



Joint HELCOM/OSPAR Guidelines for the Contracting Parties of OSPAR and HELCOM on the granting of exemptions under International Convention for the Control and Management of Ships' Ballast Water and Sediments, Regulation A-4

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#### 1. Introduction

- 1.1 Loading and discharging ballast water is an essential part of a ships operation, with ships requiring ballast water to maintain their stability, draft and manoeuvrability. Contained within this ballast water are hundreds of microscopic species that will be carried to new destinations by the ship. The vast majority of these species will not survive the journey; however, the species that do survive may establish themselves in a new environment if the biological and physical conditions are favourable. There are numerous well documented examples, from all parts of the world, of the negative effects of non-native species introduced through ballast water. Such non-native species may cause serious ecological, economic and public health impacts, particularly when they become invasive.
- 1.2 In response to this the International Maritime Organization (IMO) through its Marine Environment Protection Committee (MEPC), has over many years, been developing international legislation to prevent the harmful effects of transporting aquatic organisms in ships ballast water.

#### **IMO Ballast Water Management Convention**

- 1.3 In February 2004, a Diplomatic Conference convened by IMO adopted the "International Convention for the Control and Management of Ships' Ballast Water and Sediments" (the Convention) 1. This Convention put in place international legislation for the first time and will enter into force 12 months after it has been signed by 30 States, representing 35% of world merchant shipping tonnage. The Convention is expected to enter into force in 2014 or 2015.
- 1.4 The Convention aims to prevent the spread of harmful aquatic organisms from one region to another, by establishing standards and procedures for the management and control of ships' ballast water and sediments. Under the Convention, all ships in international traffic are required to manage their ballast water and sediments to a certain standard, according to a ship-specific ballast water management plan. All ships will also have to carry a ballast water record book and an international ballast water management certificate. The ballast water management standards will be phased in over a period of time. As an intermediate solution, ships should exchange ballast water mid-ocean. However it is expected most ships will need to install an on-board ballast water treatment system.
- 1.5 Article 3 (1) of the Convention outlines its applicability and states:

"Except as expressly provided otherwise in this Convention, this Convention shall apply to:

- (a) ships entitled to fly the flag of a Party; and
- (b) ships not entitled to fly the flag of a Party but which operate under the authority of a Party."

However the Annex to the Convention provides for Parties, under Regulation A-4, the scope to issue exemptions from Regulation B-3 (Ballast Water Management for Ships) and Regulation C-1 (Additional Measures). Regulation A-4 states:

- "1. A Party or Parties, in waters under their jurisdiction, may grant exemptions to any requirements to apply regulations B-3 or C-1, in addition to those exemptions contained elsewhere in this Convention, but only when they are:
  - a. granted to a ship or ships on a voyage or voyages between specified ports or locations; or to a ship which operates exclusively between specified ports or locations;
  - b. effective for a period of no more than five years subject to intermediate review;
  - c. granted to ships that do not mix Ballast Water or Sediments other than between the ports or locations specified in paragraph 1.1; and

<sup>&</sup>lt;sup>1</sup> http://www.imo.org/About/Conventions/ListOfConventions/Pages/International-Convention-for-the-Control-and-Management-of-Ships'-Ballast-Water-and-Sediments-(BWM).aspx

- d. granted based on the Guidelines on risk assessment developed by the Organization.
- 2. Exemptions granted pursuant to paragraph 1 shall not be effective until after communication to the Organization and circulation of relevant information to the Parties.
- Any exemptions granted under this regulation shall not impair or damage the environment, human health, property or resources of adjacent or other States. Any State that the Party determines may be adversely affected shall be consulted, with a view to resolving any identified concerns.
- 4. Any exemptions granted under this regulation shall be recorded in the Ballast Water record book."
- 1.6 Article 13 (3) of the Convention also states that:

"In order to progress further the objectives of the Convention, Parties with common interests to protect the environment, human health, property and resources in a given geographical area, in particular, those parties bordering enclosed and semi-enclosed seas, shall endeavour, taking into account characteristic regional features, to enhance regional cooperation, including through the conclusion of regional arrangements consistent with this Convention. Parties shall seek to co-operate with the Parties to regional agreements to develop harmonized procedures".

1.7 Therefore, the Helsinki and OSPAR Conventions have jointly developed these guidelines, prior to the International Convention for the Control and Management of Ships' Ballast Water and Sediment coming into force, to ensure that exemptions are granted in a constant manner that prevents damage to the environment, human health, property or resources.

Guidelines for the Contracting Parties of OSPAR and HELCOM on the Granting of Exemptions from the International Convention for the Control and Management of Ships' Ballast Water and Sediments under Regulation A-4

- 1.7 b The purpose of the Guidelines is to provide a harmonized procedure in accordance with Art. 13 (3) of the Convention for the issue of exemptions according to Regulation A-4 of the Ballast Water Management Convention for the use by OSPAR and HELCOM Contracting Parties once the Ballast Water Management Convention has entered into force. This document is not a Guideline in the sense of Regulation A-4 or any other part of the Ballast Water Management Convention.
- 1.7 c Exemptions under regulation A-4 of the Ballast Water Management Convention may only be granted by contracting parties to the Convention after its entry into force. Parties are encouraged to use the applicable parts of these guidelines in preparation for the entry into force.
- 1.8 Whilst Regulation A-4 gives parties the right to grant exemptions it also sets out the requirements for doing so, *e.g.* exemptions can be only granted for vessels operating between specified ports and locations, that an exemption shall not be effective for more than 5 years and that exemptions must be granted based on the guidelines on risk assessment developed by the IMO (G7)2. The IMO Guidelines outline three risk assessment methods that will enable Parties to identify unacceptable high risk scenarios and acceptable low risk scenarios, and advise Parties on procedures for granting and withdrawing exemptions in accordance with regulation A-4. They provide for the basis of the following HELCOM / OSPAR guidelines, which have been developed specifically for the Baltic and Northeast Atlantic regions.
- 1.9 There are three risk assessment methods outlined in the G7 Guidelines for assessing the risks in relation to granting an exemption in accordance with regulation A-4 of the Convention:
  - · Environmental matching risk assessment

<sup>&</sup>lt;sup>2</sup> http://www.imo.org/blast/blastDataHelper.asp?data id=19689&filename=162(56).pdf

- Species' biogeographical risk assessment
- Species-specific risk assessment
- 1.10 Environmental matching risk assessment relies on comparing environmental conditions between locations; species' biogeographical risk assessment compares the environmental similarity and species composition in source and destination ports/areas to identify high risk invaders, while species-specific risk assessment evaluates the distribution and characteristics of identified target species. Dependent on the scope of the assessment being performed, the three approaches could be used either individually or in any combination, recognizing that each approach has its limitations.
- 1.11 Environment matching and species' biogeographical risk assessment may be best suited to assessments between biogeographic regions. Species-specific risk assessment may be best suited to situations where the assessment can be conducted on a limited number of harmful species within a biogeographic region.
- 1.12 The HELCOM Guidance for High and Low Risk voyages<sup>3</sup>, adopted by HELCOM CP Ministers in 2010, and the North Sea Ballast Water Consultation Group Concept Issue of Exemption<sup>4</sup>, as well as work undertaken as part of the North Sea Ballast Water Management Opportunity and in the Baltic Sea Ballast Water Risk Assessment<sup>5</sup>, considered the three main approaches to risk assessment provided under the IMO guidelines G7. The reports identified that the key risk criteria for issuing exemptions within the North Sea and Baltic were limited to:
  - i) Difference in water salinity between ports/locations being visited
  - ii) Presence of non-indigenous species fulfilling certain criteria in either port/location being visited, that is, target species.
- 1.13 The HELCOM Aliens 2 project further developed a harmonized method for granting exemptions from ballast water treatment (BWMC A-4) for marine traffic. The initiative developed a detailed protocol on biological and abiotic surveys in ports of the Baltic Sea to be commonly applied when surveying the ports for the purposes of gathering the information on alien species, taking into account the need for and benefits of having a consistent approach with the North Sea region. The project also considered the procedure for selecting target species and how to structure and use the collected data to support regionally coherent and transparent decision-making on exemptions.
- 1.14 These joint OSPAR/HELCOM guidelines are based on the following common understanding:
  - a. Results from the common OSPAR/HELCOM framework are guidelines for national evaluations of applications for exemptions under Reg. A-4.
  - b. Results are non-binding. The decision on an application for exemption rests with the national authority.
  - c. If national administrations do not use, or deviate from, the results of the common OSPAR/HELCOM framework, reasons should be communicated to OSPAR/HELCOM, so that they may inform the review process of these guidelines.
  - d. Data needed under the common OSPAR/HELCOM framework should be collected according to the sampling protocol (section 4).
  - e. Subject to funding it is suggested that data should be collected by Member States or other organisation (e.g. ports). Member States and other organisations are encouraged to use projects for initial data collection.

<sup>&</sup>lt;sup>3</sup> HELCOM. 2010. HELCOM Guidance for High and Low Risk voyages. Adopted at the HELCOM Moscow Ministerial Meeting 2010 as part of the Declaration.

<sup>&</sup>lt;sup>4</sup> OSPAR (EIHA 12/3/4) - Ballast Water Exemptions in the North Sea

<sup>&</sup>lt;sup>5</sup> http://www.helcom.fi/stc/files/shipping/HELCOM RA FINAL Report.pdf

- f. If no data for a risk assessment under the common OSPAR/HELCOM framework is available from official or other sources, the applicant should collect the data according to the sampling protocol.
- g. The collected data from port surveys and on target species should be stored centrally under OSPAR/HELCOM supervision.
- h. Data should be evaluated using the common OSPAR/HELCOM framework, as a first step by an automated decision support tool, to facilitate uniform application across the regions.
- i. In an initial transitional period the guidelines are to be implemented in a flexible and practicable way by authorities in cooperation with the ship owners, the harbours and other stakeholders, taking the regulations A-4.3 of the Convention into account. This should be done in order to gain experience and to enable further development and improvement of the guidelines.
- 1.15 These joint guidelines are split into 7 sections including:
  - 1. Introduction;
  - 2. Port Survey Protocol;
  - Target Species Identification;
  - 4. Data Storage;
  - 5. Risk Assessment;
  - Decision Support Tool and;
  - 7. Administrative Procedures.

# 2. Port Survey Protocol

#### **Background**

- 2.1 This section outlines the HELCOM-OSPAR protocol for comprehensive sampling of non-indigenous species in ports. All applications aiming for a BWMC A-4 exemption in the application area, the combined HELCOM and OSPAR marine area, must carry out the port surveys following the methodology described in this section and attach the results (data sets with measurements) to the exemption application. This information should cover each stopover port on the route for which the exemption is applied.
- 2.2 Port survey is to be regarded valid for granting exemption for applicants during a period of maximum of 5 years, to be counted from the date of the first of the two sampling visits (spring). A contracting party may decide on a shorter validity for a port survey due to *e.g.* sensitiveness of the area, intensity of traffic or need for updated port survey data on non-indigenous species.
- 2.3. Port surveys for detecting non-indigenous species require sampling of several different groups of organisms: hard substrate organisms, soft bottom benthos, plankton and mobile epifauna (e.g. fish and crustaceans).
- 2.4 Following is a description of the general features and requirements but see Annex 1 for the complete protocol with all details and recommended equipment.

