three scenarios representing different vertical migration behaviour strategies were included: "passive drifter" with no active behaviour, "tidal" behaviour using stratified water currents to minimize dispersal and "counter tidal" behaviour using stratified water currents to maximize dispersal. The three scenarios represent known behaviour strategies of pelagic larvae in tidal systems. Cut-off values of 40 days of minimum dispersal time was used as a criterion for identifying ports being potentially connected.

Due to the different methodologies suggested by different studies, we here include three different methods for ranking ports based on the analysis of the potential natural dispersal of NIS:

- 1. "Cluster membership" analysis (species specific)
  - o Based on model scenario 1 (considering stepping stone dispersal)
- 2. "Dispersal probability" analysis (species specific)
  - Method 1: based on model scenario 1 0
  - Method 2: based on model scenario 2
- 3. "Dispersal duration" analysis (generic)
  - Based on model scenario 3.

Each of the methods are described in more detail below.

#### Ranking of ports based on cluster membership

The ranking of ports based on cluster membership is done using the identification and delineation of hydrographic regions, and the strengths of the connectivity between regions, as described in the previous sections. The principles of the cluster membership analysis are illustrated in Figure 20. For each port, first the number of additional ports that belong to the same hydrographic region as the port itself is counted and multiplied by the probability percentage of self-recruitment of that region. Next, the number of ports in each of the connected neighbouring hydrographic regions is counted and multiplied by the connectivity probability from the "home" region to the "destination" region. The sum of these probability weighted numbers of connected ports of all 23 species are used as the basis for ranking the ports and is referred to as the cluster membership ranking.

The use of cluster membership rather than the direct dispersal probability between ports (see later) enables us to consider stepping stone dispersal and the potential for NIS being introduced/established outside the port area itself thereby contributing to the overall connectivity between ports. The likely occurrences of NIS outside major ports are supported by studies where NIS have been registered in marinas with species diversities sometimes exceeding or diverging from those of the major ports (e.g. Ferrario et al., 2017).

38



Harbor ID	Cluster membership (species 1)			
	Count	Weighted		
1	11	6x <u>0.72</u> +3x <u>0.27</u> +2*0.01 = <b>5.15</b>		
2				

Figure 20. The principle of the cluster membership analysis. Port no. "1" is connected to 6 ports within the cluster (i.e. hydrographic region) it belongs to (red polygon). Based on the arrows indicating the BETWEEN-regions connectivity, port no. "1" is further connected to 3 ports in the "green" neighbouring region, and to 2 ports in the more distant "blue" region". In total, port no. "1" is connected 11 other ports. The weighted no. of ports (right column in the table) is calculated by multiplying the number of port connections in each region with the connectivity probability and calculating the sum. Connectivity probabilities are given by the self-recruitment percentage number in the centroid (~circle) of the region the port belongs to (e.g. 72 % for port no. "1") and the connectivity probabilities to the neighboring regions illustrated by the arrows (i.e. 27% and 1%).

#### Ranking ports based on dispersal probability

For the ranking of ports based on dispersal probability, we use the downstream dispersal probability maps (e.g. Figure 19) extracted from the connectivity probability matrices. For each of the 28 ports the downstream connectivity probability values can be directly extracted from the location of any of the other 27 ports. This is done for each species and the sum of downstream dispersal probabilities for the 23 species for each of the downstream located ports are used for ranking. Downstream probability values were extracted from positions in the connectivity grid corresponding to the approximate location of each port.

The analyses were done both for scenario 1 and scenario 2 model results. We used the original connectivity grid of the cluster analysis (40 x 24) and extracted downstream probabilities at connectivity grid cells corresponding to the port locations in the grid. The approximate locations of the 28 ports and the corresponding connectivity grid location are shown in Figure 21.



Figure 21. The connectivity grid cells associated to each of the 28 ports (red dots) used in the ranking based on dispersal probabilities between ports. Blue outline refer to grid cells of the 40 x 24 connectivity grid associated with each port location.

#### Ranking ports based on dispersal duration

For ranking ports based on dispersal duration, we used the results from dispersal modelling scenario 3. Ports are ranked according to the minimum dispersal time of simulated agents released in one port ending up in each of the other ports. Different thresholds are set, i.e. 14, 30 and 40 days, and then for each port the number of port connections is counted where connections occur within each of these thresholds. To ensure robustness of results not being influenced by outliers we use the 10 percentile of minimum dispersal time of all simulated agents connecting ports.

#### 4.2.6 Ranking of ports according to the natural dispersal potential

The ranking of ports based on the natural dispersal of pelagic life stages of NIS between the 28 major ports considered as hotspots for possible introduction of NIS as described in the previous section, will depend not only on the hydrodynamics of the area and the species specific traits, but also on the geographical proximity and distance between ports. I.e. the more ports concentrated in an area, the more likely NIS from one major port may end up in another major port by natural dispersal everything else being equal. Thus, to consider the natural dispersal potential of each major port independent of whether other major ports are located nearby, we also apply a ranking of ports based on the natural dispersal potential, indicating to which extent NIS in ports may disperse to neighbouring habitats. Here we use the results from scenario 2 where agents are released in port locations only, and we calculate for each port the sum and the mean of the downstream number of subareas in the connectivity grid where the simulated larvae will disperse to, considering all 23 NIS. We include only downstream sub areas with a dispersal probability of 0.1 % or more. The mean number of downstream sub-areas for each port is calculated including only the NIS where the simulation results show downstream dispersal different from zero.

### 4.3 Results

#### 4.3.1 Connectivity analysis results

Connectivity analysis results including maps of hydrographic regions and within and between regions connectivity probability for each of the 23 species together with potential habitat maps are shown in **Appendix 1**. Maps of downstream and upstream dispersal probabilities for each of the 28 ports and 23 species where dispersals are predicted to occur are shown in **Appendix 2**.

