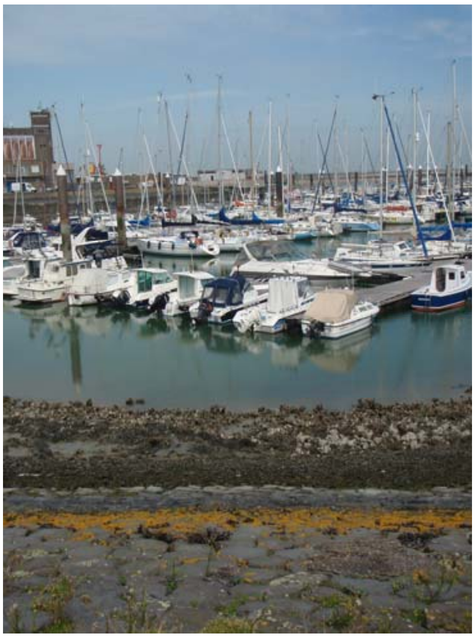
Non-indigenous species inventory of estuarine intertidal areas; a comparison of estuaries and habitats using a hard substrate transect methodology.

Pilot study within the frame of the INTERREG IV A 2 Seas project SEFINS commissioned by the NVWA.

Sander Wijnhoven[1], Anke Engelberts[1], Angela Dekker[1], *et al.*



[1]Monitor Taskforce, Royal Netherlands Institute for Sea Research (NIOZ)

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Final report September, 2015

Photos cover from left to lower right: a) View on the marina of Breskens with on the foreground an intertidal habitat gradient from high to low intertidal area; b) Hemigrapsus sanguineus, a non-indigenous species, as here collected at the site of Terneuzen; c) A quadrant in Ascophyllum nodosum dominated habitat, here at the site of Terneuzen, ready to be inventoried.

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Study commissioned by the Netherlands Food and Consumer Product Safety Authority (NVWA) of the Ministry of Economic Affairs (EZ) supervised by Sander Smolders (Office of Risk Assessment and Research). Study performed within the frame of the INTERREG IV A 2 Seas project 'Safeguarding the Environment from Invasive Non-native Species' (SEFINS), a project within the cross-border cooperation programme 2007-2013 part-financed by the European Regional Development Fund (ERDF). Study in cooperation with Morgane Barbier, Florian Rousseau & Céline Fontaine (Centre Permanent d'Initiatives pour l'Environnement (CPIE) Val d'Authie), Sharron Bosley (Wash and North Norfolk Coast European Marine Site (WNNC EMS)), Katy Owen (Norfolk County Council (NCC)), Frances Burrows (Eastern Inshore Fisheries and Conservation Authority (Eastern IFCA), Bram D'hondt, Jan Soors, Wim Jambon, Emilie Hermans, Thomas Terrie, Pieter Dhaluin, Jeroen Speybroeck, Erika Van den Bergh (Research Institute for Nature and Forest (INBO)), Arjan Gittenberger (GiMaRIS Marine Research Inventory & Strategy).

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Unless indicated differently, all used photos are taken by people involved in the project.

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Abstract

The current report describes the methodology and application of transect monitoring as a tool to inventory non-indigenous species (NIS) on hard substrate in estuarine intertidal areas. Within the frame of the SEFINS (Safeguarding the Environment from Invasive Non-native Species) project a pilot study was executed in which the efficiency of the methodology to detect and monitor the local presence of NIS was tested. Besides, the potentials of the methodology to monitor population developments and range extension of NIS and the opportunities to link NIS occurrence and abundances to environmental parameters, communities and system characteristics, were tested. Where the study focused on potential differences along the estuarine gradient and in- and outside marina comparison to allow comparison with SETL plate monitoring, also the first step towards an estuary comparison was made, inventorying 5 sites (i.e 10 transects) in the Scheldt estuary (3 sites in the Western Scheldt (the Netherlands), 2 sites in the 'Zeeschelde' (Belgium)), and 1 site (i.e 2 transects) in both the Canche estuary (France) and the Wash (England).

It was found that the methodology was effective to observe the local present NIS, where standardized random quadrant inventories allows comparison of different sites and regions whereas the search for additional species in especially a-typical habitats completes the local species list and makes sure all local NIS are detected. Although the current project was only an inventory of a limited number of sites, it was shown that in a limited timespan an extensive and valuable dataset was obtained, that included several new findings and insights on NIS distributions and NIS related to their surroundings.

A total of 10 NIS were observed, which were all present in the Scheldt estuary. Where especially the New Zealand barnacle *Austrominius modestus*, Japanese oyster *Crassostrea gigas* and Brush-clawed shore crab *Hemigrapsus takanoi* appeared to be common and have a wide distribution throughout a variety of habitats, especially the extension of the gammarid *Melita nitida* from the mesohaline to the entire polyhaline zone was so far unknown. Where the population expansions of *M. nitida* seems to come at the cost of the native *M. palmata*, also strong indications for effects of *C. gigas* and *H. takanoi* presence on respectively the blue mussel *Mytilus edulis* and the common shore crab *Carcinus maenas* distributions (as also indicated in literature: e.g. Troost, 2010; Van den Brink et al., 2012) have been found. Results from the different estuaries indicate a movement of *C. maenas* to low salinity regions in the presence of *H. takanoi*. Also for the orange-striped green anemone *Diadumene lineata* a significant range extension towards the east was observed in the Western Scheldt. Such a range extension was not expected in advance as turbidity of the watercolumn was recorded to be too high in the mesohaline zone (Faasse, 1997). *A. modestus* was actually the only NIS observed in both the Canche and the Wash, and in each of the three estuaries the species is by far the dominant barnacle. Except for the Japanese shore crab *Hemigrapsus sanguineus*, all other NIS (the quagga mussel *Dreissena bugensis*, the zebra mussel *Dreissena polymorpha*, the Chinese mitten crab *Eriocheir sinensis* and the non-indigenous amphipod *Incisocalliope aestuarius*) were only observed in the Belgian part of the Scheldt.

Striking patterns emanating from this study are the lower numbers of NIS present inside than outside marinas in accordance with higher species richness outside marinas, which indicates that marinas might be hotspots of NIS introductions but that more NIS settle in good quality environments with potential high species richness. Related to this an increase in the percentage of NIS on the total of species is observed that in the Scheldt estuary seems to go together with increasing salinity. It is expected that the observed relatively low percentages of NIS in the total of species locally present in the Canche and the Wash is the result of a relatively low connectivity (in terms of boat movements to other regions with comparable environmental conditions). It was also observed that Japanese oyster reefs at least in the more sheltered places where they are most successful do not seem to accelerate local biodiversity, as sometimes indicated, as the environmental degradation seems to be more important than the creation of additional niches. The results indicate that there are potentials to support local biodiversity and/or reduce the risk of the settlement of specific NIS by the use of different types of substrate. Also sedimentology seems to be an important indicator, even in hard substrate habitats, for the species assemblages that can be expected.

It is recommended to combine transect monitoring as a tool to inventory NIS communities, with hard substrate habitat mapping so that a scheme can be designed to monitor NIS presence and population developments that covers entire estuaries (e.g. the Scheldt estuary) with recurrent (once every few years) visits but limited yearly efforts. Such a monitoring program would be very valuable to support

and evaluate the management of (estuarine) systems and could potentially function as an early-warning system as well.

1 Introduction

The current report is presenting the results of a pilot project as commissioned by the Netherlands Food and Consumer Product Safety Authority (NVWA) of the Ministry of Economic Affairs (EZ) and performed within the frame of the INTERREG IV A 2 Seas project ‘Safeguarding the Environment from Invasive Non-native Species’ (SEFINS). As a SEFINS project the primary goal was to exchange knowledge and expertise about monitoring and inventorying non-indigenous species between SEFINS partners, so that the joint fieldwork on focal systems (i.e. estuaries) in the partner countries was one of the objectives. However besides that, the various SEFINS partners, and the NVWA regarding the Dutch focal site (the Western Scheldt) in particular, were very interested to know something about the possible presence and distribution of non-indigenous macrofaunal and macro-algal species in their estuaries. The SEFINS consortium identified an estuarine gap in both the knowledge about estuary related non-indigenous species and the monitoring efforts to identify presence, developments, distributions and impacts of non-indigenous species in estuaries (Wijnhoven, 2014). This is also the case in the countries/regions part of the Two Seas Area, which includes counties along the South and South-East coast of England, departments at the North coast of France, the Flemish coast and regions along the Dutch South-West coast (Owen, 2014). The SEFINS cluster partnership designated three focal estuaries situated in the 4 involved countries where activities of the cluster during the period 2014-2015 would focus on. These estuaries are the Scheldt estuary (Western Scheldt in the Netherlands and the Sea-Scheldt in Flanders), the Canche (France) and the Wash (England). These estuaries have in common that there is no monitoring program focusing specifically on non-indigenous macrofauna and macro-algae, although some scattered information about non-indigenous species in these systems is available as can be extracted from other monitoring and/or research activities. For the Scheldt estuary there are long-term monitoring programs on both sides of the national border, e.g. focusing on soft sediment macrofauna communities and vascular plant communities in general (Ysebaert et al., 2013). Dewarumez et al. (2011) gives an overview of all marine macrofauna and macroalgae species that might be introduced in the Artois-Picardie basin, which includes the Canche. However the report, describing the ecology, global distribution, dispersion routes and potential impacts of potentially introduced species, does not present data on numbers and/or distributions in the region. Concerning the Wash, data on macrofauna and macro-algae could be extracted from ‘The Archive for Marine Species and Habitats Data’ (DASSH, 2015). As many as 532 entries from between 1986 and 2009 could be found, which however surprisingly did not contain non-indigenous species although they were not specifically excluded from the database or whatsoever. A further overview of introduced species registers in the Two Seas Region is provided by D’hondt et al. (in prep.) within the framework of SEFINS.

The specific interest for the NVWA is to gain insight with this pilot study in the estuarine (and marine) macrofauna and macro-algae communities, with the focus on non-indigenous species, in the Western Scheldt and the other focal estuaries. The current pilot is a step towards a general methodology for inventories of hard substrate related non-indigenous species of the littoral and sublittoral zone, which should potentially be widely applicable, and therefore easy to be carried out; e.g. with limited costs and efforts. Before the start of the project the Monitor Taskforce recommended to focus on hard substrate environments as these are the least investigated (and there is an extensive soft substrate monitoring program in the Western Scheldt). Hard substrates might however be especially the habitats that harbor a large proportion of the present non-indigenous species as the total area and diversity of hard substrates is of increasing importance in Western European estuaries, especially due to the expansion of artificial substrates like dams, dikes, foreshores, piles, constructions, machinery, boats, nets, and natural hard substrate habitats are rare (the chalk formations at the Wash might be an exception). These artificial habitats might contain larger quantities of non-indigenous species as they are often open niches (Stachowicz et al., 1999; Stachowitcz & Byrnes, 2006), have large similarities to habitats in other geographical regions, and are often close to sources of introductions (e.g. ports, marinas, aquaculture facilities) (Glasby et al., 2007; Airoldi et al., 2015). Additionally certain invasive, often non-indigenous, species, like the Japanese oyster (*Crassostrea gigas*), create vast areas of new hard substrate by forming reefs or overgrowing natural habitats. Besides that potential invasive non-indigenous species are often fast colonizers of new (bare) substrates, there is a theory that species that naturally co-occur might be adapted to each other to some extent that can give such related species an advantage during settlement and/or in competition, which might be the reason that among habitat-forming non-indigenous species often several other non-indigenous species, originating from the same geographical region, can be found.

In preparation for the start of the project it was agreed to combine two promising techniques already internationally applied, so that the current project can build on earlier experiences and results can potentially be compared with inventories in other regions. The NIOZ will develop and apply the so-called 'Transect monitoring' based on the internationally applied EMBOS (pan-European Marine Biodiversity Observatory System) protocol for hard substrate monitoring (Hummel & Van Avesaath, 2014), however pin-pointed on the cost - and effort efficient inventory of non-indigenous species. GiMaRIS will apply the SETL methodology making use of settlement plates to detect settling organisms (Gittenberger, 2007). As much as possible both methodologies will be applied at the same sites so that the results of the complementary techniques can be combined. Where possible (i.e. dependent of substrate availability) the focus will be on marinas, as this is where SETL plates are already in place sometimes for several years, and these are expected to be hot spots of non-indigenous species introductions (i.e. via ship hull fouling) (Murray et al., 2011). As at least locally the aim is to detect as much non-indigenous species as possible, the proposed transect monitoring will cover the different strata in the intertidal zone and inventories will cover the locally dominant hard substrate types. Besides the standardized inventory also the possible presence of additional species in the vicinity will be inventoried so that the effectiveness of the methodology can be investigated, and the local non-indigenous species list can be made as complete as possible. Besides that marinas will be investigated, also sites in the vicinity but outside marinas will be inventoried using the transect methodology, so that possible differences in (non-indigenous) species communities can be investigated. As communities will change with the estuarine gradients (e.g. salinity, hydrodynamics, turbidity and etcetera), at least for the Scheldt estuary it is tried to cover the estuarine gradient (with observatory sites) as much as possible during this pilot. It would be nice if over time the procedure can be repeated in the estuaries of the Canche and the Wash as well. Currently the inventories in the Canche and Wash focus on one site; with in the Canche an inside – versus outside marina comparison (similar to most of the observatory sites along the Scheldt estuary), and in the Wash an artificial - versus natural hard substrate comparison. At the most upstream site in the Scheldt (the tidal fresh water to oligohaline site of Wintam, a comparison of two branches of the estuary (the lower Scheldt and the Rupel) is made.

The results obtained with Transect monitoring will be presented and discussed in this report, the results of the SETL methodology applied within the frame of SEFINS are presented and discussed in Gittenberger & Rensing (2015). A general discussion combining and comparing the two methodologies, including recommendations is given in Wijnhoven & Gittenberger (2015).

2 Material and methods

2.1 Transect monitoring

2.1.1 Selection of methodology

The aim of the pilot study is to detect the non-indigenous species present at selected sites and gather information about their distributions. It is therefore chosen to focus inventories on niches where a large percentage of the potential available non-indigenous species can be found; i.e. the hard substrate environments. Besides these hard substrate environments, certain non-indigenous species (NIS) will be more abundant in soft sediment environments. It is however generally more time consuming (e.g. due to lower species densities and vastness of potential habitats) to get a representative indication of the species living in soft sediment environments, and relatively more information about NIS of the soft sediment environments of the estuaries under investigation is already available. Additionally, although 'transect monitoring' as described here does not involve digging for specimens in often also available soft sediment at predominantly hard substrate sites, specimens present in the top layer of these soft sediments will be encountered and will be part of the inventories as well. Also the group of pelagic non-indigenous species will largely be missed with the foreseen methodology. However, a substantial part of the potential available NIS in the pelagic realm consists of pelagic stages of species that can be found on hard substrates. As indicated the current pilot study on estuarine NIS within the frame of SEFINS consists of the application of two complementary techniques of which the results of the Transect monitoring are presented and discussed here. The other technique, the application SETL plates as presented in Gittenberger & Rensing (2015), is especially a manner to sample settling NIS present in the pelagic. Similarities and differences between the two techniques and in results and patterns observed with the two techniques (i.e. the extent to which the techniques are complementary and if especially combined application is valuable tool) will be discussed in Wijnhoven & Gittenberger (2015).

One of the predetermined conditions before the start of the pilot study was that the methodology, besides that it should be effective to inventory hard substrate related NIS, it should be widely applicable in terms of that it is standardized, relative easy to perform, and cost and effort efficient. It is expected that especially (deviations from) methodologies that are already in use in a broad (i.e. international) context are promising, as they have proven themselves on several aspects already and findings are potentially comparable and/or exchangeable. It is however not necessary that a methodology is solely used for the detection of NIS, as if methodologies have broader fields of application they likely yield valuable additional information (e.g. information about habitats, communities, susceptibility, impacts, etcetera) are more frequent applied (i.e. also with other aims, but results can be used to extract information about NIS), combined application will be more efficient, and will result in a larger NIS database with a higher temporal and spatial data density. Taking this into consideration, the here called 'Transect monitoring' methodology was selected, based on the in a broad European context applied EMBOS protocol for hard substrate monitoring (Hummel & Van Avesaath, 2014). We did however deviate from the EMBOS protocol on a few aspects to improve the efficiency towards NIS detection and to reduce the efforts needed (deviations are indicated and explained in the *intermezzo* following the methodology description).

2.1.2 Specifics methodology

On beforehand a research plan has to be made, of what locations to inventory and compare. Once locations are roughly selected, eventually from behind a desk, the exact positioning of transects has to be done in the field (or after a field visit). Ideally locations for transects are chosen in such a way that the entire intertidal gradient (from the high to the low intertidal zone) is relatively easy accessible and consists of hard substratum. This means that the gradient should not be too steep (and preferably not be a vertical wall) so that you can walk the entire transect (if necessary using a rope tied on top of the transect as the hard substratum can be slippery). There might be locations where the hard substratum transitions into a soft substrate environment particularly in the lower intertidal zone. Such location is perfectly suitable if at least a certain part of the lower intertidal zone consists of hard substrate (the soft substrate parts will not be part of the monitoring). In regions where hard substrates largely lack, it is an option to monitor only a part of the intertidal gradient (the part where hard substrate is present). The methodology is build up in such a way that a spatial breakdown from large to small scale is possible, roughly going from entire systems or countries to the scale of 0,5 x 0,5 meter quadrants.

During this pilot study several monitoring sites were selected, where always two transects were inventoried that basically differed in one major (overarching) aspect, with consequences for environmental conditions as well, that could be compared on the presence of NIS.

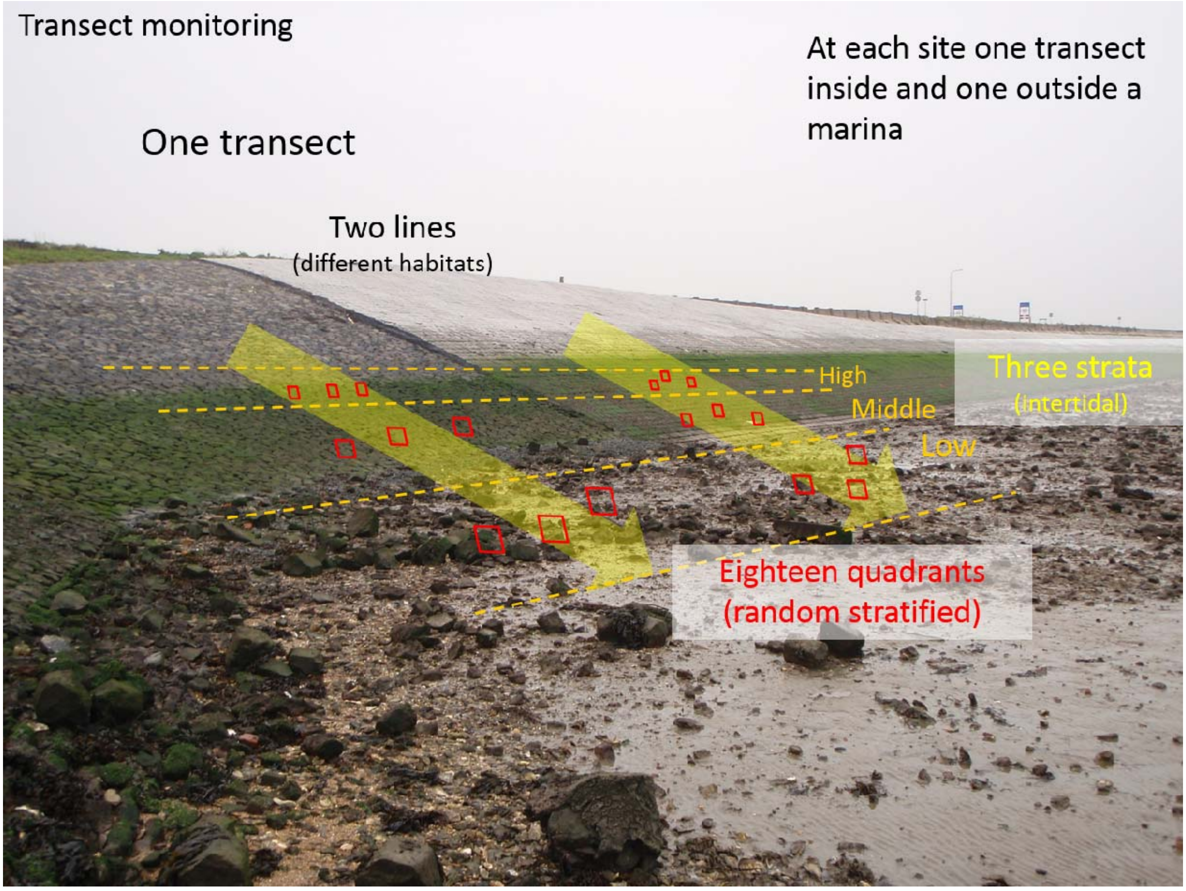


Figure 1. An example of a transect showing the ideal positioning of lines and strata and the random positioning of 18 quadrants. The initial strategy was to inventory one transect inside and one outside a marina at each site.

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 were distinguished; further called the high -, middle – and low intertidal zone. The 3 different strata were 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).
Each transect was described (few sentences in words) standard parameters were noted down and transects were captured with standard photographs making use of a digital field-form (Annex 2).
To work in a standardized way, enabling transfer to a database and relative easy breakdown of results into categories and being prepared for an extension of the 'NIS Transect monitoring database' a code consisting of standard elements is used to identify systems, transects, lines, strata and quadrants. In the digital field form those only had to be filled in once in the 'site characterization table'; codes are automatically transferred to the other tabs.
The code of a quadrant always consists of a country, estuary, observatory site, transect, day, month, year, line, stratum, replicate indication separated by indents (-); the same systematics is used for the identification of separate lines (but then without the stratum and replicate information), etcetera. These codes are also used to identify files with results or photos. An example is given in the protocol for Transect monitoring (Annex 1) and the empty digital field form (Annex 2) contains an example that can be overtyped when the form is used.
Besides location characterization by names/codes, latitude – longitude coordinates are taken in the field. Useful for data analyses and to identify relations of NIS assemblages to environmental

characteristics are the notation of approximate (as an indication is better than no value at all) year average tidal range (m) and average salinity and average low and high salinity (either measured in the field or extracted from recent literature). Also the approximate width of each distinguished stratum is estimated and noted.
To allow environment characterization and especially comparison afterwards, even when the data analyst or researcher did not inventory the transect himself (but even if he did, information to look back is very useful) every transect is characterized in a few sentences by the researcher(s) doing the inventory, and standard photos of each transect (from high to low, and from low to high) and each quadrant are provided.

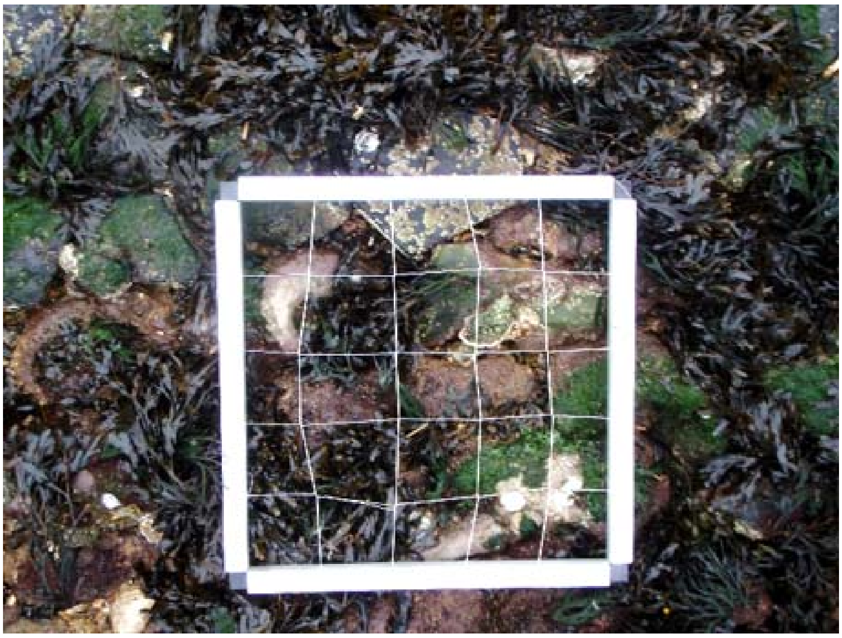


Figure 2. An example of a photo of a 'random' placed quadrant ready to be inventoried. The wiring is not essential, but can help to estimate the coverage by flora and fauna.

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 leads 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. Than 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) will be 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).

Species that cannot be identified in the field (this includes species for which the use of binoculars is necessary) are taken to the laboratory for identification afterwards, and preserved in formaldehyde if they are not identified the same day.
Additional to the inventory of 3 quadrants per stratum, the entire stratum is investigated by two researchers for 5 minutes on supplementary species. Also for these species the relative average abundance within the habitat in indicated with an A, C or R with the average coverage or abundance in the vicinity reflected on a surface of 0,25 m^2. These species are not identified as being in one of the replicates (R1, R2 or R3) but indicated with ADD (for additional species). Additional species might

complete the local list of NIS and allow to calculate a measure of efficiency of the methodology. The complete protocol as distributed to all participants before monitoring is shown in Annex 1.

Intermezzo:
In comparison to the EMBOS methodology, there were a few deviations which are the result of different aims and priorities. For EMBOS the major goal is to compare entire communities on a European scale in the best standardized way as possible. The current study aims to provide an as complete as possible representation of the non-indigenous species assemblage locally present that to a certain extent will be representative for larger areas. Therefore 'Transect monitoring' distinguishes 3 strata to better cover the entire high to low intertidal gradient than the 2 levels distinguished by the EMBOS protocol. 'Transect monitoring' also covers the dominant habitats as much as possible (2 lines through different habitats in one transect) whereas EMBOS emphasis on the number of replicates and only inventories the most dominant habitat type. The number of replicates (quadrants) per level/stratum is reduced in the 'Transect monitoring' protocol compared to the EMBOS protocol (to reduce the efforts) however due to the distinguishing of 3 strata the total number of quadrants per transects approximates the total number in the EMBOS methodology. Deviating to reduce the efforts (and therefore to increase the number of transects that can be inventoried in a certain timeframe) numbers and/or coverage per species is not determined in detail for every species; the 'Transect monitoring' protocol only distinguishes 3 abundance classes for those species present. Where EMBOS is not interested in locally deviating niches as those are not representative for the general pattern, with 'Transect monitoring' those locally deviating niches are of importance to check for additional species as the likelihood of finding additional NIS is the largest exactly there. Data coming from an EMBOS inventory can still be used for NIS inventory purposes and vice versa, however it should be taken into account that datasets should be made comparable considering the lowest level of spatial specification and abundance determination (e.g. two strata in one habitat with 3 replicates only distinguishing abundance classes).
To deviate here from the EMBOS protocol with a specific aim is not unique. Van Avesaath et al. (2014) did for instance use an on the EMBOS methodology based protocol focused on the inventory of macro-algal communities specifically related to substrate type.

2.1.3 Implementation and deviations

The current pilot study started with a rough research plan which was discussed and agreed upon in meetings with the contractor (i.e. the NVWA) and GiMaRIS so that two methodologies (Transect monitoring coordinated by the NIOZ and SETL plate inventories coordinated by GiMaRIS) would be tuned and applied at the same sites as much as possible at least at several selected localities. Therefore it was decided to do transect monitoring at several sites along the Scheldt estuarine gradient to at least get an idea about the variability in NIS assemblages with the estuarine gradients. To allow comparison and test complementarity of the two methodologies it was decided to do Transect inventories in marinas as much as possible on selected sites as this is also where SETL plates are installed or were already present. To identify whether marina environments, as marinas might be the hotspots for NIS introductions, do harbor different NIS communities compared to non-marina areas, at each site a combination of an inside and an outside marina transect was planned. It was known that this was possible at the marinas of Breskens and Terneuzen, it had to be checked and identified if there were opportunities in the Wash and the Canche where at least monitoring at one site comparable to the Netherlands was planned. It was hoped that within the frame of SEFINS also in Belgium (Scheldt estuary) one or two sites could be inventoried, and it was agreed that to achieve a nice continuum an additional site between Terneuzen and the Dutch-Belgium border would be inventoried using transect monitoring. Plans and methodology were presented and discussed with the SEFINS project partners at a SEFINS Progress Meeting and Workshop on April the 15th, 2015, in Ostend (Owen, 2015). It was agreed upon that transects would be done by people from the NIOZ and CPIE Val d'Authie at one site in the Canche estuary so that mutual expertise could be exchanged. The same accounted for the Wash, where people from the NIOZ, WNNC EMS and NCC would exchange their expertise. Due to the restriction to the same favorable tide (sufficient low water during the day) at both sites of the border in the Scheldt estuary, we did not succeed to find a date on which involved people from the NIOZ could join the monitoring by the INBO in Belgium. This is a pity but not dramatic as there has been several cooperations between the NIOZ and the INBO in projects on the Scheldt estuary, where amongst others macrofauna data have been exchanged and methodologies have been tuned before (e.g. within the frame of the Scheldt-estuary evaluation and methodology development projects; Maris et al., 2013, 2014).

In practice five sites were inventoried in the Scheldt estuary. Three sites in the Western Scheldt with at each site transects inside and outside marinas: i.e. Breskens, Terneuzen and Hansweert, inventoried by NIOZ. At Hansweert it is not really a marina, but more a small working harbor situated in the mouth of the canal (Kanaal door Zuid-Beveland). The INBO inventoried two sites: i.e. near Doel and near Wintam. Near Wintam not a deviation in inside and outside a marina was selected, but there transects in both river branches (Zeeschelde and Rupel) were inventoried. Where at Étaples (the Canche estuary) suitable locations for a transect in (or better opposite) the marina, and outside the marina were found, actually no marinas and/or suitable substrate were present near Sutton bridge (the Wash) where SETL plates were installed. At the Wash estuary therefore a site (or better two sites as transects were several miles from each other) was selected with a transect consisting of natural hard substrate and a transect consisting of artificial hard substrate (details on positioning and characteristics are given below).

In general distinguishing continuous transects that were subdivided in three strata did not give much problems. Sometimes a deviation into 4 zones or just 2 would have been more obvious, but even in those cases three zones were distinguished (i.e. by combining two of the most obvious zones or subdividing one of the most obvious zones). In some cases (e.g. one of the transects outside the marina of Terneuzen, and some of the transects in the Wash and the Canche estuaries) the hard substrate transect was intermittent by soft sediment; in which cases hard substrate patches were selected to compile a hard substrate transect with three strata. Exceptions are two transects at Doel and Étaples. At the right line of the inside marina transect at Doel there was no hard substrate present in the low intertidal zone, so that only two strata are inventoried there. The intertidal zone outside the marina of Étaples was very narrow with a limited tidal difference of approximately 2 meters only flooding for a limited period around high water due to the presence of a riffle down-stream. This means that actually only a high intertidal zone was present; therefore only one stratum was inventoried.

