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Report of the ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors (WGBOSV)

16–18 March 2015

Bergen, Norway

ICES International Council for the Exploration of the Sea

CIEM Conseil International pour l'Exploration de la Mer

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Executive summary

The 2015 meeting of the ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors (WGBOSV) was held in Bergen, Norway during 16–18 March with Anders Jelmert as Host and Sarah Bailey as Chair. There were 24 participants from 15 countries over the three days, including the joint meeting on 18 March with the Working Group on Introductions and Transfers of Marine Organisms (WGITMO). The physical participants were from Belgium, Canada, Estonia, Finland, France, Germany, Italy, Lithuania, the Netherlands, Norway, Poland, Portugal, Sweden, the United Kingdom and the United States while two participants from the Netherlands participated by videoconference, and Spain contributed by correspondence only.

The objectives of the meeting were to review and report on the status of shipping vector research with an emphasis on studies of shipping transport vectors, shipping vector management activities and risk assessment. The Group discussed sampling and analysis strategies for type approval and compliance testing of ballast water treatment technologies, as well as the effects of treated and exchanged ballast water on the aquatic environment. In addition, the Group discussed new developments in non-native species issues associated with biofouling of artificial structures in the marine environment, and in the Arctic. The Group also considered potential benefits of new molecular tools for identification, early detection and monitoring of non-indigenous species, after having explored potential for collaboration with the ICES Working Group on Integrated Morphological and Molecular Taxonomy (WGIMT). Finally, the Group reviewed the OSPAR JAMP Eutrophication Guidelines to address issues related to invasive, non-indigenous species.

The approach taken at the meeting was for each country to provide an update on the status of shipping vector research in the form of a National Report. These are provided in Annex 3 with a short summary given in the main body of the report. For the remaining Terms of Reference, more detailed presentations were given during the meeting; the report contains summaries of the presentations and subsequent discussions, with more detailed documents contained in the Annexes. To share the workload, several group members were asked to lead specific terms of reference.

The main outcome of the meeting was a better understanding of the work being carried out in different countries and the identification of future research needs and potential collaborative activities that could be undertaken to meet these needs. In addition the Group provided input to WGITMO on the revision of the OSPAR JAMP Eutrophication Guidelines that will contribute to the development of ICES advice to OSPAR. The Group noted that additional research is needed to evaluate the IMO Biofouling Guidelines and non-native species issues in the Arctic, and that molecular tools for non-native species research continues to be an emerging area of research.

1 Opening of the meeting

The meeting was opened at 09:00 on 16 March 2015. Sarah Bailey (Chair) and Anders Jelmert (Host) welcomed all participants. As there were some people new to the meeting, introductions were made around the table with everyone giving their name, institution and a brief overview of their main work in relation to the group (Annex 1).

2 Adoption of the agenda

The agenda was organized based on the Terms of Reference as given in ICES Resolution 2014/2/SSGEPI03 (see below). The agenda (Annex 2) was reviewed and there were no major changes, only slight adjustments to accommodate people participating by videoconference.

3 WGBOSV Terms of Reference

2014/2/SSGEPI03 The ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors (WGBOSV), chaired by Sarah Bailey, Canada will meet in Bergen, Norway, 16–18 March 2015, with a full day joint meeting with the Working Group on Introductions and Transfers of Marine Organisms (WGITMO) to:

a) Continue to critically review and report on the status of shipping vector research with an emphasis on studies of shipping transport vectors, shipping vector management activities and risk assessment.

b) Further discuss and evaluate sample collection, storage, and analysis strategies for type approval and compliance testing of ballast water management systems under consideration at IMO or by other regulators (e.g. U.S. Coast Guard); consider need for submission of an information paper to IMO regarding additional validation procedures related to the trial period of the Ballast Water Management Convention.

c) Further discuss and evaluate available information on the effects of treated or exchanged ballast water on the aquatic environment and provide input on strategies which could be used to increase confidence surrounding environmental safety of treated ballast water being discharged.

d) Investigate and report on new developments in non-native species issues associated with biofouling (e.g. artificial structures in the marine environment and recreational boating) (joint Term of Reference with WGITMO).

e) Investigate and report on new developments in non-native species issues in the Arctic, as a result of climate change and resource developments (joint Term of Reference with WGITMO).

f) Investigate and report (incl. via AquaNIS) on new molecular tools for identification, early detection and monitoring of non-native species, in collaboration with ICES Working Group on Integrated Morphological and Molecular Taxonomy (WGIMT).

g) Provide input to WGITMO in connection with the OSPAR 1/2015 request.

WGBOSV will report by 10 April 2015 (via SSGEPI) for the attention of SCICOM.

Supporting Information

Priority:	The Working Group review and report on the scientific and technical development in relation to ballast water and shipping vectors. As a joint working group it also follows and supports the work within IMO and IOC on these topics.
Scientific justification and relation to action plan:	WGBOSV has a long history of providing scientific support to the development of international measures to reduce the risk of transporting non-native species via shipping vectors. The group has had input into the issue of Ballast Water Sampling guidelines in several ways. The issue has been discussed at the annual meetings of the Working Group. The working group has previously submitted documents to meetings at IMO to support the development of guidelines. This type of input helps ensure that the guidelines are based on accurate scientific information and supports the implementation of the Ballast Water Management Convention.
Resource requirements:	None
Participants:	The Group is normally attended by some 25–35 members.
Secretariat facilities:	None.
Financial:	No financial implications.
Linkages to advisory committees:	SCICOM
Linkages to other committees or groups:	There is a very close working relationship with the working Group on Introductions and Transfers of Marine Organisms (WGITMO) and the Working Group on Harmful Algal Bloom Dynamics (WGHABD). There is also a link to PICES.
Linkages to other organizations:	The work of this group is closely linked to work carried out by the European Maritime Safety Agency (EMSA), the International Maritime Organization (IMO) and the Intergovernmental Oceanographic Commission (IOC).

4 Progress in relation to Terms of Reference

The sections below provide information on the progress made under each of the Terms of Reference, summaries of the more important discussions held, as well as relevant conclusions/suggestions as emerged during the meeting.

4.1 Term of Reference a)

- Continue to critically review and report on the status of shipping vector research with an emphasis on studies of shipping transport vectors, shipping vector management activities and risk assessment (ToR lead Sarah Bailey).

This Term of Reference was addressed by all meeting participants who provided information for their country according to national reporting template. This was done either via a short verbal report or in the form of a more substantial presentation. The following

sub-sections provide condensed highlights for each country. The National Reports, where provided, are contained within Annex 3. A short summary of the main points of discussion is given at the end of the section.

4.1.1 Belgium

There is currently no work on ballast water or biofouling issues in Belgium. The Ballast Water Management Convention is still in the process of being ratified despite that Belgium announced at the MEPC 64 (October 2012) in plenary that it was on the verge of ratifying the Convention. There is ongoing work studying the fouling on windmill farms where a range of species has been found, including non-natives and introduced species.

4.1.2 Canada

Shipping vector research in Canada currently focuses on risk assessments and vector management, with particular emphasis on the Arctic Region. A national risk assessment on recreational boating as a vector for introduction and spread of aquatic invasive species is in the final stages of assessment. Vector management studies include shipboard trials to examine a combination strategy (ballast water exchange plus treatment) as a means to protect low salinity waters, and examination of operational efficacy and environmental safety of ballast water management systems operated in cold (winter/Artic) conditions as well as studies to determine the effectiveness of voluntary exchange by domestic vessels transiting to the Arctic. In addition, research is underway to evaluate and select ballast water sampling and analysis methods for the purpose of compliance testing. The Canadian Aquatic Invasive Species Network (CAISN) continues to work on projects related to early detection and rapid response strategies, understanding aquatic invasive species as part of multiple stressors affecting aquatic ecosystems, and reducing uncertainty in prediction and management. In recent years, Canada has only conducted port monitoring surveys at a small number of Arctic ports - seven new species of uncertain origin (cryptogenic). National AIS regulations as part of the Fisheries Act in Canada are in the final stages of approval and are expected to come into effect in 2015.

4.1.3 Estonia

The specifically dedicated and governmentally funded non-indigenous species monitoring program was continued in 2014. Port biological monitoring (Muuga Harbour, Port of Tallinn) according to HELCOM protocol was added to the programme in 2014. The monitoring included recording of key environmental conditions (incl. CTD profiles) and sampling of phytoplankton, zooplankton, benthic infauna, fouling communities and mobile epifauna (incl. fish). One of the sub-components is to monitor high risk areas of primary invasions. In this purpose, vicinity areas of the two largest ports - Port of Tallinn and Port of Sillamäe (Gulf of Finland), were sampled. In addition, surveys in the long-term dynamics of selected key alien species were continued and the ecological impact of several NIS summarised.

4.1.4 Finland

No projects are ongoing related to the shipping vector or risk assessments at present but several project proposals have been submitted. A national group to discuss the implementation of the BWMC in Finland has been founded. It is led by the Finnish Traffic and

Safety Agency, and the Finnish Environment Institute, Ministry of Environment, Ministry of Traffic and Communication and The Finnish shipowners' association take actively part to the meetings and discussions. The ratification of the IMO's BWM Convention by Finland was again delayed and will take place in autumn 2015. No new alien species was found in Finnish waters in 2014 in the regular phyto- and zooplankton, benthos and coastal fish monitoring programs. No port monitoring was conducted in 2014.

4.1.5 France

French ministry of Environment, Sea and Transport elaborated a guide for Ballast Water Convention exemptions demands, including risk analysis, available at the entry in force of the Convention.

The impact of bio-fouling as a vector of introduced non indigenous species can be important, considering the huge number of pleasure boats visiting the marinas along French coasts.

The French oceanographic fleet will be equipped with ballast water treatment systems. A study is on its way.

4.1.6 Germany

The German Federal Maritime and Hydrographic Agency (BSH) continues approving ballast water management systems (BWMS). An agreement between BSH and NSF (NSF International, the first Independent Laboratory designated by the United States Coast Guard to evaluate and test BWMS for U.S. type approval) provides manufacturers a more economical and streamlined process for achieving two key BWMS type approvals in the industry. BWMS manufacturers now have the opportunity to obtain German and U.S. type approvals from a single test.

New projects are ongoing. The *e-CME Ballast Water* project develops an online training course for compliance monitoring and enforcement of ballast water management standards. The next training course is scheduled for mid-May 2015 at the World Maritime University, Malmö, Sweden and may be joined without attendance fee. During another project, fact sheets of non-indigenous species in Germany will be developed.

The German alien species targeted monitoring programmes continue and include sampling stations in ports along the Baltic and North Seas so that this activity fills geographical gaps in the network of German coastal monitoring stations. Results of the rapid assessments indicate that the rate of newly recorded NIS is lower along the German Baltic Sea coasts compared to the North Sea.

A species of concern but not yet known from Germany is *Didemnum vexillum.* It is found in other European countries and it may be possible that this species becomes introduced to German waters with movements of living mussels and aquaculture gear or in the biofouling of vessels.

4.1.7 The Netherlands

The Netherlands hosts three facilities involved in testing of ballast water management systems: MEA-nl (Marine Eco Analytics), IMARES (Institute for Marine Resources & Ecosystem Studies) and NIOZ (Netherlands Institute for Sea Research). The profiles of the

latter two were explained in the NL national report 2014, the profile of MEA-nl is given in this report.

The three ballast water test facilities have carried on and developed new initiatives. IMARES has further completed the building and validating of their test facility. NIOZ, during reconstruction of its harbour test site, joined WMU (Malmö, Sweden) a support project on CME for port-state control. MEA-nl tested several BWM systems for verification according to the IMO requirements, and conducted on-going research on methodologies of sampling and analysis during land-based and shipboard testing.

The project North Sea Ballast Water Opportunity (NSBWO, Interreg IV B) ended by mid-2014. Until the end, it kept up a high level of innovation in science and technology and in advanced BWM policies, while giving rise to several spin-off initiatives, such as the port-based ballast barge project of Damen Shipyards and Groningen Seaports, while the initiative of the in 2012 established MEA-nl also emerged from the project grounds. A final annual meeting was held April 2014; several more reports were finalised and one new initiative to identify hotspots of barriers to ratification resulted in an additional report.

The initiative for a port-based ballast water management unit (supported by the Wadden Fund) has resulted in a BWM unit being built by Damen Shipyards, which is now up for verification testing at the MEA-nl land-based test-site.

Species and sediments in ports have been analysed, while the Wadden Sea has been subject to an inventory for species on hard and soft substrates.

4.1.8 Norway

Toxicity tests of DBP (Disinfection By-Products) by different BWMS; Alternative Analysis Methods for 10–50 μm organisms after UV treatment; Water quality effects on G8/G9 testing; Evaluation of Culture- vs staining methods for the determination of living 10–50 10-50μm organisms; Real time monitoring ballast water with flow cytometry for the determination of living 10–50μm organisms; Risk assessment of ballast water discharge from vessels under construction transported from Turkey to Norway without any treatment system/power supply onboard, (NIVA, Stephanie Delacroix & cooperating partners.)

Sampling methods experiences from both land based and shipboard testing projects according to IMO and USCG requirements; NIVA participates to the annual Global TestNet meetings (GloBallast/IMO) for harmonization of test facilities testing procedures since 2010.

NIVA participates to the MPN-UV Group (USCG-EPA/UV technologies supplier) for validation of the MPN (culture) method for determination of the living 10–50um organisms according to USCG requirements as additional method to the FDA/CMDA method recommended in ETV protocol by EPA.

Norway delegation (incl. NIVA) participated to the IMO Correspondence Group to propose changes of the G8 guidelines to the MEPC 68 according to the specifications listed in MEPC 67. 2014/2015.

Risk assessment of ballast water discharge from vessels under construction transported from Turkey to Norway without any treatment system/power supply onboard, NIVA/DNV-GL, Stephanie Delacroix (2012–2014).

Risk assessment of the ballast water discharge by fish transport vessels in Norway according to IMO Ballast Water Convention. DNV-GL/Norwegian Maritime Directorate (2012); Risk assessment of the ballast water as fish pathogens transport vector in Norway. Norwegian Veterinary Institute/FHL (2015); The Norwegian Maritime Authority commissioned and received a consultancy report on the costs and practical effects on implementing various ballast water treatment schemes for Norwegian vessels (This report is currently only available in Norwegian).

No new alien species with suspected ship vectors to report for 2014.

4.1.9 Spain

A study, led by Dr. Eva García-Vázquez (University of Oviedo, Spain) and Dr. Anastasija Zaiko (Klaipeda University, Lithuania) addressed the applicability of metabarcoding methodology for the biosecurity surveillance, and particularly the detection of organisms in ships' ballast waters (Zaiko *et al*., 2015). Opportunities and limitations of the molecular approach were identified from taxonomical datasets rendered by two molecular markers of different degree of universality. The cost-efficacy and possible improvements were discussed for the further successful development and implementation of the approach in ballast water control and NIS surveillance.

Apart from this study, no projects specific to shipping as vector of species translocation exist at present in Spain, even though the interest is growing and proposals have been submitted.

Three new ship-mediated species are reported for Spain: i) the marine microalgae *Fibrocapsa japonica* Toriumi & Takano (Raphidophyceae), which was reported offshore in the Eastern Alboran Sea for the first time in autumn of 2006 (Fani *et al.*2014); ii) the marine nemertean *Cephalothrix cf. simula* AM-2013, which was reported for the first time in several locations along the Spanish coasts (i.e. in the North Atlantic coast of Spain in Galicia, Asturias and Cantabria, and in the Mediterranean coast in Catalonia) in a recent survey of nemertean diversity along the Iberian Peninsula coasts (Fernández-Álvarez and Machordom, 2013); and iii) the caprellid amphipod *Caprella mutica* Schurin, 1935, first reported in October 2012 in Illa d'Arousa (42.56135º N 8.95594º W) and then during 2012 and 2013 in other sites of Ria d'Arousa, Galicia, NW Spain, Atlantic coast (Almón *et al*., 2014).