# 4.3.2 Ranking of ports according to the connectivity between ports **Cluster membership ranking**

The ranking for ports based on the cluster membership analysis is shown in Figure 22. In Figure 22 (upper pane) for each port the sum of weighted number of port connections (wnos) are split into destination ports, which means the relative proportion of each downstream connected port that contributes to the overall value of wnos. In figure 22 (lower pane) is shown the same ranking, however with the sum of wnos for each port split into the contribution from each of the 23 species. The ranking shows that in general ports of Bornholm and at the west coast of Jutland have the lowest potential connection to other ports, while ports in the in Kattegat has the highest potential connection, followed by ports in Øresund, Køge bugt and Fermarn Belt. While, in general, many ports contribute to the sum of cluster memberships of each port and with some although limited variations, the contribution to the cluster membership from individual species are more diverse, with e.g. some species dominating the contribution in the central parts of Danish waters among ports with the highest rankings. Example of these are *Rapana venosa, Potamorcorbula amurensis, Palaemon macrodactulys* and *Mytilus galloprovincialis*, which are all characterized by a high dispersal potential.

#### **Dispersal probability ranking**

The ranking of ports based on dispersal probability where the underlying agent based simulations included agent emissions inside all potential species specific habitats (method 1) are shown in figure 23. Figure 23 show the sum of the average dispersal probabilities from each of the 28 ports and for each 23 species to each of the other 27 ports. In figure 23 (upper pane) each column is divided into contributions (probabilities) by individual ports to the dispersal probability to each of the 27 downstream located ports. In figure 23 (lower pane) each column is divided into contributions by each of the 23 species to the sum of dispersal probabilities to each of the 27 downstream located ports. In figure 23 (lower pane) each column is divided into contributions by each of the 23 species to the sum of dispersal probabilities to each of the 27 downstream located ports. Charts in figure 24 are similar to charts in figure 23, except that these figures are based on analysis of the dispersal connectivity probability where the underlying agent based simulations included agent emissions only in each port location.

Both methods produce similar rankings and the overall patterns in the ranking results are also to some extent similar to the cluster membership analysis result, showing the lowest natural dispersal of ports at Bornholm and the west coast of Jutland (with exception of Havneby) and the highest natural dispersal of the ports located in Kattegat, specifically in Lillebælt. However, there are some evident dissimilarities. E.g., Havneby on the west coast Jutland ranks 3rd and this is only due to a large dispersal probability to the port location of Esbjerg. Another deviation from the cluster membership analysis results is the lower number of ports contributing to the overall sum of downstream dispersal probabilities of each port. In addition, contrary to the cluster membership analysis, the contribution of the sum of downstream dispersal probabilities is not dominated by a few species.



Cluster Membership Analysis Split into species contributions



Figure 22. Results from the cluster membership analysis showing weighted number of port connections (wnos) of each of the 28 ports (x-axis). Top: Wnos split into contributions from each of the other 27 "destination" ports. Bottom: Wnos split into contributions from each of the 23 NS.







Dispersal Probability Analysis Split into Species contributions Method 2



Figure 24. Results from the dispersal probability analysis showing the ranking of ports based on downstream port connectivity probabilities of 23 marine invasive species. Based on results from larval dispersal modelling releasing larvae in port locations only, method 2.Top: Mean dispersal probability split into downstream port connection. Bottom: Mean dispersal probability split into species contributions.

#### **Dispersal duration ranking**

The ranking of ports based on the minimum dispersal duration analysis is shown in figure 25 with number of port connections representing the number of downstream ports with minimum dispersal duration less than 15, 30 and 40 days respectively. As a more robust measure of minimum dispersal duration we use the 10 percentile to minimize the effect of outliers. The ranking shown here is based on the 15 days cutoff value. The ranking based on dispersal duration deviates considerably compared to the cluster membership analysis and the dispersal probability analysis, still with the lowest ranks associated with ports of Bornholm and the west coast of Jutland, but also including ports like Aabenrå, Køge, Læsø and Studstrup that have much lower rank here.



Minimum dispersal duration - Number of connections

Figure 25. Ranking of ports based on dispersal time of simulated larvae released in each port location. Each column represents the number ports connected within a minimum dispersal time of 15, 30 and 40 days. Ranking from left to right is based on 15 days dispersal time threshold. No species-specific traits, habitat preferences etc. have been included in the larval dispersal modelling.

#### Comparison of ranking results

A summary and comparison of the results presented above is shown in table 8, Notice that the rankings of 5 out of the 28 ports (highlighted in red) are considered uncertain, due to limitations in the resolution of the hydrodynamic model in relation to fjords and inlets. These include ports of Odense, Kolding, Thyborøn, Aabenraa and Frederiksværk. The order of ports in table 8 is ranked based on the mean rank of the 4 ranking methods tested, and the range of the ranking scores (max - min) for all 4 ranking methods are compared to the range of ranking score excluding the ranking based on dispersal duration analysis. This shows that the results from the dispersal duration analysis deviates considerably from the other 3.

Table 8. Summary of the results of the different methodologies for ranking ports according to the potential natural dispersal of NIS between major Danish ports, including: 1) "Cluster membership analysis" based on agent release in all potential habitat including multiple generation stepping stone dispersal; 2) "Dispersal probability analysis method 1" based on agent release in all potential habitats including multiple generations stepping stone dispersal; 3) "Dispersal probability analysis method 2" based on agent release in port locations only including single generation dispersal; 4) "Dispersal duration" based on agent release in port locations only. "MEAN" refers to the mean of the four ranking methodologies. "Range" refers to the maximum range of ranking among methodologies for each port. "Range minus DD." refer to the maximum range of three out of four methodologies, excluding the ranking based on "Dispersal Duration". Red coloured port names are ports located in fjords or inlets where the hydrodynamic model resolution is limited and the ranking of these ports are considered uncertain.

