Biofouling as a transport vector of non-native marine species in the Dutch Delta, along the North Sea coast and in the Wadden Sea

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1. Summary

Although hull fouling is one of the main transport vectors of marine non-native species around the world, invasive species legislation tends to focus on ballast water and aquaculture related vectors. Within the present report an assessment is made of the risk of biofouling in the Netherlands, with a focus on pleasure crafts. The study was based on existing risk assessments, total species inventories, students projects, citizen science based studies and the continuous monitoring project SETL that focuses on non-native species in pleasure craft harbours. In total more non-native species were found in harbours than on dikes, shellfish beds, reefs, and in soft sediments in the Dutch Delta, along the North Sea coast and in the Wadden Sea. Harbours are therefore well-known as places where non-natives are primarily introduced into NW Europe. In addition they also function as important stepping stones for non-natives during their secondary spread within NW Europe. Not every harbour poses a similar risk, however, looking for example at the relative number of boats fouled and fouling intensities. This can be due to nearby fresh water sources as they have a deleterious effect on marine species, but also due to boat owners' and harbour master behaviour. For example, owners of relatively small motorboats with a home harbour on a Wadden Sea island have a high chance to have relatively heavy fouling on their boats while large, sailing boat owners tend to keep their hulls relatively clean by for example using more toxic anti-fouling. The IMO (International Maritime Organization) document on "Guidance for minimizing the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft" deals with this problem. However, it appears to be unknown to most boat owners and harbour masters. As a result hull fouling is at this moment, probably, the main transport vector with which non-native marine species get introduced into the Netherlands and subsequently transported along the Dutch coast. This is based on the facts that [1] harbours are, along the coast, the hotspots where most non-native species are found, [2] on average 59% of all pleasure crafts in marine harbours have fouling on their hulls, and [3] about a third of the crafts visiting Dutch harbours may introduce non-native species from abroad as they come from countries like Belgium, the United Kingdom and France, but nowadays also from other continents like America, Africa, Asia and Australia.

1. Samenvatting

Scheepshuid-aangroei betreft een van de belangrijkste transport vectoren van uitheemse marine soorten wereldwijd. Desondanks richt wetgeving met betrekking op mariene invasieve soorten zich voornamelijk op ballastwater en de aquacultuur. Binnen het huidige rapport wordt het risico van "biofouling" in Nederland onderzocht, waarbij de focus ligt op de pleziervaart. Deze studie is gebaseerd op voorgaande risicoanalyses, totale soorteninventarisaties, studentenprojecten, projecten met vrijwilligers, en het continue monitoringprogramma SETL, wat zich richt op uitheemse soorten in jachthavens. Binnen deze studies bleek dat er in jachthavens meer uitheemse soorten gevestigd leven dan op de dijken, mosselbanken, oesterriffen of in het zachte sediment in het Delta gebied, langs de Noordzee kust en in de Waddenzee. Zo fungeren havens als belangrijke tussenstations (stepping stones) voor uitheemse soorten tijdens hun secundaire verspreiding in NW Europa. Daarnaast zijn het plekken waar uitheemse soorten primair in NW Europa geïntroduceerd worden. Uit het aantal begroeide boten en de intensiteit waarmee deze begroeid zijn, blijkt dat niet iedere haven binnen Nederland een even groot risico vormt. Deze verschillen kunnen te wijten zijn aan de aanwezigheid van zoetwater uit bijvoorbeeld rivieren wat slecht is voor de overleving van mariene soorten. Verder kunnen deze verschillen verklaard worden door het gedrag van booteigenaren en havenmeesters. Zo is de kans groot dat relatief kleine motorboten met een thuishaven op een Waddenzee eiland relatief sterk begroeid zijn, terwijl een eigenaar van een relatief grote zeilboot meer geneigd is om de romp van zijn boot schoon te houden door bijvoorbeeld het gebruik van giftigere anti-fouling. De richtlijnen van het IMO (International Maritime Organization) document "Guidance for minimizing the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft" zijn opgesteld om dit probleem aan te pakken. Deze richtlijnen lijken echter niet bekend te zijn bij de meeste booteigenaren en havenmeesters. Hierdoor is scheepshuid-aangroei momenteel waarschijnlijk de belangrijkste transport vector waarmee uitheemse soorten in Nederland worden geïntroduceerd en vervolgens verder worden verspreid langs de Nederlandse kust. Dit wordt geconcludeerd gebaseerd op de feiten dat [1] havens de hotspots zijn waar de meeste uitheemse soorten worden gevonden langs de Nederlandse kust, [2] gemiddeld 59% van alle pleziervaartuigen in jachthavens aangroei heeft op de romp, en [3] ongeveer een derde van de schepen die een bezoek aan Nederlandse mariene jachthavens brengen, uitheemse soorten uit het buitenland zouden kunnen introduceren aangezien ze uit landen als België, het Verenigd Koninkrijk en Frankrijk komen, maar tegenwoordig ook uit andere continenten zoals Amerika, Afrika, Azië en Australië.

1. Zusammenfassung

Obwohl Schiffsrumpfsanwachs weltweit zu den bedeutendsten Quellen von Invasivarten im Meeresbereich gehört, konzentriert sich die diesbezügliche Gesetzgebung auf Ballastwasser und Aquakultur relatierte Aspekte. Im vorliegenden Bericht wird das Risiko von Verschmutzung in den Niederlanden abgeschätzt. Dabei wird den Vergnügungsjachten besondere Bedeutung zugemessen. Dazu steht ein Arteninventar zur Verfügung, basiert auf den Ergebnissen von Studentenprojekten, Freiwilligerarbeit, und eine anhaltende Registrierung von Invasivarten in Häfen für Vergnügungsjachten im Rahmen des SETL Projekts. Es wurden insgesamt mehr Invasivarten in Häfen festgestellt als auf den Deichen, Muschelbänken, Riffen, sowie Weichsubstraten an der niederländischen Delta, Nordseeküste und das Wattenmeer. Die Häfen können daher als wesentliche ‘stepping-stones’ bei der Verbreitung von Invasivarten betrachtet werden entlang NW Europa. Dabei gibt es jedoch wesentliche lokale Unterschiede, abhängig davon wieviele Schiffe mit Anwachs irgendwo anlegen bzw. gereinigt werden. Außerdem kann mitspielen ob Süßwasser in der Nähe vorkommt und ist das Verhalten vom Besitzer des Schiffes und vom Hafenmeister wesentlich. Besitzer eines kleinen Motorboots mit dem Heimathafen bei einer Insel im Wattenmeer zum Beispiel dürften mehr Anwachs am Schiff haben als Segler die eher die Rumpfe ihrer Boote reinigen bzw. mehr giftige Schutzmittel verwenden werden. Die IMO (International Maritime Organization) Anleitung “Guidance for minimizing the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft”, gibt nützliche Hinweise wie damit umweltfreundlich umzugehen, ist jedoch den meisten Schifsbesitzern und Häfenmeistern unbekannt. Demzufolge werden wahrscheinlich die meisten Invasivarten durch Schiffsrumpfsanwachs in die Niederlande eingeschleppt und der Küste entlang weiter verbreitet. Diese Schlußfolgerung ist basiert auf den folgenden Tatsachen: 1- der niederländischen Küste entlang sind die Häfen die ‘Hotspots’ für Invasivarten; 2- durchschnittlich 59% der Vergnügungsboote in den Meereshäfen ist mit Schiffsrumpfsanwachs verunreinigt; 3- etwa ein Drittel der Boote die in den niederländischen Häfen anlegen kommt aus Ländern wie Belgien, United Kingdom und Frankreich, wie heutzutage auch von anderen Kontinenten wie Amerika, Afrika, Asien und Australien.

1. Abstrakt

Selv om skrogbegroning er en af de vigtigste transportvektorer for ikke-oprindelige havorganismer verden over, har lovgivningen om invasive arter tendens til at koncentrere sig om ballastvand og vektorer i forbindelse med akvakultur. Som del af denne rapport blev der foretaget en vurdering af risikoen for begroning i Nederlandene, med fokus på fritidsfartøjer og baseret på risikovurderinger, fortegnelser af det samlede antal arter, studieprojekter, studier baseret på videnskabelige aktiviteter, der inddrager borgerne, samt det fortløbende overvågningsprojekt SETL, som fokuserer på ikke-oprindelige arter i fritidshavne. I alt blev der fundet flere ikke-oprindelige arter i havne end på diger, på skaldyrsbanker, på rev og i bløde sedimenter sammen den hollandske Delta, Nordsøkysten og Vadehavet. Havne fungerer derfor som vigtige springbrætter for spredningen af ikke-oprindelige arter sammen nordvest Europe. Men ikke alle havne udgør det samme risiko, når man f.eks. ser på det relative antal begroede både samt begroningens intensitet. Dette kan delvist forklares med ferskvandskilder i nærheden, men også med bådejernes og havnemestrenes adfærd. F.eks. kan ejerne af relativt små motorbåde med hjemmehavn på en vadehavsø forventes at have mest begroning på deres både, mens ejerne af store sejlbåde har tendens til at holde deres skrog relativt rent, f.eks. ved at bruge flere giftige antibegroningsmidler. IMO (International Maritime Organization) - dokumentet "Guidance for minimizing the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft" (Vejledning om minimering af overførslen af invasive akvatiske arter i form af biobegroning (skrogbegroning) for fritidsfartøjer) håndteringen af dette problem. Men de fleste bådejere og havnemestre synes ikke at være bekendt med det. Som resultat heraf er skrogbegroning på dette tidspunkt sandsynligvis den vigtigste transportvektor, hvormed ikke-oprindelige havorganismer bliver indført til Nederlandene og derefter transporteret langs med kysten. Dette er baseret på, at [1] havnene langs den hollandske kyst er de vigtigste hotspots, hvor der findes de fleste ikke-oprindelige arter, [2] i gennemsnit 59 % af alle fritidsfartøjer i søhavne har begroning på deres skrog og [3] omkring en tredjedel af fartøjerne, der besøger hollandske havne, kan indføre ikke-oprindelige arter udefra, da de kommer fra lande som Belgien, Storbritannien og Frankrig, men i dag også fra andre kontinenter som Amerika, Asien og Australien.

2. Introduction

The main primary distribution vectors of non-native species in the marine environment concern ballast water, hull fouling and shellfish transports (Gollasch, 2002; Wolff, 2005). Within the present study primary distribution vectors are defined as vectors with which non-native species are introduced in NW Europe from their region of origin (Fig. 1A). After being introduced these non-native species may be further distributed along the NW European coast with secondary distribution vectors (Fig. 1B). In many cases this occurs by travelling along with the sea currents within the pelagic life stages and within the life stages in which organisms live settled on natural floating objects like algae or wood. In addition they can also be spread by floating debris. Next to the “natural” secondary distribution vectors of these species, several anthropogenic vectors facilitate in their secondary distribution. Where ballastwater transport is usually considered as one of the main vectors with which species get primarily distributed over large distances into new regions (Fig. 1A), hull fouling and shellfish transports are considered to be vectors mainly responsible for the secondary distribution within these new regions (Fig. 1B; Gollasch, 2002). To minimize the risk of the ballast water vector, the IMO ballast water convention will go into force in September 2017. Intercontinental transports of shellfish to be released in outer waters have been responsible for a large number of primary introductions. This especially concerns Pacific oyster imports in the 1970s en 1980s (Wolff, 2005). Such intercontinental transports are nowadays prohibited (Wolff, 2005). In addition shellfish transports in NW Europe are regulated both internationally, mainly for limiting the spread of non-native parasites (EU decision 2002/300/EC: www.eur-lex.europa.eu) and nationally. Since 2010 transports within NW Europe to Dutch outer waters (the Oosterschelde) are, for example, only allowed with a permit that can be obtained when following a strict management and control system aimed at minimizing the risk of introducing nuisance species (Bleker, 2012). As hull fouling in general is not regulated it may be the main vector of both primary and secondary distribution nowadays, certainly if the risk of biofouling in the so called sea-chests of large ships

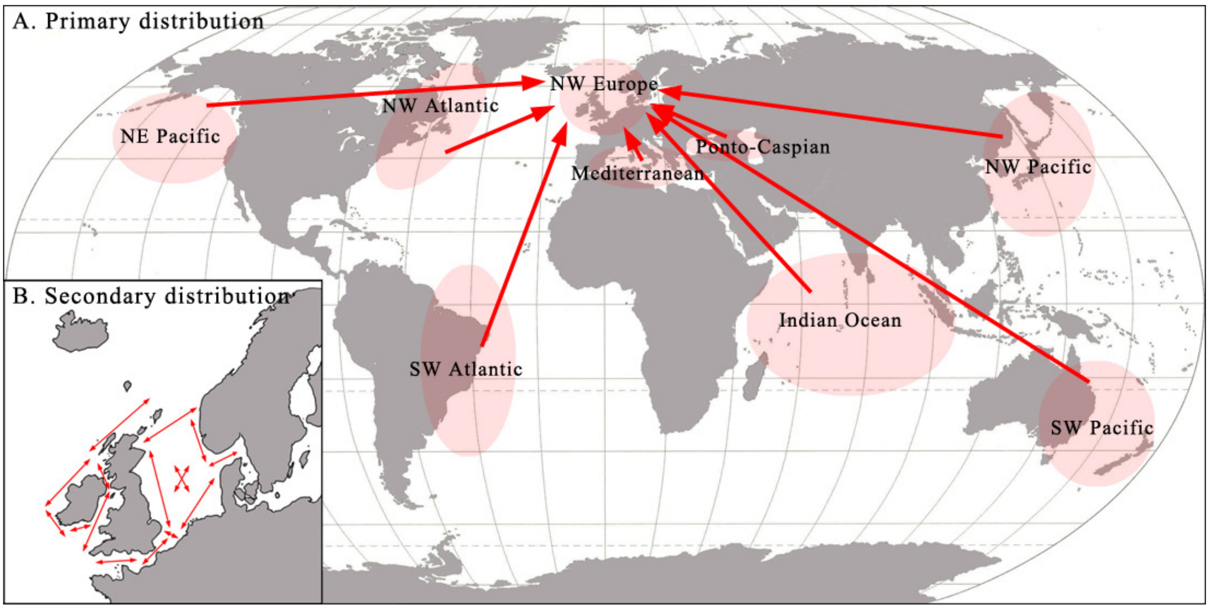

Fig. 1. [A] Primary and [B] secondary distribution of marine species into and throughout NW Europe.

is taken into account (Frey *et al.,* 2014). Biofouling on small boats has repeatedly been recognized as an important secondary transport vector of non-native marine species in Europe (Ashton *et al.,* 2006, 2014; Davidson *et al.,* 2008, 2010; Gollasch, 2002; Kauano *et al.,* 2017; Minchin *et al.,* 2006). To reduce this risk, the Netherlands government supports the IMO document on "Guidance for minimizing the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft" (IMO, 2012: http://www.imo.org/en/OurWork/Environment/Biofouling/Documents/MEPC.1-Circ.792.pdf). To what degree the protocols and advice within this guidance document are known to craft owners and harbour masters is unknown however. More in general the number of studies in Western Europe assessing the risk of hull fouling on recreational vessels remains limited (e.g. Ashton *et al.,* 2006; Nall *et al.*, 2015; Gittenberger *et al.,* 2011a). The Dutch administration would like to explore the risk of biofouling as a vector of primary and secondary distribution of non-native species in the Dutch Delta, along the North Sea coast and in the Wadden Sea. Therefore this study was issued by the Office for Risk Assessment and Research (BuRO) of the Netherlands Food and Consumer Product Safety Authority. It was done on the basis of data collected by or for GiMaRIS between 2006 and 2016. This includes, for example, an extended series of student projects focusing on hull fouling (Dekker, 2010; Freijser, 2009; Meulen, 2012, Pauli, 2014, 2015, Schie, 2011, Weger *et al.,* 2012), several citizen science based studies (in cooperation with e.g. the ANEMOON foundation and the "WaddenWerkWeekend 2016"), the continuous settlement plate project SETL, various non-native species focused surveys in the Grevelingen, Oosterschelde and Wadden Sea for the government and shellfish industry (Gittenberger *et al.,* 2010, 2011b, 2012ab, 2013a, 2014a, 2015abcde, 2016), and several port focused studies issued by Dutch governmental agencies, and shipping and ferry companies (Gittenberger *et al.,* 2014b, *in press*.).

The research questions and hypotheses that are dealt with in the present report are:

[1] What is the relative risk that a specific harbour acts as a stepping stone aiding the primary introduction and/or secondary distribution of marine non-native species in the Netherlands?

* The hypothesis is tested that not every harbour is equally likely to act as a stepping stone and that one should focus potential management and mitigation actions on those harbours that are most likely to function as stepping stones. These harbours (including the ports) may also concern the ones where species that are primarily introduced into Europe have the highest chance of survival.

[2] Are there specific habitats and sites within harbours (and ports) that pose a higher risk than others, i.e. what are the hotspots of non-native species in harbours?

* The hypothesis is tested that not every habitat within a harbour is equally suitable for the settlement of non-native species and that one should focus potential management and mitigation actions on the habitats or combination of habitats, where most non-native species are found.

[3] To what degree is the type of boat, e.g. sailing boat or motor boat, linked to the amount of hull-fouling and thereby the risk that it functions as a transport vector of non-native species?

* The hypothesis is tested that the amount of hull fouling is dependent on the type of boat.

[4] To what degree is the amount of hull fouling on a boat concentrated in certain niche areas?

* The hypothesis is tested that fouling species are found in relatively high densities in certain niche areas, like the propellor.