4.1.10 Sweden

In Sweden there are projects ongoing about recreational boating and commercial shipping where boat or ship as vector for introduction of non-indigenous are included as parts of the projects. Monitoring of biofouling are conducted with panels deployed both in marinas along the Swedish coasts (salinity gradient from 30 PSU to 3 PSU) and in Gothenburg harbour at the Swedish west coast (salinity 25 PSU -0 PSU). Currently hull cleaning of commercial ships in ports are getting attention and municipalities together with ports have localized the need of developing a method for use in hull cleaning recommendations in the aspect of non-indigenous species. Sweden is taking part in the

HELCOM/OSPAR TG BALLAST where a Joint Harmonized procedure for granting Exemptions from Ballast water treatment is being developed.

Swedish Agency for Marine and Water Management has within EC-Marine Strategy Framework Directive MSFD work for Descriptor 2 (non-indigenous species) proposed a project for monitoring of non-indigenous species in harbours and shipping lanes.

4.1.11 United Kingdom

The UK continues to participate in discussions at International Maritime Organization regarding the Ballast Water Management Convention. Orkney Islands Council continues to implement the newly approved Ballast Water Management Plan for Scapa Flow. Cefas has co-ordinated the Marine Pathways Project on behalf of Natural Resource Wales and Defra. The project has had contributions from a number of organisations from across the UK and Republic of Ireland. Work conducted by the project has included the assessment of high risk location of introduction, the development of biosecurity advise for stakeholders, the development of monitoring and surveillance programmes and tools, including assessing the distribution of certain marine non-native species, in addition to examining control measures for certain marine invasive species. The Marine Pathways Project is due to end March 2015, but the group will continue to provide advice to Defra and devolved administrations. The non-native species monitoring phase of Orkney Islands Harbour Authority's Ballast Water Management Policy initiated in 2014 and will continue on an annual basis. Completed projects that have been published in 2014 include Marine Scotland's biofouling study on commercial vessels, the Environmental Research Institute's rapid assessment of marinas and harbours for marine non-native species and the Biosecurity Plan for the Shetland Islands. New species records for the UK include the Asian shore crab (*Hemigrapsus sanguineus*) at Glamorgan and Kent in May 2014 and Quagga mussel (*Dreissena rostriformis bugensis*) in the River Wraysbury in October 2014.

4.1.12 United States

In the past year, research on ballast water as a vector of aquatic nuisance species (ANS) focused on the transport of microorganisms, while research on biofouling focused on ships' available surfaces for the transfer of organisms. Work on the treatment of ballast water proceeded in several areas: use of potable water generators, quantification of total residual oxidants (TRO), efficacy of filter systems, effects of ballast water management systems (BWMS) on ballast tanks and systems, and effects of additives used in land-based verification testing of BWMS. Compliance testing of BWMS was addressed by developing a framework for validating and verifying compliance tools as well as initiating a verification study of tools using variable fluorescence and adenosine triphosphate (ATP) to determine the number of living organisms in discharged ballast water. Regarding risk assessment, a study is underway to characterize the risk-release relationship of invasive species in the Great Lakes using meso-scale experiments and field surveys. Finally, two new species of algae were reported in the Northwest Atlantic: a green alga *Ulva laetevirens*, from New Zealand was found in Connecticut and a red alga, *Laurencia caduciramulosa*, has invaded Key Biscayne, Florida. Several species of crustaceans, fish, and bryozoans have expanded their range in the East coast. There are few formalized levels of effort to survey for new species either in ballast water or once they have arrived. Often

new species are found by scientists during their field experiments, citizens who find unusual organisms and/or citizen scientists and school age children. In the New England area, a formal Rapid Assessment Survey is conducted with taxonomic specialists who identify native and non-native species on floating docks. This captures fouling organisms and their distribution (spread) and limited information on abundance. Neither the states nor the federal government fund ongoing surveys, except through competitive grants that usually focus on genetic studies.

Summary: ToR a)

Many ICES countries are actively conducting research on shipping vectors, with the majority of projects having an Arctic focus. Few countries reported documenting new species in their waters, mainly because there are few programs in place dedicated to monitoring for the arrival of new species. There was concern by the Group that the lack of new reports could be misconstrued as a lowered risk of invasions by shipping, or celebrated as a false indicator of good environmental status. The participants agreed that any statements about invasion records should include direct descriptions of the level of surveillance effort. Finally, it was discussed that surveillance programs might be jointly initiated under funding calls related to interactive effects of non-native species and climate change.

4.2 Term of Reference b)

- Further discuss and evaluate sampling and analysis strategies for type approval and compliance testing of ballast water treatment technologies under consideration at IMO or by other regulators (e.g. U.S. Environmental Protection Agency); (ToR lead Lisa Drake).

This Term of Reference was addressed with four presentations. In addition, the Group discussed the report of the IMO correspondence group undertaking review of Guidelines for approval of ballast water management systems (G8).

Validation of Tools Used to Assess Compliance with Ballast Water Discharge Standards: Update of Research Activities (Presentation by Lisa Drake)

The presentation discussed efforts in the U.S. to develop and assess tools to measure ships' compliance with the ballast water discharge standard. As a start, to assess, validate, and select compliance tools, a framework—consisting of three parts—was discussed. The framework consists of proof-of-concept, validation and verification, and final selection stages. A case study describing the proof-of-concept stage using compliance tools employing variable fluorescence was conducted. All laboratory and field results were favourable, indicating the validation and verification stages are merited to further evaluate fluorometers as compliance monitoring tools. Indeed, an expert workshop has been held to consider the thresholds that should be used by tools using variable fluorescence, and a research project is underway to validate these tools.

Reliability of Ballastwater Testprocedures – ReBaT – RV METEOR June 2015 (Presentation by Manfred Rolke)

The Convention for the Control and Management of Ships' Ballast Water and Sediments (2004; BWMC), aims to minimize the transfer of harmful aquatic organisms with the bal-

last water from ships. The BWMC requires compliance with certain standards limiting the number of living organisms of specified size classes in a given amount of water to be released with discharge of ballast water. There exists still some doubt about the reliability of the existing compliance monitoring methods. In the meantime, some promising approaches have been developed that could be applied during a "trial period". These sampling and analysis methods need to be further studied to examine accuracy and precision, as well as to understand the differences between methods.

This project will be conducted on board a 97.5 m research vessel operated by the German Research Foundation - RV METEOR. The test voyage is planned for 4–14 June, sailing from Cape Verde to Germany. RV Meteor is equipped with a BWMS consisting of a filter and UV reactor. Multiple sampling ports have already been installed on the vessel to facilitate sample collection for compliance monitoring. In combination with different flow paths, different options for sampling will be performed covering high and low organism densities.

Ballast water sampling and analysis: The SGS Ballast Water Compliance Control Service (Presentation by Peter Stehouwer)

SGS, an international company with offices and laboratories in most major harbours in the world, developed methods for sampling and analysing ballast water on board ships in cooperation with the German Bundesamt für Seeschifffahrt und Hydrographie (BSH). For sampling of ballast water the SGS Ballast Water Sampler v02 was developed, this sampler can be run as a closed loop (filtered water is led back into the ballast water discharge line) for true isokinetic sampling. If no second port exists on the ship to lead the water back into the discharge line, the sampler can also be used in 'open' mode. In 'open' mode sampling will not be true isokinetic. The Sampler v02 also comes with different size pitot tubes to control the amount of flow through the sampler. These pitot tubes are part of a modular sampling port design that can be adapted to on board conditions. If the right valves are installed on the ship these pitot tubes can also be inserted into the ballast water discharge line without having to empty it first.

For indicative on board analysis an ATP (adenosine tri-phosphate) method was developed in cooperation with Aqua-tools and Luminultra. ATP is present in all living organisms and can therefore be used for all organisms in the IMO D-2 standard. Three variations on this method were developed, one for organisms ≥50 μm, one for organisms ≥10 <50 μm and one for total bacteria. Filtration is used to exclude organisms which are not part of the size class of interest. Additionally, for organisms ≥50 μm or ≥10 <50 μm a physical disruption step was developed to release the ATP from the cells. Additionally the ScanVIT Fluorescent In-Situ Hybridization (FISH) was developed in cooperation with Vermicon. There are two different ScanVIT methods, the BwE which detects Escherichia coli and Enterococcus and the BwV which detects *Vibrio cholerae*. This is a detailed method and the amount of equipment needed made it impractical for use on board. However, the ScanVIT method is faster and easier to use than classical microbiological incubation methods.

All these sampling and analysis methods were used in a compliance control project for the Singapore Marine Port Authorities. The ballast water on board six new-built (2013 or 2014) ships was tested for compliance with the IMO D-2 standard. Another goal of this project was to assess possible challenges to sampling and analysis on board ships. In ad-

dition to the classical methods, the new methods developed by SGS were also included in the project to have a comparison between classical and new methods. As required in the IMO regulations, the error or uncertainty of the indicative methods was calculated. However, this means that there is a range of values for which no certain compliance statement can be made. This range was referred to in the reports as 'possibly compliant'. Five out of six ships were found to be compliant with the IMO D-2 standard. There were some differences in results between detailed and indicative method, but in general they showed similar results. Also, the FISH ScanVIT method provided comparable results to the classical microbiological methods. During the on-board work some problems were identified. Ships do not always have the right types of valves installed and these valves are not always in places that can be easily accessed. Additionally, ships are generally not equipped with a second sampling point to run the sampler in closed loop. In 'open' mode large volumes of water are discharged into the bilge, which meets with some resistance from the ships' crew.

B-Box: laboratory analysis of (non)-compliance of treatment systems onboard ships (Presentation by Louis Peperzak)

How can ship-owners test if their ballast water treatment system operates as it should? Building on years of experience in measuring ballast water in type approval land-based and shipboard tests NIOZ started a service called B-box. This ballast-water-box is a specially designed cool box for transport of ballast water samples from all over the world to the NIOZ labs in Holland. B-box allows ship-owners to self-monitor the water quality of treatment systems by taking their own ballast water samples. After delivery by air couriers NIOZ performs a number of standard indicative tests for non-compliance that either measure active chlorophyll or ATP. In addition, we can measure other variables such as vital 10-50 μm organisms after CM (FDA) staining. B-box was validated during lab experiments and in shipboard trials in cooperation with Hyde Marine*. Anonymous statistical analysis of the worldwide collected data will provide scientific insight in the real world efficacy of the various ballast water treatment techniques.

*A Case Study of Ballast Water Treatment Performance Assessment During a Shipboard Trial. D.A. Wright, N.A. Welschmeyer and L. Peperzak. J. Mar. Eng. Technol. In press.

Update on status of IMO correspondence group undertaking review of guidelines for approval of ballast water management systems (G8); (Discussion led by Lisa Drake)

The IMO correspondence group discussed, at least briefly, a long list of issues at PPR2. Some of the issues were very complex and will require additional data to make a decision, while some issues were clear cut (such as transparency of testing) and decisions were easily made by the participants. The main points of the report of the IMO correspondence group included:

- that BWMS should be useful for ships in worldwide trade and any limiting conditions for operation should be identified on the type approval certificate;
- that the U.S. ETV protocol should be referenced for guidance;
- how to document deviations that occur during testing, and the effects of the deviation;

- that vendors of the BWMS need to be independent of the type approval testing;
- that test facilities should be transparent and present all data publicly, and have a recognized QA/QC system.

There was recognition that increasing the testing of BWMS does not necessarily mean additional replicates of shipboard and land based tests, but learning could be gained through modelling or bench-scale testing.

Given the short time available before the next meeting of the MEPC it was decided that it was not possible (and also not necessary) to submit a comment on the report of the correspondence group. The Group considered if any of the outstanding issues identified in the IMO correspondence group could be taken on.

Summary: ToR b)

There are now multiple sampling approaches that have been developed for validation and compliance testing, including an 'open system' approach using plankton nets and a 'closed system' approach using smaller filter apparatus which connects back to the ballast piping for isokinetic flow. Similarly, there are a variety of methods now developed for indicative analysis of samples, many of which are based on measurements of variable fluorescence. It is now necessary to conduct validation and verification studies of the different sampling and analysis approaches in order to select the best strategies. It was noted that much of the testing for compliance monitoring is focused on the ≥10 and <50 μm size class, since this size class requires less volume for testing than the greater than ≥50 μm size class. The assumption that the ≥10 and <50 μm size class is a robust indicator for compliance with all D-2 standards may hold true in all situations because this size class is more sensitive to chemical treatment methods than are larger organisms (which are generally removed by a filter in a BWMS), and because natural densities of phytoplankton can be below the requirement of the D-2 standard without any treatment. Thus, if the ≥10 and <50 μm size class is used to assess compliance, it would be imperative to know if any filtration steps occurred properly. Further assessment of both sampling and analysis approaches are needed in order to select the most appropriate methods – comparative testing of different approaches is already underway.

4.3 Term of Reference c)

- Further discuss and evaluate available information on the effects of treated or exchanged ballast water on the aquatic environment and provide input on strategies which could be used to increase confidence surrounding environmental safety of treated ballast water being discharged (ToR lead Stephanie Delacroix).

This Term of Reference was addressed with three presentations and corresponding discussion.

Disinfection by-products and ecotoxicity of ballast water after oxidative treatment – results and experiences from seven years of full-scale testing of ballast water management systems (Presentation by Stephanie Delacroix)

NIVA has since 2005 conducted testing of ballast water management systems in accordance with the IMO G8 and G9 requirements for land based and shipboard testing. Land based tests have so far been completed for several vendors utilizing different principles for ballast water treatment that makes use of active substances or not. According to IMO G8 guidelines, any change in water chemistry due to ballast water treatment should be identified and environmental risk assessment of the whole effluent treated water to be performed. The analytic load to fulfil these requirements included analyses of disinfection by-products (DBP), free residual oxidants (FRO) concentration and chronic/acute toxicity effect of treated water on test organisms over three different trophic levels (algae, crustacean and fish). All these analyses were performed according to internationally recognized standard methods. The results of these analyses performed during NIVA's full scale land-based testing of 5 different BWMS based on chlorination were reviewed and summarized for seawater and brackish water qualities. Out of the close to 100 different DBP compounds that have been included by NIVA when analysing samples of treated ballast water, 22 compounds were detected above the detection limit in at least one sample collected at the time of discharge and only eight of the DBP components were detected in more than 60% of the analysed samples. Bromoform, tribromoacetic acid and dibromochloromethane were found in nearly all analysed samples. Bromate is also a compound often detected in the samples (85% of the 39 analysed samples). The observed median and maximum concentrations of the eight most often detected DBP compounds were in the range from low μg/l to several hundred μg/l. Overall, the 5 most often detected compounds exhibited also the highest maximum concentration levels. The average and maximum concentrations of DBP observed in the full scale land-based tests done at NIVA's test facility were compared with the average and maximum DBP concentrations reported from tests done at other test facilities. This comparison indicates that the DBP levels found at NIVA were, in general, relatively high. However, the average of the maximum levels found at NIVA were always within the 90 percentile of the maximum levels reported by others, and the overall maximum level of any of the DBPs found at NIVA were below the highest reported level reported by others. The majority of the compounds on the DBP lists suggested by GESAMP-BWWG in 2009 and 2013 for analysis in connection with risk assessment of treated ballast water are among these detected compounds by NIVA. Notably, tribromoacetic acid was not among the more frequently detected DBPs, however, when found the level was often relatively high (53–240 μg/l) as compared to the others DBPs. Nevertheless, some DBP can present high toxicity in low concentration therefore the toxicity effect of some DBP was studied further. There seemed to be no clear indication that the measured DBP concentrations affected the algal toxicity neither for individual DBPs (tribromoacetic acid, dibromochloromethane, chlorate, monobromoacetic acid and tribromomethane) frequently detected in the discharged water at levels of potential environmental concern nor for the sum of all DBPs. A direct comparison between the toxicity endpoints for the individual DBPs and the highest levels at which they were found in the discharged waters further substantiated this, as their concentrations were at least a factor 25 lower than the available PNEC value. The results of a simple environmental risk assessment of the individual DBPs detected in the discharged waters were summarised. Four of the compounds (tribromoacetic acid, dibromochloro-

methane, chlorate and monobromoacetic acid) were at times found at concentrations that may pose a risk to the local aquatic environment (PEC/PNEC value >1). However PEC values are depending on the technology tested and the PNEC values dependent of the available published data, therefore these results of PEC/PNEC are just an indication from the five BWMS using active substances tested at NIVA and available literature references. As the concentration of DBPs couldn't be correlated to the toxicity effect results, the correlation between the latter with the FRO concentrations was studied. For seawater tests, the given correlation factors (R2) for the exponential fits of the data points indicate a relatively good correlation; 0.81 for the acute toxicity tests and 0.75 for the chronic toxicity tests. In our same published article, the identification of the precursors of the DBP present in the test water was further investigated. Another important factor in the risk assessment is the cocktail effect from the simultaneous release of such a large range of DBPs. The latter was further investigated by NIVA during a bench scale study with publication of the results hopefully soon.