#### General port characteristics and available species data

- 2.5. As a first preparatory step information about general characteristics, such as typical variation of abiotic conditions and patterns of port traffic, should be collected for each port to be sampled.
- 2.6. A port is considered to be a contiguous unit, separated by for example a land mass, as peninsula or distance more than 1 km from other ports or port areas. Within a port there should be a minimum of 3

sampling sites (dependent on the size of the port). A site is a separate unit within a port, such as a specific dock or a wharf. Within a site a number of replicate samples (depending of the sample type, see below) of different groups of organisms will be taken.

- 2.7. Ports often have weather stations recording wind and temperature patterns and provided they are situated in relevant locations this data can be used. If additional measurements of temperature and salinity is needed the suggestion is to use data loggers.
- 2.8. If available, existing information from national monitoring programmes or projects, should naturally also be used for planning a port survey. However, in the Baltic Sea area Estonia is the only country which carries out regular monitoring of alien species in the vicinity of ports. In addition, some individual port inventories and/or long term projects have been conducted in Poland (*e.g* Walk et al. 2011), Lithuania, Germany (Buschbaum et al. 2010) and Finland (Paavola et al. 2008).
- 2.9. In the OSPAR region some countries (e.g. Germany and Netherlands) have established monitoring activities for non-indigenous species in their waters, including in port areas. Some other rapid assessments have also been undertaken by specific projects.
- 2.10 A port information data sheet summarising all abovementioned information should be filled in together with the port authorities.

#### Number of sampling sites per port and their selection

- 2.11. The field sampling will be carried out in a number of sampling sites, or exact locations, within a port. The number of sites required for an adequate survey will depend on the size and type of port. A predetermined number of sites per port cannot be given in a general guideline, however the absolute minimum is three sampling sites per port.
- 2.12 Also, species effort/accumulation curves (see Hayek and Buzas 2010) should be presented with the results of each survey to provide proof of adequate number of sites, and samples taken in each site. Species accumulation curves can be created simultaneously with the risk assessment within the decision support tool.
- 2.13 The distribution of sampling sites in the port area should follow a stratified sampling design and attention should be given to sample all main substrate types available in the port. Special attention and increased sampling efforts should be allocated to the following high priority area types: active berths, inactive/disused wharves, channel markers, tug and pilot vessel berths and slipways (see CRIMP protocol, Hewitt and Martin 2001). Water movements within the port should also be taken into account when selecting sampling sites.
- 2.14 The first survey at a given port should be given special attention since no baseline surveys have been conducted in most Baltic or North East Atlantic ports. A brief mapping of the general underwater habitats should to be conducted in each port to assure that the survey efforts is conducted in the most abundant/relevant habitats. For the port habitat mapping visual observations (for example scuba diving, underwater cameras, echo sounders etc.) are recommended.

#### Timing of sampling

- 2.15 Due to seasonality and life cycle patterns of different life-forms and species, sampling will take place during two visits. First should take place during spring (spring bloom) and the second during late summer (summer maximum).
- 2.16 Plankton samples should be taken and analysed both during spring bloom and summer maximum (late summer). The settlement plates should be deployed when conducting the first (spring bloom plankton) sampling and taken up when conducting the second sampling to allow enough time for representative fouling organism communities to develop.

2.17 Sampling of mobile epifauna, benthic infauna and fouling organisms as well as settlement plate retrieval should be conducted simultaneously with the summer maximum sampling (between late July and September) when majority of the species are mature and identifiable.

#### **Physical parameters**

- 2.18 At each sampling site measurements on physical parameters (at minimum water temperature and salinity) should be made using a submersible data logger. In addition, water transparency should be measured using a Secchi disk.
- 2.19 In addition, if the equipment is available (for example if a CTD with additional sensors is utilised), other water properties such as turbidity and Chlorophyll-a should also be measured and reported from each sampling site.
- 2.20 Physical parameters are required to be collected during both sampling visits (spring bloom and summer maximum).

#### **Human pathogens**

- 2.21 One water sample from each sampling site should be taken for detecting the presence of bacteria according to Regulation D2 (Intestinal Enterococci, *Escherichia coli* and *Vibrio cholerae*).
- 2.22 Data from existing samples on human pathogens collected by local authorities during the same period can be used for analysis, provided that they fulfil protocol quality requirements.
- 2.23 Human pathogen samples should be collected during both sampling visits (spring bloom and summer maximum).

#### **Plankton**

- 2.24 Samples for phytoplankton and zooplankton species composition and abundance should be taken at each sampling site. One pooled phytoplankton sample (water sample), one concentrated phytoplankton sample (net sample) and two vertical zooplankton samples using nets with different mesh sizes, at each sampling site is required.
- 2.25 Nets suggested in the protocol (20  $\mu$ m, 100 200  $\mu$ m and 300 500  $\mu$ m) are hand held and have been selected to be operable from the dock.
- 2.26 Plankton samples should be collected during both sampling visits (spring bloom and summer maximum).

#### Mobile Epifauna

- 2.27 Mobile epifauna, such as crabs, should be sampled at each sampling site using light weight traps tethered to existing structures (pilings, buoys, docks). Traps are selective in nature and therefore provide only information on the presence of species or at best relative measures of species abundances. However, methodologies for sampling epifauna in the port area are very limited and for example using trawls and gillnets is impossible. Attention should be given to place traps on all available substrates (mud, sand, rocky) and catch reported accordingly.
- 2.28 Visual searches, either with remote video cameras or divers, should be conducted, if possible, at each sampling site prior to deploying the traps to assure for efficient placement and distribution of traps. Divers should swim 50 m transects along the dock/shore at several depths ranging from 0.5 m to the bottom to provide visual idea of the bottom and record presence of any non-indigenous species including epifauna.
- 2.29 Sampling of mobile epifauna is only required once during the sampling period, on the second visit (late summer).

#### **Benthic Infauna**

2.30 Grab samples: At least three samples should be taken at each sampling site located at least 15 m distance from each other using a benthic grab operable from a dock. Sediment quality can either be visually

assessed of these samples or a separate sample may be taken for sediment quality (grain size) analysis. In case of known ballast water discharge at site, additional benthic samples may be taken.

- 2.31 A satisfactory sample requires penetration to approximately 5 10 cm into the sediment. Bottom quality may limit the possibilities to obtain samples from certain sampling sites and acquiring a satisfactory sample may require several attempts. As an example, in many locations, a concrete slab has been built underneath the docks to prevent erosion. Mooring berths (walking bridges) should therefore be utilized, when possible, to reach further from the shore and obtain satisfactory grab samples.
- 2.32 Scuba samples: Benthic Infauna should be sampled using hand corers on a 50 m transect at 0 m, 25 m and 50 m distances by scuba diving. Transect of 50 m should be laid on the bottom perpendicular to the shore starting at sampling site. The transect line should be marked at 1 m intervals. Any epibenthos observed in the vicinity of transect should also be collected and distance at the transect recorded.
- 2.33 Temperature, salinity and oxygen saturation on the bottom should be measured using a submersible data logger at the start of the transect or at the grab sampling location. These data can also be obtained from site readings if the sample location is in the vicinity of the measuring location.
- 2.34 Sampling of benthic infauna is only required once during the sampling period, on the second visit (late summer).

#### Fouling organisms

- 2.35 Rapid assessment sampling protocol may be a suitable qualitative sampling method for hard substrate organisms at sampling sites with low visibility, such as typically encountered within Baltic ports, where diving may not be the best option. Existing structures within the port area will be targeted and the aim is to identify the species attached to ropes, chains, pilings and hard surfaces using hand held scraping tools and estimate the species coverage, if possible.
- 2.36 Based on test surveys, docks are often high, built on stilts and no ropes or chains are laying in the water and therefore obtaining scrape samples from the dock is close to impossible. Therefore sampling by scuba diving or snorkeling in addition to the use fouling plates (described below) is highly recommended.
- 2.37 Sampling of fouling organisms by scraping is only required once during the sampling period, on the second visit (late summer).
- 2.38 Settlement plates or settlement collectors should be used to improve the survey of fouling organisms. Fouling plates should be deployed during the first sampling visit and retrieved during the second sampling visit.
- 2.39 Scuba sampling of fouling organisms should be conducted at each sampling site if possible. In case of very low visibility or other apparent safety issues, other methods, listed above, can be used. Vertical transects should be placed on pilings, projecting steel or concrete facings of wharfs, berths, piers and dolphins. They should be inspected closely for any non-native species and quadrates sampled on set depth intervals.

#### Sample processing, analysis and data reporting

- 2.40 All samples are to be analysed by a quality assured laboratory (see Annex 1) to account for adequate taxonomic expertise.
- 2.41 At minimum, all species present in the samples are identified to the lowest taxonomic level possible. In addition, abundance of species is to be estimated using a scale from 1-5 or percentage scale. In case of finding an unknown species for the area in the survey, it should be first photographed and then preserved for further analyses (for example in 96 % ethanol for genetic analyses).
- 2.42 Data should be reported using the agreed format suitable for transferring it to the database.

#### Detailed specifications on sampling methods

2.43 A recommended example protocol is appended as Annex 1 (Detailed description of the protocol), including Suggested equipment for field sampling and a note on quality assured laboratories.

# 3. Target Species

- 3.1 In order to conduct a risk assessment for the transport of species with ballast water between harbours all organisms present as observed through port sampling, conducted as described in section 2, have to be taken into account.
- 3.2 To minimize the effort and to make the risk assessment procedure practicable a pre-selection of species that have to be assessed for their risk is necessary. The selected species are called target species.
- 3.3 With the determined target species the risk assessment model (Section 5) can be run. If more than one target species is present each one has to be evaluated with the risk assessment model.
- 3.4 Two special types of target species are included:
  - a. Known unwanted species that are known to have already generated serious problems for the environment, economy, human health, poverty or resources somewhere in the world, that have evidence of prior introduction and have a relationship with ballast water as a vector.
  - b. Species which have been comprehensively scientifically investigated for their risk potential but which have not yet caused harm.
- 3.5 For risk assessment purposes it is important to take all occurring target species into account, which include species beyond the two under 3.4. Very harmful species have in many cases already spread to all areas within their environmental limits.
- 3.6 For the continuous review and updating of the target species list a comparison of the inventories of the species present in the harbours is recommended. Species which are only in the donor port and not in the recipient port are taken into account.
- 3.7 The next step is an assessment, whether the species, which are only in the donor port, are target species or not by an evaluation and ranking. The evaluation and ranking must be carried out by experts based on criteria defined under 3.10.
- 3.8 The target species list given in Annex 2 is to be regarded as a starting point for such target species status.
- 3.9 The target species lists of OSPAR and HELCOM are to be regarded as living lists under continuous updating by HELCOM MONAS and OSPAR BDC, which means that other species can be included or species can be deleted, if further knowledge is available.
- 3.10 The target species selection criteria specified in the table below are to be used by HELCOM MONAS and OSPAR BDC to define target species status and the inclusion or exclusion of the species into the target species list in Annex 2.