[5] To what degree is the behaviour of a boat owner linked to the amount of fouling found on a boat?

* The hypothesis is tested that boat owner behaviour can vary strongly and is linked to the risk that the hull fouling on his or her boat functions as a transport vector of non-native marine species.

[6] To what degree are the suggestions for minimizing the risk of recreational crafts as transport vectors of non-native species as mentioned in the IMO guidance document (IMO, 2012), known to and applied by boat owners and harbour masters?

* The hypothesis is tested that although this IMO document is largely unknown in Dutch harbours, a most of the IMO mitigation methods and suggestions are applied at least locally to reduce fouling and thereby the risk of transporting non-native species with recreational crafts.

[7] What is the risk of hull fouling as a transport vector of marine non-native species to and within the Netherlands in comparison to other vectors like ballast water, shellfish transports and natural distribution vectors?

* The hypothesis is tested that hull fouling is the most important anthropogenic vector with which “new” non-native species get introduced to and spread throughout the Netherlands at this moment in time.

3. Methods

3.1 Harbours studied

For the present study data was used, that was collected within various GiMaRIS coordinated or linked projects between 2006 and 2016 from 73 different harbours in the Netherlands (Fig. 2; Appendix I). These projects include risk assessments, total species inventories, students projects, citizen science based studies and the continuous monitoring project SETL that focuses on non-native species in pleasure craft harbours. The risk assessements and port surveys were funded by the RWS Waterdienst of the Duch ministry of Infrastructure and Environment of the Netherlands and Office for Risk Assessment and Research, the Netherlands Food and Consumer Product Safety Authority of the Ministry of Economic Affairs.

Fig. 2. An overview of all harbours from which data is used in this report related to the risk of hull fouling as a transport vector of non-native species. A complete list of all harbours is included in Appendix I.

The data extracted from these studies include:

[1] the fouling communities and intensities in various habitats in various harbours on, for example, different kinds of pleasure crafts, floating docks, (sub)littoral zones of dikes, pillars, harbour walls, jetties and the bottom;

[2] the maintenance done by boat owners including the use of various anti-fouling types and the frequency of cleaning;

[3] the travel history of pleasure crafts;

[4] the nuisance that non-native fouling species are known to cause in harbours.

In the following paragraphs these studies are explained in more detail.

3.2 Hull Fouling on pleasure crafts

Seven student projects were done in the period of 2009-2016 coordinated by GiMaRIS, each for a duration of 6 to 12 months, focusing specifically on hull fouling of pleasure crafts in the Netherlands (Dekker, 2010; Freijser, 2009; Meulen, 2012; Pauli, 2014, 2015; Schie, 2011; Weger *et al.,* 2012). In these projects the fouling on the hulls of 1923 boats was studied. This dataset was increased further by 40 volunteers that scanned the fouling on 132 boats in the pleasure craft harbour of Terschelling during the Wadden-WerkWeekend 2016 coordinated and supervised by Saskia Moll and Vera van Berlo in cooperation with GiMaRIS ensuring that a compatible scoring methodology was used, i.e. the methodology that is described below. In total the hull fouling on pleasure crafts was studied in 12 different harbours across the Netherlands (Fig. 3).

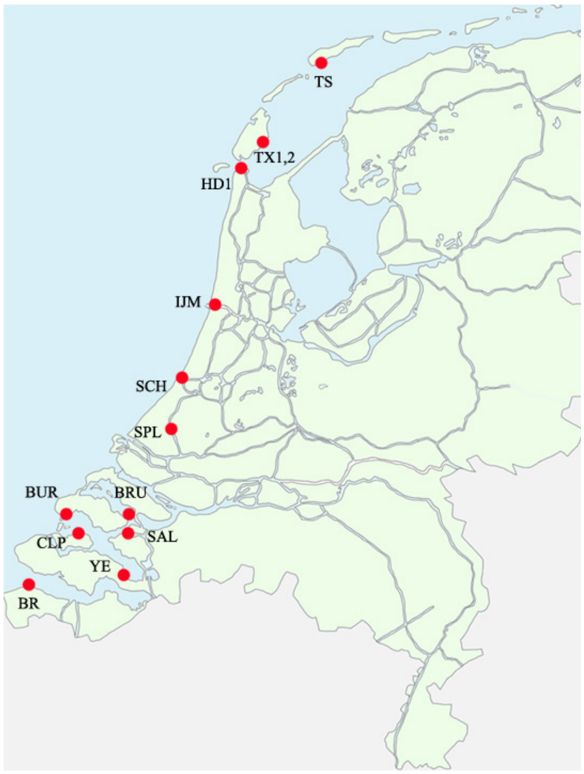

Fig. 3. An overview of all locations where the hull fouling on ships was measured in the period of 2010-2016. BR = Breskens, BRU = Bruinisse, BUR = Burghsluis, CLP = Colijnsplaat, HD1 = Den Helder, IJM = IJmuiden, SCH = Scheveningen, SPL = Schipluiden, TS = Terschelling, TX1 = Oudeschild (Home), TX2 = Oudeschild (Guests)

For assessing the fouling intensity a categorisation based on Floerl (2005) was used. The class "slime fouling only" of Floerl (2005) was ignored however, as a biofilm is hard to see in the field. The hull fouling intensities were therefore scored in the 5 classes A-E (Fig. 4; Table 1). Some of these classes were difficult to distin-

Table 1. Ranks of the ordinal fouling adapted from Floerl *et al*. (2005) that were used in this research. In the final analyses rank B and C are grouped as "Light fouling", and D and E are grouped as "Heavy fouling".

	Rank	Description	Visual estimate fouling of cover
Clean	A	No visible fouling. Hull entirely clean, no biolfim* on visible submerged parts of the hull.	0%
Light Fouling	B	Light fouling. Hull covered in biofilm and 1-2 very small patches of marcrofouling (only one taxon).	1-5 % of visible submerged surfaces
	C	Considerable fouling. Presence of biofilm, and macrofouling still patchy but clearly visible and comprised of either one single or several different taxa.	6-15 % of visible submerged surfaces
Heavy fouling	D	Extensive fouling. Presence of biofilm and abundant fouling assemblages consisting of more than one taxon.	16-40 % of visible submerged surfaces
	E	Very heavy fouling. Diverse assemblages covering most of visible hull surfaces.	41-100 % of visible submerged surfaces
*Biofilm: Thin layer of bacteria, microalgae, detritus and other particulates that is required for settlement of the larvae of many species of marine invertebrates.			

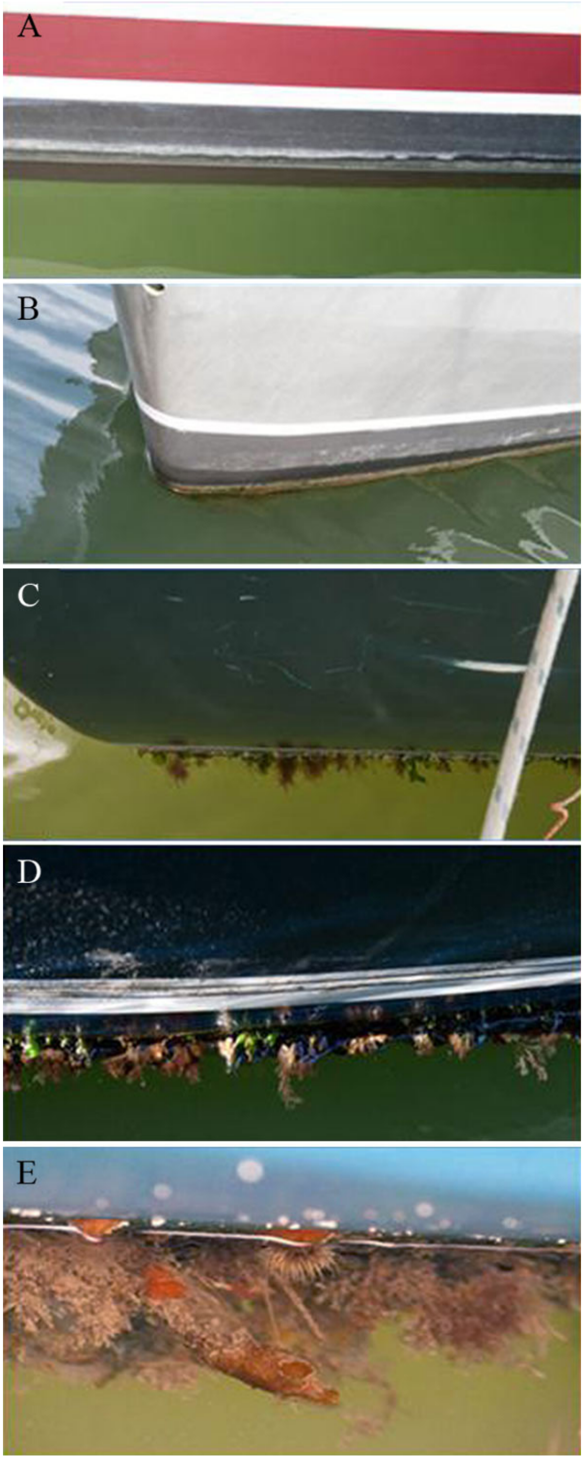

Fig. 4. Examples of the hull fouling ranks used in this study. [A] Clean hull; [B&C] Light fouling; [D&E] Heavy fouling. See Table 1.

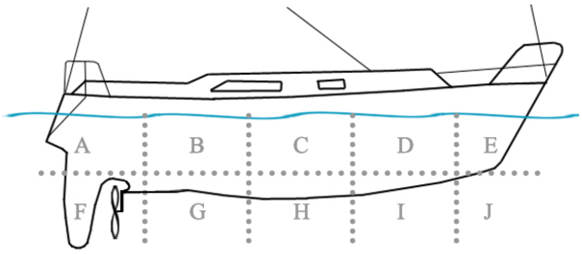

Fig. 5. Side view of a boat. The boat is divided into 10 different areas where fouling intensity (Table 1) was recorded: A-J.

guish from each other in the field causing some error because of observer variance. In the analyses of the present study it was therefore decided to use three classes: “Clean”, “Light Fouling” and “Heavy fouling” grouping classes B and C in the class “Light Fouling”, and classes D and E in the class “Heavy fouling” (Table 1).

For a selection of pleasure crafts the boat was divided in 10 areas A to J (Fig. 4) after which the fouling intensity and number of fouling species was scored for each of these areas. This was partly done based on underwater video footage taken with an underwater camera on a pole. As the areas A and F differ mostly, because of the propellor present in area F, these two areas are compared separately. To get an overview of the complete boat, the fouling was assessed in the five zones AF, BG, CH, DI and EJ (Fig. 5).

3.3 SETL

The SEtTLement-project (SETL) is a fouling community study monitoring the diversity of hard substratum related organisms with a focus on non-native species in harbours and ports.

For the start-up of the project GiMaRIS cooperated with the ANEMOON foundation (www.anemoon.org) promoting volunteer monitoring in the marine environment and has been in close contact with the Smithsonian Marine Invasions Laboratory. Their plate design was used as the basis of the SETL-project to ease worldwide comparisons to be made. The Smithsonian Marine Invasions Laboratory have been deploying plates along both coastlines of northern America and in Hawaii (Ruiz *et al.,* 2006).

The SETL project was started in 2006 and is run since then by GiMaRIS continuously in the Netherlands, locally in the USA by the Salem Sound Coastwatch (http://www.salemsound.org/PDF/SETL-1.pdf), and project based in other European countries (e.g. in the SEFINS project: www.rinse-europe.eu/sefins) and throughout the Pontocaspian region (www.pontocaspian.eu). In 2006 and 2007 a few thousand SETL plates were delivered to various European marine institutes to be deployed from the Mediterranean up to Scandinavian waters within the MarPace project (Marine Propagation Along the Coasts of Europe: www.marbef.org/projects/settlement/index.php) undertaken within the MarBEF EU Network of excellence (www.marbef.org). MarPace aimed at studying the settlement of pelagic propagules of benthic plants and animals along large-scale Pan-European transects representing spatial gradients in environmental conditions such as seawater temperature, insolation and seasonality. Using molecular techniques for assessing the presence of larvae in water this project resulted in a detailed overview of the spatial synchronies in the seasonal occurrence of larvae of Pacific oysters (*Crassostrea gigas*) and mussels (*Mytilus edulis/galloprovincialis*) in European coastal

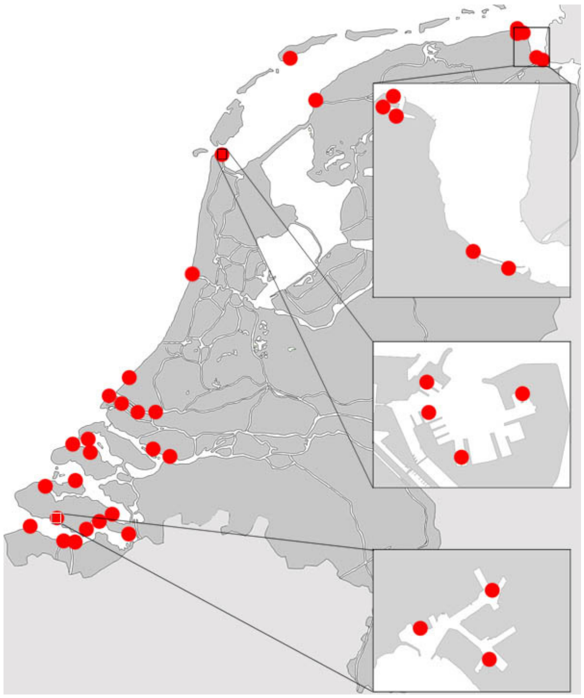

Fig. 6. Harbours in the Netherland where SETL-plates are (or have been) deployed.

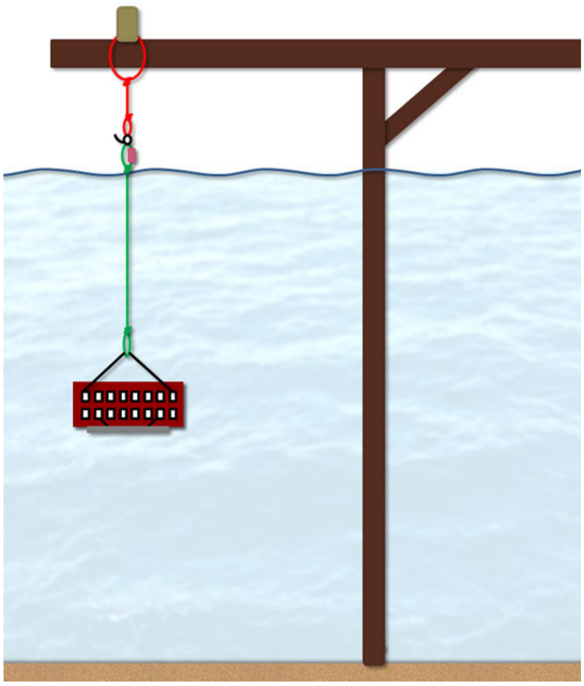

Fig. 7. Schematic view of a SETL plate hanging 1 meter under a dock.

waters (Philippart *et al.,* 2012). In 2007-2008 SETL plates were deployed in the six main ports of the Netherlands to assess the ecological quality of the water with a special focus on non-native species for the Dutch Ministry of Infrastructure and the Environment (RWS) and Deltares in the frame of the implementation of the EU Water Framework Directive and its pertinent monitoring (Gittenberger, 2008). These projects form the basis of the SETL-project as it is described for sampling the marine realm in the international manual on field recording techniques and protocols for all taxa biodiversity inventories by Templado *et al*. (2010) and has been conducted in the Netherlands since 2006 at a total of 35 sites (Fig. 6; Appendix II).

Annually about 150 plates are being deployed in the Netherlands at about 12-15 sites, mostly in pleasure craft harbours and ports. A SETL-plate consists of a 14x14x0.5 cm grey PVC plate attached to a brick to keep it horizontal, hanging from a plastic line with a metal core in the water column. It is deployed at a depth of 1 meter under the water line if it is attached to a floating object and 1 meter under the low water line in tidal areas if it is fastened to non-floating structures (Fig. 7). The downward facing side of the smooth PVC-plate was roughened with sand paper (roughness K60) to ease the attachment of fouling species. Three monthly at least three new plates per site are deployed. The already deployed plates are then taken out of the water and directly placed upside down in a tile of water showing the roughened side of the plate just submerged under the waterline. After photographing the unique label that indicates the deployment date, the plate surface is then photographed in overview and in detail. Most plates are then taken back to the lab for further analyses after three months of deployment, but some are redeployed after photographing them in the field, enabling the monitoring of the succession of fouling communities over time periods up to eight years. After dividing each plate on an overview photo into 25 grids, the presence of species, recognisable from the photos, was scored for each grid. The monitoring data from 2006 to 2017 thereby includes about 21.945.000 presence/absence records of species, i.e. 150 plates x 4 seasons x 11 years x 133 species x 25 grids.

The dataset has been used for various purposes, including biology and bioinformatics student projects and fundamental research projects. Mainly for the Dutch Office for Risk Assessment and Research (BuRO) of the Netherlands Food and Consumer Product Safety Authority, in recent years several more region focused SETL studies were done in the ports of Den Helder, Rotterdam and Vlissingen and throughout the Westerschelde. These studies are done to support the management of marine non-natives by for example assessing the potential of granting an exemption under the international convention for the control and management of ships' ballast water and sediments regulation A-4 following the HELCOM/OSPAR guidelines (HELCOM/OSPAR, 2013).