Other deviations from the protocol are more related to the practical implementation without major consequences for the results. A few deviations are listed here:

- While testing and/or working with the protocol it was experienced that at least for the current set up of the monitoring (visiting sites and substrates not recently inventoried) it was not of much use to work with a pre-prepared species-list and filled-in field-forms in the field as many species encountered were not on our list whereas the lists contained many species not observed. It can however be of use to work with such lists when transects are re-visited (e.g. for annual monitoring of same sites).
- It was observed that in the laboratory a few surprises were encountered. As an example specimens observed in one quadrant, identified the same in the field as specimens from another quadrant, appeared to be different species when identified using binoculars. In several cases two different species were found in samples taken from the field that were identified as one in the field. This makes that it is of importance to collect all species (except for larger species that cannot be misidentified) for each quadrant and collect several specimens for each species to check identifications afterwards.
- The protocol mentions the search for additional species by two researchers for 10 minutes in the entire transect. While testing the methodology it was decided to search for additional species in each stratum separately (so that more data to test the efficiency of the methodology (i.e. the inventory of 3 quadrants per stratum) were obtained). Therefore the methodology was changed into searching for additional species for a few minutes in each stratum depending on the habitat variability and the presence of additional habitats (similarity of species assemblages of three quadrants appeared to be a good indicator for the possible presence of additional species: i.e. low similarity might indicate the presence of additional species).
- It was experienced that the type of soft sediment present on, in between or near the hard substrate inventoried, might be a reasonable explaining parameter for differences in species assemblages. Although hard substrate was inventoried, there was often some sediment present which is of course not only a substrate for certain species, but also relates to the local hydrodynamics, food availability, potentials for certain types of species (e.g. filter feeders) or light and oxygen conditions. Not mentioned in the methodology, soft sediment presence and type was not always noted in the field. However, making use of the photos, local sediment presence and characteristics could generally be filled in afterwards as well.

Although the methodology was typically designed for the inventory of hard substrate macrofauna and macro-algae, also typical soft sediment macrofauna, more pelagic species and meiofauna were encountered. During the inventories, macrofauna present in the sediment top-layer (often on hard substrate, or under hard elements like boulders and shells) is taken into account, but digging for animals was not done. Pelagic fauna still alive and present in the intertidal zone that could easily be caught was identified, but shrimps in larger pools were not considered. The INBO has a lot of experience with the identification of meiofauna and often identified meiofauna to the species level. The NIOZ did collect specimens of groups like Oligochaeta, Nematoda and Insecta (larvae), but did not further identify them to reduce identification time (and as it is believed that a representative inventory of meiofauna communities asks for another standardized methodology). For the further statistical analyses and site comparisons, individual species of meiofauna were not taken into account, and only the presence of the large overarching groups was taken into account. This is similar to the tuning of the identification level for all encountered groups, where species that could generally not be identified further than the genus level are not further identified for other sites.

2.2 Research sites

2.2.1 Estuaries

Three focal estuaries situated in the four partner countries being part of the Two Seas Region were designated for this pilot study. The Two Seas Region is the center of shipping activities in Western Europe with heavy traffic through the English Channel and the two largest ports of Europe (Rotterdam and Antwerp) situated there. The region is connected with all important harbors worldwide with frequent ship movements in both directions. Additionally there is a lot of recreational boating along the coasts (including Channel crossing) in this densely populated region with marinas in many of the coastal towns. Together with aquaculture activities in each of the countries and aggregations of such activities in French Brittany and the south-western Dutch delta (Eastern Scheldt), the region is a hot-spot of NIS introductions in Europe. However, within the Two Seas Region there are also large differences in the concentrations of all kind activities and related NIS introductions and occurrences.

Figure 3. Positioning of the three focal estuaries in the Two Seas Region.

The Scheldt estuary (NL, B):

The Scheldt estuary (consisting of the Western Scheldt in the Netherlands and the 'Zeeschelde' and tributaries in Flanders) is the gate to the world port of Antwerp, but is also the entrance for large harbors like Gent and Vlissingen is a very crowded shipping route. Until recently the system was dealing with poor water quality due to the deliverance of untreated waste water, which led to increased pollutant concentrations (e.g. heavy metals), high nutrient loads and poor oxygen conditions particularly upstream. This despite the fact that the system had an important nature function especially for waders and waterfowl. The last decennia significant improvements in water quality have been made, which had their effect on biodiversity (e.g. fish, birds, marine mammal assemblages) as well, and large parts of the system are now designated as Natura2000 area. However other risks for the system, like effects of deepening of the system for shipping with impacts on amongst others hydrodynamics and turbidity, and the increasing number and dominance of NIS in the system, are very timely. For an extensive overview of the developments of the system and the near current state, see Depreiter et al., (2014). Although soft sediment habitats like sand – and mud flats are characteristic for the system, the amount and diversity of artificial hard substrate in the system is increasing.

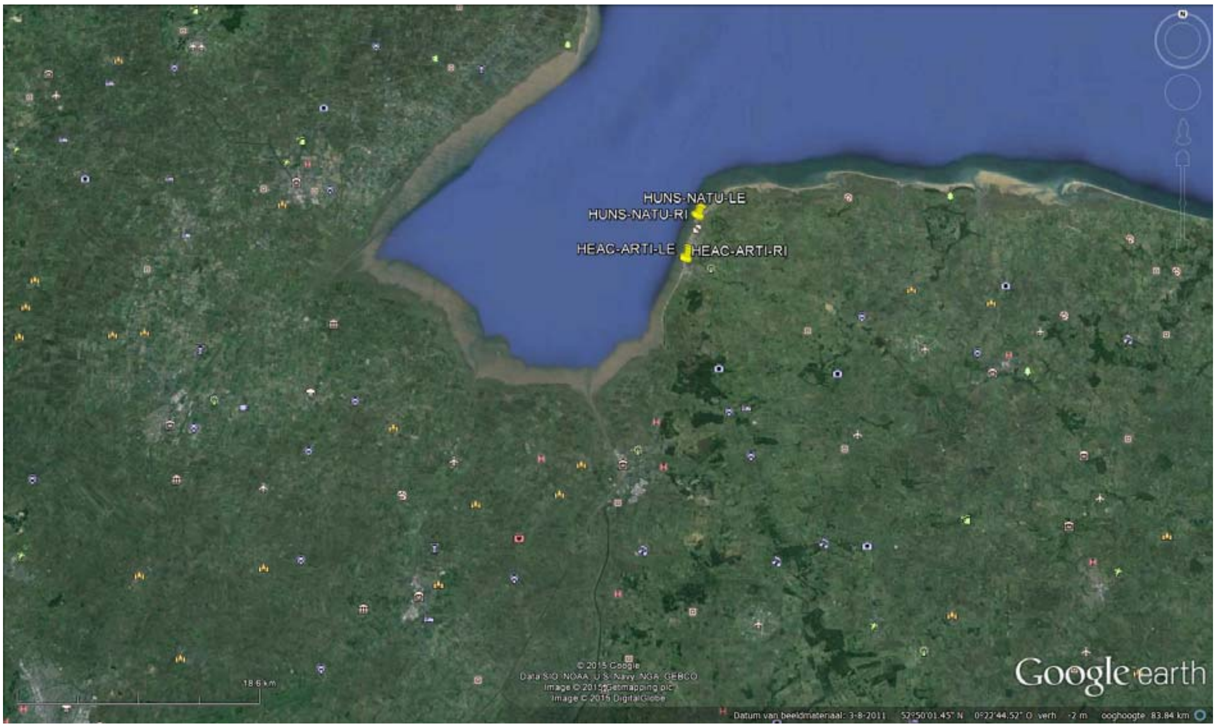


Figure 4. Positioning of the monitoring sites in the estuaries; a) Scheldt estuary with from west (estuary mouth) to south-east (oligohaline part) the research sites ‘Breskens’, ‘Terneuzen’, ‘Hansweert’, ‘Doel’ and ‘Wintam’; b) Le Canche estuary with the research site of ‘Étaples’; c) the Wash estuary with the research site of ‘Hunstanton-Heacham’.

The Canche estuary (F):
Compared to the Scheldt estuary, the Canche estuary in the north of France is a rather small system. Although, boating connections with other parts of the world are limited, two marinas are situated in the system (i.e. Le Touquet in the mouth of the system and Étaples land inwards). Although these marinas only have a few visiting places, the central positioning of the Canche among larger marinas in the north and the south and the other side of the English Channel might have resulted in the exchange of species with other regions, including NIS. An overview of the potential present NIS in the larger area of the Artois-Picardie basin is given by Dewarumez et al. (2011), however evidence for the local presence and current status of NIS in the Canche estuary is largely lacking. Typical for the estuary are sand – and mud flats and hunting ponds (to attract ducks) in the marshes (Owen, 2015).

The Wash (GB):
Although here indicated as an estuary, the fresh water inflow compared to the marine influence might be limited for the entire bay indicated as the Wash, giving it the characteristics of a marine tidal bay. The various river systems entering the Wash might however more have the characteristics of an estuary. In terms of boating connections with other parts of the world, the exchange for the Wash might be limited, but with larger ports and marinas and aquaculture in the south and the north-east of England and the promotion of recreational boating activities in the region, some visiting boats and connections with other regions can be expected. So far, only a limited number of NIS is recorded for the Wash (e.g. *Crassostrea gigas*, *Crepidula fornicata* and *Ensis directus*), but specific studies so far restrict to shellfish fishing grounds (Eastern IFCA, 2014; Owen, 2015).

2.2.2 Locations and characteristics

Breskens:
Near Breskens salinity on a yearly basis fluctuates between 21,7 and 32,6 depending on the tide, river run-off and wind direction, with an average salinity of 28,7. The average tidal range on a daily basis is approximately 4,8 meters (Rijkswaterstaat, 2009). Although these values are measured near the fairway in the Western Scheldt and there might be slight deviations in the marina of Breskens, it is expected that values will have the same order of magnitude.
Inside the marina there was a rather steep slope with a 1,5 meters high intertidal zone of bare basalt and basalt overgrown with mat-forming green-algae (*Blidingia marginata*), transitioning into a 4,5

meters middle intertidal *Fucus*-zone (predominantly *Fucus vesiculosus*) on basalt. In the lower intertidal zone (5 meters) there was rubble with tufts of *Ascophyllum nodosum* surrounded by anaerobic mud (right line). The left line consists of a slope of bare asphalt and asphalt overgrown with mat-forming green-algae (*Ulothrix flacca* and *B. marginata*) (approximately 2 meters in width), transitioning into 3,5 meter *Fucus*-zone (*F. vesiculosus* and *U. flacca*) on asphalt, continuing in a low intertidal zone (approximately 9 meters with *F. vesiculosus* and *A. nodosum* on rubble with oysters (*Crassostrea gigas*) surrounded by anaerobic mud near the low water line.

Figure 5. Positioning of the two transects consisting of a left (LE) and a right (RI) line at the site of 'Breskens' (BRES), with a transect inventoried inside (INMA) and a transect outside (OUMA) the marina.

Outside the marina, the hard substrate constructions were rather new, and there was an information sign indicating amongst others that reconstructions had taken place between 2009 and 2014. The left line was situated on a dam covered with large basalt blocks at the estuary side. The high intertidal zone (5,5 meters) was partially covered with filamentous green-algae (*B. marginata*), transitioning to a 4 meters middle intertidal zone where basalt blocks were overgrown with *Fucus spiralis*. The vast low intertidal zone (approximately 40 meters) consisted of rubble on soft sediment (muddy fine sand) substratum. The right line was situated on the more exposed extending dam at the entrance of the marina. The high intertidal zone (2 meters) consisted of green filamentous algae (*B. marginata*) on asphalt transitioning to a 4 meters middle intertidal zone with *Ulva cf lactuca* and some *Fucus vesiculosus* (also on asphalt). In the 35 meters wide lower intertidal zone, basalt blocks were overgrown with green filamentous algae (*B. marginata*). The blocks were placed on a substratum of steel slags.

Terneuzen:
A salinity of around 20,7 fluctuating between 15,5 and 23,8 is recorded the last years near Terneuzen (Rijkswaterstaat, 2009). Again, values inside the marina will approximately be the same, although not specifically measured. The tidal difference at Teurneuzen is about 5,1 meters.
The right line of the transect inside the marina (situated the farthest inside the marina) mainly consisted of asphalted rubble stones. A narrow high-intertidal zone (2 meters) was dominated by mat-forming green-algae (*Blidingia minima*), transitioning into a broader (5,4 meters) *Ascopyllum-Fucus*-zone (*A. nodosum* and *F. vesiculosus*). The low-intertidal zone (7 meters) was muddy and contained rubble stones and *Crassostrea gigas* (Japanese oyster) growth. The low intertidal zone continued with soft sediment (anaerobic mud).
The left line consisted of a with basalt-blocks paved slope. The high intertidal part (2,8 meters) of the slope was rather bare and contained only mat-forming green-algae (*U. flacca*) in the splashzone. The

middle intertidal zone (5,6 meters) was dominated by *A. nodosum* whereas the low intertidal zone (2,5 meters) consisted of muddy soft substrate with oyster (*C. gigas*) aggregations.

Figure 6. Positioning of the two transects consisting of a left (LE) and a right (RI) line at the site of 'Terneuzen' (TERN), with a transect inventoried inside (INMA) and a transect outside (OUMA) the marina.

Outside the marina the left line was positioned through a narrow (2 meters) high intertidal zone dominated by mat-forming green-algae (*U. flacca*) on a substrate of hydroblocks. The high intertidal zone transitioned into a 3 meter wide zone dominated by brown algae with *F. spiralis* on top, *F. vesiculosus* in the middle and *A. nodosum* in the lower part. The broad low intertidal zone (19 meters) consisted of rubble and debris and continued into a vast soft sediment (muddy fine sand) tidal flat.
As no perfect continues transect through other types of hard substrate was present but groynes formed the dominant hard substrates in a predominantly soft sediment environment, a transect consisting of the slope of the dike for the high intertidal zone, and the top and side of a groyne for respectively the middle and low intertidal zone, was constructed. The dike slope was paved with gravel tiles and contained almost no green algae but some *Salicornia europaea* (marsh samphire) and continued in a zone with bare basalt with green-algae (*U. flacca*); 8,5 meters altogether. The top of the groyne (2,5 meter of middle intertidal zone) consisted of cobblestone overgrown with *A. nodosum*, transitioning to rubble with oysters (*C. gigas*) in the lower intertidal zone (approximately 7 meters). The lowest part of the intertidal area is a vast soft sediment (muddy fine sand) tidal flat.

Hansweert:
Near Hansweert the salinity is not much lower than near Terneuzen: i.e. on average 19,3, fluctuating between 17 and 21,9 (Rijkswaterstaat, 2009). This is largely due to the entrance of the canal 'Kanaal door Zuid-Beveland' making the connection with the semi-enclosed saltwater tidal system of the Eastern Scheldt. Salt water coming from the Eastern Scheldt can enter the Western Scheldt through the sluices of Hansweert (and *vice versa*). The sheltered area near the sluices was selected as the 'inside marina' side near Hansweert, although the area is not really a marina but more a working harbor where boats (e.g. tugboats, small research vessels, custom authority vessels) and inland vessels dock. Salinity might therefore be slightly higher in the inside marina (INMA) transect (near the sluices) than outside the marina (OUMA) on the shore of the Western Scheldt. The tidal difference is approximately 5,3 meters.
The difference between the left and the right line of the inside marina transect is more in the details of local conditions than in the substratum. Both lines consist in the high intertidal zone of a substrate of hydroblocks with eco-toplayer largely without vegetation (some *Monostroma grevillei* in the left line, *B. marginata* in the right line) with small rubble and mud between the blocks. The middle intertidal zone is dominated by *F. vesiculosus*. The low intertidal zone consists of largly bare large rubble with mud in

between continuing to soft substrate (mud) with *C. gigas* aggregations in the lowest zone. Approximate width of the zones is 4-5 meters for the high, around 2 meters for the middle and 14-20 meters for low intertidal zones.

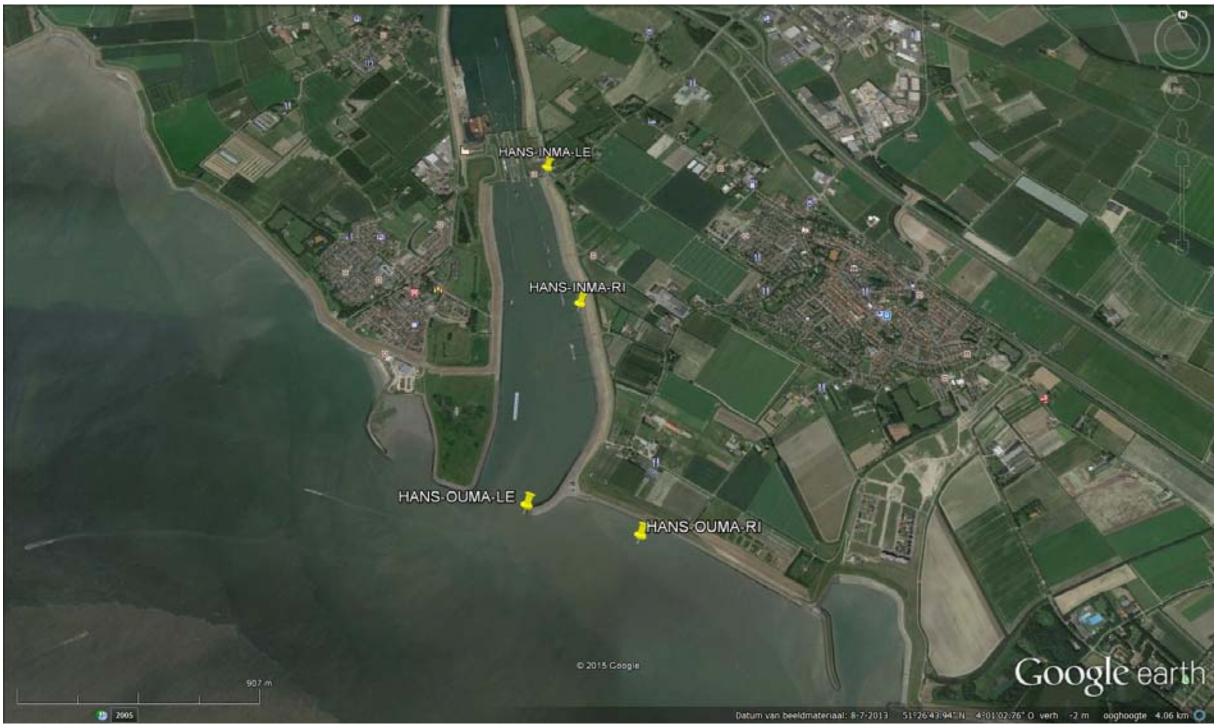


Figure 7. Positioning of the two transects consisting of a left (LE) and a right (RI) line at the site of 'Hansweert' (HANS), with a transect inventoried inside (INMA) and a transect outside (OUMA) the marina/working harbor.

The left line of the outside marina (OUMA) transect was situated on the extension of the dike/dam into the Western Scheldt. The high intertidal zone (3,5 meters) consisted of asphalted rubble, in the highest part bare, and transitioning to a coverage of mat-forming green-algae (*B. minima*) towards the middle intertidal zone. In the middle intertidal zone (3 meters) most of the asphalted rubble was bare again. The lower intertidal zone (13 meters) consisted of large rubble in the lowest part covered with oysters (*C. gigas*).
The right line consisted of bare asphalted basalt-blocks gradually transitioning to overgrow with mat-forming green-algae (*B. minima*) in the 3,5 meters high intertidal zone. The middle intertidal zone (4,5 meters) consisted of basalt-blocks (without asphalt) covered with *F. vesiculosus*. Especially around the transition from the middle to the low intertidal zone, there were several small pools formed in the crevices and in the asphalt. The 14 meters lower intertidal zone starts with a narrow strip of horizontal asphalted rubble, transitioning to a zone with large rubble in the lowest parts covered with oysters (*C. gigas*).
It is not expected that the timing of the inventories in spring/early summer had a large effect on presence of species inside the quadrants, but it has to be mentioned that at Hansweert there was about 1 month time between the monitoring of the INMA transect on May the 6th and the OUMA transect on June the 5th.

<u>Doel:</u>
The site of Doel is located on the transition from the oligohaline to the mesohaline part of the estuary. The average salinity is about 9 (mesohaline reach) however dependent of tide and river run-off it can also be a value between 2 and 16. There is a large tidal difference of about 6,8 meters (Depreiter et al., 2014).
The left line consisted of a steep, rather homogenous slope with rubble stones. The right line was also a steep, rather homogenous slope with rubble stones, however with sediment on the stones in the higher zone. The lower intertidal zone completely consisted of soft sediment (mud); therefore only two strata were inventoried. The dominant green algae especially in the high intertidal zone of the right line was *Vaucheria compacta*. The higher intertidal zone of the transect was about 2,6 meters of width, the middle zone 3,4 meters, and the lower intertidal zone approximately 4,5 meters.

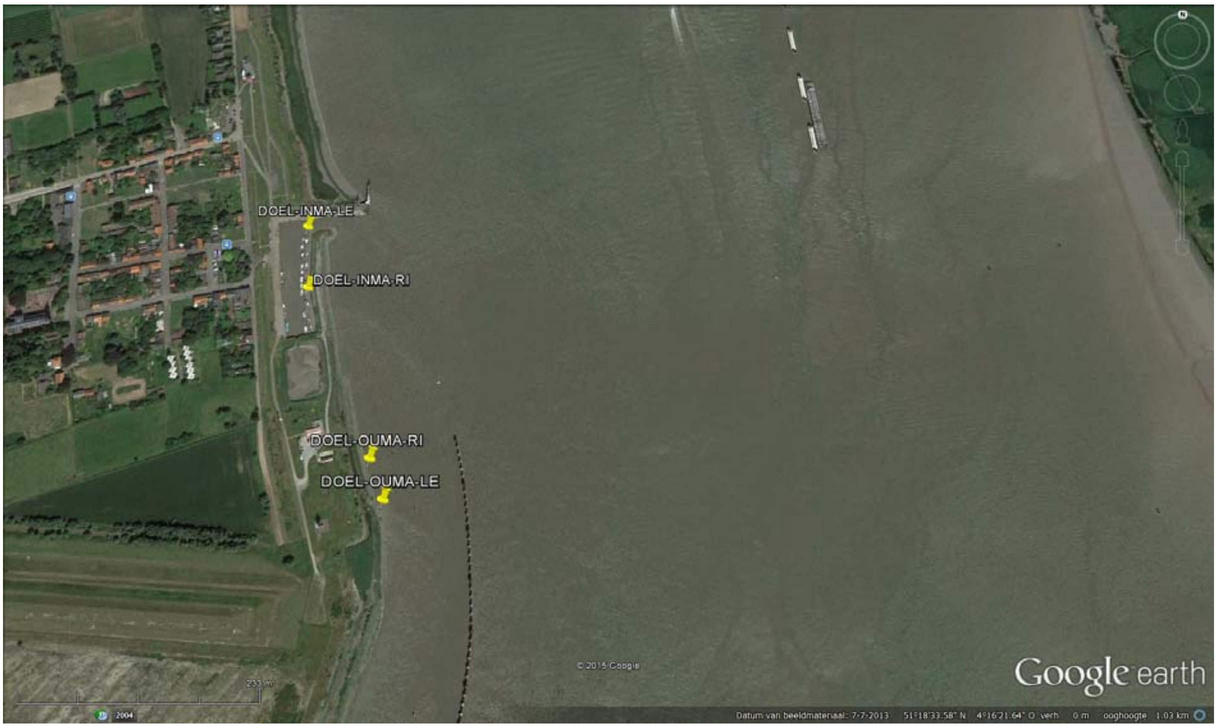


Figure 8. Positioning of the two transects consisting of a left (LE) and a right (RI) line at the site of 'Doel' (DOEL), with a transect inventoried inside (INMA) and a transect outside (OUMA) the marina.

Outside the marina the longer and less steep transect had a clear strongly distinguished zonation. In the high intertidal zone (3-7 meters) basalt blocks contained some green algae (predominantly *V. compacta*, but also *Enteromorpha sp*. The transition to the middle intertidal zone contained *Scirpus maritimus* (salt-marsh bulrush) vegetation. In the middle intertidal zone 4-12 meters of width), rubble was overgrown with mat-forming green algae (*V. compacta*). The low intertidal zone started with sandy substrate (entirely soft sediment) but continued with basalt blocks surrounded by mud in the lowest part (5 to 12 meters of width).

Wintam:

The transects near Wintam are typically situated in the low oligohaline to almost tidal fresh water reach of the Scheldt estuary. The average salinity is about 1,1, varying between 0,3 and 1,8, but the tidal range is still large: i.e. 6,5 meters (Depreiter et al., 2014).

The left line of the transect on the shore of the 'Zeeschelde' consisted of a substrate of basalt blocks and rubble; slightly steeper in the high intertidal zone, especially overgrown with green algae (*V. compacta*) in het middle and low intertidal part, and soft sediment (mud) in between the stones. The right line of the transect consisted mainly of rubble, with some larger stones in the low intertidal zone. Especially in the high intertidal zone the hard substrate was overgrown with V. compacta, whereas it was very muddy in the low intertidal zone. Whereas the left line could be distinguished in a 3 meters high intertidal zone, a 4,7 meters middle intertidal zone and a 5,3 meters low intertidal zone, the slope at the right line was less steep resulting in respectively 12, 7 and 17 meters zone width for the high - , middle – and low intertidal zones.

The transect at the Rupel consisted of basalt and rubble in a rather muddy environment, where the zonation was largely determined by the presence and abundance of green algae (*V. compacta*) on the stones. The high -, middle and low intertidal zones measured approximately 3,6, 3,7 and 4,1 meters respectively.

Figure 9. Positioning of the two transects consisting of a left (LE) and a right (RI) line at the site of 'Wintam' (WINT). As there is only small jetty opposite the transect at the shore of the 'Zeeschelde' and the most distinguishing aspect might be the positioning of the transects in different river branches with different conditions, the transects are named here after the river branch they are situated in (i.e. SCHE for the Zeeschelde and RUPE for the Rupel).

Étaples:

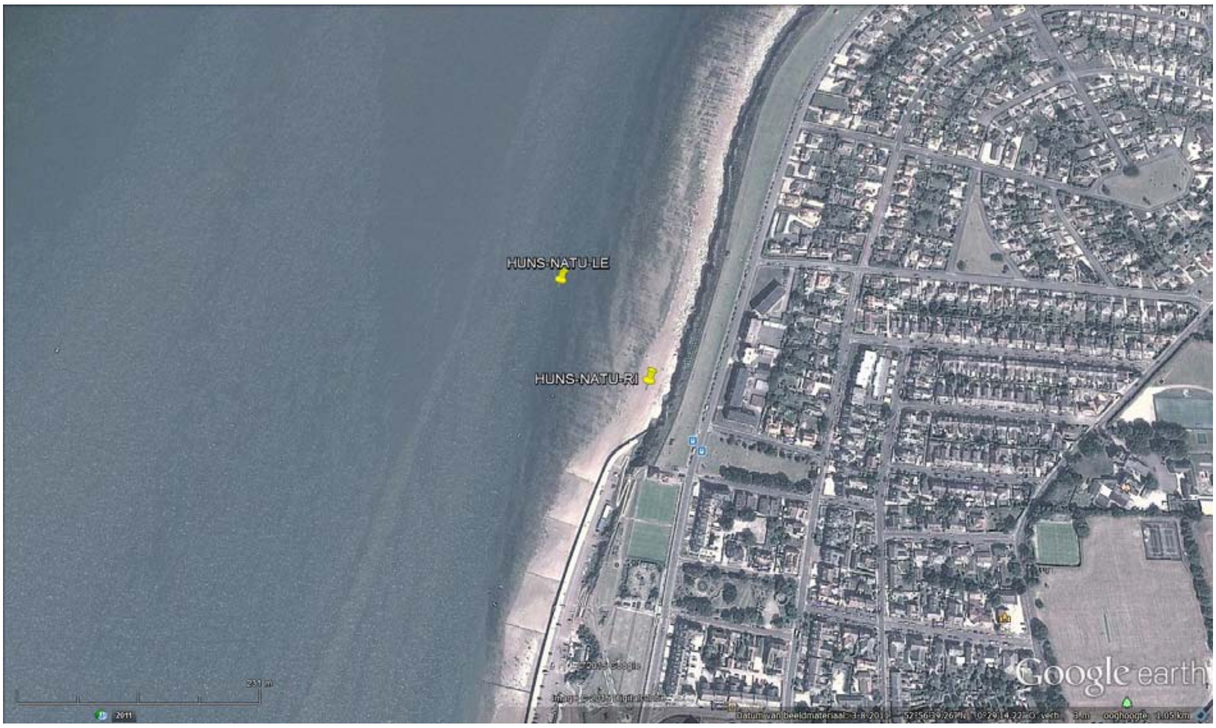
The salinity as measured during the monitoring of the transect and at the time that the SETL plates were checked, appeared to be surprisingly low, not transgressing the 0,8, with an average value of 0,5 at the transect opposite the marina (INMA). Outside the marina, upstream a riffle in the system the salinity was on average 0,2 at the time of the inventories. The tidal range is about 5,8 meters, but due to the presence of a riffle upstream of Étaples just 2 meters at the OUMA transect where water level is increased just for a limited timespan each tidal cycle. Therefore there is actually only one intertidal zone present at the OUMA transect: i.e. the high intertidal zone.

Opposite the marina (INMA transect), the high intertidal zone of the left line consists of low densities of green mat-forming algae (*B. minima*) on gravel. In the middle intertidal zone the hard substrate consists of boulders with *B. minima* and *B. marginata* growing on it. The slightly sloping high and middle intertidal zones consisting of alternating hard and soft substrate elements, continue with a steep slope of rubble with growth of some green algae (*B. minima* and *Ulva intestinalis*) in the low intertidal zone. The right line consists of a little bit of gravel with green algae in a mainly muddy fine sand (soft substrate) high intertidal zone, transitioning to a sandy soft substrate region, also a bit of gravel with green algae in a mainly fine sand (soft substrate) middle intertidal zone, continuing with a steep slope of boulders with some growth of green algae. Due to the slope the high intertidal zone and the middle intertidal zone are at both lines about 6-8 meters and 3-5 meters wide, whereas the low intertidal zone only measures about 2 meters.

Outside the marina, the shores are muddy and steep. The left line of the transect consists of a shore of approximately 3 meters in width, with big stones and some green algae (*B. minima*). The right line is a habitat of soft substrate (muddy) with algal mats on top, with only in the lower part some stones with green algae (*B. minima* and *U. intestinalis*).

Figure 10. Positioning of the two transects (inside the marina (INMA) and outside the marina (OUMA)) consisting of a left (LE) and a right (RI) line at the site of 'Étaples'.

