

HELCOM ALIENS 2

**Non-native species survey
protocols, target species
selection and risk assessment
tools for the Baltic Sea**



Helsinki Commission
Baltic Marine Environment Protection Commission

HELCOM ALIENS 2

Non-native species port survey protocols, target species selection and risk assessment tools for the Baltic Sea



Helsinki Commission

Baltic Marine Environment Protection Commission

This is the final report of the HELCOM project *Study on Biological Survey Protocols and Target Species Selection (HELCOM ALIENS 2)*. It was endorsed by HELCOM MARITIME 11/2012 (Minutes § 4.21) and subsequently approved for publishing by HELCOM HOD 39/2012 (Minutes § 3.16 LD 41).

Published by:

Helsinki Commission

Katajanokanlaituri 6 B
FI-00160 Helsinki
Finland
<http://www.helcom.fi>

Authors

Riikka Puntila, Karin Heyer, Kerstin Stelzer and Eugen Faber

Contributors (Project Core Group members)

Hermann Backer, Ulrik Berggreen, Maiju Lehtiniemi, Laura Meski, Henrik Ramstedt, Manfred Rolke and Kai Trümpler

Acknowledgements (Correspondents, Meeting participants and Field assistants)

Susanna Airola, Cecilia Andersson, Dick Brus, Jonathan David Carl, Carl Carlsson, Jan Schmidtbauer Crona, Stephan Gollasch, Lena Granhag, Wolfgang Hintzsche, Andres Jaanus, Ilmar Kotta, Erkki Leppäkoski, Daniel Masson, Daria Mróz, John Mouat, Anita Mäkinen, Henn Ojaveer, Peter Olsen, Tatjana Olson, Anna Orchard, Stina Paulin, Tiina Piltti, Johan Roos, Mattias Rust, Niklas Rönnerberg, Tamara Shiganova, Peter Anton Staehr, Holger Steinbock, Mads S. Thomsen, Philip van Wijnen, Camilla Witt, Juho Yli-Rosti and Mariusz Zabrocki.

For bibliographic purposes this document should be cited as:
HELCOM 2013. HELCOM ALIENS 2- Non-native species port survey protocols, target species selection and risk assessment tools for the Baltic Sea. 34 pp.

Information included in this publication or extracts thereof are free for citing on the condition that the complete reference of the publication is given as above.

Copyright 2013 by the Baltic Marine Environment Protection Commission – Helsinki Commission

Photo credits:

Front cover: Riikka Puntila

Number of pages: 34

Preface

Maritime transportation in the Baltic Sea region has steadily increased during the last decade. On the average 2000 ships are at sea each day and the maritime transportation of goods in the region is estimated to double by 2017. Transport and introductions of non-native species has been perceived as one of the primary threats to the coastal ecosystems worldwide and ships' ballast water has been identified as one of the main vectors transporting the species. Due to increasing shipping, increasing number of non-native species is arriving into the Baltic Sea. The invaders can induce considerable changes in the structure and dynamics of marine ecosystems and they may also cause adverse impacts on the economy or even represent risks to human health. Over 120 non-native aquatic species have been recorded in the Baltic Sea to date, and around 80 of these have established.

The HELCOM Contracting States have agreed to ratify the 2004 International Convention on the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) by 2010 or at the latest by 2013. When the BWM Convention enters into force it will be a crucial step towards the reduction of spreading of non-indigenous species. To facilitate ratification process in each country, the HELCOM Ballast Water Road Map was adopted as a part of the HELCOM Baltic Sea Action Plan. HELCOM Contracting Parties also decided that the use of ballast water exchange zones is not regarded as an efficient ballast water management tool for intra-Baltic shipping mainly due to the species' great natural dispersal ability.

According to the BWM Convention, ships will be required to implement ballast water management unless an exemption, following a risk assessment, has been granted. HELCOM Guidance on how to distinguish between high and low risk – a risk of secondary spreading of alien species through ballast water and sediments – by ships engaged in intra-Baltic voyages was adopted by the HELCOM Contracting Parties/States at the HELCOM Ministerial Meeting on 20 May 2010 in Moscow. The aim of the Guidance is to support transparent and consistent risk assessments of regional ship voyages and allow a unified Baltic Sea system on exemptions from applying ballast water management in accordance with the BWM Convention Regulation A-4. The Guidance was tested within the HELCOM project "Pilot risk assessments of alien species transfer on intra-Baltic ship voyages". However, the availability of port survey data and selection of target species for the purpose of the risk assessments were identified by the HELCOM Maritime Group as topics requiring further regional attention. Availability of data on alien species and environmental conditions in ports are a pre-requisite for carrying out reliable A-4 risk assessments.

The HELCOM ALIENS 2 - project concentrated on proposing a regionally harmonized method for granting exemptions from ballast water treatment (BWMC A-4) for marine traffic in the Baltic Sea based on the previous decisions of the Helsinki Commission. The project focused on three main themes: 1) establishing a protocol to be used in collecting information from ports in order to conduct reliable risk assessments, 2) defining the criteria for selection of target species to be used in the risk assessment, and 3) creating a harmonized decision support tool to run the risk assessments using the available data (collected by using the protocol) and target species (selected using the criteria).

This report, the final outcome of the project, describes the protocol, principles for target species selection as well as the database and decision support tool development.

Content

1. Introduction	1
2. Proposal for the HELCOM port survey protocol	2
2.1 Background	2
2.2 Existing sampling in Baltic ports.....	2
2.3 Proposal for a HELCOM port survey protocol	4
2.4 Survey design.....	4
Sampling/monitoring frequency	4
2.5 Site selection	5
2.6 Conducting the surveys	5
Port characteristics.....	5
Environmental data	5
Human pathogens	5
Plankton	6
Epifauna	7
Fouling organisms	8
Benthic infauna.....	9
Specimen handling.....	10
Sample processing, analysis and data reporting.....	10
2.7 Field tests of the proposed protocol	12
Estimates of survey costs	13
Appendix 1. Suggested equipment for field sampling.....	14
Appendix 2. List of quality assured laboratories	15
3. Proposal for harmonized Target species selection criteria	17
3.1 Background	17
3.2 Proposals of useful methods.....	19
3.3 Definition of harmonized criteria.....	19
3.4 Testing the harmonized criteria, with existing target species lists.....	22
3.5 Proposal of how to work the target species list	25
4. Developing a data/decision support tool	26
4.1 Background	26
4.2 Defining the required information for the ballast water database.....	26
4.3 Developing the data model	26
4.4 Filling database tables with data	28
4.5 Creating a template for the data input file	28
4.6 Defining a Risk Assessment algorithm.....	28
4.7 Developing and testing the web application for the NSBWO Risk Assessment.....	30
4.8 Next steps.....	31
5. Literature cited	32

1. Introduction

Transport and introductions of alien species have been perceived as one of the primary threats to the coastal ecosystems worldwide (Elton 1958, Lubchenco et al. 1991, Vitousek et al. 1996). Since the introduction of steel hulled vessels ballast has been used to stabilize the vessel and ensure its safety, en route and ballast water has been identified as one of the main vectors transporting alien species (Carlton 1985).

The International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) (IMO 2004) by the International Maritime Organization (IMO) was adopted by a consensus at a Diplomatic Conference held at IMO Headquarters in London on 13 February 2004. In the HELCOM Baltic Sea Action Plan the Contracting Parties have agreed to ratify the 2004 International Convention for Control and Management of Ships' Ballast Water and Sediments (BWM Convention) as soon as possible, but in all cases no later than 2013. Once the BWM Convention enters into force, it will be instrumental in reducing the risk of transfer of alien species to the Baltic Sea.

According to the BWM Convention, ships will be required to implement the ballast water management unless an exemption has been granted following a risk assessment to assess whether a ship is on a voyage posing a high or low risk of spreading alien species (IMO G7, MEPC.162(56)). Port States may undertake the risk assessment themselves or require the ship-owner or operator to undertake the risk assessment. Even if a Party to the BWM Convention has determined that the ship-owner or operator should undertake the risk assessment, the Party should still provide relevant information, including any application requirements, the risk assessment model to be used, any harmful species to be considered, data standards and any other required information

In the Road map towards harmonized implementation and ratification of the BWM Convention, adopted as part of the HELCOM Baltic Sea Action Plan, the Contracting Parties agreed to arrive at a unified Baltic Sea exemption system. IMO provides Guidelines for Risk Assessment under Regulation A-4 (G7) as an appendix for the Convention resolution (MEPC.162(56)). It defines the requirements for granting exemptions beginning from data quality requirements to risk assessment procedures. The 2010 HELCOM Moscow Ministerial Meeting adopted the Guidance to distinguish between unacceptable high risk scenarios and acceptable low risk scenarios – a risk of spreading of alien species by ships on intra-Baltic voyages, which follows closely IMO's guideline G7.

