Overview alien species monitoring in the Western Scheldt

Current status of monitoring efforts and presence of alien species among macrofauna and algae

by

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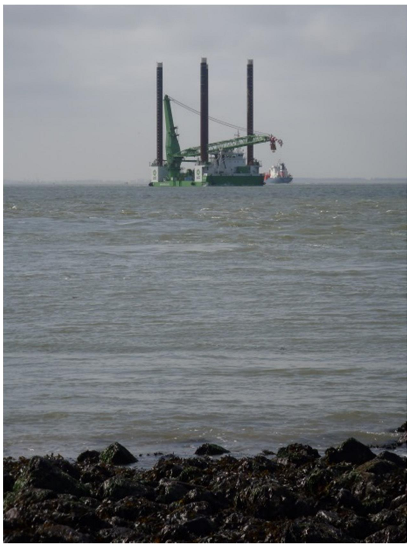

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Photos cover from left to lower right: a) Platform/ship on the Western Scheldt; b) Antwerp harbour skyline as seen from Bath; c) The intertidal community as observed in 2016 near Hoedekenskerke with amongst others the alien species *Magallana gigas*, *Austrominius modestus*, *Hemigrapsus saguineus* and *Diadumene lineata*.

Unless indicated differently, all used photos are taken by Sander Wijnhoven.

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Index

Abstract
The current report provides an overview of the monitoring efforts and the presence of alien species among macrofauna, macro-algae and plankton that have (recently) been recorded in the Western Scheldt. The basis of this study is the (ongoing) monitoring and inventory work of the companies GiMaRIS, eCOAST and Ecoauthor with a focus on alien species in the estuary (e.g. Gittenberger & Van der Stelt, 2011; Gittenberger & Rensing, 2015; Wijnhoven et al., 2015; Wijnhoven, 2016), supplemented with alien species recordings in long-term monitoring programmes within the frame of MWTL (not specifically focussed on alien species). For each of the alien species the first year of observation and their current distribution in the Western Scheldt is presented on the basis of different monitoring techniques. The number of recordings with different techniques at different sites, identify best habitats and sites (potential hotspots) for observation of different types of alien species.

Most likely primary vectors of introduction into the Western Scheldt and secondary vectors of dispersal within the estuary are identified for each species based on observations and literature. Most important vectors of primary introduction appear to be shiphull fouling and ballast water although the natural dispersal of alien species from sources in the vicinity is important as well. If ballast water regulations appear to be successful, shiphul fouling probably becomes the most important vector. Aquaculture has been of little importance so far. Plans to culture oysters in the Western Scheldt might open potentials for a range of alien species that so far have not arrived in the system, if it includes import of oysters from elsewhere. For within the Western Scheldt distribution (i.e. secondary dispersal), natural dispersal might be as important as shiphull related distribution.

Although the best strategy of early detection of new alien species and/or range extensions is highly dependent on the type of species (e.g. taxonomic group), focussing inventories on hotspots like ports, marinas and entrances/connections with other waterbodies, covering the most important (local) habitats and the estuarine gradient, is advisable. It is expected that with the monitoring of the sessile macrofauna communities and associated species in the intertidal zone and on artificial hard substrates in the shallow subtidal several exotic species will be detected at an early stage in the future as wel. However, also the soft sediment substrates and plankton sampling in the most important ports needs continuous attention to discover potential nuisance species at an early stage. Combining hard – and soft substrate sampling and sampling of the water column at the various sites during fieldwork will reduce costs.

In conclusion a total of 90 estuarine and marine alien species, of which at least 81 are still present, and an additional 9 fresh water related alien species, have been observed in the Western Scheldt during the last 25 years, as indicated in this report. Additionally several alien species were identified that can be expected in the Western Scheldt in the near future, on the basis of their presence in the vicinity or recent introductions to comparable systems in Western Europe.

Nederlandstalige samenvatting
Dit rapport geeft een overzicht van de recentelijk en/of momenteel aanwezige exoten onder de macrofauna, de macro-algen en het plankton in de Westerschelde. De studie is gebaseerd op de lopende en recente inventarisaties en monitoring speciaal gericht op het waarnemen van exoten in het estuarium, van de ondernemingen GiMaRIS, eCOAST en Ecoauthor (o.a. Gittenberger & Van der Stelt, 2011; Gittenberger & Rensing, 2015; Wijnhoven et al., 2015; Wijnhoven, 2016), aangevuld met waarnemingen vanuit de langlopende monitoring programma's in het kader van MWTL (die niet specifiek op exoten gericht zijn). Voor iedere waargenomen niet-inheemse soort

wordt het jaar van eerste observatie in de Westerschelde, alsmede de huidige verspreiding gerelateerd aan observaties met verschillende waarnemingstechnieken (hier tevens beschreven) gepresenteerd. Door middel van de waarnemingen met verschillende technieken op verschillende locaties kunnen de meest geschikte habitats en locaties (mogelijke hotspots) voor het aantreffen van verschillende types exoten worden aangeduid.

De meest waarschijnlijke primaire vectoren van introductie in de Westerschelde, alsmede de meest waarschijnlijke manieren van secundaire verspreiding binnen het systeem zijn aangeduid per soort op basis van de waarnemingen en literatuurvermeldingen. De belangrijkste vectoren van primaire introductie blijken het transport via aangroei op scheepswanden en bootjes en het transport van organismen in ballastwater te zijn. Echter ook natuurlijke verspreiding vanuit bronnen in de omgeving van de Westerschelde blijkt belangrijk te zijn. Wanneer de ballastwater regeling succesvol blijkt te zijn zal de aangroei op boten de belangrijkste transport route voor exoten worden. Tot dusver is aquacultuur nauwelijks van belang geweest (omdat het de laatste decennia ontbrak), maar plannen voor de kweek van oesters in de Westerschelde kunnen mogelijkheden creëeren voor verscheidene exoten die tot op heden nog ontbreken indien kweek ook de import van oesters uit andere systemen behelst. Natuurlijke verspreiding is naast aangroei op bootjes de belangrijkste manier van verdere (secundaire) verspreiding van de soorten binnen de Westerschelde.

Hoewel de beste strategie voor het vroegtijdig waarnemen van nieuwe exoten en/of gebiedsuitbreiding van exotenpopulaties, sterk afhankelijk is van het type exoot (o.a verschillend per taxonomische groep), wordt geadviseerd om inventarisaties toe te spitsen op zogenaamde hotspots als havengebieden, jachthaventjes en verbindingen met andere waterlichamen, waarbij de belangrijkste lokaal aanwezige habitats en de estuariene gradient wordt gedekt. Het is de verwachting dat met de monitoring van met name de sessiele macrofauna gemeenschappen en de geassocieerde soorten in intergetijde gebied en op artificiële substraten in ondiep water ook in de toekomst een groot aantal exoten vroegtijdig kan worden opgemerkt. Echter ook het zachte substraat en de plankton bemonstering in de belangrijkste havengebieden heeft standaard aandacht nog om probleemsoorten vroegtijdig op te merken. Door hard – en zacht substraat monitoring en het bemonsteren van de waterkolom op de verscheidene plaatsen te combineren tijdens veldwerk, kunnen de kosten beperkt blijven.

Zoals weergegeven in dit rapport, zijn er in totaal 90 estuariene en mariene exoten, waarvan ten minste 81 nog steeds aanwezig, en 9 extra zoetwater gerelateerde soorten, in de Westerschelde aangetroffen gedurende de afgelopen 25 jaar. Daarnaast is in dit rapport een aantal exoten geïdentificeerd, op basis van hun aanwezigheid in nabijgelegen wateren of recente introducties in vergelijkbare systemen in West Europa, waarvoor de kans groot is dat ze in de nabije toekomst in de Westerschelde zullen opduiken.

Introduction

The companies eCOAST, GiMaRIS and Ecoauthor independently monitor hard- and soft-substratum macrofauna and algae communities with a focus on alien species in the Western Scheldt. Alien species are defined in this report as non-indigenous species living outside their native distributional range, having arrived here as a result of human activity. This includes species that have arrived as a result of human activities in waters in the vicinity that might have dispersed (secondarily) in a natural way to the waters of investigation (i.e. the Western Scheldt).

The current report aims:

- To provide an up-to-date overview of the new and long-term monitoring activities in the Western Scheldt that focus on or potentially deliver information on the presence and distribution of alien species among different communities.
- And to provide an overview of the alien species currently known to be present in the Western Scheldt (with first recording, spatial distribution and most likely vectors of introduction and dispersal).

With the overview of monitoring activities potential gaps in monitored habitats can be identified. Along with an overview of the alien species currently present in the Western Scheldt, a brief selection of alien species that might invade the Western Scheldt is given (this is not an exhaustive overview, but a short list of alien species with a proven risk that they might successfully be introduced on the short term, as they have recently been introduced in comparable waters in the vicinity).

The overview consists of lists alien species of soft – and hard substratum related macrofauna (macrobenthos: infauna and epifauna), fauna present in the watercolumn (hyperbenthos and zooplankton), epibentic algae and phytoplankton.

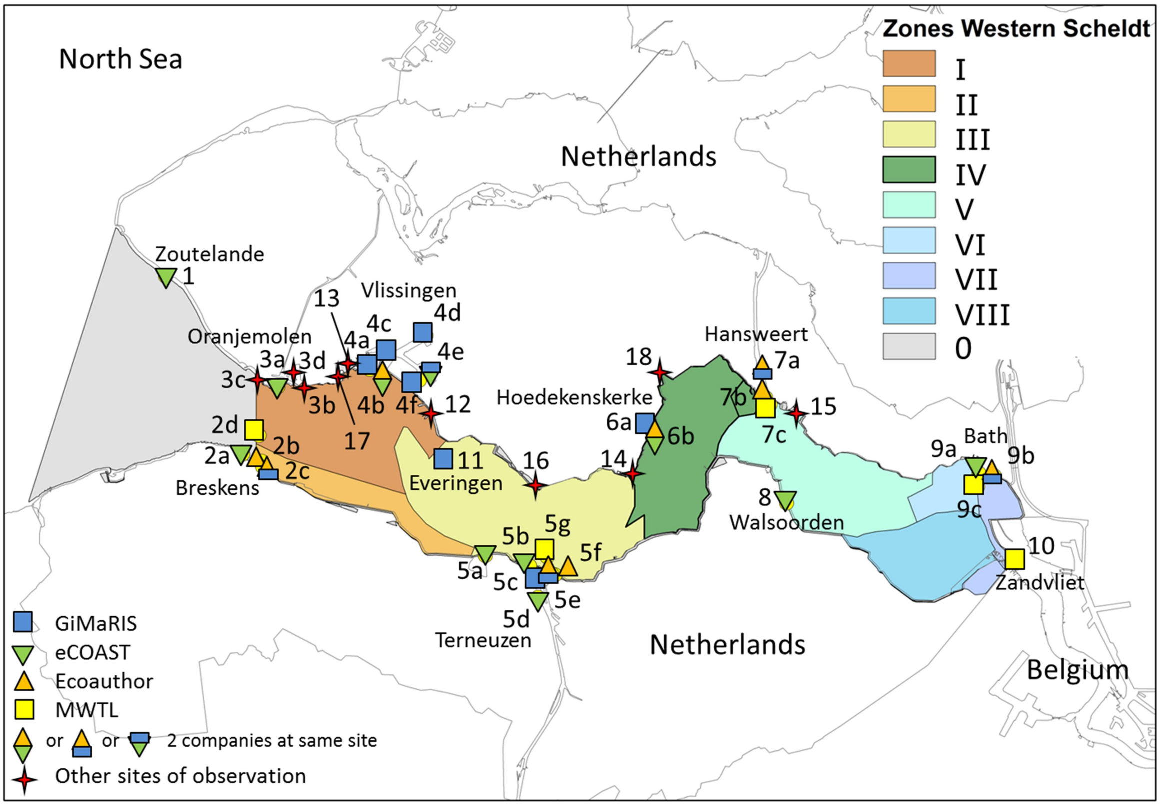
Area of investigation

The focal area for this report is the Western Scheldt, the Dutch part of the Scheldt estuary, extending from the Mouth of the estuary in the west (embordered by the line from the most western tip of Walcheren near Westkapelle to the Dutch-Belgian border near Cadzand-Bad) to the Dutch-Belgian border in the east. The research area is visualized in Figure 1. Only the area below highest high water level is considered (supratidal is excluded). The area of investigation also does not include freshwater bodies linked to the Western Scheldt. It does include the marinas and sea-ports behind sluices (like at Vlissingen) and observation sites directly behind (inside) the sluices with clear marine/estuarine conditions (salinity value above 1 psu). As the arrival of 'new' alien species is often not specifically recorded for the Western Scheldt, but for the Netherlands or the entire estuary in general; the exact location and year of appearance in the Western Scheldt might be unclear. In these cases sometimes the known appearance at Belgian sites (in the the Scheldt esyuary) is recorded in this report. Although the companies involved have monitoring programmes covering ports and marinas in the Belgian part of the estuary and along the Dutch and Belgian North Sea coast, those locations are not part of this current overview.

Current and recent monitoring projects

Table 1 gives an overview of the recent and current monitoring programmes and projects with a focus on recording the presence of alien species and additional monitoring programmes that might give insight in the presence of certain alien species.

Five monitoring programmes/projects, specifically focussed on alien species, are operational in the Western Scheldt (Table 1), two projects of which were single event inventories (port survey according to OSPAR-HELCOM protocol at Vlissingen, and marina survey at Breskens) with various techniques covering a variety of habitats.



1	Zoutelande – Breakwaters	4f	Vlissingen – Kaloot port	8	Walsoorden – Outside marina
2a	Breskens – Ferry port	5a	Terneuzen – Braakman port	9a	Bath – Embankment
2b	Breskens – Outside marina	5b	Terneuzen – West Outer port	9b	Bath – Inlet
2c	Breskens – Marina	5c	Terneuzen – Outside sluices	9c	Bath – Fairway
2d	Breskens – Fairway	5d	Terneuzen – Inside sluices	10	Zandvliet – Fairway
3a	Vlissingen – Oranjemolen	5e	Terneuzen – Marina	11	Everingen – Anchorage A
3b	Vlissingen – Outer port	5f	Terneuzen – Outside marina	12	Borssele
3c	Vlissingen – Beach	5g	Terneuzen – Fairway	13	Fort Rammekens
3d	Vlissingen – Inner port	6a	Hoedekenskerke – Marina	14	Baarland
4a	Vlissingen – Jetty west	6b	Hoedekenskerke – Outside marina	15	Kruiningen
4b	Vlissingen – Jetty east	7a	Hansweert – Outside sluices	16	Ellewoutsdijk
4c	Vlissingen – Yard	7b	Hansweert – Outside marina	17	Ritthem
4d	Vlissingen – Bijleveld port	7c	Hansweert – Fairway	18	s'Gravenpolder
4e	Vlissingen – Van Citters port				

Figure 1. Western Scheldt devided in compartments according to the Scheldt estuary evaluation methodology (Maris et al., 2014) with indication of current and recent monitoring locations of alien species. Compartments equal the macrocel division generally used to describe the sedimentology in the system; compartments 1-3 form the high polyhaline zone with year-round chloride concentrations of 10-17 g/l; compartment 4 is the low polyhaline zone with Cl concentrations of 10-17 g/l during summer but lower values at high river runoff; compartments 5-8 form the β-mesohaline zone (Cl values of 5,5-10 g/l) that also slightly extents into Flanders.