Effect of different DOC substances on organisms present in test water (Presentation by Lisa Drake)

Rigorous evaluation of ballast water management systems (BMWSs) at land-based test facilities requires that water used in testing meets minimum concentrations of dissolved and particulate material, for example, using the criteria in the U.S Environmental Technology Verification (ETV) Program's protocol for testing of BWMSs. Here, uptake water ("challenge water") can be augmented with compounds to meet these benchmarks. In this study, materials used to supplement dissolved organic matter (DOM), particulate organic matter (POM), and mineral matter (MM) used to achieve challenge water criteria were evaluated. To determine the additives' contributions to DOM and POM pools, the mass yields of *Camellia sinesis* (decaffeinated iced tea) extract and humic matter were calculated at different temperature and salinities. Additionally, the response of ambient organisms to these additives was measured in mesocosm experiments, in which changes in organism concentrations were measured after a 5-d holding time. Living organisms were grouped into three size classes: ≥50 μm (nominally zooplankton), ≥10 to <50 μm (nominally protists), and <10 μm (measured as culturable, aerobic, heterotrophic bacteria). Significant differences in concentrations between control and treatment mesocosms after 5 d were not detected for organisms in the ≥10 to <50 μm or the ≥50 μm size classes. However, bacterial concentrations increased significantly in mesocosms augmented with exogenous materials. Thus, direct impacts (or indirect impacts through increased bacterial concentrations) were not apparent among organisms in the two largest size classes. Finally, a literature review of DOM, POM, and total suspended solids concentrations in coastal waters was conducted. It revealed that the challenge water concentrations outlined in the ETV protocol are at the middle to upper range of concentrations observed in coastal and estuarine water. The mean DOM and POM concentrations in this data set typically fell short of the ETV minimum requirements, and more data are needed to fully assess the suitability of these requirements.

First MR, Robbins-Wamsley SH, Riley SC, Fisher JI, Smith JP, Drake LA (2014) Examination of additives used to augment "challenge water" used in verification testing of ballast water management systems: mass yields and biological impacts. Management of Biological Invasions 5:395–405 doi:10.3391/mbi.2014.5.4.10

Update on activities of the gesamp-ballast water working group (Presentation by Jan Linders)

The GESAMP-BWWG has been conducting Environmental Risk Assessments to assess the potential effect of ballast water management on recipient port areas. Predicted concentrations of chemicals in harbours are calculated using the MAMPEC model – developed specifically for GESAMP. The model calculates the yearly average concentration at constant daily load of ballast water in a recipient harbour. The model considers the size and connections of the harbour, the specific compound involved and the emission (concentration parameters). GESAMP also considers human exposure scenarios to the general public (beachgoers) and occupational hazards. Information for the assessment comes from the literature – acute and chronic tests for freshwater and marine aquatic environments and mammals. The Evaluation leads to a Predicted No-Effect Concentration (PNEC) or Derived No-Effect Level (DNEL). Lab toxicity tests with simulated treated ballast water are required for Basic Approval and Whole Effluent Toxicity (WET) tests at Final Approval. Depending on the amount of data available, the assessment is extrapolated to estimate error or confidence level. Sometimes expert judgement is used when the quality criteria have not been met. Literature data (tests using single compounds) are preferred over lab scale testing (multiple disinfection by-products) at Basic Approval and WET test results are preferred over literature data at Final Approval. There has been quite a debate in GESAMP-BWWG over measurement methods for total residual oxidants (TRO). The GESAMP-BWWG also considers corrosivity of relevant chemicals. There are several additional tests under discussion to improve data available for the risk assessment, but it is not feasible to modify G9 Guidelines until the Ballast Water Management Convention has entered into force. In conclusion, the methodology for G9 is reasonably in place and there is a clear structure to the environmental risk assessment strategy. The GESAMP-BWWG database became operational in 2014. In the future, it is recommended that applicants should make use of the methodology and database in submissions for Basic and Final Approval. Additional testing may be proposed when the Convention enters into force, and the risk assessment should be applied for a near ship scenario (not just inside harbours).

Discussion: ToR c)

Confidence surrounding environmental safety of treated ballast water has improved in recent years as GESAMP and test facilities have been sharing results of their evaluations. In general, under the IMO G9 guidelines, the environmental risks of treated ballast water are being evaluated adequately. The GESAMP-BWWG has identified a need to assess mixtures of components in addition to individual assessments. There is already a plan to incorporate such changes once the Convention enters into force; in addition, it will be beneficial to make G9 testing requirements mandatory after that time. The Group identified a need for monitoring of DBPs [and TRO] in ballast water being discharged during regular operations after type approval has been completed – this monitoring could be incorporated into port state control procedures [if it is not already – Sarah will raise this with Chris Wiley]. Further, it was discussed that monitoring for compounds in ports and harbours, independently from individual vessels, would generate useful data for future evaluations of impact over time, particularly if baseline conditions can be monitored before the Convention enters into force. Finally, an evaluation of the different organisms used by different test facilities would be informative. In terms of discharges of exchanged

ballast water, issues could arise related to toxic coatings inside ballast tanks, but the magnitude and prevalence of such impacts are not quantified.

4.4 Term of Reference d)

- Investigate and report on new developments in non-native species issues associated with biofouling (e.g. artificial structures in the marine environment and recreational boating); (Joint Term of Reference with WGITMO); (ToR lead Cynthia McKenzie).

This Term of Reference was addressed with two presentations and corresponding discussion. Cynthia McKenzie began by reviewing the special session on this topic at the 2014 ICES ASC. The general conclusions at the ASC supported the earlier position of our Groups - that biofouling may become more of an issue with climate change. It is now timely to work on anti-fouling strategies and addressing the IMO Biofouling guidelines. Nathalie Simard then gave a presentation updating the Groups on a project to evaluate the risk of biofouling on recreational boats as a vector in Canada. It was noted that results are available for a similar study conducted by CEFAS and partners.

Marine recreational boating risk assessment (Presentation by Nathalie Simard)

Fisheries and Oceans Canada is conducting an evaluation of the risk of recreational boating as a vector for the introduction and spread of Aquatic Invasive Species (AIS) in Canada. Studies conducted in British Columbia and Nova Scotia provided information on recreational boating as vectors in these areas but did not include several regions of Atlantic Canada. Before a national risk assessment of this vector in the marine environment was attempted, additional data for these regions was collected (2011–2014). Taken together, the complete database includes recreational boater surveys (1457), manager surveys (262) and vessel hull video/scuba (567) that were collected in Atlantic Canada (Quebec, Gulf Region, Nova Scotia and Newfoundland). These surveys were based on questionnaires and methodologies used by the studies in British Columbia and Nova Scotia. This Marine Recreational Boating Assessment is comprised of four steps which will provide an overall vector risk assessment. The first step is an assessment of potential risk which will involve a statistical comparison of the various boat types and their maintenance (e.g. storage method, use of antifouling paint, etc.) in relationship to the biofouling observed on the vessel hulls. The next step is an assessment of the movement patterns of the vessels (# destinations and #days in destination port) within and between regions. The third step is the regional AIS background of the harbours and marinas. Finally, the step four or the realized risk per boat type and region will be determined using vessels, movement and biofouling then demonstrated using a risk assessment heat matrix.

Discussion: ToR d)

Following the presentations, the Group discussed the status of the IMO Guidelines for the Control and Management of Ships' Biofouling [Resolution MEPC.207(62)]. It was noted that the IMO agreed to keep the Guidelines under review as experience is gained, but that a decision to change the voluntary Guidelines into mandatory measures would be a

long undertaking. The Group noted the research needs outlined within the Biofouling Guidelines, and agreed to contribute information to the IMO in future, when possible:

12.1 States and other interested parties should encourage and support research into, and development of technologies for:

.1 minimizing and/or managing both macrofouling and microfouling particularly in niche areas (e.g., new or different anti-fouling systems and different designs for niche areas to minimize biofouling);

.2 in-water cleaning that ensures effective management of the anti-fouling system, biofouling and other contaminants, including effective capture of biological material;

.3 comprehensive methods for assessing the risks associated with in-water cleaning;

.4 shipboard monitoring and detection of biofouling;

.5 reducing the macrofouling risk posed by the dry-docking support strips, (e.g., alternative keel block designs that leave less uncoated hull area);

.6 the geographic distribution of biofouling invasive aquatic species; and

.7 the rapid response to invasive aquatic species incursions, including diagnostic tools and eradication methods.

4.5 Term of Reference e)

- Investigate and report on new developments in non-native species issues in the Arctic (Joint Term of Reference with WGITMO); (ToR lead Anders Jelmert).

This Term of Reference was addressed with three presentations and corresponding discussion. Sarah Bailey began the work by reviewing the special session on Arctic biodiversity under climate change and other stressors at the 2014 ASC. The general conclusions at the ASC supported the earlier position of our Groups - the absence of historical data/knowledge/baselines in the Arctic hinders our ability to evaluate the current status, understand current mechanisms and make projections for the future. There was a general uncertainty if our understanding/knowledge from temperate ecosystems can be directly applied to Arctic ecosystems and a need to better understand the impacts of range extensions into the north. Finally, it was broadly recognized that bioinvasions and climate change are but two stressors currently active in the Arctic. It is expected that there will be a great demand for science advice in the near future as human activities continue to diversity and intensify in the Arctic.

Understanding ballast water as a pathway for introduction of aquatic invasive species (AIS) in the Arctic (Presentation by Nathalie Simard)

Ballast release associated with commercial shipping has been the source for a large number of invasions on a global scale and has been one of the main vectors for aquatic introductions in Canadian coastal waters. Although introductions into Arctic waters have been limited, climate warming, increased resource development and associated increases in shipping are expected to increase the risks for future introductions. We have limited understanding of the species found in the ballast water of vessels travelling into the Arctic, their interactions with native fauna, their ability to establish in the Arctic and their

impacts. Furthermore, although regulations exist for international ships, domestic vessels (within Canada only) are currently unregulated. Most Arctic domestic ballast is brought in by ice-breakers travelling year-round; these vessels conduct voluntary exchange in northern Canadian coastal waters, however there are questions regarding the effectiveness of exchange (it may actually increase risk) and how this varies by season. In order to address these questions, we are conducting research on the content of ballast water being brought into two of the main Arctic ports identified as being at risk for ballast mediated introductions: Churchill, MB and Deception Bay, QC by sampling ballast water from arriving ships for water quality, and zooplankton/phytoplankton species composition (native, non-native) and densities. We are coupling this with more specific research aimed at better understanding risks associated with the currently non-regulated domestic shipping pathway through: 1) evaluation of a subset of the above samples from ice-breaking domestic vessels that travel on a more extended basis for seasonal variation in level of risk and 2) conducting sampling and analysis of ballast on vessels prior to exchange for comparison to above post-exchange samples. Information from this study will improve our abilities to understand and manage ballast-mediated species introductions, help guide voluntary ballast management practices by industry and feed into regulatory decisions by Transport Canada. Field work has started in 2014 and will continue in 2015. Publications are expected for 2016.

Update on shipping traffic and ice conditions in the Northern Sea Route 2014 (Presentation by Anders Jelmert)

While the long-time trend for ice area / ice extent / ice volume appears to have declined rather monotonously since the eighties, the minimum ice area and –volume currently seems to have reached a (likely preliminary) minimum in the 2011/2012 seasons. The ice conditions in the 2014 navigable window were regarded as somewhat more challenging than the 2011 and 2013 seasons. The numbers of crossings over the Siberian route increased from 10 (2010) to 41 (2011) to 46 (2012) to 71 (2013), and were expected to increase further in 2014. However, the traffic statistic now shows 31 complete crossings and 20 (long) Russian domestic transports within the NSR. The vessels were quite diverse, where oil/oil products / chemical tankers being the largest vessel type, general cargo the second largest vessel type. Total tonnage of crossing vessels was 463928 Gr.Ton. The largest vessel was a "Panamax" sized vessel 41061 Gr.Ton (76160 DWT). Average voyage time for all crossings: 18.1 d. Russian domestic traffic: 20 vessels, some 200 000 Gr. Ton., Average voyage: 12 days. Despite expected increase, and only slightly more challenging ice conditions than previous years, less traffic was observed in 2014. This indicates that other factors (e.g. world economy, rates, bunker prices, SAR/Ice-breaker costs, oil exploration activities, etc.) are (and will likely be) important factors dimensioning the future Arctic traffic. A lowered-than-expected NSR traffic means that science still has an opportunity to explore important environmental issues (including e.g. baselines) before anticipated traffic increase will make these efforts more difficult.

In response to this presentation, it was noted that a commercial vessel completed the first unassisted passage of the northern route across Canada. There may be different risks along Canada's northern route since there are a number of resource (mining) companies setting up there, so there is port development as well as vessel transits.

Discussion: ToR e)

It was noted that effects of climate change which could facilitate non-native species invasions, such as decreased ice cover resulting in increased vessel traffic, are already being observed. There was discussion on the great difficulty for taxonomic identification of species and origins of phytoplankton sampled in the Arctic. It was noted that data from Canadian Arctic port surveys for invertebrates has already been uploaded to AquaNIS. The Groups then discussed the possibility that Russian Institutions should have extensive historical data that could be used to improve knowledge on baseline conditions – although likely there will be a need to translate historical materials. Although there are currently some political strains on cooperation with Russia, there are likely some avenues which could facilitate such collaboration and cooperation, such as the Arctic Council. The Groups also noted that various scientific research cruises conducted in the Arctic could be a source of baseline information. The Groups discussed an idea to initiate a workshop to bring experts on the topic together, to try to share the data to generate biodiversity baselines of the taxa most relevant to bioinvasions, improve knowledge, and made a plan to explore opportunities over the next year intersessionally, as well as to develop specific objectives and scope for such a workshop. The Groups suggested extending invitations to experts to attend the working group meetings next year. This should also involve communication with Arctic-related expert groups in ICES.

4.6 Term of Reference f)

- Collaborate with ICES Study Group on Integrated Morphological and Molecular Taxonomy (SGIMT) regarding identification, early detection and monitoring of non-native species, as appropriate (Joint Term of Reference with WGITMO); (ToR lead Sarah Bailey and Henn Ojaveer).

This term of reference was addressed by two presentations and corresponding discussion. Group member Phillipe Goulletquer solicited advice from expert colleagues, which was reviewed as a starting point for discussion. Generally, the comments identify some benefits, and some issues/warnings with the use of molecular tools for early detection:

In the past two-three decades, molecular tools have been used with success to help on three issues related to non-indigenous species: 1) Identifying non-indigenous species; 2) Tracing back the routes & processes; 3) Studying evolution in the introduction range (new range), particularly to examine hybridization with native species. In contrast, early-detection using molecular tools can be an issue because: 1) increasing lack of taxonomic expertise or reference to taxonomists; 2) species may be hardly distinguishable, particularly regarding the increased number of cryptic species (i.e. species that are morphologically similar); 3) taxonomic changes and revision and the resulting slow updating of databases (naming takes time).

There are several issues regarding identification of species that might be resolved with molecular tools:

- new non-indigenous go unnoticed because they look like native taxa, e.g. *Polysiphonia* genus (Geoffroy *et al*. 2012) and this may prevent early detection and warning; e.g. *Asterocarpa humilis* (Bishop *et al.* 2013) – see below

- new nonindigenous species go unnoticed because they are members of cryptic species complex, e.g., colonial tunicates and bryozoans - *Watersipora; Botryl-loides* sp.
- species are mistakenly described as new species (*Styela clava* first reported as a new non-indigenous taxa *Styela mammiculata*)
- Reinforcement by a species different from the targeted species (a very illustrative case study is with *Spartina densiflora* in California)

One solution is (meta) barcoding - using molecular information to identify taxa. Note that other molecular approaches are also useful, for instance population genetics and genomics to ascertain hybridization between native and non-indigenous taxa and putative emergence of successful hybrids.