	Low risk species=1	Medium risk species=2	High risk species=3
1. Dispersion potential or invasiveness	The species doesn't spread in the environment because of poor dispersal capacities and low reproduction potential	Except when assisted by man, the species doesn't colonise remote places. Natural dispersal rarely exceeds more than 1km per year. The species can however become locally invasive because of a strong reproduction potential.	The species is highly fecund, can easily disperse through active of passive means over distances > 1km/year and initiate new populations.
2. Colonisation of high conservation	Populations of the non- native species are restricted to habitats of	Populations of the non-native species are usually confined to habitats with a low or a medium	Non-native species often colonise high conservation value habitats, these are all biotopes where

value habitats	no conservation value (e.g. harbor constructions as quay walls or bank and shoreline stabilisation or pipes for cooling systems)	conservation value and may occasionally colonise high conservation value habitats	endangered species can be found. Most of the sites of a given habitat are likely to be readily colonized by the NIS when source population are present in the vicinity and makes therefore a potential threat for red-listed species.
3. Adverse impacts on native species	Data from invasion history suggest that the negative impact on native population is negligible	The non-native species is known to cause local changes (<80%) in population abundance, growth or distribution of one or several native species, especially among common and ruderal species. This effect is usually considered as reversible.	The development of the non- native species often cause local severe (>80%) population declines and the reduction of local species richness. At a regional scale, it can be considered as a factor precipitating (rare) species decline. Those non-native species form long-standing populations and their impacts on native biodiversity are considered as hardly reversible.
4. Alteration of ecosystem functions	The impact on ecosystem processes and structures is considered as negligible.	The impact on ecosystem processes and structures is moderate and considered as easily reversible. Temporary modification of water and sediment properties (e.g. algae which can be removed such as <i>Lemna</i> ) or decrease of the rate of colonisation of open habitats by species which build barriers.	The impact on ecosystem processes and structures is strong and difficult to reverse e.g. food web disruption ( <i>Crassostrea gigas</i> ) or habitat destruction ( <i>Eriocheir sinensis</i> ).
5. Effects on human health	Data from invasion history suggest that the species has weak toxic effects and no treatment is necessary	Data from invasion history suggest that the species has moderate symptoms, easily treated, no permanent damage	Data from invasion history suggest that the species has negative impact on human health, permanent damage or death
6. Effects on natural resources (e.g. fisheries)	Data from invasion history suggest that negative impact on natural resources is negligible	Data from invasion history suggest that the species has only slight negative impact on natural resources and is restricted only on single locations	Data from invasion history suggest that the species causes serious loss on aquaculture or fisheries harvest
7. Effects on property (e.g. cooling systems)	Data from invasion history suggest that the negative impact on property negligible	Data from invasion history suggest that the species has only slight negative impact on property and this is restricted only on single locations	Data from invasion history suggest that the species has high negative impact on property at many locations
8. Dispersed by ballast water or sediments	Invasion without BW, but target species now found in the harbour with the chance to dispersed further by BW	Dispersal via BW and other possibilities (stocking,)	Dispersal mainly by BW or are already found in BW or Sediments

# 4. Data Storage

- 4.1 The data collected according to the sampling protocol (Section 2), should be centrally stored in an electronic format as a database. Conditions of access to the database are discussed in paragraph 7.8. The data should be freely available for research. A burden sharing mechanism if this information is used by other applicants should be developed.
- 4.2 The system should enable the storage of data including:

- Harbour information (statistical information about environment, size and some business parameters of harbours)
- In situ measurements (abundance and biomass of species) detected in the harbours
- 4.3 The list of target species, defined using the criteria outlined in section 3 (including the option to define specific lists for different regions), as a basis for a risk assessment, should also be included in the database.
- 4.4 The database should be able to connect to existing databases in order to access additional information. However, data used in the risk assessment process should remain under the supervision of the OSPAR/HELCOM secretariats.
- 4.5 The database should be maintained by the OSPAR/HELCOM secretariats; however details of the arrangement are still to be developed.

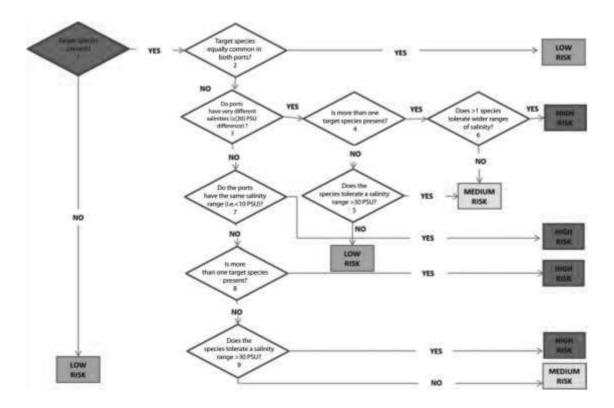
#### 5. Risk Assessment

- 5.1 Based on previous work within HELCOM<sup>6</sup> and OSPAR<sup>7</sup> a specific approach, described in this section, is recommended for risk assessments under regulation A-4 of the BWM Convention for routes with one or several ports in the application area of the OSPAR or Helsinki Conventions.
- 5.2 The eight key principles of risk assessment in the IMO Guidelines G7 are effectiveness, transparency, consistency, comprehensiveness, risk management, precautionary, science based and continuous improvement.
- 5.3 The information required to undertake a A-4 risk assessment should be supplied in line with the other section of these guidelines, *i.e.* environmental conditions and presence of non-indigenous species section 2 Port Surveys, species to be included in the risk assessment section 3 Target Species and shipping information (*e.g.* for water discharge volumes) section 7 Administrative Procedures. The absence of, or uncertainty in, any information should be considered an indicator of potential risk and the level of uncertainty should recorded in a transparent way.
- 5.4 According to the terminology of the IMO Guidelines G7, a species-specific risk assessment supported with information on environmental conditions and shipping activities is to be applied. The key risk criteria to distinguish between unacceptable, or high, risk and acceptable, or low, risk are:
  - Presence and abundance of target species in either port/location being visited by the vessel.
  - ii. Difference in water salinity between ports/locations being visited;
  - Salinity tolerance of target species present
- A risk assessment algorithm is a way to formalise risk assessment procedure through a set of binary yes/no questions based on a number of key criteria such as those defined in §5.4. The joint OSPAR-HELCOM Risk Assessment algorithm, outlined below and explained in more detail in Annex 3, includes three possible assessment results described in §5.6.

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<sup>&</sup>lt;sup>6</sup> HELCOM Guidance on high and low risk voyages – 2010 Ministerial Declaration, Pilot Risk Assessments of alien species transfer on intra-Baltic ship voyages. HELCOM Aliens Final Report.

<sup>&</sup>lt;sup>7</sup> OSPAR (EIHA 12/3/4) - Ballast Water Exemptions in the North Sea



5.6 The joint A-4 risk assessment algorithm outlined in 5.5 includes three possible risk assessment outcomes (High Risk, Medium Risk and Low Risk) which have the following implications for A-4 exemption applications:

High risk (HR):	It is highly likely that target species are distributed with ballast water and occupy a new habitat. The risk is unacceptable. <b>An exemption </b> <u>cannot be granted</u> .
Medium risk (MR):	Target species could be distributed with ballast water and might occupy a new habitat. Further review is necessary to evaluate risk. This includes <i>e.g.</i> , local conditions in the ports and salinity tolerance, temperature, behaviour as well as dispersal ability/mobility of the species. Negative impacts of related species in other ecosystems are also relevant for this review.  Based on the additional information, a decision must be reached as to whether to grant an exception permit. Individual mitigation measures other than those defined under the BWMC may be required.
Low risk (LR):	It is not very likely that target species are distributed with ballast water and occupy a new habitat. The risk is acceptable. <b>An exemption </b> can be granted.

- 5.7 It should be noted that the use of the use of risk assessment algorithms is only to aid regionally harmonised decision making and that full consideration should be given to the specific conditions in each case.
- 5.8 Based on one of the key principles of IMO Guidelines G7, "continuous improvement", the risk assessment framework and components described in this section should be kept under continuous review by the two organisations with the first assessment of the effectiveness of the risk assessment algorithm no more than two years after the entering into force of the guidelines.
- 5.9 A more detailed description of the algorithm is given in Annex 3.

# 6. Decision Support Tool

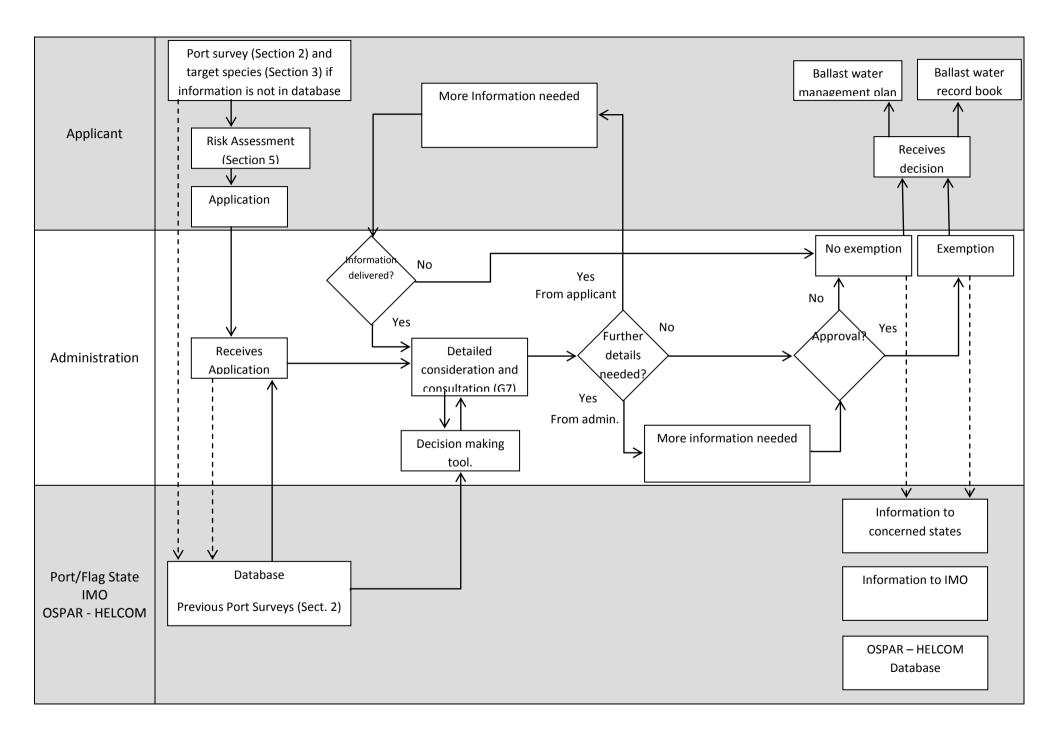
- 6.1 In order to facilitate uniform application of the common OSPAR/HELCOM guidelines across the regions the risk should be evaluated using, in a first step, an automated decision support tool available on the internet.
- 6.2 The decision support tool should be managed by the OSPAR/HELCOM Secretariats and be connected to the central database.
- 6.3 The decision support tool should highlight any differences between the sampling methods.
- 6.4 More information on a possible implementation of such a tool can be found in Annex 4.

#### 7. Administrative Procedures

- 7.1 The IMO G7 guideline identifies the basic procedure and minimum information required for granting an exemption under regulation A-4 of the Ballast Water Convention.
- 7.2 These Administrative Procedures is to be considered as additions to the G7 guidelines, and have been agreed upon by the Contracting Parties of OSPAR and HELCOM.

#### **Application Process**

7.3 To enable a Contracting Party or Parties to consider granting an exemption for a ship from the BWMC under these guidelines, it will be the responsibility of the ship owner/operator seeking the exemption to apply to the Port State(s) directly, copying in their national administration. A ship owner/operator seeking an exemption should note specifically that the procedure for seeking an exemption may take several months to conclude. An overview of the application process is described in the flowchart below.



7.4 If a ship owner/operator applies for an exemption applicable for a route where valid information is available in the database, the Contracting Party or Parties may grant the exemption without requiring new port surveys to be undertaken. The Contracting Party or Parties should also take into account the similarities of the specific ships and under which conditions and terms the existing exemption was granted. For validity of exemptions granted under these conditions see paragraph 7.9.