Port name	Cluster Member- ship	Dispersal Probability Method 1	Dispersal Probability Method 2	Dispersal duration (DD)	MEAN	Range	Range minus DD
Samsø	1	4	4	2	2.75	3	3
Fredericia	5	2	2	11	5.00	9	3
Kolding	6	1	1	13	5.25	12	5
Odense	4	9	9	4	6.50	5	5
Aarhus	2	8	5	12	6.75	10	6
Kalundborg	8	10	13	1	8.00	12	5
Copenhagen	17	3	3	15	9.50	14	14
Studstrup	3	5	10	22	10.00	19	7
Helsingør	15	11	7	8	10.25	8	8
Sjællands Odde	7	15	15	5	10.50	10	8
Omø	12	13	11	7	10.75	6	2
Aabenraa	10	6	6	24	11.50	18	4
Grenaa	11	20	20	3	13.50	17	9
Stignæs	16	16	17	6	13.75	11	1
Avedøre	21	7	14	18	15.00	14	14
Rødby	18	17	16	9	15.00	9	2
Anholt	9	21	21	16	16.75	12	12
Køge	22	14	12	20	17.00	10	10
Frederiksværk	19	22	19	10	17.50	12	3
Gedser	20	19	18	14	17.75	6	2
Havneby	26	12	8	26	18.00	18	18
Læso	14	23	22	19	19.50	9	9
Frederikshavn	13	24	24	17	19.50	11	11
Hirtshals	23	18	23	21	21.25	5	5
Thyborøn	24	25	26	23	24.50	3	2
Esbjerg	25	26	25	27	25.75	2	1
Nexø	27	27	27	28	27.25	1	0
Rønne	28	28	28	25	27.25	3	0

#### 4.3.3 Ranking of ports according to the natural dispersal potential

The results from the natural dispersal potential analysis are shown in figures 26 and 27. Figure 26 shows the total number of downstream sub-areas in the connectivity grid that each port is connected to considering all 23 NIS included in the analysis. Only downstream connectivity probability values of 0.1 % or larger are included. Bars are divided into number of downstream sub-area connections with probabilities of 0.1 - 1 %, 1 - 10 %, and > 10 %. The total number (or sum) of all downstream connections are proportional to both the number of species that may potentially spread from each port as well as to the number of areas each species may spread to. This may depend on the hydrodynamics (to the extent ocean current may convey larvae far away) the species traits (such as PLD, spawning period, etc.), the habitat preference (if there is any preferred habitat in the vicinity of the port) and/or the environmental tolerance (the extent to which the salinity conditions are within the tolerance range of the larvae).

Ports with a low number of connections may be a result of only few of the 23 NIS potentially occurring at the port location and dispersing elsewhere (like for Rønne and Nexø), or due to a limited physical dispersal despite a relatively large number of the 23 NIS with a potential occurrence at the port location and its vicinity (like Studstrup) or a combination of both (like Køge).



Figure 26. Natural dispersal potential calculated as the sum of sub-areas in the connectivity grid each port is connected to. The sum is calculated for the 23 NIS included in the analysis. Only downstream sub-areas in the connectivity grid with a dispersal probability of 0.1 % or more are included. Bars are divided into number of downstream sub-area connections with probabilities of 0.1 - 1 %, 1 - 10 %, and > 10 %. Numbers above bars indicate the number of species out the 23 NIS included in the analysis which may potential occur in the port and disperse elsewhere.

In figure 27, the number of downstream connections are shown as the mean of all NIS with a potential occurrence at each port location. Species with no potential occurrence are not considered. Thus, a low value indicates that the hydrodynamics support a limited dispersal potential, and/or the species with a potential occurrence at the port location have traits supporting only a limited dispersal potential. The latter due to a short PLD, a limited presence of preferred habitat in the region of which the port is located, and/or a dispersal potential limited by salinity gradient conditions. Thus, ports like Rønne and Nexø which are only represented by few species (6 and 5), rank higher than e.g. Studstrup represented by 19 species, indicating that hydrodynamic conditions around Bornholm are more supportive of pelagic larvae dispersal than at Studstrup.



Figure 27. Natural dispersal potential calculated as the mean of sub-areas in the connectivity grid each port is connected to. The mean is calculated for the 23 NIS included in the analysis. Species which may not have a potential occurrence in the port due to e.g. salinity intolerance or lack of preferred habitat are omitted from the calculation of the mean. Only downstream sub-areas in the connectivity grid with a dispersal probability of 0.1 % or more are included. Bars are divided into the number of downstream sub-area connections with probabilities of 0.1 - 1 %, 1 - 10 %, and > 10 %. Numbers above bars indicate the number of species out the 23 NIS included in the analysis which may potential occur in the port and disperse elsewhere.

In **Appendix 3**, cumulated downstream dispersal probability maps for each port considering one generation dispersal is presented representing the dispersal probability of an agent to disperse from a port to any of the downstream located subareas in the connectivity grid considering all species that may potentially disperse from the respective ports. To inspect the downstream and upstream dispersal probability maps of each port for each species considering stepping stone dispersal (multiple generations) are presented in the **Appendix 2**.

The ranking of the natural dispersal potential, presented in figures 26 and 27, are summarised in table 9, including a ranking based on a combination of both. The combined ranking is done by first normalising the sum and mean of the numbers of downstream sub-areas connections in the connectivity grid (normalised relative to the maximum values respectively) and then calculating the sum for each port.

In general ports located close to open waters of the Kattegat, Skagerrak and the North Sea have high dispersal potentials, while dispersal potential of ports in the western Baltic and the Inner Danish Straits have medium to low dispersal potential. Exception to these patterns are the ports of Esbjerg, Havneby and Studstrup. See discussion later.

Table 9. Ranking of major Danish ports according to the natural dispersal potential calculated as the sum and mean of the number of downstream sub-area connections in the connectivity grid (see captions of figures 31 and 32). The combined ranking is done by first normalising the sum and mean of the numbers of downstream sub-areas connections in the connectivity grid (normalised relative to the maximum values respectively) and then calculating the sum for each port. Red coloured port names are ports located in fjords or inlets where the hydrodynamic model resolution is limited and the ranking of these ports are considered uncertain.