Benefits of (meta) barcoding for species identification or early-detection are:

- Standardized and rapid procedure=> Reliability for comparison between labs.
- No need for taxonomic expertise (when routine analysis is done and upstream research work has been correctly done. See below for the "warning and cautions").
- Global databases such as BOLD (Barcoding of Life Database) and Genbank remove geographic limitations; this is a very important aspect as by definition non-indigenous species are new in a given area, and are absent from regional fauna and flora taxonomic books (but need accurate database; See below for the "warning and cautions").
- Particularly useful or needed when morphological traits are tricky to use or misleading, or when there is possible confusion between native and non-indigenous species, and to help in determining the species traits useful in the field (see an example with Sato *et al.* (2012) for discriminating between *Ciona intestinalis* species B and *Ciona intestinalis* species A - two evolutionary divergent cryptic species only recently recognized and which are two invasive species). In all the examples early detection is prevented because we do not have relevant traits to examine in the field.
- The only way when there is no morphological traits to be used and thus particularly relevant for studying small early life history stages. This is probably where most of the expectations are at the moment as we all know the importance of larvae and juveniles in introduction processes as all these stages can be easily transported by ballast waters or with aquaculture exchanges. Thus this is where there is a great challenge in early detection: track the initial founders (e.g. larvae in ballast waters).
- Work can be done using specimens that are degraded, disentangled or badly preserved. This includes, for instance, work with collection, herbarium or museum specimens to help confirm past identification or dating invasions.

Warning and Cautions

- Barcoding should not be restricted to a single marker (see on-going debates/arguments as part of the BOLD initiative). There are now more and more

evidence that using a single gene may be misleading because a gene is not a species (e.g. selective effects may have occurred on this particular gene)

- Database errors: errors in correct taxonomy can/do occur even in GenBank and BOLD (e.g. some *Botryllus* species named *Botrylloides* and vice versa)
- Lack of reference data: see the graph in Comtet *et al.* (2015) to illustrate that for many non-indigenous species, we just don't have the reference, meaning that the upstream research work has still to be done
- Samples not preserved properly (e.g. using formalin) result in poor/no DNA recovery
- Conventional barcodes is more "taxa" oriented, but there are pitfalls for metabarcoding of metazoans (Cristescu 2014)
- Requires molecular expertise and equipment
- High cost of lab analysis

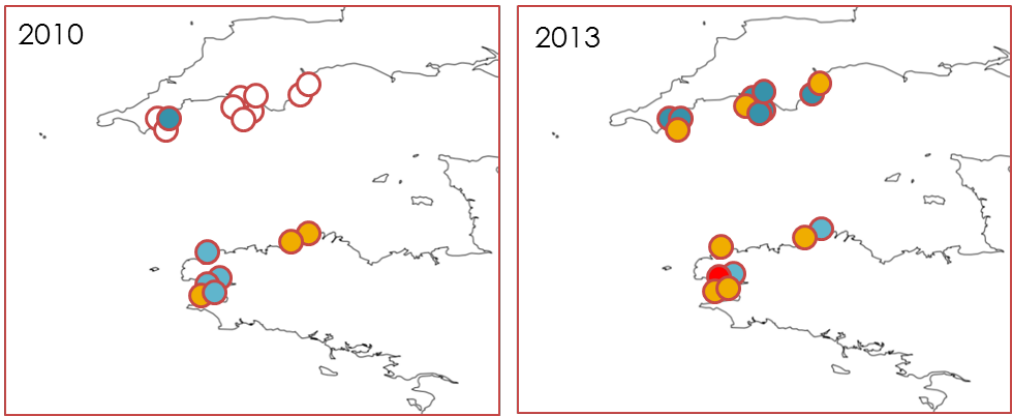
A step forward requires:

- improved protocols particularly for global analyses - to work on water samples is fine, but sampling on hard substrates or on rocks is much more difficult. In addition, there is still work to be done to assess the representativeness and certainty.
- dedicated databases, especially for metabarcoding approaches. These should not be focused on specific taxa but aim at global diversity assessment. Thus, an important need is to establish accurate and global database for barcoding.
- integrative taxonomy research. Such research should focus on designing thebarcodes.
- preservation of samples in adequate medium/buffer (e.g. ethanol). Often, the collected samples are preserved in medium not suited for genetic analysis (e.g., formalin).
- promoting and popularizing the approach with the aim to enhance collaborations between geneticists and taxonomists.

Concluding remarks: Molecular tools are sensitive to help in early warning/early detection as, for instance, they are relevant and accurate for identifying early-stages likely to be introduced in ballast water or remain unnoticed, rare specimens or badly conserved specimens. They also do not need strong taxonomic expertise and there is a standard procedure available. New techniques are currently emerging for global assessment, such as e-DNA and metabarcoding. These have proved to be very promising, however, major effort is still to be made to establish the reference database.

A simple example of detection with regular barcoding (Bishop *et al.* 2013, Bishop *et al.* 2015).

These two maps are documenting the abundance (semi-quantitative data) and site occupancy of *Asterocarpa humilis*. Morphological and molecular data were used to ascertain the species identification and later confirm its rapid spread in the English Channel.



Following review of these comments, the Groups discussed how the traditional debate over definition of a species has carried over to the results of molecular tools – with molecular tools now identifying strains of species. Significant problems can occur where taxonomic decisions, such as splitting of species, is not peer reviewed and results are not generally agreed, but results get into the literature (non-peer-reviewed books). On the one hand, there can be negative impacts if managers of non-native species have to start managing strains of species, but on the other hand, understanding of impacts can be greatly improved when invasive vs. non-invasive genotypes are recognized.

As another issue, it was noted that there could be limitations on management activities if we can only detect species by molecular tools – unless management response is also a molecular-based tool. This line of discussion re-opens the question as to whether or not post-border management is even a realistic option for the aquatic environment – and re-affirms vector management as highest priority (see also Lehtiniemi *et al.* 2015).

A presentation on molecular information in AquaNIS (by Sergej Olenin) indicated that molecular information is available for 189 species involved in 1266 introduction events:

Phylum	# of NIS
Chordata	71
Mollusca	55
Arthropoda	20
Rhodophyta	14
Annelida	9
Chlorophyta	4
Cnidaria	4
Ochrophyta	3
Bryozoa	2
Ascomycota	1
Ctenophora	1
Echinodermata	1
Magnoliophyta	1
Nemata	1

Porifera	1
Pteridophyta	1

Molecular information is available in AquaNIS for 58 species (674 introduction events) associated with vessel vectors (actual evidence of being found in samples in a particular vector from any world region):

Phylum	# of NIS
Mollusca	17
Arthropoda	10
Rhodophyta	9
Chordata	8
Annelida	3
Chlorophyta	3
Cnidaria	2
Ochrophyta	2
Ctenophora	1
Magnoliophyta	1
Nemata	1
Porifera	1

Finally, the Groups discussed how the information in molecular databases has been increasing exponentially very recently. It was noted that it is premature to consider a positive eDNA record for a new species alone as confirmation of a new record. It is still important to have a physical specimen as well because the DNA could be a failed arrival, or an error due to primer selection, etc. Molecular tools can safely be used to discriminate cryptogenic species, when you have the correct baseline sequence collection (different populations globally).

Discussion: ToR f)

Although WGBOSV and WGITMO have been open to collaboration with WGIMT, the limited taxonomic scope of WGIMT, overlapping timing of group meetings and lack of intersessional activity has prevented progress under this ToR. In order to increase access to molecular experts, WGBOSV and WGITMO discussed inviting molecular experts that specifically conduct research on non-indigneous species identification and early detection to future meetings. The Groups have also identified a dedicated person to carry this activity forward during coming years.

4.7 Term of Reference g)

- Provide input to WGITMO in connection with OSPAR 1/2015 request

A small group of experts reviewed the Eutrophication Guidelines in advance of the meeting to initiate this task. Suggested changes were then reviewed by WGBOSV and

WGITMO during the joint meeting. In general, the Groups agreed that it would be beneficial to revise the Guidelines to include monitoring for non-native species in order to meet requirements under the Water Framework Directive, Marine Strategy Framework Directive and EU Regulation on invasive alien species. The Groups discussed the importance of using consistent and well defined terminology in the Guidelines – including native vs. non-native and introduced vs. invasive species. As it can be extremely difficult to correctly identify phytoplankton to the species level, and to know the origin of species in every location, the Groups agreed that species of cryptogenic status should also be noted. In addition, the Groups felt it was important that the Guidelines should not focus only on invasive species but rather on non-indigenous species in general, since impact can be difficult to measure, invasiveness is of dynamic nature and has both spatial and regional components or, if the introduction is recent, may occur in the future after some lag time has passed. Various members of the working groups have published papers explaining terminology and factors confounding our ability to measure impact, and these could be referenced in the Guidelines directly (e.g. Galil *et al.* 2014; Ojaveer and Kotta 2015).

After this general discussion, the Groups reviewed the Guidelines paragraph by paragraph. An annotated version of the suggested changes is attached as Annex 4. Specific changes suggested include direct linkage to the various regulations and directives that require monitoring of non-indigenous species and an additional objective specific to non-indigenous species. The Groups spent some time discussing the key species referenced in section four and suggested that it may be helpful to develop a checklist of 'Species of Concern' as an annex to the Guidelines. Such a checklist would need to be specific for different locations/sea areas/sub-ecoregions, since species may be native to some areas within OSPAR region, but non-indigenous in other areas. The Group discussed the importance of setting specific criteria for inclusion of species based on distribution, abundance and previous impact. Unfortunately, it was not possible to develop a checklist within the timeframe available; if such a checklist is created in the future, it will be important to keep the list open for revisions in the future as more knowledge is gained and if new species invasions occur. The Groups discussed the list of example 'key species' currently in the Guidelines, noting that the list was very broad and might benefit from some revision. As the examples are not exclusively related to non-indigenous species, it was decided to annotate this section as needing more consideration during the development of the final advice from ICES. The Groups supported specific additions on methods suggested by the smaller group of experts, asking only that such edits be detailed precisely. Finally, the Groups suggested specific recommendations regarding the documentation and reporting of new records of non-indigenous species.

4.8 Other business

Sarah Bailey was re-elected as WGBOSV Chair for 2016–2018. New, three-year terms of reference were drafted corresponding with the next chairmanship.

Henn Ojaveer briefly described the structure of ICES SCICOM and the four Steering Groups: Ecosystem Processes and Dynamics (SGEPD), Ecosystem Pressures and Impacts (SGEPI), Integrated Ecosystem Assessments (SGIEA) and Integrated Ecosystem Observation and Monitoring Programme (SGEOM). The parent SG for both BOSV and ITMO is EPI.

The aim of EPI is to i) Understand the relationship between human activities and marine ecosystems, estimate pressures and impacts and develop science-based sustainable pathways, and ii) Provide tools and methods for assessing the relationship between marine ecosystems, their biological resources and habitats, and human societies, including how human use impacts the provision of ecosystem services. The objectives of EPI are: i) Estimate long-term trends of human impacts on marine ecosystems, ii) Understand, quantify and mitigate multiple impacts of human activity on populations and ecosystems, and iii) Provide evidence in support of the sustainable management of ecosystem goods and services.

Dependent on the incoming advice requests, BOSV and ITMO will likely get advice-related ToR's in their agendas also in future. These are related, amongst others to IMO Ballast Water Management Convention and various topics related to non-indigenous species (like MSFD D2 and IAS Regulation).

North Sea Ballast Water Opportunity Project (Presentation by Cato ten Hallers)

The North Sea Ballast Water Opportunity Project (NSBWO resulted in some major achievements, growing from 40 to 200 co-operators (2009–2014). At the start of the project, only one North Sea state (Norway) had ratified. Sharing strategies and information on the process towards ratification between countries contributed to four more ratifications (Sweden, Netherlands, Denmark and Germany); a sixth (Belgium) is close. At least six type-approved ballast water management systems have been developed within NSBWO, as well as the development of two new ballast water test sites and a contingency solution for ports, and several technical solutions for ballast water management testing technologies. Publications produced in 2014 include:

- Ballast Water Management Convention Publication, Updated (Partner BSH)
- Ballast Water Book for Children (Partner WMU)
- "Towards participatory and transparent implementation of the Ballast Water Management", author Ellen Ninaber – Interviews with ship owners; analysis of innovation processes (Partner CaTO)
- Press release: Achievements of the NSBWO project (2009-2014) (CaTO)
- Information paper to MEPC 68 (MEPC 68/INF.20) (BSH): Workshops on organism detection technologies, compliance control, monitoring and enforcement and ecotoxicity testing during land-based. Submitted by Denmark, Germany, Netherlands, Norway and United Kingdom.

Highlights from the publication by Ellen Ninaber were presented, describing five categories in embarking on an innovation trajectory – from innovators, to early adopters, to the early majority, the late majority, and laggards. In such processes a vast majority is resistant to change. UN and UN Organizations have now widely recognised the need for a more inclusive and participatory approach to policy making.

In the case of accepting the need to take up the challenge posed by the imminent ratification of the BWM Convention, it is important to recognise the differences in positions towards innovation. The policy process would benefit from support for exploring new strategies to involve sector in implementation. Examples of such support strategies to stimulate openness to innovation, can be:

- Pioneering rewards – express appreciation of the pioneer's role in the implementation of the requirements of the BWM Convention.
- Make the introduction of innovation more transparent and do-able for all parties involved.

5 Closing of the meeting

The meeting was closed at 17:00 on 18 March 2015. The Chair thanked the group for all their input and participation during the meeting and intersessionally, particularly to those members who acted as ToR lead. The Chair also thanked Anders Jelmert for hosting the meeting.

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Annex 1: List of participants

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Annex 2: Agenda

ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors
16–18 March 2014

INSTITUTE FOR MARINE RESEARCH
Nordnesgaten 501
Bergen, Norway

SUNDAY 15th MARCH

Dinner at local Restaurant – Zupperia (address Vaskerelven 12)

Meet at 19h00 in lobby of Comfort Hotel Holberg, or 19h15 at the restaurant

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MONDAY 16TH MARCH

Meet at 08.30 to allow time for setting up computers.

09.00 Opening of the meeting

- Welcoming remarks **Sarah Bailey** (Chair) and **Anders Jelmert** (Meeting Host)
- Introduction of Participants
- Review of WGBOSV Terms of Reference and Agenda
- Logistics – Report Drafting Assignments

09.25 Review Terms of Reference and Agenda

9.30 ToR a): Continue to critically review and report on the status of shipping vector research with an emphasis on studies of shipping transport vectors, shipping vector management activities and risk assessment. ***ToR lead: Sarah Bailey***

- Highlights from the National Reports, about 10-15 minutes per country:

- Belgium **Francis Kerckhof**
- Canada **Cynthia McKenzie/Nathalie Simard**
- Estonia **Henn Ojaveer**
- Finland **Lauri Urho**
- France **Phillipe Goulletquer**
- Germany **Manfred Rolke**

10.30-11.00 Morning break

- Netherlands **Cato ten Hallers**
- Norway **Anders Jelmert/Stephanie Delacroix**

- Sweden **Lena Granhag**
- UK **Lyndsay Brown**
- USA **Lisa Drake**

- General/summary discussion under ToR a)

12.30-13.30 Lunch break (not provided)

ToR b:) Further discuss and evaluate sampling and analysis strategies for type approval and compliance testing of ballast water treatment technologies under consideration at IMO or by other regulators (e.g. U.S. Environmental Protection Agency). ***ToR lead: Lisa Drake***

- Presentation: Compliance monitoring update – **Lisa Drake**

- Presentation: Compliance monitoring comparison exercise on the RV METEOR - **Manfred Rolke**

- Presentation: Update from SGS Institute – **Peter Paul Stehouwer**

15.00-15.30 Afternoon break

- Presentation: B-box "Do it yourself ballast water monitoring kit" - **Louis Peperzak** (by phone/Skype)

- Update on status of IMO correspondence group undertaking review of Guidelines for approval of ballast water management systems (G8) – **Lisa Drake**

- Consider need to submit 'comment paper' to MEPC 68 – ***deadline 20 March!***

- General/summary discussion under ToR b)

17.30 Close of first day of meeting

TUESDAY 18TH MARCH

9.00 ToR c): Further discuss and evaluate available information on the effects of treated or exchanged ballast water on the aquatic environment and provide input on strategies which could be used to increase confidence surrounding environmental safety of treated ballast water being discharged. ***ToR lead: Stephanie Delacroix***

- Presentation: Update on toxicity studies conducted at NIVA – **Stephanie Delacroix**

- Presentation: Effect of different DOC substances on organisms present in test water – **Lisa Drake**

- General/summary discussion under ToR c)

10.30-11.00 Morning break

- Return to any issues needing more discussion

- Any other business raised by members at the meeting

    - North Sea Ballast Water Opportunity Project – **Cato ten Hallers**

12.30-13.30 Lunch break (not provided)

- Election of WGBOSV Chair (tenure 2016-18)

- Drafting of 2016-18 WGBOSV ToRs (new 3-year ToRs under SCICOM)

15.00-15.30 Afternoon break

- General discussion

- Drafting of WGBOSV Report (***Final Report Due 10th April 2015***!)