<u>Hunstanton and Heacham:</u>
Although the system of the Wash is here selected as a focal estuary, the sites of Hunstanton and Heacham are actually more situated in a marine bay. There are several (smaller) rivers entering the system that have estuarine conditions upstream, but salinity is not expected to be much lowered at larger distances from these river mouths. It was expected that the salinity at Hunstanton and Heacham was at least 30. So far salinity measurements from or near these sites are not available but measurements were done at Sutton bridge, where the SETL plates were installed and which is quite far upstream the river Nene, at high water, showing a salinity of 32,6. This indicates that indeed full marine conditions can be expected at the transect monitoring sites. Tidal range in the Wash is about 4,9 meters.
At the natural hard substrate transect of Hunstanton, the intertidal zone covers a vast area from near the chalk cliffs to more than 200 meters into the system. Several parts consist however of soft sediment substrate (i.e. the beach in front of the cliffs and a large area in the lowest part of the intertidal zone). Around the average high water level bare chalk stones border the transition to a sandy beach (intermediate sand). The beach continues with a narrow strip of bare chalk stones in a sandy environment (that is still part of the about 20 meters wide high intertidal zone). The middle intertidal zone (about 30 meters wide at the left line and 70 meters wide at the right line) consists of chalk boulders (typical pattern of gullies worn in the chalk perpendicular to the water line) overgrown with green algae (mostly the 2 *Blidingia* species). The low intertidal zone consists of the same chalk boulders, however overgrown with barnacles (*Austrominius modestus*) and mussels (*Mytilus edulis*) abundantly present as well. For the left line this lower zone is about 60 meters wide, for the right line about 12 meters.
The artificial hard substrate site of Heacham consists of water works in a high dynamic environment as is indicated by the masses of coarse sand, gravel and shell grid deposited to a concrete wall. However the vast low intertidal region might be a less dynamic environment. The high intertidal zone of the left line consists of masses of shell grit deposited to a concrete high water defense structure and together with a sandy beach (coarse to intermediate sand with no hard substrate elements) has a width of approximately 28 meters. The middle intertidal zone (12 meters) consists of small boulders in a sandy (intermediate sand) environment, whereas the lower intertidal zone contains boulders in a sandy (fine sand) environment with green algae (*B. marginata* and *U. intestinalis*). The hard substrate containing lower intertidal zone is approximately 24 meters wide before a sand flat extents more than 200 meters into the sea.



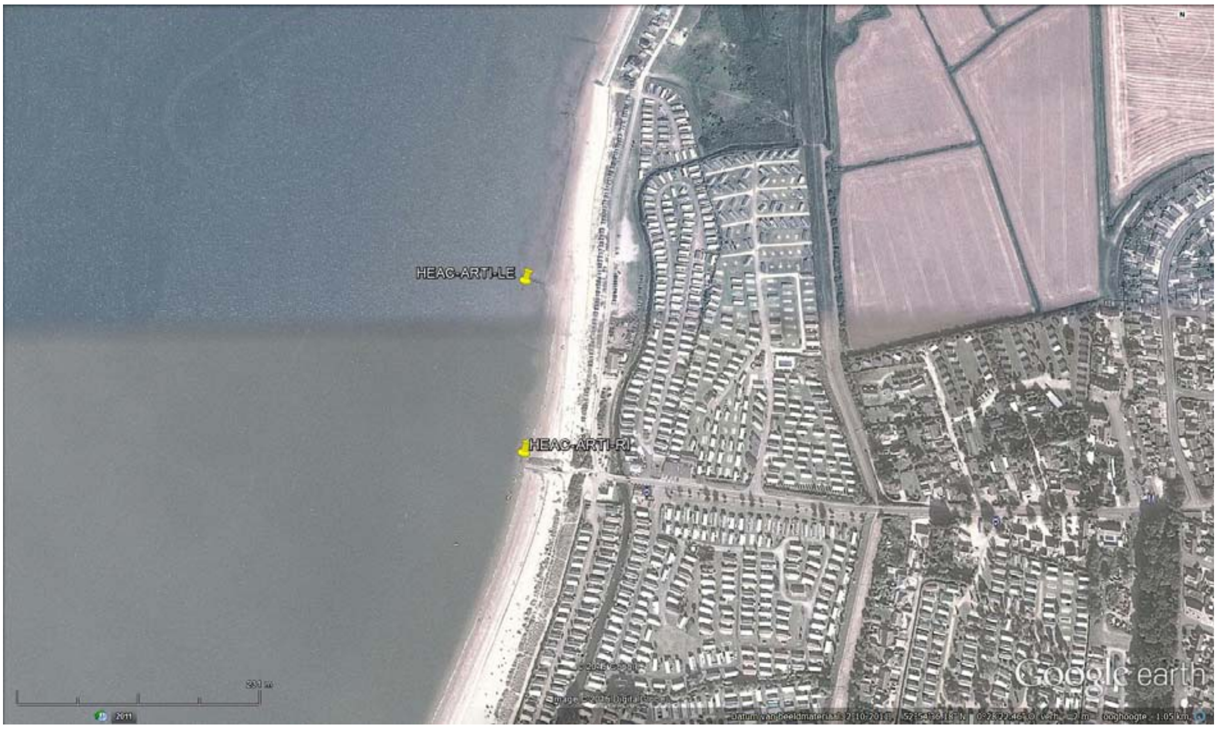


Figure 11. Positioning of the two transect on natural hard substrate (NATU) at 'Hunstanton' and the transect on artificial hard substrate (ARTI) at 'Heacham' consisting of a left (LE) and a right (RI) line.

The right line consists of large basalt blocks in the high intertidal zone guiding the stairs to the beach (21 meters). The middle intertidal zone continues with a dynamic beach with a coarse sand and gravel substrate (11 meters). The low intertidal zone (9 meters) contains a few boulders with algae (*B. marginata*, *Ulva intestinalis* and *Ulva cf lactuca*) in a largely muddy fine sand environment before a vast sand flat extent for hundreds of meters.

2.3 Data analyses and statistics

As indicated in the monitoring protocol (Annex 1) abundances of species are only recorded in terms of Rare (R), Common (C) and Abundant (A), distinguishing the categories at the transition from 2 to 3 specimens or percentage coverage (where only entire percentages are recorded) per quadrant, and at the transition from 10 to 11 specimens or percentage coverage. For statistical analyses to get average abundance indications these categories can be transformed into scores of 1, 2 and 3, so that a score of 3 for the abundance of a species at a certain site means that the species is abundant in all quadrants at that site (a score below 1 means that the species was only present in a subset of the quadrants; usually also rare in such quadrant).

All results were recorded in field data made in Excel 2013. To combine results with environmental information and site characteristics, a database was made in Access 2013. Straight-forward statistical testing and graph creation was done in Excel. To calculate diversity indicators, i.e. Margalef species richness, Pielou evenness and Shannon diversity, PRIMER 6 was used.

Principal Component Analyses (PCA; direct gradient analyses for datasets with a short gradient length) and Canonical Correspondence Analyses (CCA; indirect gradient analyses for datasets with a long gradient length) were performed after testing for the gradient length of the dataset using a Detrended Correspondence Analysis (DCA); multivariate statistics were done in Canoco for Windows 4,5. A gradient length of >3 was considered large after which dependent of whether a direct (optimal distribution of samples based on species and environmental data) or an indirect (optimal distribution of samples solely based on species data; related environmental information can be plotted afterwards) data-analyses is desirable. Data were ln(aX+1)-transformed before analyses to allow calculation with 0-values (i.e. species absence in certain quadrants).

Calculations of the expected number of NIS in a number of random quadrants were performed by calculation of the logarithmic regression according to Y=a(lnX)+b (i.e. rarefaction curves) in Excel 2013.

Recordings of additional species present in lines x strata were used as measures of the total number of species present in a line x stratum combination. This assumes that with an additional search all focal macrofauna and macro-algae species locally present (i.e. in the vicinity of the quadrants) are detected. Although there will always be a certain chance of missing species, it is expected that generally all focal species were detected; for which we adjusted the initial methodology (protocol) of searching a standardized period of time to searching till no additional species were expected anymore.

3 Results

3.1 Observed non-indigenous species

A total of 10 non-indigenous species was found during transect monitoring (Table 1). In all transects at least one NIS was found. However in the Canche and the Wash, it was always only 1 NIS, and it was also always the same species: the New-Zealand barnacle *Austrominius modestus*. This species was also found in each of the Western Scheldt transects, but lacking in the transects in the Belgian part of the Scheldt estuary. In all western Scheldt transects a set of 3 NIS were present: i.e. besides *A. modestus*, these are the Japanese oyster *Crassostrea gigas* and the brush-clawed shore crab *Hemigrapsus takanoi*. The non-indigenous amphipod *Melita nitida* was also present in each of those transects except for the outside the marina transect in Breskens. In the transects outside the marinas of Terneuzen and Hansweert a 5th NIS was present: the Japanese shore crab *Hemigrapsus sanguineus* in Terneuzen and the orange striped green anemone *Diadumene lineata*. Except for that *A. modestus* lacked in the transect outside the marina of Doel, the other 3 frequently observed NIS present in the Western Scheldt were also present in this transect near the Dutch-Belgium border. Additionally the non-indigenous amphipod *Incisocalliope aestuarius* was present there. Contrastingly in the transect inside the marina of Doel, the only NIS present was *H. takanoi*. Compared to the other transects the 2 transects near Wintam harbored a completely distinguishing NIS community consisting of the zebra mussel *Dreissena polymorpha* and the Chinese mitten crab *Eriocheir sinensis*. The quagga mussel *Dreissena bugensis* was found as an additional NIS in the transect at the shore of the Rupel.

The list of observed NIS does not contain new species for the systems (although a quick inventory of the recorded species for the Wash did not mention *A. modestus* yet: Owen, 2015).

Table 1. Overview of the observed non-indigenous species per transect.

Estuary	Site	Transect	*Austrominius modestus*	*Crassostrea gigas*	*Diadumene lineata*	*Dreissena bugensis*	*Dreissena polymorpha*	*Eriocheir sinensis*	*Hemigrapsus sanguineus*	*Hemigrapsus takanoi*	*Incisocalliope aestuarius*	*Melita nitida*
Scheldt	Breskens	Inside marina	*x*	*x*						*x*		*x*
Scheldt	Breskens	Outside marina	x	x						x		
Scheldt	Terneuzen	Inside marina	x	x						x		x
Scheldt	Terneuzen	Outside marina	x	x					x	x		x
Scheldt	Hansweert	Inside marina	x	x						x		x
Scheldt	Hansweert	Outside marina	x	x	x					x		x
Scheldt	Doel	Inside marina								x		
Scheldt	Doel	Outside marina		x						x	x	x
Scheldt	Wintam	Zeeschelde					x	x				
Scheldt	Wintam	Rupel				x	x	x				
Canche	Étaples	Inside marina	x									
Canche	Étaples	Outside marina	x									
Wash	Hunstanton -Heacham	Natural hard substrate	x									
Wash	Hunstanton -Heacham	Artificial hard substrate	x									

3.1.1. *Austrominius modestus* (New-Zealand barnacle)

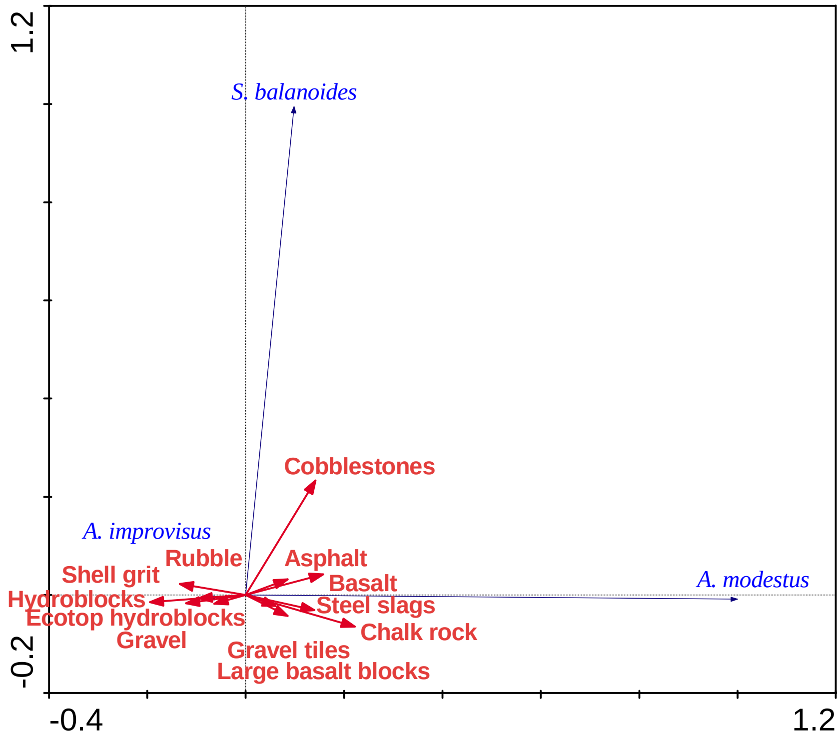
By far the most common and often abundant barnacle often dominating communities and therefore accounting for large percentages of the sessile fauna coverage is the New-Zealand barnacle

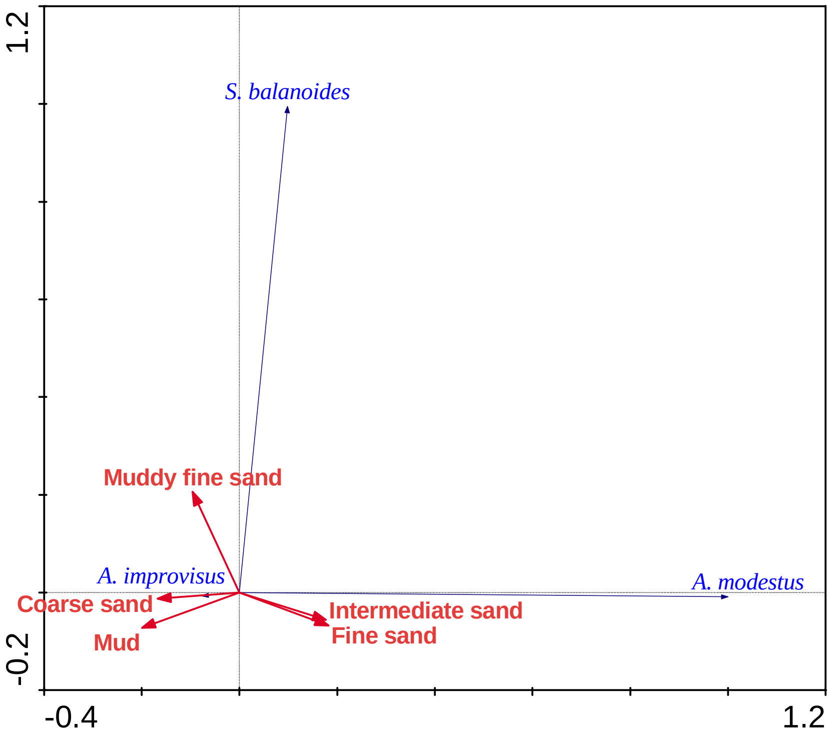
Austrominius modestus. In total four barnacle species were observed during the inventories. From these, the native *Balanus crenatus* was however only found outside quadrants on natural substrate in the high intertidal zone in the Wash (near Hunstanton), but appeared to be common at the site. For *Amphibalanus improvisus* it is uncertain whether this is a non-indigenous species. The researchers from the INBO did not indicate it as a NIS (Non-Indigenous Species) during their inventories (the species was observed near Doel and Wintam in the low intertidal zone) and Wolff (2005) indicates that it is under debate whether the species is non-indigenous. Streftaris et al. (2005) reports the species as being introduced in the North Sea region around 1850 and the species is at least known in the Netherlands from the 19th century. In this study we do not reckon the species to the NIS as such that we do not discuss the results for the species separately. The only native barnacle (for sure) found inside quadrants during this study is therefore *Semibalanus balanoides*. The species was found abundantly present at the Western Scheldt sites of Breskens and Terneuzen, always in transects outside the marinas.

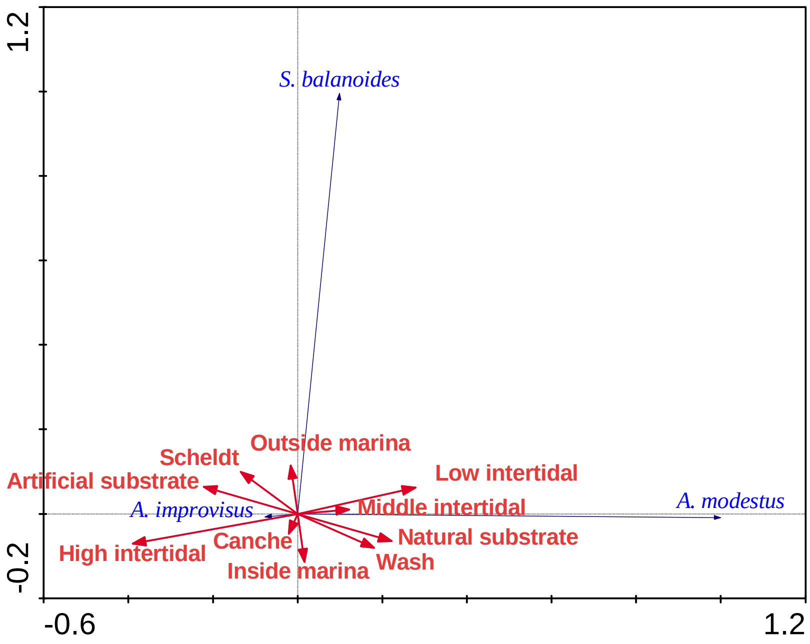
Figure 12a. *Austrominius modestus* collected during transect monitoring.

Figure 12b. *Austrominius modestus* as present in high densities on hard substrate at Heacham (the Wash).

Austrominius modestus is the most successful hard substrate related non-indigenous species found during this study. Besides that it is found to be common in each of the three estuaries, it is found throughout the salinity range as well, and it appears to be the most common and abundant barnacle as well that has taken over the role of native barnacle species. Only at the Belgian sites the species was not found; there the only barnacle observed is *Amphibalanus improvisus*, a species for which it is unclear whether it is a NIS or a native species (Kerckhof et al. 2007). Several native barnacle species were observed during the study. These were however always outnumbered by *A. modestus* in every environment with barnacles present. The New-Zealand barnacle is not a new invader, as it has arrived in Western Europe already in 1943 with first settlement in England and transportation to the European mainland with ships (ship hull fouling and transportation of larvae with ballast water) (Harms, 1999; Streftaris et al., 2005; Wolff, 2005).


1.2
-0.2
-0.4
1.2
S. balanoides
Cobblestones
A. improvisus
Rubble
Shell grit
Asphalt
Basalt
A. modestus
Hydroblocks
Ecotop hydroblocks
Steel slags
Chalk rock
Gravel
Gravel tiles
Large basalt blocks


1.2
-0.2
-0.4
1.2
S. balanoides
Muddy fine sand
A. improvisus
A. modestus
Coarse sand
Intermediate sand
Fine sand
Mud



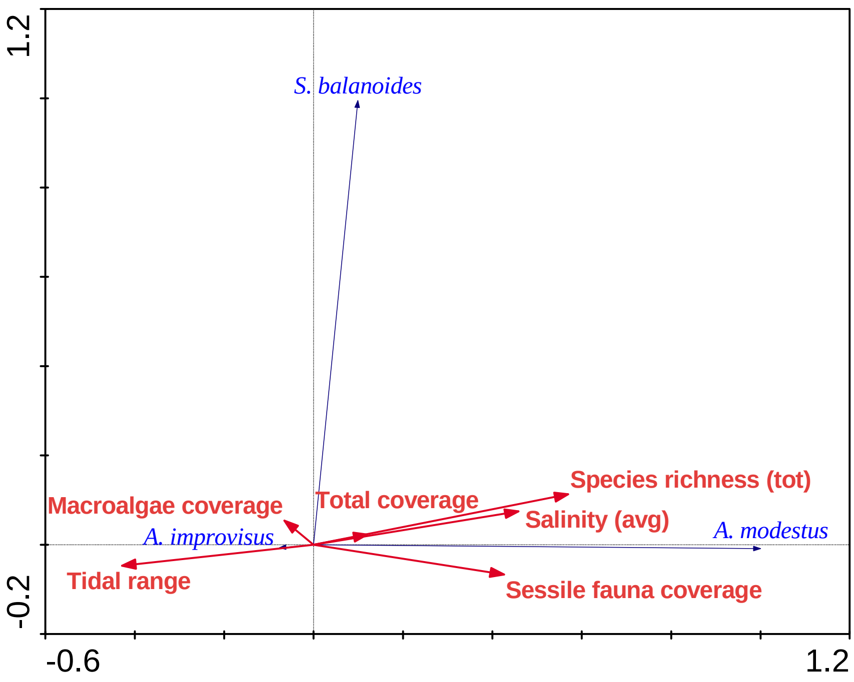


Figure 13. Results of a PCA on the relative abundance of barnacle species. Potential explaining variables are plotted afterwards (indirect gradient analysis): a) Barnacle distributions related to substrate types; b) Barnacle distributions related to sediment types; c) Barnacle distributions related to the positioning of communities; d) Barnacle distributions related to niche descriptors.

The multivariate analyses of our data (i.e. PCA results as shown in Figure 13) indicate a presence and abundance related to chalk rock (natural hard substrate) and basalt. Although *A. modestus* was present in the quadrants containing steel slags, the species was never observed on the steel slags itself (always on the basalt blocks or rubble on top of it). The relation to fine and intermediate sands indicates that the species needs a certain hydrodynamics (no stagnant water) and does not cope well with muddy conditions. As indicated by the substrate preference, *A. modestus* seems to have a preference for natural substrates, but definitely not solely. It is especially abundant in the lower intertidal zone (more than the other barnacle species). Although *A. modestus* was observed throughout the entire salinity gradient (e.g. from low salinity at the site in the Canche estuary, to high salinities at the sites near Breskens (Western Scheldt) and in the Wash, there appears to be a preference for higher salinities. Figure 14 shows the presence of *A. modestus* in near fresh water environments, but also the relative high abundance for the total of hard substrate environments at salinities above 15. It has however to be noticed that only one mesohaline site was inventoried; i.e. the transects near Doel, where the species was lacking which might have been due to other conditions like muddy and high turbidity conditions. The presence of *A. modestus* under near fresh conditions is surprising as especially the larvae are expected to need at least mesohaline conditions to develop (Harms, 1999). *A. modestus* is related to the species rich communities, which indicates that it thrives well there where a lot of species can be found, but as a habitat engineer itself (resulting in coverage of substantial parts of the available substrate) it can be concluded that *A. modestus* accelerates the local species richness (e.g. by functioning as a substrate, food source or shelter opportunity for other species). It is however expected that also other barnacle species could have such a role, but that *A. modestus* has taken over ecological niches of a series of native barnacle species. Moreover, *A. modestus* could have reduced the total biodiversity as it has replaced several barnacle species and related fauna, and is now dominating a variety of habitats that used to harbor different species (e.g. Kerckhof et al., 2007).

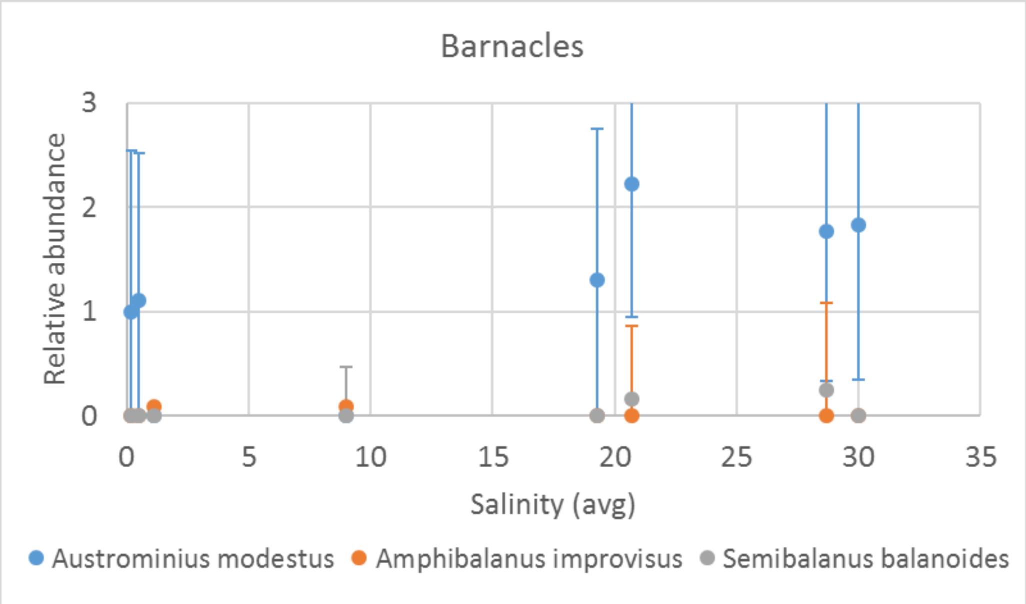


Figure 14. Average (± standard deviation) relative abundance of barnacle species related to salinity (psu). The relative abundance scale goes from absence (0) to rare (i.e. 1: only 1 or 2 specimens per quadrant) to common (2: between 3 and 10 specimens per quadrant) to abundant (3: more than 10 specimens per quadrant).

(*The recording of a salinity of 30 in this graph (i.e. the English sites) is uncertain, but ii is expected that average salinity is at least 30 but probably higher there).

3.1.2 *Crassostrea gigas* (Pacific oyster)

In terms of the impact, *Crassostrea gigas* might be the most prominent non-indigenous species encountered during this study as it has the capacity to entirely modify habitats. The species is therefore also classified as a high impact species in terms of risk for biodiversity, risk for ecosystems, risk for ecosystem services and other risks due to effects for commercial fisheries, aquaculture and infrastructures, amongst others in the expert panel assessment of potential invasive species for the Netherlands (Verbrugge et al., 2015). The ecosystem engineer was frequently observed during our inventories where it was especially present in the lower intertidal zone. Often the populations of the species extended beyond the hard substrate into soft sediment environments where at certain sites entire oyster reefs are present. When little hard substrate could be observed outside the oysters themselves, these patches were not considered in the present study, but at these sites there was always a transition from hard to soft substrate with plenty of oysters present on hard substrate as well (so in such cases, *C. gigas* will have been recorded as abundant in at least one of the low intertidal quadrants as well). Although the species is present in reasonable numbers at least in parts (i.e. the Gat Sand mussel beds) of the Wash (Eastern IFCA, 2014) and recorded just east of the Wash (Hughes, 2008), and *C. gigas* is also frequently found just north and south of the Canche (Dewarumez et al., 2011) the Pacific oyster was only found in the Scheldt estuary during our study. In the Canche estuary, salinity was definitely too low at our research sites. It is unclear if the species has settled in the vast soft sediment areas of the mouth of the Canche yet.

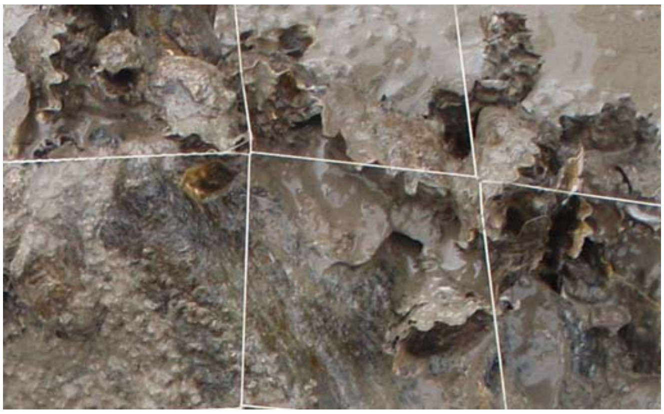
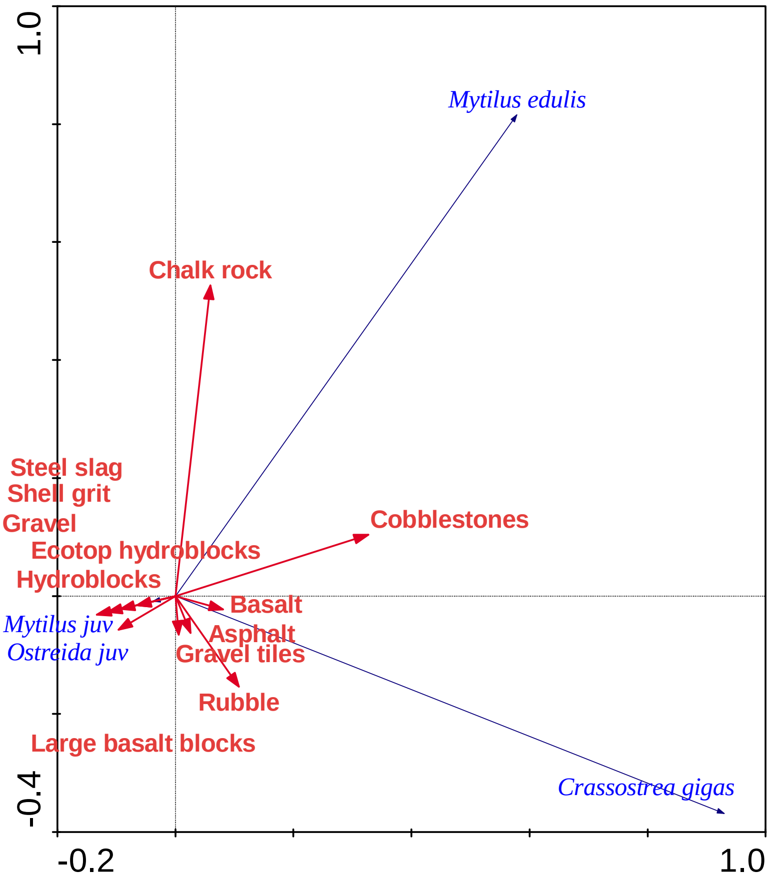


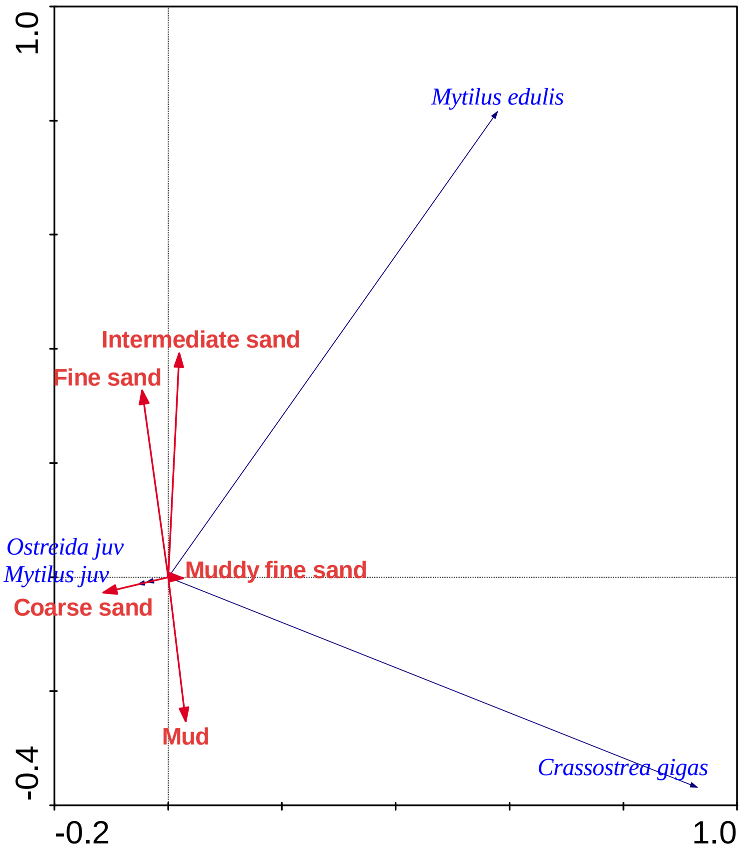
Figure 15a. *Crassostrea gigas* in a muddy environment.