	Ranking based on:				
Port Name	SUM of				
	downstream downstream		Combined		
	sub-areas	sub-areas			
Omø	3	1	1		
Samsø	2	2	1		
Hirtshals	4	3	3		
Frederikshavn	1	7	4		
Odense	6	4	5		
Thyborøn	7	8	6		
Rødby	11	5	7		
Grenå	8	9	8		
Læsø	5	13	9		
Kalundborg	10	10	10		
Sjællands Odde	9	11	10		
Stignæs	14	6	10		
Aarhus	12	16	13		
Anholt	13	15	13		
Helsingør	15	14	15		
Gedser	20	12	16		
Kolding	16	17	17		
Aabenraa	17	20	18		
Copenhagen	21	18	19		
Fredericia	18	21	19		
Frederiksværk	19	23	21		
Rønne	27	19	22		
Avedøre	23	24	23		
Esbjerg	22	25	23		
Havneby	24	26	25		
Nexø	28	22	25		
Køge	25	27	27		
Strudstrup	26	28	28		

### 4.4 Discussion

#### 4.4.1 Connectivity between ports

The analyses carried out on ranking of ports according to the natural dispersal of NIS illustrate that different methods could produce different results. Especially the dispersal duration analysis leads to a ranking deviating markedly from the other two methodologies. Species specific habitat preferences and salinity tolerances are of particular importance in the transition waters between the North Sea and the Baltic Sea where salinity gradients are large and highly dynamic in space and time, and where the spatial distribution and extent of the potential species specific habitats of NIS at the same time varies considerably. Ignoring the relative complex dynamics between species specific traits, habitat preferences and environmental tolerance when trying to predict the potential dispersal of NIS in Danish marine waters thus may provide an incomplete and biased result. However, for NIS where no information exists except an estimate on the pelagic larvae duration, dispersal duration analysis as presented here may be useful to estimate the number of ports that may potentially export NIS to other ports within the region. If such information is available, it is however recommended to apply a species specific approach.

The two other methods tested here, i.e. the cluster membership analysis and the port dispersal probability analysis, show comparable results in their ranking of the 28 ports based on analysis of the 23 species, however, the two methods still deviate notably when identifying which port and which species that contributes to the overall ranking. Thus the rankings based on these two methodologies have different sensitivity to changes in the number of ports or species included in the analysis. While the ranking based on the cluster membership analysis is more robust to changes in the number of ports, the ranking based on the dispersal probability is more robust to changes in the number of species.

While the cluster membership analysis attempts to cover the spatial and temporal dynamics of the potential dispersal of a given NIS considering multiple stepping stone dispersal processes in the entire study area (not limited to the ports, but to all potential habitats), the port dispersal probability analysis focuses narrowly on the dispersal probability between ports ignoring any possible presence of the NIS outside port locations. Thus, the two methodologies represent two extremes: the former using an "ideal" set of assumptions trying to aggregate all possible dispersal connections in an area, and the latter strictly looking at dispersal probabilities from port to port

To facilitate the decision making process, we recommend to prepare dynamic spreadsheets where species and/or ports can be selected or deselected and the ranking subsequently automatically updated. The dynamic spreadsheets can easily be extended to add weights to each of the included species for instance to account for invasion potential.

#### 4.4.2 Downstream dispersal potential

In general, the dispersal potential calculated as the sum of downstream connections show relatively large differences between ports, with ports located towards open waters of Kattegat, Skagerrak and the North Sea having the highest dispersal potential and with ports located towards the more brackish water of the Baltic Sea having lower dispersal potential. Exceptions to this pattern are the ports of Esbjerg and Havneby, both located in the tidal flats of the Wadden Sea, and Studstrup located in the bottom of Århus bay (Kalø Vig). Both areas are

subject to limited water exchange to the surrounding waters due to tidal cycles of very shallow areas (Esbjerg and Havneby) or due to the geographical delimitation of a bay (Studstrup). The otherwise dominant pattern is partly due to the relatively larger number of NIS with higher salinity tolerance. The distribution of salinity tolerance ranges of the 23 species in this study (see: Hansen and Christensen 2018, Appendix 1) is somewhat consistent with the general pattern of the species diversity expected across salinity gradients in the marine environment such as the salinity range between Bornholm (~ 8 PSU) towards the Inner Danish Straits, Kattegat and Skagerrak (28-30 PSU). According to Smyth & Elliott (2016) species diversities are highest in marine environments with high salinities, and similarly in pure (or close to pure) freshwater environments. On the other hand species diversity in estuarine systems of salinity ranges from ca. 8 PSU and above, are dominated by marine species tolerating lower salinities rather than freshwater species tolerating higher salinities. Exceptions are euryhaline species specifically adjusted to thrive in brackish conditions, especially in conditions of 5-7 PSU.

Ports like Rønne and Nexø rank as ports with an intermediate dispersal potential when looking at the calculated mean number of downstream sub-area connections, only including those species where a potential dispersal might occur due to tolerated salinity conditions and/or habitat preferences (figure 27). This means that the number species with a dispersal potential is limited, but for those species that may have dispersal potential in each of the ports, the dispersal area are similar to the mean of the dispersal potential of all ports in the analysis.