In the Netherlands the presence of 133 species, of which 38 non-native, was scored on SETL plates over the years. Immediately after the start in 2006, a species was recorded as new to the Netherlands in the Wadden Sea (Gittenberger & Schipper, 2008). Further illustrating the potential as an early detection method of non-native species, in the same year the westernmost sighting was done of the invasive Ponto-caspian quagga mussel *Dreissena rostriformis bugensis* within the Haringvliet a few months after its first sighting in the Netherlands (Schonenberg & Gittenberger, 2008). Samples from SETL plates were furthermore used for the extended genome analyses of the invasive carpet sea squirt *Didemnum vexillum* (Velandia-Huerto *et al.,* 2016), the quantification of the competition for space between non-native and native fouling species (Gittenberger & Moons, 2011), and the study of dominance and presence of non-natives in species communities in their pioneer stages in comparison to later succession stages (Lindeyer & Gittenberger, 2011). In 2009 the SETL-dataset was used for the first non-native species focussed survey of the Wadden Sea (Gittenberger *et al.,* 2010), which was subsequently used for a risk analysis of hull fouling on small to medium sized boats as an import vector of non-native species in the Wadden Sea (Gittenberger *et al.,* 2011a).

As most of the SETL-plates are deployed from floating docks just alongside pleasure crafts, the project provides invaluable data on species communities that may settle on these boats. Because the hulls of boats are relatively often cleaned, the species settling on them will mainly concern pioneer species that are also found on settlement plates that have been deployed for several months. Fouling species that become more dominant in later succession stages are recorded mostly on floating docks and on settlement plates that have been deployed for several years. These communities would be expected to grow on boats that are not cleaned over longer periods of time. In the present report we will test these hypotheses on the basis of the SETL data available comparing communities recorded on boat hulls with communities on SETL-plates, floating docks, and other structures (Gittenberger & van der Stelt, 2011). In addition it will be assessed in which harbours most non-native fouling species are found assuming that this number provides an indication of the risk that a specific harbour poses as a stepping stone for non-native species expanding their range along the Dutch coastline.

3.4 Total species inventories

During total species inventories done in the Wadden Sea (Gittenberger *et al.,* 2010, 2012a, 2015a), in the port of Rotterdam (Gittenberger *et al.,* 2014b) and in the Delta region (e.g. Gittenberger & van der Stelt, 2011, Gittenberger *et al., in press*), the diversity of fouling species on floating docks was recorded at 26 different locations (Fig. 8) between 2009-2016 (Fig. 9). This dataset was used to assess [1] which harbours in the Netherlands host most non-native species, and [2] to what degree fouling species communities on floating docks resemble communities recorded on settlement plates and communities recorded on pleasure craft hulls.

In addition to floating docks, many more habitats, including soft sediments were searched for non-native species within these inventories. The surveys for example in the ports of Rotterdam and Vlissingen (Sloehaven) were conducted according to the HELCOM/OSPAR protocol (Gittenberger *et al.,* 2014, *in press*., HELCOM/OSPAR, 2013). Hereby in total 336 samples were taken at 149 different sampling locations in three research areas within the port of Vlissingen Sloehaven (Gittenberger *et al., in press*.; Fig. 10) and 257 samples were taken at 118 locations within four research areas in the port of Rotterdam (Gittenberger *et al.,* 2014; Fig. 10). The habitats that were searched are illustrated in figure 11. This was done using, among other methods, Chinese crab traps, Gee's minnow traps, fouling plates (SETL), sub-littoral scrape samples of pilings and docks, visual inspections of dike transects and petit ponar samples. Similar methods were used during the non-native species focused inventories done in the Dutch Wadden Sea in 2009, 2011 and 2014 (Gittenberger *et al.,* 2010, 2012a, 2015a).

The data of these studies has been combined to detect hotspots of non-native marine species along the Dutch coast, comparing harbours, with dikes outside of harbours, soft sediments and shellfish beds and reefs. The total number of non-native species found in harbours, is furthermore compared with the total number of non-natives that was found in the shellfish associated species inventories (SASI's) carried out in the Oosterschelde, Grevelingen and Wadden Sea between 2010 and 2016 (Gittenberger *et al.,* 2011b, 2012b, 2013a, 2014a, 2015bcde, 2016). Finally it was assessed in which habitats within harbours most non-natives are recorded.

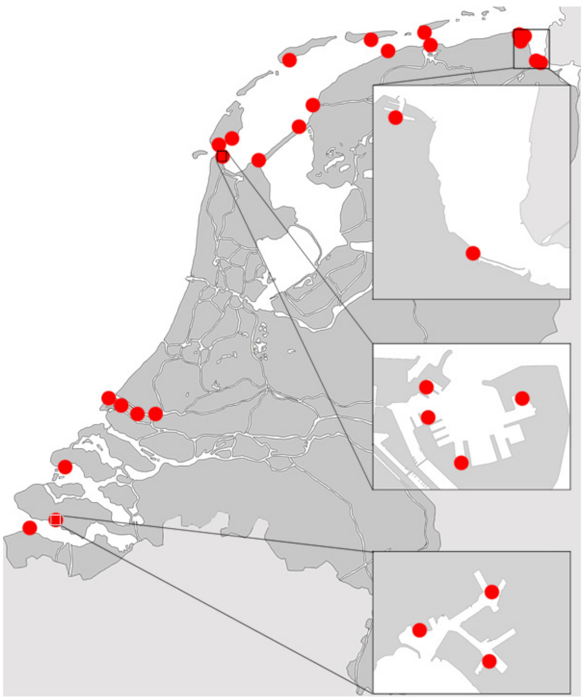

Fig. 8. All locations of harbours in the Netherland where floating docks have been monitored using snorkeling and/or scrape techniques.

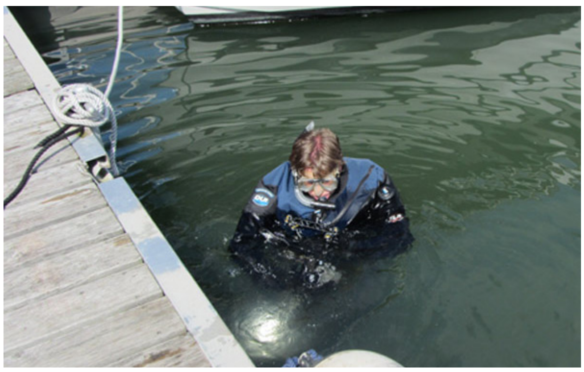

Fig. 9. A floating dock is being inspected by a snorkeller.

Similar total inventories in ports following the HELCOM/OSPAR protocol have been conducted in other European countries, e.g. in Hull, UK (Gittenberger *et al*., 2015fg) and the Baltic (e.g. Ojaveer *et al.,* 2013). Similar inventories as were done in the Dutch Wadden Sea were also done in the German Schlegwig Holsteijn Wadden Sea (Gittenberger *et al.,* 2013b). The latter dataset is used in this report in to compare the situation in the Netherlands with neighbouring countries.

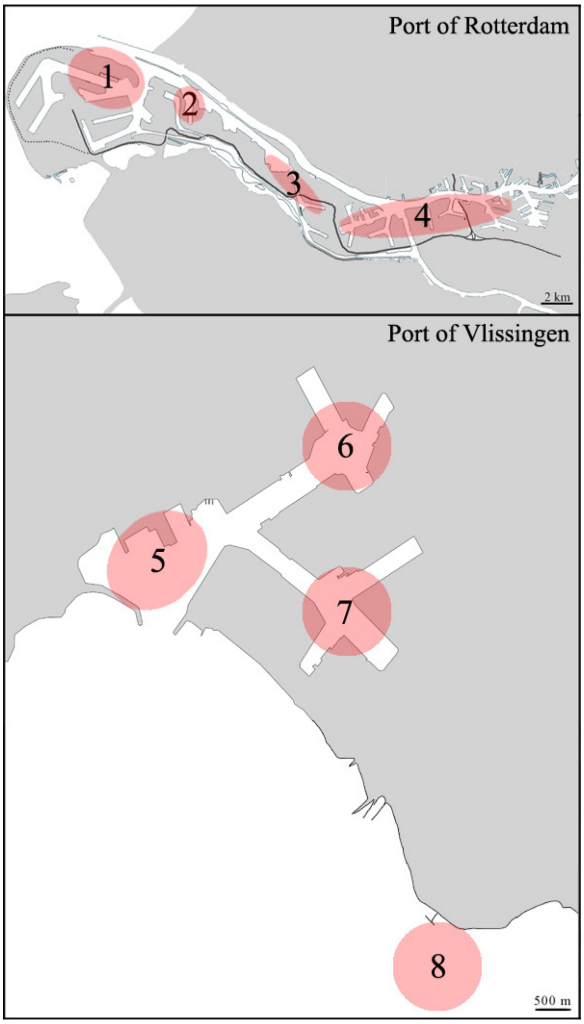

Fig. 10. Research areas of the ports of Rotterdam and Vlissingen (Sloehaven) for species inventories according to OSPAR-HELCOM protocol. [1] Rotterdam 8e Petroleumhaven, [2] Rotterdam Beneluxhaven, [3] Rotterdam Brittanniëhaven, [4] Rotterdam 1e Eemhaven, [5] Vlissingen Ritthemsestraat, [6] Vlissingen Westhofhaven, [7] Vlissingen Kaloothaven, [8] Everingen.

3.5 IMO-guidelines and questionnaires

Between 2009 and 2016, during five student projects supervised at GiMaRIS, harbour masters and pleasure craft owners were interviewed (Dekker, 2010; Freijser; 2009, Meulen; 2012, Pauli, 2014, 2015; Schie, 2011). To estimate the annual economic cost of fouling caused by native and non-native species in harbours, 38 harbour masters across the Netherlands were interviewed. In addition, 143 boat owners were interviewed with questions regarding their cleaning activities and travel behaviour.

All questionnaires collected within these studies were reanalysed to assess [1] to what degree harbour master and boat owner behaviour is linked to the risk of hull fouling as transport vector of non-natives, and [2] to what degree the IMO guidelines for minimizing the transfer of invasive aquatic species as biofouling with recreational crafts (IMO, 2012), are followed in Dutch harbours.

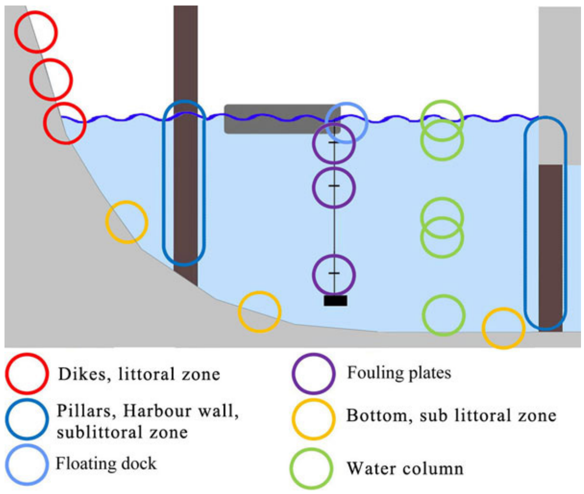

Fig. 11. Habitats sampled in the Sloehaven & Rotterdam. Red: dike in the littoral zone; dark blue: pillars and harbour walls in the sub-littoral zone; light blue: floating dock; purple: fouling plate construction; yellow: bottom; green: water column.

4. Results & Discussion

4.1 The relative risk of a harbour acting as a steppingstone

The hypothesis was tested that not every harbour is equally likely to act a stepping stone and that one should focus potential management and mitigation actions on those harbours that are most likely to function as stepping stones. It was first assessed in which habitats within a harbour one can find species communities that closely resemble species communities that are found on boat hulls. Based on the species communities recorded in various habitats (accumulated from all studies: Appendix III), a MDS (Multidimensional scaling) plot was made giving an indication of the resemblances between species communities recorded in these habitats (Fig. 12). This illustrates that species communities found on floating docks and SETL-plates are more similar to the species communities that are found on the hulls of pleasure crafts, than for example species communities recorded in other habitats like dikes, vertical structures, or shellfish areas (Fig. 11). The communities recorded on hulls are different from those that were recorded in the monitoring projects on SETL plates and floating docks, mainly because much less species were found on hulls, i.e. 16 species in comparison to respectively 39 and 44 species (Appendix III). This is probably due to the fact that SETL plates were designed to attract as many species as possible, and floating docks are rarely cleaned. We therefore hypothesize in the present study that they give an indication of the non-native species that can settle on hulls in a worst case scenario.

The numbers of non-native species recorded on respectively SETL-plates and floating docks, was therefore used to get an indication of the risk that a harbour acts as a stepping stone for non-native species.

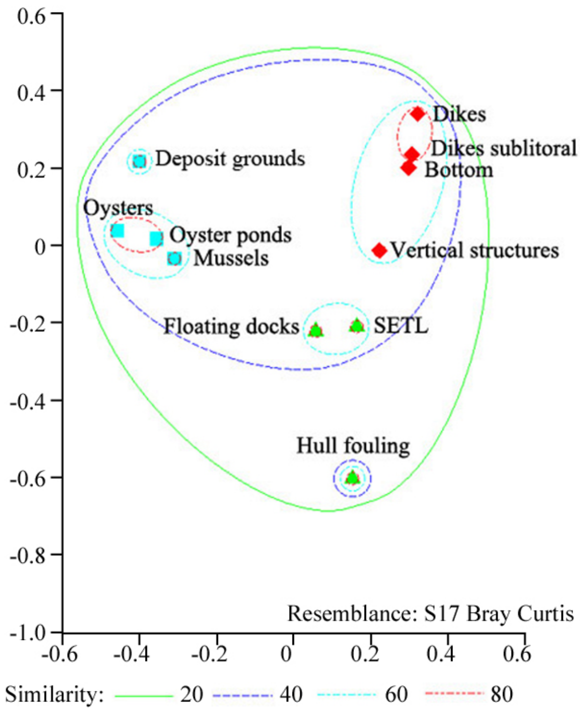

Fig. 12. MDS plot of the resemblances between species communities recorded in different habitats. The distance between two dots indicates how similar the species communities are. Blue squares are shellfish related habitats; green triangles are floating objects; red diamonds are non-floating structures.

In total 132 different species were found on SETL plates between 2006 and 2016. Of these species, 39 are non-native for the Netherlands. The total number of non-native species recorded at each SETL location is given in figure 12. Notice that the black circles represent SETL-locations where both non-native algae and animals are recorded, while the red circles represent locations where only animals were recorded. As the species communities scored on SETL-plates are living in the shadows on the underside of the plate, not many algae live there: The highest number of non-native algae is found in Vlissingen Ritthemsestraat (3 species). At the other locations with algae, only 1 or 2 non-native algae were found. Most non-native species recorded for a location were found there within a few years. The fact that not all SETL-locations were monitored for the same number of years, may cause some differences in total number of species scored however.

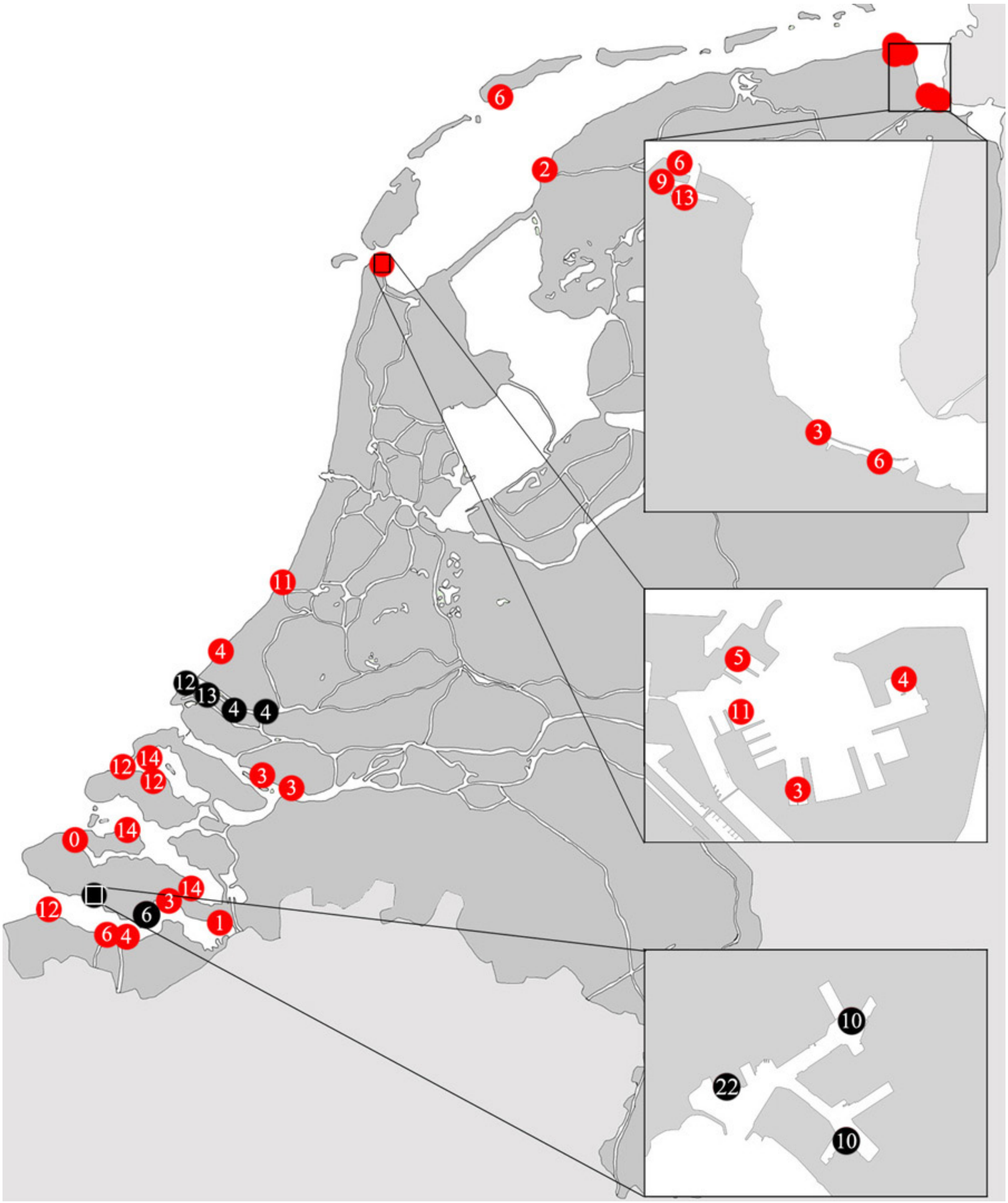

Fig. 13. All locations of harbours in the Netherland where SETL-plates are (or have been) deployed. The numbers inside the circles represent the total number of non-native species found on the SETL plates per locations. Locations with a black circle indicate that non-native algae species found on the plates are scored as well, while these are not scored for the red circles. Not all locations have been monitored for the same time period (Appendix II).