The port survey at Vlissingen that was commissioned by the Office for Risk assessment and Research, was specifically meant to give a complete picture of the presence of alien species in the port area (Sloehaven) and therefore covers all habitats present and related species groups as much as possible (Rapid Assessment Survey (RAS) according to OSPAR-HELCOM protocol was used). The marina survey in Breskens focused on various hard substratum surfaces and types in the intertidal zone extended with the shallow subtidal zone in the form of a floating dock survey during maintenance. The set-up, with a lot of replicates and/or covering entire gradients, was suited to identify gradients and habitat preferences of species.

Table 1. Overview of monitoring projects and programmes focussing on, or potentially providing, information on the presence of alien species in the Western Scheldt, with their sampling characteristics, focal habitats and most probable vectors of introduction/dispersal that are under investigation.

Monitoring projects	**Years, timing and frequency**	**Type of inventory and number of sites/samples (Site codes as in Fig. 1)**	**Inventoried habitats**	**Most likely vectors of dispersal for observed alien species**
SETL-plate monitoring – GiMaRIS (e.g. Gittenberger & Rensing, 2015; Appendix 1a).	2007- now, 4 times a year.	Port of Vlissingen (site 4c) since 2007 and Breskens marina (site 2c) since 2009. Terneuzen (sites 5c, 5e) since 2005, and Hoedekenskerke (site 6a), Hansweert (7a) and Bath (9b) since 2016. In total circa 70 plates in the Western Scheldt.	Water column at 1 m of depth (below water line if attached to floating objects; below low water line if attached to non-floating structures). Specifically organisms that are not exposed to direct sunlight.	Ballast water (epibenthic species with pelagic phase), shiphull fouling and natural dispersal of settling stages after introduction in marinas and ports.
OSPAR-HELCOM port survey – GiMaRIS (HELCOM/OSPAR , 2013).	2016, two sampling events, i.e. in spring and in summer.	SETL-plates in port of Vlissingen (sites 4c, 4d, 4e, 4f) at a depth of 1, 3 and 7 m of depth.	Water column at 3 depths. Specifically organisms that are not exposed to direct sunlight.	Ballast water (epibenthic species with pelagic phase), shiphull fouling and natural dispersal of settling stages after introduction in marinas and ports.
		Plankton sampling in port of Vlissingen (sites 4c, 4d, 4e, 4f) in spring and summer.	Water column.	Ballast water related and by currents (secundary distribution) into harbours introduced species of small unicellular organisms (‘phytoplankton’), ‘larger’ one- and multicellular organisms (phytoplankton) and for zooplankton.
		Crab traps in the port of Vlissingen (sites 4c, 4d, 4e, 4f) in summer: Chinese crab traps and Gee’s minnow traps.	Horizontal soft and hard substrate bottom.	Ballast water (mobile fauna with pelagic phase) and natural dispersal of mobile epifauna foraging near the bottom.
		Littoral zone inventories of dikes in the port of Vlissingen(sites 4c, 4d, 4e).	Intertidal hard substratum inventories of clearly distinguishable zones.	Ballast water (epibenthic species with pelagic phase) and natural dispersal.
		Subtidal scrape samples of vertical surfaces in the port of Vlissingen: from floating docks (sites 4c, 4d, 4e), pilings (4c, 4e) and harbour walls (4e).	Shallow subtidal vertical hard substrata (floating docks, pillars and harbour walls).	Ballast water (epibenthic species with pelagic phase), shiphull fouling and natural dispersal.
		Petit ponar grabs in the port of Vlissingen (sites 4c, 4d, 4e, 4f).	Subtidal soft sediment, pebbles or shells substratum.	Ballast water (benthic species with pelagic phase) and natural dispersal.
		Hand dredge sampling in the port of Vlissingen (sites 4c, 4d, 4e, 4f.	Subtidal soft sediment environments.	Ballast water (benthic species with pelagic phase) and natural dispersal.

Monitoring projects	Years, timing and frequency	Type of inventory and number of sites/samples (Site codes as in Fig. 1)	Inventoried habitats	Most likely vectors of dispersal for observed alien species
		Drop down camera: video recording in the port of Vlissingen of subtidal harbour wall (sites 4c, 4e), pillar (4c, 4d, 4e), floating dock (4d).	Subtidal vertical hard substratum (floating docks, pillars and harbour walls).	Ballast water (epibenthic species with pelagic phase), shiphull fouling and natural dispersal.
Marina survey Breskens – GiMaRIS (Gittenberger & Van der Stelt, 2011).	2008-2009, single sampling event in early winter 2008 and summer 2009.	Floating dock survey during maintenance in marina of Breskens (site 2c) in 2008: Complete inventory of floating docks lifted out of the water for maintenance after 14 years, including detail photograph inventory.	Shallow subtidal hard substratum (floating dock) just below the water line.	Especially shiphull fouling related species.
		Iron harbour wall survey in marina of Breskens (site 2c) in 2009: Presence-absence scoring in intertidal zone.	Vertical intertidal hard substratum (iron wall) in marina covering entire gradient.	Especially shiphull fouling related species.
		Rip-rap dike survey in marina of Breskens (site 2c) in 2009: Presence-absence in intertidal zone.	Intertidal hard substratum (dike) covering entire gradient.	Especially shiphull fouling related species.
		Wooden pilings survey in marina of Breskens (site 2c) in 2009: Presence-absence scoring in intertidal zone.	Intertidal hard substratum (dike) covering entire gradient.	Especially shiphull fouling related species.
Inventories of seaports and surroundings - eCOAST (Appendix 1b).	2014-continuing, annually, 2 times a year.	In (vicinity of) harbours and reference locations, 11 localities : sites 1, 2a, 3, 4b, 4e, 5a, 5b, 5d, 6b, 8, 9a), 51 sampling events over 2014-2016.	Sub- and intertidal hard substrata, including the microhabitats *Ulva* spp., branched red algae, branched Hydrozoa/Bryozoa, barnacles, mussel clumps, silt tubes on the substratum, silt under Japanese oysters and rocks and vertical walls (if present).	Especially shiphull related species, but also ballast water species and species that arrived after secondary natural distribution.
Monitoring within frame of tidal sandflat suppletions – eCOAST.	2016, single sampling event in august, with extention in 2017.	162 intertidal and 84 subtidal sampling locations in two types of ecotopes.	High-dynamic and low-dynamic sub- and intertidal soft sediment habitats.	Especially established species that might have introduced via ballast water introduction, but especially secondary natural distribution.

Monitoring projects	**Years, timing and frequency**	**Type of inventory and number of sites/samples (Site codes as in Fig. 1)**	**Inventoried habitats**	**Most likely vectors of dispersal for observed alien species**
Transect monitoring – Ecoauthor (Commissioned by the NVWA) (Wijnhoven et al., 2015; Wijnhoven, 2016; Appendix 1c).	2015-2017, yearly in summer:	6 transect locations: total of 12 transect lines: total of 108 quadrants per year.	Dominant habitat types on intertidal hard substratum (embankments).	Dependent of location especially species introduced via shiphull fouling, ballastwater transport, shellfish transport: But especially natural dispersal after introduction in harbours, marinas and connected waterbodies.
	2015, within the frame of SEFINS under the flag of the NIOZ.	Hansweert – Terneuzen – Breskens (sites 2b, 2c, 5e, 5f, 7a, 7b).	Intertidal hard substratum (embankments inside and in vicinity of (outside) marinas.	
	2016-2017, executed by Ecoauthor.	Bath – Hansweert – Hoedekenskerke – Terneuzen – Vlissingen – Breskens (sites 2b, 4b, 5f, 6b, 7b, 9b).	Intertidal hard substratum (embankments inside and in vicinity of (outside) marinas.	
Macrozoobenthos monitoring within frame of MWTL (Commissioned by RWS-CIV) (e.g. ScheldeMonitor, 2017a).	1990-now, yearly:			Especially species introduced via ballastwater and shellfish transport and especially secondary natural dispersal: however generally first observed when already rather common.
	1990-2008, BIOMON sampling executed by the NIOO-CEME.	120 random stratified (depth strata) samples in three extensive areas (sample sites in all indicated zones of Fig. 1), handcores in intertidal zone and boxcores in subtidal zone, yearly in spring and autumn (e.g. Sistermans et al., 2009).	Sampling of soft sediment depth strata (intertidal (<2m), 2-5 m, 5-8 m, >8 m below NAP), in three extensive zones (East, Central, West).	
	2009-2016, 2009-2012 executed by NIOO-CEME / NIOZ-Yerseke, 2013-2016 executed by Eurofins/Aquasense/ Grontmij, Koeman & Bijkerk, Bureau Waardenburg, Habitat Advies (data for 2015-2016 not available yet).	195 to 200 random stratified (to ecotopes) soft sediment samples (boxcores in subtidal and handcores in intertidal zone) covering the entire Western Scheldt, taken every year (2009-2012) or every 2 years (2013-2016) in autumn (Escaravage et al., 2013; Verduin et al., 2016).	Sampling of 13 different soft sediment ecotopes (subtidal and intertidal) according to ZES.1 (Bouma et al., 2005).	

Monitoring projects	**Years, timing and frequency**	**Type of inventory and number of sites/samples (Site codes as in Fig. 1)**	**Inventoried habitats**	**Most likely vectors of dispersal for observed alien species**
Phytoplankton monitoring within frame of MWTL (Commissioned by RWS-CIV) (e.g. ScheldeMonitor, 2017b).	1990-continuing, executed by Koeman & Bijkerk (data for 2016 not available yet).	Monthly (two times a month during summer months) sampling of the water column for community analyses (cell counts) at four stations at Hansweert geul – Schaar van Ouden Doel – Terneuzen Boei 20 – Vlissingen Boei SSVH (sites 2d, 5g, 7c, 9c), in combination with salinity measurement.	Water column of the fairway.	Especially species introduced via ballastwater.
Zooplankton monitoring within frame of MWTL (Commissioned by RWS-CIV) (e.g. ScheldeMonitor, 2017c).	2011-2013, executed by CNRS-EcoLab.	Monthly from March till November, filtration of 300 liter over 50 µm at 5 stations for community analyses based on genera (only a few mesozooplankton species are determined to species level) at Zandvliet (border) – Bath – Hansweert – Terneuzen Boei 20 – Breskens SSVH (sites 2d, 5g, 7c, 9c, 10).	Water column of the fairway.	Especially species introduced via ballastwater (but most species will be missed due to taxonomic level of identification).

The three other projects are covering several years (Seaport and surroundings, Transect Monitoring and SETL-plates project) are multi-site inventories covering the estuarine gradient of the Western Scheldt which are planned to continue in 2017. These programmes are specifically meant to cover the estuarine gradient and the different related habitats. They do not focus on monitoring the entire estuary but on the expected hotspots of introduction, i.e. locations where introductions are more likely than elsewhere due to ballast water release and/ or longer stay of ships. The monitoring sites are therefore positioned around ports, marinas, in/outlets and connections to other waterbodies. These are very suitable to identify spatial distribution patterns, enlarging the chance of finding alien species shortly after introduction (distributed over the west-east range of the estuary) and allowing to analyse changes in time in introductions, distributions and population expansions.

To some extent, monitoring programmes without a special focus on alien species can be of use to gather information on the presence of alien species as well. The MWTL soft substratum macrobenthos monitoring programme can be supplementary as it involves a large number of samples taken every three years (yearly in the past) in habitats generally not covered by the alien species specific inventories. This means that especially for subtidal polychaete species and a few common bivales it can be of use as those are generally missed by the other programmes. It has, however, to be noticed that the MWTL programme does not cover the hotspots for alien species (e.g. in and around ports and marinas and hard and artificial structures). The MWTL programme is specifically meant to provide information on densities, biomass and diversity of the most common species in the vast soft substratum areas, and is therefore not the most suitable tool for early detection. This is shown in Table 2 where despite of the long duration (over 25 years) of extensive monitoring within the framework of MWTL, most first observations of alien species and their distributions are known from inventories focussing on alien species. A general pattern is that 'newly' introduced species, if present in subtidal, predominantly soft sediment habitat, appear in the MWTL monitoring only after several years.

The monitoring within the framework of sandflat suppletions, for which no data are available yet, is of the same kind as the MWTL macrobenthos monitoring, with the same chance of finding new alien species. As a rapid detection programme of alien species the MWTL-monitoring appears to be unsuitable at present as the data may become available at least 2-3 years after the monitoring took place, which means that the period between introduction and reporting is often at least 5 years or more. (E.g. only the data up to 2014 could be made available for the present study). Several alien phytoplankton species in the Western Scheldt are detected for the first time with the MWTL phytoplankton monitoring programme. This is largely because except for the OSPAR-HELCOM monitoring in the port of Vlissingen in 2016 there has been little attention for phytoplankton, and the MWTL monitoring is an extensive sampling programme in place for over 25 years.

As for phytoplankton, there has not been much specific attention for alien species among the zooplankton. The monitoring of zooplankton is not part of any continuous programme within MWTL at the moment, and identification does not go to the species level for most groups, which makes that a limited number of alien species among the zooplankton has been detected so far.

Different methodologies focussing on different habitats

The dikes consist of hard substratum as do the pillars and harbour walls. Depending on the ecological niche of species, they may prefer the high, middle or low intertidal zone. The subtidal can be zonated depending on light conditions (especially for green algae) and wave action. It may also be that the settling organisms themselves provide new habitat for others (i.e. ecosystem engineers), providing surface, structure and potentially food, which attracts other species. Dependending on sedimentation processes, hard substratum communities can also harbor infauna communities, especially in less steep environments like dikes (compared to walls and pillars). E.g. certain mudworms (*Polydor*a, *Boccardia*) live in mud accumulated between and under Pacific oysters. Inventories in the intertidal zone typically consist of visual inspections and/or collection of organisms.