Figure 15b. Typical *Crassostrea gigas* reef growing on the hard elements in the low intertidal zone near Hansweert.

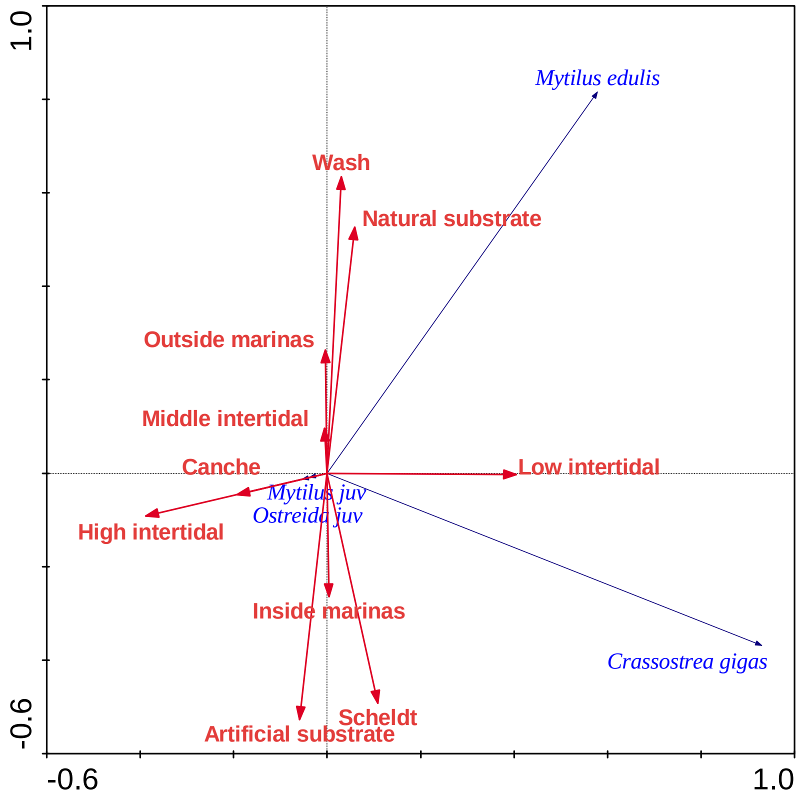
Several *Crassostrea* species have been introduced into Europe throughout the last centuries for aquaculture purposes. Although *C. angulata*, introduced from Taiwan via Portugal to Western Europe during the 1930s to 1960s might still be present locally, by far the largest part of the *Crassostrea* in Western Europe will be *C. gigas* by now (Wolff, 2005). The species was directly introduced in the Netherlands from Japan in 1964 (Drinkwaard, 1999; Troost, 2010). It was expected that the species could not reproduce in the Dutch waters, but the first young specimens were observed in 1971, and after that in 1975, and especially in 1976 and 1982 masses of larvae were produced and released in the Eastern Scheldt. In the meanwhile the species was also introduced to Wales, Belgium and several places along the French coast (Dewarumez et al., 2011). It took however till the 1990s before the species was first recorded in the Scheldt estuary; its presence in the Western Scheldt dates back at least to 1993 (Wijnhoven & Hummel, 2009), and first recordings in the Belgian part are from 1997 (as *C. angulata*) (Ysebaert et al., 2000). Nowadays the species is present in huge numbers throughout the entire Western Scheldt, but population sizes (as the average size of the animals) decreases land inwards with salinity. It seems that the species succeeds to reproduce in the eastern part of the Western Scheldt at permanent salinities below 18, but it is unclear how far into the estuary the species can reach. With decreasing water runoff from the Scheldt tributaries and a larger salt intrusion due to the deepening of the Scheldt estuary it looks like *Crassostrea* populations will expand further into Belgium.



1.0
-0.4
-0.2
1.0
Mytilus edulis
Chalk rock
Steel slag
Shell grit
Gravel
Ecotop hydroblocks
Hydroblocks
Cobblestones
Basalt
Mytilus juv
Ostreida juv
Asphalt
Gravel tiles
Rubble
Large basalt blocks
Crassostrea gigas



1.0
-0.4
-0.2
1.0
Mytilus edulis
Intermediate sand
Fine sand
Ostreida juv
Mytilus juv
Muddy fine sand
Coarse sand
Mud
Crassostrea gigas



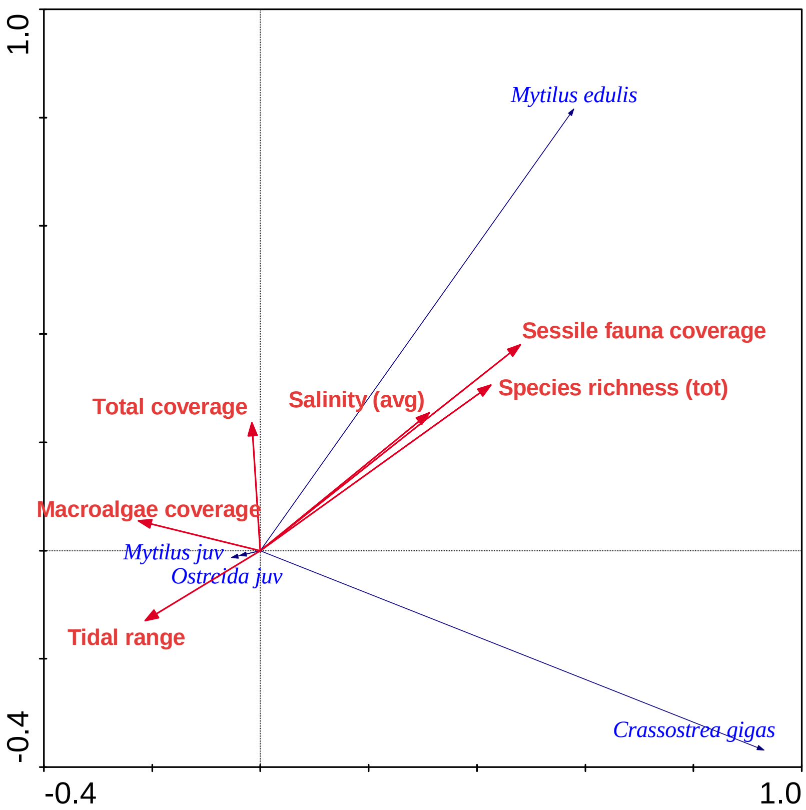


Figure 16. Results of a PCA on the relative abundance of hard substrate bivalve species. Potential explaining variables are plotted afterwards (indirect gradient analysis): a) Bivalve distributions related to substrate types; b) Bivalve distributions related to sediment types; c) Bivalve distributions related to the positioning of communities; d) Bivalve distributions related to niche descriptors.

Besides the numerous recordings in high densities at the Western Scheldt sites during this study, the species was indeed also found near Doel at the transect outside the marina in the lower intertidal zone in two quadrants, where it appeared to be common in one of them. With multivariate statistics the distribution pattern of the species was analyzed together with the only real hard substrate bivalve observed in quadrants; the native blue mussel *Mytilus edulis*. It is interesting to compare the distribution patterns of these species as it is well known that where mussel populations appeared to be abundantly present before (e.g. on piles and wooden constructions) these are now largely replaced by Pacific oysters. Two other groups consisting of juvenile specimens were included in the analyses; juvenile *Mytilus* and juvenile Ostreidae. It cannot be ruled out that these specimens belonged to other species (e.g. *Ostrea edulis*) but it is very unlikely that these specimens did not belong to the two in the analyses included species.
A relation of *C. gigas* with substrate types is not so strong, which means that several hard substrate types will do to grow on. But compared to *M. edulis*, *C. gigas* was more often found on cobblestones and rubble (Figure 16). *M. edulis* is especially related to chalk rock, exactly the natural hard substrate environment of the Wash where *C. gigas* is absent. There is a series of substrate types where adult specimens of both *Crassostrea* and *Mytilus* were lacking and where only occasional juveniles were found (i.e. hydroblocks with or without ecotop, gravel, shell grid, and steel slags). Reasons for the lack of adults of *C. gigas* are that these substrates are predominantly situated in the high and middle intertidal zone, that a substratum of shell grid was only present in the Wash and that the presence of shell grid might indicate high hydrodynamics as well, and a substratum of gravel was only present in the Canche. On steel slags no animals were found, but there was also no growth of *C. gigas* on the basalt blocks on top of those, although those were situated in the low intertidal zone. It can be sighted (Figure 16) that *C. gigas* is more abundant at the more muddy end of the sediment spectrum whereas *M. edulis* is more related to fine and intermediate sand. It is not solely that *C. gigas* prefers muddy environments, but the reefs do also have a stagnation effect on the water. Together with the massive production of phaeces and pseudo-phaeces by the oysters this results in environments getting muddier especially in sheltered places and in the transition zone from hard substrate (often a steeper slope) to soft sediment (where the slope flattens). Besides the preference for the low intertidal zone, *C. gigas* was more frequently found inside marinas than outside, and especially *M. edulis* is related to the transects outside the marinas. Although it is often mentioned that *Crassostrea* reefs might accelerate local biodiversity, this is not what could be observed at the intertidal research sites in the Scheldt estuary. Some species (like *Hemigrapsus takanoi*) do well in the reef structures but those appear to be small numbers of species that are often even more abundant when the oysters are less dominant in the environment. This probably has to do with the muddy conditions in which the oyster aggregations are found at least at a large part of the sites visited in this study. In our comparison it is especially *M. edulis* that is related to the species rich environments with high sessile fauna coverage (*M. edulis* itself is part of this coverage, but it is generally *A. modestus* that is responsible for the largest part of the faunal coverage). *C. gigas* is negatively related to macroalgae coverage. On the one hand macroalgae are generally less important in the lower intertidal parts with rather muddy conditions, but on the other hand, these might be due to *Crassostrea* presence as there were several examples of algal rich lower intertidal zones (e.g. in the Wash and Canche) in the absence of *Crassostrea*.

3.1.3 *Diadumene lineata* (orange-striped green anemone)

This anemone species is already introduced in Western Europe from Japan at the end of the 19th century. The first observation of the species was near Plymouth in 1896 where it probably arrived via ship hull transport. Although also oyster transport as a vector is suggested, in the Netherlands the species was first found near Den Helder. After several records related to the North Sea coast, recordings from delta waters arrive; e.g. lake Veerse Meer in 1968, before the first observations for the Eastern Scheldt (the center of oyster culture in the Netherlands) are done in 1986. This suggests that oyster transport is not the initial vector. Nowadays the species is abundantly present in the Eastern Scheldt (Wolff, 2005). In the Western Scheldt the species was so far only known from a few places; i.e. the sheltered outer marina of Vlissingen and near Borssele. Faasse (1997) mentions that there might be too much mud in the water column for the species to live east of Borssele. Wolff (2005) refers to M.A. Faasse (personal communication) with a sighting near Baarland (which is more to the east in the Western Scheldt). We can now extend the range further inland with sightings near Hansweert at the transect called outside marina, which is just around the corner of the Eastern Scheldt – Western Scheldt canal (which could be the expansion or transportation route as well). The species was found in both transects, in the lower intertidal zone as rare outside the quadrants, but abundantly present in some of the quadrants of the middle intertidal zone. As also indicated by Faasse (1997), the species was found on basalt and especially in the crevices that sometimes harbored water, but often the specimens were found fallen dry. In the transect where the species was only found in one quadrant being rare, the substrate consisted of asphalted rubble, where the specimens were found in a small pool in a hole in the asphalt. Besides *D. lineata* also *D. cincta* and *Actinia equina* were found at the site where *D. lineata* was abundantly present, but both other species were found to be less abundant (rare in the quandrants). *D. cincta* might be a non-indigenous species as well, Wolff (2005) gives for the status 'probably an exotic NIS', but the area of origin is possibly Atlantic, and natural range extension is a possibility. We therefore do not consider it a NIS here, but also for this species the only site where it was observed during this study was the site described here (Hansweert, outside marina).

Figure 17a. *Diadumene lineata* in the laboratory (the typical orange stripes are vaguely visible).

Figure 17b. *Diadumene lineata* in the field.

Figure 17c. *Diadumene lineata* in its typical habitat near Hansweert.

The only other observations of Anthozoa are from the Wash (transects at Hunstanton) where on natural hard substrates the native *Sagartia troglodytes* was found a few times. So far there are no recordings of non-indigenous Anthozoa from the Wash, although the species is recorded to be common in British brackish waters and Williams (1973) records the species specifically for Norfolk as well.
It has to be mentioned that there is another sighting of *D. lineata* already from 1995 in the vicinity; i.e. in a brackish water lake inside the sea dike near Kruiningen (Den Inkel) (Faasse, 1997), but an exchange of specimens from here seems to be unlikely. A range extension further upstream in the Western Scheldt might be a possibility if slightly sheltered basalt habitat is available, as the species tolerates salinities to at least below 12 (and probably lower as it is not expected that the salinity at Den Inkel reaches 12). It is also known that the species can suddenly settle, but also disappear after a while, after which it can pop-up at another site.

3.1.4 *Dreissena bugensis* (quagga mussel)

Figure 18a. Lege klep van *Dreissena bugensis.*

Figure 18b. *Dreissena polymorpha* (upper) and *D. bugensis* (lower). (http://fl.biology.usgs.gov)

The quagga mussel is a NIS that origins from the Black Sea region and that has profited from the connection (i.e. with canals) of the various river systems. Until the 1940s it was restricted to the Ukraine after which a gradual range extension into Russia could be observed. Although the quagga mussel was already observed in North America in 1991, and probably introduced already in 1986 via larvae in ballast water, the species was restricted to Eastern Europe till 2004, with the most westward occurrence in Romania (the Danube). Then, the first observation of the species in Western Europe was done in 2006 in the Hollandsch Diep in 2006 (a freshwater section of the Rhine and Meuse estuary). It is very likely that ballast water transport from either the Black Sea region or North America has been the vector (Van Emmerik, 2014). After that the range extensions in Western Europe went very fast. In 2007 the species was amongst others already present in the Meuse near the German border, the lake Volkerak-Zoommeer, close to the province of Zeeland, and lake IJsselmeer in the north of the Netherlands. First sightings in Germany (river Main and river Rhine) are also from 2007. In 2009 the first record was done in Belgium in the Albert Canal, and at least since 2012 the species is present in the Scheldt estuary around the Dutch-Belgium border (Matthews et al., 2014). Quagga mussels are found in all kind of fresh waters like rivers, streams and lakes, but especially in canals and harbors with a preference to settle on stone. Regarding the salinity tolerance, the species is generally found within the fresh water reach, but can survive under oligohaline conditions up to a salinity of 5 (Matthews et al., 2014). The quagga mussel was indeed found outside the quadrants in the Rupel transect near Wintam. The species was found there together with *D. polymorpha*. Although at the site found to be rare, it is expected that the species nowadays can be found on several sites along the Scheldt tributaries.
First observations of *D. bugensis* were in France in the rivers Moselle and Meuse in 2011 (Matthews et al., 2014). In 2012 the species was still restricted to the north-eastern part of the country, but the

current situation is unclear to us. During the inventories in the Canche estuary we did not observe any dreissenid specimens whereas the conditions (especially upstream, i.e the outside marina transect) might have been suitable. The small estuary of the Canche seems to be rather unconnected via freshwater and import of larvae in ballast water is for the Canche very unlikely. We do therefore not expect *D. bugensis* to be present there, yet.
In the UK the first observations of the quagga mussel are from 2014 in tributaries of the river Colne, near Egham, Surrey (NNSS, 2015a). So far the species seems to be restricted to this area, and therefore it is expected that *D. bugensis* is not present in or near the Wash estuary, yet. However, the fast range extensions on European mainland are not a good sign for England. The oligohaline parts of the Wash estuary were not investigated with transect monitoring during this study.
D. bugensis seems to take over from *D. polymorpha* (see below), though at several places they maintain to co-exist. Although the improvement of the visibility due to the high filter capacity of the large populations of the species in several waterbodies as a positive effect, competition with native bivalves like Unionidae species and the impoverishment of the communities on a large scale as the majority of communities become *Dreissena* dominated, is a major thread.

3.1.5 *Dreissena polymorpha* (zebra mussel)

Dreissena polymorpha expanded from the Ponto-Caspian region to the Baltic region through freshwater canals already in the 18th century. Besides ‘natural’ expansion after the opening of the Oginsky canal, the species was also aided by transport of fouling communities on ships and timber rafts. The species was first observed in the Baltic in the Curonian Lagoon in 1803 (Wolff, 2005). After that the species was introduced in several Western European countries via timber transport overseas, for instance already observed in 1824 in Germany and England (Werkgroep Exoten, 2015). Nowadays the species is present all over Europe (bordered by temperature in the north at the Scottish border, southern Sweden and just north of Estonia, and in the south to north-eastern Spain, northern Italy and the north-east of the Balkan) in freshwater lakes, canals and rivers from fresh water up to oligohaline conditions (Wolff, 2005; Zaiko & Olenin, 2006).
Nowadays the species has to compete with the recently introduced *D. bugensis* (see above), although at certain places they seem to co-exist. It is not expected that there are still many places in Western Europe suitable for *D. polymorpha* that are not invaded yet.
Sites visited during this study that might be suitable for the species are those in the oligohaline to freshwater part of the Scheldt estuary and in the Canche estuary. It is surprising that we did not find specimens or any traces of *D. polymorpha* in the Canche estuary. Although salinity measurements indicated very low salinities, the communities indicated a salt influence. It is however unclear to us if salinity near Étaples can increase to levels unsuitable for *D. polymorpha*. It is expected that *D. polymorpha* is present more upstream in the Canche estuary or its tributaries.
D. polymorpha, although indicated as rare and only present outside the quadrants, was found in both transects near Wintam (i.e along the Rupel and along the Scheldt). In the Scheldt estuary it might be the case that *D. polymorpha*, as well as *D. bugensis*, can profit from improving water quality. On the other hand, high turbidity can suppress population expansions, and further salt intrusion can lead to an upstream movement of the most seaward populations in the Scheldt estuary in future.

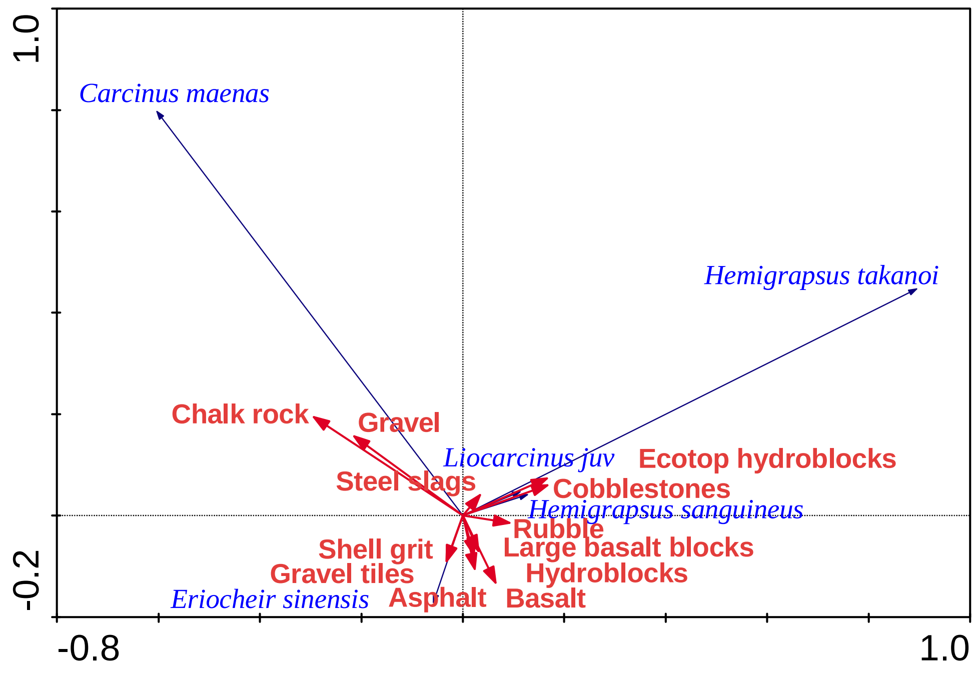
3.1.6 *Eriocheir sinensis* (Chinese mitten crab)

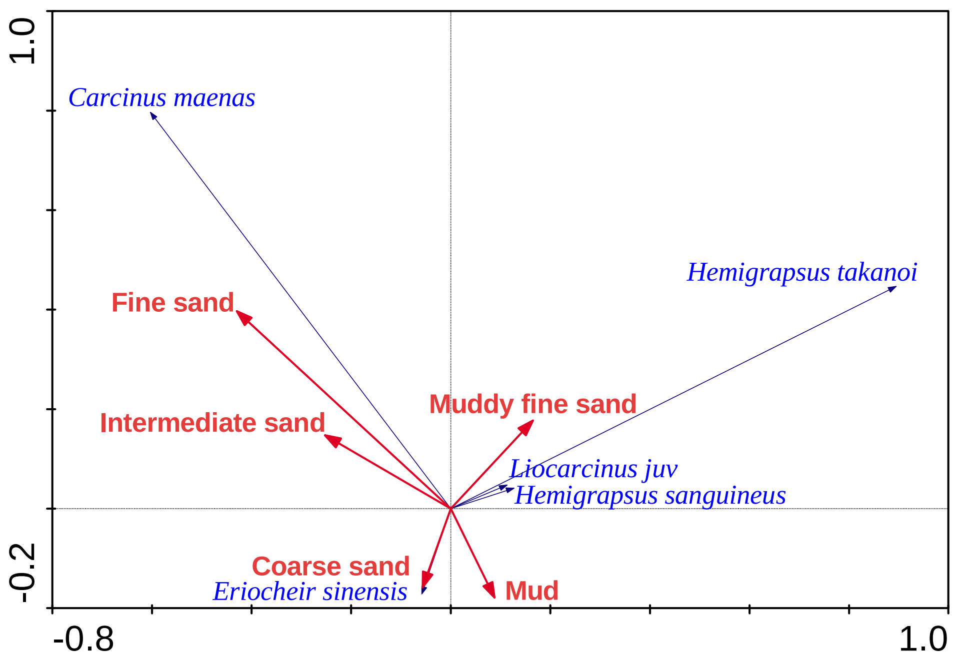
Eriocheir sinensis was introduced via ballast water (i.e. the larvae) from East Asia in Western Europe. First observations were in a tributary of the Weser (Germany) in 1912 from where populations gradually expanded (Dittel & Epifanio, 2009). As adults walk down the rivers, from fresh water to the sea to reproduce, the species reached Dutch waters in 1931. From there the populations expanded fast over entire coastal and riverine Netherlands, with a presence of the species in the Dutch delta waters probably already in 1934 (first sightings in the estuaries of Zeeland including the Western Scheldt in 1935-1936) (Wolff, 2005). The species is not only restricted to the coastal seas, estuaries and rivers but goes over land to lakes and gravel pits as well. The species is nowadays distributed all over the Western European coasts from the Baltic to south-west France and in east England, and beyond with populations in Portugal and Spain. Besides large abundances in the Elbe and Weser, the Thames is one of the high density populations (Gollash, 2006). From the Western Scheldt it is known that the species can be observed often half buried in soft sediment at the transitions from hard to soft substrate in subtidal and intertidal zones, but adults are generally found buried in subtidal soft sediments (in both marine and freshwater environments). The species can be easily observed on grids

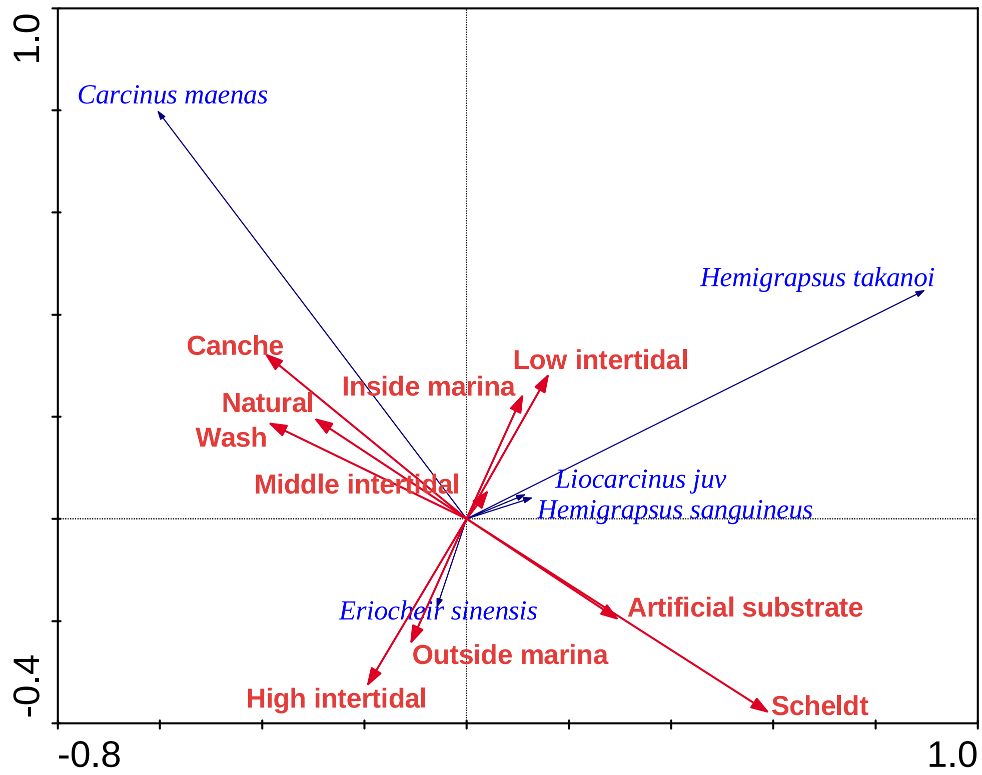
of cool water inlets, and is often found in fykes and nets. Observations in the mesohaline to polyhaline intertidal zones of estuaries can be expected, but densities are generally not that high.
During the inventories the Chinese mitten crab was frequently found near Wintam in the transect at the Scheldt, where it was present in quadrants in each of the strata. In a quadrant in the high intertidal zone several specimens were found (i.e. the species was scored as common), and this was also the case in one high intertidal quadrant at the Rupel. Figure 20 shows that the relation of E. sinensis with potential explaining variables is not so strong as for species like Carcinus maenas and Hemigrapsus takanoi. But besides the obvious relations with artificial substrate, outside marinas and the Scheldt (species only found at Wintam) the species appeared to be more abundant in the high intertidal zone and less common in the lower intertidal zone. Further E. sinensis is found in species poor communities (almost) without sessile fauna on the substratum.

Figure 19. *Eriocheir sinensis* as observed near Wintam.

It is unclear if rigorous changes in the population size of this NIS can be expected in the Scheldt estuary, but at high densities they will compete for food and space especially with other Brachyurans (for instance the native *Carcinus maenas*). In Asia the species is found to be an intermediate host for the human lung fluke parasite, but the parasite has never been reported from Europe.
The species was not observed during our inventories in the Wash, but the chance of finding the species in a marine intertidal zone with just two transects is not so high if densities are not that high. The fact that densities are high in the Thames estuary and that observations of the species are from the entire English east coast suggests that the species might be present in the Wash estuary as well. Also the Canche estuary is situated in a region where the Chinese mitten crab is present, and Dewarumez et al. (2011) does report the species specifically for the Canche estuary as well. Although the environment near Étaples seemed to be suitable to find the species as well, the only Brachyuran species that was found was the common shore crab *Carcinus maenas*.


1.0
-0.2
-0.8
1.0
Carcinus maenas
Hemigrapsus takanoi
Chalk rock
Gravel
Liocarcinus juv
Ecotop hydroblocks
Steel slags
Cobblestones
Hemigrapsus sanguineus
Rubble
Shell grit
Large basalt blocks
Gravel tiles
Hydroblocks
Eriocheir sinensis
Asphalt
Basalt


1.0
-0.2
-0.8
1.0
Carcinus maenas
Hemigrapsus takanoi
Fine sand
Muddy fine sand
Intermediate sand
Liocarcinus juv
Hemigrapsus sanguineus
Coarse sand
Eriocheir sinensis
Mud



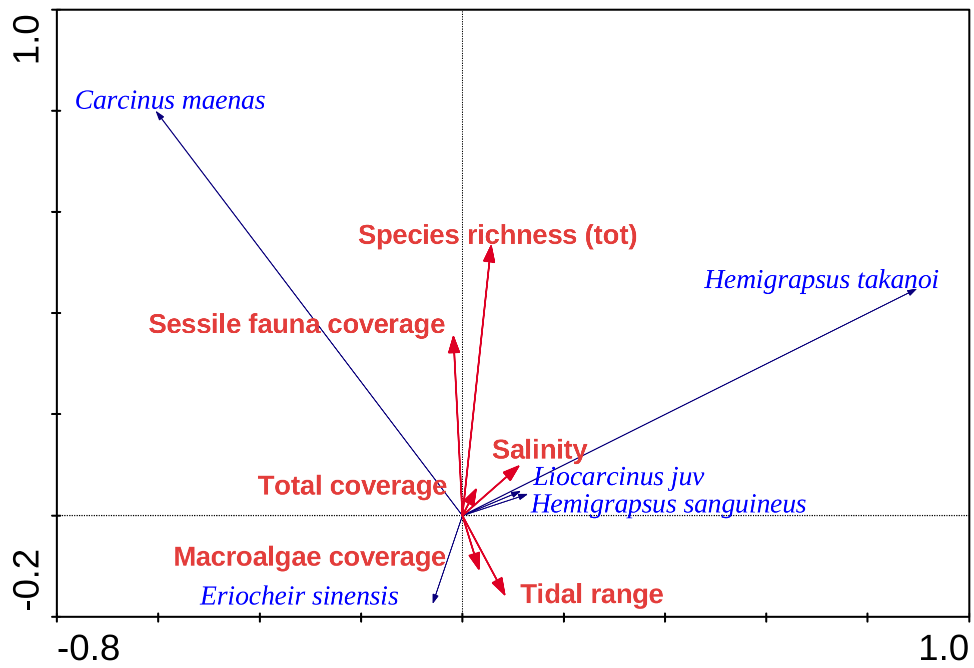


Figure 20. Results of a PCA on the relative abundance of Brachyura species. Potential explaining variables are plotted afterwards (indirect gradient analysis): a) Brachyura distributions related to substrate types; b) Brachyura distributions related to sediment types; c) Brachyura distributions related to the positioning of communities; d) Brachyura distributions related to niche descriptors.