#### Information to be provided

- 7.5 Information should be provided as set forth in G7. In addition the shipowners/operators should provide information as specified below, upon application within the OSPAR and HELCOM regions.
- 7.6 A copy of the Ship's approved Ballast Water Management Plan should be submitted. Exemptions have to be marked on Ballast Water Management Plan and the plan has to be reapproved after granted exemption.

#### 7.7 Port Information:

a) The applicant should provide at least the information required in section 2, either by submitting data or by using data already available in the database, subject to a burden sharing mechanism. Information on the characteristics of ports which the ship will be visiting should be provided in line with section 2 of the guidelines on Port Surveys and be submitted in the agreed format as included in Annex 1.

#### 7.8 Species Information:

- b) Information on the presence of non-indigenous species should be collected in line with section 2 of the guidelines on Port Surveys and be submitted in the agreed format as included in Annex 1.
- c) Upon submission the ownership of the submitted information will be transferred to the public authorities. A burden sharing mechanism if this information is used by other applicants should be developed.

#### Granting of the exemption

- 7.9 An exemption shall be granted for a maximum of 5 years but no longer than the time period specified by paragraph 2.2 when the port surveys are regarded valid. The approval may contain seasonal and time-specific or other restriction within the time of validity. The intermediate review as suggested in G7 is included in the grant. A recipient port State may require several reviews to be taken during the period the exemption is granted for, but more frequent than annual reviews generally should not be required.
- 7.10 The intermediate review should be based on any new information on the basis of the exemption granted including but not limited to: presence of non-indigenous species, introduction pathways for NIS, changes in physical conditions in the port. To check that the requirements of the exemption has been followed, the intermediate review may include also history of the vessel's voyages (eg. on the basis of log book records) after the exemption was granted.
- 7.11 Where the Contracting Party or Parties in receipt of the application decide on the exemption the shipowner /operator should be notified as soon as possible.
- 7.12 A recommended model for an exemption should be developed for these guidelines in order to ensure the uniformity throughout the HELCOM and OSPAR regions.

#### **Communication of Information**

- 7.13 Relevant contact details for receipt of applications should be submitted to the HELCOM and OSPAR Secretariats by the Contracting Party/Parties for publication on their respective websites.
- 7.14 The decision of the recipient Contracting Party should, in addition to the recipients outlined in G7, be communicated to HELCOM and/or OSPAR as soon as possible before the effective date of the exemption.
- 7.15 If national administrations do not use, or deviate from, the results of the common OSPAR/HELCOM

framework, reasons should be communicated to OSPAR/HELCOM, so that they may inform the review process of these guidelines.

#### Withdrawal of an exemption

- 7.16 In addition to the circumstances outlined in G7, which may result in the withdrawal of an exemption granted under regulation A-4 of the Convention, the following circumstances should also be considered as reasons for a temporary or permanent withdrawal of an exemption. This would include emergency situations or breaches of the exemption conditions such as:
  - A new target species(s) are found in a port where an exemption is granted.
  - Mixing ballast water or sediments between ports or locations other than those outlined in the exemption.

# Annex 1: Detailed description of the Port Survey Protocol

#### Introduction

The HELCOM-OSPAR protocol for comprehensive sampling of alien species in ports has been constructed based on globally and nationally used port sampling protocols (Hewitt and Martin 2001, Inglis et al. 2006, Power et al. 2006, Buschbaum et al. 2010). Methods have already been used in port sampling and therefore allow both standardization and comparability of the data. In addition, all methods used in national monitoring programmes, HELCOM Combine, OSPAR JAMP, or existing port monitoring programs were taken into account.

European Union's Marine Strategy Framework Directive requires monitoring of alien species as well. Ports are mentioned as one of the priorities. However, MSFD monitoring will concentrate on monitoring certain indicators and these data may not fulfill the quality standards for risk assessments. In addition, in MSFD monitoring data would likely be collected from only few hot-spot ports in each country.

Typically surveys of biota include sampling of several different groups of organisms: hard substrate organisms, soft bottom benthos, plankton and mobile epifauna (e.g. fish). All these species groups should be surveyed in a comprehensive sampling protocol. The protocol focuses on groups of organisms that are relatively easy to collect from the quays. Therefore, some organism groups such as meiofauna (including juvenile forms of macrobenthos organisms) are not taken into account in this study. When new sample analysis methods, such as DNA based methods, are available they should be used in addition to increase the detection of non-indigenous species.

As an example, the CRIMP protocol was originally created for baseline surveys in Australian ports in 1995 with the goal to determine the scale of marine invasions as well as to determine the efficacy of survey methods (Hewitt and Martin 1996). An updated version of the survey protocol was published in 2001 following five years of implementation in practice (Hewitt and Martin 2001). The protocol was adopted by IMO GloBallast program for their port surveys. CRIMP protocol relies heavily on scuba diving transects, scuba sampling and visual censuses, which may not be feasible in all ports. Therefore, CRIMP is merely used as an outline for the protocol and scuba methods may be replaced with surface operated methods.

Qualitative surveys, such as Rapid Assessment Survey, provide evaluations of presence of alien species and may be useful in assessing change in spatial distribution of species (e.g. Pederson et al. 2003, Cohen et al. 2005, Ashton 2006). Quantitative methods such as CRIMP (Hewitt and Martin 2001) require more time for field sampling and sample processing. They, however, also provide more detailed data on the abundance of the species which may be required for risk assessment (Hayes and Hewitt 2000).

#### Existing sampling in Baltic and North Sea ports

Currently ongoing national sampling programs or data from previous sampling projects can be utilized in developing the protocol if they exist. Regular monitoring is limited to Estonia. In addition, some individual port surveys and long term projects have been conducted in Poland (e.g Walk et al. 2011), Lithuania, Germany (Buschbaum et al. 2010) and Finland (Paavola et al. 2008). These data, obtained from prior surveys and monitoring in for example in Port of Tallinn will be utilized in determining the efficiency of the proposed sampling protocol in detecting alien species in the ports.

In the OSPAR region several countries (e.g. Germany and Netherlands) have established monitoring activities for non-indigenous species in their waters, including in port areas. Some other rapid assessments have also been undertaken by specific projects.

#### Proposal for a HELCOM-OSPAR port survey protocol

The proposed protocol is developed based on CRIMP sampling protocol (Hewitt and Martin 2001), rapid assessment protocols (Pederson et al. 2003, Cohen et al. 2005, Buschbaum et al. 2010) and aligned with HELCOM and OSPAR monitoring protocols (HELCOM Combine manual, OSPAR CEMP Monitoring Manual) where applicable. Sampling methods were tested over late summer and fall 2012 in Finland and Estonia and the final survey protocol has been modified based on experiences from the field testing. Detailed list of materials and equipment needed for the field sampling is included in Appendix 1.

Level of detail required depends if the data obtained from the surveys will be used only for the risk assessment or for other purposes as well (for example for fulfilling MSFD requirements). For risk assessment only, the most important information is presence and absence of non-indigenous species and their abundance on a scale from 1-5 (or on a percentage scale). For MSFD purposes more details, such as abundance of native species is needed. Level of detail depends obviously heavily on the available resources as well.

All samples are to be analysed by a quality assured laboratory (Appendix 2) to account for adequate taxonomic expertise. In case of finding an unknown species for the area in the survey it should be first photographed and then preserved for further analyses (for example in 96% ethanol for genetic analyses).

ISO/IEC quality assured laboratories are rare. However, other proofs of quality assurance are accepted as well. For example participation in HELCOM quality assurance projects such as ZEN QAI and PEG intercalibration are considered adequate assurance of quality. In addition, any laboratory approved by national administrations can be considered quality assured.

### Survey design

Ports are highly variable environments and provide a number of different habitats for non-native species. Therefore sampling should follow stratified sampling design (Hayek and Buzas 2010). Special attention and increased sampling efforts should be allocated to high priority area types, listed in Table 1(modified from Hewitt & Martin 2001).

Within each port several sites representing a wide range of environmental characters (incl. considering different salinities, water velocities and substrates) should be sampled. At minimum, three sites in each port should be sampled. In case of a port being very large of apparently providing a wide range of habitats, the number of sites should be increased. And consequently, if port is very small, the number of sites can be decreased accordingly. Based on the data obtained from the test surveys, the minimum number of sites required will be updated.

Species effort (accumulation) curves (e.g. Hayek and Buzas 2010) should be presented with the results of each survey to provide proof of adequate sampling effort both in terms of sites and number of samples taken. Since no baseline surveys in the Baltic Sea ports have been conducted, more attention should be given to the first survey at each port. Visual observations and mapping of the general underwater habitats in each port are highly recommended to assure for aiming survey efforts in the most likely sites even if scuba sampling is not possible (for example utilizing underwater cameras, echo sounds etc.).

All different types of hard substrates present in the port (such as concrete, rock, wood, metal and plastic) should in any case be surveyed at each site (Paavola et al. 2008). Also, a minimum of three replicate samples at each site should be taken. Similarly, all different kinds of soft substrate (sand, gravel, mud, clay etc.) in the port area should be sampled and at minimum of three benthic samples at each site should be taken.

#### Sampling/monitoring frequency

Port survey is to be regarded valid for granting exemption for applicants during a period of maximum of 5 years, to be counted from the date of the first of the two sampling visits (spring). A contracting party may decide on a shorter validity for a port survey due to e.g. sensitiveness of the area, intensity of traffic or need for updated port survey data on non-indigenous species.

Due to seasonality and life cycle patterns of some species, survey for mobile epifauna, fouling organisms, and benthic infauna should be conducted between late July and September when majority of the species are mature and identifiable. Plankton samples should be taken and analysed during spring bloom and summer maximum (late summer), which can be combined with performing the rest of the survey. When taking the spring bloom plankton sample, settlement plates should also be deployed simultaneously. Plates can be retrieved when conducting the survey in the late summer.

#### Site selection

Survey is to be designed properly prior to the execution. Port authorities can often provide useful information on the port characteristics such as ballast release locations and most frequently visited berths. Also, survey should be conducted without disturbing port activities and port authorities provide information on selecting such sites. Sites should be selected to represent a range of abiotic conditions and aimed to cover high priority areas (Table 2).

Table 1. Priority of sampling location types based on Hewitt and Martin 2001

Port area	Priority		
Commercial shipping facilities in port			
active berths	1		
inactive/disused wharves	1		
channel markers	1		
tug and pilot vessel berths	1		
slipways	1		
dredge disposal and spoil grounds	2		
breakwaters, groynes etc.	3		

#### Conducting the survey

#### Port characteristics

Information about port characteristics, such as abiotic conditions and port traffic, should also be collected. Port information data sheet (Field data sheet 1) should be filled out in cooperation with the port authorities and by using available data.

Ports often have weather stations recording wind and temperature patterns. Temperature and salinity loggers would be an easy and cost effective addition for recording water properties in the port area and ports are encouraged to install such devices.

#### **Environmental data**

At each site environmental data (minimum requirement being temperature and salinity) should be collected using a submersible data logger and water transparency measured using a Secchi disk. If equipment allow (for example a CTD is available), other water properties such as turbidity and Chlorophyll-a should also be measured. Environmental data will be collected on during both sampling visits (spring bloom and summer maximum).

#### Field sampling

Environmental data should be recorded using Field data sheet 2. GPS location of each of the sampling site should be recorded using WGS84 coordinate system. Salinity at different depths and salinity changes due to tidal effects should be given due consideration in sampling design. Water salinity and temperature should be measured at least at three meter intervals from 1m depth to bottom at each site, taking into account the potential effect of tides and characteristics of the port. If possible, also dissolved oxygen, turbidity and Chlorophyll-a should be measured. Wind speed and direction, air temperature and cloud cover should also

be noted. Water transparency should be measured using a Secchi disk. Sediment type and fractions can be assessed visually from the benthic grab samples or taking a separate sediment sample.