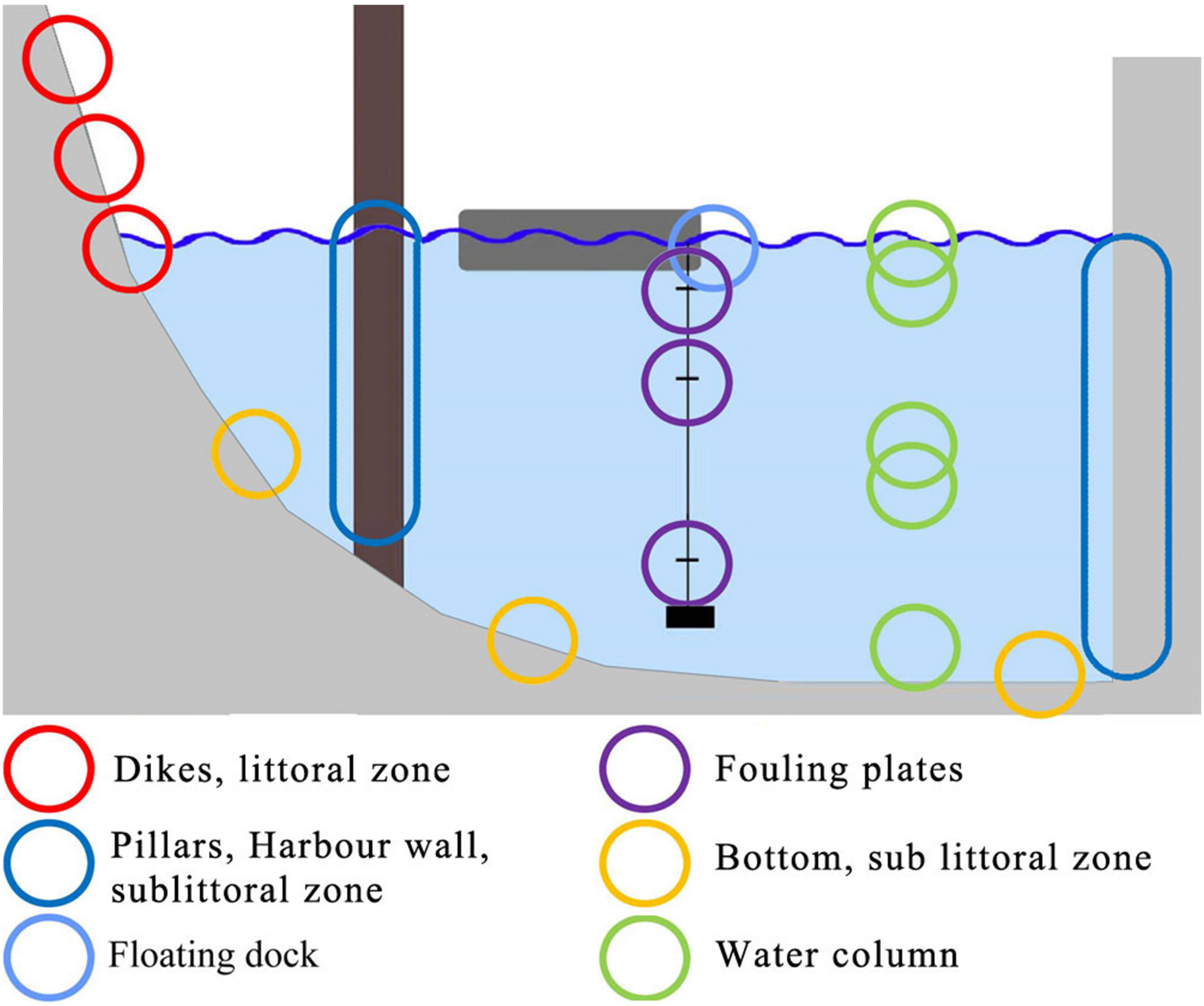


Figure 2. Overview of types of habitats present in a port or marina environment that can be covered with different monitoring techniques. At least several of these habitats are also present at monitoring sites outside marinas.

For pillars and walls this is actually the same, although they might be less accessible. In such cases scrape samples are a solution, where assemblages are collected (scraped) and sorted/identified in a lab. A specific type of] visual investigation is by making a photograph, which is quick, non-destructive and allows identification long after the fieldwork. However, especially specimens covered by others might be missed. A solution can be combining photography and 'destructive' collection of specimens.

Subtidal inventories of hard substratum habitats can be visual or with scrape samples as well, but might ask for snorkeling, scuba diving or ROV-inspection, especially below 2 meters of depth (when working with tools on a stick it becomes more difficult). Settling alien species are typically related to shiphull and ballast water transport. Sites of first observation will be reasonably indicative for the supposed (either initial or secundary) vector of introduction. Floating docks are a special case of hard substratum as their positioning in the waterlevel is generally constant. Those docks are ideal to observe shiphull related introductions, as the ships themselves use them as berth, which makes species exchange just a small step.

Intertidal and subtidal soft sediment habitats typically contain infauna communities and mobile epifauna species at the surface that can be collected with all kinds of corers, grabs, dredges, etc. In the intertidal zone samples can be taken by hand. In the subtidal there may be the need for boats and ships that function further from shores. In ports and marinas sampling can be done with small handheld devices (e.g. Petit ponar) or by scuba-diving. Typically, alien species that can be found there are related to hull fouling or ballast water transport of larvae and juvenile stages (for certain species also adults). When hard elements are present in the soft sediment environments, also hard substratum related epifauna and macroalgae appear, associated with related hyperbenthos. In such soft substratum environments the use of cameras can be on option.
A special way of observing settlement of hard substratum related subtidal species is by providing artificial substratum (e.g. SETL-plates). The chance of finding 'new' species in an early stage is probably larger on empty substrata, especially when a complete dominance of green algae is prevented, avoiding direct sunlight on certain surfaces. The technique typically focusses on species with a pelagic phase and is effective to show local reproduction or release of organisms via ballast water or from fouling on shiphulls.

In the water column, phytoplankton, zooplankton, pelagic macrofauna, hyperbenthos (typically near the bottom) and larvae of epi- and infauna can be collected. Many of these species and their occurrences show strong seasonality and might be indicative for reproduction. Typical methodologies for monitoring are all kind of nets focussing on different sizes of the organisms, different water depths and mobility of organisms. Again the site of (first) observation is often indicative of whether introductions are ballast water or shiphull related or involve secundary distribution from sources in the vicinity. Besides this active trapping of specimens, also passive traps can be used, like lighttraps for mobile pelagic organisms and baited traps typically for larger mobile epifauna (like crab traps).

Table 2a. Overview of alien species present (or recently having been present) in the Western Scheldt with indication of year and site of first observation (including observation methodology) and current distribution. If the current distribution is indicated with 'Unknown' the species to our knowledge has not been observed in the last decade.

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indicated in Fig. 1**	**Specific habitat**
Amphipoda						
Ampithoe valida	2014 (Faasse, 2015)	Intertidal hard substratum inventories	4a	Inventories of seaports and surroundings	4a	Intertidal mainly on green algae
Caprella scaura	2016 (Faasse, subm. manuscript)	Intertidal hard substratum inventories	12	Intertidal hard substratum inventories	12	Subtidal hard substratum
Caprella mutica (Japanese skeleton shrimp; harig spookkreeftje)	1997 (Faasse, unpublished data)	Investigation cooling water discharge	12	SETL-plates	4c, 4d, 4e, 4f	Artificial hard substrate in water column
				OSPAR-HELCOM scrape samples (floating dock, pillar/harbour wall)	4c, 4d, 4e, 4f	Subtidal hard substratum
Incisocalliope aestuarius (estuariene poliepvlo)	1991 (Faasse & van Moorsel, 2003; Wolff, 2005)	Boxcore monitoring	VII	Boxcore monitoring	VII	Subtidal soft sediment with hard elements
				Inventories of seaports and surroundings	6b, 8	Subtidal hard substrate
				Subtidal collection by SCUBA-diving	IV, VII	Subtidal hard substratum
Jassa marmorata	1990 (Cattrijsse et al.,1993; Faasse & Van Moorsel, 2000)	Hyperbenthic sledge	0	According to literature	2c, 3b, 3c, 12, 13	Subtidal soft sediment and intertidal hard substratum
				SETL-plates	4c	Artificial hard substratum in water column
				OSPAR-HELCOM scrape samples (floating dock, pillar/harbour wall)	4c, 4d	Subtidal hard substratum
				OSPAR-HELCOM petit ponar grab	4c	Subtidal soft sediment with hard elements

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indi-cated in Fig. 1**	**Specific habitat**
Melita nitida (elegante honingvlokreeft)	1998 (Faasse & van Moorsel, 2003)	Intertidal hard substratum inventories	9a	Inventories of seaports and surroundings	5a, 5b, 5d, 6b, 8, 9a	Intertidal and shallow subtidal under boulders
				Transect Monitoring	2b, 2c, 4b, 5f, 6b, 7b, 9b	Intertidal under boulders
				OSPAR-HELCOM petit ponar grab	4e, 4f	Subtidal soft sediment
				Subtidal collection by SCUBA-diving	IV, VII	Subtidal soft sediment with hard elements
				Boxcore and handcore monitoring	V, VII, VIII	Subtidal soft sediment with hard elements
Ptilohyale littoralis	2014 (Faasse, 2014)	Intertidal hard substratum inventories	4b, 5a	Inventories of seaports and surroundings	3a, 4b, 4e, 5a	Intertidal under boulders
Bacillariophyceae						
Asterionellopsis glacialis	Unknown (Wolff, 2005), 1990 (MWTL fytoplankton)	Phytoplankon sampling	Unknown	MWTL phytoplankton sampling	2d, 7c, 9c	Water column
Coscinodiscus wailesii	Probably 1978 (Wolff, 2005), 1990 (MWTL fytoplankton)	Phytoplankon sampling	Unknown	MWTL phytoplankton sampling	2d, 7c	Water column
Odontella longicruris	2013 (Van Wezel et al., 2015)	Fytoplankon sampling water column	2d	MWTL phytoplankton sampling	2d, 7c	Water column
Odontella sinensis	Probably early 20th century (Wolff, 2005), 1990 (MWTL fytoplankton)	Fytoplankon sampling water column	Unknown	MWTL phytoplankton sampling	2d, 7c	Water column
Pleurosigma simonsenii	1991(MWTL fytoplankton), might have been present since the mid 70s (Wolff, 2005)	Phytoplankon sampling	2d, 7c, 9c	MWTL phytoplankton sampling	2d, 7c, 9c	Water column
Pseudochattonella sp.	2012 (Mulderij et al, 2013)	Immuno-chemical detection	2d	MWTL phytoplankton sampling	2d	Water column

Species per class	First observation in the Western Scheldt			Current distribution		
	Year	Methodology	Site	Methodology	Sites as indicated in Fig. 1	Specific habitat
Thalassiosira hendeyi	1995 (MWTL fytoplankton)	Fytoplankon sampling water column	7c, 9c	MWTL phytoplankon sampling	2d, 7c, 9c	Water column
Thalassiosira nordenskioeldii	1991 (MWTL fytoplankton)	Fytoplankon sampling water column	2d, 7c	MWTL phytoplankon sampling	2d, 7c	Water column
Thalassiosira punctigera	1992 (MWTL fytoplankton)), might have been present since the early 80s (Wolff, 2005)	Fytoplankon sampling water column	2d, 7c, 9c	MWTL phytoplankon sampling	2d, 7c, 9c	Water column
				OSPAR-HELCOM phytoplankton	4c, 4d, 4e, 4f	Water column
Bivalvia						
Ensis leei (Atlantic jack knife clam; Amerikaanse zwaardschede)	1988 (Wijnhoven & Hummel, 2009)	Boxcore monitoring	II, III	Boxcore monitoring	I, II, III, IV, V, VII, VIII	Subtidal soft sediment
				OSPAR-HELCOM hand dredge	4c	Subtidal soft sediment
Magallana gigas (Pacific oyster; Japanse oester)	Sporadic during 1980s, increasing during 1990s (Drinkwaard, 1999)	Intertidal observations by professionals and hobbyists	0	Inventories of seaports and surroundings	1, 2a, 3a, 4b, 4e, 5a, 5b, 6b, 8, 9a	Intertidal and subtidal hard substratum
				Transect Monitoring	2b, 2c, 4b, 5e, 5f, 6b, 7a, 7b, 9b	Intertidal hard substratum
				Inventory of intertidal soft sediment and Boxcore monitoring	III, IV, V, VI, VII, VIII	Subtidal soft sediment with hard elements
				SETL-plates (also OSPAR-HELCOM SETL)	2c, 4c, 4e, 5c, 6a	Artificial hard substratum in water column
				OSPAR-HELCOM drop-down camera	4c, 4d, 4e, 4f	Subtidal hard substratum
				OSPAR-HELCOM hand dredge	4c	Subtidal soft sediment with hard elements

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indicated in Fig. 1**	**Specific habitat**
(*M. gigas* continued)				OSPAR-HELCOM dike, littoral zone	4c, 4d, 4e, 4f	Intertidal hard substratum
				OSPAR-HELCOM scrape samples (floating dock, pillar/ harbour wall)	4c, 4e, 4f	Subtidal hard substratum
				OSPAR-HELCOM petit ponar grab	4c, 4d	Subtidal soft sediment with hard elements
				Floating dock survey during maintenance	2c	Subtidal hard substratum
				Iron harbour-wall survey Breskens	2c	Intertidal hard substratum
				Rip-rap dike survey Breskens	2c	Intertidal hard substratum
Mya arenaria (softshell clam; strandgaper)	Probably 13th century (Wolff, 2005)	Unknown	Unknown	Boxcore and handcore monitoring	I, II, III, IV, V, VI, VII, VIII	Subtidal and intertidal soft sediment
				OSPAR-HELCOM hand dredge	4c, 4d	Subtidal soft sediment
Mytilopsis leucophaeata (Conrad's false mussel; brakwatermossel)	Around 2000 (Faasse & Van Moorsel 2003)	Subtidal hard substratum inventories	VII	Inventories of seaports and surroundings	5d	Intertidal and subtidal hard substratum
Petricola pholadiformis (false angelwing; Amerikaanse boormossel)	Probably early 20th century	Unknown	Unknown	Boxcore and handcore monitoring	I, II, III, IV, V, VI, VII, VIII	Subtidal soft substratum: especially in peat and clay
Ruditapes philippinarum (Manila clam; Filippijnse tapijtschelp)	2014 (data eCOAST)	Intertidal hard substratum inventories	4b,4e	Inventories of seaports and surroundings	4b, 4e	Intertidal mixed sediment
Teredo navalis (naval shipworm; paalworm)	<1985 (De Bruyne et al., 2013)	Unknown	Unknown	Inventories of seaports and surroundings	8, 9a	Intertidal and subtidal hard substratum: in wood
Bryozoa						
Bugula stolonifera (vogelkopmosdiertje)	2016 (GiMaRIS data)	Scrape samples (pillar/harbour wall)	4c	OSPAR-HELCOM scrape samples (pillar/harbour wall)	4c	Subtidal hard substratum

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indicated in Fig. 1**	**Specific habitat**
Smittoidea prolifica	2015 (GiMaRIS data)	SETL-plates	2c, 4c	SETL-plates	2c, 4c	Artificial hard substratum in water column
				OSPAR-HELCOM petit ponar grab	4d	Subtidal soft sediment with hard elements
Tricellaria inopinata (onverwacht mosdiertje)	2016 (GiMaRIS data)	SETL-plates	4c, 4d, 4e, 4f	OSPAR-HELCOM SETL-plates	4c, 4d, 4e, 4f	Artificial hard substratum in water column
Cirripedia						
Amphibalanus amphitrite (paarsgestreepte zeepok)	2014 (Faasse, 2014)	Intertidal hard substratum inventories	12	Inventories of seaports and surroundings	Unclear if still present	Intertidal and subtidal hard substratum
Amphibalanus improvisus (bay barnacle; brakwaterzeepok)	17th century (Kerckhof & Cattrijsse, 2001)	Museum material	Antwerpen (Western Scheldt unclear)	Inventories of seaports and surroundings	4b, 5a, 5b, 5d, 6b, 8, 9a	Intertidal and subtidal hard substratum
				Transect monitoring	4b, 6b, 9b	Intertidal hard substratum
				SETL-plates	2c, 4c, 5e, 5c, 6a, 7a	Artificial hard substratum in water column
				MWTL boxcore and handcore monitoring	IV, V, VI, VII	Subtidal soft sediment with hard elements
Austrominius modestus (New-Zealand barnacle; Nieuw-Zeelandse zeepok)	1949 (Den Hartog, 1953)	Intertidal observations by professionals and hobbyists	3(?)	Inventories of seaports and surroundings	1, 2a, 3a, 4b, 4e, 5a, 5b, 5d, 6b, 8	Intertidal and subtidal hard substratum
				Transect monitoring	2b, 2c, 4b, 5e, 5f, 6b, 7a, 7b	Intertidal hard substratum
				SETL-plates (also OSPAR-HELCOM SETL)	2c, 4c, 4d, 4e, 4f, 5c, 5e, 6a	Artificial hard substratum in water column
				OSPAR-HELCOM Chinese crab trap	4c, 4e	Artificial hard substratum in water column