3.1.7 *Hemigrapsus sanguineus* (Japanese shore crab)

Figure 21. *Hemigrapsus sanguineus* as observed near Terneuzen.

The Japanese shore crab probably came to Europe via ballast water transport, although further range extensions besides natural dispersal of larvae might be related to oyster transports and ship hull fouling (Epifanio, 2013). The species was first observed in 1999 almost at the same time in France (Le Havre) and the Netherlands (Eastern Scheldt). The population in France seemed to have settled at least one year earlier as breeding adults were present in 1999 (Wolff, 2005). In France the species appears to be especially successful along the north and east coast of Normandy, and more to the north (e.g. in Belgium) the densities decrease (Dauvin, 2009). However, the species has dispersed up to the Saxony coast in Germany, but also the second source in the Eastern Scheldt will have helped. During a survey of the intertidal zone in the Eastern Scheldt and just outside the Eastern Scheldt at the North Sea coast in 2011, *H. sanguineus* appeared to be abundantly present especially at the most exposed sites. *H. sanguineus* was dominating the communities at the North Sea coast, was present in about similar numbers as *H. takanoi* just inside the storm surge barrier in the Eastern Scheldt and was found in low numbers at several sites in the Eastern Scheldt where *H. takanoi* completely dominated the communities (Van den Brink et al., 2012). *H. sanguineus* is therefore also expected on the shores in the mouth of the Western Scheldt and probably inland as well, however the current status in the Scheldt estuary is unclear.
About the Canche estuary it is very likely that *H. sanguineus* is present at the coast of the English Channel, however the lack of hard substrate in large parts of the estuary and a strong decrease of the salinity land inwards might prevent the settlement of *H. sanguineus* land inwards in the estuary. In the UK the records for *H. sanguineus* are at present restricted to the Channel islands (since 2009) and south Wales and Kent since 2014. Although the Wash estuary might be suitable for *H. sanguineus*, it is not very likely that the species is present there yet (NNSS, 2015b).
During the transect monitoring the Japanese shore crab was indeed not observed at the sites in the Canche and the Wash, but the species was present in the transect outside the marina of Terneuzen. This is indeed a more exposed site where the species was found in two quadrants in the different

lines, both situated in the lower intertidal zone. In both quadrants also *H. takanoi* was present, and in one of the quadrants *H. takanoi* was found to be common (see below). Moreover, at this site *H. takanoi* was found in almost all quadrants in the lower and the middle intertidal zone and generally common. Taking the hydrodynamics into account, one would expect *H. sanguineus* in the outside marina transect at Breskens as well, it was however not observed there. As only two specimens were found in the entire study, a relation with environmental parameters is not very clear, and it seems that *H. sanguineus* is in the same niche now as *H. takanoi* (Figure 20; this is probably not the case when more transects in the poly- and mesohaline zones of the Scheldt estuary will be inventoried and more specimens of *H. sanguineus* are found).
When present in higher densities *H. sanguineus* will compete with the native shore crabs and *C. maenas* in particular, for which this can be a serious risk as observed in North America (NNSS, 2015b).

3.1.8 *Hemigrapsus takanoi* (brush-clawed shore crab)

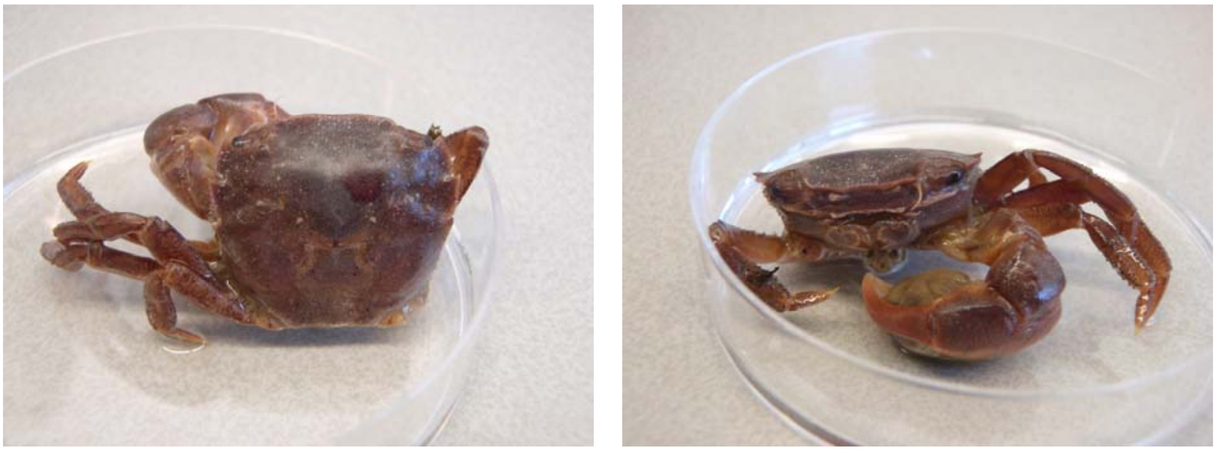


Figure 22. *Hemigrapsus takanoi* (few legs missing) collected during transect monitoring.

Hemigrapsus takanoi was often called *H. penicillatus* at the sites where it first arrived in Europe. As also *H. penicillatus*, which first arrived in Bremerhaven in 1993 found as hull fouling on a Japanese vessel, is present in Europe, the early arrival and expansion of the *H. takanoi* populations is uncertain. Looking back, it looks like the first observation of *H. takanoi* is from La Rochelle (France). After that the species was observed in 1997 in Le Havre and some more observations along the French north coast in 2005 and 2006. In the meanwhile the brush-clawed shore crab was also found for the first time in the Netherlands in the Eastern Scheldt in 2000 after which more specimens were found. It looks like *H. takanoi* must have been present already in 1999, after which its populations expanded rapidly within a few years over the entire Eastern Scheldt, and since 2001 also in the Western Scheldt (Wolff, 2005) at least as far as the Biezelingsche Ham (on the approximate border between the poly- and the mesohaline zone). In Belgium the species was first observed in 2003 near Oostende. How far into the Scheldt estuary the species does reach is so far unclear, but the species was detected a few times in soft sediment samples from the Western Scheldt in 2007 and 2008 that were taken almost at the Belgian border (Van den Brink et al., 2012). This might be an indication that during those years the species had reached Belgium via the Scheldt as *H. takanoi* is especially expected on hard substrates. As indicated, it is likely that *H. takanoi* came across the Canche estuary at the sea side already. It is a matter of whether a suitable habitat is present in the mouth of the estuary where it is predominantly soft sediment environment, taking into account that the species also prefers more sheltered places. Suitability of the system then also depends on the salinity tolerance of the species. Mingkid et al. (2006) record that *H. takanoi* cannot go through successful metamorphosis at salinities of 20 or lower, but Duenas (2013) records a salinity tolerance in the range of 7-35 for at least the adults.
The first observations of *H. takanoi* in England are from recent date; the species is found for the first time in 2013 and now present in Essex and Kent (Wood et al., 2015). It is therefore unlikely that the species is already present in the Wash estuary, but the system is at risk of an invasion in the future.
No *H. takanoi* was found at the sites in the Canche estuary, where the salinity was probably too low. Suitable habitats might be limited in the Canche estuary for flourishing populations. Indeed also no observations of *H. takanoi* in the Wash estuary. But the species was present in several transects and

often common to abundant in the Scheldt estuary, including the transects at Doel (Belgium). If the recorded salinity tolerance of larvae as recorded by Mingkid et al. (2006) is correct, it must be the juvenile specimens that float inland in large quantities. Average salinity at Doel is still within the tolerance range of the adults. Figure 20 indicates that *H. takanoi* is more often found on cobblestones and on and in between hydroblocks with an ecotop toplayer, than the native concurrent species *Carcinus maenas*. *C. maenas* was especially related to the chalk rock and gravel substrates, and for instance not to basalt substrates. Where *C. maenas* was typically related to the fine and intermediate sand substrates (e.g. slightly more exposed sights), *H. takanoi* is most common at the sites with muddy fine sand. *Hemigrapsus takanoi* is found especially inside marinas (i.e. more sheltered and often more muddy) and in the lower intertidal zone. *C. maenas* is here typically related to natural substrate as this was only present in the Wash estuary, where *H. takanoi* was absent, and *C. maenas* abundantly present. However, both species are more related to species rich communities. As indicated by Van den Brink et al. (2012), although *H. takanoi* at least in the Netherlands does not seem to be the reason of the dramatic decrease in the *C. maenas* populations, *H. takanoi* definitely competes with the juvenile and smaller *C. maenas* specimens for the same niches, and seems to be more successful in that. Besides a moderate risk for biodiversity, Wijnhoven et al. (2015) estimates the species to be a moderate risk to ecosystems as well, due to large disturbing effects of digging activities (more than the native shore crab *C. maenas*) in habitats of vulnerable species like *Auriculinella bidentata* and *Nephasoma minuta* in the Netherlands. It is worthwhile to monitor shore crab population developments at sites not invaded by *H. takanoi* yet where *C. maenas* still flourishes, like in the Wash, and to investigate the distribution over the entire salinity range. The situation in the Canche (where *C. maenas* is abundantly present at low salinities) might give an indication of the refuge for *C. maenas* that might be in the oligohaline reach (Figure 23). The near absence of *C. maenas* in the Belgian part of the Scheldt might indicate that here there are other reasons for the decrease of the *C. maenas* populations (*C. maenas* did not succeed to escape or survive there in large quantities). Whether there are also opportunities for *C. maenas* at the high salinity end of the continuum as suggested by Figure 23, is uncertain. There it is probably more a matter of hydrodynamics, as exposed sites are often also the more saline sites. It has to be taken into account that at these sights the risk might be *H. sanguineus*.

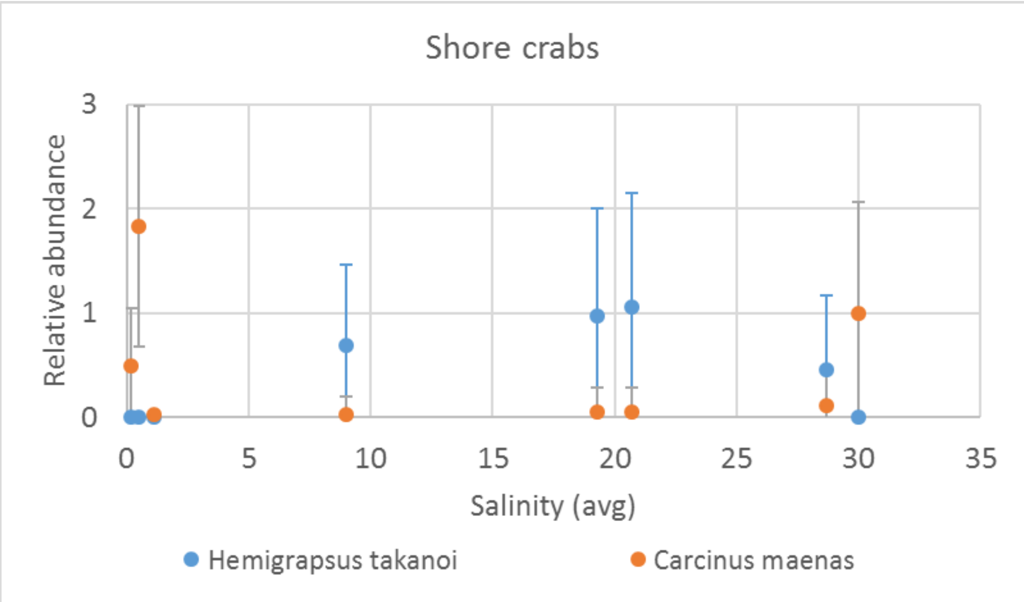


Figure 23. Average (± standard deviation) relative abundance of shore crab species related to salinity. The relative abundance scale goes from absence (0) to rare (i.e. 1: only 1 or 2 specimens per quadrant) to common (2: between 3 and 10 specimens per quadrant) to abundant (3: more than 10 specimens per quadrant).

3.1.9 *Incisocalliope aestuarius*

(No vernacular name in English: ‘estuariene poliepvlo’ in Dutch)

Incisocalliope aestuarius is a non-indigenous amphipod that originally comes from the American Atlantic coast. The first European observation was from the Belgian part of the Scheldt in 1996, but it appeared to be present in the eastern part of the Western Scheldt already since 1991, where it was not recognized at that time. The species probably came via ballast water or hull fouling (Wolff, 2005). To our knowledge the species is still restricted to Belgium and the Netherlands, and the Scheldt estuary in particular, outside its native range. It seems therefore that the species is not a risk of getting invasive. This might be as it seems to inhabit a very specific niche related to hydrozoans. Further the species seems to be restricted in Europe to the mesohaline realm where it inhabits to lower intertidal and subtidal zones (Faasse & Van Moorsel, 2003). However Faasse & Van Moorsel (2003) indicate also that in its native region the species is found at salinities ranging from 10 to 33 and indicate that the species is abundantly present around Baarland and Hoedekenskerke, which is actually around the transition from the mesohaline to the polyhaline zone. Further they also record *I. aestuarius* for Walsoorden and Bath where it is found to be rare. At the Belgian side of the border its range extents till Doel. Indeed, during the transect monitoring for this study *I. aestuarius* was found near Doel, where it was (solely) observed outside the quadrants in the transect outside the marina. As indicated by Faasse & Van Moorsel (2003) as well, it is not expected that the species significantly extends its distribution range in the Scheldt estuary and it is unlikely that the species suddenly appears in the relatively isolated (in terms of shipping connectivity) Canche and Wash estuaries.

Figure 24. *Incisocalliope aestuarius*.
http://home.kpn.nl/faassema/introduced%20crustacea.html

3.1.10 *Melita nitida*

(No vernacular name in English: ‘elegante honingvlokreeft’ in Dutch)

Also *Melita nitida* is a non-indigenous species with its origin at the North American Atlantic coast that probably arrived in Europe via ballast water or hull fouling. Also for this species the first European observation was done in the Scheldt estuary in 1998 near Bath, which is just at the Dutch side of the border. At that site it appears to be abundantly present, but the species was also occasionally found at Baarland and Walsoorden (i.e. therefore along the entire mesohaline spectrum) (Faasse & Van Moorsel, 2003). Faasse & Van Moorsel (2003) indicate that the species is typically found in the lower intertidal and subtidal zones, especially under boulders and among Pacific oysters. To identify the niche of the species we did a PCA where it was compared with the other amphipod species observed. Although several native amphipod species appeared to have a very specified niche on bases of our observations (e.g. *Orchestia mediterranea*, *Apohyale prevostii*, *Chaetogammarus marinus*, *Corophium* *volutator*) as indicated by the long arrows in Figure 26, the distribution of *Melita nitida* appeared to be difficult to characterize (short arrow). In Figure 26 the example of relations with substrate types is shown. But as *M. nitida* could not be well distinguished from the other amphipods although the species was frequently observed, this might indicate that the species has a very broad tolerance range and acts opportunistically inhabiting environments with limited competition.

Figure 25. *Melita nitida* collected during transect monitoring.

Melita nitida was found during our inventories in a total of eleven quadrants at 4 sites (6 transects) along the salinity gradient in the Scheldt estuary. The species was present at an average salinity of 9 at Doel (rare in 2 quadrants) up to an average salinity of almost 29 at Breskens (common in one of the quadrants), and was abundantly present at least locally at Hansweert and Terneuzen. This is a significant range extension in the Scheldt estuary covering the entire polyhaline zone as well, compared to the recordings of Faasse & Van Moorsel (2003) which were also only 4 years after introduction. In contrast to the situation described in 2003 where the native *Melita palmata* was identified as common to abundantly present at most sites along the entire transect from Rhittem to Bath, this species appeared to be absent now, and was only found at Doel. The interesting aspect was that at Doel, *M. nitida* was found occasionally in the transect outside the marina where no *M. palmata* was found, whereas in the transect inside the marina, *M. nitida* was absent but *M. palmata* was abundantly present. The native *M. palmata* was during the study also observed in one of the transects with natural hard substrate in the Wash estuary where it appeared to be abundantly present at least locally, where there are no indications that *M. nitida* is present in the estuary.
M. nitida was always found on a substrate of rubble, i.e conform the observations by Faasse & Van Moorsel (2003) who named the substrate preference ‘boulders’. In 8 of the 11 quadrants where *M. nitida* was found, *Crassostra gigas* appeared to be common to abundantly present. The elegant honey amphipod was generally found in the lower intertidal zone but at Hansweert also in the middle intertidal zone; often in an environment containing mud or muddy fine sand.
The observed above described findings indicate that *Melita nitida* is an opportunistic species that behaves as an invader and seems to outcompete the native *Melita palmata* at least in the Scheldt estuary. So far the number of sightings of *M. nitida* outside the Scheldt estuary is still limited, but the species is already present in the estuarine North Sea Canal (Netherlands) (Faasse & Van Moorsel,

2003), has extended to the Belgium coast since 2003 (Dewarumez et al., 2011) and is in 2010 also observed for the first time in Germany in the mesohaline part of the Kiel Canal (Reichert & Beermann, 2011). We now know that the species also has the potential to go beyond the mesohaline zone.

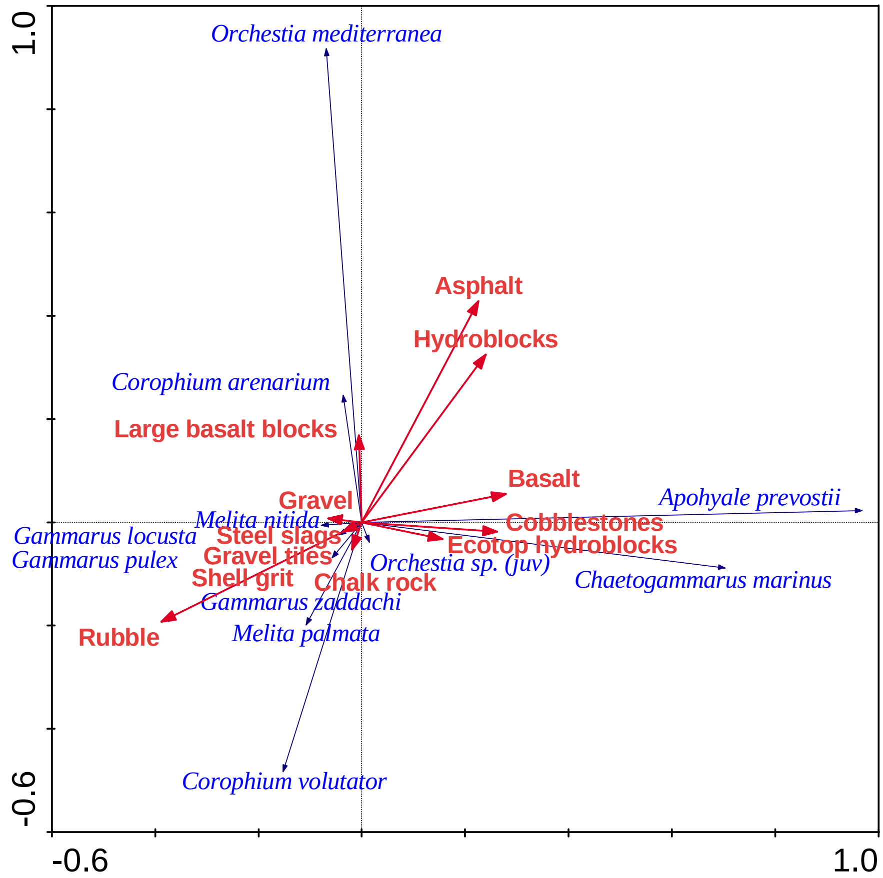


Figure 26. Results of a PCA on the relative abundance of amphipod species. Potential explaining variables are plotted afterwards (indirect gradient analysis): Amphipod distributions related to substrate types.

As the current study focuses on NIS and the PCA was not very indicative for *M. nitida* (the only amphipod NIS found in the quadrants) no further potential relations with potential explaining variables were investigated by use of a PCA.

3.2 NIS in relation to their surroundings

When species compositions of quadrants are compared making use of a direct gradient analyses (as the gradient length is long a Canonical Correspondence Analysis, CCA, is used) so that as relevant indicated environmental parameters are taken into account for the optimal distribution of the quadrants) it becomes clear that the communities of the Scheldt are more different from the other two estuaries, than the communities of the Wash and the Canche do differ from each other. Within the Scheldt there is a clear separation between the Belgian and the Dutch sites (Figure 27). It appears that there is a set of species typical for the Belgian sites, and there is a set of species that forms more or less a gradient from the Dutch communities via the English communities to the French communities. Only a limited number of species appears to be present at both Belgian sites and one of the other countries their sites. At first sight this is surprising as one would expect an important role of salinity as the most distinguishing parameter determining the communities. Although perhaps not thé most distinguishing parameter, salinity still plays an important role. Several species are especially present at high salinity values, especially in the Netherlands whereas others are typical for low salinities as found in Belgium.

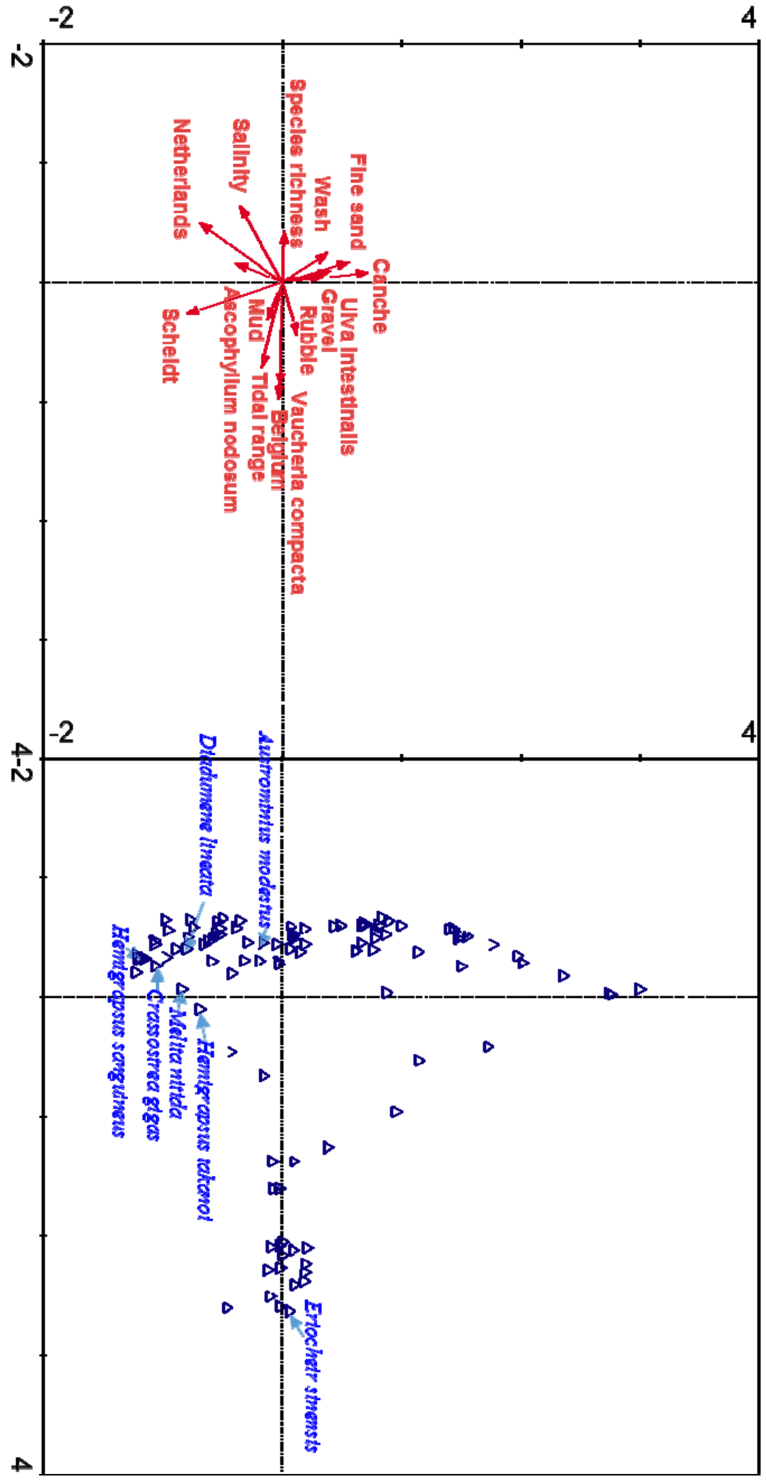


Figure 27. Results of a CCA based on the quadrant data: all species (125 taxa) and environmental data (selection of 56 parameters). The positioning of the non-indigenous species is indicated in the plot. Only the most relevant ‘environmental parameters’ for the species distributions are indicated.

Here it is surprising that the low salinity sites situated in the Canche estuary are not that much determined by low salinity species, and this is actually an observation we did already at first sight in the field (several 'marine' species were observed). Several algae species were rather typical for certain sites as indicated in Figure 27. Of the identified substrate types, only a limited number seemed to harbor typical communities, like there are the gravel sites (especially present in England and France, and the rubble sites, especially found in Belgium). A substrate type like chalk did not appear to contain a very distinguishing community, neither did asphalt. Although as much as possible hard substrate environments were sampled, those containing fine sand contained different communities from those containing mud. Besides the substratum as such, sediment type also reflects the hydrodynamic situation on the sites and the suspended matter content.
For this study it is especially of interest where the non-indigenous species can be found. Except for *Eriocheir sinensis* (in this study only observed at a Belgian site) all other NIS were at least also found in the Netherlands, and generally in relatively more quadrants and/or more abundant than in the other countries. Therefore the NIS cluster near the Dutch sites often at higher salinities, but also at the sites where in total most species can be found (species-rich sites). *Austrominius modestus* is the only NIS observed in the Wash and the Canche, and is also very common in the Western Scheldt. *Hemigrapsus takanoi* and *Melita nitida* are common in the Western Scheldt, but were also found at the mesohaline sites of Doel in Belgium. It has to be noticed that some non-indigenous species (e.g. *Dreissena bugensis*, *D. polymorpha* and *Incisocalliope aestuarius*) were only observed outside the quadrants. Observations from outside the quadrants are not taken into account in the CCA, but all these NIS missed with the quadrants are observed at Belgian sites, i.e. confirming deviating communities from the other countries.

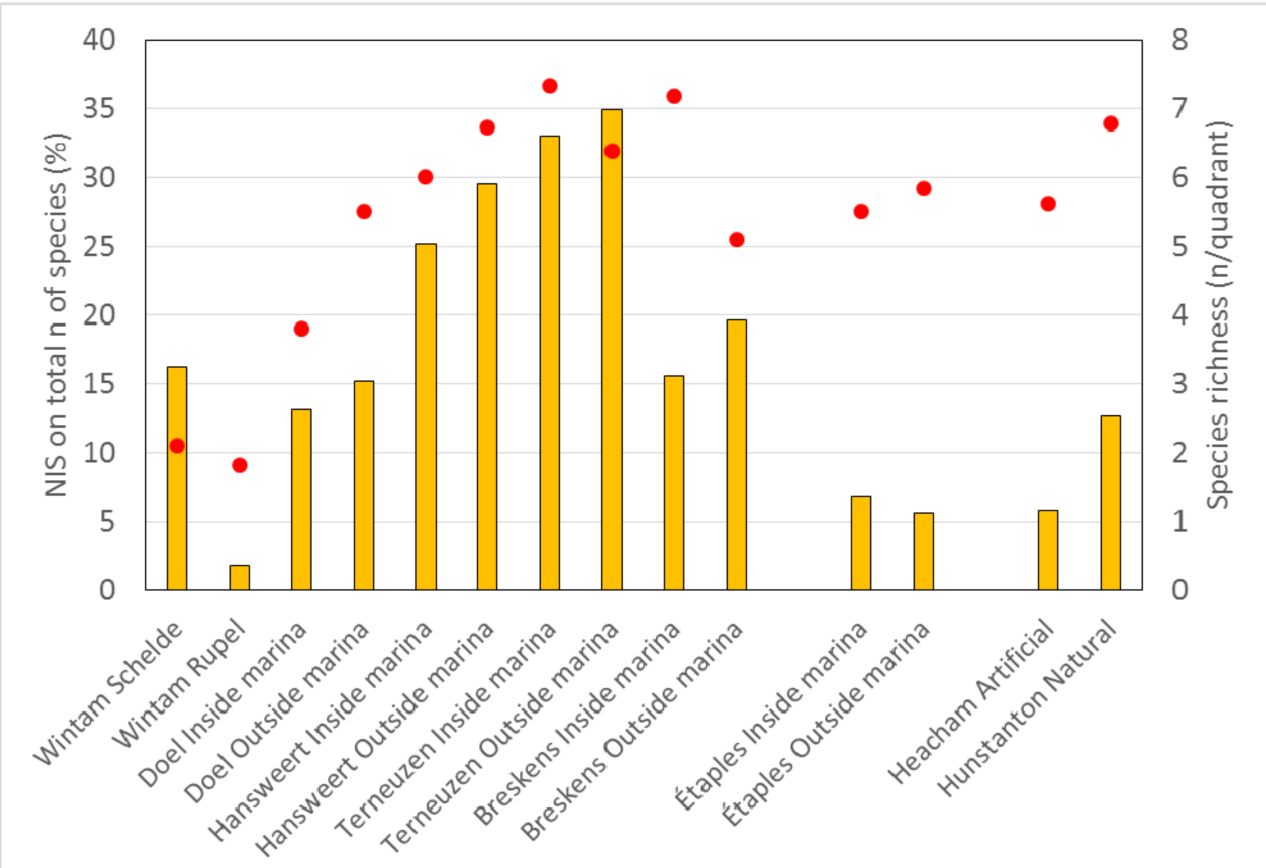


Figure 28. Comparison of the average percentage of non-indigenous species in the total of species per quadrant and the species richness shown as average total number of species per quadrant for each transect.

