Extended Rapid Assessment Survey of non-indigenous species – a tool for detecting trends in marine introductions

Introduction

The human-mediated introduction and establishment of non-indigenous species (NIS) in the marine environment are of worldwide concern. Nowadays many nations undertake notable efforts to prevent or at least minimize new imports of exotic alien species. Other than terrestrial NIS, marine invaders are almost impossible to eradicate and the rising temperatures in the world's oceans often facilitate their establishment in temperate regions since many of them originate from warmer coasts. The increasing awareness concerning NIS is mirrored in the Marine Strategy Framework Directive of the European Commission (EU-MSFD) which aims to achieve and maintain a good environmental status by 2020. The description of the goal also takes into consideration species introductions (descriptor 2) as a measure of marine ecosystem health, focusing in particular on invasive species. Invasive alien species (IAS) are defined as spatially expanding NIS which may threaten biological diversity, impact the environment, ecosystem services, human health and economy. As a prerequisite the knowledge of the status quo in coastal areas is essential and an inventory of NIS and their temporal development is crucial. Based on this, a standard monitoring scheme is needed for early detection of new introductions as well as following the spread and distribution pattern of already established NIS.

German Federal and State agencies developed standardized guidelines for the monitoring of NIS that generate comparable results when studies are conducted by various investigators in different regions. Specific monitoring programs for NIS did not exist along German seashores before. Introduced species were detected in the past only in the context of common monitoring programs for benthic and pelagic organisms, or by chance. A preliminary approach started in 2009, when the states of Schleswig-Holstein and Lower Saxony initiated a monitoring project on introduced species along German coasts.

It is almost impossible to detect all alien species present at a site of concern on a reasonable time and cost basis, but several attempts have been made worldwide to deal with the subject and different methods have been developed, applied and evaluated.
Rapid Assessment surveys (RAS) have proven to be suitable and practicable tools and have been tested on many coasts (e.g. Pederson et al. 2003, Arenas et al. 2006, Minchin 2007, Nall et al. 2014, Bishop et al. 2015, Collin et al. 2015). Given the taxonomic expertise needed they are comparatively easy to conduct with limited resources, they are time and cost effective and do not necessarily require elaborate equipment tools. RAS record merely the presence of NIS at defined locations. Because of the broad range of life forms looked at, a time consuming determination of their precise abundances or coverage is neglected and their occurrences are at most semi-quantitatively estimated. However, a comprehensive taxonomic knowledge covering native and non-indigenous species alike is absolutely essential. Results from RAS become even more significant when combined with a settlement panel program, applied at different sites under defined conditions. It increases the likelihood of detecting small and rare fouling organisms or species living in deeper water. It is an approach towards quantitative information about frequency and abundance of species occurences, linking the NIS monitoring program to the benthic part of the HELCOM/OSPAR port monitoring survey scheme which is aimed at the Ballast Water Management Convention.

The resulting data will be used in the application of general 'NIS Trend Indicators'. The indicators compare NIS diversity at defined temporal intervals to a baseline, evaluating the present status relative to earlier situations.

We here describe a Rapid Assessment protocol which we perform along German coastlines since 2009 (Darr et al. 2014). Additionally, from 2016 onwards we intend to couple the RAS with a settlement panel program, thus monitoring non-indigenous and cryptogenic marine and brackish water species. As the introduction of many NIS is linked to shipping activities, most NIS arrive in ports and harbors. Therefore the emphasis of our efforts is placed on these assumed hotspots. We also investigate the vicinity of culture plots of oysters and blue mussels, since NIS often travel as hitch-hikers on aquaculture imports. Artificial hard structures outside harbors like stony breakwaters, embankments or jetties are included in the sampling activity as well as sedimentary tidal flats and beaches nearby.
Suitable localities along the German North and Baltic Sea coast were chosen for annual surveys of non-indigenous and cryptogenic species. Repeated investigations led to improved strategies, so that the survey design has been optimized with respect to the choice of sampling locations and precise sites in areas of concern. The investigations comprise of field studies, net samples and settlement panels as well as analyses in the lab to identify smaller species.
The described procedure represents the current protocol for German coasts but is generally suitable for the development of monitoring programs in other areas.

Selection of study sites

In a first attempt we determined a net of 14 sampling locations along the North Sea and Baltic Sea shore which in 2016 will be extended to 17 stations as a result of intense debates of experts and recent investigations (Fig. 1).