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indi-cated in Fig. 1**	**Specific habitat**
(A. Modestus continued)				OSPAR-HELCOM drop-down camera	4c	Subtidal hard substratum
				OSPAR-HELCOM dike, littoral zone	4c, 4d, 4e, 4f	Intertidal hard substratum
				OSPAR-HELCOM scrape samples (pillar/harbour wall)	4c, 4d, 4e, 4f	Subtidal hard substratum
				Floating dock survey during maintenance	2c	Subtidal hard substratum
Cnidaria						
Blackfordia virginica	2014 data (eCOAST)	Intertidal hard substratum inventories	6b, 9a	Inventories of seaports and surroundings	6b, 9a	Intertidal hard substratum
Diadumene lineata (orange-striped green anemone; groene golfbrekeranemoon)	1996 (Faasse, 1996; Wolff, 2005)	Intertidal hard substratum inventories	3b, 12	Inventories of seaports and surroundings	6b, 8	Intertidal and subtidal hard substratum
				Transect Monitoring	2c, 6b, 7b	Intertidal hard substratum
				According to literature	5(?), 14	Intertidal and subtidal hard substratum
Garveia franciscana (rope grass hydroid; berevachtpoliep)	Around 2000 (Faasse & Van Moorsel 2003)	Subtidal hard substratum inventories	VII		Unclear if still present	Intertidal and subtidal hard substratum
Copepoda						
Acartia tonsa (langsprietroeipoot-kreeft)	1967 (Bakker & De Pauw, 1975)	Mesozoo-plankton sampling	8, 9(?), 10	MWTL mesozooplankton sampling	Unclear (Genus present at 2d, 5g, 7c, 9c, 10)	Water column
Mytilicola intestinalis (red worm disease; rood darmroeipootkreeftje)	Probably 1950 (Wolff, 2005)	Probably monitoring of mussel plots	Former Braak-man inlet in commer-cial mussel plots	MWTL boxcore monitoring	III	In shellfish (mussels and oysters)

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indi-cated in Fig. 1**	**Specific habitat**
Pseudodiaptomus marinus (Pacifisch eenoogkreeftje)	2011 (MWTL mesozooplankton data; Brylinski, 2012)	Mesozoo-plankton sampling	5g	MWTL mesozooplankton sampling	5g, 7c, 9c, 10	Water column
Ctenophora						
Mnemiopsis leidyi (warty comb jelly; Amerikaanse ribkwal)	2006 (Faasse & Bayha, 2006)	Collection with plankton net	12 (but commonly present in estuary)	Plankton net	I, II, III, IV, V, VI, VII, VIII	Water column
				Inventories of seaports and surroundings	12	Water column and strandings in intertidal zone
				Transect monitoring	2c	Water column and strandings in intertidal zone
				OSPAR-HELCOM Gee's minnow trap	4c, 4d	Water column
				OSPAR-HELCOM Chinese crab trap	4c	Water column
				OSPAR-HELCOM drop-down camera	4c, 4d, 4e, 4f	Water column
				OSPAR-HELCOM hand dredge	4c, 4d, 4e, 4f	Water column
				OSPAR-HELCOM scrape samples (floating dock)	4d	Water column
				OSPAR-HELCOM gelatineus zooplankton sampling	4c, 4d, 4e, 4f	Water column
				OSPAR-HELCOM petit ponar grab	4c, 4d	Water column
Decapoda						
Eriocheir sinensis (Chinese mitten crab; Chinese wolhandkrab)	1935 (Wolff, 2005)	Unknown	Unknown	Inventories of seaports and surroundings	9a	Intertidal and subtidal hard substratum
				Transect monitoring	15	Intertidal hard substratum

Species per class	First observation in the Western Scheldt			Current distribution		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indi-cated in Fig. 1**	**Specific habitat**
(*E. sinensis* continued)				SETL-plates	2c	Artificial hard substratum in water column
Hemigrapsus sanguineus (Japanese shore crab; blaasjeskrab)	2004 (Faasse, 2004)	Intertidal hard substratum inventories	6b	Inventories of seaports and surroundings	1, 2a, 3a, 4b, 4e, 5a, 5b, 6b, 8, 9a	Intertidal and subtidal hard substratum
				Transect monitoring	2c, 4b, 5f, 6b, 7b, 9b	Intertidal hard substratum
				OSPAR-HELCOM Chinese crab trap	4d	Subtidal soft sediment with hard elements
				OSPAR-HELCOM dike, littoral zone	4d, 4e, 4f	Intertidal hard substratum
Hemigrapsus takanoi (brush-clawed shore crab; penseelkrab)	2001 (Faasse et al., 2002)	Intertidal observations by professionals and hobbyists	17	Inventories of seaports and surroundings	2a, 3a, 4b, 4e, 5a, 5b, 5d, 6b, 8, 9a	Intertidal and subtidal hard substratum
				Transect monitoring	2b, 2c, 4b, 5e, 5f, 6b, 7a, 7b, 9b	Intertidal hard substratum
				Boxcore monitoring	I, V, VIII	Subtidal soft sediment with hard elements
				According to literature	12, 16, 17, 18	Intertidal and subtidal hard substratum
				SETL-plates (also OSPAR-HELCOM SETL)	2c, 4c, 5c, 6a, 9b	Artificial hard substratum in water column
				OSPAR-HELCOM Gee's minnow trap	4d, 4e, 4f	Subtidal soft sediment with hard elements
				OSPAR-HELCOM Chinese crab trap	4d, 4e, 4f	Subtidal soft sediment with hard elements
				OSPAR-HELCOM dike, littoral zone	4d, 4e, 4f	Intertidal hard substratum
				OSPAR-HELCOM scrape samples (pillar/ harbour wall)	4c	Subtidal hard substratum

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indi-cated in Fig. 1**	**Specific habitat**
Palaemon macrodactylus (Oriental shrimp; rugstreepsteurgarnaal)	1999 (d'Udekem d'Acoz et al., 2005)	Scrape sample from pontoon in harbour	8	Inventories of seaports and surroundings	5d, 8	Watercolumn near hard substratum and intertidal pools
				Boxcore monitoring	V, VII	Water column
				Handnet / scrape samples (According to literature)	5e, 6a, 7a, 8, 16	Water column near hard substratum
Rhithropanopeus harrisii (Zuiderzee crab; Zuiderzeekrabbetje)	Around 2000 (Faasse & Van Moorsel 2003)	Subtidal hard substratum inventories	VII	Inventories of seaports and surroundings	5d	Intertidal and subtidal hard substratum
Dinophyceae						
Alexandrium ostenfeldii	2010 (MWTL phytoplankton)	Phytoplankon sampling	7c	MWTL phytoplankon sampling	7c	Water column
Prorocentrum triestinum	Unknown (Wolff, 2005), 1990 (MWTL fytoplankton)	Fytoplankon sampling water column	Unknown	Phytoplankton sampling water column	2d, 7c, 9c	Water column
Scrippsiella trochoidea	1990 (MWTL fytoplankton)	Fytoplankon sampling water column	2d, 7c, 9c	Phytoplankton sampling water column	2d, 7c, 9c	Water column
Gastropoda						
Crepidula fornicata (slipper limpet; muiltje)	Early 1930s (Wolff, 2005)	Unknown	Unknown	Inventories of seaports and surroundings	1, 3a, 5b	Intertidal and subtidal hard substratum
				Boxcore monitoring	I, III, IV, V	Subtidal soft sediment with hard elements
				OSPAR-HELCOM dike, littoral zone	4c	Intertidal hard substratum
Hexapoda						
Telmatogeton japonicus (marine splash midge; Japanse dansmug)	2016 (data eCOAST)	Intertidal hard substratum inventories	3a, 6b, 9a	Inventories of seaports and surroundings	3a, 6b, 9a	Under filamentous green algae and in sediment on hard substratum

Species per class	First observation in the Western Scheldt			Current distribution		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indicated in Fig. 1**	**Specific habitat**
Isopoda						
Synidotea laticauda (brakwaterpissebed)	2009 (Faasse, 2011)	Handnet sampling	8 (at Doel in 2005: Soors et al., 2010)	Inventories of seaports and surroundings	8	Subtidal hard substratum: especially among seaweeds and on ropes
Nemertea						
Cephalothrix cf. simula (oranje snoerworm)	2015 (data eCOAST)	Intertidal hard substratum inventories	3a	Inventories of seaports and surroundings	3a	Intertidal (soft sediment on) hard substratum: especially among oysters
Phaeophyceae						
Sargassum muticum (Japanese sargasso weed; Japans bessenwier)	Unclear	Unknown	Might be present at Vlissingen	Unclear	Unclear if present at Vlissingen	Subtidal hard substratum and floating in the water layer
Undaria pinnatifida (wakame Japanse kelp)	2016 (data GiMaRIS)	Observation during SETL-plate check	2c	Observation during analysis of SETL-plates	2c	Sighted on the hull of a resident pleasure craft
Polychaeta						
Alitta virens (king ragworm; zager)	1963 ? (Wijnhoven & Hummel, 2009)	Handcore monitoring	IV	MWTL handcore and boxcore monitoring	0, I, II, III, IV, V, VIII	Intertidal and subtidal soft sediment
Aphelochaeta marioni (*Tharyx* sp. A in MWTL reports)	Common in 1963 (Wolff, 1973)	Van Veen grab monitoring	IV	MWTL boxcore and handcore monitoring	I, II, III, IV, V, VI, VII, VIII	Intertidal and subtidal soft sediment
Boccardia proboscidea	2013 (Kerckhof & Faasse, 2014)	Intertidal hard substratum inventories	12	Inventories of seaports and surroundings	2a, 3a, 12	Intertidal soft sediment on hard substratum: especially among oysters
Boccardiella hamata	2013 (Kerckhof & Faasse, 2014)	Intertidal hard substratum inventories	3a	Inventories of seaports and surroundings	1, 2a, 3a, 4b, 4e, 5a, 5b, 6b, 8	Intertidal soft sediment on hard substratum: especially among oysters
Ficopomatus enigmaticus (Australian tubeworm; trompet-kalkkokerworm)	1974 (Vaas, 1975)	Inventory of hard substratum in marina	3d	Inventories of marinas and surroundings	4e, 5a, 5b, 5d, 6b, 8, 9a	Intertidal and subtidal hard substratum

Species per class	First observation in the Western Scheldt			Current distribution		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indicated in Fig. 1**	**Specific habitat**
(*F. enigmaticus* continued)				MWTL boxcore and handcore monitoring	III	Intertidal and subtidal soft sediment with hard elements
				SETL-plates	4c, 5c, 6a	Artificial hard substratum in the water column
				OSPAR-HELCOM hand dredge	4c	Subtidal soft sediment with hard elements
Marenzelleria viridis (is probably *M. neglecta*: Van Moorsel et al., 2010) (Oostzeegroenworm)	1995 (Wolff, 2005)	Boxcore and handcore monitoring	VII	MWTL boxcore and handcore monitoring	III, IV, V, VI, VII, VIII	Intertidal and subtidal soft sediment
Microphthalmus similis	1988 (Wijnhoven & Hummel, 2009)	Boxcore monitoring	I, III	MWTL boxcore and handcore monitoring	I, III, V	Intertidal and subtidal soft sediment with hard elements
Neodexiospira brasiliensis	2002 (Faasse, unpublished data)	Intertidal hard substratum inventories	4e	Inventories of seaports and surroundings	4b, 4e	Intertidal hard substratum
				SETL-plates	2c, 4c, 5e	Artificial hard substratum in the water column
Pileolaria berkeleyana	2015 (eCOAST data)	Intertidal hard substratum inventories	4e	Inventories of seaports and surroundings	4e	Intertidal hard substratum: especially under boulders and oyster shells
Polydora sp. (Unknown species of alien origin)	2016 (eCOAST data)	Intertidal hard substratum inventories	9a	Inventories of seaports and surroundings	9a	In intertidal oyster shells
Proceraea cornuta	1995 (Wijnhoven & Hummel, 2009)	Boxcore monitoring	I	MWTL boxcore monitoring	I, III	Subtidal soft sediment with hard elements
Streblospio benedicti	2009 (MWTL macrobenthos)	Boxcore	VII	MWTL boxcore monitoring	0, I, II, III, IV, V, VI, VII, VIII	Subtidal soft sediment
Syllidia armata	2013 (Verduin et al., 2014)	Boxcore monitoing	I	MWTL boxcore monitoring	I	Subtidal soft sediment with hard elements

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indicated in Fig. 1**	**Specific habitat**
Porifera						
Haliclona xena (paarse buisjesspons)	2009 (GiMaRIS data)	SETL-plates	2c, 4c	SETL-plates	2c, 4c	Artificial hard substratum in the water column
				Floating dock survey during maintenance	2c	Subtidal hard substratum
Mycale micracanthoxea (encrusting sponge grijze korstspons)	2013 (GiMaRIS data)	SETL-plates	4c	SETL-plates	2c, 4c	Artificial hard substratum in the water column
				Inventories of seaports and surroundings	4b, 4e, 5a	Subtidal hard substratum
Sycon scaldiense (harige zakspons)	2008 (GiMaRIS data)	Floating dock survey during maintenance	2c	Floating dock survey during maintenance	2c	Subtidal hard substratum
				SETL-plates	2c, 4c	Artificial hard substratum in the water column
Rhaphidophyceae						
Fibrocapsa japonica	1991 (Vrieling et al., 1995)	Immuno-chemical detection	2d, 7c	MWTL phytoplankton sampling	2d, 7c	Water column
Heterosigma akashiwo	2002 (MWTL phytoplankton)	Phytoplankon sampling	2d, 7c	MWTL phytoplankton sampling	2d, 7c, 9c	Water column
Rhodophyceae						
Antithamnionella spirographidis	2016 (GiMaRIS data)	OSPAR-HELCOM Sloehaven	4c, 4d, 4e, 4f	OSPAR-HELCOM SETL-plates	4c, 4d, 4e, 4f	Artificial hard substratum in the water column
Caulacanthus ustulatus (also identified as *C. okamuae*)	Between 2005 and 2014 (Stegenga & Karremans, 2014)	Intertidal observations by professionals and hobbyists	17	Transect Monitoring	4b	Intertidal (pools) hard substratum
				Inventories of seaports and surroundings	4b	Intertidal (pools) and subtidal hard substratum
Dasya sessilis	2015 (eCOAST data)	Intertidal hard substratum inventories	5d	Inventories of seaports and surroundings	5d	Intertidal (pools) and subtidal hard substratum