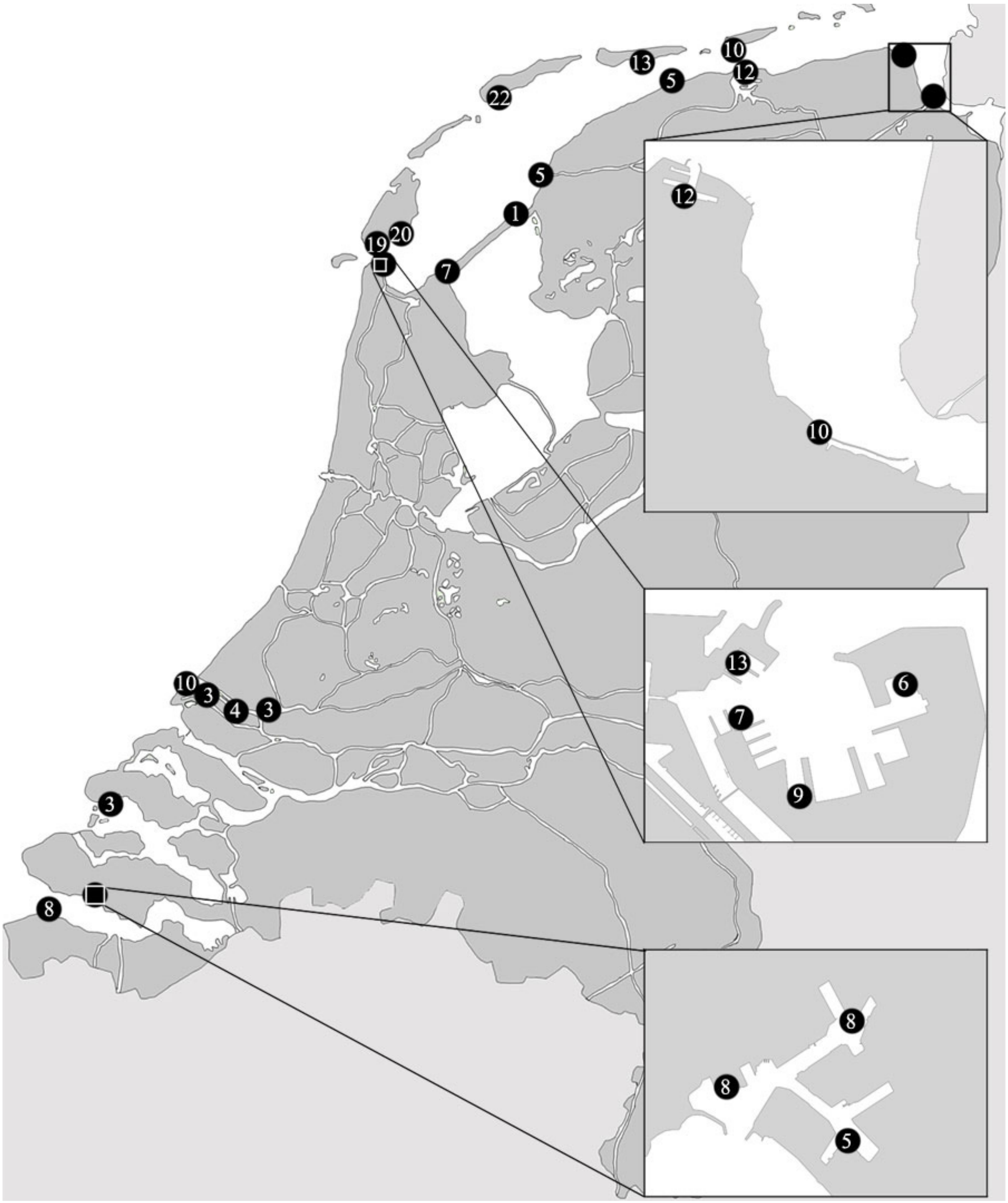

Fig. 14. All locations of harbours in the Netherland where floating docks have been monitored using snorkeling and/or scrape techniques. The numbers inside the black circles represent the total number of non-native species (including algea) found on the floating docks per locations.

Most non-native species were recorded close to the entrance of the Sloehaven, Vlissingen. On the SETL-plates that are deployed there since 2007 in total 22 non-native species were found while only 12 and 13 non-natives were found in the port of Rotterdam where the plates have also been deployed since 2007. The main reason for this difference appears to be the freshwater influx from the rivers into the port of Rotterdam. Such a freshwater source is absent in the port of Vlissingen. This hypothesis is supported by the fact that the non-native species like the sea-squirts *Botrylloides violaceus* and *Didemnum vexillum*, which are present in Vlissingen but absent in Rotterdam, are known to be relatively sensitive to low salinities (Gittenberger, 2010; Gittenberger & Moons, 2011). That the presence of a freshwater source is reducing the number of non-native species is further supported by the decreasing number of species found on SETL-plates from the North Sea in the west to the brackish port of Antwerp outlet in the east in the Westerschelde (Fig. 13). In the Oosterschelde, a sea-inlet that is disconnected from the rivers, similar numbers of non-native species (14) are recorded on SETL-plates from west to east. In general it can therefore be concluded that more non-native species are found on SETL-plates in more saline harbours.

A similar pattern was found on the floating docks. In the port of Rotterdam the number of non-native species declines from 10 to 3 going from North Sea in the west up to the river in the east (Fig. 14). In most harbours on the western Wadden Sea islands, which are more saline, almost double the number of non-native species is found in comparison to the Wadden Sea harbours along the main coast (Fig. 14). A total of 178 different fouling species have been found on floating docks, of which 43 are non-native for the Netherlands There are some distinct differences between the species found on floating docks and those found on SETL-plates. This can partly be explained by the fact that algae do grow well in the sunlight on the sides of floating docks, but do not grow well on the monitored underside of SETL-plates. In addition most SETL-plates have only been deployed for three months, while most floating docks have been in the water for years at the moment that they are monitored. Therefore pioneer species are mainly scored on SETL-plates and species that become more dominant in later succession stages are mainly scored on floating docks. The difference between floating docks and SETL-plates in the pleasure craft harbour of Breskens is described in more detail by Gittenberger & van der Stelt (2011). The differences between fouling species communities in their pioneer stages and later succession stages is described in more detail by Lindeyer & Gittenberger (2011) on the basis of SETL-plates throughout the Netherlands that were deployed for the first time in various seasons, and were checked for species after 3, 6, 9, and 12 months.

Based on the number of non-native species found on SETL-plates and floating docks, there is no significant difference between the numbers of non-native species present in large ports like the port of Rotterdam, which are > 100 km^2 in size in comparison to a pleasure craft harbour of about 1 km^2 in size. In several cases more non-natives were found in a single pleasure craft harbour than in such a port. This illustrates the relatively high impact that a pleasure craft harbour can have. It is furthermore concluded that harbours with more saline waters are most likely to act a stepping stones for the secondary spread of non-native species. In the Netherlands this concerns mostly the harbours in the western part of the Westerschelde and the harbours on the islands in the western part of the Wadden Sea. In the Grevelingen and Oosterschelde similar numbers of non-native species are found on the SETL-plates and floating docks, but based on the questionnaires it appears that a relatively lower percentage of pleasure crafts in these waters travel to the open sea. The risk that these harbours act as stepping stones for non-natives is therefore assumed to be slightly less.

4.2 High risk habitats within harbours

Within pleasure craft harbours four main habitats are present where species are found: floating docks, vertical structures (like a harbour wall or a pole), dike and bottom (usually soft sediments). Combining all data from harbours monitored during the three non-native species focused inventories in the Dutch Wadden Sea (Gittenberger *et al.,* 2010, 2012a, 2015a), the total number and average number of both non-native and native species scored in these habitats was calculated (Fig. 15). The highest number of non-native species and native species is found on floating docks, respectively 40 and 151 (Fig. 15A). The highest average number of non-native species recorded, is also found on floating docks, i.e. 5.2 (Fig. 15B). Although on average virtually no non-native species are found on the bottom, i.e. 0.7 (Fig. 15B), in total 16 non-native species (Fig. 15A) were found on the bottoms in the pleasure craft harbours of the Dutch Wadden Sea.

In conclusion, aiming at finding most non-native species in a pleasure craft harbour with the least amount of effort (costs), one should search the floating docks, but if one aims at finding all non-native species present, you also need to search the other habitats, including the bottom.

The species surveys in the port of Rotterdam and the port of Vlissingen Sloehaven gave a slightly different result (Figs 16-17). There, in total, a similar number of non-native species was found in all habitats with the exception of dikes where slightly less non-natives were found (Figs 16A, 17A). On average, however, most non-native species were found within these ports on either the floating docks (Rotterdam: Fig. 16A) or on the SETL-plates (Vlissingen Sloehaven: Fig. 17A).

This illustrates that aiming at finding most non-native species in a port with the least amount of effort (costs), one should search the floating docks and/or SETL-plates, but if one aims at finding all non-native species present, one should certainly also search the other habitats present. For illustration purposes the results of the plankton monitoring in both ports was also included in the figures 16 and 17. Although by far most species are found in the plankton, the number of non-natives is relatively the lowest (Figs 16A, 17A).

The fact that, at least on average, most non-native species are found on SETL-plates and floating docks, the habitats that most closely resemble ship hulls (Fig. 12), supports the hypothesis that these ports and harbours probably act as important stepping stones in the distribution of non-native species. Especially in pleasure craft harbours, keeping floating docks clean by regular maintenance, is therefore a potential mitigation method that is expected to considerably reduce this risk.

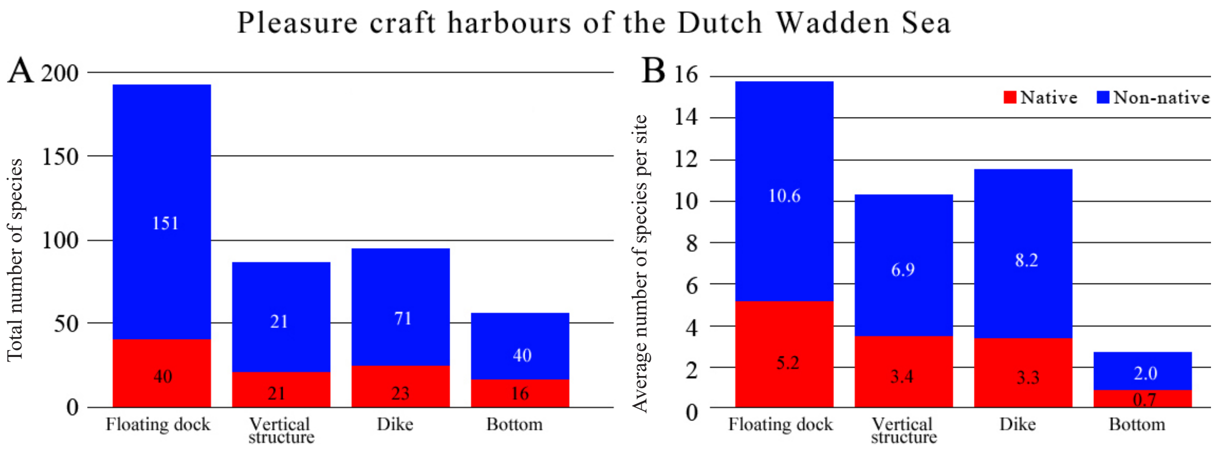

Fig. 15. Dutch Wadden Sea. Species scored in various habitats in 12 harbours. [A] Total number of native (blue) and non-native (red) species. [B] Average number of native and non-native species (Gittenberger *et al.,* 2010, 2012a, 2015a).

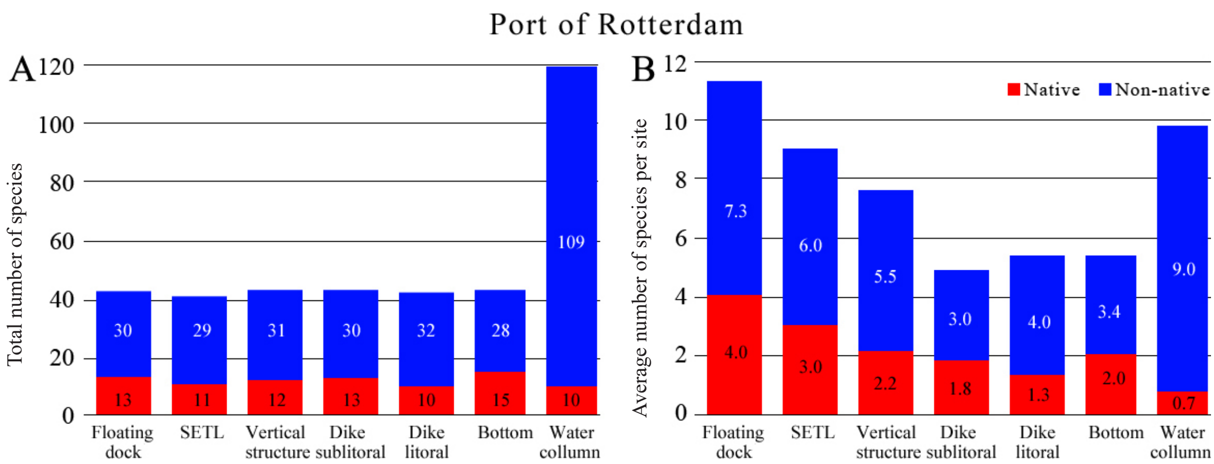

Fig. 16. Port of Rotterdam. Species scored in various habitats in the port. [A] Total number of native (blue) and non-native (red) species. [B] Average number of native and non-native species (Gittenberger *et al.,* 2014b).

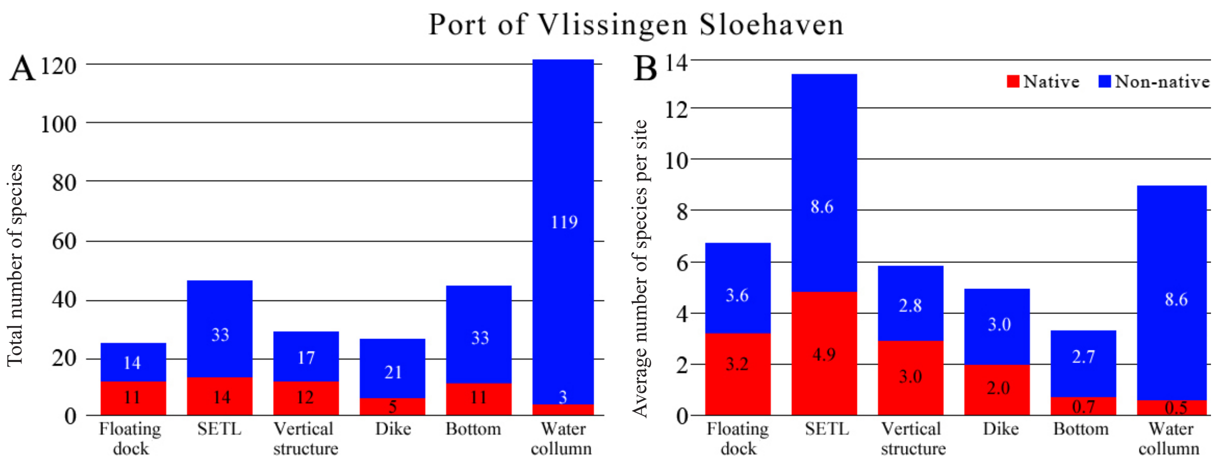

Fig. 17. Port of Vlissingen Sloehaven. Species scored in various habitats in the port. [A] Total number of native (blue) and non-native (red) species. [B] Average number of native and non-native species (Gittenberger *et al., in press*).

4.3 High risk vessels

For the harbour of Breskens, a harbour in the Westerschelde close to the North Sea (Fig. 3), a dataset was made available by the harbour master describing all visitors between 2002 and 2010. On the basis of 15631 records in this dataset (Gittenberger *et al.,* 2011a), it was concluded that 36% of the visitors had a home port located outside the Netherlands. Most of these boats were from Belgium (16.6 %), the United Kingdom (11.2%), Germany or France (5.7 %). About 1 % (n=85) of the pleasure crafts visiting Breskens travels throughout European waters over distances > 1000 km. A selection of boats that visited Breskens have their home ports in remote areas like the USA, Canada, Mexico, Sierra Leone, Nigeria, South Africa, Japan, Australia and New Zealand (Gittenberger *et al.,* 2011a). As Breskens is a relatively small pleasure craft harbour in the Netherlands, this data indicates that one should consider hull fouling on pleasure crafts as a potential primary import vector of marine non-native species from other continents into NW Europe.