Fig. 1: Sampling locations for non-native species in German coastal waters in 2016.

In order to cover the entire German coastline, we focus on assumed hotspots of introductions, based on potential vectors and hydrographic features:

Ports and harbors. Shipping is by far the most important NIS introduction vector, in particular ballast water and fouling of ship hulls. Primary introductions occur in big ports with transoceanic cargo vessels, cruise ships, ferries or navy vessels, but these locations are often logistically difficult if not impossible to reach for sampling. If direct sampling in the harbor of concern proves to be impracticable we concentrate on smaller harbors and marinas nearby where sampling is more feasible. Furthermore, introductions from adjacent European coasts and dispersal by recreational boats crossing borders occur presumably more often than direct primary introductions, turning leisure crafts into important vectors for secondary introductions and spread.

We ignored the major ports of Hamburg and Bremen because of their distance to the North Sea and freshwater conditions. The vast majority of the ballast water arriving in these ports is marine and these species are unlikely to establish in freshwater environments. We sample the Kiel Canal, which connects the North and the Baltic Sea only at the beginning (harbors of Brunsbüttel and Kiel) but we regard the entire canal as an introduction hotspot and important route for the exchange of species between the North and Baltic seas.

Aquaculture sites. In German waters few aquaculture sites exist. Merely south of List harbor Pacific Oysters are fattened in the intertidal, and north of Hörnum harbor (both on the island of Sylt, North Sea) blue mussels are cultured commercially in the subtidal and have been stocked in the past with seed mussels from The Netherlands and Great Britain. Although these are comparatively small patches, a variety of alien species have proven to occur in or near these areas (Reise 1998, Wolff & Reise 2002), making them hotspots for NIS.

Hydrographic factors. Vectors are the most important factor to assess the likelihood of a NIS introduction. Additionally, we consider salinity as an important feature for the survival and establishment of alien species. Therefore, fully marine sites in the North Sea (e.g. List, Hörnum, Wilhelmshaven) as well as brackish water estuaries (Emden, Brunsbüttel), harbors of decreasing salinity in the Baltic (Flensburg, Wismar, Rostock) and sites with oligohaline conditions (Oderhaff) were selected (Fig. 1).

Diversity of habitats. At each location, we look at the (local) diversity of habitats on site. We regard the following habitat types as preferred sampling sites:

- **Floating pontoons, quays and artificial hard structures within harbors:**
  Floating pontoons in marinas have proven to be priority sites for monitoring purposes (Arenas et al. 2006). They offer suitable habitats for sessile organisms and are readily accessible to investigations. Often submerged boat equipment like buoys, hanging ropes or fenders provide extra space to settle. Because floating docks stay off the seafloor, benthic predators like crabs or starfish have limited access which may ease the establishment of NIS. Mooring posts and quay walls are additional substrates for sessile species. Swimming or drifting fauna is often associated with fouling communities for foraging or hiding.
  Poles, jetties and quays are suitable places for anchoring settlement panels.
- **Stony groynes, breakwaters and jetties outside harbors:**
  Harbors and marinas are mostly protected by hard structures, which are often favorable habitats for algae or hiding places for macrofauna. Hydrographic conditions are different from those inside harbors and the interspaces between rocks or stones offer shelter to larger organisms like crabs.

- **Sedimentary flats or beaches:**
  Newly introduced endobenthic organisms are difficult to find if not sufficiently abundant. A visual search for epibenthic species is conducted as well as an inspection of the sediment surface for any traces or hints that might uncover the presence of endobenthic infauna.
  Sediment is sampled and sieved to detect soft bottom infauna. Also, the supralittoral zone is checked for the potential occurrence of organisms, e.g. gammarid amphipods.
- **Oyster culture plots and their vicinity are investigated only near List (island of Sylt)**

Logistics. The majority of our sampling sites are easily reachable and accessible. Of the newly added sampling sites some harbors require a permit from local authorities. Since many marinas are not open to the public we check in advance whether entering and working on the pontoons is possible without difficulties. If access turns out to be impossible the harbor is rejected.
Two earlier sampling stations in the Baltic were replaced during the ongoing project, because occurrence of NIS in these two sites was low and habitat diversity was limited (Lubmin, Greifswald).