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indicated in Fig. 1**	**Specific habitat**
Dasysiphonia japonica	2015 (eCOAST data)	Intertidal hard substratum inventories	4b	Inventories of marinas and surroundings	4b	Intertidal and subtidal hard substratum
				OSPAR-HELCOM hand dredge	4d	Subtidal soft sediment with hard elements
				OSPAR-HELCOM SETL-plates	4c, 4d, 4e, 4f	Artificial hard substratum in the water column
Neosiphonia harveyi (Harvey's siphon weed; violet buiswier)	Unclear, because not recognized as such (possibly around 2010) (Stegenga & Karremans, 2014)	Intertidal observations by professionals and hobbyists	I	OSPAR-HELCOM SETL-plates	4c	Artificial hard substratum in the water column
Tunicata						
Aplidium glabrum (glanzende bolzakpijp)	2009 (GiMaRIS data)	SETL-plates	2c, 4c	SETL-plates	2c, 4c	Artificial hard substratum in the water column
				OSPAR-HELCOM scrape samples (floating dock)	4c, 4d, 4e, 4f	Subtidal hard substratum
Botrylloides violaceus (colonial sea squirt; slingerzakpijp)	2000 (Faasse & De Blauwe, 2000)	Inspection of pontoons in marina	4e	Inventories of seaports and surroundings	4e	Subtidal hard substratum
				SETL-plates (also OSPAR-HELCOM SETL)	2c, 4c, 4d, 4e, 4f, 5e	Artificial hard substratum in the water column
				OSPAR-HELCOM scrape samples (floating dock, pillar/ harbour wall)	4c, 4d, 4e, 4f	Subtidal hard substratum
				OSPAR-HELCOM drop-down camera	4c, 4d, 4e, 4f	Subtidal hard substratum
				Floating dock survey during maintenance	2c	Subtidal hard substratum
Didemnum vexillum (compound sea squirt; druipzakpijp)	2014 (GiMaRIS data)	SETL-plates	4c	SETL-plates (also OSPAR-HELCOM SETL)	4c	Artificial hard substratum in the water column

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indicated in Fig. 1**	**Specific habitat**
(*D. vexillum* continued)				OSPAR-HELCOM scrape samples (floating dock)	4d	Subtidal hard substratum
				OSPAR-HELCOM drop-down camera	4e	Subtidal hard substratum
				Inventories of marinas and surroundings	3	Subtidal hard substratum
Molgula manhattensis (sea grape; ronde zakpijp)	18th century (Wolff, 2005)	Unknown	Unknown	Inventories of marinas and surroundings	5b, 8, 9a	Intertidal and subtidal hard substratum
				SETL-plates (also OSPAR-HELCOM SETL)	2c, 4c, 4d, 4e, 5c, 5e, 6a, 7a	Artificial hard substratum in the water column
				OSPAR-HELCOM scrape samples (floating dock)	4d	Subtidal hard substratum
				OSPAR-HELCOM Chinese crab trap	4d	Artificial hard substratum in the water column
				MWTL boxcore monitoring	V	Subtidal soft sediment with hard elements
Perophora japonica (colonial sea squirt; Japanse zakpijp)	2014 (GiMaRIS data)	SETL-plates	4c	SETL-plates (also OSPAR-HELCOM SETL)	4c, 4d	Artificial hard substratum in the water column
Styela clava (leathery sea squirt; knotszakpijp)	2000 (Faasse & De Blauwe, 2000)	Inspection of pontoons in marina	4e	Inventories of seaports and surroundings	4e	Intertidal and subtidal hard substratum
				SETL-plates (also OSPAR-HELCOM SETL)	2c, 4c, 4d, 4e, 4f	Artificial hard substratum in the water column
				OSPAR-HELCOM scrape samples (floating dock)	4c, 4d, 4e, 4f	Subtidal hard substratum
				OSPAR-HELCOM drop-down camera	4c, 4d	Subtidal hard substratum
				OSPAR-HELCOM petit ponar grab	4c	Subtidal soft sediment with hard elements
				Floating dock survey during maintenance	2c	Subtidal hard substratum

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**	**Site**	**Methodology**	**Sites as indi-cated in Fig. 1**	**Specific habitat**
Ulvaceae						
Codium fragile (sponge seaweed ; vertakt viltwier)	< 1983 (Stegenga & Mol, 1983)	Intertidal hard substratum inventories	0	OSPAR-HELCOM phytoplankton	4c, 4d, 4e, 4f	Floating in the water layer
Ulva australis (sea lettuce; zeesla)	Late 1990s (Stegenga & Mol, 2002)	Intertidal hard substratum inventories	unknown	Transect monitoring	2b, 2c, 4b, 5e, 5f, 6b, 7a, 7b	Subtidal and intertidal hard substratum
				OSPAR-HELCOM SETL-plates	4d	Artificial hard substratum in the water column

Table 2b. Alien species that have been present in the Western Scheldt (with indication of year and site of first observation, including observation methodology) for which the current distribution is 'Unknown' or the species is probably not present anymore, as the species to our knowledge has not been observed in the last decade.

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**		**Year**	**Methodology**	
Monocorophium sextonae (Sexton's slijkgarnaal) (Amphipoda)	1998 (Faasse & Van Moorsel, 2000)	Subtidal hard substratum inventory (Scuba diving)	17		Unknown	Subtidal soft substratum with hard elements
Corethron pennatum (Bacillariophyceae)	1993 (MWTL fytoplankton)	Phytoplankon sampling	2d	MWTL phytoplankton sampling	Unknown	Water column
Proboscia indica (Bacillariophyceae)	1993 (MWTL fytoplankton)	Phytoplankon sampling	2d	MWTL phytoplankton sampling	Unknown	Water column
Megabalanus coccopoma (titan acorn barnacle; grote rose zeepok) (Cirripedia)	2006 (Van Nieulande et al., 2006)	Cooling water inlet	12	According to literature	Unknown	Subtidal hard substratum
Alexandrium tamarense (Dinophyceae)	1990 (MWTL phytoplankton)	Phytoplankon sampling	2d	MWTL phytoplankon sampling	Unknown	Water column
Karenia mikimotoi (Dinophyceae)	1990 (MWTL fytoplankton)	Fytoplankon sampling water column	2d, 7c	Phytoplankton sampling water column	Unknown	Water column
Protoceratium reticulatum (Dinophyceae)	1991 (MWTL fytoplankton)	Fytoplankon sampling water column	7c	Phytoplankton sampling water column	Unknown	Water column
Syllis gracilis (Polychaeta)	1998 (Wijnhoven & Hummel, 2009)	Boxcore monitoring	III	MWTL boxcore monitoring	Probably nowadays absent	Subtidal soft sediment with hard elements
Chattonella marina (var. antiqua) (Rhaphidophyceae)	1991 (Vrieling et al., 1995)	Immuno-chemical detection	2d, 7c	MWTL phytoplankton sampling	Unknown	Water column

Table 2c. Fresh water species with limited tolerance towards increased salinity: only present at and near fresh water inlets.

Species per class	**First observation in the Western Scheldt**			**Current distribution**		
	Year	**Methodology**		**Year**	**Methodology**	
Chelicorophium curvispinum (Caspian mud shrimp; Kaspische slijkgarnaal) (Amphipoda)	End of 90s (Faasse & Van Moorsel, 2000)	Inter-/subtidal hard substratum inventories	9b		Unknown	In subtidal and intertidal sediment on hard substratum
Gammarus tigrinus (tijgervlokreeft) (Amphipoda)	Around 2000 (Faasse & Van Moorsel 2003)	Subtidal hard substratum inventories	VII	Inventories of seaports and surroundings	5d	Subtidal hard substratum
Dreissena bugensis (quagga mussel: quaggamossel) (Bivalvia)	Unknown	Unknown	Unknown	Inventories of seaports and surroundings	5d	Subtidal hard substratum
Dreissena polymorpha (gewone driehoeksmossel) (Bivalvia)	Unknown	Unknown	Unknown	Inventories of seaports and surroundings	5d	Subtidal hard substratum
Physella acuta (acute bladder snail; puntige blaashoren) (Gastropoda)	2016 (Wijnhoven, 2016)	Transect monitoring	9b	Transect monitoring	9b	Intertidal hard substratum
Cordylophora caspia (freshwater hydroid; brakwaterpoliep) (Cnidaria)	2015 (data eCOAST)	Intertidal hard substratum inventories	5d	Inventories of seaports and surroundings	5d	Subtidal hard substratum
Neogobius melanostomus (round goby; zwartbek-grondel) (Pisces)	Unknown	Unknown	Unknown	Inventories of seaports and surroundings	5d	Subtidal hard substratum
Laonome calida (Polychaeta)	Around 2009-2012 (Capa et al., 2014)	Artificial substratum	5d		Unknown	Artificial hard substratum in the water column
Sinelobus vanhaareni (Tanaidacea)	2007 (Van Haaren & Soors, 2009)	?	5d	Inventories of seaports and surroundings	5b, 5d	Subtidal and intertidal soft sediment and hard substratum

Alien species present in the Western Scheldt
Table 2 gives an overview of all alien species identified in the Western Scheldt during the listed monitoring programmes. For several species the current distribution is indicated as 'Unknown'. This generally means that the species has been observed in the Western Scheldt, however not during the last 10 years. This can mean that the species is still present but typically present in poorly or so far not inventoried habitats or areas (these species are listed with the alien species known to be pesent in Table 2a), or that the introduced species did not establish a self-sustaining population and has likely disappeared (species for which it is expected that this is the case are indicated in Table 2b). A total of 90 estuarine and marine alien species have been identified to have been present during the last 25 years in the Western Scheldt of which proably at least 81 species are still present. Besides, nine additional species have been observed in the estuary (at salinities above 1 psu) which are however typical fresh water species, not expected to be able to maintain populations under estuarine or marine conditions (Table 2c). It largely depends on the taxonomic group whether there is a reliable overview of the current distribution in the Western Scheldt.

Pathways and vectors; primary introduction and secondary dispersal
Table 3 gives an overview of the most likely vector of primary introduction into the Western Scheldt and the most likely way of secondary dispersal. It is clear that the vector of primary introduction of species into the Western Scheldt might be different from the vector of primary introduction in the Netherlands or Western European waters. In many cases locations where alien species have been introduclsewhere in the Netherlands or Western Europe can act as a source for introduction into the Western Scheldt.

Several alien Amphipods are present in the Western Scheldt, which to a large part probably have been introduced via shiphull fouling and in several cases via ballast water (Table 3). Besides shiphull fouling, natural distribution is likely an important factor for secondary distribution, although not frequently recorded as such in literature. This is shown by observations of alien amphipods outside ports and marinas shortly after the first observations (Table 3). With the recent new monitoring initiatives, specifically in the intertidal zone, several new species have been found. Species are typically introduced in marinas and ports, where potentially also predominantly subtidal species could be collected with scrape samples, SETL-plates and possibly small grabs.

The Bacillariophyceae, Dinophyceae and Rhaphidophyceae (unicellular organisms) are all picked up with the MWTL phytoplankton monitoring. The vector of introduction is likely ballast water, although primary introduction with currents (probably also the main vector of secondary dispersal) from sources in the vicinity could also have led to their introduction into the Western Scheldt. It is expected that phytoplankton monitoring in ports can lead to the early detection of additional species.

Bivalves are a diverse group in terms of introduction vector to the Western Scheldt. There are several cases of shiphull and ballast water introductions, besides natural dispersal, which is likely the major way for secondary distribution. Most species are not recent introductions and are therefore also picked up by the MWTL macrobenthos monitoring. For hard substratum related species, however, the inventories of artificial and hard substrata, inter- and subtidal, around marinas and seaports are of importance.

Table 3a. Overview of alien species in the Western Scheldt with the most likely vector of primary introduction (1) and the most likely vector of secondary dispersal (2) in the Western Scheldt. Vectors of introduction and of secondary distribution are based on expert judgement (of the authors) if no information was available from literature.

Species	**Vector of introduction**				**Species**	**Vector of introduction**			
	Shiphull fouling	**ballast water**	**natural dispersal**	**aquaculture**		**Shiphull fouling**	**ballast water**	**natural dispersal**	**aquaculture**
Amphipoda					**Dinophyceae**				
Ampithoe valida	1,2		2		*Alexandrium tamarense*		1	1,2	
Caprella scaura	1,2	1	2		*Alexandrium ostenfeldii*		1	1,2	
Caprella mutica	1,2	1	2		*Karenia mikimotoi*		1	1,2	
Incisocalliope aestuarius	1	1	2		*Prorocentrum triestinum*		1	1,2	
Jassa marmorata	1		1,2		*Protoceratium reticulatum*		1	1,2	
Melita nitida		1	1,2		*Scrippsiella trochoidea*		1	1,2	
Monocorophium sextonae	1		2		**Gastropoda**				
Ptilohyale littoralis	1	1	2		*Crepidula fornicata*			1,2	
Bacillariophyceae					**Hexapoda**				
Asterionellopsis glacialis		1	1,2		*Telmatogeton japonicus*	1,2		2	
Corethron pennatum		1	1,2		**Isopoda**				
Coscinodiscus wailesii		1	1,2		*Synidotea laticauda*	1,2	1	2	
Odontella longicruris		1	1,2		**Nemertea**				
Odontella sinensis		1	1,2		*Cephalothrix cf. simula*	1	1	2	
Pleurosigma simonsenii		1	1,2		**Phaeophyceae**				
Proboscia indica		1	1,2		*Sargassum muticum*			1,2	
Pseudochattonella sp.		1	1,2		*Undaria pinnatifida*	1,2		2	
Thalassiosira hendeyi		1	1,2		**Polychaeta**				
Thalassiosira nordenskioeldii		1	1,2		*Alitta virens*		1	2	
Thalassiosira punctigera			1,2		*Aphelochaeta marioni/Tharyx* sp. A	1	1	2	
Bivalvia					*Boccardia proboscidea*	1,2	1	2	
Ensis leei		1	1,2		*Boccardiella hamata*	1,2	1	2	
Magallana gigas			1,2		*Ficopomatus enigmaticus*	1,2		2	
Mya arenaria		1	2		*Marenzelleria viridis / M. neglecta*		1	2	
Mytilopsis leucophaeata	1		2		*Microphthalmus similis*	1	1	2	
Petricola pholadiformis			1		*Neodexiospira brasiliensis*	1,2	1	2	
Ruditapes philippinarum		1	2		*Pileolaria berkeleyana*	1,2		2	
Teredo navalis	1		1,2		*Polydora sp.*	1,2	1	2	
Bryozoa					*Proceraea cornuta*	1	1	2	
Bugula stolonifera	1,2		2		*Streblospio benedicti*		1	2	
Smittoidea prolifica	1,2		2		*Syllidia armata*	1,2			
Tricellaria inopinata	1				*Syllis gracilis*	1		2	

Species	**Vector of introduction**				**Species**	**Vector of introduction**			
	Shiphull fouling	**ballast water**	**natural dispersal**	**aquaculture**		**Shiphull fouling**	**ballast water**	**natural dispersal**	**aquaculture**
Cirripedia					**Porifera**				
Amphibalanus amphitrite	1	1	2		*Haliclona xena*		1	2	
Amphibalanus improvisus	1		2		*Mycale micracanthoxea*		1	2	
Austrominius modestus	1		1		*Sycon scaldiense*		1	2	
Megabalanus coccopoma		1	2		**Rhaphidophyceae**				
Cnidaria					*Chattonella marina (var. antiqua)*		1	2	
Blackfordia virginica	1	1	2		*Fibrocapsa japonica*		1	2	
Diadumene lineata	1,2		2		*Heterosigma akashiwo*		1	2	
Garveia franciscana	1,2		2		**Rhodophyceae**				
Copepoda					*Antithamnionella spirographidis*	1,2		2	
Acartia tonsa		1	2		*Caulacanthus ustulatus*	1,2		2	
Mytilicola intestinalis			1,2	1,2	*Dasya sessilis*	1,2		2	
Pseudodiaptomus marinus		1	2		*Dasysiphonia japonica*	1,2		2	
Ctenophora					*Neosiphonia harveyi*	1,2		2	
Mnemiopsis leidyi		1	1,2		**Tunicata**				
Decapoda					*Aplidium glabrum*	1,2		2	
Eriocheir sinensis		1	1,2		*Botrylloides violaceus*	1,2		2	
Hemigrapsus sanguineus	1,2	1	2		*Didemnum vexillum*	1,2		2	
Hemigrapsus takanoi	1,2	1	2		*Molgula manhattensis*	1,2		2	
Palaemon macrodactylus		1	2		*Perophora japonica*	1,2		2	
Rhithropanopeus harrisii	1,2	1	2		*Styela clava*	1,2		2	
					Ulvophyceae				
					Codium fragile	1		2	
					Ulva australis		1	1,2	

The bryozoans are all recently noticed introductions that normally arrive via shiphull fouling, and reproduce in seaports and marinas, for which SETL-plates and scrape samples in and around marinas and seaports are the most suitable methodology for early detection. It is very likely that new species for this group will appear in the near future.