The distances that boats travel is directly correlated with the length of the boat (Fig. 18). This is based on a relationhip between number of boats in a certain length class and the distance of their home ports to Breskens (χ^2 = 123.608, df = 8, p < 0.00005; Fig. 18). Hereby most, but not all, pleasure crafts that travel distances of > 2000 km concern boats with lengths of >10 m (Figs 18-19). The average length of boats that travelled from a destination that is more than 5000 km away from Breskens is 14.87 ± 1.64 m (n=23). This significantly larger (37%) than the average length of pleasure crafts that visited Breskens, i.e. 10.88 ± 0.15 m (n=15337). Also boats that travelled 1500 km are significantly larger (13.6 ± 1.15 m, n= 52) than the average boat size. These records support the assumption that longer journeys are usually done in larger pleasure craft boats.

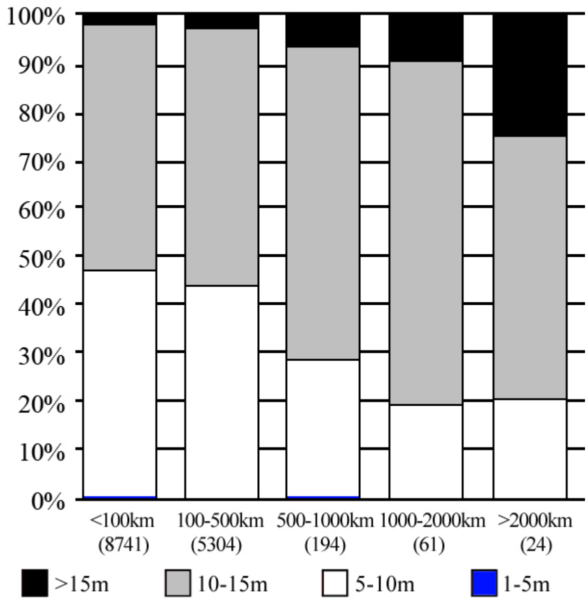

Fig. 18. The x-axis shows the distance between home ports and Breskens for boats that visited Breskens between 2002-2010 (n=14324). These boats are divided into 5 classes: Home ports located <100km from Breskens (n= 8741), at 100-500 km distance (n = 5304), at 500-1000 km distance (n= 194), at 1000-2000 km distance (n=61) and > 2000 km distance (n=24). The y-axis shows boat length categories: 1-5m, 5-10m, 10-15m and >15m.

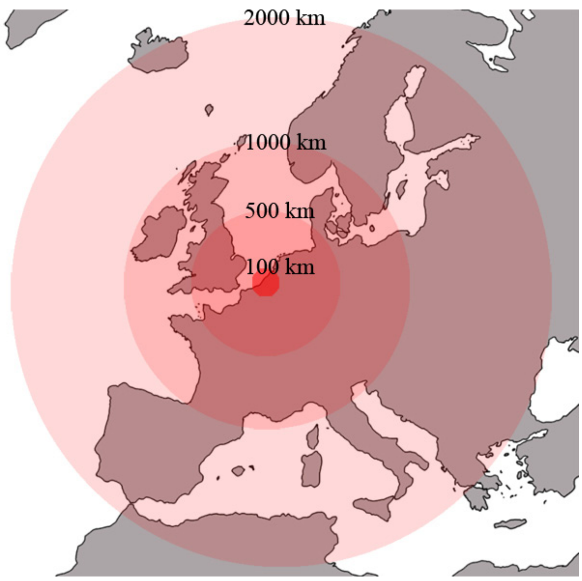

Fig. 19. Map of Europe to get insight into where visitors of Breskens come from (Fig. 18).

The risk of transporting non-natives with larger boats is reduced to some degree by the fact that larger boats more often have clean hulls and less intense fouling (Fig. 20; $\chi^2 = 14.1$, df = 4, $p < 0.007$). Still about 43% of the larger boats (>15 m) is fouled however, while in all studies combined on average 59% of all pleasure crafts (1205 boats) is fouled. Respectively 92% and 73% of the smaller boats in the size classes 1-5 m and 5-10 m are fouled.

Especially over smaller distances, e.g. concerning the secondary spread of non-natives (Fig. 1B) within NW Europe (< 500 km, Figs 18-19), it is concluded that the risk that they travel along within the fouling on the hulls of relatively small pleasure crafts (<10 m) is considerably higher than the risk that they get transported by larger boats. As larger boats (>10 m) in general travel longer distances than smaller boats, larger boats are probably the main primary transport vectors with which non-natives get introduced from their region of origin into new regions (Fig. 1A), i.e. by journeys worldwide and throughout the whole of Europe (> 500 km, Figs 18-19).

Within harbours, most pleasure crafts (>99%) concern either sailing ships or motor boats. These two boat types differ significantly ($\chi^2 = 158.709$, df = 2, $p < 0.00005$) in the amount of fouling and the fouling intensities present (Fig. 21). As virtually all, i.e. 96% of the pleasure crafts (1008 out of 1048 boats) are made from polyester, the material that these boats are made of cannot explain the difference. About 75% of all motorboats (567 out of 755 motorboats) are fouled and about 17% of all motorboats have "heavy fouling" on their hulls. In comparison, slightly less than 50% of the sailing boats (560 out of 1208 sailing boats) is fouled and in total about 10% of all sailing boats have "heavy fouling" on their hulls. It can, therefore, be concluded that the risk that non-native species travel along within the fouling on the hulls of motorboats is distinctly higher than the risk that they travel along within the fouling on the hulls of sailing boats. It should not be disregarded however that still half of all sailing boats are fouled and they therefore remain to be a vector of risk (Fig. 21).

In conclusion the highest risk vessels concerning the primary introduction of non-native species into NW European waters are expected to be relatively large (>10 m) motorboats. Relatively small (< 10 m) motorboats are expected to be the highest risk vessels responsible for the secondary distribution of non-native species throughout NW European waters.

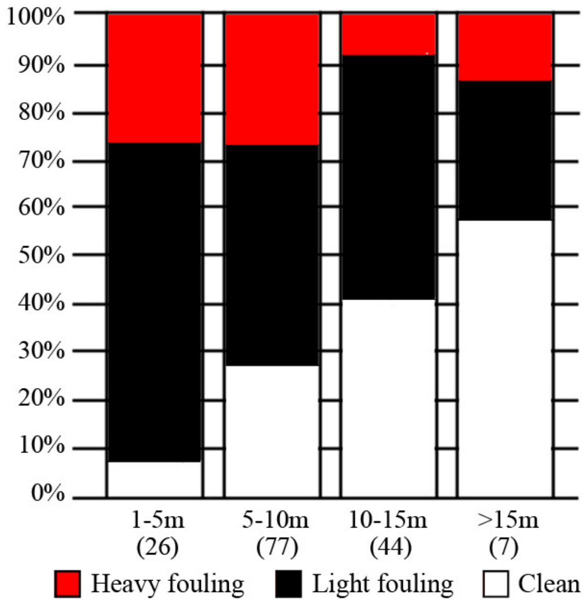

Fig. 20. An overview of the different hull fouling types found on pleasure crafts of different lengths. Lengths are grouped into 4 classes, between 1 and 5 meters, 5 – 10 meters, 10 – 15 meters and larger than 15 meters. The y-axis shows the relative number of ships that were clean, lightly fouled and heavily fouled. The total number of ships studied is indicated below the columns.

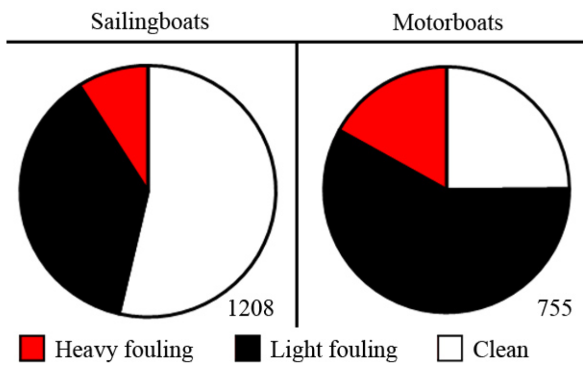

Fig. 21. The percentage of sailing boats (n=1208) and motorboats (n=755) that was clean, lightly fouled or heavily fouled. The total number of ships studied is indicated below the pie diagrams.

4.4 Micro habitats on vessels.

A total of 125 areas on boats were videoed on 62 boats with an underwater camera on a pole to assess the fouling intensity and the number of species in each area.

No significant differences were found between the fouling intensities recorded in the 10 different areas A to J (Fig. 22A) separately, and combined in the five zones from the back to the front of the boat, i.e. AF, BG, CH, DI and EJ (Fig. 22B, χ^2 = 4.756, df = 8, p =0.7833). There was also no significant difference in fouling intensities recorded within the shallower (ABCDE) and deeper (FGHIJ) parts of a boat (χ^2 = 2.600, df = 2, p =0.2726). Assuming that the propellor (in area F, Fig. 22A) would be more intensively fouled, area A was compared with area F but no significant difference was recorded (χ^2 = 0.488, df = 2, p =0.7835). In conclusion, if a boat is fouled, the fouling intensity as recorded in the present study, is the same in all areas.

Although the fouling intensities do not differ significantly, the number of species recorded on the hull does differ per area. On average more species are found at the front of the boat (area EJ) and at the back (area AF), than in between (areas BG, CH and DI)(Fig. 22C). In the back, significantly more species (P (X > 15) = 0.0345), i.e. 15 species, are found around the propellor or motor in area F than on the hull at the surface in area A, i.e. 8 species.

In conclusion, the highest risk areas on a boat concern the front of a vessel and the propellor or motor at the back (Figs 23-24).

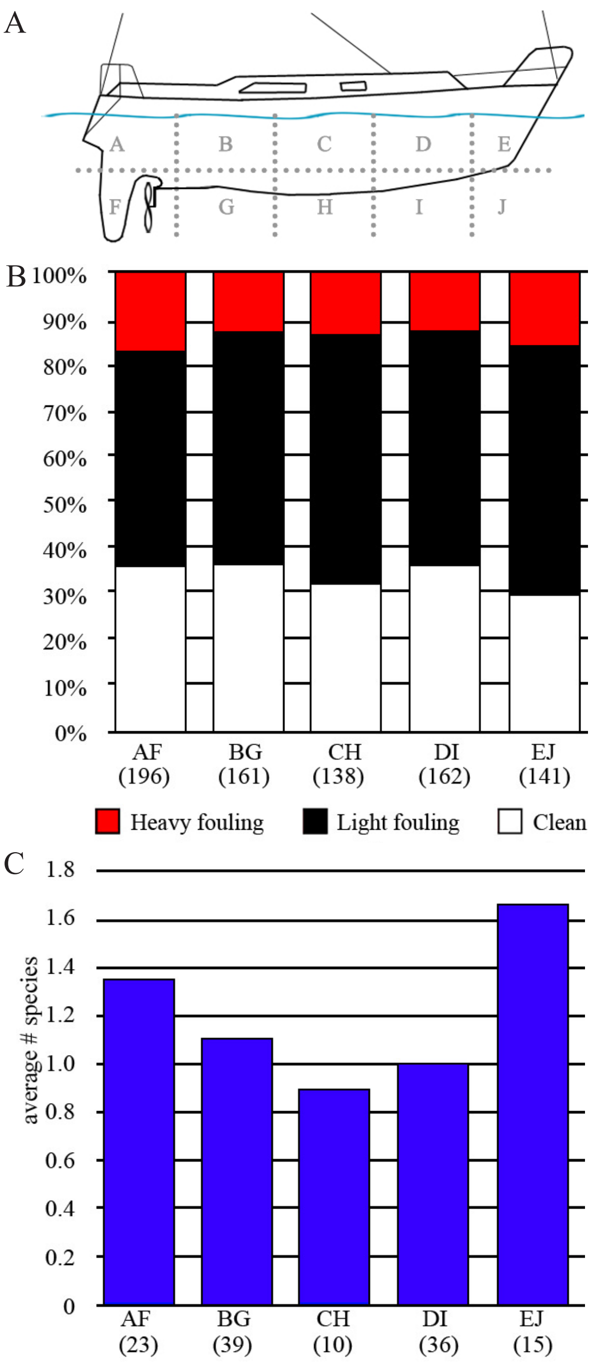

Fig. 22 [A] The sideview of a boat which is divided into different areas (Fig. 4), [B] the percentage of boats found to be either clean, with light fouling or heavy fouling in 5 different areas, [C] the average number of species found in these areas.

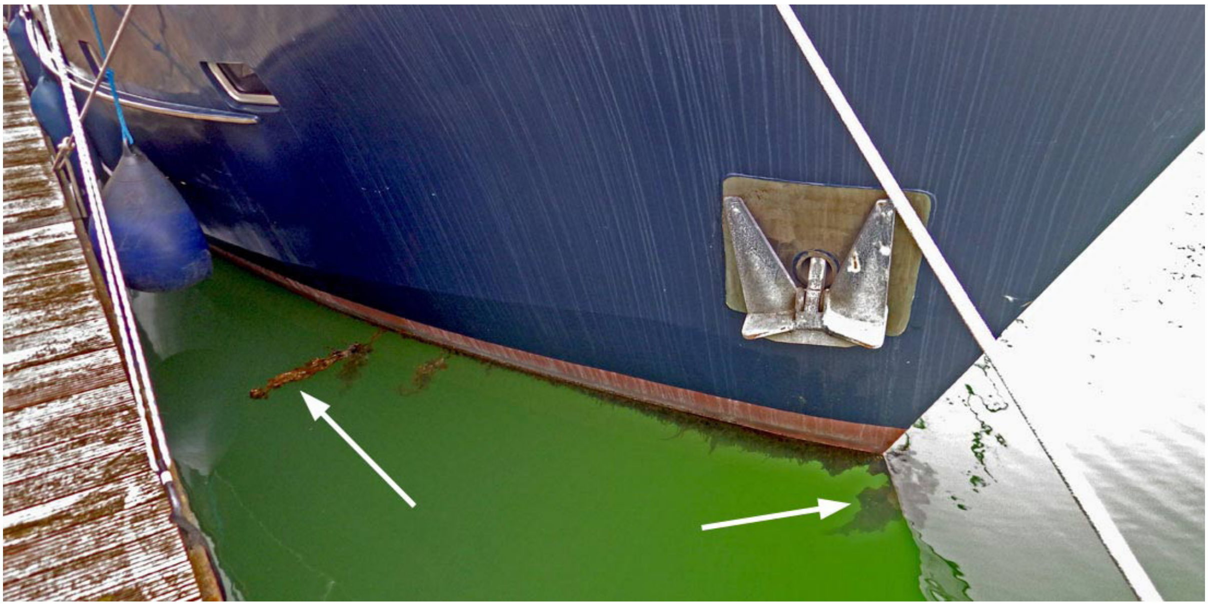

Fig. 23. The invasive Asian kelp species *Undaria pinnatifida* (white arrows) attached to the hull of a sailing boat in the harbour of Breskens.

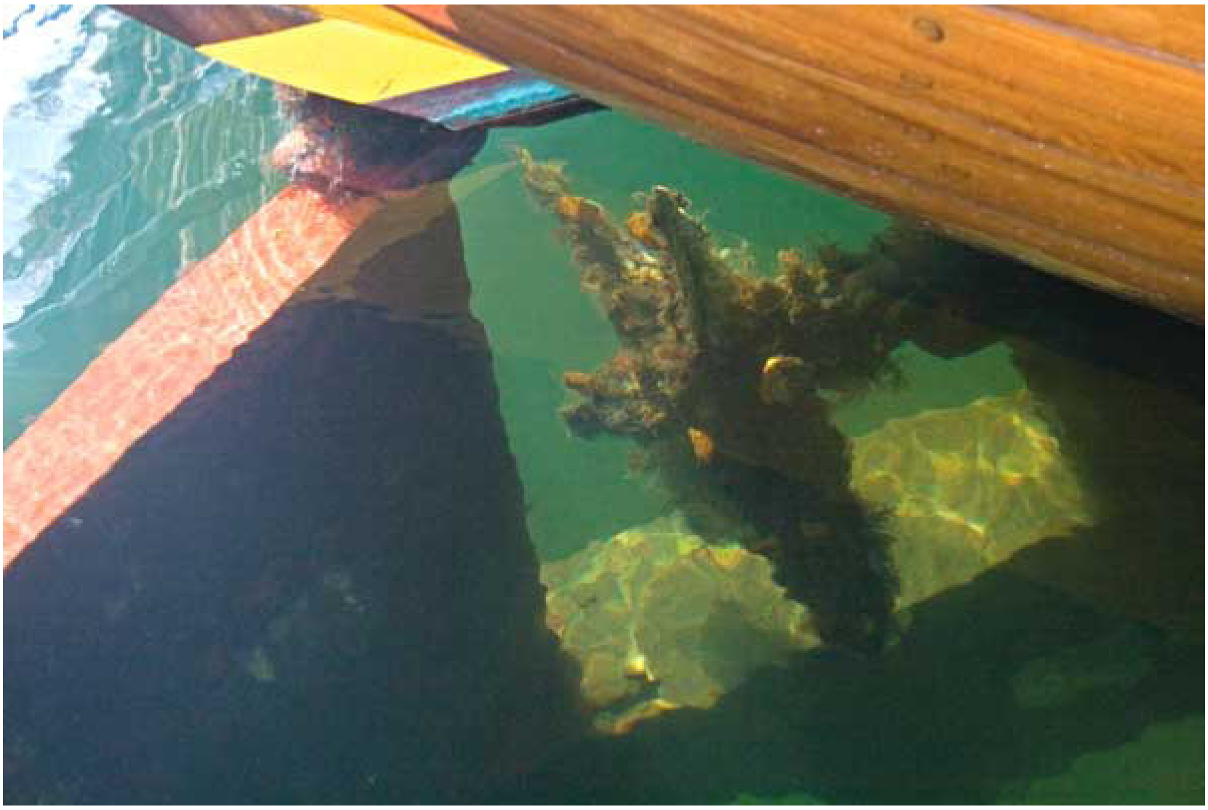

Fig. 24. A propeller of a boat in Breskens, heavily overgrown with fouling species. The sides of the boat are relatively clean compared to the propeller.