During 2011 the Guidance was tested within the HELCOM project "Pilot risk assessments of alien species transfer on intra-Baltic ship voyages" (Gollasch et al. 2011), with good results. However, the availability of port survey data and selection of target species for the purpose of the risk assessments were identified by HELCOM MARITIME as topics requiring further regional attention. Availability of data on alien species and environmental conditions in ports are a pre-requisite for carrying out reliable A-4 risk assessments.

Also, in order to create a regional dataset of port profiles with biological data on alien species, it is essential that the same sampling protocol is followed within the surveys carried out in the Baltic Sea. Moreover, HELCOM MARITIME highlighted the need to cooperate with the North Sea countries to put in place a consistent exemption regime.

The aim of the HELCOM Aliens 2 project was to propose a regionally harmonized method for granting exemptions from ballast water treatment (BWMC A-4) for marine traffic in the Baltic Sea based on the previous decisions of the Helsinki Commission. The project focused on three main themes. The first theme concentrated on establishing a protocol to be used in collecting information from ports in order to conduct risk assessments. The second theme concentrated on defining the criteria for target species selection to be used in the risk assessment. The third theme concentrated on creating a harmonized decision support tool to run the risk assessments using the available data (collected by using the protocol) and target species (selected using the criteria).

2. Proposal for the HELCOM port survey protocol

Riikka Puntila

2.1 Background

This proposal for a HELCOM protocol for comprehensive sampling of alien species in ports has been constructed based on globally and nationally used protocols (Hewitt and Martin 2001, Inglis et al. 2006, Power et al. 2006, Buschbaum et al. 2010), which 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 programmes 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 programme for their port surveys. CRIMP protocol relies heavily on scuba diving transects, scuba sampling and visual censuses, which are not feasible in the Baltic Sea ports. Therefore, CRIMP is merely used as an outline for the protocol and scuba methods are replaced with surface operated methods.

Qualitative surveys, such as Rapid Assessment Survey, provide evaluations of the 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).

2.2 Existing sampling in Baltic ports

Currently ongoing national sampling programmes or data from previous sampling projects can be utilized in developing the protocol if they exist. Currently existing and ongoing sampling in ports or in their vicinity is presented in Table 1. 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, for example, in the Port of Tallinn will be utilized in determining the efficiency of the proposed sampling protocol in detecting alien species in the ports.

Table 1. Existing sampling in Baltic ports or their vicinity. Please note that monitoring is currently only carried out in Estonia.

Country	Sample	Port	Sample type	Started	Frequency	Responsible party
Estonia	Phytoplankton	Muuga Bay (Port of Tallinn)	Analysis according to HELCOM methodology	1997	One or more times per month Monthly (Mar/Apr-Oct)	Estonian Marine Institute, University of Tartu
	Zooplankton	Muuga Bay (Port of Tallinn)	Juday net. Analysis according to HELCOM methodology	Early 1990s	One or more times per month Monthly (Mar/Apr-Oct)	Estonian Marine Institute, University of Tartu
	Abiotic data	Muuga Bay (Port of Tallinn)	CTD, water transparency, nutrients	Early 1990s	One or more times per month Monthly (Mar/Apr-Oct)	Estonian Marine Institute, University of Tartu
	Macrozoobenthos	Muuga Bay (Port of Tallinn)	Ekman, van Veen samplers. Analysis according to HELCOM methodology	1997	One or more times per month Monthly (Mar/Apr-Oct)	Estonian Marine Institute, University of Tartu

2.3 Proposal for a HELCOM port survey protocol

The proposed protocol is developed based on the CRIMP sampling protocol (Hewitt & Martin 2001), rapid assessment protocols (Pederson et al. 2003, Cohen et al. 2005, Buschbaum et al. 2010) and aligned with HELCOM monitoring protocols (HELCOM COMBINE Manual) where applicable. Sampling methods were tested over late summer and fall 2012 and the final survey protocol was modified based on experiences from the field testing.

The level of detail required depends on whether the data obtained from the surveys will be used only for the risk assessment or for other purposes as well (for example MSFD). 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. The level of detail depends obviously 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).

2.4 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 & Buzas 2010). Special attention and increased sampling efforts should be allocated to high priority area types, listed in Table 2 (modified from Hewitt & Martin 2001).

Within each port several sites representing a wide range of environment (incl. considering different salinities, water velocities and substrates) should be sampled. At a minimum, three sites in each port should be sampled. In case of a port being very large or apparently providing a wide range of habitats, the number of sites should be increased. And consequently, if a 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) curve (e.g. Hayek & Buzas 2010) should be drawn following each survey to assure for adequate sample size. 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 of the general underwater features 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 in the port area should be sampled and at a minimum, three benthic samples at each site should be taken. The list of equipment needed for the field sampling is attached as Appendix 1.

Sampling/monitoring frequency

Following the baseline survey, monitoring utilizing the same protocol should be conducted at fixed intervals. Exemptions from BWMC can be granted for up to five years. However, IMO recommends reviewing granted exemptions at periods of 12 to 36 months (IMO G7 MEPC.162(56)). A review can include a new survey if deemed necessary and the review frequency may be decided based on, for example, traffic in the port.

Due to seasonality and life cycle patterns of some species, survey for epifauna, hard substrates and benthos should be conducted between late July and September, when the 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.

2.5 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 2. Priority of sampling location types based on Hewitt & Martin 2001

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

2.6 Conducting the surveys

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.

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 allows (for example a CTD is available), other water properties such as turbidity and Chlorophyll-a should also be measured.

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. Water salinity and temperature should be measured at least at bottom, 7 m, 3 m and 1 m depths at each site. 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 the IMO D-2 bacteria. Identification of intestinal enterococci, *Escherichia 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 once 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

A water sample of 500 ml from at approximately 30 cm depth should be taken at each site. The 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.

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 cm³/ 100 cm³ 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. The 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 the 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. The tow rate should be adjusted to approximately 1 m/s and the 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). 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.

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 different 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 weighed 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

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.

Field sampling

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



Figure 2. Scraping tool for sampling fouling organisms

In most locations, it is not possible to obtain samples further than arms reach. When structures can be lifted to the dock (for example ropes and chains), 3 x 0.10 m² quadrates should be digitally photographed and scrape samples should be taken at depths of 0.5 m, 3.0 m, 7.0 m and the bottom. Otherwise, samples should be taken at all suitable locations reaching down or snorkeling.

Hand net equipped with a scraping blade (Fig. 2) can be rinsed in a bucket filled with water and when finished with a scraping location, the sample can be sieved with 0.5mm sieve. After sieving,

the sample can be transferred to a ziplock bag, placed in a cooler and transported to the quality assured laboratory for analysis. When snorkeling, the sample can be scraped with a hand scraper straight into the ziplock bag. Prior to transport, samples can be preserved in 4 % formalin solution, frozen or follow specific instructions from the analyzing laboratory.

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, fouling plate method (described below) and obtaining samples by snorkeling are highly recommended.

Settlement plates or settlement collectors (Marshall & Cribb 2004) should be used to improve the survey of fouling organisms (Fig. 3). Three plates (sanded grey PVC-plates, 15 x 15 cm) should be deployed to the above mentioned structures or nearby them to depths 7 m, 3 m and 1 m for at least 1.5 - 2 months. When retrieving the plates, the plates should be separated from the rope and all pieces placed in separate labeled plastic bags. The rope should be placed in a separate bag and the brick inspected visually. Prior to transport, the plates should be photographed and placed in a cooler. Plates can be preserved in 4 %

formalin solution (or 96 % ethanol if genetic analysis will follow) (or follow specific instructions from the analysing laboratory) and transported to the quality assured laboratory for analysis.

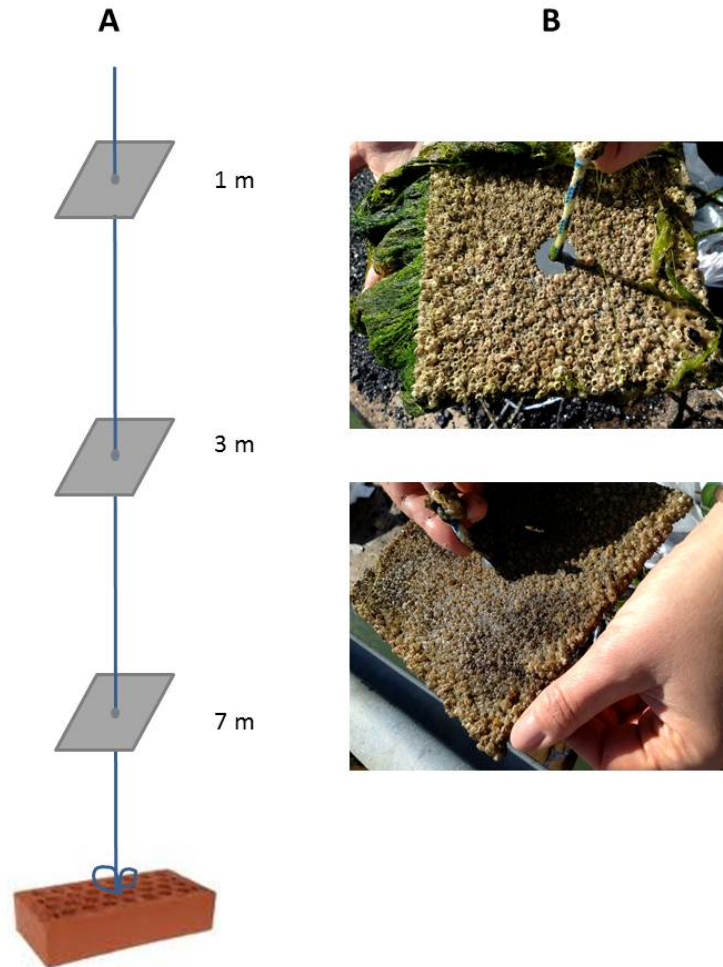


Figure 3. Suggested setup for fouling plates (A) and retrieved fouling plates (B) after 1.5 month soak time.