Also cirripeds are a group for which new species can be expected, although *Austrominius modestus* might have taken in a range of niches. Like for bryozoans, shiphull fouling and reproduction in marinas is the most likely vector of introduction of cirripeds into the Western Scheldt. However, natural distribution of larvae or dispersal via floating material and ballast water from sources in the vicinity can be expected as well as being the primary way of introduction, besides that it is the way of secondary dispersal within the Western Scheldt. The combination of inventories in the vicinity of seaports and marinas in the intertidal zone, scrape samples for the shallow subtidal and SETL-plates to collect settling larvae is a good combination to monitor the Cirripedia assemblages.

Table 3b. Overview of alien fresh water related species observed in the Western Scheldt with limited tolerance towards increased salinity: only present at and near fresh water inlets. Most likely vectors of primary introduction (1) and the most likely vectors of secondary dispersal (2) in the Western Scheldt are indicated. *=via 'new' watershed connections.

Species	**Vector of introduction**			
	Shiphull fouling	**ballast water**	**natural dispersal**	**aquaculture**
Chelicorophium curvispinum (Amphipoda)	1,2		1*	
Gammarus tigrinus (Amphipoda)	1,2	1	1,2	
Dreissena bugensis (Bivalvia)	1		1*	
Dreissena polymorpha (Bivalvia)	1		1*	
Cordylophora caspia (Cnidaria)	1		1*	
Physella acuta (Gastropoda)			1,2	
Neogobius melanostomus (Pisces)			1*	
Laonome calida (Polychaeta)		1	2	
Sinelobus vanhaareni (Tanaidacea)	1,2	1	2	

Cnidarians are likely primarily introduced via shiphull fouling or natural distribution from sources in the vicinity, although ballast water related dispersal can be expected for certain species as well. Inter- and subtidal inventories in the vicinity of marinas and seaports are the most likely methodology to detect them (although certain species of anemones and hydropolyps could appear below the shallow subtidal as well). To detect medusae and also Ctenophora (typically linked to ballast water transport), plankton sampling in ports would be the ideal methodology for detection.

Alien copepods likely arrive via ballast water and/or natural dispersal from sources in th vicinity. As no specific monitoring for zooplankton is in place it is likely that at the moment already species are overseen, which might be detected by zooplankton monitoring in port areas.

Introduction of decapods is typically via ballast water, although natural dispersal from sources in the vicinity is certainly of importance, while for several species also shiphull fouling plays a role. Although most species are expected to be found with inventories of the intertidal and shallow subtidal habitats, species can be found with a variety of techniques including the directed technique of crab trapping. For shrimps handnetting might also be an option.

At present gastropods likely arrive via natural dispersal, as long as aquaculture is not present in the Western Scheldt. Species can likely be detected with hard substratum inventories in intertidal and shallow subtidal zones.

The distribution of Hexapoda is likely shiphull related, besides natural distribution of adults from sources in the vicinity as the primary way of introduction. Species of Hexapoda can be found in the intertidal zone.

The introduction of isopods might be shiphull or ballast water related or will occur by natural distribution from sources in the vicinity, both primarily and secondarily. Intertidal species will likely be picked up with intertidal inventories, but some species might ask for subtidal inventory below the shallow subtidal zone.

At present, there is little attention for hard substratum related Nemertea and Polychaeta, especially those living in soft sediments accumulated between oysters, so that group of alien species might be missed. Soft sediment related polychaetes might in the long term be observed via the MWTL macrobenthos monitoring programme, although sampling in port regions (hotspots of introduction) might result in more immediate discovery of new species. The introduction of species is expected to occur via ballast water but in many cases also shiphull fouling and/or natural distribution of larvae.

The introduction of Phaeophyceae to the Western Scheldt is expected to occur basically via floating material (primary introduction via natural distribution from sources in the vicinty) and shiphull fouling in the absence of aquaculture in the Western Scheldt. To find species, the entrances of the canals in the estuary and the marinas and ports are the most probable locations.

Porifera are likely introduced via ballast water and hull fouling and might be first observed in port regions. SETL-methodology, but also inspection of other materials in the water column seems to be the way to detect new species and their distributions.

New Rhodophyceae species (due to the fine and small habitus of many species) are likely introduced in the Western Scheldt via shiphull fouling (or floating in from resources in the vicinity). With inventories of the intertidal zone and the shallow subtidal in and around ports and marinas, most species are likely detected. SETL-plates will also help to detect species.

Tunicates are typically related to shiphull related introductions. Therefore, in the absence of aquaculture in the Western Scheldt, inventories in and near marinas and seaports in the subtidal zone are the best way for early detection: i.e. scrape samples, SETL-plates, inspection of other materials in the water column and camera’s beyond the shallow subtidal area.

Ulvophyceae might be introduced in various ways and are expected in the intertidal and shallow subtidal zone (in the Western Scheldt). Intertidal inventories and scrape samples seem to be most suitable for detection.

As indicated, many alien species are primarily introduced in adjacent waters before they are ‘primarily introduced’ in the Western Scheldt from these sources. Common sources for introduction of alien species in the vicinity of the Western Scheldt are the Dutch delta waters and the Eastern Scheldt in particular, the North Sea coastal zone and due to the currents the Belgian ports and marinas in particular, and the upstream parts of the Scheldt estuary in Belgium and due to similar salinity conditions the Antwerp port area in particular. At present there is no aquaculture activity in the Western Scheldt. Although several species are known to have been primarily introduced in the Netherlands and in the Eastern Scheldt in particular via shellfish transports, this is not a vector of introduction into the Western Scheldt at present. This might of course change in the future if oyster or mussel culture is initiated (again) in the Western Scheldt and this also includes imports without proper management. In that case a new route for alien species introductions is created with potentials for a range of species not present in the Western Scheldt yet. As the culture of especially oysters in the Western Scheldt is currently considered, a steeper increase in the number of alien species present in the estuary in the near future might become reality.

Potential future introductions and developments in alien species populations
Table 4 gives some examples (but not an exhaustive overview) of alien species present in the vicinity that have a reasonable chance to appear in the near future in the Western Scheldt. The overview in table 4 shows that with monitoring focussed on alien species, each year several new species will be detected. Besides that, there are several introduced species in the Western Scheldt that are so far restricted to certain areas. For these species it would be wise to keep an eye on their distribution and effect on native communities. In the future eDNA sampling and metabarcoding may be useful in detection and monitoring of alien species that are expected or have been introduced already in connected waters (both waters adjacent to the Western Scheldt or waters with frequent shipping movements to the Western Scheldt). Although these molecular methods may have an added value to detect the possible presence of specific single species (e.g. Owen et al., 2015), they have not proven their value yet in literature as effective tools for the rapid detection of the settlement of the wide range of non-native species in marine open systems that can be recorded with the in this report described monitoring methods. This is amongst other reasons because molecular techniques do not differentiate between dead and alive material (dead material present in treated ballast water can still be detected). Also gametes and larvae that arrive in the Western Scheldt (e.g. with currents or ballast water) but have no chance of survival or settlement due to the environmental conditions (e.g. climate) might be detected. Additionally, certain species groups (like macro-algae) are less well detected with molecular techniques as their cells are rather persistent.

Table 4. Horizon scan of alien species that might be found in the Western Scheldt in te near future (or that are already present but have not been detected yet).

Species per class	Reason	Suitable methodology for detection	Most likely vectors of introduc-tion	Expected impact
Amphipoda				
Caprella equilibra	Recent northward dispersal from northern France to Belgium (Kerckhof, 2016)	Inventories of seaports and surroundings (subtidal), SETL plates, scrape samples, transect monitoring	Natural distribution	Moderate ecological impact
Grandidierella japonica	Recent introduction to Europe (SE England, France: Jourde et al., 2013)	Boxcore and handcore	Ballast water	Moderate ecological impact
Bacillariophyceae				
Stephanopyxis palmeriana	Present in Dutch delta waters (MWTL Phytoplankton sampling)	Phytoplankton sampling	Natural distribution of cells, ballast water	Low ecological impact
Bivalvia				
Rangia cuneata	Present at Antwerpen at least since 2005 (Verween et al., 2006)	Inventories of marinas and surroundings, transect monitoring, scrape samples	Natural distribution of larvae, ballast water	Moderate impact as fouling organism
Bryozoa				
Biflustra sp.	Spreading all over the world; unconfirmed records from the Western Scheldt exist	Inventories of seaports and surroundings, transect monitoring, scrape samples	Shiphull fouling, ballast water	Low impact
Pacificincola perforata	Present in Eastern Scheldt and Lake Veere (Faasse et al.,	Inventories of marinas and surroundings (subtidal), SETL plates,	Ballast water	Low impact

	2013).	scrape samples		
Copepoda				
Mytilicola orientalis	Common in Eastern Scheldt and can withstand low salinity	Inventories of musselbeds	Shiphull fouling, accidental shellfish transport	Moderate economic impact
Dinophyceae				
Prorocentrum cordatum	Present in Dutch delta waters (MWTL Phytoplankton sampling)	Phytoplankton sampling	Natural distribution of cells, ballast water	Potential ecological and health impact (moderate), although human illness not confirmed
Entoprocta				
Barentsia ramosa	Present at Doel (Belgium) and in Nieuwe Waterweg (Faasse, 2006); similar habitats in eastern part of westen Scheldt	Inventories of marinas and surroundings (subtidal), SETL plates, scrape samples	Shiphull fouling, ballast water	Low impact
Isopoda				
Ianiropsis serricaudis	Present in Dutch delta waters (i.e. Eastern Scheldt) (Hobbs et al., 2015); similar habitats in mouth of Western Scheldt	Inventories of marinas and surroundings, scrape samples	Shiphull fouling, ballast water	Moderate ecological impact
Platyhelminthes				
Euplana gracilis	Present in Dutch delta (i.e. Eastern Scheldt, Lake Veere when still brackish) (Faasse & Ligthart, 2007); similar habitats in Western Scheldt	Inventories of marinas and surroundings, transect monitoring	Natural distribution of larvae, ballast water, shiphull fouling	Low ecological impact

Conclusions & discussion

Impact of species groups

Several alien species (e.g. most representatives of the macroalgae, Tunicata, Cirripedia, Porifera, and some Polychaeta) are known as spatial competitors with native species for substrata. This includes the overgrowing of native communities and the outcompetance of vulnerable species. Besides expected impacts on native species diversity, such shifts in species composition will potentially also impact foodwebs, nutrient cycling and even the abiotic constitution of the environment (of which impacts are difficult to generalize). Potential large ecological impacts of alien species population expansions can come with economic consequences as well. Examples of such nuisances are the extensive growth of alien species populations on recreational sites (e.g. Pacific oyster reefs and growth of macro algae), competition with - and fouling of commercial shellfish stocks, and fouling of ship's hulls and cooling water systems. In all cases the alien nuisance species might lead to reduced yields and income and high costs can be involved with prevention - and cleaning measures.

Natural - and aquacultural shellfish stocks are not only at risk of competition for space, but can directly be impacted by predation and/or infestation as well. Copepoda (mussel parasites) are known for parasitism in Bivalvia, and Gastropoda (oyster drills) and certain Decapoda (*Hemigrapsus*) for predation on Mollusca. A group of boring Polychaeta (mudworms: *Polydora*, *Boccardia*) is known for weakening of shells of commercial and other mollusks. Furthermore, there is competition for food that might impact various species of commercial interest. Examples are alien bivalve populations consuming large quantities of fytoplankton and Ctenophora (*Mnemiopsis leidyi*) preying on zooplankton and fish larvae (Finenko et al., 2015).
Several alien species, like the Pacific oyster *Magallana gigas* and the Atlantic jack-knife clam *Ensis leei*, are considered ecosystem engineers. Besides providing new habitat and potentials for new species, such alien ecosystem engineers might provide especially atypical habitat. In that case such new habitats are often considered an alteration of the natural situation and might provide opportunities for additional alien species as well. Also polychaetes (*Polydora*, *Pseudopolydora* and *Ficopomatus*) are able to smother native communities and alter habitats. Amphipoda, Isopoda and Polychaeta often have important roles in foodwebs due to their high densities and intermediate position in food chains. It is however difficult to estimate the impact of such species. The ecological impact of different types of unicellular organisms and phytoplankton is largely unclear as well. Some species have the potential to significantly influence plankton size distributions and/or total biomass which might have a large impact on the foodweb (higher trophic levels). Especially Dinophyceae potentially produce toxins that might impact organisms at different levels in the foodchain, including in some cases consequences for human health (e.g. Katsanevakis et al., 2014).

<u>Vectors</u>
Although ballast water may become relatively less important in the future thanks to legislation, hull fouling and ballast water appear at present equally important vectors in the Western Scheldt (Table 3a). Hull fouling of recreational boats is mainly a cause of secondary introductions. This can best be monitored in marinas. Hull fouling and ballast water of commercial ships may cause primary introductions as well. This can best be monitored in or near commercial ports. Secondary spread by natural means (rafting, swimming, floating) may result in introductions outside ports and marinas, which can be monitored at any location in the Western Scheldt, as it is impossible to indicate hotspots for this. Presently aquaculture is not present in the Western Scheldt and therefore plays no role as a transport vector for alien species into and throughout the system. Aquaculture can however become an important vector of introduction for a range of species for which chances of introduction are very small at present, when plans to culture oysters in the Western Scheldt include oyster imports from elsewhere.

We record 90 alien species from the Western Scheldt estuary and associated lock gates and harbours, and an additional 9 alien fresh water species (tables 3a and 3b). With respect to the total number of species known from the estuary this is a considerable number. We estimate that for the entire Western Scheldt about 1 in 8-9 species is alien. Although at present it seems that there are large differences between taxonomic groups (large share alien among predominantly sessile macrofauna and low share among zooplankton), this might also indicate that certain groups are underinvestigated. Also especially in the eastern part of the estuary, where the number of native species is much lower than in the western part, the proportion of benthic alien species recorded is relatively high, one third or more (data eCOAST). The same holds for harbours. Still almost every year additional alien species are recorded from the Western Scheldt (table 2a). The occurrence of alien species is certainly not restricted to harbours; many occur in other habitats as well, as indicated in the current overview for the Western Scheldt.