#### 4.4.3 Remarks

Like any other ranking methodology, the results presented here of the potential natural dispersal of NIS in Danish marine waters are sensitive to the underlying assumptions for larval dispersal modelling, connectivity analysis and ranking methodologies. A number of issues should be mentioned to be kept in mind in the interpretation and application of ranking results in a management context. These include:

- Water temperature is not included explicitly in the larval dispersal modelling. A few of the species included in the analysis could be excluded due to potentially limiting temperature conditions. Species potentially limited by larval tolerance include *Arcuatula senhousia*.
- 2. Water temperature, in particular winter minimum temperature, is not included in the habitat mapping. Species potentially limited by cold water temperatures during winter include: *Callinectes sapidus*, *Hemigrapsus sanguineus*, *Mytilopsis leucophaeata* and *Rapana venosa*.
- 3. The natural dispersal from and to ports located in fjords where the spatial resolution of the hydrodynamic model is limited are considered uncertain. These ports include Odense, Kolding, Aabenraa, Frederiksværk and Thyborøn.
- 4. A number of factors affecting natural dispersal and especially the success of recruitment are not included, such as reproductive potential, larval survival, intra and interspecific competition affecting the success of population establishment and maintenance, etc.

### 4.5 Conclusions

The aim of this section was to rank major Danish ports according to the natural dispersal of nonindigenous species (NIS). We applied agent-based modelling to simulate the potential natural dispersal of 23 selected NIS. Based on simulation results we analysed the connectivity between the 28 major Danish ports (or ports locations) using three different methods. We also analysed the dispersal potential from each port to other part of the marine system.

The overall conclusions of the ranking of ports according natural dispersal and the connectivity between ports are:

- Ports with the highest ranking are ports located in the western and southern part of Kattegat, and in the Danish straits of Lillebælt, Storebælt and Øresund, while the ports with the lowest rankings are located on Bornholm, along the west coast of Jutland and in the northern parts of Kattegat.
- The connectivity between ports depends on which analytical method is used.
- The "cluster membership" analysis and the "port connectivity probability" analysis provide comparable rankings when considering all 23 NIS, however with the former being more sensitive to which species are included or excluded in the analysis, and with the latter being more sensitive to which ports are included or excluded in the analysis.
- The ranking of ports based on "cluster membership" analysis represents an ideal situation with NIS potentially being present in, or introduced to, any suitable habitat and preferred salinity conditions. This method may be considered in a decision making process where the location of introduction or presence of the NIS is unknown and may not be limited to major ports, or where the potential future longer term development in NIS dispersal need to be considered.
- The ranking of ports based on "port connectivity probability" analysis focuses narrowly on the dispersal of NIS from individual ports. This method may be considered in the decision-making process evaluating the potential dispersal of NIS introduced to, or being present at, individual ports.
- The dispersal duration analysis may be considered when no species specific data exist on species traits, habitat preference and/or salinity tolerance, but this method is not recommended as basis for decision making in general.
- The geographical proximity of the 28 major ports included in the analysis is positively correlated to the ranking of the 28 major ports. Any change in port activity, or criteria, for classifying ports as major ports, may change the number of ports included in the analysis, and hence, potentially affect the ranking of port presented here.

The overall conclusions of the ranking of ports according natural dispersal potential to other parts of the marine territory are:

- The natural dispersal potential is highest in ports located towards the open water of Kattegat, Skagerrak and the Norths Sea, except the ports of Esbjerg, Havneby and Studstrup because of local hydrodynamic conditions.
- While the overall dispersal potential of the ports of Rønne and Nexø are limited due to a limited number of NIS with a potential occurrence in these port locations (6 and 5 out of 23 respectively), the dispersal potential of the species a potential occurrence are of a magnitude similar to the mean of all 28 ports.
- The salinity tolerance among the 23 NIS selected for the current study are considered to reflect the general salinity tolerance gradient of estuarine systems, i.e. with a decreasing species diversity with decreasing salinity (in the salinity ranges 8 – 30 PSU covered by the study area).
- The ranking of ports based on the dispersal potential may be used in the decision making process where the extent to which individual ports may export NIS to other marine areas outside each of the 28 major port location need to be considered including potential habitats and minor ports.

# 5. Comparison of the different rankings

In the previous sections we have presented the results from the ranking of 28 major Danish ports and port locations according to: 1) shipping activities; 2) natural dispersal probability and connectivity between ports; and 3) the natural dispersal potential of each port to neighbouring habitats. Each ranking can be evaluated independently and provide a basis for future risk assessments where risk of primary or secondary introduction needs to be evaluated. A comparison of the three different rankings can be used to identify ports or port locations where the risk for both primary and secondary introductions of NIS is high. These areas may be considered as the major hotspots for introduction of NIS in the Danish marine territory.

A comparison of the different rankings are shown in table 10. The order of the ports in table 10 is done simply by ranking the ports according to the sum of the three rankings presented in the previous chapters; however, this is done for comparison only, with no specific principle for such ranking.

Table 10. Summary and comparison of the results of the different rankings of ports according to 1) shipping activities; 2) natural dispersal probability (~connectivity) between ports; and 3) the natural dispersal potential of each port. "Combined" refers to a simple ranking based on the sum of the three rankings. Port names with red colour are ports located in fjords or inlets where the hydrodynamic model resolution is limited and the ranking of these ports are considered uncertain.

PORT	Shipping activity	Port connectivity	Dispersal potential	Combined
Aarhus	5	4	13	1
Kalundborg	4	10	10	2
Copenhagen	1	7	19	3
Fredericia	7	2	19	4
Hirtshals	3	24	3	5
Kolding	12	1	17	5
Odense	19	6	5	5
Samsø	28	3	1	8
Frederikshavn	6	23	4	9
Grenå	10	17	8	10
Omø	25	11	1	11
Thyborøn	8	25	6	12
Aabenraa	13	9	18	13
Rødby	19	15	7	14
Stignæs	16	16	10	15
Helsingør	16	12	15	16
Avedøre	19	8	23	17
Sjællands Odde	27	13	10	17
Esbjerg	2	26	23	19
Anholt	22	18	13	20
Continues on next page				

#### Continued ...

PORT	Shipping activity	Port connectivity	Dispersal potential	Combined
Studstrup	22	5	28	21
Frederiksværk	14	22	21	22
Køge	11	19	27	22
Læsø	27	21	9	22
Rønne	10	27	22	25
Gedser	25	20	16	26
Havneby	25	14	25	27
Nexø	22	28	25	28

In figure 28, we have made a comparison of the results from the three ranking analyses of the 28 major port locations showing proportional normalised values of the output of each analysis. Results from the shipping activity analysis in terms of PRA scores are normalised to values between 0 and 1, with 1 representing the maximum score. Results from the analysis of natural dispersal probability between ports is shown as an average of the results from 2 out 3 analysis (table 6) but excluding results from the "dispersal duration" analysis. All analysis outputs are normalised to values between 0 and 1 prior to averaging. Finally, the results from the downstream dispersal potential analysis calculated as both the sum and as the mean of the number of downstream sub-areas in the connectivity grid each port is connected to considering the 23 NIS, is included, and similarly, normalised to values between 0 and 1, with 1 representing the maximum value of each data set.