4.5 Influence of boat owners' behaviour on the risk of spread of non-native species

In total 59% of all pleasure crafts (2055 boats) studied, were fouled. This is a similar percentage as was calculated by Ashton et al. (2006) for harbours in Scotland. There also 59% of all yachts were found to have fouling. Along the coast of the USA Zabin *et al*., (2014) found even higher percentages, i.e. 80% of the boats were fouled. Within the Netherlands these percentages can also differ strongly between harbours as is illustrated in figure 25. Figure 25 gives an overview of the relative number of pleasure crafts that were fouled in the summer with either light fouling or heavy fouling in eight harbours. Especially in the Wadden Sea harbours of Oudeschild and Terschelling a relative high number (>75%) of the boats was fouled. These also concern the harbours where relatively most boats were found to have "heavy fouling" (>25%). Within the Wadden Sea the pleasure craft harbour of Den Helder was also studied. Less than 25% of the boats there were fouled. This may partly be due to some freshwater entering the harbour, but may also be due to the fact that only people working for the Dutch ministry of defence are allowed to have their boat there. It is the largest military port of the Netherlands. They may be more keen on keeping their boats clean than the people that visit the Wadden Sea islands or own a pleasure craft there.

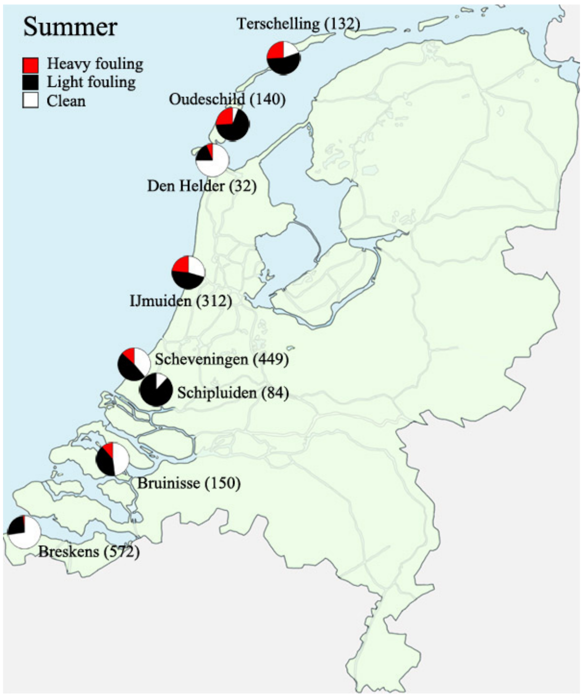

Fig. 25. The percentages of hull fouling types found at different harbours in the Netherlands. A red colour indicates heavy hull fouling, black indicates light hull fouling and white indicates a clean hull. Between brackets is the number of pleasure crafts investigated at each harbour. In Oudeschild only the resident area was taken into account.

The number of fouled boats in the harbour of Oudeschild on Texel differs significantly between the residents and passers/visitors areas ($\chi^2 = 45.01$, df = 1, $p < 0.0005$). There virtually all boats in the residents part of the harbour were fouled, while only about 50% of the boats visiting the harbour had fouling on their hulls (Fig. 26). Residents having more fouling on their boats than visitors of a harbour was also found for the harbour of Scheveningen, positioned centrally in the Netherlands, although there the difference was not as extreme and not significant ($\chi^2 = 3.21$, df = 2, $p = 0.201$) (Fig. 26). Although the presence of an influx of freshwater may explain some of these differences, this does not explain the difference between the number of fouled boats in the Wadden Sea island harbours (>75%) and the equally saline harbour of Breskens where only about 25% of the boats were fouled in summer (Figs. 25, 27). In Breskens the hulls of the boats are probably more actively cleaned or the visitors of this harbour in general have boats with cleaner hulls. This is confirmed by the fact that in Breskens more boats were found to be fouled in spring than in summer. The boats monitored in spring were probably the ones that were not taken out of the water in winter time explaining the relatively extreme fouling. The fact that the absolute number of boats that were heavily fouled declines in Breskens from spring to summer, can be explained by the fact that boat

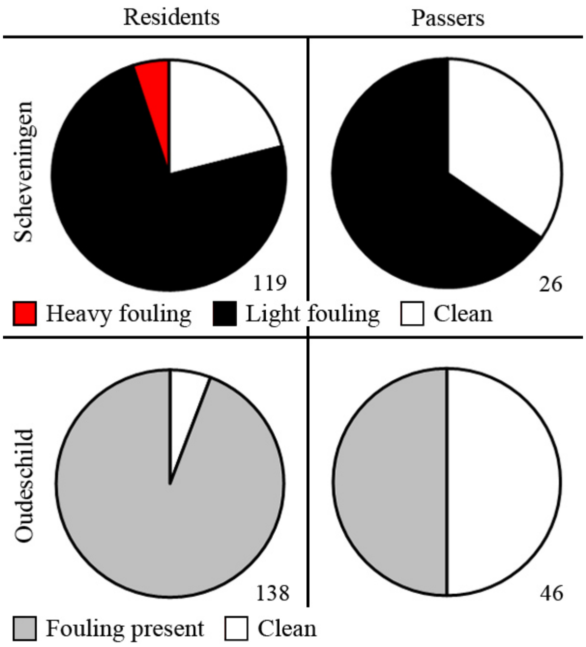

Fig. 26.The percentages of boats that were clean, lightly fouled and heavily fouled in the residents and passers areas within the harbours of Scheveningen and Oudeschild. For Oudeschild it was only noted whether fouling was present or not. The number of pleasure crafts investigated is indicated below each pie diagram.

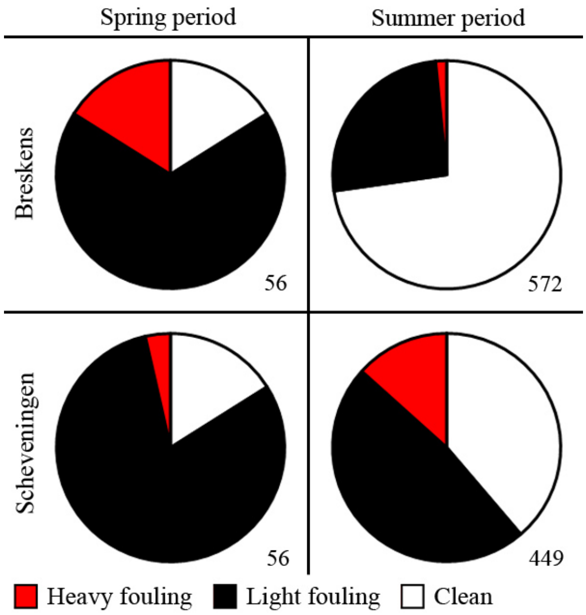

Fig. 27. The percentages of boats that were clean, lightly fouled and heavily fouled in the harbour of Breskens in spring (n=56) and in summer (n=572) and in Scheveningen in spring (n= 56) and summer (n=449). The number of pleasure crafts investigated is indicated below each pie diagram.

owners clean the hulls of their boats in that period. That a higher number of boats are fouled in spring in Scheveningen probably also concerns boats that were left there in winter time, illustrating that taking a boat out of the water in the winter, will strongly reduce the amount of fouling (Fig. 27).

A relatively high percentage of all boats was also found to be fouled in the harbour of Schipluiden (>80%) but there none of the boats was "heavily fouled". This is probably due to the fact that Schipluiden is a freshwater harbour.

4.6 IMO Guidelines

The IMO document on "Guidance for minimizing the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft" (IMO, 2012) provides a good insight in the potential risk of hull fouling on recreational crafts and how to mitigate that risk. In total 143 boat owners and 42 harbour masters were interviewed in the Netherlands over the period of 2009 – 2015 (Dekker, 2010; Freijser, 2009; Meulen, 2012; Pauli, 2014, 2015; Schie, 2011; Weger *et al.,* 2012). For boat-owners 13 surveys were done in Breskens, 83 in Scheveningen, 2 in Bruinisse, 7 in IJmuiden, 31 in Oudeschild and 7 in Schipluiden (Fig. 25). Even though these interviews did not have the specific goal to test the awareness of the IMO document itself, none of the boat-owners or harbour masters mentioned it or gave any indication that they knew of its existence.

The focus of this study was to assess to what degree the content of the document is already known and to what degree the guidelines described are followed. This is done below by going through the various topics described in the IMO document.

Why is the Transfer of Biofouling organisms a problem?

One of the first chapters of the IMO guidelines emphasizes that the transfer of biofouling organism can threaten fresh, brackish and marine environments and economic and cultural activities.

Focusing on the economic impact, in 2009, 38 harbour masters of harbours in the Netherlands were interviewed in order to get an estimation of what the total economic impact of fouling species are for the harbours in the Netherlands. About 55% (n=21) of these harbour masters indicated that they encounter problems related to fouling. The annual costs related to fouling amounted annually to at least €512.000 in harbours. About 23 percent (€120.000) of these costs is caused by invasive fouling species, more specifically the Pacific oysters (*Crassostrea gigas*). The incidental (non-yearly) costs amounted to about €95.000 of which €23.000 was directly caused by invasive fouling species. Harbour masters indicated that these expenses were caused for the most part by transporting and cleaning of floating docks on land, cleaning of poles, pillars and dam walls, cleaning of drainage outlets, pumps and cool water systems, the dredging of areas infested with Pacific Oysters and underwater cleaning using divers (Freijser, 2009).

The main problem that boat owners are aware of when talking about hull fouling, is the fact that fouling slows down the boat and takes a lot of time and effort to clean. None of the boat owners interviewed indicated that they were aware of the potential environmental impact of hull fouling. Most are aware of the potential negative impact of the use of toxic anti-fouling on the environment.

What influences the amount of biofouling on recreational crafts?

In total 77 boat owners were asked what type of anti-fouling they used to reduce the amount of fouling on their boat hulls. These surveys were done in Scheveningen (28), Bruinisse (2), IJmuiden (7), Oudeschild (31) and Schipluiden (7). Table 2 gives an overview of the type of antifouling used. Out of 77 boat owners, only 2.6% indicated that they did not use antifouling on their crafts and 16.9% were not sure what type of antifouling they used. Although 23.4% used non-toxic antifouling, 36.4 % indicated that they used biocides, 18.2% used antifouling with copper and 2.6% of the boat owners used zinc or tin. (Table 2). Two boat owners indicated that they used "Milking grease"('Uierzalf', consisting of petroleum jelly with an disinfectant agent, triclosan) as an alternative anti-fouling. They indicated that it is better than traditional antifouling and doesn't have to be re-applied as often. Although they may assume that this is a non-toxic antifouling, triclosan acts as a biocide. Therefore this method is considered as a biocide antifouling method and grouped as such in this study.

Table 2. Results of the boat owner questionnaires concerning boat maintenance (2011-2012).

Type of antifouling	# Boats	Motor Boat	Sailing Boat	Average length (m)	Average speed (km/h)	Average draft (m)	Average cleaning p/y
Unknown	13 (16.9%)	7	6	9.5	19.5	0.9	1.6
None	2 (2.6%)	1	1	7.5	6.5	0.4	1
Non-toxic	18 (23.4%)	5	13	9.3	15.2	1.2	1.4
Biocides (undefined)	28 (36.4%)	7	21	10.5	16	1.4	1.2
Biocides with copper	14 (18.2%)	0	14	10.4	11.6	unknown	0.6
Biocides with zinc	1 (1.3%)	1	0	11.4	13	unknown	0.5
Biocides with copper & tin	1 (1.3%)	0	1	12.5	unknown	unknown	0.5
Total	**77 (100%)**	**21**	**56**				

An apparent difference between motorboat owners and sailing boat owners, is that sailing boat owners tend to use more toxic anti-fouling, i.e. 14 sailing boat owners indicated that they use anti-fouling with copper, while none of the motorboat owners indicated the use of copper based anti-fouling paints (Table 2).

Although the average cleaning frequency per year for boat owners using different anti-fouling types did not differ significantly, some trends appeared to be present. For example, the group of boat owners that did not know what kind of antifouling they used, had the highest average frequency of hull cleaning practices per year (1.6 times/year, n=13). The group with the second highest cleaning frequency were the non-toxic antifouling users with an average of 1.4 times a year (n=18). Biocide users had a cleaning frequency of 1.2 (n=28) and users that used copper, zinc and even tin in their antifouling had the lowest cleaning frequency of 0.61 (n = 16). These differences may be representative for the effectiveness of the different anti-biofouling types. It also indicates that most boat owners clean their boats more often than once per year, i.e. during the winter period that most boats are taken out of the water.

In general it is concluded that owners using non-toxic antifouling paints clean their boats more as is suggested within the IMO guidelines. There were no indications however, that the IMO advice was followed to use certain designs or constructions to prevent fouling on more susceptible areas such as rudders, propellers and propeller shafts.

How can biofouling be minimized?

As is suggested by IMO a good maintenance regime is important to minimize biofouling accumulation. From the surveys in the Netherlands it is concluded that about 64% of the boat owners (48 out of 75) hauled their crafts out of the water at least once a year after which they are cleaned with high pressure hoses. This percentage is not representative for all harbours however. For example, in the harbour of Scheveningen on 21 January 2017, 269 boats were still in the water, which is 94% of the total capacity of the harbour (285 docking places). Of these boats 15 were 1-5 meter, 136 were 5-10 meter, 108 were 10-15 meter and 10 boats were > 15 meter in size. In total 34% (92) were motorboats and 66% (177) were sailing boats. These numbers indicate that most boats in Scheveningen are not dry-docked for winter storage. This may be because it is too expensive as little space for dry-docking appears to be present around the harbour. The fact that most boats are not dry docked, may also at least partly explain the relatively high percentage of boats fouled (~75 %, Figs 25-27) in this harbour. For comparison, the harbour master of pleasure craft harbour in Yerseke in the Oosterschelde, indicated that 95% of the boat owners cleaned their boats there on land when they were stalled just before the winter period.

Boat owners that indicated that they only travel in salt water cleaned their crafts on average 1.7x per year (n=19), which was considerably higher than boat owners that operated recreational crafts in both marine and fresh waters (average cleaning frequency = 1.1, n =28) and fresh water only (average cleaning frequency = 0.8, n = 6). This supports the suggestion by IMO that hulls with marine fouling can be cleaned by travelling to fresh water areas. This is based on the fact that fresh water usually has a deleterious effect on marine species.

It is concluded that taking boats out of the water in winter time and switching between freshwater and more saline harbours, minimized the amount of hull fouling. Whether this is done differs greatly between harbours varying from dry-docking virtually all boats in winter time to virtually no boats in winter time.

Is one anti-fouling coating system acceptable for all crafts?

In total 57% of the boat owners interviewed used biocides, with or without copper (44 out of 77). Hereby there is a trend that sailing boat owners use more biocide anti-fouling than motorboat owners, respectively 64% (n=56) and 38% (n=21). Notable furthermore is that 14 sailing boat owners indicated that they used copper in their antifouling, while none of the motorboat owner did so. The main reason for this difference may be that the questionnaires indicated that sailboat owners in general appear to more keen to minimize the drag of their crafts than motorboat owners.

The IMO guidelines indicate that one should take the construction material into account when choosing the anti-fouling type. In the Netherlands 96% of the boats (n= 1048; Table 2; Fig. 3) is made from polyester. The number of boats made from other materials was too low in the present study to get an indication on whether or not other antifouling types were used for other materials (like wood).

How can biofouling be minimized in niche areas?

No information was obtained in this study that specific antifouling coating systems were applied on different niche areas as is suggested in the IMO guidelines. However, 23 boat owners were asked if they encountered frequently fouled niche areas and how they dealt with this. Out of these 23 boat owners 8 said that they experienced differences in fouling intensity in specific niche areas, mentioning specifically the propeller, propeller shaft, rudder and back side of the boat. Of the 8 boat owners, 7 used scuba-diving gear to physically clean these specific areas in water ranging between 1 to 4 times per year. They all concerned the owners of sailing boats.

In conclusion, some boat owners are aware of niche areas with increased fouling. They are mostly sailing boat owners that clean these area in water with scuba-gear.

What about debris after cleaning?

The harbour masters of Burghsluis, Sint Annaland, Yerseke and Colijnsplaat were asked the question what was done with the chemical and physical debris after cleaning. All of them answered that they stored the water and debris in separate tanks, which were moved and cleaned by a specialized company, as is also suggested by IMO.