If the percentage of NIS in the total of species as observed inside inventoried quadrants is compared, large differences can be found along the salinity transect in the Scheldt estuary. This percentage appears to be relatively low in the freshwater part, increasing with salinity to the polyhaline zone where it reaches more than 30 % in Terneuzen, and then again lower (between 15 and 20 %) at the mouth of the estuary in Breskens. It has to be noticed that at the Scheldt site the NIS percentage is more than 15 %, and that only at the Rupel site this is just about 2 %. It also has to be noticed that we are talking

about only one NIS present in the quadrants of Wintam in both transects: *Eriocheir sinensis*. This means that less quadrants with *E. sinensis* were observed at the Rupel site, where the average total number of species observed per quadrant is about the same as at the Scheldt site. The increase in the percentage of NIS in the communities goes together with an increase in the total number of species, however the increase in NIS is steeper than that of the native species. Although the average total number of species observed inside the marina of Breskens is in line with the numbers found at Terneuzen, outside the marina of Breskens the species richness was much lower. It might be that the lower percentage of NIS at Breskens is due to a higher salinity (i.e. that it is a typical pattern observed along a salinity gradient). However total species richness indicates that there might be other aspects involved as well, leading to the observed pattern. It appears that especially at Breskens the hard substrate has been renewed recently (including that amongst others steel slags have been used outside the marina). To draw conclusions about typical patterns with salinity and/or the effect of the age of the top layer of artificial hard substrate areas, additional research is needed (i.e. more sites along the salinity transect and knowledge about the exact age of the structures).
Interesting is the aspect that the percentage of NIS appears to be often slightly higher outside marinas than inside marinas (except for Étaples). Again this seems to be in line with the total species richness, except for the deviating site of Breskens. It might be concluded that NIS do, like other species, need good conditions and are therefore more often found at sites with higher species richness. At these sites with relative good conditions, NIS however appear to be relatively more successful in settling than native species, compared to the sites with poorer conditions. Then there is Étaples with only one NIS (*Austrominius modestus*) present, and on average a low percentage of NIS in the communities (slightly more than 5 %). This low percentage might be due to the relative isolated position in terms of ship movements from elsewhere into the estuary combined with that the boats coming in arriving at low mesohaline conditions generally do not come from similar conditions elsewhere. Additionally, there is little connectivity with other riverine areas in the freshwater to mesohaline zone (no canals).
Interesting to see that NIS are also doing better in the communities of the natural transect in the Wash than the artificial one, where average species richness is lower. Again we are looking at a pattern due to the presence of the only NIS found during the inventories in the Wash: *A. modestus*. The non-indigenous barnacle was present in a large percentage of the quadrants at Hunstanton. Comparing the Wash with an estuary where loads of non-indigenous specimens are released on a daily basis, and where it is only a matter of succeeding to settle and reproduce, one can estimate that if connectivity with other parts of the world increases for the Wash, or if NIS gradually succeed to expand from hotspots (i.e. international ports and marinas) in the north and especially the south of the UK, the NIS percentage in the communities might gradually go in the direction of 30 %.
Findings on hard substrate are in line with those of the soft sediment communities monitoring program in the Western Scheldt where the percentage of NIS per sample is on average about 25 % (Monitor Taskforce own data).
It has already been mentioned in the introduction of this report that particularly artificial substrates might be perfect habitats for a variety of species. As plenty of often empty niches are available as structures can be of recent origin and native hard substrate communities are not that rich as at least in systems like the Scheldt estuary there is originally almost no natural hard substrate available, artificial hard substrate environments might be more susceptible to NIS. However, the current study shows that at least the environmental conditions should potentially be suitable for a variety of species; the likelihood of the presence of one or several NIS is increased in habitats where high species richness can be expected. The opposite also appears to be true with an extreme example from this study where the rather unsuitable habitat containing steelslag appeared to be very species-poor and did also not contain NIS at all (which is an exception for the range of habitats present in the Scheldt estuary).

4 Discussion

4.1 Efficiency of the methodology

The aim of the methodology was to detect all non-indigenous species that were locally present (i.e. within a transect covering an intertidal zone over approximately 100 meters) on hard substrate. The choice for hard substrate was made as it was expected that this would lead to the detection of several NIS that might be overlooked by extensive soft substrate monitoring programs in place at least in the Scheldt estuary. Hard substrate is expected to be a habitat hardly investigated whereas the potentials for NIS to settle are plenty and increasing due to the continuous construction and/or renewal of artificial substrates. Additionally it was chosen to focus on intertidal zones as those are characteristic for estuaries, contain a variety of different habitats within small areas (e.g. tidal gradients, hydrodynamic exposure, substrate types, etcetera), relatively easy to access and therefore relatively quick to inventory. We are aware that with this choice the predominantly subtidal species and pelagic species are largely missed (for those species other techniques are needed). What we tested is whether we could apply a standardized methodology, observing nearly all locally present non-indigenous species, get an indication of their abundance and collect additional valuable information about the habitats and communities so that eventually connections and relations can be made, and that local information can be extrapolated to other regions or give valuable information for further (focused) research, predictions and/or policy. We therefore chose to work with three intertidal strata and the inventory of quadrants within those, after which surroundings were checked for additional species. The quadrants give the standardized information for statistical analyses and comparisons.

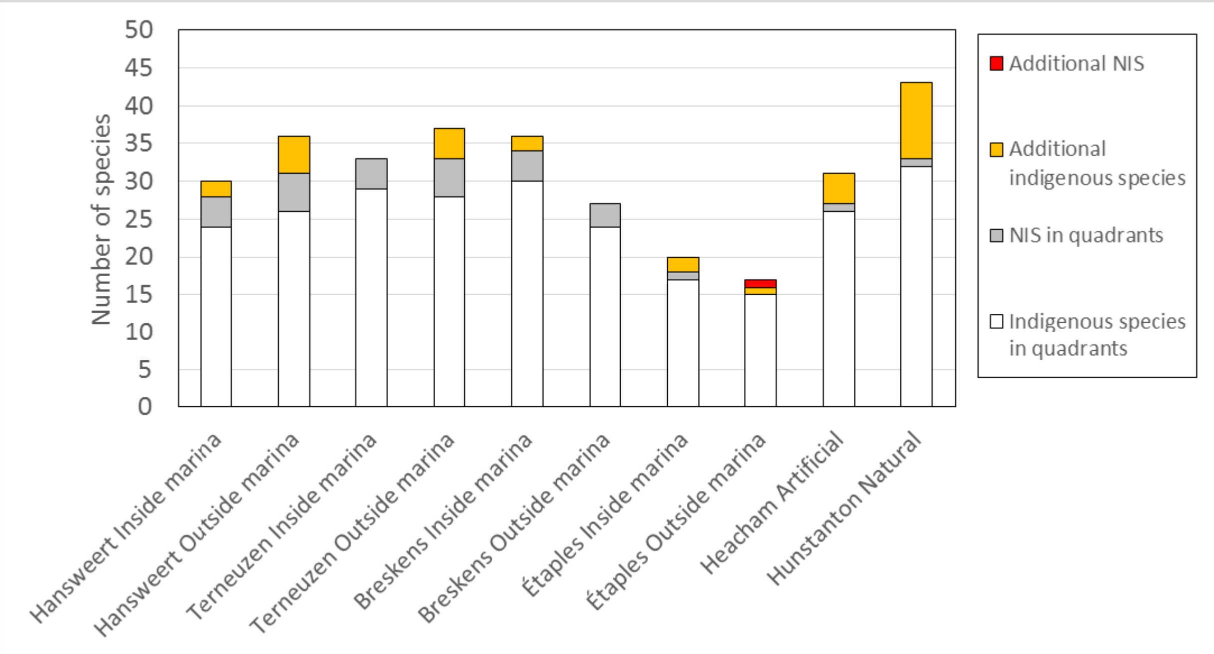
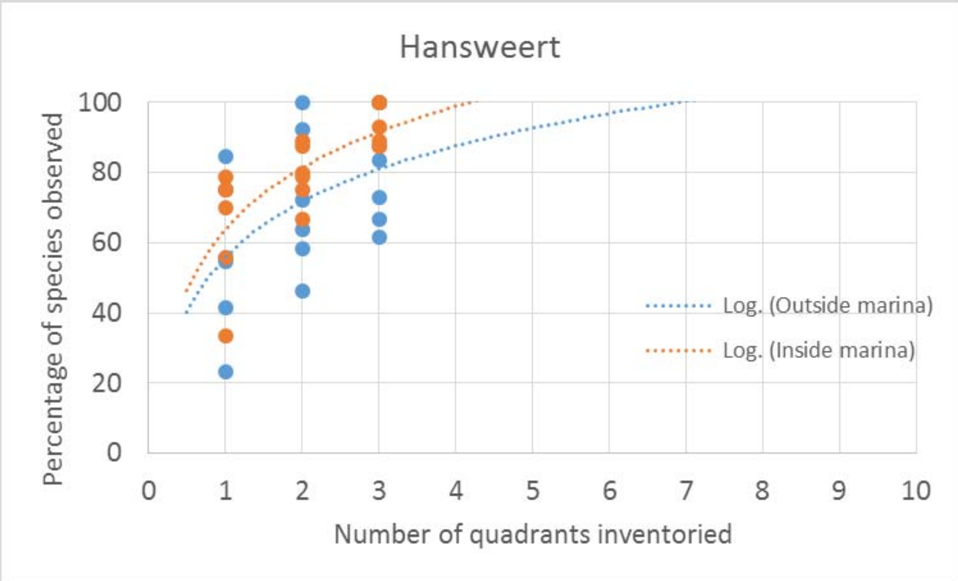
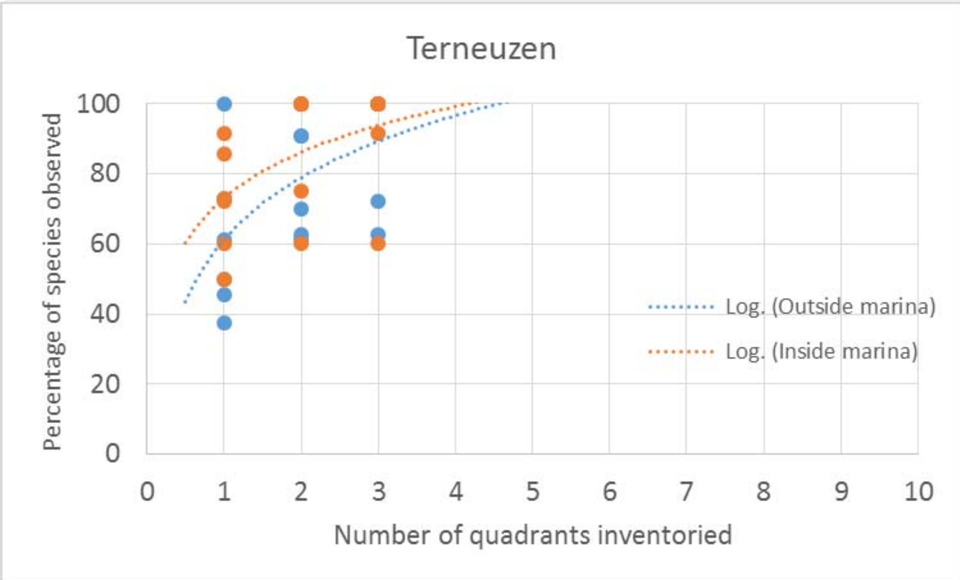


Figure 29. Total number of species observed in entire transects distinguishing the indigenous – and non-indigenous species, and indicating the number of species only observed outside quadrants (additional).

The choice for three intertidal zones generally works very well. At most sites three zones can be distinguished, and additional zones are not necessary. Exceptions are however sites with abrupt changes in substrate within distinguished strata (generally the high or middle intertidal zone), where substrate types harbor very different communities. Also the lower intertidal zone could sometimes be split in two, especially when an oyster reef was present or a predominantly soft substrate zone with hard elements. On the other hand, there were cases where only two clear zones could be distinguished (generally due to the lack of hard substrate in one of the zones), or even one (like at the Canche outside the marina) as there the upcoming tide was limited due to a riffle in the system. As already done during this pilot, we suggest to only distinguish 1 or 2 zones if no (more) differences in habitat structure can be distinguished. In case of the presence of 4 or 5 clearly distinguished zones, it would be wise to take 3 quadrants in each of the zones, stick to a deviation in 3 intertidal levels, but

distinguish habitat types in the name (e.g. low intertidal basalt and low intertidal oyster or middle intertidal basalt and middle intertidal asphalt).
The results of the inventories show that inventorying 3 quadrants per zone is generally sufficient to collect all NIS locally present in that zone and get an idea about their abundances (Figure 29). Only in the transect outside the marina of Étaples a non-indigenous species (i.e. *Austrominius modestus*) was found solely outside the quadrants. It has to be mentioned that this is exactly the transect where due to the limited intertidal difference and rather steep slope only one intertidal zone was distinguished and therefore in the entire transect only 6 quadrants instead of 18 were inventoried. It appears that in practice occasionally a NIS is missed for a certain intertidal zone in a transect, when only 6 quadrants in a stratum are inventoried, as indicated by an occasional observation of a NIS among the additional species. However these additional NIS were then always found in quadrants of other strata of the same transect, resulting in a complete list of NIS for each of the transects solely based on the quadrant information. One can even debate whether such an additional NIS in a certain stratum is typical for that zone or actually is a species usually observed in another stratum where it is observed in the quadrants.





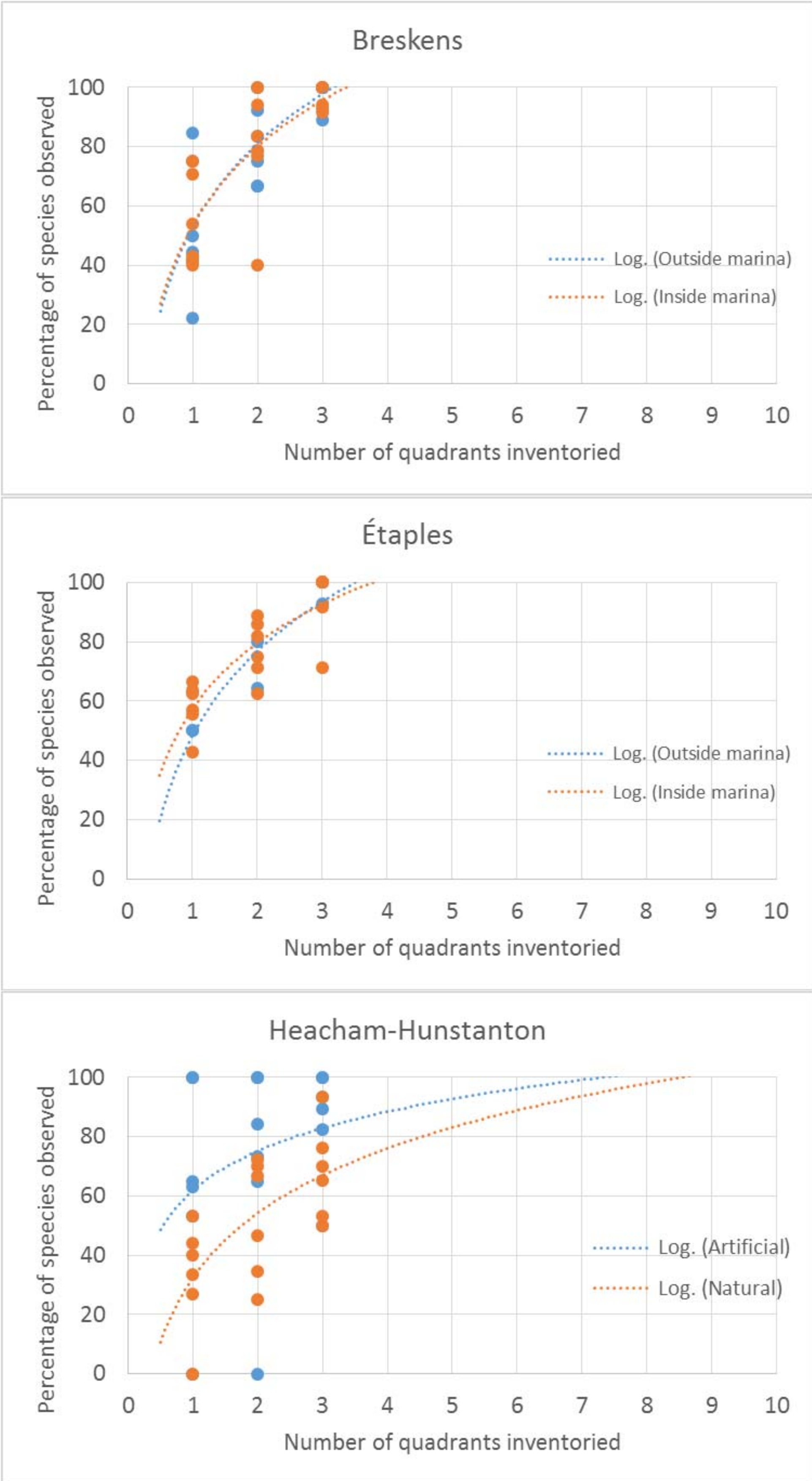


Figure 30. Trends in total number of species observed per habitat (line x stratum combination) with the inventory of an additional quadrant.

Taking all species (non-native and native) into account, generally only one or two additional species are found for each stratum x line combination (i.e. additional to only 3 quadrants). Figure 30 shows the percentage of species relative to the total number of species present, found with either 1, 2 or 3 quadrants for each of the inventoried transects and the calculated average trend showing the effect of inventorying additional quadrants. With 3 quadrants always at least 50 % of the species in a certain stratum x line combination are found, in the Western Scheldt at least 60 % of the species are found and in Breskens more than 88 % of the species was always observed with 3 quadrants. That in most cases even a much higher percentage of the total available species in a line x stratum combination is found with 3 quadrants is indicated by the trends showing that on average more than 80 %, more than 90 % and even more than 95 % of the species was found in this way at respectively Hansweert, Terneuzen and Breskens. Also at Étaples the effectivity was very high, only at the transects in the Wash, and the natural strata and lines in particularly, the efficiency was somewhat lower. Trends indicate how much quadrants are on average necessary to observe all species available within a line x stratum combination, but it has to be taken into account that the most important aspect of the monitoring is to find all species present where it is of lesser importance in which habitat they are found. Figure 29 already showed that it is about 0 to 5 species per transect that are overlooked when solely inventorying quadrants (i.e. 18 quadrants if 3 strata can be distinguished), which equals 0 to <14 % of the species (except for the natural substrate at Hunstanton where almost 25 % of the species was missed). However, the most important goal is not to detect all species, but all NIS. As NIS make up 11 to 14 % of the species on the total of species in a transect in the Western Scheldt, and only 2 to 6 % in the Wash and the Canche, in a worst case scenario: 0,25 x 14 % = less than 4 % of the NIS locally present (< 2 % for the Western Scheldt) is missed by inventorying quadrants according to the transect monitoring methodology. Taking into account that during this study never more than 5 NIS in the same transect were observed, the chance of missing one of them is rather small. Unfortunately, at Étaples (outside the marina) we did miss a NIS with the transects; however this is exactly the transect where not 18 but only 6 quadrants were inventoried. One can therefore debate whether it is essential to always inventory more than 6 quadrants, even if only one intertidal zone is present. As inventorying the quadrants is actually a way to standardize the methodology allowing comparison of communities and NIS assemblages in particular with their abundance indications, it is more straightforward to maintain the search for additional species as part of the methodology. Moreover as small deviating habitat parts can exactly be the niche for additional species and NIS in particular (and those do generally by chance not end up exactly in a random quadrant). As it can be exactly locally rare NIS living in these a-typical habitats that can become problems in the future.
At Hunstanton a rather large number of species was missed with the quadrant inventories, and it is just that NIS are uncommon there, that they are not among the species initially missed. Such sites are exceptions where clearly more than three zones are present, or very heterogeneous environments with many different habitats as actually only observed in the Wash on natural hard substrate. There it is of importance that in all common habitats 3 quadrants are inventoried.
The two Belgian sites are not considered in the efficiency analyses as there the additional species are scored for the entire line (from the high to the low intertidal zone) as a whole, which is according to the original protocol as can be found in Annex 1, but does not allow analyses as presented here. We are however aware of the fact that exactly at these sites in total 3 NIS were observed only outside the quadrants. It is unclear whether this is the result of the possible presence of more than 6 important habitat types (which does not seem to be the case on first sight), whether they are related to a-typical elements in the transect, or whether they undermine our likelihood calculations as presented. The additional NIS however confirm the importance of searching for additional species as part of the methodology.

An additional remark has to be made: One assumption that has been made in the above calculations is that with the search for additional species actually all species present in the transect were collected. It is agreed that there is always a chance of missing species in the search for additional ones. The largest chance of missing species is however not, not seeing them, but not recognizing them! In the first place this can happen in the field where you think that you have a certain species that after checking in the laboratory appears to be another species (which makes you doubt about the presence of the one or the other species or even co-occurrence in one of the other quadrants). It is therefore highly recommended to collect several specimens (if present) of each seemingly species in the field, unless there can be no doubt about the identification with the naked eye.

We did not expect already on forehand that a total of 5 sites, consisting of 10 transects, which makes 20 lines through various substrate types would deliver us all NIS present in the intertidal zone of the

Scheldt estuary. However, we are certain that a line gives a representative view for that type of substrate considering that the salinity and hydrodynamics do not differ too much. Taking that into consideration it is of importance to do a habitat mapping (or collect the information about the substrates used for dike sections) along the entire Scheldt estuary. Doing that, this could mean that a certain line is representative for kilometers of dike, after which the number of inventories necessary to get a near complete view of all hard substrate NIS present in the intertidal zone can be calculated. After all it will always be a trade-off between observing the next additional NIS and the efforts you want to invest.

4.2 Estuary comparison

Although in both estuaries of the Canche and the Wash only two transects at approximately one site (i.e. restricted to a certain salinity) were inventoried whereas for the Scheldt estuary we did get a better view of the entire salinity transect, it can already be concluded that there are some striking differences between the systems concerning the presence of NIS.

Non-indigenous species play a prominent role in the communities of the Scheldt estuary and the ecosystem in its entirety. Figure 28 showed the larger percentages of NIS species present in the communities of the Scheldt estuary than in those of the other two estuaries where actually only one species was found (*Austrominius modestus*). In both cases the limited number of NIS found, probably has to do with a limited connectivity with hotspots of NIS with similar conditions. It has to be seen if we would come to the same conclusion if we had inventoried the high salinity part of the Canche as it seems that marine NIS can easily enter the mouth of the estuary in a natural way from the south and the north where several species are present in the vicinity. The Wash would be a nice example to study the entire salinity gradient as it seems to be relatively NIS free, but risks are approaching from all sites (marine NIS are more and more recorded from international ports and marinas in the north and in particular the south of the English east coast). Also several NIS have been recorded recently in the English freshwater systems, and also in England all larger systems (including the Wash) are connected with a network of canals and waterways.

Going more to the level of taxonomic groups and species, it is clear that several groups have their invaders in the Scheldt estuary. The current data show the examples of competition between Pacific oysters and native blue mussels, competition between *Hemigrapsis takanoi*, *H. sanguineus* and *Eriocheir sinensis* as invaders and the native *Carcinus maenas*, the emerging patterns around the *Melita* competitors (*M. nitida* and the native *M. palmata*), and we will probably find similar competition when we focus on the other less abundantly observed NIS during this study. For these cases of competition it is interesting to make the comparison with the uninvaded system of the Wash (we did already see communities with large abundances of *Mytilus edulis*, *C. maenas* flourishing and *M. palmata* abundantly present locally) and look at the entire salinity gradient. We might already have observed native species remaining in refuges as observed for *C. maenas* in the Canche. Only for the barnacle communities we see similar patterns in each of the systems: Complete dominance of the barnacle communities by *Austrominius modestus*. That might be a warning if connectivity increases and NIS are not taken care of.

In the overall analyses of NIS assemblages in relation to their surroundings (Figure 27), typically the Belgian communities are deviating more from all the other communities than for instance the Dutch, French and English do from each other. Although we do not expect a large difference in methodology used at the different sites, we can not rule out a slight observer effect that must be in different identification of similar species and/or whether groups are identified to the same level. We cannot rule out an observer effect as no joint inventory has taken place between the INBO and the NIOZ (as both were dependent of the same tide for their monitoring) and no specimens have been exchanged during the project. It has to be noticed that it is however very unlikely that such an observer effect has influenced the NIS assemblages observed.

4.3 Towards the monitoring of entire systems

The current pilot study resulted in observations of 10 non-indigenous species in the Scheldt estuary of which one (*A. modestus*) was the only NIS observed in both the Canche and the Wash. Although the current study did not result in the observation of new NIS for the systems (although *A. modestus* was not recorded in the quick listing of NIS for the Wash yet; Owen, 2015), at least for 2 species significant range extensions within the Scheldt estuary were observed. So far, *Melita nitida* was not recorded for the polyhaline zone of the Western Scheldt yet, whereas now the species is found to be common to abundantly present at all Western Scheldt sites, and besides that also present in Doel. This clearly at the expense of the native *Melita palmata*. Also the observation of *Diadumene lineata* is a significant

range extension eastwards in the Western Scheldt, although the population might origin from the Eastern Scheldt as well. In case of the anemone an increasing impact or risk for other species is not directly expected as the species is already present in the Dutch delta waters for several decennia and does not seem to have had a large impact before, where it is a common phenomenum that the species suddenly appears at certain sites and disappears again after a while. This shows that already with the inventory of a limited number of sites clear patterns and results are obtained on population developments of NIS is estuaries. The results are also of importance to identify patterns and developments in competition between more common non-indigenous species (of which we might think that know a lot) and native species. Examples are the appearing niche segregation in *Hemigrapsus* and *Carcinus* populations, and patterns in the distribution of *A. modestus*. The findings do also shine an important light on the discussion whether Japanese oyster reefs might enhance (local) biodiversity and whether species rich habitats are less susceptible towards NIS settlement (to be clear in both cases the answer is no!), and give indications about what relative uninvaded communities might look like and whether there are differences between natural and artificial hard substrates in susceptibility towards NIS. What we can also learn from this study is that different types of substrate harbor different species asemblages which also accounts for the NIS present in the system. Playing with different substrates in system management might enhance native species population developments and reduce the success and/or potential impact of NIS.
To be clear, these are all results of a pilot study with a limited number of sites involved only visted once.
The methodology has been proven to give a good and accurate view of locally present non-indigenous species assemblages and the communities and environments they are in. To extent monitoring to estuary broad inventories to achieve a listing of all NIS present on hard substrates in the intertidal zones of an estuary, it is of importance to get a view of all available hard substrate types and their distributions within the estuary. In that way the representativety of a transect and the number of transects necessary for a near-complete view can be determined. Recurrent vists in time (e.g. via a scheme of visits every few years) eventually via a roulating system so that every year several but not all sites are visited can result in an efficient monitoring tool to identify developments in NIS communities, dispersal and impacts. Such a monitoring can be used as an early warning system although the frequency of visits and number of sites determines how effective transect monitoring as an early-warning tool is. The standardized methodology has proven here to be efficient in system or habitat comparisons, for impact studies and to improve our understanding of fundamental processes. It is expected that transect monitoring can be an efficient tool for water/system management evaluation as well.

4.4 Recommendations

Several recommendations can be made, but it is of importance to see them within the scope of future plans concerning NIS research, awareness, policies, treatments and prevention of introduction. So first the aim of additional research should be clearified, and then recommendations can be made how to prolong and/or adjust the current research using transect monitoring as the central methodology in NIS observation.

4.4.1 Recommendations to fine-tune the methodology:

- **Distinguishing habitat types if strata are clearly subdivided**To fine-tune the methodology it is recommendated to sometimes distinguish two different habitats that are both inventoried with 3 quadrants if one of the strata clearly consists of 2 different types. This would result in now and than transect lines consisting of 12 instead of 9 quadrants.

- **Inventory a minimum of 6 quadrants per transect line**
  However in cases where only 1 or 2 strata can be distinguished it is fine to reduce the number of strata, although a minimum of 6 quandrants to be inventoried per transect line is recommended (to reduce the likelihood of missing species by chance).

- **Always search for additional species**
  It is recommended to maintain the additional search for species, especially as certain microhabitats like small pools in holes in the substrate or a-typical substrate elements like wood or debris are likely not part of the random quadrant inventory, but can exactly be where

locally rare NIS live and it can be exactly these species that can become problems in the future.

- **Always a joint inventory when with more partners**
  Although the methodology as such is not that difficult and rather straight-forward, it is recommended to at least do a koint inventory if more partners are involved, as it can be just small aspects that are interpreted in a different way that might reduce the comparability of results.

- **Collect several specimens of each species for each quadrant**
  It is also recommended to collect several specimens of each seeming species in the field for each quadrant, as specimens might belong to other or different species after a closer look in the laboratory. If more specimens are collected, even in the case of those belonging to different species, some kind of abundance indication can be given.

- **Exchange of specimens between partners**
  Similarly it is recommended to store those specimens at least till the end of the project and take pictures of all ‘new’ species. If more laboratories are involved in the identification it is recommended to exchange at least some potential problematic species.

4.4.2 Recommendations towards SEFINS

The results showed that already a monitoring program with just a few transects can give valuable information about the NIS present in systems, their distributions, habitat preferences and potential impacts. Therefore for SEFINS it is recommended to put a monitoring in place in phase 3 that also covers the salinity gradients present in the Canche and the Wash as well. Moreover as already with the inventory of just one site in the Canche and the Wash, the estuary comparison gives interesting results related to what communities look like with a limited impact of NIS. By putting a recurrent monitoring program in place, which can make use of an alternation of sites to be inventoried in different years to minimize the efforts, insights in developmental patterns for NIS populations can be gained.
If it is concluded that not much information is available about soft sediment communities, and soft sediment related NIS in particular, it can be considered to extent the inventory to soft sediment environments as well.

For further recommendations on combining techniques to come to an efficient monitoring tool for the future see Wijnhoven & Gittenberger (2015).

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6 Annexes

6.1 Annex 1. SEFINS Protocol Transect monitoring.

SEFINS Protocol Transect monitoring

Based on the EMBOS (pan-European Marine Biodiversity Observatory System) protocol for hard substrate monitoring.

Selection of observatory site:
The methodology is typically designed for the monitoring of non-indigenous species, and the (indigenous) communities in which they are found, in hard substratum environments, that can be either artificial or of natural origin, of the intertidal zone. Ideally locations for transects are chosen in such a way that the entire intertidal gradient (from the high to the low intertidal zone) is relatively easy accessible and consists of hard substratum. This means that the gradient should not be too steep (and preferably not be a vertical wall) so that you can walk the entire transect (if necessary using a rope tied on top of the transect as the hard substratum can be slippery). There might be locations where the hard substratum transitions into a soft substrate environment particularly in the lower intertidal zone. Such location is perfectly suitable if at least a certain part of the lower intertidal zone consists of hard substrate (the soft substrate parts will not be part of the monitoring). In regions where hard substrates largely lack, it is an option to monitor only a part of the intertidal gradient (the part where hard substrate is present).
For the SEFINS pilot study the focus will be on sites where transects can be done inside marinas and outside marinas (in the vicinity of the selected marinas). The combination of a transect inside and outside a marina is called a site (a site consists of 2 transects). If in certain regions no marinas with suitable hard substrate can be found, another site with for instance artificial and natural hard substrate can be selected. Ideally sites will be selected where also SETL plates are installed (so that the results of the 2 complementary techniques can potentially be combined). If not, no problem; we will do with whatever is available, as this is still a pilot study.