The current study suggests that with the monitoring efforts of the last years (indicative those of 2015-2016) it seems that a large share of the potentially successful settling new alien species introduced in the Western Scheldt is detected within a few years after introduction. It is however too early to draw this conclusion for sure as there is no experience with similar amounts of monitoring efforts specifically to detect exotic species in the Western Scheldt in the past, and it is possible that additional species (currently overseen) are discovered the coming years. Focussing on potential hotspots, covering the most important habitats along the entire estuarine gradient, as largely done last years, optimalizes the chance of observing new species in an early phase before they become abundant and widespread. As an indication; from all 19 species observed for the first time in the Western Scheldt during 2014-2016, for 15 the distribution is so far restricted to one location (defined as the cluster of sites indicated with the same number in Fig. 1), 2 species have been observed at 2 and 2 species have been observed at 3 locations. At the same time monitoring with different techniques in different habitats covering the estuarine gradient allows to study population developments and interactions with (native) communities and habitats. Ideally this is done annually and preferably in several seasons as population sizes and species compositions in temperate regions vary both seasonally and annually, depending for example on winter temperatures and early spring settlement success. Such studies focusing on the impact of aliens on populations and communities are amongst others of value towards assessments of the risk of secondary distribution to elsewhere, assessments of risk of potential impacts, and options for management, isolation and/or eradication (although eradication is generally not an option). Although monitoring is always a trade-off between efficiency and costs, the current study shows that reducing the monitoring frequency to every other year, means that approximately 6,3 species (average 2014-2016) are missed that are likely still restricted to one location but that might potentially expand to other locations and can become nuisance species. (Monitoring once every three years might lead to not noticing a dozen of species that potentially can lead to problems). Increasing the monitoring frequency to several times a year does not significantly increase the chance of early observation of species, as most species will most likely be detected at maximum individual and/or population size at the end of summer (HELCOM/OSPAR, 2013).

Recommendations

Of importance is however the continuity of the monitoring efforts. SETL-plate monitoring (GiMaRIS) Transect monitoring (Ecoauthor) and Inventories of seaports and surroundings (eCOAST) are planned or in progress in 2017 as well, but should be consolidated for the coming years. The benthos and phytoplankton monitoring within the frame of MWTL is expected to continue the coming years as well, although the taxonomic identification to species level (crucial if samples are used for the detection of alien species) of phytoplankton samples is continuously under debate. The OSPAR-HELCOM port survey and the marina survey (respectively at Vlissingen and Breskens) have been proven very valuable in delivering information about the presence and distribution of alien species, including several new species, as those surveys meant an intensification of monitoring efforts at the most important hotspots in the Western Scheldt (harbour areas) including additional habitats (e.g. bottom, sublittoral, water column) not inventoried during the 'regular' monitoring projects. We recommend to plan recurrent port surveys in the major port areas of Vlissingen and Terneuzen (and possibly in dialogue with Flemish government and port authorities in Antwerp as well); indicative once every three years. Besides, as pontoons concern hotspots of alien species within marinas and ports (Gittenberger et al., 2017), it would be wise to plan pontoon inventories in dialogue with marina authorities during planned maintenance in the larger marinas. In addition plankton sampling in sheltered areas (i.e. marinas and ports) should be part of the regular monitoring, moreover as there is no zooplankton monitoring programme in place at the moment.

Together with zooplankton, benthos of soft sediments and sublittoral hard substrata (except pontoons etcetera) in harbours are not well covered by current regular monitoring. In neighbouring countries several alien species within these groups have been discovered. Plankton samples, grab and hand-dredge samples in harbours could remedy this hiatus. To optimally cover all habitats along the estuarine gradient, it is suggested to include besides marinas and surroundings, also both sides of dock sluices and freshwater influxes and cooling water discharges in a regular monitor programme in the Western Scheldt. As shown by ‘historic’ alien species observations and to cover the entire gradient, it is important to make sites as Borssele, the marina and former port area of Vlissingen, Cadzand-Bad, Ellewoutsdijk or Baarland and Paal part of the monitoring, possibly by replacing monitoring sites now visited by two companies.

Within the hotspots it is important to monitor as many habitats as possible. Many alien species are associated with specific habitats. Alien mud worms are often present only in mud between oysters, certain crustaceans are associated to hydrozoan polyps, others to algae. A number of benthic alien species is found in the littoral zone, others are restricted to buoys and pontoons, which highlights the importance of monitoring different substrata. With every reduction in the number of habitats to be monitored, a couple of alien species potentially present will be overlooked. Hard substrate related species, and hull fouling associated species in particular, can be expected especially on harbour walls, pillars and buoys, as those (almost) directly make contact with the boats. Scrape samples and intertidal inventories potentially provide information on all species present, although certain microhabitats might ask for a more detailed look. In harbor areas it would be wise to select at least a certain type of plankton sampling methodology and to take some soft sediment grab samples.

Such a monitoring scheme would provide the information on the arrival, distribution and potential impact (if information on native communities is gathered as well) of alien species covering all macrofauna and algae groups in the Western Scheldt, suitable to function as an early detection system to allow management and/or isolation of alien populations if necessary. Besides, the proposed monitoring scheme would provide valuable information on the presence or absence of alien species in harbour - and port areas of importance towards the ballastwater regulation and the possibility to ship between local harbours making use of the temporal exemptions with regard to the ballastwater treatment regulations.

As hull fouling is expected to be the most important vector for introductions in the near future, it is wise to focus alien species monitoring on sessile macrofauna communities and associated species, as is the case the last 2-3 years. Besides the intertidal also the shallow subtidal artificial hard substrates should be part of the standard monitoring. It has to be noticed that hard substrates also have the best potentials for management of alien species populations once they have been detected. Additional plankton monitoring (phytoplankton and zooplankton including the gelatinous component) in major port areas on a regular base could results in the detection of several new species. Soft sediment sampling in major port areas is of importance as it concerns an important habitat largely overlooked at present, where potentially high impact species could be detected in an early phase.

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Definitions

- Alien species:	Species living outside their native distributional range, having arrived here as a result of human activity.
- Gee’s minnow trap	Special cage-like trap for catching mobile epifauna on and near the bottom.
- Hotspot:	In the context of exotic species a site where several exotic species are likely introduced (primary introductions) for the first time.
- Monitoring project:	Single or multiple occasion’s inventory of in this case (amongst others) alien species.
- Monitoring programme:	Recurrent inventories (e.g. every year or every three months) of in this case (amongst others) alien species.
- Petit ponar:	Small handhold deployed (from the shore or a small raft) grab to sample subtidal ‘soft’ sediment benthic communities in shallow waters.
- Primary introduction:	A species introduced in a different biological entity for the first time. It is possible that species are introduced several times or continuously coming from other regions; that is still ‘primary introduction’. Talking about the Western Scheldt, several species are primarily introduced in waters in the vicinity which can then act as a source for primary introduction into the Western Scheldt.
- Seaport and surroundings inventories:	Monitoring programme of eCOAST inventorying alien species presence and distributions in and near seaports or marinas
- Secondary dispersal:	Dispersal and or distribution of a species primarily introduced in the region (in this case dispersal of the alien species from the primary introduction source in the Western Scheldt).

- SETL-plates:	Standard PVC plates used by GiMaRIS to inventory the settlement of pelagic phases of alien species, especially in ports and marinas.
- Transect monitoring:	Standardized methodology in use by Ecoauthor to inventory intertidal benthic communities and alien species in particular.
- Vectors (of introduction):	The dominant pathways of how alien species disperse to other regions.

Abbreviations

- BIOMON:	Benthos monitoring as part of the MWTL monitoring programme, from 1991-2008 called BIOMON (Biologische Monitoring)
- BuRo:	Office for Risk assessment and Research, division of the NVWA
- CIV:	Centrale Informatie Voorziening: Central Information division of Rijkswaterstaat
- eCOAST:	An internationally-operating environmental service company performing independent marine and maritime research, in the fields of ecology, economy, environment and energy, and the focus on COASTal and marine ecosystems.
- Ecoauthor:	Company of Sander Wijnhoven focussing on projects related to Scientific Writing & Ecological Expertise: performing deskstudies, reporting & publishing and doing applied research and monitoring for clients.
- GiMaRIS:	Gittenberger Marine Research, Inventory & Strategy solutions is a company focussing on consultancy and monitoring projects in temperate Atlantic seas, the Ponto-Caspian and Indo-Pacific waters.
- HELCOM:	Baltic Marine Environment Protection Commission - Helsinki Commission: is the governing body of the Convention on the Protection of the Marine Environment of the Baltic Sea Area, known as the Helsinki Convention.
- MWTL:	Monitoring van de Waterstaatkundige Toestand des Lands: Monitoring of the water status of Dutch waters: long-term monitoring programme commissioned by Rijkswaterstaat.
- NIOO-CEME:	Nederlands Instituut voor Ecologisch Onderzoek – Centrum voor Estuarine en Mariene Ecologie: Netherlands Institute of Ecology – Centre for Estuarine and Marine Ecology; research station in Yerseke nowadays part of the NIOZ
- NIOZ:	Koninklijk Nederlands Instituut voor Onderzoek der Zee: Royal Netherlands Institute for Sea Research
- NVWA:	Nederlandse Voedsel en Waren Autoriteit: Netherlands Food and Consumer Product Safety Authority
- OSPAR:	The mechanism by which 15 Governments & the EU cooperate to protect the marine environment of the North-East Atlantic, named OSPAR because of the original Oslo and Paris Conventions.
- RAS:	Rapid Assessment Survey
- RWS:	Rijkswaterstaat
- SEFINS:	'Safeguarding the Environment From Invasive Non-native Species', an INTERREG IV A 2 Seas project
- WVL:	Water, Verkeer en Leefomgeving: Water, Traffic and Environment, division of Rijkswaterstaat

Appendices

Appendix 1a. SETL-plate protocol.

Protocol SETL monitoring

A more detailed description of the SETL-project can be found in Gittenberger et al. (2017): 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. It 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 Ponto-caspian region (www.pontocaspian.eu). It was started in close cooperation with the Smithsonian Marine Invasions Laboratory in the US. Their plate design is used as the basis of the SETL-project to ease worldwide comparisons. 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 at present. 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 settlement plates that have been deployed for several years. In the Westerschelde SETL-plates are being monitored in the Port of Vlissingen since 2007, in Breskens marina since 2009, in Terneuzen since 2015, and in Hoedekenskerke, Hansweert and Bath since 2016.

Gittenberger, A., Wesdorp, K.H. & M. Rensing, 2017. Biofouling as a transport vector of non-native marine species in the Dutch Delta, along the North Sea coast and in the Wadden Sea. GiMaRIS report 2017_09: 45 pp.

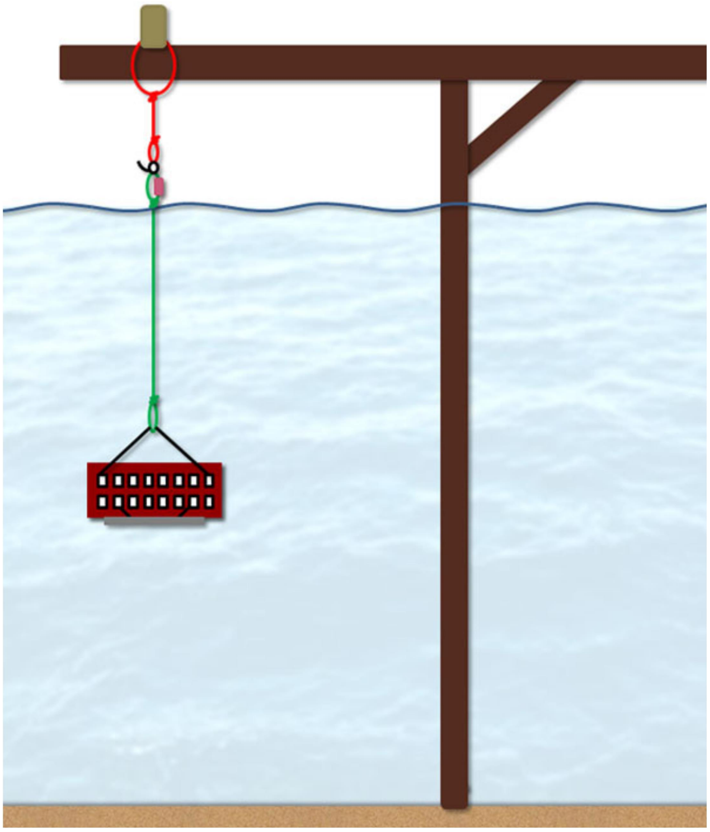

Fig. 1. Schematic view of a SETL plate hanging 1 meter under a dock (figure taken from Gittenberger et al., 2017).

Appendix 1b. Inventories of seaports and surroundings protocol.

Material and methods eCOAST inventories of seaports and surroundings.

On site

On each site the percentage coverage of dominant organisms is estimated and noted. 8 quadrants of 20 x 20 cm are randomly placed and a picture is taken. Next, an inventory is performed of the organisms recognisable by naked eye. These are occurrences are noted on the spot. Organisms that are not recognisable by naked eye are collected. Finally, of all microhabitats, samples are taken. If present, samples are taken of *Ulva spp*., of branched red algae, like *Ceramium*, of branched *Hydrozoa/Bryozoa*, barnacles, mussel clumps, of silt tubes on the substrate, and of silt beneath Japanese oysters. If present rocks can be turned, both the top and the underside of the rock is inspected and sampled.
On sites with a vertical dock 4 samples are taken with a scrape sampler from the bottom (up to 1.5 m deep) up to the water surface.
Samples are fixated in F-solv.

From 2015 onwards present Japanese oysters *Magallana gigas* were measured in the first 5 (of 8) quadrants. These lengths were later converted to Dry Weight (without shell) using an allometric relation. Since 2015 salinity is also measured per site. Also, in surroundings of all harbours additional sites were added to the sampling program (marked red in table 1) as well as a set of sites outside of (the surroundings of) harbours in the Western Scheldt.

In the laboratory

Sediment is washed from the samples over a 0.5 mm sieve. Inorganic material, like empty shells, is removed. Organic material is fixated in alcohol 70%.
Seaweed samples require a different protocol. The weeds are flushed over a 0.5 mm sieve. The material on the sieve is saved in alcohol 70%; the weeds are fixed in F-Solv.
Small samples (200 ml) are sorted completely, and all organisms are identified. Samples with a larger volume of substrate (like seaweeds) are only saved for later analysis.