#### **Human pathogens**

One water sample from each site should be taken for detecting the presence of IMO D-2 bacteria both during both sampling visits (spring bloom and summer maximum). Identification of intestinal enterococci, *Eschcericia coli* and *Vibrio cholera* are of special concern. Samples may also be collected by local authorities and these data can be used instead if they exist and fulfill protocol quality requirements.

Sampling pathogens only twice a year provide poor information on pathogen abundances in the port. Therefore, pathogen sampling should be included in the local monitoring to assure for frequent sampling required for detecting the IMO D-2 bacteria in the ports.

#### Field sampling

Water sample of 500 ml from at approximately 30 cm depth should be taken at each site. Analysing laboratory may require additional samples or larger sample volumes. Sampling should follow the guidance described in the EU Bathing Water Directive 2006/7/EC. Sample depth, water depth at the site, and other relevant information should be noted using the Field data sheet 3. To prevent overlapping measurements and excess work, the pathogen sample can be taken at the same location as the environmental data sampling.

#### **Plankton**

Samples for phytoplankton and zooplankton species composition and abundance should be taken at each sampling site. Nets suggested in the protocol are hand held and have been selected to be operable from the dock. One pooled phytoplankton sample, one concentrated phytoplankton sample and two vertical zooplankton samples using nets with different mesh sizes, at each site is required. Both zooplankton and phytoplankton samples are to be taken during both sampling visits (spring bloom and summer maximum).

#### Field sampling

Samples of *phytoplankton* should be collected by obtaining a 250 ml water sample pooled from three locations at least 15 m apart at each site. Samples (0.5-1.0 l) should be taken at each location at the surface (1 m depth) and 5 m depth. Additionally, a concentrated vertical sample using a small hand held 20 µm plankton net should be taken. The specific dimensions of the net used as well as a comprehensive description of the sampling procedure should be recorded in the field data sheet 3 with other relevant information. Three tows, 10 to 15 m apart should be conducted to ensure for adequate sample. Haul and tow rates should not exceed 0.25-0.30 m/s. Clear, colourless iodine-proof bottles with tightly fitting screw caps should be used as containers. Samples should be preserved in acid Lugol solution  $(0.25-0.5 \text{ cm}^3/100 \text{ cm}^3 \text{ sample})$  and placed in a cooler for transport to the analysing laboratory. (Follow HELCOM COMBINE manual Annex 6: Guidelines concerning phytoplankton species composition, abundance and biomass, when applicable)

A vertical zooplankton sample should be collected with a standard 100 - 200 µm mesh free-fall dropnet or similar at each site. Three tows, 10 to 15 m apart should be conducted to ensure for adequate sample. Mesh size depends on the size range of zooplankton in the area and needs to be reported with the data. In addition, a sample of larger zooplankton organisms including gelatinous species should be obtained using a net with mesh size 300 - 500 µm by conducting three tows 10 to 15 m apart. The specific dimensions and mesh size of the net used as well as a comprehensive description of the sampling procedure should be recorded in the Field data sheet 3 with relevant abiotic information. Tow rate should be adjusted to approximately 1 m/s and net stopped 1 m before the bottom. A flow meter can be mounted on the mouth of the web for quantification of the water volume sampled. Details of the sampling procedure, gear used and number of tows in addition to any other relevant information should be noted on the field data sheet and reported in the provided excel sheet. Samples should be placed in sample jars or bottles and in a cooler. Samples should be preserved in 4 % formalin solution prior to transport to the analyzing laboratory or follow the instructions given by the analyzing laboratory. Gelatinous species should be examined immediately after

collection without preservation. If the species identification is unknown, a digital photo should be taken. (Follow HELCOM COMBINE manual Annex C-7 Mesozooplankton, when applicable)

#### **Epifauna**

Mobile epifauna, such as crabs, should be sampled at each site using *light weight traps* tethered to existing structures (pilings, buoys, docks). Sampling may occur only on the second sampling visit (late summer). Traps are selective in nature and therefore provide only relative measures of species abundances. However, methodology for sampling epifauna in the port area is very limited and for example using trawls and gillnets is impossible. Attention should be given to place traps on all available substrates (mud, sand, rocky) and catch reported accordingly. Traps should be baited with locally abundant fish.

*Visual searches*, either by divers or using drop down video equipment, should be conducted at each site prior to deploying the traps to assure for efficient placement and distribution of traps. Divers should swim 50 m transects along the dock/shore at several depths ranging from 0.5 m to the bottom to provide visual idea of the bottom and record presence of any non-indigenous species including epifauna.

#### Field sampling

Two types of traps should be used when sampling mobile epifauna, Chinese crab traps (for example Fukui-designed box traps 63 cm x 42 cm x 20 cm, with 1.3 cm mesh netting, sold in many countries under various names) and minnow traps (for example Gee-minnow trap, 42 cm long and 23 cm wide with 6.4 mm netting and 2.5 cm mouth)(Fig. 1). Minnow traps have been more effective for catching small fish and proven also effective for catching small crabs (such as mud crabs) and shrimp (Pitkänen 2012). Crab traps (box traps) catch larger invertebrates such as *Eriocheir sinensis* and some larger fish species more effectively.

Traps should be baited using locally available fish and should be weighted either by placing rocks (approx. 1 kg) inside (minnow traps) or attaching a 1-2 kg lead weight on their frame (box traps). Traps should be tethered securely to wharves and/or dolphins or other structures. Three traps of both trap type at each site should be deployed for at least 48 h and the soak time (minutes) reported with the catch. Dimensions of the trap type used and bait species used should be reported as well.

After retrieving the traps or conducting trawling or other similar sampling, the catch should be identified and placed in zipper storage bags in a cooler. Depth and location (GPS coordinates) of the sampling as well as gear and soak time and substrate type should be recorded (Field data sheet 3). Later in the laboratory, species identification should be verified (or samples prepared for identification by a quality assured laboratory), measured, weighed, prepared and preserved. Fish and larger invertebrates can be frozen, smaller invertebrates preserved in 4 % formalin solution.





**Figure 1.** Traps suggested to be used in sampling of epifauna (Chinese crab trap on left, Gee's minnow trap on right)

#### Fouling organisms

Scuba sampling of fouling organisms should be conducted at each site if possible. In case of very low visibility or other apparent safety issues, other methods can be used (described below).

Rapid assessment sampling protocol may be a suitable qualitative sampling method for hard substrate organisms at sites of low visibility, such as Baltic ports where diving is not an option. Existing structures within the port area will be targeted and the aim is to identify the species attached to ropes, chains, pilings and hard surfaces using hand held scraping tools and estimate the species coverage, if possible. Sampling of fouling organisms by scraping can be conducted on the second sampling visit only (late summer). Based on test surveys, docks are often high, built on stilts and no ropes or chains are laying in the water and therefore obtaining scrape samples from the dock is frequently close to impossible. Therefore sampling by scuba diving or snorkeling in addition to the use fouling plates (described below) is highly recommended. Settlement plates or settlement collectors (Marshall and Cribb 2004) should be used to improve the survey of fouling organisms (Figure 3). Fouling plates should be deployed during the first sampling visit and retrieved during the second sampling visit.



Figure 2. Scraping tool used in sampling of fouling community

#### Field sampling

#### Scraping

Pilings or projecting steel facings of *wharfs, berths, piers and dolphins* are accorded as high priority in CRIMP protocol (Table 2). At least three pilings or similar structures should therefore be sampled from these abovementioned locations at each site. The first piling should be located about 10 meters from the end of the structure to eliminate any edge effect and other pilings at equal distance (10 - 15 meters) from each other. On *breakwaters, groynes, rockwall facings and natural rocky reefs* three sampling sites should similarly be placed 10 – 15 meters apart. *Hulks (wrecks)* are often hotspots for NIS and therefore should be included in the sampling in a similar manner.

The selected pilings should be vertically inspected and sampled by <u>scuba divers</u>. Three replicate 0.10 m<sup>2</sup> quadrates should be digitally photographed and scrape sampled at depths of 0.5 m, 3.0 m, 7.0 m and close to the bottom. The area of a quadrate can be scraped to the piling surface using a hand-held scraper tool and after taking the photo sample can be scraped straight into pre-labeled zipper bags.

Similarly, on rocky shores or breakwaters three vertical transects should be inspected and sampled as described above. While conducting the sampling, qualitative visual surveys for detecting non-indigenous species should be conducted in the area.

In some locations, diving is not possible due to poor visibility or other apparent safety hazards. Samples should then be obtained by snorkeling by reaching down to 0.5-1 meters depth and by lifting any detached structures such as ropes and chain to the dock. From ropes, quadrates at depths of 0.5 m, 3.0 m, 7.0 m and the bottom should be digitally photographed and scrape samples should be taken if possible. In addition a hand net equipped with a scraping blade (Figure. 2) can be used when obtaining scrape samples from the dock. When scraping, sample falls into the mesh bag and it can be rinsed into a bucket filled with water. When finished with scraping, sample can be sieved with 0.5mm sieve and transferred into a zipper bag. Sampled area should be estimated and reported in the Field data sheet 3.

Samples are to be placed in cooler and transported to the quality assured laboratory for analysis. Prior to transport, samples can be preserved in 4 % formalin solution, frozen or follow specific instructions from the analyzing laboratory.

#### Settlement plates

In the test surveys, the fouling plate units were deployed for approximately six weeks. However, in the HELCOM-OSPAR Port Sampling Protocol, the suggested deployment takes place simultaneously with spring bloom plankton sampling and retrieval with late summer sampling event. Soak time will therefore be at minimum 3 months.

Fouling plate units should be deployed in a location where they will not be disturbed by for example port traffic. Units should be tied securely to the dock structures so that the first plate is submerged at approximately 1 m depth. If the water depth at the site is less than 8 m, the deepest plate may be removed and brick tied at suitable depth for the site. The unit should always remain in a vertical position and the rope should be tight.

Fouling plate units should be retrieved simultaneously with the summer maximum sampling (HELCOM Port Survey Protocol). However, based on the test survey, only six weeks soak time was adequate to acquire a representative fouling community on the plates (Figure 3 B).

When retrieving the units, they should be pulled on the dock as carefully as possible to prevent losing any organisms such as mobile epifauna. The whole unit should be placed on a plastic sheet (or an opened plastic bag) and rope and brick separated from the plates. The plates should be photographed and placed in labeled resealable plastic bags prior to transport. The brick and the rope should be packed to a separate bag. The plates should be kept moist by adding some sea water in the bags. All detached organisms should be collected and placed into a separate labeled ziplock bag. All fouling plate unit's parts should be placed into a cooler and transported to the laboratory as soon as possible.

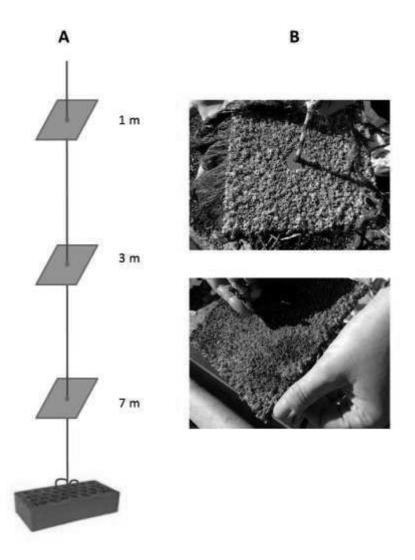


Figure 3. Suggested setup for fouling plates (A) and retrieved fouling plates (B) after 1.5 month soak time.