Benthic infauna

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. A satisfactory sample requires penetration to approximately 10 cm into the sediment.

Field sampling

Grab samples should be taken using a hand operated benthic grab, operable from a dock. Temperature, salinity and oxygen saturation on the bottom should be measured using a submersible data logger. These data can also be obtained from site readings if the sample location is in the vicinity of the measuring location. Other relevant information as well as the



Figure 4 Benthic sample obtained using an Ekman Grab.

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/dm³ 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. The 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 a minimum, all non-indigenous species are to be identified to the lowest taxonomic level possible and their abundance estimated using a scale from 1-5 or percentage scale. If

resources allow, 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 provided excel sheets (file available at the HELCOM Meeting Portal).

Human pathogens

The sample analysis and processing should follow the EU Bathing Water Directive 2006/7/EC and analysis should be conducted by a quality assured laboratory. The analysis of *Cholera* bacteria may require a specialized laboratory. Following the sample analysis, presence and abundance (concentration) of the IMO D-2 bacteria are to be reported using the provided excel sheet.

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 using the provided excel sheet.

Zooplankton samples should be analysed according to HELCOM COMBINE Manual Annex C-7: Mesozooplankton. At a 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 provided excel sheet.

Mobile epifauna

A 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 provided excel sheet.

Hard substrates

Scrape samples should be qualitatively analyzed 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 provided excel sheet. 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, weighed and preserved. Observed species and, if possible, their coverage and biomass per unit of area should be reported using the provided excel sheet.

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 on 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 provided excel sheet.

2.7 Field tests of the proposed protocol

During August-September 2012 the described survey protocol was tested in the Ports of Turku (FIN), Naantali (FIN) and Muuga (EST) and modified based on experiences gained. The aim of these surveys was to test the suitability of the proposed selected methods to port conditions as well as evaluate associated costs of the surveys protocol. In Turku and Naantali three working days and in Muuga (Port of Tallinn) one day was allocated to carry out surveys. Port authorities were contacted well in advance at the end of June about conducting the surveys and the required permissions to the port areas were obtained. In all cases, ports warmly welcomed testing the protocol. Also, at the end of June three sets of settling plates were deployed in the ports of Turku and Naantali (see Fig. 3), one set at each sampling site.

The Port of Turku

The Port of Turku was surveyed on 15–17 August. Three sampling sites were selected to represent a range of different conditions (salinity, type of traffic, etc.) in the port; however, all sites were classified as priority 1 sites (Table 2). The first site was located in the vicinity of frequent passenger ferry traffic, the second one in the vicinity of a cargo terminal and the third one in the oil port. In these sites all abovementioned samples were taken and fouling plates retrieved (20 August). The sampling team consisted of 2 to 3 persons and field sampling took the total of 13.5 working hours (33 man hours). Transport of pathogen samples to the laboratory cut the second work day short, otherwise all sampling could have been finished in two working days. However, for epifaunal sampling traps should be deployed for at least two days. Assuring for adequate soak time traps were deployed over the weekend.

The Port of Naantali

Survey in the Port of Naantali was conducted on 20 – 22 August. Similarly to Turku, three sites were located in the port area representing various features (type of traffic, bottom type). The first site was located on a coal dock, the second one on a ferry dock and the third one on a less frequently visited mooring berth. The sampling team consisted of 2 to 3 persons and sampling took a total of 8 h (19.5 man hours). Distances between the sites were significantly shorter than in Turku, which largely explains the difference in working hours. The traps were deployed on Monday, 20 August and retrieved on Wednesday, 22 August. The soak time for traps was less than in Turku, but adequate to obtain a representative catch.

Over all, very few issues were encountered while sampling. Perhaps the most significant issue was related with the benthic sampling and the fact that the grab requires soft substrate which was occasionally difficult to find. The port authority representative in Turku informed that in most ports and port locations the bottom underneath the docks is covered with a concrete slab to prevent erosion. However, in these situations mooring berths are very useful in reaching further from the dock to the soft substrate and allow easier benthic sampling. Therefore, utilizing mooring berths in benthic sampling, whenever they are available, is highly recommended. Secondly, sampling of fouling organisms was only conducted from arms reach (maximum depth penetration was 1.5 m). As described in many Rapid Assessment papers, ports did not have any debris (chains, ropes) floating in the water. In fact, only two

tires and one rope were found in the water. Therefore, obtaining samples from them was minimal. Fouling plates are a very cost effective sampling method and should therefore be used whenever possible to obtain samples of fouling species from various depths. Snorkeling may be a suitable sampling method in ports, especially when using a dry suit.

Pathogen sample processing was slightly complicated in Finland. Apparently, only one laboratory in the whole country is able to analyse cholera bacteria from the water samples. Availability of laboratories for testing ballast appears also to be poor, based on a recent project by the Traffic Safety Agency (Trafi) in Finland.

The Port of Tallinn (Muuga Bay)

The port survey protocol was also tested in the Port of Tallinn on 12.9.2012 in collaboration with scientists from Estonian Marine Institute, University of Tartu local scientist, Dr. Henn Ojaveer. Sampling went even more smoothly than in Turku and Naantali, mainly thanks to the very professional field personnel. This was due to previous experience and knowledge on port sampling, carried out in the same three sites during the mid-2000's (Ojaveer & Kotta 2006). The sampling itself took only about 3.5 hours for four people (14 man hours) excluding the retrieval of the traps (approximately 1.5 h).

Sample analyses

All samples collected in the survey are currently being analyzed by quality assured laboratories (SYKE Marine Research Centre, Estonian Marine Institute). Species data will be reported when analyses are finished and used in testing the decision support tool.

Estimates of survey costs

Cost of the surveys will be determined by 1) sending a query to consulting companies in different countries and 2) estimating the exact cost of the field samplings conducted within the project. Working hours and costs of the sample processing will be finalized when the most costly part of the survey, the laboratory analyses, is finished. However, based on the sampling experiences, the sampling cost should be approximately 10 000 € total (port with 3 sites).

Appendix 1. 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
 - o Small hand hauled 20 µm net for phytoplankton (450 mm long with 250 mm mouth)
 - o 100 – 150 µm (or smaller) free fall drop net for zooplankton (for example 400 - 700 mm opening)
 - o 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
 - o 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)
 - o 1 – 2 l ziplock bags for the obtained samples
- Traps
 - o 6 x Collapsible Chinese crab trap
 - 6 x 2 kg lead weights
 - Cable ties (for attaching the lead weights to the traps)
 - o 9 x Shrimp trap (Box or cylinder, 2mm plastic mesh, 150-200mm high, 400-500mm long)
 - Rocks (approx. 1 kg) inside the traps for weight
 - o Approximately 250 m of rope for tethering the traps
 - o 1 l ziplock bags for the catch
 - o Bait fish
- Ekman grab or similar hand-operated benthic grab
 - o 0.5 mm sieve
- Jars (1 l) 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

Appendix 2. List of quality assured laboratories

Quality assured laboratories include any laboratory qualified with ISO/IEC 17025 standard or its predecessors (ISO 9000, EN-45001). In addition, following laboratories are involved in HELCOM Quality Assurance Programmes for phytoplankton (PEG) and zooplankton (ZEN) and are considered as Quality Assured.