Linking voyage plans to anti fouling maintenance.

No indication was given in this study that cleaning or maintenance of the anti-fouling coating systems, and hull and nice areas were coordinated or linked in any way with the planning of a voyage or trip as is suggested in the IMO guidelines.

What about in-water cleaning?

About 23% (n=17) of the boat owners interviewed (75 of which 28 in Scheveningen, 2 in Bruinisse, 7 in IJmuiden, 18 in Oudeschild Guest, 13 Oudeschild Home and 7 Schipluiden) indicated that they used in-water cleaning on fouling species. Cleaning methods used were brushes, extendable brushes, sponges. No information was gathered whether or not local authorities were contacted before cleaning or how the physical and (possible) chemical debris was disposed of as is suggested within the IMO guidelines.

Of the boat owners that used in-water cleaning 24% (n=4) used non-toxic anti-biofouling and 53% percent used toxic biofouling. This ratio of antifouling usage is about the same as for all of the interviewed (n=75, Table 2).

In conclusion there is no indication that users of non-toxic biofouling do in-water cleaning more often than other boat owners as is recommended by IMO.

Is recording biofouling activities important?

There is no information in this study that any of the boat owners kept his/her craft's biofouling management information in one place, such as a logbook as is recommended by IMO.

General conclusions IMO guidelines

The present report indicates that most recommendations of the IMO-Guidelines will indeed minimize the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft. Even though there are no indications that the IMO-guidelines were known among the boat owners and harbour masters, most IMO recommendations are applied, but not consequently in all harbours. This includes for example [1] the recommendation to dry-dock and clean boats at least once a year (65.3% of the boat owners in the present study do this), [2] the advice to increase hull cleaning frequency when using non-toxic biofouling (33.3% of the boat owners in the present study do this), [3] the suggestion to minimize fouling in niche areas by appling an appropiate coating system (none of the boat owners in the present study indicated that they used a specific coating system for niche areas) and [4] the recommendation to store water and debris after cleaning on land in separate tanks, making certain that non-native species do not get flushed back into the water (the interviews with harbour masters indicated that this is probably done in all harbours).

Some IMO recommendations did not appear to be followed by any boat owner or harbour master in the Netherlands. Examples are the advice [1] to adjust the design or construction of areas that are more susceptible to fouling such as rudders and propellers, or [2] to apply more effective anti-fouling coating systems in these areas. As boat owners did indicate that rudders and propellers need to be cleaned more often, these IMO- recommendations would probably be effective. The IMO-guidelines give some examples on how to implement these recommendations describing for example how one can minimize biofouling on an anode by inserting a rubber backing pad between the anode and the hull (IMO, 2012).

Other recommendations made by IMO that were not followed by boat owners and harbour masters in the present study are for example [1] the use of logbooks to store biofouling management information and [2] the application of a specific cleaning and fouling maintenance plan looking at anti-fouling systems, hulls and niche areas prior to planning large trips or voyages.

The present study indicates that most recommendations in the IMO guidelines will indeed minimize the risk of non-native species being transported within the biofouling on pleasure craft hulls. They are applied by only a selection of boat owners and harbour masters seemingly because of a lack of awareness. Increased awareness of these recommendations in the Netherlands is therefore expected to reduce the risk that aquatic invasive species are distributed by pleasure crafts.

4.7 Hull fouling as a transport vector of non-native species

Based on three non-native species inventories in the Dutch Wadden Sea in 2009, 2011 and 2014 (Gittenberger *et al.*, 2010, 2012a, 2015a) and two similar inventories in 2012 and 2013 in the Schleswig Holstein Wadden Sea (Gittenberger *et al.,* 2013b) it can be concluded that harbours concern the hotspots in the Wadden Sea where most non-native species are found (Fig. 28). In the Netherlands this is also the habitat in which most native species are found while in the Schleswig Holstein Wadden Sea most natives are found on the bottom. This may be linked to the fact that more hard substratum, i.e. rocks and pebbles, can be found in the Schleswig Holstein region and in general more species are found on hard substrata than in soft sediments. In both the Dutch and German Wadden Sea much less species are found in shellfish areas than in harbours, and the species that are found in shellfish areas mostly concern species associated with Pacific oyster reefs (Gittenberger *et al*., 2010, 2012a, 2013b, 2015a). Shellfish aquaculture mostly focuses on mussels in the Dutch Wadden Sea.

Although shellfish transports have played a distinct role in the introduction of non-native species in the past, this role has been reduced considerably in recent times. Most of these imports of non-native species were associated with Pacific oysters (*Crassostrea gigas*) that were imported in European waters from the Pacific and American coasts in the 1970s and 1980s (Wolff, 2005). Such transports are not allowed anymore and transports within NW European waters are nowadays regulated with permits aiming at minimizing the chance of transporting non-native species. The hull fouling vector is not regulated, however, and is, therefore, probably the main vector with which marine non-native species get introduced and spread throughout the Netherlands and NW European waters. This is supported by the fact that about 60 % of the pleasure crafts are fouled, harbours are the hotspots along the coastline where most non-native species are recorded, and the floating docks that are used by the boats are the hotspots within harbours where most non-natives are found. In addition heavy fouling is commonly recorded on pleasure crafts in the Wadden Sea as is illustrated in figure 29. Up to about 25% of the pleasure crafts in the

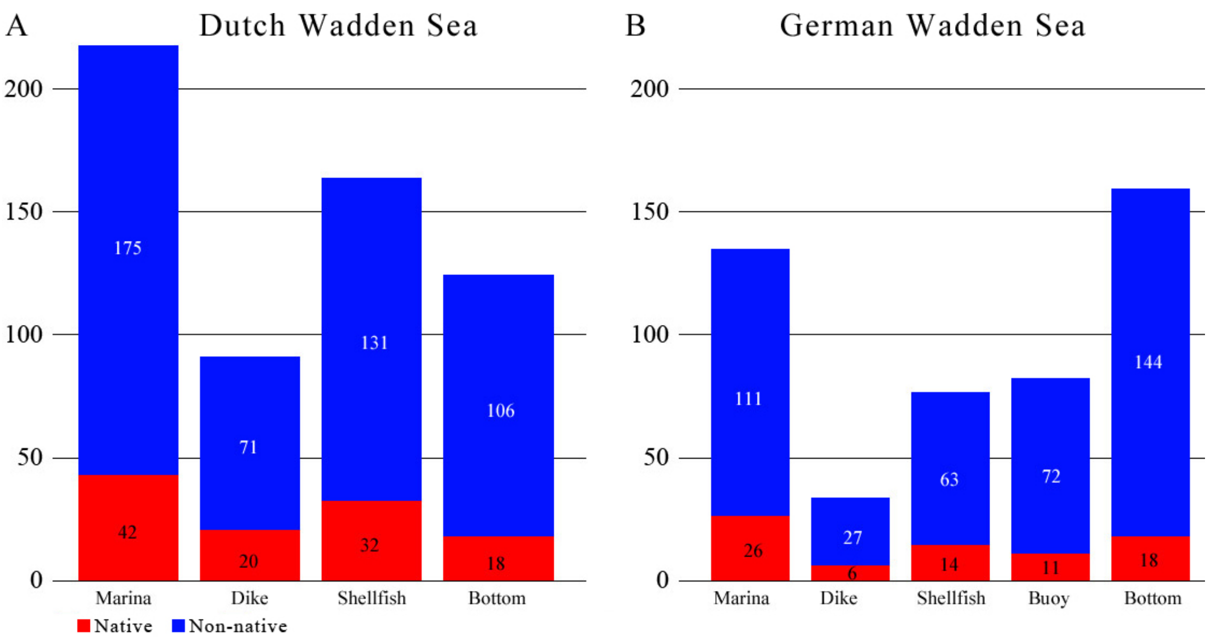

Fig. 28. Total number of species found within different habitats in [A] the Dutch Wadden Sea (Gittenberger *et al.,* 2010, 2012a, 2015a) and [B] the German Schleswig Holstein Wadden Sea (Gittenberger *et al.,* 2013b).

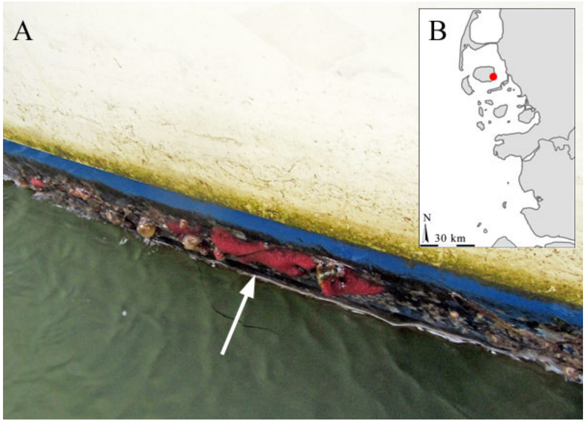

Fig. 29. Hull of a pleasure craft in the harbour of Wyk, German Wadden Sea, photograped during a non-native species focussed survey (Gittenberger *et al,* 2013b). The arrow illustrates the non-native red colonial sea-squirt *Botrylloides violaceus* among other fouling organisms. This species was first recorded for the German Wadden Sea during this expedition.

Wadden Sea have such heavy fouling in summer time (Harbours of Texel and Terschelling in Fig. 25). This fouling consists of full-grown animals and algae that can be transported in-water along the NW European coast. Pleasure crafts commonly travel throughout NW Europe and less often over even longer distances (Figs 18-19). Especially within NW European waters hull fouling is therefore expected to be the main transport vector of non-natives with probably the exception of natural dispersion. Many marine non-natives can travel over vast distances with the sea currents within their pelagic life stages or settled life stages on floating algae and debris. Ballast water is also considered to be an important transport vector of marine non-natives. However, as non-native species can be distributed throughout NW European waters with their pelagic life-stages by natural dispersion, the impact of ballast water as a transport vector of mainly organisms in their pelagic life stages becomes less important. In addition ballast water release is already regulated and will become even more strictly regulated when the IMO ballast water convention comes into force in September 2017. Hull fouling therefore remains as the least regulated vector of marine non-native species.

4.8 Examples of invasive species distributed by bio fouling.

Especially in recent years, possibly aided by relatively warm winters, the spread of invasive non-native species via biofouling on boats has become quite apparent.

The carpet sea squirt *Didemnum vexillum* for example was first recorded in the Netherlands in 1991. After that it remained rare until 1996. It rapidly became abundant in the Dutch Delta (Oosterschelde and Grevelingen) after the extremely cold winter of 1995-1996 followed by a relatively warm winter in 1997 (Gittenberger, 2007). Since then its spread in the Netherlands appeared to have halted until it was first recorded in the Wadden Sea in and in the vicinity of the pleasure craft harbour of Terschelling in 2008/2009 (Fig. 30; Gittenberger *et al.*, 2010). Subsequentely it was recorded in 2011 on the hull of a pleasure craft in the harbour of Oudeschild, Texel (Fig. 31; Gittenberger *et al.,* 2012a). In 2014 it was found to be abundant in the pleasure craft harbours of Texel, Vlieland and Terschelling and in the Wadden Sea on oyster reefs just in front of these harbours (Gittenberger *et al.*, 2015a). In 2014 it was recorded for the first time in the Westerschelde on a SETL-plate deployed from a floating dock in the port of Vlissingen. In 2016 it had spread throughout the port (Gittenberger *et al., in press*). Although over the years a variety of habitats has been searched in the Wadden Sea and Westerschelde for non-native

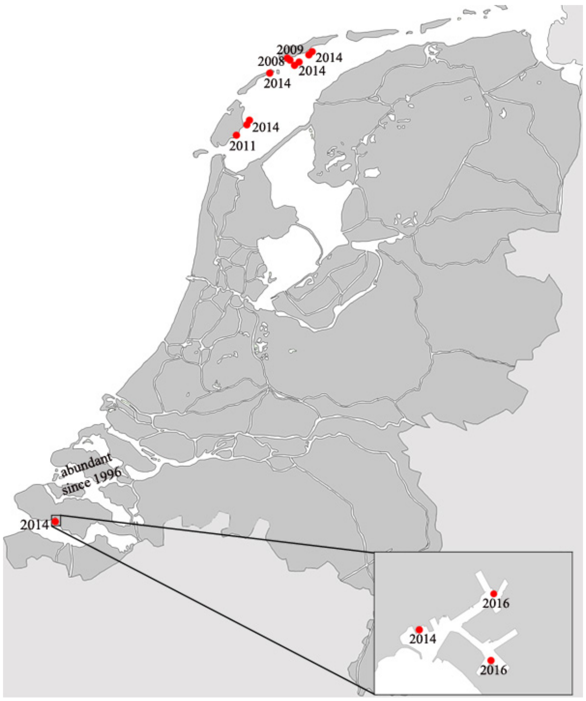

Fig. 30. The first records of the carpet sea-squirt *Didemnum vexillum* (Fig. 31) after its introduction in the Oosterschelde in 1991 where it remained rare until 1996.

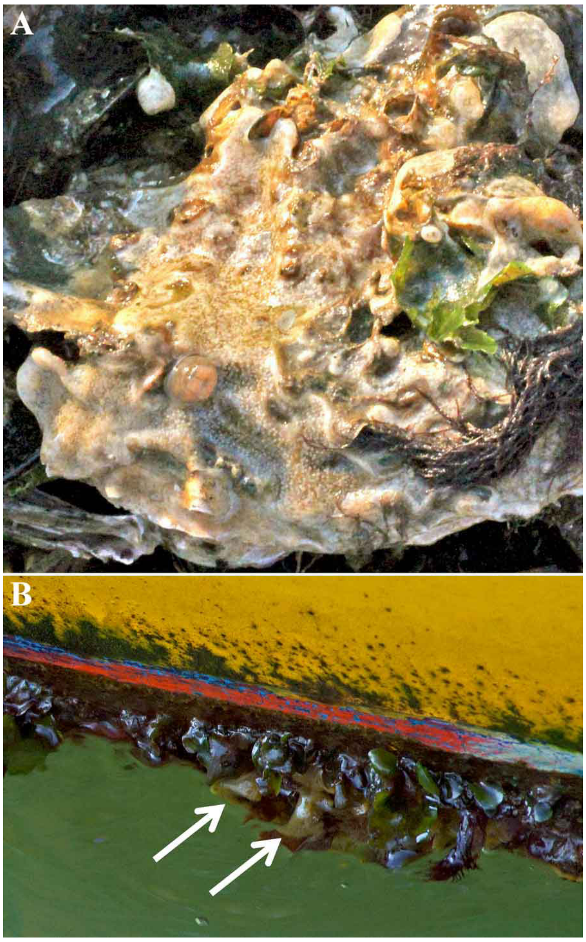

Fig. 31. *Didemnum vexillum*. [A] A colony on a Pacific oyster (*Crassostrea gigas*) found on a dike near the pleasure craft harbour of Terschelling. [B] The white arrows indicate the colony of *D. vexillum* on the hull of a sailing boat in the pleasure craft harbour of Oudeschild (located in the area of the harbour that is designated for residents). Several other colonies were found on this boat. (Gittenberger *et al*. 2011)

species (Gittenberger *et al.,* 2010, 2011, 2014), *D. vexillum* was first found in these regions in the vicinity of harbours and ports. These records therefore indicate that hull fouling is the most probable vector with which this invasive species was introduced to the Wadden Sea and the Westerschelde (Fig. 30).

Fig. 30. The first records of the carpet sea-squirt *Didemnum vexillum* (Fig. 31) in various regions within the Netherlands.

Another example of an invasive non-native species being recently spread by hull fouling concerns the kelp Wakame, *Undaria pinnatifida*. It was first recorded in 1999 (Stegenga, 1999) in the Oosterschelde where it quickly became abundant, especially in the pleasure craft harbours (*pers. obs.*, Fig 32). In 2008 it was first recorded in the Wadden Sea in the same year and at the same place where also *Didemnum vexillum* was first recorded for that region, i.e. in the pleasure craft harbour of Terschelling (Fig. 33; Ruijter, 2008). There it remained abundant on the floating docks (Gittenberger *et al.,* 2010, 2012a). In 2014 a single specimen was found settled on a Pacific oyster reef just south of Terschelling (Gittenberger *et al*., 2015a). In 2016 it was first recorded in the Wadden Sea pleasure craft harbour of Vlieland (Ruijter, 2016). This illustrates the potential stepping stone function of pleasure craft harbours. In addition, it was first recorded in 2016 for the Westerschelde, where several close to a meter long kelp leaves were growing on the hull of one of the resident pleasure crafts in the harbour of Breskens (Fig. 23).

Fig. 32. Wakame (*Undaria pinnatifida*) on the hull of a pleasure craft in the harbour of Burghsluis Oosterschelde.