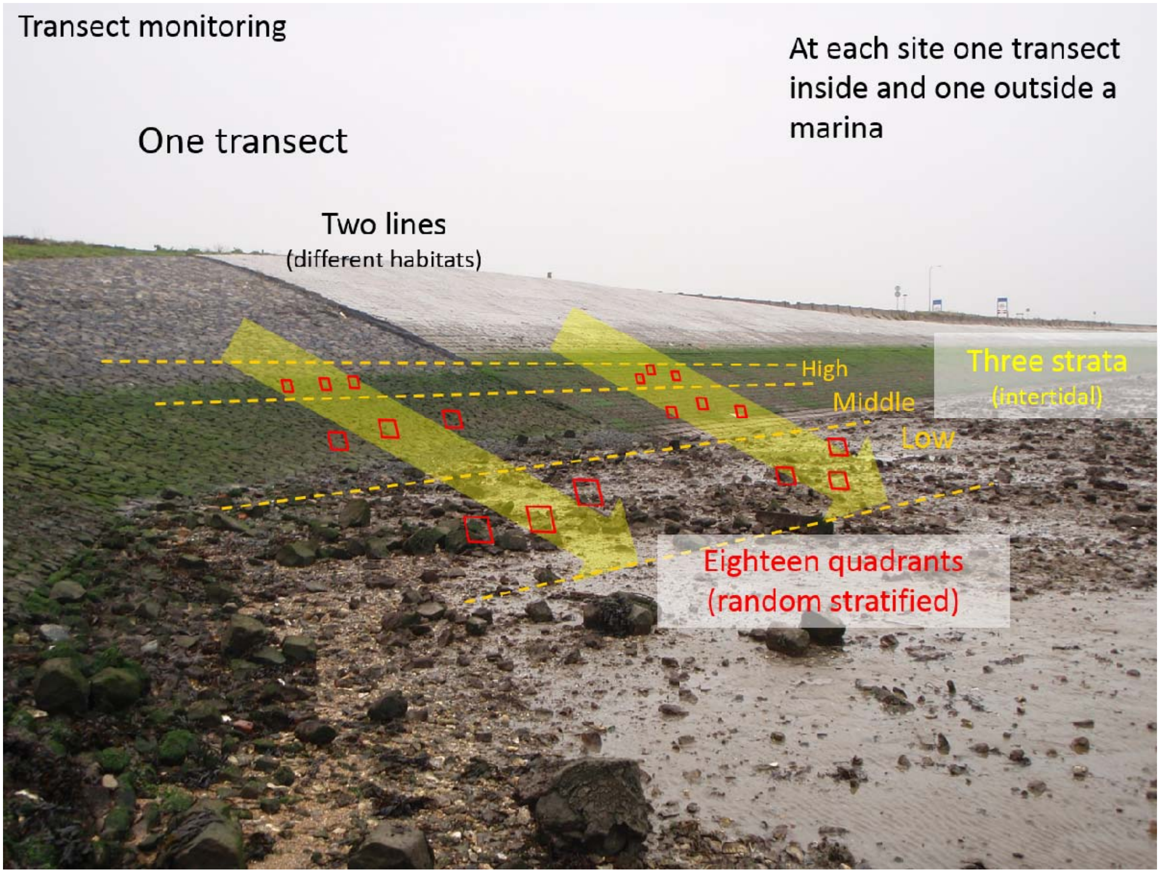


Fig. 1. An example of a transect showing the ideal positioning of lines and strata and the random positioning of 18 quadrants.

Selection of transects:
A transect consists 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 Fig. 1 as an example).

Selection of strata:
Habitats also differentiate with the height in the intertidal zone (e.g. exposure time). For each transect 3 intertidal strata will be distinguished; further called the high -, middle – and low intertidal zone. The 3 different strata will be distinguished by visual observation, dividing the hard substrate gradient in 3 zones by the 2 most distinguishing imaginary horizontal lines (and draw borders at the lowest low water level and the highest high water level) (see Fig. 1 as an example).

Characterization of sites and transects:
For each transect some standard characteristics and parameters will be noted down and transects will be captured with standard photographs. For the characterization of a transect write down or fill in the digital field-form as can be found as an attachment. As weather conditions can be suboptimal and it is likely that some of the paper-works can get wet, we recommend to use a water resistant notebook to write down your findings in the field (on which you do already write the parameters you have to note in the field before you leave; collected data can be filled in in the digital field-form (excel-file) after returning .

What will be noted down for each transect are:

- Country, Estuary and CODE
(e.g. The Netherlands, Scheldt and NL-SCHE; other options EN-WASH and FR-CANC)

- Observatory site name and CODE
(e.g. Terneuzen and TERN or Breskens and BRES)

- Transect name and CODE
(*e.g. Inside marina and INMA, Outside marina and OUMA or Natural chalk and NACH)*

- Names of observers with abbreviation of their institute/organization
(e.g. Sander Wijnhoven, NIOZ or Sharron Bosley, WNNSCEMS)

- Date of inventory
(e.g. 15-06-2015)

- Coordinates of the center of the transect (Latitude - Longitude)
(e.g. 51°20'24 N - 3°49'45 E)

- Tidal range in meters (approximate average year values between highest high water and lowest low water level if available, otherwise an indication from a tidal table will do)
(e.g. 5,8 m)

- Average salinity and salinity range (approximate average year values can be given if available, otherwise a measurement as indication will do)
(e.g. 22 with range 20,5 – 23,5)

- Approximate width of each intertidal hard substrate zone from high to low intertidal (high – middle – low) as measured in the field
(e.g. 9 – 24 – 31 m)

- Give a short description of the transect in terms of type of substrate, positioning, surroundings and type of habitat
(e.g. transect on basalt slope of embankment inside a sheltered marina with rubble in the lower intertidal zone, largely overgrown with brown and green algae; just opposite of floating jetties with several yachts)

- Provide a map with the positioning of the transect (e.g. as a dot on a google earth map) and call the file according to the above described coding using indents: *e.g. NL-SCHE-TERN-INMA-15-06-2015-Map*

- Provide photograph(s) with an overview of the transect (preferably two: one from the viewpoint at the low water line (or where the hard substrate starts) and one from the viewpoint above the high water line) and call the file according to the above described coding using indents: *e.g. NL-SCHE-TERN-INMA-15-06-2015-photo1*
(It is possible to give appropriate names to photos after you return from the field, but be sure that you write down photo numbers and their contents in the field so that you know what is on it and where they were taken).

Inventory of transects:
In each transect 18 quadrants measuring 0,5 by 0,5 meters will be inventoried (see Fig. 2 as an example of a quadrant; the wiring is not essential but can help to estimate the coverage by flora and fauna; you can prepare a quadrant of any material you like, as long as the inside measures 0,5 by 0,5 meters). The quadrants are equally distributed over the 2 lines and 3 strata which leads to 3 inventoried quadrants (randomly placed) for each line x stratum combination (the methodology is therefore randomly stratified). Each quadrant is named according to a standard CODE following the above mentioned CODE for the transects and completed with:

- an indication for the line (from the low water line viewpoint either left (LE) or right (RI)
- an indication of the stratum (either high intertidal (HI), middle intertidal (MI) or low intertidal (LI)
- and a replicate number (of your choice for a quadrant either replicate 1 (R1), 2 (R2) or 3 (R3)

As an example a quadrant can therefore be named: NL-SCHE-TERN-INMA-15-06-2015-LE-HI-R1. We use such a standardized name as it can easily be split up in all separate components which is the start of a database, and this pilot can easily be extended with a multitude of quadrant inventories using the same nomenclature.

First for each quadrant the coverage of the substrate by flora and fauna is noted (the total surface includes the sites of boulders, etc, so the total surface in 3D environments can be more than just visible from above in a photo):

- Provide percentage of coverage by the total of species (flora and fauna) without removing any canopy.
- Provide percentage of coverage by macroalgae alone without removing canopy and provide names of the dominant ones (those covering more than 20 %) if applicable (also look for species underneath the top coverage as there can also be species belonging to the dominant ones).
- Provide percentage of coverage by (sessile) macrofauna (including under the canopy) and provide names of the dominant ones (those covering more than 20 %) if applicable.

Than in each quadrant all species (macrofauna and macroalgae, clearly visible to the naked eye) will be noted with an indication of its abundance or coverage for which we only use 3 categories:

- 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).

To speed up the inventory process in the field, a species checklist can be prepared before going into the field, so that on the site only A's, C's and R's have to be noted on a field data sheet prepared for each separate quadrant.
Of course it is not of use to make an extensive check list, so expected very uncommon species should not be on the checklist (and can be noted on the list when occasionally found).
It is likely that certain species cannot be identified in the field. Those should be noted with a name indicating the type of species (e.g. gammarid 1, red algae 3, etc.) and should be collected in already prepared storage bags or containers with a clear indication of the quadrant on it or in it; for taxonomic identification afterwards in the laboratory. It is an option to make numbered bags (preferably numbers on it and numbers on water resistant paper in it), so that you only note the number of the bag and what is in it in the field (write it down with the notes belonging to the quadrant you are inventorying).
If you suspect that in the field unidentifiable specimens might belong to more species, the shared abundance should be noted and several specimens should be collected for identification afterwards so that from the subsample the relative abundance of each species can be identified (e.g. several specimens of *Bathyporeia* 1 (A) will be collected which might be *Bathyporeia sarsi* (A) and *Bathyporeia pilosa* (F).
If it is unlikely that specimens are identified the same day, those should be preserved with formaldehyde before storage.

A photo is taken of each quadrant perpendicular on the surface so that the quadrant fills most of the picture (see for example Fig. 2).

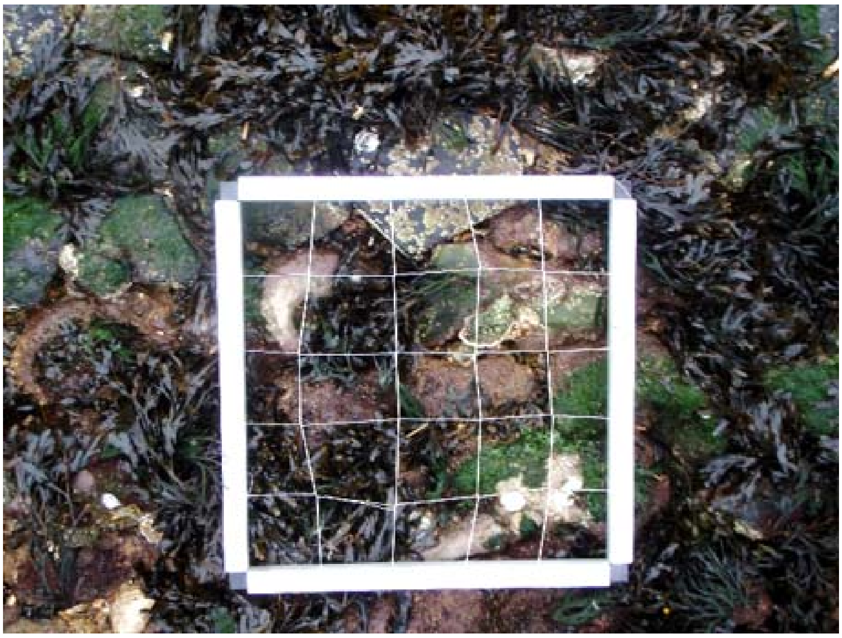


Fig. 2. An example of a picture of a randomly placed quadrant.

Additionally the entire stratum is investigated by two researchers for 5 minutes on supplementary species. Take into account that this also accounts for the lower intertidal zone (so do at least this part at the time that it is still possible with respect to the tide). The additional species are noted as would be done with a quadrant (with indication of relative average abundance with A, C or R measured for the average coverage or abundance in the vicinity reflected on a surface of 0,25 m^2.

The list of additional species should be coded as 'ADD', which for example leads to CODE like: *NL-SCHE-TERN-INMA-15-06-2015-LE-HI-ADD.*

Please identify species as much to the species level as possible: is case of difficulties take pictures and/or contact me to send specimens for identification by specialists of the Monitor Taskforce of the NIOZ.

We hope to see your results filled in in the digital field-forms (one form for each transect) before June the 23rd (2015) so that we can use them in the statistical analyses for the SEFINS report.

Success, best wishes, Sander Wijnhoven

For questions contact me at +31-113-577357 or sander.wijnhoven@nioz.nl

Packing list for fieldwork:

- Photo camera + spare batteries
- Quadrant (0,5 x 0,5 m)
- Tape measure (e.g. 30 m)
- Sufficient long rope for difficult to access and/or slippery sites
- (Pre-prepared) notebook
- Pencil (+ spare one) that work under wet conditions
- Waterproof marker (+ spare one)
- Field clothing (warm and dry, e.g. raincoat and good shoes or boots for slippery surfaces)
- Bags and/or containers (to collect specimens) and waterproof labels (numbered) to put in the bags
- Formaldehyde if samples cannot be transported to the laboratory the same day
- Identification keys and/or booklets
- GPS
- Watch
- Magnifying glass
- Light-colored bins (photo tray?) to observe collected specimens
- GSM (in case of emergency; and tell a colleague where and when you are going in the field)
- Tweezers
- Towel
- Pocket knife
- Sieve (e.g. 1 mm mesh size)
- Spray bottle
- Backpack
- Permission to do fieldwork (might be necessary for certain areas)

6.2 Annex 2. Digital field forms as provided before the start of the inventories

Field forms consisted of 3 tabs.

a) Site characterization

Site characterization							
	Country	Estuary	Observatory site	Transect name	Date of inventory		
Full name	Netherlands	Scheldt	Hansweert	Inside Marina	day	month	year
CODE	NL	SCHE	HANS	INMA	6	5	2015
	Name 1	Name 2	Name 3	Name 4	Name 5	Name 6	Name 7
Observers	Anke Engelberts	Sander Wijnhoven					
Institute/Organization	NIOZ-MON	NIOZ-MON					
	LE:				RI:		
Coordinates	Degrees (°)	Minutes (')	Seconds (")	Direction (N,S,E,W)	Degrees (°)	Minutes (')	Seconds (")
Latitude				N			
Longitude				E			
Tidal range (m)							
	average	low	high				
Salinity (PSU)							
	high	middle	low		high	middle	low
Width of zone (m)							
Transect description							

b) Field data sheet

Only the table for the left line is shown (there is a similar table for the right line), and the table continues so that additional species can be added.

Field data sheet	*To list species and their relative abundance*											
CODE	NL-SCHE-HANS-INMA-6-5-2015											
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD
CODE	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH
Coverage (%)												
Total species coverage												
Macroalgae coverage												
Sessile fauna coverage												
Dominant macroalgae												
Spec 1												
Spec 2												
Spec 3												
Spec 4												
Spec 5												
Dominant macrofauna												
Spec 1												
Spec 2												
Spec 3												
Spec 4												
Spec 5												
Species	(A/C/R)											

c) Photo list

Only the table for the left line is shown (there is a similar table for the right line), and the table continues so that additional species can be added.

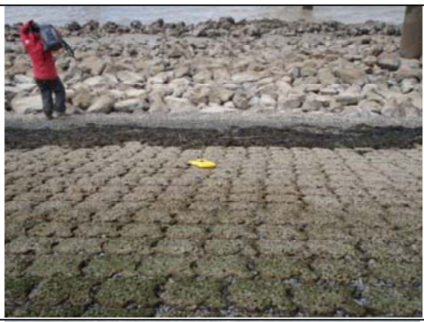
Photo list	*To list photo numbers*											
CODE	NL-SCHE-HANS-INMA-6-5-2015											
Overview high to low	LE:											
Overview low to high	LE:											
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD
CODE	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH	NL-SCH
Detail												
Additional (give indication, e.g. species name)												

6.3 Annex 3. Overview of the inventoried lines (left and right line through different habitats) forming a transect, with a view from the high to the low intertidal zone and vice versa.

Transect line (half of a transect)	View from high to low intertidal.	View from low to high intertidal.
Breskens - Inside marina – Left line (BRES-INMA-LE)		
Breskens - Inside marina – Right line (BRES-INMA-RI)		

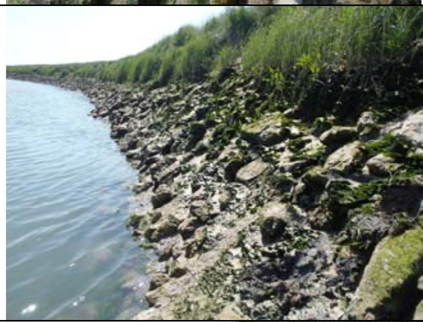
Breskens - Outside marina – Left line (BRES-OUMA-LE)		
Breskens - Outside marina – Right line (BRES-OUMA-RI)		
Terneuzen - Inside marina – Left line (TERN-INMA-LE)		

Terneuzen - Inside marina – Right line (TERN-INMA-RI)		
Terneuzen - Outside marina – Left line (TERN-OUMA-LE)		
Terneuzen - Outside marina – Right line (TERN-OUMA-RI)		
Hansweert - Inside marina – Left line (HANS-INMA-LE)		

Hansweert - Inside marina – Right line (HANS-INMA-RI)		
Hansweert - Outside marina – Left line (HANS-OUMA-LE)		
Hansweert - Outside marina – Right line (HANS-OUMA-RI)		

Doel - Inside marina – Left line (DOEL-INMA-LE)		
Doel - Inside marina – Right line (DOEL-INMA-RI)		
Doel - Outside marina – Left line (DOEL-OUMA-LE)		
Doel - Outside marina – Right line (DOEL-OUMA-RI)		
Wintam - Zeeschelde – Left line (WINT-SCHE-LE)		

Wintam - Zeeschelde – Right line (WINT-SCHE-RI)		
Wintam - Rupel – Left line (WINT-RUPE-LE)		
Wintam - Rupel – Right line (WINT-RUPE-RI)		
Étaples - Inside marina – Left line (ETAP-INMA-LE)		

Étaples - Inside marina – Right line (ETAP-INMA-RI)		
Étaples - Outside marina – Left line (ETAP-OUMA-LE) (*From left to right and right to left respectively, instead of from high to low and low to high)		
Étaples - Outside marina – Right line (ETAP-OUMA-RI) (*From left to right and right to left respectively, instead of from high to low and low to high)		
Hunstanton – Natural hard substrate – Left line (HUNS-NATU-LE)		

Hunstanton – Natural hard substrate – Right line (HUNS-INMA-RI)		
Heacham – Artificial hard substrate – Left line (HEAC-ARTI-LE)		
Heacham – Artificial hard substrate – Right line (HEAC-ARTI-RI)		

6.4 Annex 4. Species list

Nr	Taxon	Scientific name	Kingdom	Phylum	Class	Order	Family	Genus	Name analyses	NIS
1	Acari	Acari	Animalia	Arthropoda	Arachnida	SUBCLAS-Acari	SUBCLAS-Acari	SUBCLAS-Acari	SUBCLAS-Acari	
2	Actinia equina	Actinia equina	Animalia	Cnidaria	Anthozoa	Actiniaria	Actiniidae	Actinia	Actinia equina	
3	Aglaothamnion tenuissimum	Aglaothamnion tenuissimum	Plantae	Rhodophyta	Florideophyceae	Ceramiales	Callithamniaceae	Aglaothamnion	Aglaothamnion tenuissimum	
4	Alcyonidium condylocinereum	Alcyonidium condylocinereum	Animalia	Bryozoa	Gymnolaemata	Ctenostomatida	Alcyonidiidae	Alcyonidium	Alcyonidium condylocinereum	
5	Alitta succinea	Alitta succinea	Animalia	Annelida	Polychaeta	Phyllodocida	Nereididae	Alitta	Alitta succinea	
6	Amphibalanus improvisus	Amphibalanus improvisus	Animalia	Arthropoda	INFRACLASS-Cirripedia	Sessilia	Balanidae	Amphibalanus	Amphibalanus improvisus	
7	Apohyale prevostii	Apohyale prevostii	Animalia	Arthropoda	Malacostraca	Amphipoda	Hyalidae	Apohyale	Apohyale prevostii	
8	Arenicola marina	Arenicola marina	Animalia	Annelida	Polychaeta	CLAS-Polychaeta	Arenicolidae	Arenicola	Arenicola marina	
9	Ascophyllum nodosum	Ascophyllum nodosum	Chromista	Ochrophyta	Phaeophyceae	Fucales	Fucaceae	Ascophyllum	Ascophyllum nodosum	
10	Ascophyllum sp. (juv)	Ascophyllum	Chromista	Ochrophyta	Phaeophyceae	Fucales	Fucaceae	Ascophyllum	GEN-Ascophyllum	
11	Assiminea grayana	Assiminea grayana	Animalia	Mollusca	Gastropoda	Littorinimorpha	Assimineidae	Assiminea	Assiminea grayana	
12	Aster tripolium	Aster tripolium	Plantae	Tracheophyta	PH-Tracheophyta	Asterales	Asteraceae	Aster	Aster tripolium	
13	Austrominius modestus	Austrominius modestus	Animalia	Arthropoda	INFRACLASS-Cirripedia	Sessilia	Austrobalanidae	Austrominius	Austrominius modestus	1
14	Autolytinae	Autolytinae	Animalia	Annelida	Polychaeta	Phyllodocida	Syllidae	SUBFAM-Autolytinae	SUBFAM-Autolytinae	
15	Balanus crenatus	Balanus crenatus	Animalia	Arthropoda	INFRACLASS-Cirripedia	Sessilia	Balanidae	Balanus	Balanus crenatus	
16	Bathyporeia guilliamsoniana	Bathyporeia guilliamsoniana	Animalia	Arthropoda	Malacostraca	Amphipoda	Bathyporeiidae	Bathyporeia	Bathyporeia guilliamsoniana	
17	Bembidion sp.	Bembidion	Animalia	Arthropoda	Insecta	Coleoptera	Carabidae	Bembidion	GEN-Bembidion	
18	Blidingia marginata	Blidingia marginata	Plantae	Chlorophyta	Ulvophyceae	Ulvales	Kornmanniaceae	Blidingia	Blidingia marginata	

19	Blidingia minima	Blidingia minima	Plantae	Chlorophyta	Ulvophyceae	Ulvales	Kornmanniaceae	Blidingia	Blidingia minima	
20	Brachyura (juv)	Brachyura	Animalia	Arthropoda	Malacostraca	Decapoda	INFRAORDO-Brachyura	INFRAORDO-Brachyura	INFRAORDO-Brachyura	
21	Bugulidae	Bugulidae	Animalia	Bryozoa	Gymnolaemata	Cheilostomatida	Bugulidae	FAM-Bugulidae	FAM-Bugulidae	
22	Campanularia sp.	Campanularia	Animalia	Cnidaria	Hydrozoa	Leptothecata	Campanulariidae	Campanularia	GEN-Campanularia	
23	Carcinus maenas	Carcinus maenas	Animalia	Arthropoda	Malacostraca	Decapoda	Portunidae	Carcinus	Carcinus maenas	
24	Ceramium cf virgatum	Ceramium virgatum	Plantae	Rhodophyta	Florideophyceae	Ceramiales	Ceramiaceae	Ceramium	Ceramium virgatum	
25	Cerastoderma edule	Cerastoderma edule	Animalia	Mollusca	Bivalvia	Veneroida	Cardiidae	Cerastoderma	Cerastoderma edule	
26	Chaetogammaru s marinus	Chaetogammaru s marinus	Animalia	Arthropoda	Malacostraca	Amphipoda	Gammaridae	Chaetogammaru s	Gammarus marinus	
27	Chaetomorpha linum	Chaetomorpha linum	Plantae	Chlorophyta	Ulvophyceae	Cladophorales	Cladophoraceae	Chaetomorpha	Chaetomorpha linum	
28	Chironomidae	Chironomidae	Animalia	Arthropoda	Insecta	Diptera	Chironomidae	FAM-Chironomidae	FAM-Chironomidae	
29	Chondrus crispus	Chondrus crispus	Plantae	Rhodophyta	Florideophyceae	Gigartinales	Gigartinaceae	Chondrus	Chondrus crispus	
30	Cirratulidae	Cirratulidae	Animalia	Annelida	Polychaeta	Terebellida	Cirratulidae	FAM-Cirratulidae	FAM-Cirratulidae	
31	Collembola	Collembola	Animalia	Arthropoda	Collembola	CLAS-Collembola	CLAS-Collembola	CLAS-Collembola	CLAS-Collembola	
32	Corophium arenarium	Corophium arenarium	Animalia	Arthropoda	Malacostraca	Amphipoda	Corophiidae	Corophium	Corophium arenarium	
33	Corophium volutator	Corophium volutator	Animalia	Arthropoda	Malacostraca	Amphipoda	Corophiidae	Corophium	Corophium volutator	
34	Crangon crangon	Crangon crangon	Animalia	Arthropoda	Malacostraca	Decapoda	Crangonidae	Crangon	Crangon crangon	
35	Crassostrea gigas	Crassostrea gigas	Animalia	Mollusca	Bivalvia	Ostreoida	Ostreidae	Crassostrea	Crassostrea gigas	1
36	Cyathura carinata	Cyathura carinata	Animalia	Arthropoda	Malacostraca	Isopoda	Anthuridae	Cyathura	Cyathura carinata	
37	Diadumene cincta	Diadumene cincta	Animalia	Cnidaria	Anthozoa	Actiniaria	Diadumenidae	Diadumene	Diadumene cincta	
38	Diadumene lineata	Diadumene lineata	Animalia	Cnidaria	Anthozoa	Actiniaria	Diadumenidae	Diadumene	Diadumene lineata	1
39	Diptera	Diptera	Animalia	Arthropoda	Insecta	Diptera	ORDO-Diptera	ORDO-Diptera	ORDO-Diptera	
40	Dreissena	Dreissena	Animalia	Mollusca	Bivalvia	Veneroida	Dreissenidae	Dreissena	Dreissena bugensis	1

	bugensis	bugensis								
41	Dreissena polymorpha	Dreissena polymorpha	Animalia	Mollusca	Bivalvia	Veneroida	Dreissenidae	Dreissena	Dreissena polymorpha	1
42	Electra pilosa	Electra pilosa	Animalia	Bryozoa	Gymnolaemata	Cheilostomatida	Electridae	Electra	Electra pilosa	
43	Electridae	Electridae	Animalia	Bryozoa	Gymnolaemata	Cheilostomatida	Electridae	FAM-Electridae	FAM-Electridae	
44	EMPTY		EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	EMPTY	
45	Enteromorpha sp.	Enteromorpha	Plantae	Chlorophyta	Ulvophyceae	Ulvales	Ulvaceae	Enteromorpha	GEN-Enteromorpha	
46	Eriocheir sinensis	Eriocheir sinensis	Animalia	Arthropoda	Malacostraca	Decapoda	Varunidae	Eriocheir	Eriocheir sinensis	1
47	Erpobdella octoculata	Erpobdella octoculata	Animalia	Annelida	Clitellata	Arhynchobdellida	Erpobdellidae	Erpobdella	Erpobdella octoculata	
48	Erythrotrichia carnea	Erythrotrichia carnea	Plantae	Rhodophyta	Compsopogonophyceae	Erythropeltidales	Erythrotrichiaceae	Erythrotrichia	Erythrotrichia carnea	
49	Flustridae	Flustridae	Animalia	Bryozoa	Gymnolaemata	Cheilostomatida	Flustridae	FAM-Flustridae	FAM-Flustridae	
50	Fucus sp. (juv)	Fucus	Chromista	Ochrophyta	Phaeophyceae	Fucales	Fucaceae	Fucus	GEN-Fucus	
51	Fucus spiralis	Fucus spiralis	Chromista	Ochrophyta	Phaeophyceae	Fucales	Fucaceae	Fucus	Fucus spiralis	
52	Fucus spiralis (juv)	Fucus spiralis	Chromista	Ochrophyta	Phaeophyceae	Fucales	Fucaceae	Fucus	Fucus spiralis	
53	Fucus vesiculosus	Fucus vesiculosus	Chromista	Ochrophyta	Phaeophyceae	Fucales	Fucaceae	Fucus	Fucus vesiculosus	
54	Fucus vesiculosus (juv)	Fucus vesiculosus	Chromista	Ochrophyta	Phaeophyceae	Fucales	Fucaceae	Fucus	Fucus vesiculosus	
55	Gammarus locusta	Gammarus locusta	Animalia	Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammarus	Gammarus locusta	
56	Gammarus pulex	Gammarus pulex	Animalia	Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammarus	Gammarus pulex	
57	Gammarus zaddachi	Gammarus zaddachi	Animalia	Arthropoda	Malacostraca	Amphipoda	Gammaridae	Gammarus	Gammarus zaddachi	
58	Glaux maritima	Glaux maritima	Plantae	Tracheophyta	PH-Tracheophyta	Ericales	Primulaceae	Glaux	Glaux maritima	
59	Hediste diversicolor	Hediste diversicolor	Animalia	Annelida	Polychaeta	Phyllodocida	Nereididae	Hediste	Hediste diversicolor	
60	Helophorus sp.		Animalia	Arthropoda	Insecta	Coleoptera	Helophoridae	Helophorus	GEN-Helophorus	
61	Hemigrapsus sanguineus	Hemigrapsus sanguineus	Animalia	Arthropoda	Malacostraca	Decapoda	Varunidae	Hemigrapsus	Hemigrapsus sanguineus	1

62	Hemigrapsus takanoi	Hemigrapsus takanoi	Animalia	Arthropoda	Malacostraca	Decapoda	Varunidae	Hemigrapsus	Hemigrapsus takanoi	1
63	Heterosiphonia sp.	Heterosiphonia	Plantae	Rhodophyta	Florideophyceae	Ceramiales	Dasyaceae	Heterosiphonia	Heterosiphonia	
64	Idotea granulosa	Idotea granulosa	Animalia	Arthropoda	Malacostraca	Isopoda	Idoteidae	Idotea	Idotea granulosa	
65	Incisocalliope aestuarius	Incisocalliope aestuarius	Animalia	Arthropoda	Malacostraca	Amphipoda	Pleustidae	Incisocalliope	Incisocalliope aestuarius	1
66	Jaera (Jaera) albifrons	Jaera (Jaera) albifrons	Animalia	Arthropoda	Malacostraca	Isopoda	Janiridae	Jaera	Jaera (Jaera) albifrons	
67	Lanice conchilega	Lanice conchilega	Animalia	Annelida	Polychaeta	Terebellida	Terebellidae	Lanice	Lanice conchilega	
68	Lekanesphaera rugicauda	Lekanesphaera rugicauda	Animalia	Arthropoda	Malacostraca	Isopoda	Sphaeromatidae	Lekanesphaera	Lekanesphaera rugicauda	
69	Lepidochitona cinereus	Lepidochitona cinereus	Animalia	Mollusca	Polyplacophora	Chitonida	Lepidochitonidae	Lepidochitona	Lepidochitona cinereus	
70	Ligia oceanica	Ligia oceanica	Animalia	Arthropoda	Malacostraca	Isopoda	Ligiidae	Ligia	Ligia oceanica	
71	Limonium vulgare	Limonium vulgare	Plantae	Tracheophyta	PH-Tracheophyta	Caryophyllales	Plumbaginaceae	Limonium	Limonium vulgare	
72	Liocarcinus sp. (juv)	Liocarcinus	Animalia	Arthropoda	Malacostraca	Decapoda	Polybiidae	Liocarcinus	GEN-Liocarcinus	
73	Lipura maritima	Lipura maritima	Animalia	Arthropoda	Collembola	CLAS-Collembola	Neanuridae	Lipura	Lipura maritima	
74	Littorina littorea	Littorina littorea	Animalia	Mollusca	Gastropoda	Littorinimorpha	Littorinidae	Littorina	Littorina littorea	
75	Littorina obtusata	Littorina obtusata	Animalia	Mollusca	Gastropoda	Littorinimorpha	Littorinidae	Littorina	Littorina obtusata	
76	Littorina saxatilis	Littorina saxatilis	Animalia	Mollusca	Gastropoda	Littorinimorpha	Littorinidae	Littorina	Littorina saxatilis	
77	Lumbricidae	Lumbricidae	Animalia	Annelida	Clitellata	Crassiclitellata	Lumbricidae	FAM-Lumbricidae	FAM-Lumbricidae	
78	Macarorchestia roffensis	Macarorchestia roffensis	Animalia	Arthropoda	Malacostraca	Amphipoda	Talitridae	Macarorchestia	Macarorchestia roffensis	
79	Macoma balthica	Macoma balthica	Animalia	Mollusca	Bivalvia	Veneroida	Tellinidae	Macoma	Macoma balthica	
80	Melita nitida	Melita nitida	Animalia	Arthropoda	Malacostraca	Amphipoda	Melitidae	Melita	Melita nitida	1
81	Melita palmata	Melita palmata	Animalia	Arthropoda	Malacostraca	Amphipoda	Melitidae	Melita	Melita palmata	
82	Monostroma grevillei	Monostroma grevillei	Plantae	Chlorophyta	Ulvophyceae	Ulotrichales	Gomontiaceae	Monostroma	Monostroma grevillei	