Institutes involved in PEG intercalibration (laboratories involved in also ZEN QA are labeled accordingly):

Leibniz-Institut für Ostseeforschung,
Warnemünde *ZEN QA*
Seestraße 15
DE-18119 Warnemünde
GERMANY

State Agency for Agriculture,
Environment and Rural Areas Schleswig-Holstein
Subdepartment Coastal Waters
Hamburger Chaussee 25, 24220 Flintbek,
GERMANY

GEOMAR
Düsternbrooker Weg 20
24105 Kiel
GERMANY

WEAQ AB
Doktorsgatan 9 D
SE-26252 Ängelholm
SWEDEN

Department of Systems Ecology *ZEN QA*
Stockholm University
Svante Arrhenius väg 21 A
SE-10691 Stockholm
SWEDEN

UMF/Umeå universitet *ZEN QA*
Norrbyn
SE-91020 Hörnefors
SWEDEN

SMHI Oceanographic Unit *ZEN QA*
Sven Källfelts gata 15
SE-42671 Västra Frölunda
SWEDEN

Department of Marine Research of
Environmental Protection Agency *ZEN QA*
Taikos 26
LT-91141 Klaipeda
LITHUANIA

Coastal Research and Planning Institute
Klaipeda University
H. Manto 84
LT-92294 Klaipeda
LITHUANIA

Estonian Marine Institute *ZEN QA*
Tartu University
Mäealuse 14
EE-12618 Tallinn
ESTONIA

Inga Lips, Head of Marine ecology lab
Marine Systems Institute
Tallinn Univestity of Technology
Akadeemia Rd. 15A
12618 Tallinn
ESTONIA

Latvian Institute of Aquatic Ecology *ZEN QA*
Marine Monitoring Centre
Daugavgrivas 8
LV-1048 Riga
LATVIA

Finnish Environment Institute (SYKE) *ZEN QA*
Marine Research Centre
Erik Palmenin Aukio 1
PBox 140
FI-00251 Helsinki
FINLAND

Maritime Institute in Gdańsk
Department of Aquatic Ecology
Abrahama 1, 80-307 Gdańsk,
POLAND

Institutes involved in ZEN QAI project

Sea Fisheries Institute
POLAND

Department of Oceanography and Baltic Sea Monitoring
POLAND

Zoological Institute, RAS
RUSSIA

3. Proposal for harmonized Target species selection criteria

Karin Heyer

3.1 Background

Most risk assessments for granting exemptions of ballast water treatment are based on target species. A problem arises in the fact that different countries use different target species lists and different criteria for selecting target species due to a lack of harmonized criteria for the selection process. Therefore, one of the project tasks was to define harmonized criteria for target species selection and to test the developed criteria with already existing target species lists.

The steps for defining target species selection criteria were

1. Compilation of existing criteria for defining target species
2. Proposals of useful methods
3. Definition of harmonized criteria
4. Testing the harmonized criteria, with existing target species lists
5. Next steps

Currently points 1 and 2 are completed and a proposal of selection criteria is made (see below).

Compilation of existing criteria for defining target species

Many methods exist to assess whether an alien species will become invasive or not (Table 1). Some methods are qualitative (GABLIS, Norwegian Black list, Classification key for Neophytes) and some semi-quantitative. No method available is truly quantitative.

All methods to define the risk of an alien species to become invasive are based on expert judgment and therefore always include a degree of subjectivity, which depends on the interest group of the experts. Some methods are not useful, because they do not fulfill all of the criteria defined by IMO G7 (these include ISEIA, UK FISK, GABLIS, Norway Black list) (Table 1). The assessments used by GB and Ireland are based on many questions, and it seems that more information is required than is available. Furthermore, the species can be ranked based on their harmfulness only if the outputs of the risk assessments are scores, which allows comparisons between species. German/Austrian, Norwegian and Swiss approaches, which are listing systems, do not allow this.

Based on a review, the Swedish (adapted ISEIA protocol (ISEIA 2009)) and the Australian (Hayes et al. 2005) methods seem to be the most useful tools for assessing the invasiveness of the non-indigenous species (see below).

Table 1: Compilation of methods for defining target species

Country	IMO Criteria	Belgium	Sweden	UK FISK	UK GB Assesm.	Germany/ Austria	Ireland	Norway	Switzerland	Australia	US/ Canada/ Mexico
Name		ISEIA	ISEIA and additional criteria	FISK	GB Ass,	GABLIS,	Invasive species Risk Assessment Ireland	2007 Norwegian Black List,	Classification key for Neophytes,	Hazard assessment protocol	Tri-national Risk Assess. Guidelines for Aquatic alien invasive species
output		score	score	score	score	list	score	list	list	score	score
Questions		4	8	49	prelim. screening +51	5	10	prelim. screening +4	10	14	prelim. screening
Taxa		all	all	Fresh w. Fisk, inv.	all	all	all	all	Only plant	all	Aquatic non-indigenous
Vector	Evidence of prior introduction	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Environment	Demonstrated impacts on environment, and strength and type of ecol. Interaction	yes	yes	yes	yes	yes	yes	yes	yes	yes	yes
Economy	Demonstrated impacts on economy incl. property and natural resources	no	yes	no	yes	no	yes	no	yes	yes	yes
Human health	Demonstrated impacts on human health	no	yes	no	yes	no	yes	no	yes	yes	yes

3.2 Proposals of useful methods

Sweden inserted the assessment of the impacts on economy and human health to the Belgian ISEIA protocol (ISEIA 2009), assuring that the adapted method fulfills the IMO G7 requirements (Tables 1 and 2). Sweden has already used this adapted method for defining their target species for the North Sea and Baltic coast (Table 3).

Also, the semi-quantitative Australian approach (HAYES et al 2005), fulfills all requirements of the IMO G7 (Table 1). The method takes into account the **invasion potential** (ship movements, weighed by journey duration) and the **impact potential** (Table 4; Human health, impact on ecology and economy). The determination of the harmfulness of the non-indigenous species is accomplished by a **hazard ranking**, by plotting the invasion potential against impact potential.

3.3 Definition of harmonized criteria

It is proposed here that the HELCOM target species lists should be constructed by adopting the Belgian ISEIA method with the Swedish adaption along with the Australian method. In the Australian system the experts have to score the potential or actual impacts on human health, economic values and environmental values of a certain species. The scores can obtain a value on a scale between 0 (no impact) and 1 (high impact), and the uncertainty of the assessment can be calculated. However, there are no specific definitions about low, medium or high risk as in the Belgium approach (Table 2). This is one reason that outcomes of the impact assessments for the same species vary significantly between different experts. For that reason Hayes et al. (2005) proposed that the assessors are allowed to see the responses of others allowing them the opportunity to discuss and re-evaluate their scores. Furthermore, it seems to be useful to define the impact criteria as in the Belgian approach (Table 2). For the final hazard ranking of the harmfulness of non-indigenous species the Australian Risk assessment takes into account the duration of the voyage, which is an advantage of the method.

Table 2: Assessment criteria proposed by Belgium (ISEIA protocol) and the additional points included by Sweden.

	Low risk=1	Medium risk=2	High risk=3
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 or passive means over distances > 1km/year and initiate new populations.
Colonisation of high conservation value habitats	Populations of the non-native species are restricted to man-made habitats (low conservation value)	Populations of the non-native species are usually confined to habitats with a low or a medium conservation value and may occasionally colonise high conservation value habitats	Non-native species often colonise high conservation value habitats (i.e. most of the sites of a given habitat are likely to be readily colonized by the species when source population are present in the vicinity) and makes therefore a potential threat for red-listed species.
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.
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.	The impact on ecosystem processes and structures is strong and difficult to reverse.
Effects on human health	Criteria inserted by Sweden, assessment criteria has to be defined		
Effects on natural resources (e.g. fisheries)	Criteria inserted by Sweden, assessment criteria has to be defined		
Effects on property (e.g. cooling systems)	Criteria inserted by Sweden, assessment criteria has to be defined		
Dispersed by ballast water or sediments	Criteria inserted by Sweden, assessment criteria has to be defined		

Table 3: Swedish assessment of the harmfulness of Swedish Baltic non-indigenous species based on the Belgium ISEIA protocol with the additional criteria included by Sweden.

	dispersions potential of the NIS	colonisation of high conservation area	impacts on native species	impacts on ecosystem functions	effects on Human Health	effects on natural resources	effects on property	dispersed by ballast water or sediments	SUM
Species name									(max value 24)
<i>Cercopagis pengoi</i>	3	3	3	3	2	3	1	3	21
<i>Ensis directus</i>	3	3	3	3	3	0	1	3	19
<i>Mnemiopsis leidyi</i>	3	3	3	3	0	3	0	3	18
<i>Eriocheir sinensis</i>	2	3	2	2	2	2	2	3	18
<i>Balanus improvisus</i>	3	3	2	2	1	0	3	2	16
<i>Neogobius melanostomus</i>	3	3	3	2	0	2	0	3	16
<i>Prorocentrum minimum</i>	3	3	2	2	1	2	0	3	16
<i>Marezzelleria spp</i>	3	2	2	3	0	1	0	3	14
<i>Potamopyrgus antipodarum</i>	3	3	2	2	0	0	0	3	13
<i>Anquillicoloides crassus</i>	3	3	3	1	0	3	0	0	13
<i>Oncorhynchus mykiss</i>	3	3	3	1	0	3	0	0	13
<i>Hemimysis anomala</i>	3	3	1	3	0	0	0	2	12
<i>Evadne anonyx</i>	3	3	1	1	0	1	0	3	12
<i>Acartia tonsa</i>	3	3	1	1	0	0	0	3	11
<i>Chara connivens</i>	2	3	2	1	0	0	0	3	11
<i>Chaetoceros concavicornis</i>	3	1	2	1	0	2	0	2	11
<i>Mya arenaria</i>	3	3	1	1	0	1	0	2	11
<i>Fucus evanescens</i>	2	3	2	2	0	0	0	1	10
<i>Odontella sinensis</i>	3	1	1	1				3	9
<i>Cordylophora caspia</i>	2	1	1	2	0	0	1	1	8
<i>Telmatogeton japonicus</i>	2	2	1	1	0	0	1	1	8