5. Conclusion

Although hull fouling is generally considered to be one of the main transport vectors of marine non-native species around the world, invasive species legislation tends to focus on ballast water and aquaculture related vectors. Within the present report an assessment was made of the risk of hull fouling in the Netherlands, with a focus on pleasure crafts. It is concluded that hull fouling is probably, at this time, the main transport vector with which non-native species are imported into and transported throughout the Netherlands, with exception of natural dispersion. This is based on the conclusions that [1] harbours are, along the Dutch coast, the hotspots where most non-native species are found, [2] on average 59% of all pleasure crafts in marine harbours has fouling on its hull, and [3] about a third of the crafts visiting Dutch harbours can introduce new species from abroad as they come from countries like Belgium, the United Kingdom and France, but nowadays also from other continents like America, Africa, Asia and Australia. Several possibilities may exist to manage this risk as the amount of hull fouling recorded on pleasure crafts is linked to [1] a selection of "high risk harbours" that tend to be positioned in relatively saline waters and [2] a "high risk boat type", i.e. motorboats tend to be more fouled than sailing boats. This is probably so because sailing boat owners are more concerned to keep the hulls of their boats clean by using more toxic anti-fouling. Although the potential impact of toxic anti-fouling on the environment is well known among harbour masters and boat owners, the impact of hull fouling as a transport vector of non-native species, is not. The IMO (International Maritime Organization) document on "Guidance for minimizing the transfer of invasive aquatic species as biofouling (hull fouling) for recreational craft" may be useful to reduce this risk, but appears to be unknown to most boat owners and harbour masters. As mainly sailing boat owners are keener to minimize the drag of their craft, some of the suggestions within the IMO-document are followed, however. To what degree these suggestions are followed varies greatly between harbours throughout the Netherlands. This can subsequently be linked to the number of boats fouled, the fouling intensities and the number of non-native species recorded in these harbours.

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Appendix I

A list of 73 locations sampled between the period of 2006 and 2016. This list includes their codes used in this research and corresponding geographical coordinates.

Code	Harbour name	Location	Coordinates.
AME	Stichting Yachtharbour "Het Leyegat" Ameland	Wadden Sea	53.438177, 5.776101
AQU	Aquadome	Grevelingen	51.739034, 3.825856
ARM	WSV Arnemuiden	Veerse meer	51.510198, 3.702962
BAT	Bath	Westerschelde	51.399042, 4.218611
BH	Yachtharbour Brouwershaven	Grevelingen	51.729379, 3.913205
BOM	Bommenede	Grevelingen	51.731667, 3.972952
BR	Breskens	Westerschelde	51.395771, 3.570649
BRE	Breezanddijk	Wadden Sea	53.019728, 5.202860
BRU	Bruinisse	Grevelingen	51.671520, 4.083927
BUR	Burghsluis	Oosterschelde	51.674461, 3.755290
CLP	Colijnsplaat	Oosterschelde	51.602709, 3.849481
COC	De Cocksdorp	Wadden Sea	53.157881, 4.877581
DEL	Delfzijl Yachtharbour	Wadden Sea	53.331022, 6.930926
DOE	Den Oever	Wadden Sea	52.934642, 5.039188
DOS	Yachtharbour Den Osse	Grevelingen	51.740072, 3.890982
DOV	Den Oever Vishaven	Wadden Sea	52.937424, 5.031692
EEM	Eemshaven	Wadden Sea	53.443955, 6.825242
GOE	WSV Goeree	Grevelingen	51.797881, 3.933935
HAN	Hansweert	Westerschelde	51.454192, 4.010020
HAR	Harlingen Seaport	Wadden Sea	53.175616, 5.414044
HD1	Den Helder Bevesierweg (Dock 4)	Wadden Sea	52.962078, 4.779422
HD2	Den Helder Nieuwe Haven (Dock 19/20)	Wadden Sea	52.954515, 4.786337
HD3	Den Helder Nieuwe Haven (Dock 66/77)	Wadden Sea	52.961104, 4.795844
HD4	Den Helder Nieuwe Haven (Dock 12)	Wadden Sea	52.958930, 4.780828
HOE	Hoedekenskerke	Westerschelde	51.420165, 3.914948
HOL	Holwerd	Wadden Sea	53.395515, 5.878429
HOM	Hompelvoet	Grevelingen	51.776099, 3.946346
HOR	t Horntje	Wadden Sea	53.005794, 4.793289
IJM	IJmuiden	North Sea	52.457930, 4.559744
JSI	Yachtharbour W.S.V. Scharendijcke	Grevelingen	51.739089, 3.846892
KAM	Kamperland Harbour	Veerse meer	51.558131, 3.689254
KAT	Marina Kats	Oosterschelde	51.574158, 3.890484
KOR	Kortgene Delta Marina	Veerse meer	51.552948, 3.809762
KWZ	Kornwerderzand	Wadden Sea	53.074796, 5.335717
LEZ	Lezardrieux (France)	Atlantic Ocean	48.787816, -3.102694
LO	Lauwersoog harbour	Wadden Sea	53.405125, 6.207320
MOL	Molwerk	Wadden Sea	53.007807, 4.762774
MPZ	Marina Port Zelande	Grevelingen	51.758810, 3.855297
MSI	Marina Stellendam (inside)	Haringvliet	51.819814, 4.051406

Code	Harbour name	Location	Coordinates.
MSS	Marina Stellendam Fishermen harbour (sea side)	Haringvliet	51.821959, 4.039472
NEU	WSV Neuzen	Westerschelde	51.336069, 3.814972
NIE	Nieuwstad	Wadden Sea	53.404006, 6.884871
NU	Numansdorp	Hollands Diep	51.717237, 4.436847
OD	"Punt van Goeree" Ouddorp	Grevelingen	51.779044, 3.882422
RO1	Rotterdam 8e Petroleumhaven	North Sea	51.967950, 4.064695
RO2	Rotterdam Beneluxhaven	North Sea	51.952790, 4.125229
RO3	Rotterdam Brittanniëhaven	North Sea	51.895806, 4.239898
RO4	Rotterdam 1e Eemhaven	North Sea	51.889043, 4.418231
ROO	Roompot Marina	Oosterschelde	51.591584, 3.718753
SAL	Sint Anna-land	Oosterschelde	51.602607, 4.108165
SAS	Sas van Goes	Oosterschelde	51.538945, 3.927593
SCH	Scheveningen	North Sea	52.095843, 4.266540
SMO	Schiermonnikoog Yachtharbour	Wadden Sea	53.469839, 6.166350
SPL	Schipluiden	Schipluiden	51.972007, 4.308544
SWB	Sail- and windsurf school "Brouwersdam"	Grevelingen	51.754191, 3.852347
TIE	Tiengemeten	Haringvliet	51.754170, 4.317485
TNJ	Terneuzen Yachtharbour	Westerschelde	51.339836, 3.827441
TS	Terschelling	Wadden Sea	53.364925, 5.220875
TSC	t Schor	Wadden Sea	53.397812, 5.991237
TSL	Terneuzen Sluis	Westerschelde	51.338557, 3.818464
TX1	Oudeschild (home)	Wadden Sea	53.044155, 4.856929
TX2	Oudeschild (guest)	Wadden Sea	53.044155, 4.856929
VE	Yacht club Veere	Veerse meer	51.549760, 3.668636
VEM	Veerse Meer	Veerse meer	51.580740, 3.629555
VL1	Vlissingen Ritthemsestraat	Westerschelde	51.448875, 3.588234
VL2	Vlissingen Engelandweg	Westerschelde	51.460927, 3.685541
VL3	Vlissingen Westhofhaven	Westerschelde	51.470703, 3.713053
VL4	Vlissingen Kaloothaven	Westerschelde	51.445333, 3.708115
VLL	Stg. Aanloophaven Vlieland	Wadden Sea	53.296264, 5.091626
WMD	Yachtharbour Wemeldinge	Oosterschelde	51.515644, 4.004704
WPD	WSV Wolphaartsdijk	Veerse meer	51.546675, 3.817498
YE	Yerseke	Oosterschelde	51.496689, 4.054273
ZZ	Yachtharbour Zierikzee	Oosterschelde	51.643854, 3.912591

Appendix II

Locations of the SETL project in the Netherlands, including GPS coordinates and time period.

	Harbour name	**Harbour location**	**GPS coordinates**	**SETL (from-until)**
1	Aquadome	Grevelingen	51.739034, 3.825856	2006-2008
2	Bath	Westerschelde	51.399042, 4.218611	2016-2017
3	Bommenede	Grevelingen	51.731667, 3.972952	2008-2017
4	Breskens	Westerschelde	51.395771, 3.570649	2009-2017
5	Colijnsplaat (Floating dock)	Oosterschelde	51.602709, 3.849481	2006-2017
6	Colijnsplaat (Jetty)	Oosterschelde	51.603401, 3.850774	2006-2017
7	Delfzijl Adele	Wadden Sea	53.316304, 6.981278	2016-2017
8	Delfzijl yachtharbour	Wadden Sea	53.331022, 6.930926	2016-2017
9	Den Helder Bevesierweg (Dock 4)	Wadden Sea	52.962078, 4.779422	2007-2017
10	Den Helder Nieuwe Haven (Dock 12)	Wadden Sea	52.958930, 4.780828	2015-2017
11	Den Helder Nieuwe Haven (Dock 66-77)	Wadden Sea	52.961104, 4.795844	2015-2017
12	Den Helder Nieuwe Haven (Dock 19-20)	Wadden Sea	52.954515, 4.786337	2015-2017
13	Eemshaven Beatrixhaven	Wadden Sea	53.456059, 6.832089	2016-2016
14	Eemshaven Julianahaven	Wadden Sea	53.452657, 6.813770	2016-2016
15	Eemshavens Emmahaven	Wadden Sea	53.443955, 6.825242	2007-2017
16	Hansweert	Westerschelde	51.454192, 4.010020	2016-2017
17	Harlingen	Wadden Sea	53.175616, 5.414044	2010-2010
18	Hoedekenskerke	Westerschelde	51.420165, 3.914948	2016-2017
19	Hompelvoet	Grevelingen	51.776099, 3.946346	2008-2017
20	IJmuiden	North Sea	52.457930, 4.559744	2007-2017
21	Numansdorp	Hollands Diep	51.717237, 4.436847	2006-2017
22	Rotterdam 1e Eemhaven	North Sea	51.889043, 4.418231	2014-2014
23	Rotterdam 8e Petroleum haven	North Sea	51.967950, 4.064695	2007-2017
24	Rotterdam Beneluxhaven	North Sea	51.952790, 4.125229	2007-2017
25	Rotterdam Brittanniëhaven	North Sea	51.895806, 4.239898	2014-2014
26	Scheveningen	North Sea	52.095843, 4.266540	2010-2010
27	Terneuzen (Sluice)	Westerschelde	51.338557, 3.818464	2007-2008
28	Terneuzen (Yachtharbour)	Westerschelde	51.339836, 3.827441	2016-2017
29	Terschelling	Wadden Sea	53.364925, 5.220875	2010-2010
30	Tiengemeten	Haringvliet	51.754170, 4.317485	2006-2017
31	Veerse Meer	Veerse meer	51.580740, 3.629555	2006-2006
32	Vlissingen Kaloothaven	Westerschelde	51.445333, 3.708115	2016-2016
33	Vlissingen Ritthemsestraat	Westerschelde	51.448875, 3.588234	2007-2017
34	Vlissingen Westhofhaven	Westerschelde	51.470703, 3.713053	2016-2016
35	Yerseke	Oosterschelde	51.496689, 4.054273	2006-2011

Appendix III

Non-native species (macro fauna and flora) recorded during various non-native species inventories in the Netherlands. Note that there are large differences in the number of locations searched and number of samples taken for each of the habitats. * Only locations in the Port of Rotterdam and Vlissingen are included.

		Harbours								Cultivation parcels		Shellfish related	
	Indication of # locations	**8**	**35**	**25**	**7***	**6***	**4***	**7***	**7***	**300**	**268**	**1**	**3**
	Indication of # samples	**150**	**7000**	**35**	**55**	**53**	**7**	**68**	**66**	**1724**	**268**	**158**	**80**
Scientific name	**Group**	**Hull fouling**	**SETL**	**Floating docks**	**Vertical structure**	**Dikes**	**Dikes sublittoral**	**Watercollumn**	**Bottom**	**Mussels**	**Oysters**	**Oyster ponds Yerseke**	**Deposit grounds**
Codium fragile	Algae (Chlorophyta)	1	1	1						1	1	1	
Ulva australis	Algae (Chlorophyta)		1	1	1	1	1		1	1	1	1	1
Colpomenia peregrina	Algae (Ochrophyta)									1	1		
Sargassum muticum	Algae (Ochrophyta)			1						1	1	1	1
Stictyosiphon soriferus	Algae (Ochrophyta)										1	1	
Undaria pinnatifida	Algae (Ochrophyta)	1		1						1	1	1	1
Acrochaetium catenulatum	Algae (Rhodophyta)									1			
Agardhiella subulata	Algae (Rhodophyta)									1	1	1	1
Antithamnionella spirographidis	Algae (Rhodophyta)		1	1						1	1		
Bonnemaisonia asparagoides	Algae (Rhodophyta)		1										
Bonnemaisonia hamifera	Algae (Rhodophyta)											1	
Ceramium sungminbooi	Algae (Rhodophyta)			1									
Ceramium tenuicorne	Algae (Rhodophyta)		1	1		1	1		1				
Chylocladia verticillata	Algae (Rhodophyta)										1		
Cryptopleura ramosa	Algae (Rhodophyta)									1			
Dasya baillouviana	Algae (Rhodophyta)		1										
Dasya sessilis	Algae (Rhodophyta)										1		
Dasysiphonia japonica	Algae (Rhodophyta)		1	1	1	1	1		1	1	1	1	1
Erythroglossum laciniatum	Algae (Rhodophyta)									1			
Gelidium vagum	Algae (Rhodophyta)										1	1	
Gracilaria vermiculophylla	Algae (Rhodophyta)									1	1		
Grateloupia turuturu	Algae (Rhodophyta)									1	1	1	
Lomentaria hakodatensis	Algae (Rhodophyta)									1			
Neosiphonia harveyi	Algae (Rhodophyta)		1	1						1	1	1	
Polysiphonia senticulosa	Algae (Rhodophyta)									1	1	1	
Alitta virens	Annelida									1			
Bispira polyomma	Annelida			1								1	
Ficopomatus enigmaticus	Annelida		1	1	1	1	1		1			1	
Neodexiospira brasiliensis	Annelida		1										
Polydora ciliata	Annelida		1	1									
Sthenelais boa	Annelida									1			
Aplidium glabrum	Ascidiacea	1	1	1						1	1	1	
Botrylloides violaceus	Ascidiacea	1	1	1	1					1	1	1	1
Clavelina lepadiformis	Ascidiacea	1		1									
Corella eumyota	Ascidiacea		1										
Didemnum vexillum	Ascidiacea	1	1	1	1					1	1	1	1

Scientific name	Group	Hull fouling	SETL	Floating docks	Vertical structure	Dikes	Dikes sublittoral	Watercollumn	Bottom	Mussels	Oysters	Oyster ponds Yerseke	Deposit grounds
Diplosoma listerianum	Ascidiacea	1	1	1	1					1	1	1	1
Molgula manhattensis/socialis	Ascidiacea	1	1	1			1		1	1	1	1	
Perophora japonica	Ascidiacea		1							1		1	
Styela clava	Ascidiacea	1	1	1	1		1		1	1	1	1	1
Bugula neritina	Bryozoa	1	1	1									
Bugulina stolonifera	Bryozoa	1	1	1	1					1			
Fenestrulina delicia	Bryozoa									1	1		1
Pacificincola perforata	Bryozoa										1	1	1
Smittoidea prolifica	Bryozoa		1						1	1	1		
Tricellaria inopinata	Bryozoa		1	1						1	1	1	1
Ammothea hilgendorfi	Chelicerata									1	1		
Cordylophora caspia	Cnidaria		1	1	1				1				
Diadumene cincta	Cnidaria		1	1	1					1	1	1	
Diadumene lineata	Cnidaria			1						1	1	1	
Gonionemus vertens	Cnidaria			1									
Nemopsis bachei	Cnidaria							1					
Amphibalanus improvisus	Crustacea	1	1	1	1	1	1		1	1			
Austrominius modestus	Crustacea	1	1	1	1	1	1		1	1	1	1	1
Caprella mutica	Crustacea	1	1	1	1					1			
Eriocheir sinensis	Crustacea		1	1									
Hemigrapsus sanguineus	Crustacea		1	1	1	1	1			1	1	1	
Hemigrapsus takanoi	Crustacea		1	1	1	1	1		1	1	1	1	1
Ianiropsis serricaudis	Crustacea											1	
Jassa marmorata	Crustacea		1	1	1	1			1	1			
Melita nitida	Crustacea								1				
Palaemon macrodactylus	Crustacea			1						1	1		
Rhithropanopeus harrisii	Crustacea		1	1	1	1	1		1				
Mnemiopsis leidyi	Ctenophora				1			1	1	1	1	1	
Acanthocardia paucicostata	Mollusca												1
Corbicula fluminalis	Mollusca						1		1				
Crassostrea gigas	Mollusca	1	1	1	1	1	1		1	1	1	1	1
Crepidula fornicata	Mollusca		1	1	1	1	1		1	1	1	1	1
Dreissena bugensis	Mollusca		1	1					1				
Dreissena polymorpha	Mollusca		1	1					1				
Ensis leei	Mollusca			1					1	1	1		
Gibbula cineraria	Mollusca									1	1	1	1
Glycymeris glycymeris	Mollusca												1
Mya arenaria	Mollusca								1	1			
Ocenebra inornata	Mollusca									1	1	1	1
Rangia cuneata	Mollusca								1				
Ruditapes philippinarum	Mollusca									1	1	1	1
Celtodoryx ciocalyptoides	Porifera										1	1	1
Haliclona (Soestella) xena	Porifera		1	1						1	1		1
Hymeniacidon perlevis	Porifera			1							1	1	
Leucosolenia somesii	Porifera			1									
Sycon scaldiense	Porifera	1	1	1							1	1	
Totals per habitat		**16**	**39**	**44**	**20**	**12**	**14**	**2**	**23**	**48**	**44**	**38**	**23**