17.30 Close of second day of meeting

WEDNESDAY 19TH MARCH
JOINT MEETING WITH WGITMO

09.00 Opening of the joint meeting

- Welcoming remarks from **Sarah Bailey, Henn Ojaveer** (Co-Chairs) and **Anders Jelmert** (Meeting Host)

- Introduction of participants

- Review of joint WGBOSV/WGITMO Terms of Reference and Agenda

09.30 ICES update: Henn Ojaveer

09.45 ToR g): Provide input to WGITMO in connection with OSPAR 1/2015 request.
ToR lead: Lyndsay Brown

Review of draft OSPAR JAMP Eutrophication Guidelines on phytoplankton species composition

ICES is requested to advise OSPAR on the revision of the OSPAR JAMP Eutrophication Guidelines which will be revised by experts from Germany, The Netherlands and Sweden.

ICG-EUT 2014 concluded, and HASEC 2014 endorsed, that these guidelines were in need of a review. The guidelines should be revised to reflect new knowledge about phytoplankton and needs within (directives such as) the EU Marine Strategy Framework Directive (MSFD) and the Water Framework Directive (WFD).

It is the intention of the revision that the existing aims described in the guidelines1 will be supplemented with the following:

- to identify harmful algae species and blooms in line with MSFD Descriptor 5.
- to identify invasive (non-indigenous) species in line with MSFD Descriptor 2.
- to monitor effects of ocean acidification as e.g. on coccolithophorids (e.g. *Emiliania huxleyi*) in line with Descriptor 1 in MSFD.

The revised guidelines should incorporate coming monitoring and measurement techniques such as (but not limited to) spectrofluorometry, flow cytometry and qualitative observations of foam production, and should make use of existing standards, such as EN 159722 and EN 152043 and reflect developments within the OSPAR ICG – COBAM which is working on biodiversity monitoring and assessment. Data handling issues, such as the format required for reporting to ICES, should also be addressed.

WGITMO [jointly with WGBOSV] should address the issues related to invasive (non-indigenous) species and relevant monitoring and measurement techniques as mentioned above.

10.30-11.00 Morning break

- General/summary discussion under ToR g)

11.45 ToR d): Investigate and report on new developments in non-native species issues associated with biofouling (e.g. artificial structures in the marine environment and recreational boating) ***ToR lead: Cynthia McKenzie***

- Short overview of special session at ICES Annual Science Conference – **Cynthia McKenzie**
- Presentation: Biofouling on recreational boating - **Nathalie Simard**
- Presentation: TBD

- General/summary discussion under ToR d)

12.30-13.30 Lunch break (not provided)

- **ToR e)** Investigate and report on new developments in non-native species issues in the Arctic, as a result of climate change and resource developments ***ToR lead: Anders Jelmert***

- Short overview of special session at ICES Annual Science Conference – **Sarah Bailey**

- Presentation: Examining risk of domestic vessels moving ballast water from St. Lawrence River to Canadian Arctic ports – **Nathalie Simard**

- Presentation: Update on shipping traffic and ice conditions in the Northern Sea Route – **Anders Jelmert**

- General/summary discussion under ToR e)

15.00-15.30 Afternoon break

15.30 ToR f) Investigate and report (incl. via AquaNIS) on new molecular tools for identification, early detection and monitoring of non-native species, in collaboration with ICES Working Group on Integrated Morphological and Molecular Taxonomy (WGIMT) ***ToR lead: Maiju Lehtiniemi*** (by correspondence)

- General/summary discussion under ToR f)

16.15

- Location of next meeting and joint 2016-18 WGBOSV/WGITMO ToR's

- Any Other Business/Wrapping up of joint meeting

17.00 End of WGBOSV

Annex 3: National reports

Canada

Submitted By:

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SUMMARY

Shipping vector research in Canada currently focuses on risk assessments, and vector management, with particular emphasis on the Arctic Region. A national risk assessment on recreational boating as a vector for introduction and spread of aquatic invasive species is in the final stages of assessment. Vector management studies include shipboard trials to examine a combination strategy (ballast water exchange plus treatment) as a means to protect low salinity waters, and examination of operational efficacy and environmental safety of ballast water management systems operated in cold (winter/Artic) conditions as well as studies to determine the effectiveness of voluntary exchange by domestic vessels transiting to the Arctic. In addition, a study has been recently completed to evaluate the response of freshwater organisms to vital stains used to assess compliance with ballast water discharge standards. The Canadian Aquatic Invasive Species Network (CAISN) continues to work on projects related to early detection and rapid response strategies, understanding aquatic invasive species as part of multiple stressors affecting aquatic ecosystems, and reducing uncertainty in prediction and management. Recent surveys of Arctic ports have identified seven new species of uncertain origin (cryptogenic). National AIS regulations as part of the Fisheries Act in Canada are in the final stages of approval and are expected to come into effect in 2015.

FULL REPORT

A TRANSPORT VECTORS

Understanding ballast water as a pathway for introduction of aquatic invasive species (AIS) in the Canadian Arctic (2013–2017)

Freshwater Institute, Fisheries and Oceans Canada, Winnipeg, Manitoba, Kimberly Howland: Kimberly. Howland@dfo-mpo.gc.ca
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Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, Burlington, Ontario, Sarah Bailey: sarah.bailey@dfo-mpo.gc.ca
Andre Rochon and Gesche Winkler, UQAR-ISMER, Rimouski, Quebec

The primary objectives of this project are to: 1) Characterize diversity and propagule pressure of zooplankton and phytoplankton in the ballast of vessels in Arctic Ports (Churchill and Deception Bay); 2) Evaluate seasonal changes in risks associated with ballast being brought into the Canadian Arctic; 3) Determine effectiveness of current voluntary exchange practices for Arctic domestic vessels. The project will provide analyses of species composition and abundance for both domestic and international shipping pathways, seasonal analyses of risks and recommendations regarding current voluntary exchange. Information from this study will improve our abilities to understand and manage ballast-mediated species introductions, help guide voluntary domestic ballast management practices by industry and feed into regulatory decisions by Transport Canada. Ballast samples of ships arriving in ports of Churchill and Deception Bay were collected in 2013 and 2014. Experimental testing of different exchange locations vs control (no exchange) was conducted on 3 voyages of the domestic vessel MV Arctic in 2015. Sample analyses are underway and further sampling/experimental work is planned for 2015.

Sea chests as a potential vector for aquatic invasive species along Canadian coasts

Maurice Lamontagne Institute, Fisheries and Oceans Canada, Mont-Joli, Quebec, Canada
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Sea-chests, recesses built into the hull of a vessel, have been recently identified as hotspots for fouling organisms. In this study, we examined the types and abundances of taxa found in sea-chests of commercial vessels, and investigated whether vessel specifications and voyage histories influenced the nature and extent of sea-chest fouling. Eighty-two sea-chests were sampled from 39 commercial vessels while in dry dock on the West or East Coast of Canada. Overall, 80% of the vessels showed evidence of sea-chest fouling, and 46% harboured at least one non-indigenous species. In total, 299 unique taxa were recorded, including a number of non-indigenous and cryptogenic organisms that collectively made up 20.5% and 14.4% of the taxa sampled from West and East Coast vessels, respectively. Additional results suggested that in-service period (i.e., duration since last sea-chest cleaning) and vessel origin (i.e., domestic versus international) may, in part, determine the nature and extent of sea-chest fouling. By contrast, vessel size and port duration were unable to explain taxonomic richness or abundance of fouling organisms in sea-chests. Taken together, these findings highlight the role of sea-chests as an important vector responsible for the introduction and spread of a variety of taxa, including aquatic invasive species, but also suggest that the factors that influence sea-chest fouling in commercial vessels are complex. Further research, aimed at better understanding the determinants of sea-chest fouling and the efficacy of anti-fouling systems, would help further refine management strategies and reduce the risks associated with sea-chest fouling. Results of this study have been published (Frey *et al.* 2014).

B VECTOR MANAGEMENT

Examination of ballast water exchange plus treatment to achieve enhanced protection of low salinity waters

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The most effective way to manage nonindigenous species (NIS) is to prevent their introduction via vector regulation. Soon, international ships will be required to meet numeric ballast discharge standards using ballast water treatment (BWT) systems and ballast water exchange (BWE), currently required by several countries, will be phased out. However, there are concerns that BWT systems may not function reliably in fresh and/or turbid water. A land-based evaluation of simulated 'BWE plus BWT' versus 'BWT alone' demonstrated potential benefits of combining BWE with BWT for protection of freshwater ecosystems (Briski *et al.* 2013). We conducted ship-based testing to compare the efficacy of 'BWE plus BWT' versus 'BWT alone' on voyages starting with freshwater ballast in partnership with Stephan Gollasch (Germany) and Matej David (Slovenia) in 2013 and 2014. We tested the hypotheses that there is an additional effect of 'BWE plus BWT' compared to 'BWT alone' on the reduction of plankton, and that taxa remaining after 'BWE plus BWT' will be marine (low risk for establishment at freshwater recipient ports). Our study found that BWE has significant additional effect on the reduction of plankton, and this effect increases with initial abundance. As per expectations, 'BWT alone' tanks contained higher risk freshwater or euryhaline taxa at discharge, while 'BWE plus BWT' tanks contained mostly lower risk marine taxa unlikely to survive in recipient freshwater ecosystems. Results have been submitted for peer review in a scientific journal.

Examination of operational efficacy and environmental safety of ballast water management systems in cold waters

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The use of Ballast Water Management Systems (BWMS) on board ships will be required soon to meet national and international standards aimed to reduce the risk of ballast-mediated invasions. Type approval testing of BWMSs is typically conducted at summer temperatures when plankton density is highest, despite the fact that ships operate globally at all times of the year. Extreme temperatures, such as those encountered in winter in the Great Lakes or the Arctic, could impact treatment efficacy through changes in biological metabolic rates or chemical reaction rates. To better understand the effect of temperature on ballast water treatment processes, we are conducting chemical (chlorine) and physical (UV-C) tests to examine efficacy at different temperatures. Organisms from two size classes (>10 to <50µm and >50µm) are being studied using epifluorescence, culture techniques and microscopy. This work is being conducted in partnership with the

IMARES Research Institute of the Wageningen University & Research Centre (Netherlands), and will be published in the scientific literature in due course.

Evaluating efficacy of a ballast water filtration system for reducing spread of aquatic species in freshwater ecosystems

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Numerous commercial ballast water treatment systems have been, or are being, developed to prevent future aquatic invasions. However, most treatment systems are being designed for the many vessels undertaking long transoceanic voyages in marine waters rather than the relatively few vessels operating on short voyages in freshwater, such as those in the Laurentian Great Lakes. We conducted testing of the biological efficacy of a 40 μm ballast water filtration unit through shipboard trials. We tested the hypotheses that i) filtration will significantly reduce abundance of zooplankton greater than 50 μm in size but not phytoplankton 10 to 50 μm in size; ii) filtration will reduce zooplankton abundances in ballast water below International Maritime Organization discharge standards, but not those of phytoplankton; and iii) filtration will alter the community composition of zooplankton, non-randomly reducing invasion risk of larger taxa. Ballast water samples were collected using a before-after experimental design. Our study showed that filtration significantly reduced abundance of copepods and cladocerans, but not of juvenile dreissenid veligers and rotifers. Contrary to our expectation, phytoplankton densities were also significantly lower after the treatment. Filtration altered relative abundance of zooplankton, but did not reduce introduction risk of any taxonomic group due to the small juvenile stages and dormant eggs which passed through the treatment. While we do not rule out filtration as a ballast water treatment option for zooplankton in the future, our tests indicate further development is required for meaningful reduction of invasion risk. Results were published in 2014 (Briski *et al.* 2014).

C METHODS FOR SAMPLING AND ANALYSIS

Evaluating the response of freshwater organisms to vital staining

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Proposed international regulations for ballast water management will require enumeration of viable plankton in ballast water. In this study, the efficacy of vital stains fluorescein diacetate (FDA) and FDA+5-chloromethylfluorescein diacetate (CMFDA) was evaluated with freshwater macroinvertebrates, zooplankton, and phytoplankton. Macroinvertebrates were cultured in laboratory, while plankton were collected from Hamilton Harbour and ballast tanks of commercial vessels. Organisms were subjected to various treatments (i.e., heat, NaClO, and NaOH) to establish efficacy of stains for viable and non-viable organisms. No significant difference in accuracy rate was found between stains, regardless of treatment, within groups of organisms, indicating that the addition

of CMFDA is superfluous in the sample region studied. False positive errors, in which dead organisms fluoresced similarly to live organisms, occurred in most groups and were significantly different between test groups. The FDA/FDA+CMFDA vital staining methods provide useful tools for viability analysis of freshwater phytoplankton, soft-bodied invertebrates and zooplankton, and may be used for viability analysis of the ≥ 10 μm to < 50 μm size fraction in compliance testing of ballast water. However, viability analysis of larger freshwater crustaceans with vital stains should be undertaken with caution. Results were published in 2014 (Adams *et al.* 2014).

D RISK ASSESSMENT

NSERC Canadian Aquatic Invasive Species Network II

Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario

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The national network consisting of some of the world's leading researchers, explorers and innovators in the field of aquatic invasive species has received funding for an additional five years (2011-2016). A coordinated set of comprehensive studies are planned, directed at four research themes pertaining to Aquatic Invasive Species (AIS): i) early detection strategies; ii) rapid response strategies; iii) AIS as part of multiple stressors affecting aquatic ecosystems; and iv) reducing uncertainty in prediction and management. Selected projects under each theme are meant to further understanding of the AIS issue, with particular focus on early detection and rapid response. What's more, CAISN will be conducting its first series of research efforts in the Arctic, where increased shipping, due in part to climate change, has put it at greater risk than ever before for aquatic invasion. It is also worthwhile to note that numerous publications resulting from the shipping vector research conducted during CAISN I are now available. See www.caisn.ca for more information on CAISN II research projects and links to publications from CAISN I.