Table 4: Marine pest impact categories used in the Australian approach

Human health:	1) Human health
Economic:	1) Aquatic transport, 2) Water abstraction/nuisance fouling, 3) Loss of aquaculture/commercial/recreational harvest, 4) Loss of public/tourist amenity, 5) Damage to marine structures/archaeology
Environmental:	1) Detrimental habitat modification, 2) Alters trophic interactions in communities and food-webs of ecosystem, 3) Dominates/out competes and limits resources of native species, 4) Predation on native species, 5) Introduces/facilitates new pathogens, parasites or other NIS, 6) Alters biogeochemical cycles, 7) Induces novel behavioral or eco-physiological responses, 8) Genetic impacts: hybridization and introgression, 9) Herbivory

3.4 Testing the harmonized criteria, with existing target species lists

For risk assessment purposes for granting exemptions of ballast water treatment the target species list for the Baltic Sea must include two types of species:

- 1) for the intra-Baltic shipping routes, those non-indigenous species are of interest which do not occur in the total Baltic area, but have a wide salinity tolerance so that there is a risk to be transferred from one port to another and
- 2) for the inter-Baltic shipping routes, those non-indigenous species are of interest which do not occur in the Baltic area, but have the ability to tolerate the specific salinity and temperature conditions in the Baltic Sea.

HELCOM already compiled two different lists (HELCOM MARITIME 7/2008), one with non-indigenous species, which already occur in the Baltic area (HELCOM LIST OF NON-INDIGENOUS AND CRYPTOGENIC SPECIES IN THE BALTIC SEA (VERSION 2)) and a draft target species list (DRAFT HELCOM TARGET SPECIES LIST (VERSION 2)) with species, which do not occur in the Baltic area.

Furthermore, HELCOM compiled a list of the worst non-indigenous species in the Baltic Sea (Table 5). The criteria for the selection of these species except for Sweden (see above) are not known and therefore not harmonized. The vector of all species was shipping and nearly all species are established in the Baltic Sea. Moreover, most of the species occur already in the whole area of the Baltic Sea. Therefore, since there is no further risk of transporting new species with ballast water within the Baltic Sea, conducting Risk assessments based on this list is not useful. It would be a more useful approach to construct a list for each port with the **most unwanted** species. For example, Finland and Estonia named *Beroe ovata*, and *Proterorhinus marmoratus*, Finland additionally also *Corbicula fluminea* and Estonia *Neogobius iljini* in a previous HELCOM questionnaire.

On the basis of the two HELCOM lists (HELCOM MARITIME 7/2008), a proposal of a target species list will be compiled in this project. The list of non-indigenous species which occur already in the Baltic Sea is the basis for defining target species, especially concentrating on species only occurring in some restricted parts in the Baltic (assessment of intra-Baltic

shipping routes) and the HELCOM list of the draft target species (most unwanted ones) is the basis for finding target species for the assessment of inter Baltic shipping routes.

Compiling the ecological requirements of each species is a very time consuming task, since for many species no fact sheets or easily obtainable information exist. The information is obtained from different databases or original research papers and source of the information is documented. Many of the species on both lists, lack published ecological information. Therefore, the first step was to focus on species mentioned by Hayes & Sliwa 2003, Hayes et al. 2005 and Hayes et al. 2008 after a detailed review of the literature on the worst non-indigenous species of the world and transported by shipping. In the next step, their abiotic requirements were checked for whether they could find comparable abiotic conditions in the Baltic area (Table 6). For example the species *Balanus eburneus*, *Beroe ovata*, *Blackfordia virginica* and *Hemigrapsus takanoi* tolerate a wide range of salinities and are therefore possible candidates as target species for the Baltic Sea. *Asterias amurensis* and *Rapana venosa* on the other hand, require higher salinities and are able to live only in the western part of the Baltic Sea (Table 6). *Alexandrium monilatum* requires warm temperatures and is for this reason excluded from the target species.

For the intra-Baltic shipping routes the HELCOM non-indigenous species list has to be checked for target species, occurring only in restricted parts of the Baltic Sea.

Table 5: Draft compilation of the worst non-indigenous species in the Baltic Sea (BS) without harmonized criteria. With exception of *Eriocheir sinensis* all species are established in the Baltic Sea; all species have shipping as vector. (EE= Estonia, FI= Finland, LT= Lithuania, PL=Poland, SE= Sweden).

Rank	Species	Listed by	Rank by countries respectively	occurrence
1	<i>Cercopagis pengoi</i>	FI, LT, PL, SE, EE	5, 2, 1, 1, 2	to 13 PSU
2	<i>Mnemiopsis leidyi</i>	FI, PL, SE, EE	1, 6, 3, 1	total BS up to salinities > 4‰
3	<i>Neogobius melanostomus</i>	FI, LT, PL, SE, EE	7, 1, 7, 4, 7	east part of the BS 0 to 10 PSU
4	<i>Prorocentrum minimum</i>	FI, LT, SE, EE	4, 3, 4, 4	total BS
5	<i>Dreissena polymorpha</i>	FI, LT, PL, EE	3, 7, 3, 3	fresh and brackish inland estuaries and bays (Both., Finish Gulfs) of the BS
6	<i>Eriocheir sinensis</i>	LT, PL, SE,	4, 4, 3	all coastal areas and bays for spawning migrate in higher salinities
7	<i>Marenzelleria spp</i>	LT, PL, SE, EE	8, 5, 5, 8	in all coastal habitats of the BS
8	<i>Dikerogammarus</i>	FI, PL, EE	8, 2, 6	Inland Germany to Poland

	<i>villosus</i>			
9	<i>Balanus improvisus</i>	LT, PL, SE, EE	6, 9, 4, 9	in all coastal habitats of the BS
10	<i>Gammarus tigrinus</i>	FI, LT, EE	9, 5, 5	total BS
11	<i>Potamopyrgus antipodarum</i>	LT, PL, SE,	10, 9, 6	total BS

Table 6: Draft compilation of some potential target species for the Baltic Sea (BS) for a risk assessment for inter-Baltic shipping routes.

	Salinity	Temperature	Potential target species for the Baltic
<i>Alexandrium monilatum</i>	10-18 PSU (Black Sea and Sea of Azov) 30-40 PSU (Native area)	warm water species	no
<i>Asterias amurensis</i>	18,7-41 PSU	0-25	Only for the western part of the BS
<i>Balanus eburneus</i>	5-35 PSU		yes
<i>Beroe ovata</i>	7-35 PSU		yes
<i>Blackfordia virginica</i>	3-40 PSU (native area), 3-10 PSU (Black Sea and Sea of Azov); 3-18 PSU(Caspian Sea)	tolerates wide range of temperatures	yes
<i>Hemigrapsus sanguineus</i>	tolerates wide range of salinities; >10PSU		Only for the western part of the BS
<i>Hemigrapsus takanoi/penicillatus</i>	brackish, estuarine and marine habitats, 1-17PSU (annual mean 9PSU) Scheldt near Belgian Border		yes
<i>Hydroides dianthus</i>	marine		no
<i>Pfiesteria piscicida</i>	brackish	temperate to subtropical estuarine systems	yes

<i>Pseudo-nitzschia seriata</i>	10-33PSU Amursky Bay, East coast of Russia		Only for the western part of the BS
<i>Rapana venosa</i>	15-35 PSU		Only for the western part of the BS
<i>Thalassiosira nordenskioldii</i>	10-18 PSU (Black Sea and Sea of Azov) already found in the Kattegat		yes

3.5 Proposal of how to work the target species list

Exemption from ballast water treatment can be granted if the shipping route between the donor and the recipient port is a low risk route. Low risk is assumed if

- the species compositions of the two ports are identical or
- no target species that could pose a risk for the port of destination occurs in the donor port

The steps to assess, whether this shipping route is of high or low risk, are

- 1) to compare the species compositions of both ports based on the results of the proposed port surveys;
- 2) to determine the species, which appear only in the donor port and not in the recipient port;
- 3) to assess, whether the species, which are only in the donor port, are a target species or not. The assessment should be carried out on the basis of the proposed target species list or if the species is not already in the list by applying the criteria for the determination of target species;
- 4) to use the risk assessment tool in order to determine whether this shipping route is of high or of low risk provided a target species is identified;
 - a. The species of concern are classified as non-target species and thus this shipping route is regarded as a low risk route.