83	Myosotella denticulata	Myosotella denticulata	Animalia	Mollusca	Gastropoda	[unassigned] Pulmonata	Ellobiidae	Myosotella	Myosotella denticulata	
84	Myosotella myosotis	Myosotella myosotis	Animalia	Mollusca	Gastropoda	[unassigned] Pulmonata	Ellobiidae	Myosotella	Myosotella myosotis	
85	Myrianida sp.	Myrianida	Animalia	Annelida	Polychaeta	Phyllodocida	Syllidae	Myrianida	GEN-Myrianida	
86	Mytilus edulis	Mytilus edulis	Animalia	Mollusca	Bivalvia	Mytiloida	Mytilidae	Mytilus	Mytilus edulis	
87	Mytilus edulis (juv)	Mytilus edulis	Animalia	Mollusca	Bivalvia	Mytiloida	Mytilidae	Mytilus	Mytilus edulis	
88	Nematoda	Nematoda	Animalia	Nematoda	PH-Nematoda	PH-Nematoda	PH-Nematoda	PH-Nematoda	PH-Nematoda	
89	Nemertea	Nemertea	Animalia	Nemertea	PH-Nemertea	PH-Nemertea	PH-Nemertea	PH-Nemertea	PH-Nemertea	
90	Neomolgus littoralis	Neomolgus littoralis	Animalia	Arthropoda	Arachnida	Trombidiformes	Bdellidae	Neomolgus	Neomolgus littoralis	
91	Oligochaeta	Oligochaeta	Animalia	Annelida	Clitellata	SUBCLAS-Oligochaeta	SUBCLAS-Oligochaeta	SUBCLAS-Oligochaeta	SUBCLAS-Oligochaeta	
92	Orchestia mediterranea	Orchestia mediterranea	Animalia	Arthropoda	Malacostraca	Amphipoda	Talitridae	Orchestia	Orchestia mediterranea	
93	Orchestia sp.	Orchestia	Animalia	Arthropoda	Malacostraca	Amphipoda	Talitridae	Orchestia	GEN-Orchestia	
94	Orchestia sp. (juv)	Orchestia	Animalia	Arthropoda	Malacostraca	Amphipoda	Talitridae	Orchestia	GEN-Orchestia	
95	Ostreidae (juv)	Ostreidae	Animalia	Mollusca	Bivalvia	Ostreoida	Ostreidae	FAM-Ostreidae	FAM-Ostreidae	
96	Pagurus bernhardus	Pagurus bernhardus	Animalia	Arthropoda	Malacostraca	Decapoda	Paguridae	Pagurus	Pagurus bernhardus	
97	Patella aspera		Animalia	Mollusca	Gastropoda	SUBCLAS-Patellogastropoda	Patellidae	Patella	Patella aspera	
98	Pelvetia canaliculata	Pelvetia canaliculata	Chromista	Ochrophyta	Phaeophyceae	Fucales	Fucaceae	Pelvetia	Pelvetia canaliculata	
99	Peringia ulvae	Peringia ulvae	Animalia	Mollusca	Gastropoda	Littorinimorpha	Hydrobiidae	Peringia	Peringia ulvae	
100	Phragmites australis	Phragmites australis	Plantae	Tracheophyta	PH-Tracheophyta	Poales	Poaceae	Phragmites	Phragmites australis	
101	Phyllodocidae	Phyllodocidae	Animalia	Annelida	Polychaeta	Phyllodocida	Phyllodocidae	FAM-Phyllodocidae	FAM-Phyllodocidae	
102	Polydora cornuta	Polydora cornuta	Animalia	Annelida	Polychaeta	Spionida	Spionidae	Polydora	Polydora cornuta	
103	Polysiphonia elongata		Plantae	Rhodophyta	Florideophyceae	Ceramiales	Rhodomelaceae	Polysiphonia	Polysiphonia elongata	

104	Polysiphonia fucoides	Polysiphonia fucoides	Plantae	Rhodophyta	Florideophyceae	Ceramiales	Rhodomelaceae	Polysiphonia	Polysiphonia fucoides	
105	Porphyra purpurea	Porphyra purpurea	Plantae	Rhodophyta	Bangiophyceae	Bangiales	Bangiaceae	Porphyra	Porphyra purpurea	
106	Psammoryctides barbatus	Psammoryctides barbatus	Animalia	Annelida	Clitellata	Haplotaxida	Tubificidae	Psammoryctides	Psammoryctides barbatus	
107	Pygospio elegans	Pygospio elegans	Animalia	Annelida	Polychaeta	Spionida	Spionidae	Pygospio	Pygospio elegans	
108	Rhizoclonium riparium	Rhizoclonium riparium	Plantae	Chlorophyta	Ulvophyceae	Cladophorales	Cladophoraceae	Rhizoclonium	Rhizoclonium riparium	
109	Rhizostoma pulmo	Rhizostoma pulmo	Animalia	Cnidaria	Scyphozoa	Rhizostomeae	Rhizostomatidae	Rhizostoma	Rhizostoma pulmo	
110	Rhodophyta		Plantae	Rhodophyta	PH-Rhodophyta	PH-Rhodophyta	PH-Rhodophyta	PH-Rhodophyta	PH-Rhodophyta	
111	Sagartia troglodytes	Sagartia troglodytes	Animalia	Cnidaria	Anthozoa	Actiniaria	Sagartiidae	Sagartia	Sagartia troglodytes	
112	Salicornia europaea	Salicornia europaea	Plantae	Tracheophyta	PH-Tracheophyta	Caryophyllales	Amaranthaceae	Salicornia	Salicornia europaea	
113	Scirpus maritimus	Scirpus maritimus	Plantae	Tracheophyta	PH-Tracheophyta	Poales	Cyperaceae	Scirpus	Bolboschoenus maritimus	
114	Semibalanus balanoides	Semibalanus balanoides	Animalia	Arthropoda	INFRACLASS-Cirripedia	Sessilia	Archaeobalanidae	Semibalanus	Semibalanus balanoides	
115	Spartina maritima	Spartina maritima	Plantae	Tracheophyta	PH-Tracheophyta	Poales	Poaceae	Spartina	Spartina maritima	
116	Spionidae	Spionidae	Animalia	Annelida	Polychaeta	Spionida	Spionidae	FAM-Spionidae	FAM-Spionidae	
117	Spirorbis (Spirorbis) spirorbis	Spirorbis (Spirorbis) spirorbis	Animalia	Annelida	Polychaeta	Sabellida	Serpulidae	Spirorbis	Spirorbis (Spirorbis) spirorbis	
118	Staphylinoidea		Animalia	Arthropoda	Insecta	Coleoptera	SUPERFAM-Staphylinoidea	SUPERFAM-Staphylinoidea	SUPERFAM-Staphylinoidea	
119	Tipulidae	Tipulidae	Animalia	Arthropoda	Insecta	Diptera	Tipulidae	FAM-Tipulidae	Tipulidae	
120	Trombidioidea	Trombidioidea	Animalia	Arthropoda	Arachnida	Trombidiformes	SUPERFAM-Trombidioidea	SUPERFAM-Trombidioidea	SUPERFAM-Trombidioidea	
121	Ulothrix flacca	Ulothrix flacca	Plantae	Chlorophyta	Ulvophyceae	Ulotrichales	Ulotrichaceae	Ulothrix	Ulothrix flacca	
122	Ulva cf clathrata	Ulva clathrata	Plantae	Chlorophyta	Ulvophyceae	Ulvales	Ulvaceae	Ulva	Ulva clathrata	
123	Ulva cf lactuca	Ulva lactuca	Plantae	Chlorophyta	Ulvophyceae	Ulvales	Ulvaceae	Ulva	Ulva lactuca	

124	Ulva intestinalis	Ulva intestinalis	Plantae	Chlorophyta	Ulvophyceae	Ulvales	Ulvaceae	Ulva	Ulva intestinalis	
125	Ulva sp.	Ulva	Plantae	Chlorophyta	Ulvophyceae	Ulvales	Ulvaceae	Ulva	GEN-Ulva	
126	Vaucheria compacta	Vaucheria compacta	Chromista	Ochrophyta	Xanthophyceae	Vaucheriales	Vaucheriaceae	Vaucheria	Vaucheria compacta	

6.5 Annex 5. Results per Transect

Field data sheet	*To list species and their relative abundance*											
CODE	NL-SCHE-BRES-INMA-22-5-2015											
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD
Species	(A/C/R)											
Blidingia marginata	A	A	A				A					
Fucus sp. (juv)			A									
Fucus vesiculosus					A	A	A		C	A	C	
Fucus spiralis												
Blidingia minima							A		R			
Ascophyllum nodosum									A	C	A	
Ulothrix flacca	A	A	A		A	A	A		C	C	C	
Ulva cf lactica							A					
Porphyra purpurea									R		R	
Littorina littorea									C	C	R	
Littorina obtusata												
Apohyale prevostii					R	C						
Chaetogammarus marinus						C				R		
Orchestia mediterranea												
Orchestia sp. (juv)												
Lipura maritima			A		A	A	A					
Myosotella myosotis												
Myosotella denticulata												
Austrominius modestus					A	C			A	A	A	
Tipulidae												
Melita nitida										C		
Hemigrapsus takanoi									C		R	
Peringia ulvae			R			R	A					
Carcinus maenas										R		
Crassostrea gigas									R			
Brachyura (juv)												
Mytilus edulis												
Patella aspera												
Allita succinea												
Neomolgus littoralis					R	R	R					
Oligochaeta						R						
Chironomidae					R		R					

Field data sheet	*To list species and their relative abundance*												
CODE	NL-SCHE-BRES-INMA-22-5-2015												
Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												
Blidingia marginata	A	A	A					R		C	R		
Fucus sp. (juv)		R											
Fucus vesiculosus					A	A	A		R				
Fucus spiralis					A								
Blidingia minima					A	C	C		C				
Ascophyllum nodosum					A	A	A		A		A		
Ulothrix flacca											R		
Ulva cf lactica						R							
Porphyra purpurea									R				
Littorina littorea	A	A	A		A		C			R	C		
Littorina obtusata					C	A							
Apohyale prevostii		R	A		A	C	C						
Chaetogammarus marinus					A	C							
Orchestia mediterranea	R		R										
Orchestia sp. (juv)						R							
Lipura maritima	A	A	A		A	A	A		A	A	C		
Myosotella myosotis				R									
Myosotella denticulata	R												
Austrominius modestus		A	A		A	A	A			A	R		NIS
Tipulidae		R											
Melita nitida													NIS
Hemigrapsus takanoi		R			R	C	R			C	R		NIS
Peringia ulvae			R										
Carcinus maenas					R		R						
Crassostrea gigas						C	C		A	A	A		NIS
Brachyura (juv)						R	R						
Mytilus edulis								R		R			
Patella aspera												R	
Allita succinea											R		
Neomolgus littoralis													
Oligochaeta													
Chironomidae													

CODE	NL-SCHE-BRES-OUMA-21-5-2015												CODE	NL-SCHE-BRES-OUMA-21-5-2015												
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												Species	(A/C/R)												
Blidingia marginata	A	A	A		A	A	A		A	A	A			A	A	A				A		A	A	A		
Blidingia minima									A					A				A	A							
Ulothrix flacca										A	A															
Fucus spiralis					A	A	A																			
Fucus sp. (juv)	A		A											A		A										
Ulva cf lactuca			A			R												A	A	A						
Porphyra purpurea										C	C											A	A	C		
Fucus vesiculosus								R										C	A	A						
Neomolgus littoralis		C	R																							
Ligia oceanica		R																								
Hemigrapsus takanoi		C					R				C												R	R		NIS
Orchestia mediterranea		R														C										
Apohyale prevostii					C	R												A	A	C						
Lipura maritima			R			A	C		C									A		A						
Austrominius modestus			A		A	A	A		A	R								A	A	A		A	A	A		NIS
Semibalanus balanoides										A	A							A								
Crassostrea gigas							C		C																	NIS
Liocarcinus sp. (juv)											R															
Brachyura (juv)																		A	R	R			R			
Littorina littorea										R								R								
Mytilus edulis (juv)										C								R								
Carcinus maenas																							R			
Tipulidae																			R							
Chironomidae																		R								

Field data sheet	*To list species and their relative abundance*												**Field data sheet**	*To list species and their relative abundance*												
CODE	NL-SCHE-TERN-INMA-19-5-2015												CODE	NL-SCHE-TERN-INMA-19-5-2015												
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												Species	(A/C/R)												
Blidingia minima														A	A	A			A	A						
Fucus sp. (juv)														C	A	A										
Ascophyllum nodosum					A	A	A		A	A	A							A	A	A			C			
Ascophyllum sp. (juv)	R		R																							
Fucus vesiculosus								R										A	A	A						
Ulva cf lactuca																			R							
Porphyra purpurea									A	C	C															
Monostroma grevillei									C										R							
Ulothrix flacca	A	A	A																							
Littorina littorea				R			R		A	C	C			A	A	A		A	A	A		A		A		
Littorina obtusata					R	R																				
Corophium arenarium														A												
Jaera (Jaera) albifrons					R	R	R		R	R				A		A		R	A	A						
Lekanesphaera rugicauda							R																			
Ligia oceanica														A		A										
Peringia ulvae														A	C	A			R	R						
Orchestia mediterranea														A	R	A										
Hemigrapsus takanoi						R			A	A	C				R			R	A	R		A		A		NIS
Lipura maritima															C			A	A	R						
Austrominius modestus	A	A	C		A	A	A		A	A	A							A	A	A		A	A	A		NIS
Crassostrea gigas				R	R	R	C		A	A	A							A		C		A	R	A		NIS
Mytilus edulis									C									R		R		R		R		
Apohyale prevostii						R	A											R	R	R						
Chaetogammarus marinus					R		R											R								
Melita nitida																						A				NIS
Carcinus maenas									R										R							
Oligochaeta						R									R			R		A						
Electra pilosa									C																	
Polydora cornuta										R								R	A							

Field data sheet	*To list species and their relative abundance*												**Field data sheet**	*To list species and their relative abundance*												
CODE	NL-SCHE-TERN-OUMA-20-5-2015												CODE	NL-SCHE-TERN-OUMA-20-5-2015												
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												Species	(A/C/R)												
Ulothrix flacca	A	A	A												A											
Blidingia marginata	A	A	A																							
Fucus spiralis					A	A	R																			
Fucus spiralis (juv)	A	A	A																							
Blidingia minima					A												R									
Fucus vesiculosus						R	A					R						C	C							
Fucus vesiculosus (juv)											R															
Ascophyllum nodosum						R						C						A	A	A			A			
Pelvetia canaliculata						R																				
Ulva cf lactuca											R						R									
Chondrus crispus												R														
Littorina littorea	A	C	A		A	A	A		A	A	A			A	A	A		A	A	A		A	A	A		
Oligochaeta	A	C	A																							
Apohyale prevostii	C				C	C	A											C	C	R						
Melita nitida									C		R													R		NIS
Orchestia mediterranea	R	A	A		A																					
Hemigrapsus takanoi						R	R		R	C	R							C	R	C		C	C	C		NIS
Austrominius modestus						R	C		A	A	A			A		A		A	A	A		A	A	A		NIS
Chaetogammarus marinus							R													C						
Crassostrea gigas									C	A	C			C				A	A	A		A	A	A		NIS
Hemigrapsus sanguineus									R													R				NIS
Mytilus edulis									C		C							A	C	C			C			
Jaera (Jaera) albifrons									R																	
Peringia ulvae									R																	
Semibalanus balanoides									A										A							
Electra pilosa									R		R															
Cerastoderma edule												R														
Patella aspera												R														
Alitta succinea																								R		
Lipura maritima															C				R							
Cirratulidae																								R		
Salicornia europaea																	R									

Field data sheet	*To list species and their relative abundance*												**Field data sheet**	*To list species and their relative abundance*												
CODE	NL-SCHE-HANS-INMA-6-5-2015												CODE	NL-SCHE-HANS-INMA-6-5-2015												
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												Species	(A/C/R)												
Fucus sp. (juv)	R	A	A											C	A	C										
Fucus vesiculosus					A	A	A											A	A	A						
Monostroma grevillei	A	A	A				C																			
Aglaothamnion tenuissimum								R																		
Blidingia marginata														A	A	A		A								
Erythrotrichia carnea										R																
Peringia ulvae	A	A	A		A	A	A							C	A	A		A		C						
Littorina littorea	R	C	A		C	R	C				A				R					R		A	A	C		
Corophium volutator		A	R		C	R	R		A	A	A													C		
Oligochaeta		A	A		R	C	C																	R		
Lekanesphaera rugicauda		R			C		R							R		R										
Carcinus maenas					R	R																				
Hemigrapsus takanoi					R	R					C			C	R	R		A	C	C		C	A			NIS
Chaetogammarus marinus					R													A	C	C						
Apohyale prevostii					R	R												A	C							
Orchestia mediterranea														C	R	R										
Ostreidae (juv)					R																					
Melita nitida																							A	R		NIS
Crassostrea gigas									C	A	A										R	A	A	C		NIS
Austrominius modestus										A	A											C	R	C		NIS
Alitta succinea																						C	R			
Mytilus edulis																						R				
Spirorbis (Spirorbis) spirorbis																						C				
Chironomidae							R																			
Myosotella myosotis	R																									
Aster tripolium				R													R									

Field data sheet	*To list species and their relative abundance*												**Field data sheet**	*To list species and their relative abundance*												
CODE	NL-SCHE-HANS-OUMA-5-6-2015												CODE	NL-SCHE-HANS-OUMA-5-6-2015												
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												Species	(A/C/R)												
Blidingia minima	A	A	A		A	A	A			A	R			A	A	A		A	A	A		A				
Fucus sp. (juv)	C														A	A										
Fucus vesiculosus			C		R	C	C											A	A	A					R	
Porphyra purpurea									A		R										R					
Ulva cf lactuca					A		A														R					
Ceramium virgatum																									R	
Littorina littorea				R	R	C	R		R	C				C	C	A		A	A	A			A	C		
Lipura maritima		R			C	A	A			A	A			C	C			R	R				A	A		
Orchestia mediterranea	R	C	C		R	R	R								A	A					R					
Chironomidae															R											
Neomolgus littoralis	R	R	R													R						C				
Myosotella myosotis																	C									
Myosotella denticulata																R										
Hemigrapsus takanoi						R			C	C							R	R	R	R		C	C	A		NIS
Carcinus maenas																	R									
Austrominius modestus	A				A	A	A		A	A	A						R	A	A	A		A	A			NIS
Mytilus edulis					C		R		A	C							R	C								
Diadumene lineata					R													C	A	A					R	NIS
Diadumene cincta																			R							
Chaetogammarus marinus																			C							
Apohyale prevostii																			C	C						
Melita nitida					R	R																				NIS
Actinia equina																				R						
Crassostrea gigas	R				A	C	A		A	A	A									C		C	A	R		NIS
Alitta succinea																								C		
Jaera (Jaera) albifrons																									C	
Brachyura (juv)							R																			
Ligia oceanica				C																						
Bembidion sp.				R																						
Pygospio elegans																		R								
Oligochaeta																		R								

Field data sheet	*To list species and their relative abundance*												**Field data sheet**	*To list species and their relative abundance*												
CODE	BE-SCHE-DOEL-INMA-21-5-2015												CODE	BE-SCHE-DOEL-INMA-21-5-2015												
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												Species	(A/C/R)												
Vaucheria compacta	A	A	A		A	A	A			R				A	A	A		A	C	C						
Ulva sp.		C	A																							
Corophium volutator									A	C	R					C										
Melita palmata					C	C	C		A	R	A															
Hemigrapsus takanoi		R			R	R	C		R	R	R							R								NIS
Hediste diversicolor									R	R	C							C	C	C						
Acari		R	C		R				R	R																
Ligia oceanica	R	C	R						R					R	R											
Assiminea grayana	R	R	R						R																	
Orchestia sp.			R																							
Lekanosphaera rugicauda															R											
Carcinus maenas																R										

CODE	BE-SCHE-DOEL-OUMA-21-5-2015												CODE	BE-SCHE-DOEL-OUMA-21-5-2015												
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												Species	(A/C/R)												
Vaucheria compacta	A	A	C		A	A	A		R	R	R			A	A	A		A	A	A		A	A	A		
Enteromorpha sp.		C	A		A	A	A		R		R			R	A	A		C	R	R		R	R	A		
Assiminea grayana	A	A												C	A			C								
Orchestia sp.	A	A			C		A																			
Ligia oceanica	A	A																								
Helophorus sp.	R				R																					
Hediste diversicolor	R		R			R																				
Cyathura carinata	R																									
Staphylinidae		R																								
Lekanosphaera rugicauda	A	A	R		A	C	A		C					A	A	A						A				
Hemigrapsus takanoi			R		R				R							R			C	C		R	C	C		NIS
Acari							R							R		R										
Collembola											A															
Melita nitida									R	R																NIS
Corophium volutator											R				R	C		R				R		A		
Gammarus zaddachi																						A				
Amphibalanus improvisus																						C	R			
Crassostrea gigas																						C	R			NIS
Jaera (Jaera) albifrons															R	R		A	R			A	A			
Incisocalliope aestuarius												R														NIS
Scirpus maritimus		A	A																							
Aster tripolium		C																								
Glaux maritima			R																							

Field data sheet	*To list species and their relative abundance*											
CODE	BE-SCHE-WINT-SCHE-22-5-2015											
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD
Species												
Vaucheria compacta	A	A	A		A	A	A		A	A	A	
Phragmites australis	C											
Lumbricidae	C	A	R			A						
Erpobdella octoculata	R											
Octolasion tyrtaeum	R											
Eriocheir sinensis			C		R		R					
Psammoryctides barbatus			R									
Oligochaeta												
Dreissena polymorpha								R				
Collembola								R				
Diptera												
Lekanosphaera rugicauda												
Gammarus zaddachi												

Field data sheet	*To list species and their relative abundance*												
CODE	BE-SCHE-WINT-SCHE-22-5-2015												
Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												
	A	A	A		A	A	A		R	R	R		
		C	R										
		R	R			R			R				NIS
	R						A					R	
	R												
					R								
												R	

Field data sheet	*To list species and their relative abundance*											
CODE	BE-SCHE-WINT-RUPE-22-5-2015											
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD
Species												
Vaucheria compacta	A	A	A		A	A	A			A	C	
Rhodophycaea									C	A		
Enteromorpha sp.												
Lumbricidae	R	R										
Oligochaeta												R
Eriocheir sinensis												
Amphibalanus improvisus												R
Diptera				R								
Collembola				R								
Dreissena polymorpha												R
Dreissena bugensis												R

Field data sheet	*To list species and their relative abundance*												
CODE	BE-SCHE-WINT-RUPE-22-5-2015												
Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												
	A	A	A		A	A	A		A	A	A		
					R	R	R			A	A		
	R	R	C										
									R	R	R		
	C												NIS
													NIS
													NIS

Field data sheet	*To list species and their relative abundance*											
CODE	FR-CANC-ETAP-INMA-10-6-2015											
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD
Species	(A/C/R)											
Blidingia minima	A	A	A		A	A	A		A	A	A	
Blidingia marginata						A	A					
Ulva intestinalis	A								A		A	
Chironomidae	R				C							
Lipura maritima		R			A	A	A		A	A	C	
Hediste diversicolor		R	R						C	C	C	
Carcinus maenas				C	C	C	C		A	A	R	
Macarorchestia roffensis				R								
Orchestia mediterranea												
Oligochaeta					C	R	R		C	A	C	
Austrominius modestus					A	A			R	A	R	
Bembidion sp.						R				R		
Trombidioidea							C				C	
Gammarus pulex							R					
Gammarus zaddachi									C	R		
Lekanesphaera rugicauda												
Corophium volutator					R						R	
Melita palmata												R
Staphylinoidea												

Field data sheet	*To list species and their relative abundance*												
CODE	FR-CANC-ETAP-INMA-10-6-2015												
Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												
Blidingia minima	A	A	A		A	A	A		A	A	A		
Blidingia marginata													
Ulva intestinalis									A	A	A		
Chironomidae					R								
Lipura maritima	R	R	R								R		
Hediste diversicolor		R	R								C		
Carcinus maenas	A	A	A		C	C			A	C	C		
Macarorchestia roffensis													
Orchestia mediterranea		R											
Oligochaeta	A	A	A			C			R	C	C		
Austrominius modestus						A			A		A		NIS
Bembidion sp.			R		R								
Trombidioidea	R												
Gammarus pulex													
Gammarus zaddachi													
Lekanesphaera rugicauda		R											
Corophium volutator											R		
Melita palmata													
Staphylinoidea							R						

Field data sheet	*To list species and their relative abundance*											
CODE	FR-CANC-ETAP-OUMA-11-6-2015											
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD
Species	(A/C/R)											
Blidingia minima	A	A	A									
Rhizoclonium riparium												
Ulva intestinalis			C									
Bembidion sp.	C	R	R									
Oligochaeta	R											
Nematoda												
Lekanesphaera rugicauda	R	C										
Carcinus maenas		R	R									
Austrominius modestus		A	A									
Lipura maritima		R										
Hediste diversicolor												
Gammarus zaddachi												
Trombidioidea												
Orchestia mediterranea			R									
Spartina maritima												
Limonium vulgare	R											

Field data sheet	*To list species and their relative abundance*												
CODE	FR-CANC-ETAP-OUMA-11-6-2015												
Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												
	A		A										
	A												
	A	A											
	R												
	R	A	R										
	R												
			A										
	R												
				R									NIS
		C	R										
		A											
			R										
			R										
			R										

Field data sheet	*To list species and their relative abundance*												**Field data sheet**	*To list species and their relative abundance*												
CODE	EN-WASH-HUNS-NATU-15-6-2015												CODE	EN-WASH-HUNS-NATU-15-6-2015												
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												Species	(A/C/R)												
Blidingia marginata		A	C		A	A	A			A				C	R			A	A	A		A	R			
Blidingia minima											R							A	A	A				R		
Chaetomorpha linum															A											
Monostroma grevillei																				C					R	
Fucus spiralis					A	A	R			A	A								A	A		C		A		
Heterosiphonia sp.					R																					
Ceramium cf virgatum								R							R				R					R		
Polysiphonia elongata																									R	
Polysiphonia fucoides																									R	
Carcinus maenas			R					C	C	C	C			R	C			C	R	R		A	C	C		
Mytilus edulis				R		C			A	A	A			R		R		C	C	A		A	A	A		
Littorina littorea				R	A	A	R		A	A	A						R	A	C	R		A	A	A		
Littorina saxatilis														R	R											
Austrominius modestus					A	A	A		A	A	A				A	A		A	A	A		A	A	A		NIS
Balanus crenatus																	C									
Alitta succinea																							R			
Spionidae																	R									
Autolytinae											R								R						R	
Electridae																		A								
Apohyale prevostii						R												R								
Melita palmata							A														C					
Oligochaeta									R									R						R		
Flustridae																			C							
Lipura maritima																			A	A						
Bugulidae									C												R		R			
Campanularia sp.											R								R	R				R		
Gammarus locusta											R								R						R	
Cerastoderma edule																					R				R	
Arenicola marina								C													A					
Lanice conchilega									A	A	A										C	A		A		
Pygospio elegans																								R		
Polydora cornuta																								R		
Jaera (Jaera) albifrons								R				R						R								
Idotea granulosa																									R	
Neomolgus littoralis									R																	
Lepidochitona cinereus								R																		
Nematoda																								R		
Phyllodocidae																								R		
Sagartia troglodytes								R			R										R					
Peringia ulvae																		R		R						
Pagurus bernhardus																									R	
EMPTY	x																									
Rhizostoma pulmo								R																	R	

Field data sheet	*To list species and their relative abundance*												**Field data sheet**	*To list species and their relative abundance*												
CODE	EN-WASH-HEAC-ARTI-15-6-2015												CODE	EN-WASH-HEAC-ARTI-15-6-2015												
Line (LE/RI)	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	LE	Line (LE/RI)	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	RI	
Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	Stratum (HI/MI/LI)	HI	HI	HI	HI	MI	MI	MI	MI	LI	LI	LI	LI	
Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	Replicate (R1/R2/R3)	R1	R2	R3	ADD	R1	R2	R3	ADD	R1	R2	R3	ADD	
Species	(A/C/R)												Species	(A/C/R)												
Blidingia minima						A	A																			
Blidingia marginata									A	A	A							C				A	A	A		
Ulva intestinalis						R	R		A	A	A											A	A			
Ulva cf clathrata							R		A																	
Ceramium cf virgatum									R																	
Ulva cf lactuca																		C						A		
Mytilus edulis					C	A	C		C	C	C											C	C	C		
Austrominius modestus					A	A	A		A	A	A											A	A			NIS
Bugulidae									R																	
Peringia ulvae					C	R	R		C	R	C											C				
Gammarus locusta									C															C		
Carcinus maenas					A	A	A		R	C												R	R	R		
Cerastoderma edule								R	R		R											R	R	R		
Alcyonidium condylocinereum												R													R	
Lepidochitona cinerea							R				R											R	C	R		
Littorina littorea					A	A	A			C	C											A	A	A		
Hediste diversicolor					R	R	R	R		R	R															
Macoma balthica						R		R		C	R											R	R			
Pygospio elegans							R			R																
Oligochaeta					R		C																			
Neomolgus littoralis			R		R									C	C	A										
Orchestia meditteranea														C	C	C										
Macarorchestia roffensis				R																						
Lipura maritima														C		R										
Myrianida sp.												R														
Nematoda																						R				
Nemertea																								R		
Bathyporeia guilliamsoniana																									R	
Melita palmata																									R	
EMPTY	x	x																	x	x						
Crangon crangon									R																	