#### Benthic infauna

Benthic Infauna should be sampled on a 50 m transect using scuba diving. Transect of 50 m should be laid on the bottom perpendicular to the shore starting at sampled pilings. The transect line should be marked at 1 m intervals. Any epibenthos observed in the vicinity of transect should also be collected.

At least three grab samples should be taken at each site located at least 15 m distance from each other using a benthic grab operable from a dock. Sediment quality can either be visually assessed of these samples or a separate sample may be taken for sediment quality analysis. In case of known ballast water discharge at site, additional benthic samples may be taken. Bottom quality may dominate the possibility to obtain samples from certain sites and acquiring a satisfactory sample may require several attempts. In many locations, a concrete slab has been built underneath the docks to prevent erosion. Mooring berths (walking bridges) should therefore be utilized, when possible, to reach further from the shore and obtain satisfactory grab samples. Satisfactory sample requires penetration to approximately 10 cm into the sediment.

Temperature, salinity and oxygen saturation on the bottom should be measured using a submersible data logger at the start of the transect. These data can also be obtained from site readings if the sample location is in the vicinity of the measuring location. Sampling of benthic infauna may occur only on the second sampling visit (late summer).

#### Field sampling



**Figure 4.** Benthic sample obtained by using an Ekman Grab.

When diving is possible, benthic Infauna should be sampled on a 50 m transect by using benthic core tubes (0.025 m² hand corers). Corers should be pushed to a depth of 20 to 25 cm if possible. At each site, three inner cores (0 m at the transect) and three outer cores (50 m at the transect) should be taken. In case of apparent variability, more cores may be taken for example at 25 m on the transect. At each distance, cores may be pooled. They should be transferred to 0.5 mm mesh bags and rinsed under water or transported to the surface for sieving, depending on the conditions.

If diving is not an option, grab samples may be taken instead using a hand operated benthic grab, operable from a dock. Relevant information such as description of the site as well as name and specific dimensions of the sampler used should be recorded on the Field data sheet 3.

Samples should be sieved with a 0.5 mm sieve, transferred to sample jars, preserved in buffered 4% formaldehyde solution (1 part 40% formaldehyde solution and 9 parts water) or alcohol (70%), or follow specific instructions by the analysing laboratory and

placed in a cooler for transport to the analysing laboratory as soon as possible. In the laboratory, samples may be stained using Rose Bengal (1 g/dm<sup>3</sup> of 40% formaldehyde). (Follow HELCOM Combine manual Annex C-8 Soft bottom Macrozoobenthos, when applicable).

#### Specimen handling

All sampled materials should be placed in a cooler and transported to the laboratory for sorting as soon as possible. Preservation or narcotization should take place immediately, never later than 8 hours from collection.

Preservation guidance may be given by the analyzing laboratory and may include:

- Formalin stock (1:1 propylene glycol-formalin) diluted to seawater 1:9 for most of the species
- Hexamin buffered formalin, diluted to 4 %
- Ethanol (96% for genetic analyses)
- Formaldehyde solution and 9 parts water and stained with Rose Bengal (1 g/l of 40 % formaldehyde) for benthic samples

#### Sample processing, analysis and data reporting

All samples should be processed and species identified or identification confirmed by a quality assured laboratory. Executing party should contact the local laboratories prior to the sampling to obtain any specific instructions, equipment and/or materials concerning sample preservation and handling.

At minimum, all species are identified, non-indigenous species to the lowest taxonomic level possible and their abundance estimated using a scale from 1-5 or percentage scale. All species are to be identified to the lowest taxonomic level possible and their number/biomass in the sample counted. Data should be reported

using the agreed format suitable for transferring to the database (Files for field recording (pdf) and recording for entry into the database (Excel) are embedded below).





#### **Human pathogens**

Sample analysis and processing should follow the EU Bathing Water Directive 2006/7/EC and analysis should be conducted by a quality assured laboratory. Analysis of *Cholera* bacteria may require specialized laboratory. Following the sample analysis, presence and abundance (concentration) of IMO D-2 bacteria are to be reported using the agreed format suitable for transferring to the database.

#### **Plankton**

Sample processing and species identification should be conducted by a quality assured laboratory according to their best practices and should follow the HELCOM COMBINE manual Annex C-6: Guidelines concerning phytoplankton species composition, abundance and biomass. At a minimum, all non-indigenous species should be identified and their abundance estimated on a scale 1-5 (1=rare, 5=very abundant) or on percentage scale. Phytoplankton species composition and abundance per sampled volume should be reported using the provided excel sheet. Data should be reported as number of individuals or estimated abundance of each species per sampled volume and reported using the agreed format suitable for transferring to the database.

Zooplankton samples should be analysed according to HELCOM COMBINE manual Annex C-7 Mesozooplankton. At minimum, all non-indigenous species should be identified and their coverage estimated on a scale 1-5 (1=rare, 5=very abundant) or on percentage scale. Species composition should be reported as species abundance or estimated abundance per sampled volume using the agreed format suitable for transferring to the database.

#### Mobile epifauna

Quality assured laboratory or local authorities should confirm species identification from the preserved samples and/or photographs. Otherwise, data can be reported by the executing party. Catch per time interval per a trap (CPUE) should be reported using the agreed format suitable for transferring to the database.

#### Hard substrates

Scrape samples should be qualitatively analysed by local experts or quality assured laboratory. Observed species and if possible their coverage and dry biomass per unit of area should be reported using the agreed format suitable for transferring to the database.

Settlement plates should be analysed by local experts or a quality assured laboratory. Identifying the organisms is easiest when the plates are fresh. If the analysis is delayed, possible preservation methods include 4% formaldehyde, freezing or ethanol. Ethanol tends to deteriorate the coloring of the organisms and therefore the other two are preferred.

All non-indigenous species should be identified and their coverage estimated on a scale of 1-5 (1=rare, 5=very abundant) or on a percentage scale. If resources allow, all species should be identified. After identification, sub-sections of the plates (3-5, depending of variability observed) should be scraped, species sorted, weighed and preserved. Observed species and, if possible, their coverage and biomass per unit of area, should be calculated and reported.

The rope and brick should be analysed first visually and all organisms identified. Both should also be rinsed thoroughly above a 1 mm sieve. All organisms from the sieve should also be identified. Similarly, settlement plates should be analysed by local experts or quality assured laboratory. All non-indigenous species should be identified and their coverage estimated on a scale 1 - 5 (1=rare, 5=very abundant) or on percentage scale. If resources allow, all species should be identified. After identification, sub-sections of the plates (3 – 5, depending of variance observed) should be scraped, species sorted, weighted and preserved. Observed species and if possible their coverage and biomass per unit of area should be reported using the agreed format suitable for transferring to the database.

#### Soft substrates

Samples should be analysed and processed by a quality assured laboratory following guidelines from HELCOM Combine manual Annex C-8 Soft bottom macrozoobenthos. All non-indigenous species in the samples should be identified and their abundance estimated a scale 1-5 (1=rare, 5=very abundant) or on percentage scale. If resources allow, all individuals should be identified counted and their biomass weighed. Results should be reported as abundance or scaled abundance and biomass per unit of volume of sediment using the agreed format suitable for transferring to the database.

## Appendix 1: Field Sampling Equipment

#### Suggested equipment for field sampling

- 1000 ml and 500 ml sterile transparent glass bottles for pathogen samples (usually provided by the analyzing laboratory)
- Water sampler
- Plankton nets
  - Small hand hauled 20 µm net for phytoplankton (450 mm long with 250 mm mouth)
  - $100 150 \,\mu\text{m}$  (or smaller) free fall drop net for zooplankton (for example 400 700 mm opening)
  - 500 µm dropnet for larger zooplankton (for example 3 4 m long with a 700 mm opening)
- 500 ml transparent glass bottles for zooplankton samples
- 250 ml transparent glass bottles for phytoplankton samples
  - Lugol solution
- Clean funnel and a bail (for water samples)
- Scrapers for RAS (handheld, mesh bag attached or hand held scrapers for sampling by snorkeling)
  - 1 − 2 l ziplock bags for the obtained samples
- Traps
  - 6 x Collapsible Chinese crab trap
    - 6 x 2 kg lead weights
    - Cable ties (for attaching the lead weights to the traps)
  - 9 x Shrimp trap (Box or cylinder, 2 mm plastic mesh, 150-200 mm high, 400-500 mm long)
    - Rocks (approx. 1 kg) inside the traps for weight
  - Approximately 250 m of rope for tethering the traps
  - 1 I ziplock bags for the catch
  - Bait fish
- Ekman grab or similar hand-operated benthic grab
  - 0.5 mm sieve
- Jars (1 I) for benthic samples
- Alcohol and/or formaldehyde solution (at minimum 2 l per 3 sites)
- Buckets (rope attached to one for obtaining rinsing water)
- 3 large coolers with cold blocks
- YSI logger or CTD
- Secchi disc
- Digital camera and a GPS device

- Permanent markers
- Labeling tape for the sample containers
- Scuba equipment
- Mesh bags (0.5 mm)
- 50 m transect line, labeled at 1 m intervals
- 0.10 m<sup>2</sup> quadrate frame(s)
- · Camera in an UW housing
- Hand corers

# Appendix 2: Criteria for quality assured laboratories

Quality assured laboratories may include any laboratory qualified with ISO/IEC 17025 standard or its predecessors (ISO 9000, EN-45001). Laboratories that are involved in HELCOM Quality Assurance Programs for phytoplankton (PEG) and zooplankton (ZEN) or meet the requirements of the OSPAR JAMP guidelines on quality assurance for biological monitoring<sup>8</sup> are also considered quality assured. In addition, any laboratory approved by national administrations can be considered quality assured.

<sup>&</sup>lt;sup>8</sup> JAMP guidelines on quality assurance for biological monitoring in the OSPAR area Ref. No. 2002-15

# Annex 2: Target Species list

The target species list embedded below was developed as an initial list however the target species lists of OSPAR and HELCOM should always be consulted as they are living lists under continuous updating by HELCOM MONAS and OSPAR BDC, which means that other species can be included or species can be deleted, if further knowledge is available.



2013 3 Kopie von Target list\_amendem

# Annex 3: Detailed explanations for Risk Analysis Algorithm

#### **Definitions:**

BD Background data

EM Environmental matching risk analysis component

SpS Species-specific risk analysis component

1.1 1st level question (BD): Target species present? (1)

No No Target Species present: low risk

Yes Next level

1.2 2nd level question (BD): Target species equally common in both ports? (2)

Yes Target Species are very common in places of BW exchange.

No problem if some more are added: low risk

No Next level; includes cases in which only very few of the target species are present in one port, but the species is common in the other

1.3 3rd level question (EM): Do ports have very different salinities (≥ 30 PSU)? (3)

For the majority of organisms it would not be possible for all life stages to survive in waters with a difference of more than 30 PSU, and therefore the answer **yes** could mean low risk. To be on the safe side, even in this case a set of additional questions have to be answered for a final risk assessment.

Yes Branch of the 3rd level

No Next level

1.4 Branch of the 3rd level (BD): Is more than one target species present? (4)

The answers to this question lead to a species-specific **(SpS)** examination.

- If there is only one target species, the question is whether it tolerates a salinity range >30 PSU. (5)
  - o If the answer is **no**, then the species will not be able to survive or reproduce in the new environment and the risk is regarded as acceptable.
  - If the answer is yes, then this species could establish itself in the environment. Because it is
    only one species, the risk is regarded as medium, and further criteria must be taken into
    account.
    - If there are more than one target species that tolerates a salinity range >30 PSU?
       (6)
  - If the answer is yes the risk is regarded as unacceptable.
  - If the answer is **no**, then, as above, one species could establish itself in the environment.
     Because it is only one species, the risk is regarded as medium, and further criteria must be taken into account.
- 1.5 5th level (BD): Do the ports have the same salinity range? (7)

This question takes into account the salinity ranges defined in table 1. The marine environment is divided into three categories based on their salinity: saline, brackish and fresh water.