For step 3 it is necessary to have a careful and well defined target species list. At the end of this project a preliminary list of target species based on expert judgment with harmonized criteria will be given. Next steps

- Continuing with compiling a potential target list for inter- and intra-Baltic shipping routes
- Ranking the target species with the above mentioned method. The assessment of the species should be done by experts.

4. Developing a data/decision support tool

Eugen Faber and Kerstin Stelzer

4.1 Background

The third task of the HELCOM Aliens 2 project was to propose a harmonized decision support tool to run the risk assessments using a list of selected target species and the data collected with the survey protocol. Such a decision support tool provides a simple interface to conduct the risk assessment for transporting target species in ballast water between ports. The prototype of the decision support tool is a web application that uses data on species assemblages in donor and recipient ports and creates an output calculating the level of risk (low, medium and high) of transporting harmful alien species between them. The level of risk is determined by the comparison on the presence of target species in the ports and their salinity tolerance, and the comparison of the ecological/physical situation in the ports, such as salinity.

An Oracle database is used as backend of the decision support tool containing different in situ measurements (abiotic and biotic) and a decision algorithm has been implemented for the risk assessment calculation.

The prototype of the tool includes the following information components:

- Port profiles (statistical information about the environment, size and some business parameters of harbours)
- In situ measurements (abundance and biomass of species) found in the harbours
- Lists of target species, defined for different regions
- Risk assessment algorithm

The development of the prototype has been performed iteratively in the following steps:

- Collection and analysis of information about the parameters needed in the HELCOM ballast water database (HBW-DB)
- Development of the data model
- Filling database tables with data
- Creation of a template for the measurement data as input file for database import
- Defining a Risk Assessment algorithm
- Developing, testing and deploying of a web application for the NSBWO Risk Assessment.

4.2 Defining the required information for the ballast water database

Working group members discussed and decided the structure of the required information and the parameters, such as port characteristics and field measurements for the database. The information was used as a basis for the data model development and for structuring the excel template for measurement data (input file format for database import).

4.3 Developing the data model

The database Oracle11 was installed in Brockmann Consult (Germany). The first draft of the data model was developed; all required tables were created and during the real data import adopted to new additional requirements.

The current data model consists of 7 tables:

- *Harbour* – statistical information from harbours
- *Region* – regions for port and target species definition
- *Species* – information about all species (HELCOM LIST)
- *Region_species_target* - target species setting
- *Environment* – in situ measurements, environment parameters
- *Sampling* – in situ measurements, parameter for sampling
- *Results* – in situ measurements, number and biomass of species

The table “region_species_target” enables identification of species as target species for each region. Currently there are two regions included in the database: Baltic Sea and North Sea.

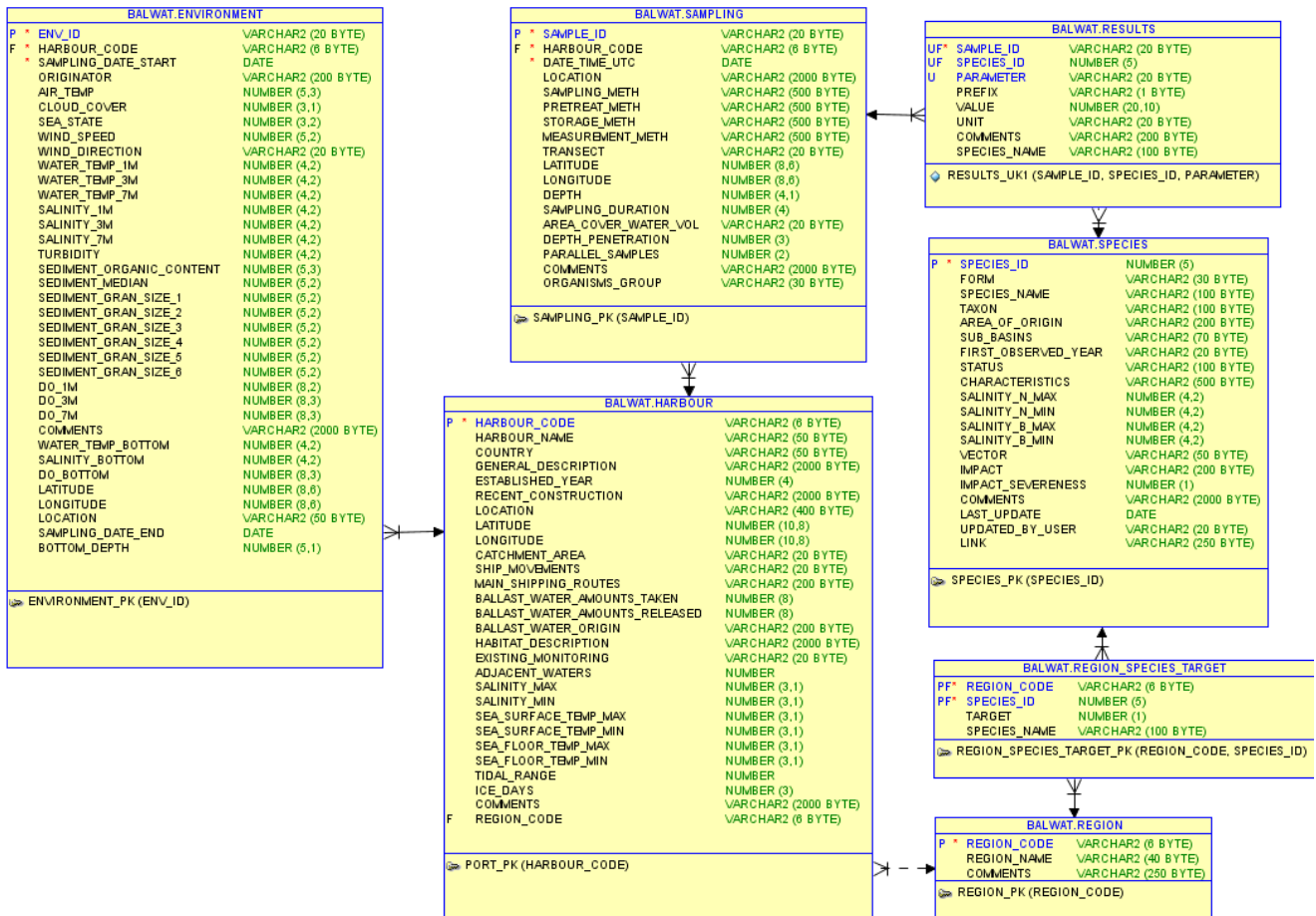


Figure 1: Adopted data model

4.4 Filling database tables with data

All database tables were filled at first with some test data:

- Table *species* was filled with data from the HELCOM list of non-indigenous species.
- Table *region_species_target* was filled with data from publication Heyer 2012 following the discussion with the project members
- Table *harbour* was filled with data from publicly available information about the harbours
- Tables for the measurement data were also filled with test data from Heyer 2012.

Also, as samples analyses are finished, real in situ data from Turku and Naantali (Finland) have been delivered from field sampling (see page 14) and successfully imported into the database.

4.5 Creating a template for the data input file

The Excel template for in situ measurement was developed and discussed within the project. The real data from samplings conducted in Turku and Naantali were delivered in this format and were easily imported. All field measurement data in the future will be prepared and controlled by HELCOM and will be delivered to Brockmann Consult for import into the database. There are three sheets in the template: 1) environment, 2) sampling and 3) results (Fig. 5).

env_id	harbour	date	air temp.	cloud cover	sea state	wind speed	wind direction	sediment analysis, organic content	sediment analysis, Median	sediment analysis >1mm	sediment analysis <1-0,5mm	sediment analysis <0,5-0,25mm	sediment analysis <0,25-0,125mm	sediment analysis <0,125-0,063mm	sediment analysis <0,063mm	water temp. 1 m	water temp. 3 m	water temp. 7 m	water temp. bottom
			°C	%	m	m/s	Grad	g	µm	% Dry weight	% Dry weight	% Dry weight	% Dry weight	% Dry weight	% Dry weight	°C	°C	°C	°C
1	HH	22.02.2001																	
2	HH	22.02.2003																	
3	HH	22.02.2004																	
4	HH	22.02.2005																	

env_id	sampl_id	date_time UTC	location	organisms group	duration of sampling	area covered or water volume filtrated	depth penetration	Parallel sample per position	sampling method	pretreat method	storage method	method of measurement	transect	latitude	longitude	depth	comments
					min	m² m³	cm							decimal degrees	decimal degrees	m	
1	1	22.02.2001 09:22		Human pathogens		xx l											
2	2	22.02.2001 10:22		Phytoplankton		xx l											
3	3	22.02.2001 12:22		Zooplankton		xx l											
4	4	22.02.2001 16:22		Mobile epifauna		xx			Traps								
51	51	03.06.2001 10:45		Hard substrates		xx											
51	51	03.06.2001 12:45		Soft substrates		xx											
51	51	03.06.2001 16:45		Mobile epifauna		xx			Trawl or dredge								

sampl_id	species	parameter	prefix	value	unit	comments
1	Eschercheria coli		<		2 cfu/ml	
3	1 Vibrio cholera					
4	2 Actinoptychus undulatus	count		25070	number/litre	
5	2 Rotifera	count			number/litre	
7	7 Abra prismatic	count		20	number/m2	
8	7 Cerastoderma edule	count		100	number/m2	
9	7 Cerastoderma edule	biomass		0,5	mg/m2	

Figure 2: The three sheets in the Excel template for data import

4.6 Defining a Risk Assessment algorithm

Some existing Risk Assessment algorithms were analysed and discussed by specialists within the project. The algorithm proposed by the NSBWO (Nord Sea Ballast Water Opportunity) was selected in to be tested and implemented in the prototype of the decision support tool (Fig. 7). In the finalized product, other risk assessment algorithms may also be included.