Figure 28. Map showing the results for each of the 28 ports from the shipping activity preliminary risk assessment (blue), the analysis of natural dispersal probability (~connectivity) to other ports (orange), and the analysis of the downstream natural dispersal potential (green). The downstream natural dispersal potential is calculated as the sum (dark green) and as a mean (light green) of the number of downstream sub-areas in the connectivity grid each port is connected considering the 23 non-indigenous species. The size of the bars are normalised relative to the maximum value of each category. For details, see text.

The shipping activity results can be interpreted as the extent to which each port may be at risk for receiving NIS from other locations via shipping. The natural dispersal connectivity results can be interpreted as the relative risk or potential of each port to export NIS to other major Danish ports. In addition, the dispersal potential results can be interpreted as the relative risk of ports exporting NIS to the surrounding marine areas in general, not only considering the 28 major ports.

The ports with the highest combined ranking include the ports of Aarhus, Kalundborg, Copenhagen and Fredericia, and these ports are ranked high in two out of three different rankings including shipping activities and dispersal probability to downstream ports. The dispersal potential of these ports, although with an intermediate or low ranking (table 10) are still at a proportionally high level (figure 28) due to relatively small differences in dispersal potential between most ports in this analysis, apart from ports of e.g. Rønne, Nexø, Køge and Studstrup. Thus, the four highest ranked ports can be regarded as major hotspots among the Danish ports with a combination of both relative high chance of primary introduction of NIS and a subsequent potentially efficient secondary dispersal to other marine habitats and both major and minor ports.

Ports like Esbjerg, Thyborøn, Hirtshals and Frederikshavn rank high in the PRA of shipping activities and "high to intermediate" in the ranking based on dispersal potential to neighbouring

habitats, but the dispersal probability to other major port are limited. These ports can be considered as major hotpots for primary introduction of NIS, but with more limited potential for sustaining secondary introduction to other major Danish ports.

Ports with ferry activities like Samsø, Sjællands Odde, Læsø, Anholt, Omø, Rødby and Gedser rank low in the PRA of shipping activities, however with high dispersal probability to other ports as well as dispersal potential to other marine habitats. With the frequent ferry connections to major cargo ports, these ports could be considered as hot spots for secondary introduction of NIS enhancing stepping stone dispersal of NIS.

The remaining ports in connection to Kattegat and the inter Danish straits have shipping activities ranking from low to intermediate, however they all (except Studstrup) are characterised with medium to high dispersal probabilities to other major ports and medium to high dispersal potential to other marine habitats. Thus, Kattegat and inner Danish straits can be regarded as the most interconnected area in the Danish marine waters, and it is likely that NIS occurring in one port (or a habitat) may disperse to other parts of this area, either by natural dispersal or facilitated by local ferry routers (and potentially leisure boats).

Ports of Rønne, Nexø, Studstrup, Esbjerg and Havneby are all characterised by a limited dispersal probability to other major ports, and/or limited dispersal potential to neighbouring habitats either due to hydrodynamic limitations (Studstrup, Esbjerg and Havneby) or due to the limited of NIS expected to potentially occur in these ports (Nexø and Rønne).

Ports of Odense and Kolding rank among the top 7 in table 10, however these ports (as for Frederiksværk, Aabenraa and Thyborøn) are located in fjords (or inlets) and the resolution of the hydrodynamic model is very limited in these areas. The actual rating considering dispersal probability to other ports and dispersal potential to neighbouring habitats may be relative robust considering locations corresponding to the outlets of the fjords and inlets of which the ports are located. However, it is more uncertain to which extent this will be the case at the actual port location sometimes many kilometres inward the fjord system. To address the natural dispersal of these ports and particularly the exchange of pelagic larvae in and out of the fjords and inlets will require the use of local models with a higher spatial resolution. For most of these fjords and inlets local models exists and can be considered in future projects (Anders Erichsen DHI, personal communication).

# 6. References

Andersen, J.H., Pedersen, S.A., Thaulow, J., Stuer-Lauridsen, F., Cochrane, S. 2014. Monitoring of non-indigenous species in Danish marine waters. Background and proposals for a monitoring strategy and a monitoring network. Danish Nature Agency. 55 pp.

Anderson LG, Rocliffe S, Haddaway NR, Dunn AM. 2015. The Role of Tourism and Recreation in the Spread of Non-Native Species: A Systematic Review and Meta-Analysis. PLoS One. 2015;10(10):e0140833. Published 2015 Oct 20. doi:10.1371/journal.pone.0140833

Ashton, G., C. Zabin, I. Davidson, and G. Ruiz. 2012. Aquatic Invasive Species Vector Risk Assessments: Recreational vessels as vectors for non-native marine species in California. Prepared for Ocean Science Trust. 75 pp. Final Report July 2012 Submitted to the California Ocean Science Trust Funded by the California Ocean Protection Council. By: The Aquatic Bioinvasion Research & Policy Institute. A Partnership between Portland State University & the Smithsonian Environmental Research Center.

Barnes, D. 2002. Biodiversity: Invasions by marine life on plastic debris. Nature 416: 808-809.