Assessment of ecological risks for ballast water exchange in the Canadian Arctic

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Transport Canada Marine Safety (TCMS) requested scientifically defensible advice as the basis for national ballast water regulations regarding Alternate Ballast Water Exchange Zones (ABWEZs) for ships in ballast destined for ports in waters of the Canadian Arctic. Under current regulations, ABWEZs have been designated in the Hudson Strait and Lancaster Sound regions of the eastern Canadian Arctic for foreign vessels travelling to the Port of Churchill or the Northwest Passage, respectively, in the event that foreign vessels bound for Arctic ports need to conduct emergency ballast water exchange within the Canadian Exclusive Economic Zone (EEZ). In this study, ecological risks of Aquatic Invasive Species introduction were assessed for the Eastern Arctic where designated zones cur-

rently exist (Stewart and Howland 2009; http://www.dfo-mpo.gc.ca/CSAS/Csas/Publications/ResDocs-DocRech/2009/2009_008_e.pdf. The Beaufort Sea was also evaluated to determine if there were any suitable locations for ABWE (Fissel *et al.* 2013; http://www.dfo-mpo.gc.ca/Csas-sccs/publications/resdocs-docrech/2012/2012_149-eng.pdf . Recent assessments included oceanographic modeling of particle dispersion along major ship tracks in relation to climate and depth, together with the identification of areas of ecological, economic and/or cultural significance; associated publications are currently in press (DFO 2015) or in review (Stewart *et al.* 2015) and expected to be available online (http://www.dfo-mpo.gc.ca/csas-sccs/index-eng.htm) within the next 6 months.

Climate related changes in marine invertebrate communities and Aquatic Invasive Species (AIS) risk in the north

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The objectives of this study are to improve the existing baseline information on coastal marine invertebrate communities and to spatially model future climate change-related invasion risk using hydro-climatic variables in the Canadian Arctic. More specifically we are: 1) Developing an inventory of existing biota and environmental conditions in areas of the Arctic with the highest risk for introduction by non-indigenous species (high use ports); 2) Comparing species lists from current biodiversity studies in high risk port areas with historical survey information in order to identify new species and to evaluate if new records are most likely to represent introductions, range expansions or increased survey effort in a given location; 3) Assessing the relative risks for future AIS incursions across the Canadian Arctic; 4) Predicting species-specific potential spatial distributions (habitat matches) and assessing the probability of establishment for a subset of higher risk AIS under various climate changes scenarios using ecological niche modelling based on known environmental ranges for these species. A publication related to objectives 1 and 2 was completed in 2014 and is available online: http://www.aquaticinvasions.net/2014/issue3.html. A draft manuscript related to objectives 3 and 4 has been completed and is expected to be published during 2015.

An investigation of the risk posed by marine recreational boating as a vector in the introduction and spread of aquatic invasive species in Canada

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Science advice was requested to assess the risk of recreational boating as a vector for the introduction and spread of Aquatic Invasive Species (AIS) in Canada. A recreational boating survey project (2012–2014) in Atlantic Canada was recently completed (McKenzie *et al.*2014). Using this data along with data from the Pacific region, a national marine recreational boating risk assessment will be conducted. The Marine Recreational Boating Assessment is comprised of four steps which will provide an overall vector risk assessment. The first step is an assessment of potential risk which will involve a statistical comparison of the various boat types and their maintenance (e.g. storage method, use of antifouling paint, etc.) in relationship to the biofouling observed on the vessel hulls. The next step is an assessment of the movement patterns of the vessels within and between regions. The third step is the regional AIS background of the harbours and marinas. This information will be provided by the DFO AIS monitoring program using biofouling plate data from 2009 to 2014. Step four or the realized risk per boat type and region will be determined using vessels, movement and biofouling then demonstrated using a risk assessment heat matrix. This risk assessment is scheduled for completion by November 2015.

E IMPACTS OF INTRODUCED SPECIES

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

New to Arctic Canada (cryptogenic origin; for details see Goldsmit et. al 2014):

1) Aricidea cf. hartmani, Polychaete, found in Churchill, MB and Deception Bay, QC

2)Dipolydora socialis group, Polychaete, found in Deception Bay, QC

3) Lumbrineris cf. zatsepini, Polychaete, found in Deception Bay, QC

4) Owenia borealis, Polychaete, found in Iqaluit, NU

5) Paraonides Nordica, Polychaete, found in Churchill, MB and Deception Bay, QC, and Iqaluit, NU

6) Onisimus sextoni Group, Crustacean, found in Deception Bay, QC

7) Heterostigma sp., Ascidian, found in Deception Bay, QC

G OTHER RELEVANT INFORMATION

6th GEF-UNDP-IMO GloBallast R&D Forum and Exhibition on ballast water management will be held in Canada from 14 to 16 October 2015.

H REFERENCES

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Estonia

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SUMMARY

The specifically dedicated and governmentally funded non-indigenous species monitoring program was continued in 2014. Port biological monitoring (Muuga Harbour, Port of Tallinn) according to HELCOM protocol was added to the programme in 2014. The monitoring included recording of key environmental conditions (incl. CTD profiles) and sampling of phytoplankton, zooplankton, benthic infauna, fouling communities and mobile epifauna (incl. fish). One of the sub-components is to monitor high risk areas of primary invasions. In this purpose, vicinity areas of the two largest ports - Port of Tallinn and Port of Sillamäe (Gulf of Finland), were sampled. In addition, surveys in the long-term dynamics of selected key alien species were continued and the ecological impact of several NIS summarised.

FULL REPORT

A TRANSPORT VECTORS

PhD project by L. Rostin on 'Changes in benthic littoral vegetation as a reflection of shifting Baltic environment caused by climatic shifts and human influence'. It also involves research on biofouling of artificial substrata such as windmill pillars.

B VECTOR MANAGEMENT

C METHODS FOR SAMPLING AND ANALYSIS

D RISK ASSESSMENT

The specifically dedicated and governmentally funded alien species monitoring program, started in 2010, was continued in 2014. The alien species monitoring consists of three major sub-components: 1) monitoring of high risk areas of primary invasions; 2) tracking long-term performance of selected most important alien species and 3) evaluation of ecological and socioeconomic impacts caused by alien species. Monitoring of high risk areas of primary invasions – vicinity of ports – has been conducted in Port of Tallinn (since 2010) and Port of Sillamäe (since 2012), both located in the Gulf of Finland. Several stations were sampled in port vicinity and also in more distant localities called also as reference sites. Importantly, all data and annual reports are freely available, though unfortunately written in Estonian only (Anon. 2015).

Port biological monitoring (Muuga Harbour, Port of Tallinn) according to HELCOM protocol was added to the programme in 2014 with sampling being carried out in spring,

summer and autumn. The monitoring included recording of key environmental conditions (incl. CTD profiles) and sampling of phytoplankton, zooplankton, benthic infauna, fouling communities and mobile epifauna.

E IMPACTS OF INTRODUCED SPECIES

Ecological impact of NIS is being summarised and documented for the alien species monitoring purposes. The report includes information for the following NIS: the cirriped *Balanus improvisus,* the gibel carp *Carassius gibelio*, cladocerans *Cercopagis pengoi* and *Evadne anonyx*, the zebra mussel *Dreissena polymorpha*, the Chinese mitten crab *Eriocheir sinensis*, the amphipod *Gammarus tigrinus*, the polychaete *Marenzelleria neglecta*, the mud crab *Rhithropanopeus harrisii* and the round goby *Neogobius melanostomus*. The most impacting NIS in the Estonian coastal sea (due to wide-scale and very abundant presence) are *M. neglecta* and *N. melanostomus* However, *R. harrisii* and *G. tigrinus* can be locally very abundant, and therefore their ecological impacts might be substantial, but restricted to a few localities currently (Anon. 2015; Nurkse *et al.* 2105). No economic impacts evaluations were performed.

In the pan-Baltic scale, information on the ecological impacts of NIS is relatively limited (no information is available for about 1/3 of the most widespread NIS) with the benthic invertebrates *Marenzelleria* spp. and *Dreissena poymorpha* having the highest impact (Ojaveer and Kotta 2015).

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

An as-yet-undescribed, non-indigenous, polychaete species was found at very high densities in the north-eastern Gulf of Riga, Pärnu Bay in 2012. The species belongs to the sabellid genus *Laonome* Malmgren, 1866, but it could not be assigned to any of the previously described species. To date, the species has established a stable population after surviving a notably cold winter (2012/2013). The abundance of *Laonome* sp. exhibited strong seasonal variation, peaking between July and November. Besides seasonality, the quantity of decomposed microalgae in the sediment and wave exposure best explained the variation in abundance. This non-indigenous polychaete may potentially modify sediment morphology and chemistry and disrupt the natural infaunal communities. *Laonome* sp. could displace or even completely eliminate some species currently present in the study area and beyond if it spreads; however, it could also facilitate currently-present species through the provision of alternative substrate and/or food (Kotta *et al.*, 2015).

G OTHER RELEVANT INFORMATION

The IMO's BWMC is still not yet ratified.

H REFERENCES

Anon 2015. Operational monitoring of Estonian coastal sea. Estonian Marine Institute, University of Tartu. Final report, Tallinn.

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Finland

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SUMMARY

No projects are ongoing related to the shipping vector or risk assessments at present but several project proposals have been submitted. A national group to discuss the implementation of the BWMC in Finland has been founded. It is led by the Finnish Trafic and Safety Agency, and the Finnish Environment Institute, Ministry of Environment, Ministry of Traffic and Communication and The Finnish shipowners' association take actively part to the meetings and discussions. The ratification of the IMO's BWM Convention by Finland was again delayed and will take place in autumn 2015. No new alien species was found in Finnish waters in 2014.

FULL LIST OF PROJECTS

A TRANSPORT VECTORS

B VECTOR MANAGEMENT

C METHODS FOR SAMPLING AND ANALYSIS

D RISK ASSESSMENT

E IMPACTS OF INTRODUCED SPECIES

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

No new records of ship-mediated species in Finland 2014.

G OTHER RELEVANT INFORMATION

An ad hoc national group to discuss the implementation of the BWMC in Finland has been founded. It is led by the Finnish Trafic and Safety Agency, and the Finnish Environment Institute, Ministry of Environment, Ministry of Traffic and Communication and The Finnish shipowners' association take actively part to the meetings and discussions.

H REFERENCES

France

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With help from Amelia Curd & P. Goulletquer.

SUMMARY

French ministry of Environment, Sea and Transport elaborated a guide for Ballast Water Convention exemptions demands, including risk analysis, available at the entry in force of the Convention.

The impact of bio-fouling as a vector of introduced non indigenous species can be important, considering the huge number of pleasure boats visiting the marinas along French coasts.

The French oceanographic fleet will be equipped with ballast water treatment systems. A study is on its way.

FULL LIST OF PROJECTS

A TRANSPORT VECTORS

The WMU project (Malmö World Maritime University) on Arctic navigation risk assessment has had a limited cooperation in 2014. Contact with the Murmansk biological station which would have been directly involved in the project has stopped.

B VECTOR MANAGEMENT

C METHODS FOR SAMPLING AND ANALYSIS

D RISK ASSESSMENT

The guide initiated by the Ministry of Ecology, Sea and Transport about Ballast convention Exemptions has been completed and is presently in the final review stage by the Ministry's departments (Title: "Guide pour la réalisation des évaluations des risques d'invasion d'espèces non indigènes par les eaux de ballast"; guide for non-indigenous species ballast water-introduced risk assessment)

This report provide guidelines to ship owners to prepare a full implementation of the IMO convention on ballast water. Moreover, it provides informations sources regarding the Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD). It aims to avoid the discrepancies among demands by setting a minimum list of mandatory standards. This work anticipates the entry into force of the Ballast Management Convention as a main objective. It can be considered as the last step before the full implementation of the Ballast Water Management Convention - a set of national regulations will be necessary to apply this convention.

Largely based on North Sea Ballast Water Initiative work (obviously cited) for international standardisation reasons, the same risk assessment protocol is used. This approach has been adapted to the French context considering the MSFD ecoregions and based upon data from both directives (environmental conditions & list of exotic species). The list of non indigenous species known in the French coastal waters is presented for Mediterranean Sea, Bay of Biscay and Channel-North Sea. Different sampling protocols (e.g., coastal waters, ports, ship's ballasts) to be used for data collection (data necessary for the ship owners requesting exemption) are presented.

E IMPACTS OF INTRODUCED SPECIES

During the "Sea Tech Week" international conference in Brest (France) (Oct 2014), a specific session on biofouling was organized. A presentation on the introduction vectors of invasive species in France was carried out including biofouling vectors. An interesting point was the biofouling of recreational boats and the relative importance of marinas in France: the assessment provided various figures including 370 marinas, listing more than 226 000 moorings. Recreational boating concerns 4 million people. More than 980 000 boats are spending days on those marinas on a yearly basis. This is a considerable vector of non indigenous species introduction. It should be emphasized than less than 1/5th of the boats are dry-docked during the winter, meaning that most of the boats are permanently floating, representing a suitable habitat for biofouling. A concomitant research project in the English Channel (Bishop *et al.* 2015) has demonstrated that each sailing boat hosts 2 to 3 exotic species, concluding that this vector is presently well underestimated, as well as the Ferries vectors (biofouling & ballasts). The study by Bishop *et al.* (2015) was carried out as part of the Marinexus project (http://www.marinexus.org; leader, M. Cock, Station Biologique de Roscoff) an interreg IV A project launched at the beginning of January 2010. It demonstrated a high prevalence of non-indigenous species in marinas of the Western English Channel with very similar lists of NIS in Brittany and Devon-Cornwall.

Moreover, several side effects of exotics were illustrated: *Crepidula fornicata* (e.g.), a food competitor to cultured shellfish, represents a 70 000 ton stock biomass in the Marennes Oleron Bay. In this area, management options remove 1500–2000 tons a year to limit the population and resulting side effects: by way of example, 1 ton of *Crepidula* eats the same quantity of phytoplankton than 400kg oysters. In the Gulf of Saint Malo, this species has reached more than 200 000 metric tons of biomass.

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

Two non-indigenous hydrozoans, Lovenella assimilis (Browne, 1905) and Eucheilota menoni (Kramp 1959), have had their presence in the Eastern part of the English Channel and North Sea confirmed since 2007 (Brylinski *et al.*, 2015). Their taxonomy is under review, and their vectors of introduction have been identified as ballast water and ship fouling.

G OTHER RELEVANT INFORMATION

Most of the French oceanographic fleet (10 research vessels) is managed by IFREMER-GENAVIR. After the full entry into force of Ballast Water Convention, those ships will be obliged to treat their ballast waters, volumes, depending on the ship size, ranging from 50m^3 to 1500 m^3. As existing ships, the problem is rather complex: those ships have been built with optimization without any space for a treatment system. The challenge is now to equip them in the years to come at a reasonable cost. An on-going study will address this issue.

H REFERENCES

Bishop, J.D.D., Wood, C.W., Lévêque, L., Yunnie, A.L.E., & Viard, F. 2015. Repeated rapid assessment surveys reveal contrasting trends in occupancy of marinas by non-indigenous species on opposite sides of the western English Channel." *Marine Pollution Bulletin*: (early-view on line: http://dx.doi.org/10.1016/j.marpolbul.2014.11.043).

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Germany

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SUMMARY

The German Federal Maritime and Hydrographic Agency (BSH) continues approving ballast water management systems (BWMS).

An agreement between BSH and NSF (NSF International, the first Independent Laboratory (IL) designated by the United States Coast Guard (USCG) to evaluate and test ballast water management systems (BWMS) for U.S. type approval) provides manufacturers a more economical and streamlined process for achieving two key BWMS type approvals in the industry. Ballast water management systems (BWMS) manufacturers now have the opportunity to obtain German and U.S. type approvals from a single test.

New projects are ongoing. The *e-CME Ballast Water* project develops an online training course for compliance monitoring and enforcement of ballast water management standards. The next training course is scheduled for mid-May 2015 at the World Maritime University, Malmö, Sweden and may be joined without attendance fee.

During another project fact sheets of non-indigenous species in Germany will be developed.

The German alien species targeted monitoring programmes continue and include sampling stations in ports along the Baltic and North Seas so that this activity fills geographical gaps in the network of German coastal monitoring stations. Results of the rapid assessments indicate that the rate of newly recorded NIS is lower along the German Baltic Sea coasts compared to the North Sea.

A species of concern but not yet known from Germany is *Didemnum vexillum.* It is found in other European countries and it may be possible that this species becomes introduced to German waters with movements of living mussels and aquaculture gear or in the biofouling of vessels.