The main decision points in the algorithm are based on the salinity in the respective ports, on the salinity tolerance of target species and on the occurrence of different target species in the start and destination ports. The decision-step “Do species have the ability for natural spread?” is a very important one, but up till now the necessary information does not exist (Heyer 2012). Therefore this decision point was not implemented.

An extension of the risk assessment algorithm with additional decision criteria that has been proposed in the framework of the HELCOM project "Risk Assessment of alien species transfer on intra-Baltic Ship voyages (Gollasch et al. 2012)" should be taken into account provided that the relevant data are available in order to run the extended algorithm.

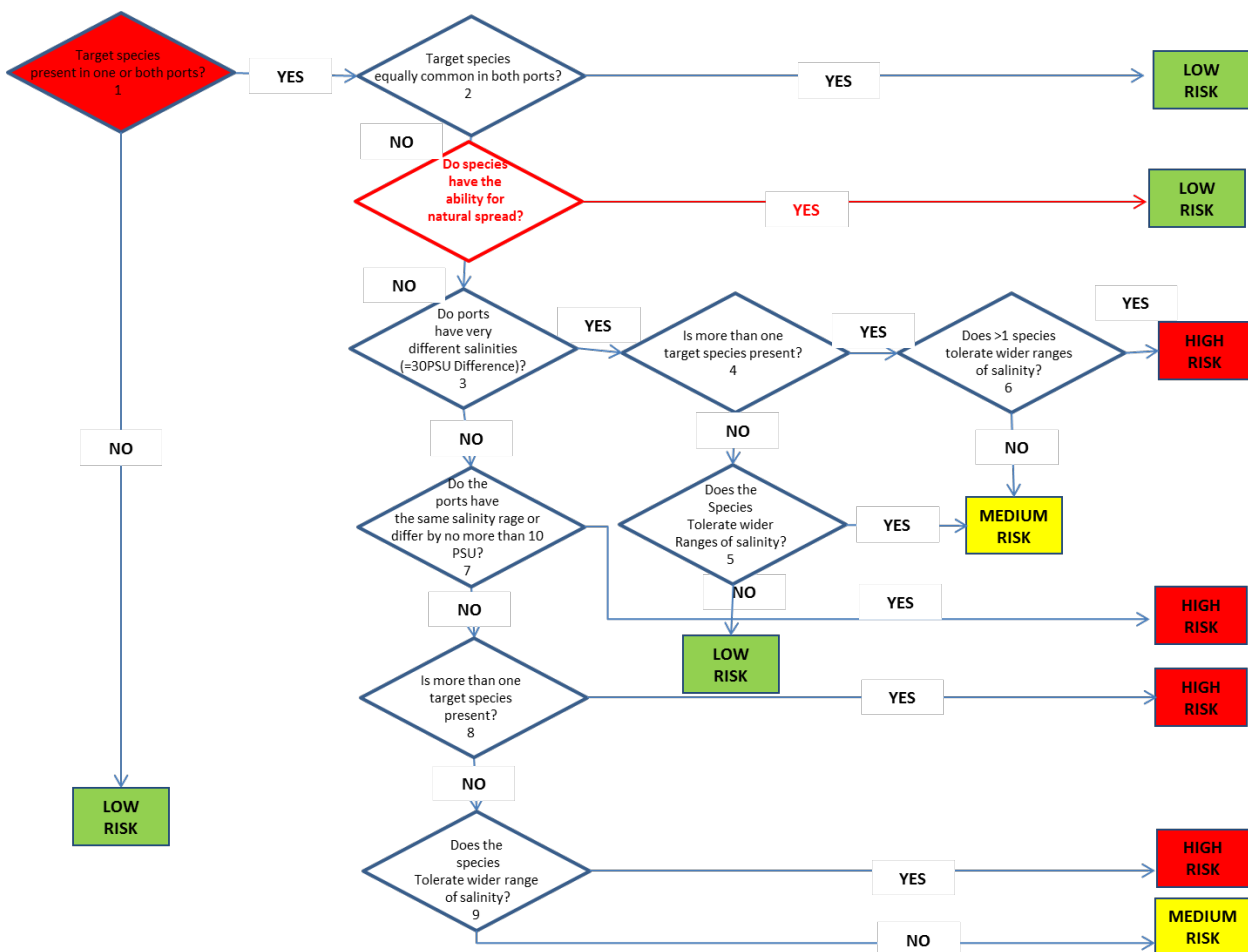


Figure 3: Risk Assessment algorithm

4.7 Developing and testing the web application for the NSBWO Risk Assessment

The risk assessment tool was implemented as a web application and is available under: www.brockmann-consult.de/ballast_water_RA/ (Fig. 3). Password and user id may be requested from the project team.

The NSBWO Risk Assessment algorithm contains 9 decision points and 11 possible decisions. The available data did not supply enough combinations of different salinities for all 11 possible decisions. Therefore, some fake ports with invented in situ measurements were inserted in the database. After successful tests the fake information will be deleted. The data gathered from publications will, however, remain in the database.

Results of the Risk Assessment were tested successfully with all currently available data.

The Oracle Application Express (APEX 4.1) technology was used for the development and implementation of the web application and it can be easily deployed on any Oracle-server. The web application is currently hosted by Brockmann Consult.

The web application for the Risk Assessment consists of 8 tabs that show all components of the decision support tool: description of the Risk Assessment algorithm (1), the data model (2), information about all species (3) and the target species (4) with links to known fact sheets, information about ports with Google-Map-links (5), measurement data (6&7) and the interface to the Risk Assessment itself (8).

All data are search- and exportable; the “interactive reports” provide a good possibility for simple analytical calculations and representations.

Like every database-based web application, the Risk Assessment can restrict access to a part or detail of the information. Currently there are three users that have access to the application: admin, bw_reader and bw_writer.

The bw_reader can only see selected information and is able to use the Risk Assessment interface.

The bw_writer can additionally add/edit port and species information.

Currently, importing of the measurement data is restricted to the database administrator only.