<u>RAS sampling and settlement panels</u>

All RAS fieldwork is conducted during late summer to early fall (end of July to October) because then the abundances of most species are highest. We try to maintain a chronological order of the sampling stations each year for comparable results. Sampling in tidal areas is done at low tide, especially the inspection of tidal flats or beaches. The investigation of floating pontoons is generally independent from the tidal status but we prefer to sample with the incoming tide in order not to miss larger planktonic organisms (medusae) that are driven by currents and drift near the surface.

Depending on the station surveyed and the number of researchers involved, the sampling time differs, but the minimum time spent is 90 minutes. Often it takes up to 3 hours or more to inspect all habitats thoroughly. Large harbors with more complex structures and many jetties require a more extensive search and sampling procedure than marinas with only few pontoons, smaller sedimentary patches and less rocky structures.

Careful visual inspections of artificial hard structures, sediment surfaces or vegetation reveal the majority of macrofauna and -flora of reasonable/detectable size. Species are listed and if possible the frequency of their relative occurrence is roughly estimated to dominant, abundant, rare or present. If the taxonomic status of a species is certain, none or only few individuals are collected. Since fouling communities on the surface of floating pontoons tend to include a variety of small-sized species, we take scratch samples with a scraper from different sites off the substrates for identification in the lab. We collect clustered mussels from the side, from underneath or between the docks and try to cover as many different physical conditions as possible: more or less exposed to sun, currents or wind, freshwater inflow, tidal level, substrate type etc.. Associated swimming and drifting fauna is sampled with a dip net or sieve with a 1 mm mesh. Plankton samples are (so far) not taken due to limited funding and taxonomic expert availability.

When macroscopic inspections of all habitats in question yield no additional NIS for approximately 15 minutes, we regard the survey as complete. Whereas the exact pattern of scratch and sediment samples will vary from harbor to harbor, care must be taken to repeat the same pattern in each harbor year after year to achieve comparable result in the long run. Therefore, a complete documentation of each site is necessary.

Settlement panels are deployed in spring/early summer and left in situ for 3 - 4 months. PVC Panels with a size of 150 x 150 x 5 mm and a central hole (15 mm in diameter) are used. This allows fixing three panels at one rope with defined distances using sailor knots. At one end of the rope a weight is used to stabilize the experimental treatment in the water column. The rope is fixed at artificial harbor structures (walls, pontoons etc.) in a way that the panels are positioned in defined water depths (1 m below the surface, at half the water depth, and 1 m above the bottom respectively). After the experimental period, settlement panels are carefully collected, stored individually in plastic bags with sea water and transported immediately to the lab in a cooler. If the number of settled specimen on a panel turns out to be too high to be processed in a reasonable time, panels are fixed in alcohol or formalin/seawater.

Identification in the lab

All RAS samples as well as settlement panels are carefully examined in the lab with the aid of stereomicroscopes and microscopes. The species number and density of individuals from settlement panels are quantified. Specimens are identified to the lowest taxonomic level possible (usually the species level). Identification is done according to established taxonomic keys and current neobiota literature. Identifications of rare or novel species are sometimes extremely time consuming and may require correspondence to taxonomic experts.
Species of concern are fixed in formalin/seawater or alcohol and stored. For future genetic analysis of controversial species storage in alcohol may be necessary.

Conclusions

1. Standardized Rapid Assessment surveys (RAS) combined with a settlement panel program offer an effective and sufficient method to detect introduced macroscopic species at coastal sites.
2. RAS require a minimum of equipment (no boats, diving gear etc.) and of time (one visit per site and year), panel tests one or two additional visits.
3. However, a comprehensive taxonomical expertise is needed and the knowledge of alien species' habitat preferences.
4. Site-specific procedures have to be repeated each year, therefore systematic documentation and mapping of each site is mandatory.
5. Settlement panel investigations enhance the likelihood of detecting small or rare species or organisms occurring in a water depth not accessible to sampling with a dip net.
6. Standardized RAS coupled with settlement panel investigations at hotspots of introduced species allow for early detections of new NIS and can reveal temporal trends in the number of NIS per site.
7. All collected data on NIS need to be summarized in a common central database for introduced and cryptogenic species, which is currently achieved by the implementation of a national registration platform for non-native organisms in German marine waters. AquaNIS, the database on aquatic non-indigenous and cryptogenic species, will be supported with the NIS data generated during the surveys.

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