To be on the safe side, the limits of the categories should overlap: if the two locations are not in the same salinity range according to table 1 but have a difference in salinity of less than 10 PSU, they should be regarded as being in the same range.

- Yes Species of concern enter an area that has comparable conditions and are likely to survive: unacceptable risk
- No Next level
- 1.6 6th level (BD): Is more than one target species present? (8)
  - Yes More than one target species is released into an environment that differs in salinity from the origin by less than 30 PSU: unacceptable risk
  - No Next level
- 1.7 7th level (SpS) Does the target species tolerate a salinity range >30 PSU? (9)
  - Yes If the physiological salinity tolerance of the target species is high, the species is likely to survive: unacceptable risk.
  - No If the salinity tolerance of the target species is very narrow (e.g. 5 PSU) it can be assumed that the species has no chances survival. Nonetheless a medium risk that requires further assessment is assumed. Note that it is not sufficient that the salinity tolerance is smaller than the difference of salinity between source and recipient area, as there is a potential for species adaptation.

Table 1: Classification of Salinity

Classification of Salinity according the EU Water Framework Directive		PSU	PSU	Classification for risk assessment
euhalin	Marine, salinity is equal to the salinity in the ocean	> 30	> 18	saline water
polyhalin	Salinity is not much lower than salinity in the ocean	18 to < 30	7 10	Samic Water
mesohalin		5 to < 18		
oligohalin	Very low salinity, mainly in the inner coastal waters with a high amount of freshwater intake, like in lagoons	0.5 to < 5	0.5 – 18	brackish water
fresh water		< 0.5	0 – 0.5	fresh water

# **Annex 4: Decision Support Tool**

#### Introduction

The goal of the Decision Support Tool is to provide a simple interface to a risk assessment for translocation of target species in ballast water between harbours. It bases on a risk assessment algorithm, which uses the information about occurrence of target species and their characteristics for assessing the riskiness that they will survive and spread in the recipient harbour. Therefore a well-structured organization of the port sampling data and the species information is required.

#### User interface

The prototype of the decision support tool is a web application that uses a start and a destination harbour as input and calculates three level of risk (low, medium and high) for a transfer between them as output. Different levels of explanations for the resulting risk assessment are provided.

The design is flexible and scalable. This means it is possible to integrate changes with little effort in the data structure and in the web application. It is possible to import data from the field measurements with standard database tools.

# Status and Contents of the database and respective data.

The prototype of the Risk Assessment Tool includes the following information components:

- Harbour profiles (statistical information about environment, size and some business parameters of harbours)
- In situ measurements (abundance and biomass of species) detected in the harbours.
- Lists of target species (optionally defined for different regions)
- · Risk Assessment Algorithm

All parameters that should be sampled and that can be saved in the database for species, harbours and field measurements are listed in Annex 1. For this purpose, an Oracle11 database was created by Brockmann Consult GmbH, the respective data model is shown in Annex 2. Within the Alien-2 project, the data base was filled with data gained form literature and with first sample data from two harbours. It contains the following data information:

**Harbour profiles:** All harbour data were collected from literature sources. There are 9 harbours currently in the database:

Finland: Hamina

Kokkola Turku

Naantali

Germany: Hamburg

Kiel

Belgium: Antwerp

Lithuania: Klaipeda

Sweden: Gotheburg

In situ measurements: There are three kinds of in-situ data in the database:

- Data from literature sources (11 samplings)
- Test data for some extreme cases (fake, only for test of RA algorithm)
- Real data from finish harbours Turku and Naantali delivered from aliens-2 project (6 sampling with 111 samples with 870 species captures)

**Lists of target species:** The latest version of target species list from aliens-2 project was implemented in the database. There are 316 species, 112 of them were defined as target species. Only 31 target species were observed in real in-situ data.

### Web application as interface to the data and the decision tool

The web application for the prototype Decision Support tool was developed with Oracle Application Express (APEX). APEX is a modern Rapid Application Development Technology that provides very efficient development of scalable web applications and deployed of them in any Oracle environment.

The web application is deployed on a webserver at Brockmann Consult and is available for authorized users under address:

http://www.brockmann-consult.de/ballast water RA.

Two user accounts exist currently in the HELCOM-DB:

- **bw\_reader** with possibility only to view data and decision tool: password balwat
- **bw\_writer** with additionally possibility to edit data in species- and harbor-tables.

#### It is possible currently in the Decision Support Tool:

- View all saved data in the database
- Change the data representation
- Use some analytical function and create simple diagrams online
- Export data in csv format
- Edit/insert/delete information for port profiles and species (for authorized users only)
- Use the risk assessment tool including information about the decision
- Visualize the species accumulation curves for Naantali and Turku for six organisms groups
- Link to detailed information for species through links to other databases ( not complete )
- See a picture with the RA Algorithm description
- · See the database data model

It is possible for the tools developer to create additional views on the data very quickly.

# Example from web application for the Risk Assessment Tool

#### Views on the sampling data and target species

The following figures show the different views on the sampling data with examples for data queries, harbour sheets and species accumulation curves.

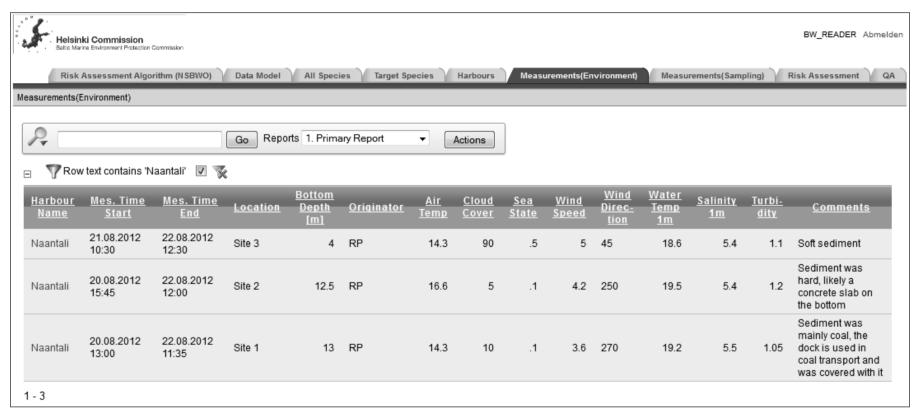


Figure 5: Sampling data; query for all sampling form Naantali

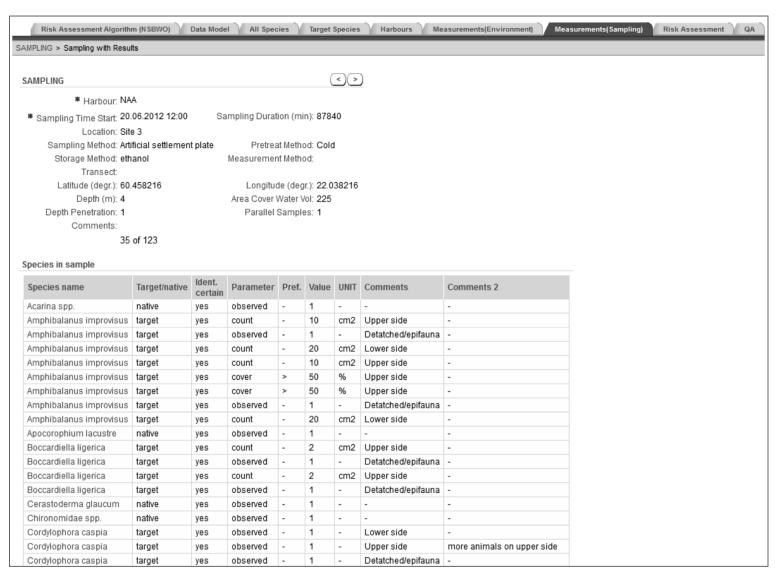


Figure 6: Sampling with details in Naantali on 20.062012

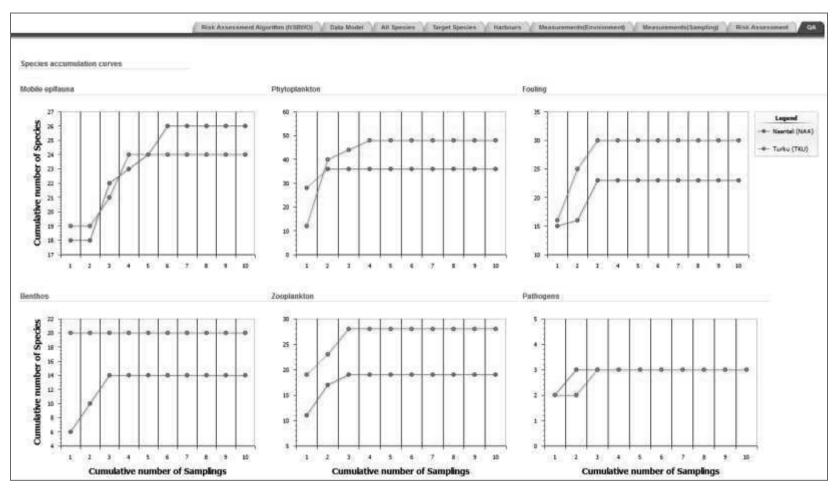


Figure 7: Species accumulation curves for Turku and Naantali (6 organisms group)

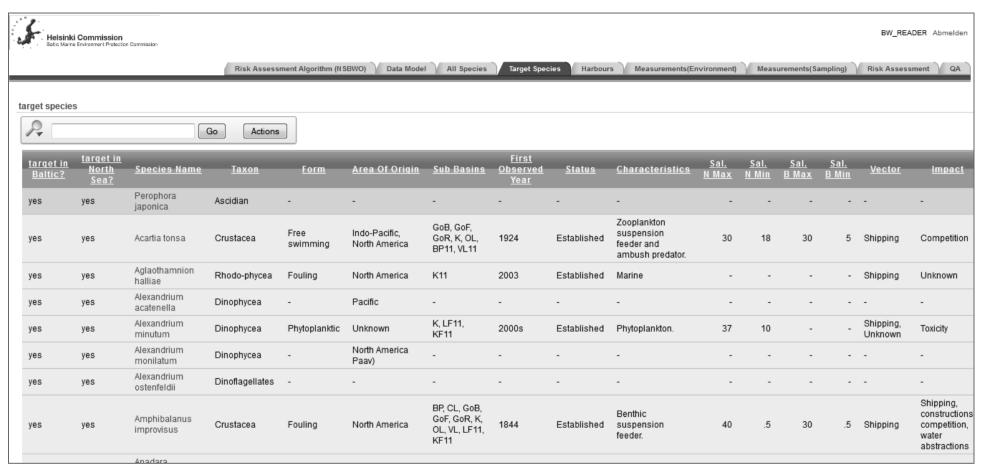


Figure 8: Target Species

#### Views on the Risk Assessment Tool

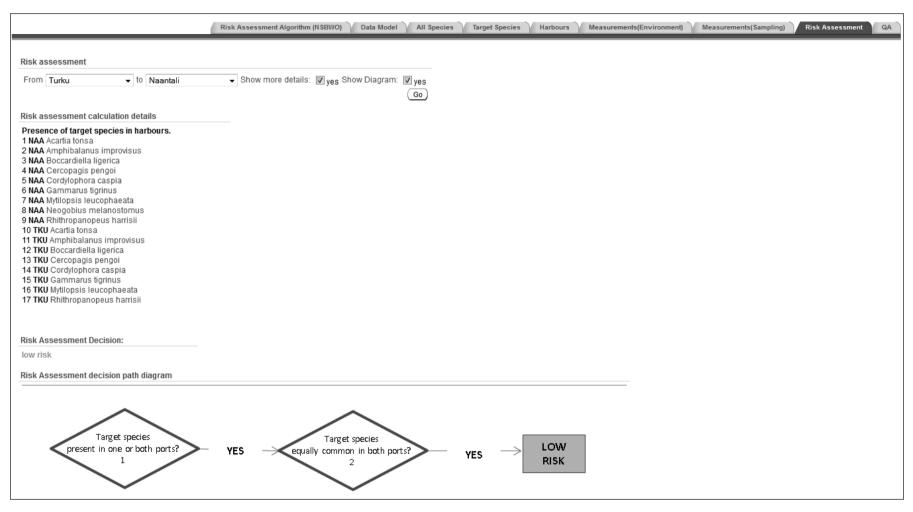


Figure 9: Risk assessment for travelling from Turku to Naantali: low risk (all target species observed in Turku were observed in Naantali too)