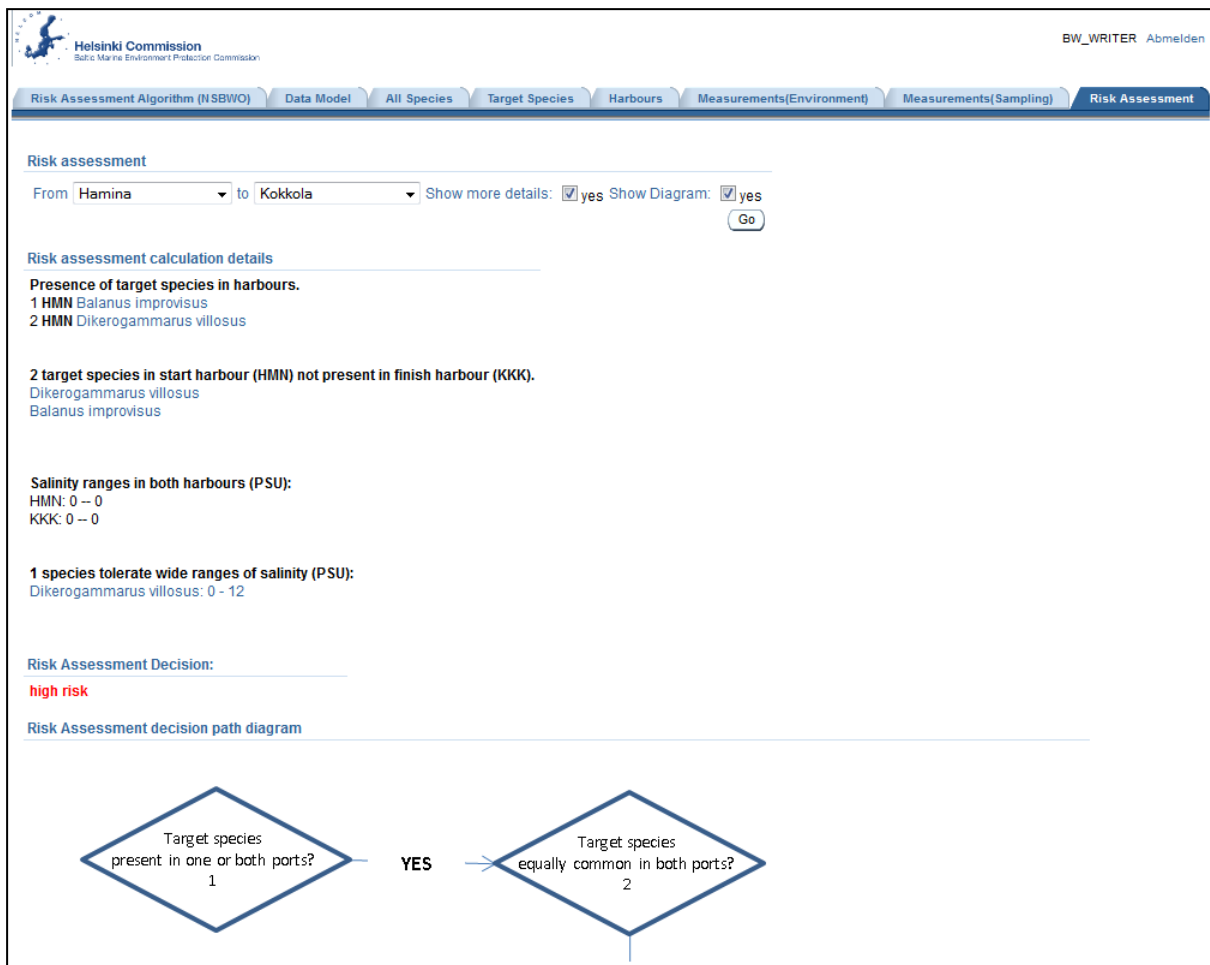


Figure 4: Web application for the Risk Assessment

4.8 Next steps

The database will be further developed by importing more data as more ports will be sampled and more old samples will be analysed. The modular structure of the prototype allows further extensions and improvements. The accessibility of the application, via the internet and the organization of user rights, provides the opportunity to serve a wide range of users.

5. Literature cited

- Ashton, G. 2006. Rapid assessment of the distribution of marine non-native species in marinas in Scotland. *Aquatic Invasions* 1:209–213. doi: 10.3391/ai.2006.1.4.3.
- Buschbaum, C., R. Karez, D. Lackschewitz, and K. Reise. 2010. Rapid assessment of neobiota in German coastal waters. HELCOM Indicator Fact Sheets and development of the HELCOM core set of indicators. St. Petersburg, Russia.
- Carlton, J. T. 1985. Transoceanic and interoceanic dispersal of coastal marine organisms: the biology of ballast water. *Oceanogr. Mar. Biol. Ann. Rev.* 23:313–372.
- Cohen, A. N., L. H. Harris, B. L. Bingham, J. T. Carlton, J. W. Chapman, C. C. Lambert, G. Lambert, J. C. Ljubenkov, S. N. Murray, L. C. Rao, K. Reardon, and E. Schwindt. 2005. Rapid Assessment Survey for Exotic Organisms in Southern California Bays and Harbors, and Abundance in Port and Non-port Areas. *Biological Invasions* 7:995–1002. doi: 10.1007/s10530-004-3121-1.
- Elton, C. S. 1958. *The ecology of invasions by animals and plants*. University of Chicago Press, Chicago.
- Gollasch, S., M. David, and E. Leppäkoski. 2011. Pilot risk assessments of alien species transfer on intra-Baltic ship voyages. Pages 1–98.
- Hayek, L.-A. C., and M. A. Buzas. 2010. *Surveying Natural Populations: Quantitative Tools for Assessing Biodiversity*. Page 592 Second edi. Columbia University Press, New York. Retrieved from http://books.google.fi/books?id=nn9t329w8z0C&printsec=frontcover&source=gbs_ge_summary_r&cad=0#v=onepage&q&f=false.
- Hayes, K. R., and C. L. Hewitt. 2000. Risk assessment framework for ballast water introductions Volume II. Page 198.
- Hayes, K., Sliwa, C., 2002. Identifying potential marine pests - a deductive approach applied to Australia. *Mar.Poll.Bull.* 46, 91-98.
- Hayes, K., Sliwa, C., Migus, S., McEnnulty, F., Dunstan, P. 2005. National priority pests: Part II Ranking of Australian marine pests - final report - marine.csiro.au
- Hayes, K.R., McEnnulty, F., Sutton, C., Cooper, S., 2008. Ballast Water Decision Support System (DSS) Service Level Agreement (SLA) - Part III: Appendix A.
- Heyer 2012: Compiling and testing of biological risk assessments for the invasion of alien species with ballast water (in Prep).
- Hewitt, C. L., and R. B. Martin. 1996. *Port Surveys for Introduced Marine Species - Background Considerations and Sampling Protocols*. Page 40. Hobart, TAS, Australia.
- Hewitt, C. L., and R. B. Martin. 2001. Revised protocols for baseline port surveys for introduced marine species: survey design, sampling protocols and specimen handling. Centre for Research on Introduced Marine Pests. Technical Report No. 22.

IMO. 2004. International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM). London, UK.

Inglis, G., N. Gust, I. Fitridge, O. Floerl, C. Woods, B. Hayden, and G. Fenwick. 2006. Port of Taranaki Baseline survey for non-indigenous marine species.

ISEIA protocol, Version 2,6 from 7/12/2009;
http://ias.biodiversity.be/documents/ISEIA_protocol.pdf

Lubchenco, J., A. M. Olson, L. B. Brubaker, S. R. Carpenter, M. M. Holland, S. P. Hubbell, S. A. Levin, J. A. MacMahon, P. A. Matson, J. M. Melillo, H. A. Mooney, C. H. Peterson, H. R. Pulliam, L. A. Real, P. J. Regal, and P. G. Risser. 1991. The Sustainable Biosphere Initiative: An Ecological Research Agenda: A Report from the Ecological Society of America. *Ecology* 72:371. doi: 10.2307/2937183.

Marshall, A. J., and H. N. Cribb. 2004. Monitoring for marine pests in Raffles Bay, Cobourg Peninsula. Page 11. Darwin, Australia.

Ojaveer, H., and J. Kotta (Eds.). 2006. Alien invasive species in the north-eastern Baltic Sea : population dynamics and. Estonian Marine Institute Report Series No. 14, University of Tartu, Estonia.

Paavola, M., A. O. Laine, M. Helavuori, and P. Kraufvelin. 2008. Profiling four brackish-water harbours: zoobenthic composition and invasion status. *Boreal Environment Research* 13:159–175.

Pederson, J., R. Bullock, J. Carlton, J. Dijkstra, N. Dobroski, P. Dyrinda, R. Fisher, L. Harris, N. Hobbs, G. Lambert, E. Lazo-Wasem, A. Mathieson, M.-P. Miglietta, J. Smith, J. I. Smith, and M. Tyrrell. 2003. MARINE INVADERS IN THE NORTHEAST Rapid Assessment Survey of Non-native and Native Marine Species of Floating Dock Communities. Page 29.

Pitkänen, V. K. 2012. VIERASLAJIEN PYYDYSKOKEILU Mustatäplätokon, liejutaskuravun ja sirokatkaravun kustannustehokkaimmat pyyntimenetelmät Helsingin, Turun ja Naantalin merialueilla vuonna 2011. Turku University of Applied Sciences.

Power, A., M. Mitchell, R. Walker, M. Posey, T. Alphin, and C. Belcher. 2006. Baseline Port Surveys for Introduced Marine Molluscan, Crustacean and Polychaete Species in the South Atlantic Bight.

Vitousek, P. M., C. M. D'Antonio, L. L. Loope, R. Westbrooks, and C. M. D'Antonio. 1996. Biological Invasions as Global Environmental Change. *The American Naturalist* 84:468–478. US DEPT INTERIOR, HALEAKALA NATL PK, HI USA ANIM & PLANT HLTH INSPECT SERV, USDA, WASHINGTON, DC USA. Retrieved from ISI:A1996VC02700013.

Walk, M., H. Modrzejewska, and G. Szydłowska-Herbut. 2011. Alien Species Introduction Risk Assessment on the Basis of Quality of Ballast Water Discharged at the Port of Gdynia. Page 45.

HELSINKI COMMISSION

Baltic Marine Environment Protection Commission
Katajanokanlaituri 6 B, FIN-00160 Helsinki, Finland

Phone: +358 (0)207 412 649, fax +358 (0)207 412 645

e-mail: info@helcom.fi

<http://www.helcom.fi>