FULL LIST OF PROJECTS

A TRANSPORT VECTORS

Ballast Water Opportunity

The Interreg IVB funded project Ballast Water Opportunity (BWO) was terminated in May 2014 and it was concluded that its objectives were met and that the North Sea region considerable benefitted from BWO activities. Germany was responsible for WP2 (BSH) and WP4 (GoConsult). One of the last project results, which were only available in Janu-

ary 2015, was a report addressing possible improvements of the IMO test guideline for ballast water management systems (Guidelines G8). This report may be found at the ResearchGate account of Stephan Gollasch:

https://www.researchgate.net/profile/Stephan_Gollasch

Vectors of Change in Oceans and Seas Marine Life, Impact on Economic Sectors (VECTORS)

This project was terminated in January 2015. BOSV may be interested to note a comprehensive project report on BWM related risk assessments and decision support systems authored by Matej David and Stephan Gollasch. For more information visit:

http://www.marine-vectors.eu/

NIS Port-monitoring for MSFD baseline and HELCOM

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In coordination with the other federal states of Germany with a coastline in the Baltic or North Sea, GEOMAR (Kiel) as contractor of the State Agency for Agriculture, Environment and Rural Areas (LLUR) will survey ports and marinas in Schleswig-Holstein for NIS in 2013 and 2014. The main purpose is to find all NIS that already invaded the German coastal waters, which will serve a baseline for an MSFD-indicator that will assess the environmental status by monitoring new invaders (on top of the baseline). A second purpose is a test application of the HELCOM "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". The monitoring will include various methods and biota (scraping, settlement plates, traps, grabs, plankton samples, invertebrates, algae, pathogens, heavy metals and water parameters).

Collect and assessment of non indigenous species in coastal waters of Mecklenburg-Western Pommerania according to MSFD and HELCOM"".

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Aim of the project is to test two methods (rapid assessment, HELCOM protocol 2013), to gain background data for further assessments and to develop monitoring strategies.

Duration: August 2013 to March 2015

Rapid-assessment of non-native species in German Coastal Waters including further development of the trend indicator.

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Naturschutzfachliche Invasivitätsbewertungen für in Deutschland vorkommende gebietsfremde Wirbellose, Pilze und Pflanzen.

The project is ongoing, for Bundesamt für Naturschutz (BfN), Bonn, Germany. The ongoing project is coordinated by Wolfgang Rabitsch, Umweltbundesamt GmbH, Vienna, Austria. The anticipated outcome includes fact sheets of non-indigenous species in Germany.

B VECTOR MANAGEMENT

Certification and performance tests of ballast water management systems

The Federal Maritime and Hydrographic Agency (BSH) is responsible for the type approval of ballast water management systems (BWMS) in Germany. Various BWMS are at different stages in the approval process.

An agreement between BSH and NSF (NSF International, the first Independent Laboratory (IL) designated by the United States Coast Guard (USCG) to evaluate and test ballast water management systems (BWMS) for U.S. type approval) provides manufacturers a more economical and streamlined process for achieving two key BWMS type approvals in the industry. Ballast water management systems (BWMS) manufacturers now have the opportunity to obtain German and U.S. type approvals from a single test

NSF International, the first Independent Laboratory (IL) designated by the United States Coast Guard (USCG) to evaluate and test ballast water management systems (BWMS) for U.S. type approval, can now provide BWMS testing to Germany's Federal Maritime and Hydrographic Agency (Bundesamt für Seeschifffahrt und Hydrographie (BSH)) requirements, which are based on International Maritime Organization (IMO) Guidelines. BWMS manufacturers may now submit for required type approvals to NSF International IL and in parallel to BSH using the same set of test data. This will reduce test costs and approval time and allow ships to navigate to U.S. and international shipping destinations.

USCG type approval of onboard BWMS is required for vessels entering and discharging treated ballast water in U.S waters to prevent the spread of non-native aquatic species in lakes, rivers and coastal waters. Additionally, the national type approvals for Germany offered through BSH meet the IMO requirements for ships throughout the rest of the world.

GoConsult continued to test BWMS on board of commercial vessels according to the IMO G8 guidelines.

Recently GoConsult and Dr. Matej David Consult d.o.o. formed a strategic partnership to conduct on board performance tests of BWMS to assure the high quality and credibility of the shipboard performance test results.

Further, application documents were submitted to become a member in a US Coast Guard approved "Independent Laboratory". The Independent Laboratory consortium developed under the umbrella of the Dutch company Control Union and was launched to meet the US test requirements for BWMS.

GloBal TestNet

A formal group of organizations involved in testing for the certification of BWMS was established as the "GloBal TestNet" to facilitate increased standardization and harmonization of test procedures and information exchange. The group met in December 2014 to contribute to the IMO debate on improving the G8 test guideline of BWMS.

Shipboard tests of ballast water exchange plus treatment

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Elizabeta Briski

As mentioned last year this project is carried out to test the efficacy of ballast water exchange in combination with and without ballast water treatment. Three test voyages were conducted and the results are currently worked out.

C METHODS FOR SAMPLING AND ANALYSIS

Ballast water management for Adriatic Sea protection (BALMAS)

Stephan Gollasch is involved as external expert in the BALMAS project. The project integrates all necessary activities to enable a long-term, environmentally efficient and financially and maritime transport sustainable implementation of ballast water management measures in the Adriatic Sea. BALMAS established a common cross-border system linking all Adriatic research, experts and national responsible authorities in order to avoid the unwanted risks to the environment and humans from the transfer of harmful aquatic organisms and pathogens through the control and management of ships' ballast waters and sediments. Ballast water sampling and organism detection methods for compliance testing were evaluated in laboratory and on board of a commercial vessel.

For more details see: http://www.balmas.eu/ or contact Dr. Matej David at:

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The e-CME project – Compliance, monitoring and enforcement as required by the IMO Ballast Water Management Convention

The aim of the e-CME ballast water project is to ensure that all North Sea Region countries are ready to perform CME in agreement with the BWMC when it enters into force. It will provide education that involves hands-on training as well as e-learning/internet CME modules. The e-learning modules will be freely available from late spring 2015. The training and education will be prepared specifically for Port State Control officers but may also be used by ship crews, Maritime Administrations and Maritime University students. Three key training modules will be prepared:

1) The background module will inform why there is a need for an international Ballast Water Management Convention (BWMC).

2) The ballast water sampling module will explain the need for sampling as required by the BWMC (indicative and detailed sampling).

3) The ballast water sample analysis module will explain how samples need to be analysed according to the BWMC (indicative and detailed sample analysis).

The next training course is planned at the World Maritime University (project coordinator) in May 2015. The course is for free. For more information, please contact Dr. Josefin Madjidian at:
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Update on Sampling for Compliance of the IMO's Ballast Water Management Convention

This training workshop was held in October 2014 at the European Maritime Safety Agency (EMSA), Lisbon, Portugal. The objective was to train port State control officers and other experts on ballast water sampling options (indicative and in detail) and sample analysis (indicative and in detail). Stephan Gollasch and Matej David were invited as high level experts and gave several presentations. In addition a hands-on sampling event was conducted and the sample was processed with the participants for phytoplankton and zooplankton.

D RISK ASSESSMENT

The **German alien species targeted monitoring programmes** as reported last year continue and include sampling stations in ports along the Baltic and North Seas. The samplings are conducted annually between August and October (Buschbaum pers. comm.). However, not all habitats and all species groups are monitored in the same level of detail. Most efforts focus on benthos and to a lesser degree on plankton. Recent monitoring activities in Germany filled geographical gaps in the network of coastal monitoring stations. Results of the rapid assessments indicate that the rate of newly recorded NIS is lower in the Baltic Sea compared to the North Sea.

BALSAM (2013–2015)

BALSAM (Testing new concepts for integrated environmental monitoring of the Baltic Sea) is a Pilot Project for enhancing the capacity of the Baltic Sea member states to develop their monitoring programmes. The project started on 1 October 2013 and will run until the end of May 2015. It consists of 6 Work Packages (WP). Focusing on gaps, BALSAM will provide recommendations for marine monitoring in the Baltic, especially for mammals and seabirds, non-indigenous species and benthic habitats.

WP4: Non-indigenous species: This Work Package is focused on the monitoring of the non-indigenous species occurring in the Baltic. It aims to provide recommendations to harmonize the monitoring and sampling methods for these species to meet the needs of

the MSFD as well as the Ballast Water Management Convention (BWMC) of the International Maritime Organization (IMO).

WP4 experts will also test monitoring methods for alien species distribution by conducting sampling in ports using the Joint HELCOM/OSPAR Guidelines on the granting of exemptions under the International Convention for the Control and Management of Ships' Ballast Water and Sediments, Regulation A-4. This activity started with a workshop in October 2013, where the sampling methodology was introduced to the participants. The testing of sampling and analysis will be carried out during summer and autumn 2014 in Estonia, Latvia and Poland.

The Work Package is led by the HELCOM Secretariat and consists of five partners: Estonian Marine Institute (EMI), University of Gdansk (UG), Federal Maritime and Hydrographic Agency (BSH), Latvian Institute of Aquatic Ecology (LIAE) and the Finnish Environment Institute (SYKE).

BSH contributes in close cooperation with the consultants Brockman Consult and Karin Heyer to migrate the "Decision support tool" to the HELCOM Secretariat and to further develop the criteria for the determination of "target species" for risk analysis for exemptions from ballast water management

E IMPACTS OF INTRODUCED SPECIES

No new information.

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

The planktonic copepod ***Oithona davisae*** was first recorded in Germany in 2008 (Sylt Island near List) and was found since then several times. The species identification was confirmed in 2014. It also seems to be able to reproduce in German waters as copepodite stages, males and females with egg sacks were found in 2014 (Cornils & Wend-Heckmann 2015).

One adult individual of the tropical ***Auxis rochei*** (bullet tuna) was caught 29. July 2014 in the Kiel Fjord as reported by a fisherman.

Rangia cuneata (Bivalvia) was first recorded 2013 in the Kiel Canal near Brunsbüttel (Lackschewitz *et al.* (in prep.)).

Pileolaria berkeleyana (Polychaeta) was first found at Helgoland in 2013 (Lackschewitz *et al.* (in prep.)).

The most up-to-date list of alien species in German coastal waters may be found at www.aquatic-aliens.de

Not yet seen species

One species of concern is *Didemnum vexillum*. This is found in European countries, but not yet known from the German coast. It may be possible that this species becomes introduced to German waters with movements of living mussels and aquaculture gear or in the biofouling of vessels.

G OTHER RELEVANT INFORMATION

The new Regulation of the European Parliament and of the Council on the prevention and management of the introduction and spread of invasive alien species was adopted and published in the Official Journal on 4 November 2014. It will already enter into force on 1 January 2015. The new regulation addresses invasive alien species comprehensively to protect biodiversity and ecosystem services, as well as to minimize and mitigate the human health or economic impacts of these species. The regulation addresses prevention, early warning and rapid response, as well as management. A list of invasive alien species of European Union concern will be drawn up and managed with Member States using risk assessments and scientific evidence.

This Regulation includes wording on biofouling and ballast water:

A1 *(21) A large proportion of invasive alien species are introduced unintentionally into the Union. It is therefore crucial to manage the pathways of unintentional introduction more effectively. Action in this area should be gradual, given the relatively limited experience in this field. Action should include voluntary measures, such as the actions proposed by the International Maritime Organisation's* ***Guidelines for the Control and Management of Ships' Biofouling****, and mandatory measures. Action should build on the experience gained in the Union and in Member States in managing certain pathways, including measures established through the* ***International Convention for the Control and Management of Ships Ballast Water and Sediments*** *adopted in 2004. Accordingly, the Commission should take all appropriate steps to encourage Member States to ratify that Convention.*

A2 The Regulation is available at:
http://ec.europa.eu/environment/nature/invasivealien/index_en.htm

The work in the "**Platform for Information Exchange on Neobiota**" continues. It was established in the framework of the "Federal and Federal States Marine Monitoring Programme" the national body concerning the duties arising from national and international obligations. Involved in the group are representatives from different federal agencies, federal state agencies and research facilities. One of the key activities of this group is to address the Good Environmental Status (GES) for the EU Marine Strategy Frameworks Directive (MSFD).

Development of a Non Indigenous Species Trend Indicator for the MSFD in Germany, OSPAR, HELCOM and beyond

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The Marine Strategy Framework Directive (MSFD; 2008/56/EC) stipulated a set of "Descriptors" to assess the state of the marine environment. The goal of Descriptor 2 states that "Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems". The COMMISSION DECISION of 1 September 2010 on

criteria and methodological standards on good environmental status of marine waters (2010/477/EU) then lead to the development of a NIS Trend Indicator in Germany.

In 2009 a research and development project started to investigate potential introduction pathways, mainly harbors, with a "Rapid Assessment" monitoring program, that targets the macrofauna and -flora. The limitation to a single compound of the marine environment was deemed necessary, because other aspects like plankton or meiofauna are much harder or even impossible to identify to species level. Also, the rapid assessment was identified as the most cost-effective monitoring, with a potential to be established in a large number of European countries. Based on the NIS monitoring, a Trend indicator was developed to measure the rate of the introduction of new species to defined locations.

The indicator works with three parameters: species, inventory and dispersal. Most important is the species parameter, which measures the number of new arrivals to a location in a given time period. This parameter only is assessed in the result of the indicator, and the number is set back to zero at the beginning of the next period. Otherwise, a region with a high number of NIS not reaching GES (Good Environmental Status) would not be able to get any better. The inventory parameter records the whole set of NIS at a location over time and the dispersal parameter the spread of NIS from one location to another. Both give very relevant information for the state of the NIS population, but are not assessed for the indicator result to avoid a dilution of the indicator message.

At the combined Coreset-Cobam meeting in Gothenburg on 01.10.2014 ways for the development of coherent or integrated OSPAR and HELCOM MSFD Indicators were explored. The indicator best suited for a common approach was identified as the NIS Trend indicator. The experts present at the workshop agreed to try to develop a unified indicator concept. As a first step, the German concept was integrated to the OSPAR indicator (lead UK with Paul Stebbing, CEFAS) and the resulting "NIS3 Trends in arrival of new non-indigenous species" was adopted for promotion to "common" indicator in OSPAR regions II, III, and IV at BDC on 03.03.2015.

The next step is the integration of the OSPAR concept to the provisional HELCOM CORESET II Trend indicator, which, if agreed on, should be finished by mid-March and discussed by the relevant HELCOM groups in May. The task managers have been contacted with a proposal in late February. In June 2015 both HELCOM and OSPAR indicators are presented for final adoption at the HOD meetings of the Regional Sea Conventions. Finally, due to its basic and straight-forward concept the NIS Trend Indicators can also be adapted to the other RSCs (a representative of the Barcelona Convention already has expressed an interest during the Gothenburg workshop)and to marine regions outside of the EU.

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The Netherlands

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SUMMARY

The Netherlands hosts three facilities involved in testing of ballast water management systems: MEA-nl (Marine Eco Analytics), IMARES (Institute for Marine Resources & Ecosystem Studies) and NIOZ (Netherlands Institute for Sea Research). The profiles of the latter two were explained in the NL national report 2014, the profile of MEA-nl is given in this report.

The three ballast water test facilities have carried on and developed new initiatives.

IMARES has further completed the building and validating of their test facility. NIOZ, during reconstruction of its harbour test site, joined WMU (Malmö, Sweden) a support project on CME for port-state control.

MEA-nl tested several BWM systems for verification according to the IMO requirements, and conducted on-going research on methodologies of sampling and analysis during land-based and shipboard testing.

The project North Sea Ballast Water Opportunity (NSBWO, Interreg IV B) ended by mid 2014. Until the end it kept up a high level of innovation in science and technology and in advanced BWM policies, while giving rise to several spin-off initiatives, such as the port-based ballast barge project of Damen Shipyards and Groningen Seaports, while the initiative of the in 2012 established MEA-nl also emerged from the project grounds.

The last annual meeting was held April 2014. Several more reports were finalised and one new initiative to identify hotspots of barriers to ratification resulted in an additional report.

The initiative for a port-based ballast water management unit (supported by the Wadden Fund) has resulted in a BWM unit being built by Damen Shipyards, which is now up for verification testing at the MEA-nl land-based test-site.

Species and sediments in ports have been analysed, while the Wadden Sea has been subject to an inventory for species on hard and soft substrates.