Baetens K., Gittenberger A., Barbut L., Lacroix G. (2018). Assessment of the ecological implications when installing an SRA between Belgium and the Netherlands. Final project report. Royal Belgian Institute of Natural Sciences. Operational Directorate Natural Environment, Ecosystem Modelling. 70 pp.

Berg P, Poulsen J W, 2012. Implementation Details for HBM. DMI Technical Report, No. 12-11, Copenhagen.

Chapman, J. W., T. W. Miller and E. V. Coan. 2003. Live seafood species are recipes for invasion, Conservation Biology 17: 1386-1395.

Clarke Murray, C, Pakhomov E A and Therriault T W, 2011. Recreational boating: a large unregulated vector transporting marine invasive species. Diversity and Distributions, 17: 1161-1172. doi:10.1111/j.1472-4642.2011.00798.x

Christensen A, 2008, Bank resolved prognoses of sandeel fishing potential in the North Sea". Final report for the project "Fiskeriudsigt for tobis i Nordsøen på bankeniveau. (FIUF, 2005-2007).

Christensen A, Mariani P, Payne M R, in review. A generic framework for individual-based modelling and physical-biological interaction. Environmental Modelling and Software.

Corell H, Moksnes PO, Engqvist A, Döös K, Jonsson PR (2012) Depth distribution of larvae critically affects their dispersal and the efficiency of marine protected areas. Mar Ecol Prog Ser 467:29-46. https://doi.org/10.3354/meps09963

Davidson I, Ashton G, Zabin C & Ruiz G 2012. Aquatic Invasive Species Vector Risk Assessments: The role of fishing vessels as a vector for marine and estuarine species in California. Final Report July 2012. Submitted to the California Ocean Science Trust. Funded by the California Ocean Protection Council. By: The Aquatic Bioinvasion Research & Policy Institute. A Partnership between Portland State University & the Smithsonian Environmental Research Center.

Ferrario, J., Caronni, S., Occhipinti-Ambrogi, A., Marchini, A. 2017. "Role of Commercial Harbours and Recreational Marinas in the Spread of Non-Indigenous Fouling Species." Biofouling 33 (8): 651–60. https://doi.org/10.1080/08927014.2017.1351958.

Florin, A. B., Mo, K., Svensson, F., Schagerström, E., Kautsky, L., & Bergström, L. (2013). First records of Conrad's false mussel, *Mytilopsis leucophaeata* (Conrad, 1831) in the southern Bothnian Sea, Sweden, near a nuclear power plant. Bioinvasions Records, 2(4), 303–309. https://doi.org/10.3391/bir.2013.2.4.07

Fofonoff, P.W., G.M. Ruiz, B. Steves, and J.T. Carlton. 2003. In ships or on ships? Mechanisms of transfer and invasion for nonnative species to the coasts of North America. Pp. 152-181. In Invasive species, vectors and management strategies. G.M. Ruiz and J.T. Carlton eds. Island Press, Washington D.C.

Fowler, A., A. Blakeslee, J. Canning-Clode, and W. Miller. 2015. Opening Pandora's bait box: A potent vector for biological invasions of live marine species. Diversity and Distributions 22:1-13.

Geburzi, J. C., and McCarthy, M.L. 2018. "How Do They Do It? – Understanding the Success of Marine Invasive Species." Youmares 8 – Oceans Across Boundaries: Learning From Each Other, 109–24. https://doi.org/10.1007/978-3-319-93284-2\_8

Gollasch, S., Leppakoski, E. 1999. Initial Risk Assessment of Alien Species in Nordic Coastal Waters. Copenhagen, Denmark: Nordic Council of Ministers.

Gollasch, S., Leppakoski, E. 2007. "Risk Assessment and Management Scenarios for Ballast Water Mediated Species Introductions into the Baltic Sea." Aquatic Invasions 2 (4): 313–40. https://doi.org/10.3391/ai.2007.2.4.3.

Gollasch, S. n.d. Adjustable calculation form to quantify the amount of ballast water transported in ballast tanks and discharged annually. www.gollaschconsulting.de/download/bwcalc.xls

Grosholz, E., R. E. Crafton, R. E., Fontana, J. Pasari, S. Williams, and C. Zabin. 2012. Aquatic Invasive Species Vector Risk Assessments: An Analysis of Aquaculture as a Vector for Introduced Marine and Estuarine Species in California. Prepared for Ocean Science Trust. 77 pp.

Hallegraeff, G. M. & Bolch, C. J. 1991. Transport of toxic Dinoflagellate cysts via ship's ballast water. Mar. Poll. Bull. 22, 27-30.

Hallegraeff, G. M. & Bolch, C. J. 1992. Transport of diatom and dinoflagellate resting spores in ships' ballast water: implications for plankton biogeography and aquaculture. J. Plankton Res. 14, 1067-1084.

Hansen, F. T. & Christensen, A. (2018-a). Same-Risk-Area Assessment Model (SRAAM). User's man-ual. Version 2.0. DTU Aqua report no. 332-2018

Hansen, F. T. & Christensen, A. (2018-b). Same-Risk-Area Case-study for Kattegat and Øresund. Final report. DTU Aqua report no. 335-2018. National Institute of Aquatic Resources, Technical University of Denmark. 37 pp. + appendices.

Helcom/Ospar 2015. Joint Harmonised Procedure for the Contracting Parties of OSPAR and HELCOM on the granting of exemptions under International Convention for the Control and Management of Ships' Ballast Water and Sediments, Regulation A 4. HELCOM/OSPAR TG BALLAST 5-2014.

Kern, K., Stuer-Lauridsen, F. 2009. Ballast Water Discharges in Denmark. LITEHAUZ, Danish Agency for Spatial and Environmental Planning (BLST).