Locations

Table 1. Sampling sites. Red sites sampled since jan/feb 2015

Harbour	site	coördinates	Salinity (ppt)	Deviant sampling method
Rotterdam	Slag de Beer	51°58'43.38"N 4° 3'54.10"O	20-26	
Rotterdam	Nieuwe Waterweg	51°57'26.92"N 4° 9'7.17"O	11-23	
Rotterdam	Calandkanaal	51°57'24.33"N 4° 8'59.85"O	22-28	
Rotterdam	Hartelkanaal	51°54'11.56"N 4°12'27.31"O	4-11	
Rotterdam	Londenhaven	51°53'39.65"N 4°13'46.07"O	22-30	
Rotterdam	Rozenburg	51°54'43.40"N 4°14'36.08"O	6-14	Scrape-sampler
Antwerpen	Doel	51°18'41.02"N 4°16'5.66"O	3-15	
Antwerpen	Doeldok	51°15'44.21"N 4°16'10.99"O	6-13	Scrape-sampler
Antwerpen	Fort Liefkenshoek	51°14'59.70"N	0.5-14	

		4°18'59.03"O		
Antwerpen	Galgenweel	51°12'32.98"N 4°22'13.73"O	3-5	Scrape-sampler
Vlissingen	Havendam oost	51°26'55.72"N 3°40'38.27"O	25-30	
Vlissingen	Van Cittershaven	51°26'54.26"N 3°42'58.74"O	25-30	
Terneuzen	Westbuitenhaven	51°20'37.43"N 3°48'32.61"O	22-27	
Terneuzen	Braakmanhaven	51°20'55.97"N 3°46'10.39"O	23-29	
Terneuzen	Kanaal Gent-Terneuzen	51°19'34.66"N 3°49'2.13"O	2-9	Scrape-sampler
Zeebrugge	Westelijke havendam	51°20'23.09"N 3°10'30.06"O	31-34	
Zeebrugge	Verbindingsdok	51°19'31.07"N 3°13'35.93"O	28-31	Scrape-sampler
Zeebrugge	Boudewijnkanaal	51°17'2.91"N 3°12'31.61"O	21-29	Scrape-sampler
Zeebrugge	Zwankendamme	51°18'43.18"N 3°12'6.07"O	26-31	Scrape-sampler
Oostende	Oostelijke havendam	51°14'13.00"N 2°55'26.29"O	20-28	
Oostende	Visserijdok	51°14'15.26"N 2°56'4.47"O	29-34	
Oostende	Spuikom	51°13'41.88"N 2°56'43.04"O	29-34	Scrape-sampler
Oostende	Vlotdok/Doksluis	51°13'11.40"N 2°56'52.56"O	1-3	
Hoedekenskerke		51°25'9.04"N 3°54'59.75"O	20-25	
Bath		51°23'59.56"N 4°12'23.04"O	9-20	
Walsoorden		51°22'53.36"N 4° 2'10.18"O	13-23	
Breskens		51°24'7.23"N 3°33'7.89"O	30-33	
Oranjemolen		51°26'21.48"N 3°34'55.45"O	28-33	
Zoutelande		51°29'58.44"N 3°28'46.11"O	30-34	

Table 2. Sampling dates and number of sites.

Sampling date	Number of sites
aug/sept 2014	17
jan/feb 2015	29
sept/okt 2015	29
mar/apr 2016	29
july 2016	29

Description sites

Rotterdam Slag de Beer: This site is located near the rivermouth of the Nieuwe Waterweg. In the tidal zone rock-filling is present from the high waterline to the low waterline.

Rotterdam Nieuwe Waterweg: This site is located on the side of a dam with a lighthouse. Because of river discharge and tide salinity is relatively low and oscillating, the water flow is strong also because of ship-movements. In the tidal zone rock-filling is present from the high waterline to the low waterline. In the high-littoral zone asphalt is present.

Rotterdam Calandkanaal: This site is located near 'Rotterdam Nieuwe Waterweg' on the other side of the dam, in the Calandkanaal. Salinity is higher here, because of the lack of river discharge. Water movement is also not strong. The tidal zone is mostly covered with rocks; the high-littoral with a stone coverage of the dyke, the mid-littoral with spread rocks and the low-littoral up to below the low waterline with rock filling.

Rotterdam Hartelkanaal: At this site rock filling is very coarse and is present from the high waterline to the low waterline. Water movements are strong, because of ship-movements.

Rotterdam Londenhaven: The tidal zone is mostly covered with rocks; the high-littoral with a stone coverage of the dyke, the mid-littoral with spread rocks and the low-littoral up to below the low waterline with rock filling. There is almost no water movement.

Rotterdam Rozenburg: On this site there is hardly any rock filling, but sheet-piling which is sampled with a scrape sampler. There is considerable water movement form river discharge and ships.

Antwerpen Doel: This site is located at the seaside at Doel. The high-littoral is covered with stones on the dyke. From the mid-littoral to below the low waterline rock filling is present as well as sheet-piling. Presence of salt marsh vegetation. There is considerable water movement form river discharge and ships.

Antwerpen Doeldok: The Doeldok is separated by a sluice from the Zeeschelde. There is no rock filling present, just sheet piling. There is almost no water movement.

Antwerpen Fort Liefkenshoek: At this site upstream of Doel rock filling is very coarse and is present from the high waterline to the low waterline. Water movements are strong, because of ship-movements and river discharge. Between rock filling in the low-littoral zone there is high presence of silt.

Antwerpen Galgenweel: This site is a brackish pond next to the Zeeschelde. Just marginal presence of rock filling. Sandy subtrate. At the spillway sheet piling is present. Alongside the bank reed is present and below the waterline sometimes *Ruppia*. There is almost no water movement.

Vlissingen Havendam oost: This site is located at the mouth of the harbour of Vlissingen-Oost. The tidal zone is mostly covered with rocks; the high-littoral with a stone coverage of the dyke, the mid-littoral with spread rocks and the low-littoral up to below the low waterline with rock filling. There is almost no water movement.

Vlissingen van Cittershaven: This site is located in the centre of the harbour of Vlissingen–Oost. The tidal zone is mostly covered with rocks; the high-littoral with a stone coverage of the dyke with asphalt, the mid-littoral with spread rocks and the low-littoral up to below the low waterline with rock filling. There is almost no water movement.

Terneuzen Westbuitenhaven: This site is located just outside the mouth of the canal between Gent to Terneuzen. In the high-littoral a stone coverage of the dyke is present, in the mid-littoral down to below the low waterline he embankment is steep with an incomplete coverage of rock filling. There is considerable water movement because of tide and ships.

Terneuzen Braakmanhaven: This site is located just inside the Braakmanhaven. In the high-littoral a stone coverage of the dyke is present, in the mid-littoral down to below the low waterline the embankment is steep with an incomplete coverage of rock filling. There is some water movement because of ships.

Terneuzen Kanaal: This site is located just inside de sluices on the canal from Gent to Terneuzen. Only sheet piling present.

Zeebrugge Westelijke havendam: This site is fully exposed to the North sea on the outside of the harbour dam of Zeebrugge. The complete tidal zone is covered with concrete blocks.

Zeebrugge Verbindingsdok: This site is in the innerharbour of Zeebrugge. There is hardly any rock filling present, just sheet piling. There is almost no water movement.

Zeebrugge Boudewijnkanaal: This site is located upstream of the harbour of Zeebrugge. There is no rock filling present, just sheet piling, a low concrete wall and a rock bottom.

Zeebrugge Zwankendamme: This site is located upstream of the harbour of Zeebrugge. There is no rock filling present, just sheet piling, a low concrete wall and a rock bottom.

Oostende Oostelijke havendam: This site is located at the inside of the mouth of the harbour of Ostend. Rock filling is very coarse and present from the high waterline to the low waterline. Ships cause water movements regularly.

Oostende Visserijdok: This site is separated from the mouth of the harbour of Ostend by sluices. There is some rock filling present around the low waterline. No water movements.

Oostende Spuikom: This site is located in a shallow pond, separated by sluices from the harbour of Ostend. Around the waterline there are some loose rocks, whereas the bank is enforced by a steel network filled with rocks. The bottom of the pond is sandy.

Oostende Doksluis: This site is located upstream from the harbour of Ostend. A ponton (vlotdok) a sampled with a scrape sampler up to sept/okt 2015, while the rocks on the embankment are sampled from mar/apr 2016. Op de locatie Vlotdok zijn tot en met najaar 2015 schraapmonsters van een ponton genomen.

Hoedekenskerke: The tidal zone is mostly covered with rocks; the high-littoral with a stone coverage of the dyke, the mid-littoral with spread rocks and the low-littoral up to below the low waterline with rock filling. There is considerable water movement because of tide and ships.

Bath: The tidal zone is mostly covered with rocks; the high-littoral with a stone coverage of the dyke, the mid-littoral and the low-littoral up to below the low waterline with filling. The filling consists of steel and/or phosphor slag. Between filling there is considerable presence of silt. There is considerable water movement because of tide and ships.

Walsoorden: The tidal zone is mostly covered with rocks; the high-littoral with a stone coverage of the dyke, the mid-littoral and the low-littoral up to below the low waterline with rock filling. Between rock filling there is considerable presence of silt. There is considerable water movement because of tide and ships.

Breskens: This site is located on the inside of the eastern Veerhavendam. The tidal zone is mostly covered with rocks; the high-littoral with a stone coverage of the dyke, the mid-littoral and the low-littoral up to below the low waterline with rock filling. Between rock filling there is considerable presence of silt. There is almost no water movement.

Oranjemolen (Vlissingen): This site is located westernly outside the harbour of Vlissingen. Samples are taken along a pier. The tidal zone is mostly covered with rocks; the high-littoral with a stone coverage of the dyke, the mid-littoral and the low-littoral up to below the low waterline with coarse rock filling. A sandy bottom is present around the pier. There is considerable water movement because of tide and ships.

Zoutelande: This site is located on the border between the North Sea and the Western Scheldt. Samples are taken from coarse rock filling at the end of a branch line more or less covered/surrounded by sand. There is considerable water movement because of tide and ships.

Appendix 1c. Transect monitoring protocol.

Protocol Transect monitoring v2016

Below a basal description of the Transect monitoring methodology (further details and a protocol can be found in Wijnhoven et al. (2015)):
Transects always consist of 2 lines (perpendicular on the shore and/or waterline) preferably through two different types of habitat (preferably the dominant hard substrate habitats) situated in each other's vicinity (indicative 50 to 100 meters from each other). Habitats are often determined by different types of hard substrate and/or whether or not (different) macro-algae grow there (see Figure 1 as an example). Additionally habitats differentiate with the height in the intertidal zone (e.g. exposure time). For each transect 3 intertidal strata are distinguished; further called the high -, middle – and low intertidal zone. The 3 different strata are distinguished by visual observation, dividing the hard substrate gradient in 3 zones by the 2 most distinguishing imaginary horizontal lines (and bounded at the lowest low water level and the highest high water level) (see Figure 1 as an example).

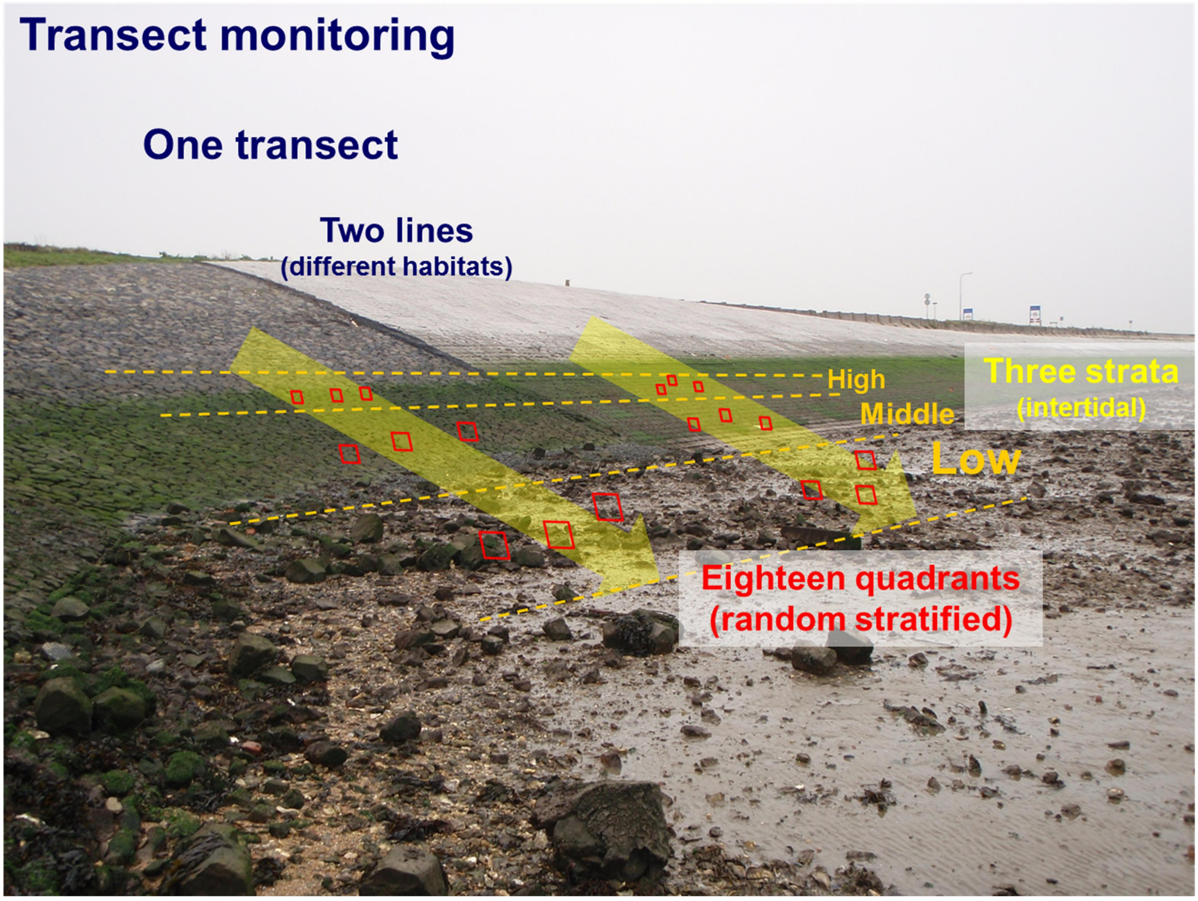

Figure 1. An example of a transect showing the ideal positioning of lines and strata and the random positioning of 18 quadrants.

The actual standardized NIS inventory in done in 0,5 by 0,5 meter quadrants (Figure 2 is an example of such a quadrant random positioned in the field). Quadrants are random placed equally distributed over the 2 lines and 3 strata which lead to 3 inventoried quadrants for each line x stratum combination to achieve a randomly stratified methodology. Although it is called random positioning, quadrants are placed as such that they give a representative view of the hard substrate habitat; i.e. if a habitat consist of reasonable areas with algae and areas without, it is

made sure that both are included in the set of 3 random samples in that habitat (therefore 'random' between quotation marks).
At first a photo of the quadrant like shown in Figure 2 is taken. Then the inventory of the quadrant consists of an estimation of the total and the separate coverage (in %) of the total 3D surface by flora and fauna. Additionally dominant species (i.e. those covering more than 20 % of the total surface are noted as a habitat descriptor. This can include several species of macro-algae and sessile fauna with a total percentage coverage of even more than 100 % as they can cover each other.
In each quadrant all species (macrofauna and macro-algae, clearly visible to the naked eye) are noted with an indication of their abundance or coverage for which we only use 3 categories to speed up the inventory proces:
Abundant: More than 10 % cover or more than 10 specimens present (indicated with A).
Common: More than 2 % cover or more than 2 specimens present (indicated with C).
Rare: Less than 2 % cover and only 1 or 2 specimens present (indicated with R).
Additional to the inventory of 3 quadrants per stratum, the entire stratum is investigated for approximately 10 minutes on supplementary species (or slightly longer if habitats not covered by the quadrants are present).
From each quandrant several specimens that cannot be identified with the naked eye are collected for taxonomic identification afterwards (it is important that several specimens are collected when different species can be present at the same site; so that it can be identified whether one or more species in which relative abundances are present. These specimens are stored in 96% ethanol. Larger and sessile organisms are also photographed in the field if possible if they might be damaged with collection. Actiniaria are first stored in mentol before storage in ethanol.

Wijnhoven, S., Engelberts, A., Dekker, A. et al. (2015) Non-indigenous species inventory of estuarine intertidal areas; a comparison of estuaries and habitats using a hard substrate transect methodology. Pilot study within the frame of the INTERREG IV A 2 Seas project SEFINS commissioned by the NVWA. Monitor Taskforce Publication Series 2015-07, NIOZ-Yerseke.