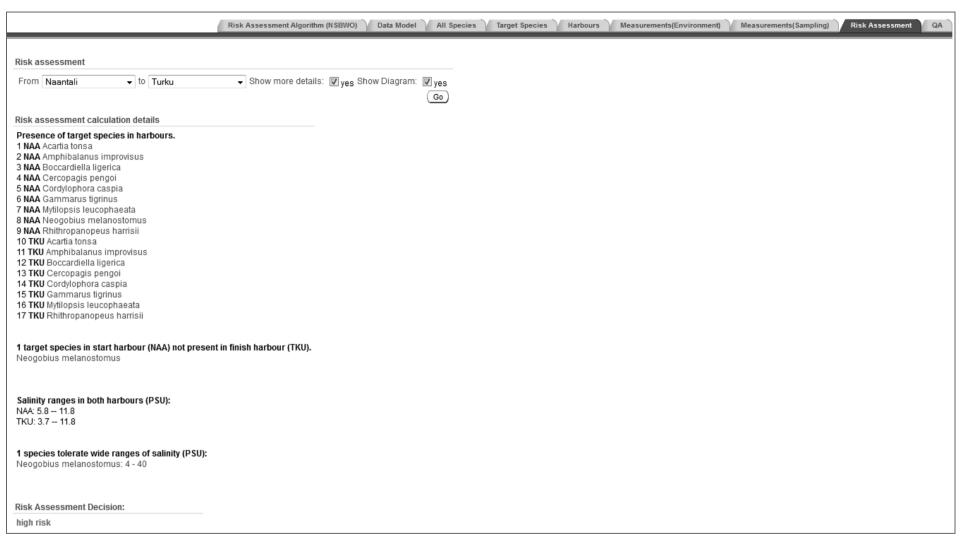


Figure 10: Risk assessment for travel from Naantali to Turku: high risk (one target species Neogobius melanostomus founded in Naantali was not observed in Turku, see explanation diagram below)

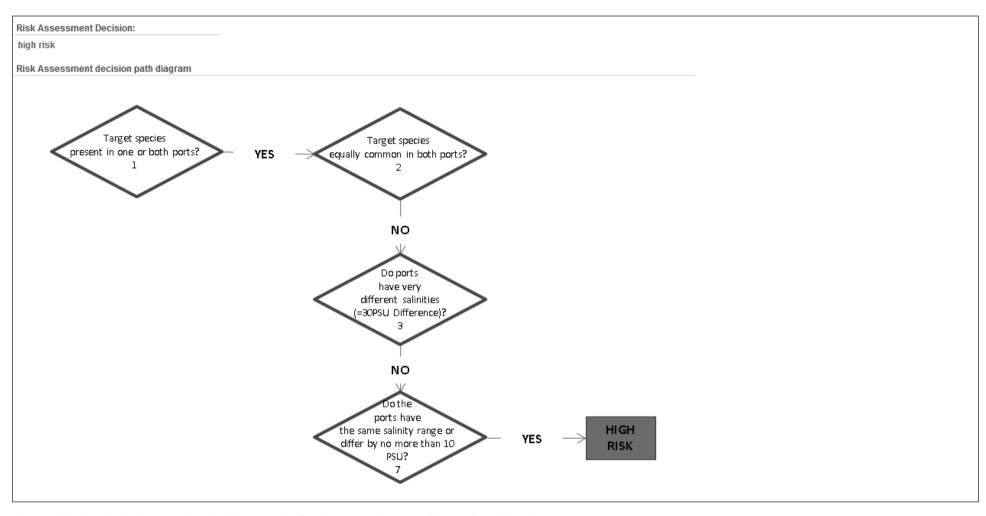


Figure 11: Detailed view on the decisions made fort he route between Naantali and Turku

# Proposals for further development of database and web application

The prototype for the risk assessment tool demonstrates the support for the application of exemption granting for ballast water management systems. However, some points are still open and should be addressed accordingly. Here is a list of improvements that would be possible to realize in future activities.

- Import data from additional harbours
- · Regular update of target species list
- Assimilation of alternative RA-algorithms
- Add data for the decision knot "Do species have the ability for natural spread?" and implement it in the web application
- Add a harbour criterion: is enough data collected for RA?
- Add reports with statistical analyses of the data for end users
- Add connections with other databases

#### References

Gollasch, S.; David, M., Leppäkoski, E. (2011): Pilot risk assessments of alien species transfer on intra-Baltic ship voyages.- Final Report V. 1.3, HELCOM Projet No. 11.36

Heyer, K. (2012): Compiling and testing of biological risk assessments for the invasion of alien species with ballast water.

Puntila, R. (2012): Final Report Aliens-2 Project

#### Content of the data base

#### Species parameters (column names in database table species):

- 1. FORM
- 2. SPECIES\_NAME
- 3. TAXON
- 4. AREA\_OF\_ORIGIN
- 5. SUB BASINS
- 6. FIRST\_OBSERVED\_YEAR
- 7. STATUS
- 8. CHARACTERISTICS
- 9. SALINITY\_N\_MAX
- 10. SALINITY\_N\_MIN
- 11. SALINITY\_B\_MAX
- 12. SALINITY\_B\_MIN
- 13. VECTOR
- 14. IMPACT
- 15. IMPACT\_SEVERENESS
- 16. COMMENTS
- 17. LAST\_UPDATE
- 18. UPDATED\_BY\_USER
- 19. LINK
- 20. SYNONYM 1
- 21. SYNONYM 2

#### Harbour parameters (column names in database table harbour):

- 1. HARBOUR\_NAME
- 2. COUNTRY

- 3. GENERAL\_DESCRIPTION
- 4. ESTABLISHED\_YEAR
- 5. RECENT\_CONSTRUCTION
- 6. LOCATION
- 7. LATITUDE
- 8. LONGITUDE
- 9. CATCHMENT\_AREA
- 10. SHIP MOVEMENTS
- 11. MAIN\_SHIPPING\_ROUTES
- 12. BALLAST\_WATER\_AMOUNTS\_TAKEN
- 13. BALLAST\_WATER\_AMOUNTS\_RELEASED
- 14. BALLAST\_WATER\_ORIGIN
- 15. HABITAT\_DESCRIPTION
- 16. EXISTING MONITORING
- 17. ADJACENT\_WATERS
- 18. SALINITY\_MAX
- 19. SALINITY\_MIN
- 20. SEA\_SURFACE\_TEMP\_MAX
- 21. SEA\_SURFACE\_TEMP\_MIN
- 22. SEA\_FLOOR\_TEMP\_MAX
- 23. SEA\_FLOOR\_TEMP\_MIN
- 24. TIDAL\_RANGE
- 25. ICE DAYS
- 26. COMMENTS

#### In-situ data environment parameters (column names in database table environment):

- 1. SAMPLING\_DATE\_START
- 2. SAMPLING\_DATE\_END
- 3. ORIGINATOR
- 4. LATITUDE
- 5. LONGITUDE
- 6. LOCATION
- 7. BOTTOM\_DEPTH
- 8. AIR TEMP
- 9. CLOUD\_COVER
- 10. SEA STATE
- 11. WIND SPEED
- 12. WIND\_DIRECTION
- 13. WATER\_TEMP\_BOTTOM
- 14. WATER TEMP 1M
- 15. WATER\_TEMP\_3M
- 16. WATER\_TEMP\_7M
- 17. SALINITY BOTTOM
- 18. SALINITY 1M
- 19. SALINITY\_3M
- 20. SALINITY 7M
- 21. TURBIDITY
- 22. SEDIMENT\_ORGANIC\_CONTENT
- 23. SEDIMENT MEDIAN
- 24. SEDIMENT\_GRAN\_SIZE\_1
- 25. SEDIMENT GRAN SIZE 2

- 26. SEDIMENT\_GRAN\_SIZE\_3
- 27. SEDIMENT\_GRAN\_SIZE\_4
- 28. SEDIMENT\_GRAN\_SIZE\_5
- 29. SEDIMENT\_GRAN\_SIZE\_6
- 30. DO\_BOTTOM
- 31. DO\_1M
- 32. DO\_3M
- 33. DO\_7M
- 34. COMMENTS

#### In-situ data sampling parameters (column names in database table sampling):

- 1. DATE\_TIME\_UTC
- 2. LOCATION
- 3. SAMPLING\_METH
- 4. PRETREAT\_METH
- 5. STORAGE METH
- 6. MEASUREMENT\_METH
- 7. TRANSECT
- 8. LATITUDE
- 9. LONGITUDE
- 10. DEPTH
- 11. SAMPLING DURATION
- 12. AREA\_COVER\_WATER\_VOL
- 13. DEPTH\_PENETRATION
- 14. PARALLEL\_SAMPLES
- 15. COMMENTS
- 16. ORGANISMS GROUP

#### In-situ data species observation parameters (column names in database table result):

- 1. PARAMETER
- 2. SPECIES\_NAME
- 3. IDENTIFICATION\_CERTAIN
- 4. NATIVE\_SPECIES
- 5. PREFIX
- 6. VALUE
- 7. UNIT
- 8. COMMENTS
- 9. COMMENTS\_2

#### Data Model

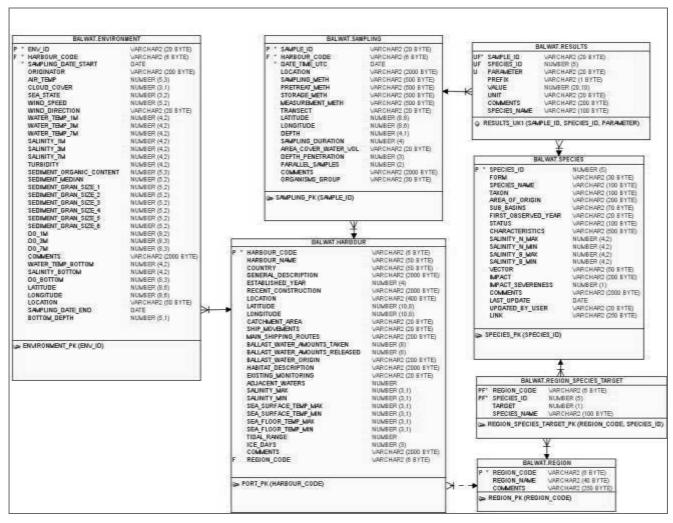


Figure 12: Data model