Laine, A.O.,Matilla, J., Lehikoinen, A. 2006. First record of the brackish water dreissenid bivalve *Mytilopsis leucophaeata* in the northern Baltic Sea. Aquatic invasions 1:38-41, <u>https://doi.org/10.3391/ai.2006.1.1.9</u>

Laine, A. O., Urho, L. 2007. National Report Finland, 2006. In ICES Working Group on Introduction and Transfers of Marine Organisms (WGITMO) Report 2007 ICES CM 2007/ACME:05.

MEPC 2017. Resolution mepc.289(71) (adopted on 7 July 2017) 2017 guidelines for risk assessment under regulation a-4 of the BWM convention (g7).

Minchin, D., Gollasch, S., Cohen, A. N., Hewitt, C. L., & Olenin, S. (2009). Characterizing Vectors of Marine Invasion. In G. Rilov & A. J. Crooks (Eds.), Biological Invasions in Marine Ecosystems (pp. 109–116). Berlin: Springer Berlin Heidelberg. http://doi.org/10.1007/978-3-540-79236-9\_5

R Core Team, 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <u>http://www.R-project.org/</u>.

Rata, V., Gasparotti, C., Rusu, L. 2018. Ballast Water Management in the Black Sea's Ports. Journal of Marine Science and Engineering. <u>https://doi.org/10.3390/jmse6020069</u>

Rossi V, Ser-Giacomi E, López C, Hernández-García E, 2014. Hydrodynamic provinces and oceanic connectivity from a transport network help designing marine reserves. Geophysical Research Letters 41, 2883-2891 (2014). DOI: 10.1002/2014GL059540

Rosvall M & Bergstrom C T, 2008. Maps of random walks on complex networks reveal community structure, Proc. Natl. Acad. Sci. U.S.A., 105(4), 1118–1123.

Simard N, Pelletier-Rousseau M, Clarke Murray C, McKindsey C W, Therriault, T W, Lacoursière-Roussel A, Bernier R, Sephton D, Drolet D, Locke A, Martin J L, Drake D A R, and McKenzie C H 2017. National Risk Assessment of Recreational Boating as a Vector for Marine Non-indigenous Species. DFO Can. Sci. Advis. Sec. Res. Doc. 2017/006. vi + 95 p.

Smyth K & Elliott M, 2016. Effects of changing salinity on the ecology of the marine environment. 10.1093/acprof:oso/9780198718826.003.0009

Stuer-Lauridsen F, Drillet G, Hansen F T, Saunders J, 2018. Same Risk Area: An area-based approach for the management of bioinvasion risks from ships' ballast water. Marine Policy. Accepted. DOI: 10.1016/j.marpol.2018.05.009

Stuer-Lauridsen F, Hansen F T, Overgaard S B, 2016. Same Risk Area Concept.Procedure and Scientific Basis. Final report. By Litehauz Aps for ITERFERRY and Danish Nature Agency.

Williams, S., R. E. Crafton, R. E.Fontana, E. D. Grosholz, J. Pasari, and C. Zabin. 2012. Aquatic Invasive Species Vector Risk Assessments: A Vector of the Aquarium and Aquascape ('Ornamental Species') Trades in California. Prepared for Ocean Science Trust. 87 pp.

# Appendix 1. Hydrographic regions and habitat maps

Hydrographic regions and potential habitat maps for each of the 23 non-indigenous marine. The delineation of hydrographic regions are based on simulated larvae dispersal with agents release in all potential habitats, and considering multiple generation (5 years) stepping stone dispersal. For details on the interpretation of maps, see the methodology section of the main report.

# Contents of appendix 1

Arcuatual senhousia	. 64
Asterias amunrensis	. 65
Austrominius modestus	. 66
Bugula nertina	. 67
Bugulina simplex	. 68
Callinectes sapidus	. 69
Crassostrea gigas	. 70
Didemnum vexillum	. 71
Ensis americanus	. 72
Eriocheir sinensis	. 73
Ficopomatus enigmaticus	. 74
Hemigrapsus sanguineus	. 75
Hemigrapsus takanoi	. 76
Hydroides dianthus	. 77
Laonome calida	. 78
Marenzelleria viridis	. 79
Mytilopsis leucophaeata	. 80
Mytilus galloprovincialis	. 81
Paleomon macrodactylus	. 82
Potamocorbula amurensis	. 83
Rangia cuneata	. 84
Rapana venosa	. 85
Rhithropanopeus harisii	. 86

### Arcuatual senhousia



### Asterias amunrensis



### Austrominius modestus



# Bugula nertina



# Bugulina simplex



# Callinectes sapidus



# Crassostrea gigas



### Didemnum vexillum


#### Ensis americanus



#### Eriocheir sinensis



## Ficopomatus enigmaticus



## Hemigrapsus sanguineus



## Hemigrapsus takanoi



## Hydroides dianthus



#### Laonome calida



#### Marenzelleria viridis



## Mytilopsis leucophaeata



## Mytilus galloprovincialis



## Paleomon macrodactylus



#### Potamocorbula amurensis



## Rangia cuneata



## Rapana venosa



## Rhithropanopeus harisii



## Appendix 2. Dispersal Probability Maps

Appendix 2 can be found in a separate file on DTU Aqua's website aqua.dtu.dk. Click on the link to download Appendix 2: <u>https://www.aqua.dtu.dk/-/media/Institutter/Aqua/Publikationer/Rapporter-352-400/369-2020-</u> <u>Appendix-2-Species-dispersal-probability-maps.ashx</u>

# Appendix 3. Cumulated downstream dispersal probability maps

Cumulated dispersal probability maps (downstream only) of all 23 non-indigenous marine species for each of the 28 major Danish ports, considering single generation dispersal. Dispersal probabilities are based on agent based model simulations with agents released in port locations only. Colour legends (see below) are linear and relative to the largest value (red colour) in each map. Hatched light blue areas represent dispersal probability values less than 0.1 %.











Technical University of Denmark

DTU Aqua Kemitorvet 2800 Kgs. Lyngby

www.aqua.dtu.dk