FULL LIST OF PROJECTS

A TRANSPORT VECTORS

1. The North Sea Ballast Water Opportunity Project (NSBWO) ended by 30 June, after five and a half year of innovative activities on almost all aspects related to Ballast water management in view of promoting ratification and implementation of the Ballast Water

Management Convention (IMO, 2004). The project has more than fulfilled its initial goals and resulted in several spin-off projects.

During its last year the project achievements were:

Meetings:

- 3st IMarEST Ballast Water Technology Conference, March 2014, London, UK (partner IMarEST)
- Annual Meeting (AM14), 10 and 11April, Texel, Netherlands (Partner NIOZ/ Partner CaTO)

Workshops:

- Achievements Workshop, AM 14, 10-11 April (CaTO)

Publications;

- BWMC Publication, Updated (Partner BSH)
- Ballast Water Book for Children (Partner WMU)
- Towards participatory and transparent implementation of the Ballast Water Management, author Ellen Ninaber (CaTO)
- Press release Achievements of the NSBWO project (2009-2014) (CaTO)
- Information paper to MEPC 68 (MEPC 68/INF.20) Workshops on organism detection technologies, compliance control, monitoring and enforcement and ecotoxicity testing during land-based. Submitted by Denmark, Germany, Netherlands, Norway and United Kingdom. (BSH)

Course development:

- E-learning for CME (WMU)
-
- NSBWO web site (www.northseaballast.eu)
- The NSBWO web site will continue to operate from its present host until the end of 2015; thereafter the material will be hosted by the World Maritime University (Malmö, Sweden).
-

2. Other projects under this heading, not related to the NSBWO project:

A study on sediment analysis of short-sea shipping arrivals in Groningen Seaports has been conducted together with R. van der Meer of MIWB. Dr. M. Veldhuis (m.veldhuis@mea-nl.com) took the lead in this project on behalf of MEA-nl. Results and experiences during a Port Survey according to the protocol of OSPAR/HELCOM performed in Rotterdam Harbour, resulted in amendments to the OSPAR/HELCOM protocol.

Species composition of the hard substrates and soft sediments in the Wadden Sea and its harbours were inventoried. The results will be reported in the near future. Drs. A.A.J. Smolders (Sander.Smolders@vwa.nl) co-ordinated the latter two studies on behalf of NVWA.

B VECTOR MANAGEMENT

The Netherlands hosts three facilities involved in testing of ballast water management systems and components thereof:

MEA-nl (Marine Eco Analytics), IMARES (Institute for Marine Resources & Ecosystem Studies) and NIOZ (Netherlands Institute for Sea Research). The latter two were described in detail in the NL report 2014, below specifics of MEA-nl are given.

1. Test facility for ballast water management systems MEA-nl bv, an independent test and research facility for R&D on transfer of aquatic invasive species, has its own test-barge. The barge, stationed in Den Oever, in the vicinity of both saline and fresh water, can access for testing Lake IJssel (IJsselmeer) for fresh water and the Dutch Wadden Sea for saline water. The barge serves to conduct land-based testing of BWM systems in accordance with the IMO regulations. Apart from land-based activities, MEA-nl has been involved in ship-board testing and a number of R&D activities concerning BWM as well as issues related to compliance monitoring.

MEA-nl is one of the partners in a project of the Dutch "Waddenfonds" called: "Waddenfonds project voor de ontwikkeling en realisatie van een barge voor de behandeling van zeeschepen" (Wadden Fund project to develop and realise a barge for the treatment of sea-going vessels). The other project partners are: Damen, Groningen Seaports, Van Gansewinkel, Wagenborg, Imares and CaTO Marine Ecosystems.

During verification testing of BWM systems according to the IMO requirements, MEA-nl has been conducting on-going research on the methodology of sampling and sample analysis. This was done both during land-based and shipboard test runs, both of which were performed in 2014. The results were presented in the test reports, during meetings and as MEA-nl's contribution to the IMO correspondence group for revision of guideline G8 at the IMO meeting PPR 2. F. Fuhr (f.fuhr@mea-nl.com) took the lead in the G8 revision process.

Apart from verification tests of complete BWM systems, MEA-nl was engaged in testing of components of BWM systems (e.g. UV-reactors) and filters. Results of the tests were communicated with national administrations to facilitate a way forward towards a testing regime for components.

M. Veldhuis (m.veldhuis@mea-nl.com) took the lead in the following studies:

- Study to quantify organisms in residual water and sediment of ballast tanks after discharge, the results of which have been reported to NSF International (USA).
- -Study on applicability of chlorination installations for BWM systems at low salinities, which was conducted at salinities down to 0.5 PSU.
- Support studies for the development of emerging technologies for dredging companies and port reception facilities, which were conducted and or finalized in the reporting period.Study on population dynamics of heterotrophic bacteria in ballast water tanks.

2. Test facility for BWM, IMARES has further completed the building of their test facility and performed validation tests for physical parameters, such as volumes and pumping capacity and rates.

3. Test facility NIOZ participated in a Project e-CME Ballast Water, intended as an electronic (internet) course for control, monitoring and enforcement (CME) for port-state control (PSC), Maritime Administrations, Ship crew and Maritime University students. The project was initiated and led by the World Maritime University (WMU, Malmö, Sweden). The project aims to ensure that all North Sea Region countries are ready to perform CME in agreement with the BWMC once it enters into force. The first of the two editions of the course has been held at NIOZ (Texel, 3-4 March 2015); the second is scheduled at WMU (Malmö, 20-21 May 2015). The project will finalise 22 May 2015.

C METHODS FOR SAMPLING AND ANALYSIS

An ongoing study to develop new methods for compliance control in co-operation with the RUG (Groningen) for zoo-plankton has been initiated (still on-going). F. Fuhr (f.fuhr@mea-nl.com) took the lead on behalf of MEA-nl.

Sampling and analysis studies led by M.Veldhuis:

- A study to improve the analytical methods for phytoplankton has been initiated and is still on-going.
- A study for the development of a standard sampling tool has been initiated and is still on-going.
- A comparative study of different staining methods has been initiated and is still on-going.

D RISK ASSESSMENT

In the course of MEA-nl's standard operations, samples were monitored for changes in water quality and biological composition at Den Oever harbour, in the Wadden Sea and in Lake IJssel (IJsselmeer). The sampling frequency per station varied, since sampling was done irregularly, depending on the test schedule for the BWM systems.

E IMPACTS OF INTRODUCED SPECIES

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

No new records have been released

G OTHER RELEVANT INFORMATION

Meetings:

Annual Meeting 2014 North Sea Ballast Water Opportunity Project (01/01/2009 – 30/06/2014), Texel, the Netherlands, 10-11/04/2014.

IMARES and MEA-nl, both member of the Global Test Net, participated in the 2014 meeting (Plymouth, 9-11 December 2014). For MEA-nl F.Fuhr took the lead in this activity together with E. Brutel de la Rivière (e.brutel@mea-nl.com) and C. ten Hallers (cato@catomarine.eu); For IMARES K. Kaag (klaas.kaag@wur.nl) took the lead. MEA-nl and CaTO also participated in the relevant IMO meetings (MEPC 66 and 67 and PPR 1 and 2).

Papers presented at relevant meetings:

International Conference on advanced technologies of BW and biofouling (Chennai-India,; 4-7 March 2014):

- F. Fuhr, M. Veldhuis, E. Brutel de la Rivière, I. van der Star; Challenges and solutions from certification testing and their implications for the practical implementation of ballast water management.

IMarEST Ballast Water Panel at Oceanology International (London, 13 March 2014).

- C.C. ten Hallers-Tjabbes, A. Radford, B.Mackenzie, S. Simpson, Emerging non-chemical ballast water systems and environmental acceptability

WGBOSV 2014 (Palanga, 17-19 March).

- C.C. ten Hallers-Tjabbes, A. Radford, B.Mackenzie, S. Simpson, Ballast water management by acoustic means; notes on environmental acceptability

3rd Ballast Water Technology Conference IMarEST; (London, 27-28 March 2014):

- I. van der Star, M. Veldhuis, F. Fuhr, E. Brutel de la Rivière, C. Ten Hallers-Tjabbes; Inter comparison of viability methods (i.vanderstar@mea-nl.com).
- C.C. ten Hallers-Tjabbes, A. Radford, B.Mackenzie, S. Simpson, Ballast water management by acoustic means; notes on environmental acceptability.

Workshop Quick tests for ballast water quality, BBE (Kiel, 02 June 2014):

- M. Veldhuis; Comparison of different fluorometric methods for ballast water tests.

SOWOS (Hamburg, 08 November 2014):

- M. Veldhuis; Ballast Water Management Convention- What is the ultimate goal and how to reach it.

BWMTech (London, 08-09 December 2014).

- M. Veldhuis; Overview of 10 years testing BWMS; what have we learnt so far.

WGBOSV 2014 (Palanga, 17-19 March).

- C.C. ten Hallers-Tjabbes, A. Radford, B.Mackenzie, S. Simpson, Ballast water management by acoustic means; notes on environmental acceptability
- C.C. ten Hallers-Tjabbes, A. Radford, B.Mackenzie, S. Simpson, Ballast water management by acoustic means; notes on environmental acceptability

IMarEST Ballast Water Panel at Oceanology International (London, 13 March 2014).

- C.C. ten Hallers-Tjabbes, A. Radford, B.Mackenzie, S. Simpson, Emerging non-chemical ballast water systems and environmental acceptability.

NSBWO Annual Meeting 2014 (Texel, 10-11 April)

- J. Boon, S. Kaçan, L. Peperzak, J. Madjidian, C. ten Hallers: Project presentations - highlights of five of the six Work Packages.
- Press message: Achievements of five and a half year NSBWO; Outcome of the Achievements workshop.
- E. Ninaber: Study Hotspots of resistance to ratification of the BWM Convention.

NSBWO-Network at MEPC 67 (London, October 2014)

- E. Ninaber: Towards a participatory and transparent implementation of the Ballast Water Management Convention
- C.C. ten Hallers-Tjabbes: Experiences from Port's project to meet challenges in BWM by m

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Norway

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SUMMARY

Toxiciy tests of DBP (Disinfection ByProducts) by different BWMS; Alternative Analysis Methods for 10-50 μm organisms after UV treatment; Water quality effects on G8/G9 testing; Evaluation of Culture- vs staining methods for the determination of living 10-50 10-50μm organisms; Realtime monitoring ballastwater with flowcytometry for the determination of living 10-50μm organisms ;Risk assessment of ballast water discharge from vessels under construction transported from Turkey to Norway without any treatment system/power supply onboard (NIVA, Stephanie Delacroix & cooperatining partners.)

Sampling methods experiences from both land based and shipboard testing projects according to IMO and USCG requirements; NIVA participates to the annual Global TestNet meetings (GloBallast/IMO) for harmonization of test facilities testing procedures since 2010.

NIVA participates to the MPN-UV Group (USCG-EPA/UV technologies supplier) for validation of the MPN (culture) method for determination of the living 10-50um organisms according to USCG requirements as additional method to the FDA/CMDA method recommended in ETV protocol by EPA.

Norway delegation (incl. NIVA) participated to the IMO Correspondence Group to propose changes of the G8 guidelines to the MEPC 68 according to the specifications listed in MEPC 67. 2014-2015.

Risk assessment of ballast water discharge from vessels under construction transported from Turkey to Norway without any treatment system/power supply onboard, NIVA/DNV-GL, Stephanie Delacroix (2012-2014)

Risk assessment of the ballast water discharge by fish transport vessels in Norway according to IMO Ballast Water Convention. DNV-GL/Norwegian Maritime Directorate (2012); Risk assessment of the ballast water as fish pathogens transport vector in Norway. Norwegian Veterinary Institute/FHL (2015); The Norwegian Maritime Authority commissioned and received a consultancy report on the costs and practical effects on imple-

menting various ballast water treatment schemes for Norwegian vessels. (This report is currently only available in Norwegian)

No new alien species with suspected ship vectors to report for 2014.

A TRANSPORT VECTORS

Biology of Ballast Water

(NIVA's research projects in 2014-2015):

Toxicity effect of Disinfection By-Products (DBP) cocktail discharged into seawater by different BWMS using active substances, NIVA, Stephanie Delacroix (2014).

Alternative analysis method for the 10-50µm organism group after UV treatment, NIVA, Stephanie Delacroix (2015).

Water quality effect on G8/G9 treatment systems biological efficiency, NIVA, Stephanie Delacroix (2014-2016).

Evaluation of the culture methods vs. staining methods for the determination of living 10-50µm organisms, NIVA, August Tobiesen (2014-2016).

Realtime monitoring ballastwater with flowcytometry for the determination of living 10-50µm organisms, HSH-NIVA (2012-2014) (http://www.ballastflow.com/)

Risk assessment of ballast water discharge from vessels under construction transported from Turkey to Norway without any treatment system/power supply onboard, NIVA, Stephanie Delacroix (2012-2014)

Compilation of available commercial test kits for compliance testing according to Vessel General Permit (VGP) 2013 and testing of different indicative analysis methods under development for compliance testing according to IMO requirements (Port State Control). NIVA, Stephanie Delacroix, 2014-2016.

B VECTOR MANAGEMENT

Ballast Water Treatment

(NIVA's Ballast Water Testing Facility):

Approved by DNV-GL for both land-based testing and shipboard testing according to IMO and USCG requirements in freshwater, brackish water and seawater. Land-based testing of 12 different BWMS and shipboard testing of 6 different BWMS according to IMO requirements since 2005. Some research/learned experiences related to pilotscale and labscale land based for testing of different BWMS, NIVA, Stephanie Delacroix, several months each project, type approval final report for approval. (Customer's property results) http://www.ballasttech-niva.no/

C METHODS FOR SAMPLING AND ANALYSIS

Ballast Water Sampling

Sampling methods experiences from both land based and shipboard testing projects according to IMO and USCG requirements, NIVA

Testing of SGS sampling facility prototype at NIVA's test facility during ongoing land-based testing of BWMS. NIVA, Stephanie Delacroix (2012)

D RISK ASSESSMENT

Risk assessment of ballast water discharge from vessels under construction transported from Turkey to Norway without any treatment system/power supply onboard, NIVA/DNV-GL, Stephanie Delacroix (2012-2014)

Risk assessment of the ballast water discharge by fish transport vessels in Norway according to IMO Ballast Water Convention. DNV-GL/Norwegian Maritime Directorate (2012).

Risk assessment of the ballast water as fish pathogens transport vector in Norway. Norwegian Veterinary Institute/FHL (2015).

While not a risk assessment as such, The Norwegian Maritime Authority commissioned and received a consultancy report on the costs and practical effects on implementing various ballast water treatment schemes for Norwegian vessels. (This report is currently only available in Norwegian)

Rapid Coastal Surveys, method testing/comparisons in S. Norway. P.I. V. Husa, I.M.R. (Reports not ready).

E IMPACT OF INTRODUCED SPECIES

No studies to report

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

No report of new non-indigenous species in Norway in 2014.

- Little dedicated NIS fieldwork for target –and doorstep species in 2014
- Rebounce of dense *M. leidyi* swarms along S. Norwegian coasts during summer/autumn 2014 (Low numbers the previous years)

G OTHER RELEVANT INFORMATION

Ballast Water Legislation/Regulations

NIVA participates to the annual Global TestNet meetings (GloBallast/IMO) for harmonization of test facilities testing procedures since 2010.

NIVA participates to the MPN-UV Group (USCG-EPA/UV technologies supplier) for validation of the MPN (culture) method for determination of the living 10-50um organisms according to USCG requirements as additional method to the FDA/CMDA method recommended in ETV protocol by EPA.

Norway delegation (incl. NIVA) participated to the IMO Correspondence Group to propose changes of the G8 guidelines to the MEPC 68 according to the specifications listed in MEPC 67. 2014-2015.

Norway delegation (incl. NIVA) participated to the IMO Correspondence Group to finalize the development of a BWM circular on ballast water sampling and analysis for Port State Control. 2012

Fewer than expected transits over Northern Sea Route (NE Atlantic-Siberia-Pacific, or vice versa) in 2014: 31 full transits (2014), compared to 72 (2013).

H REFERENCES

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Ware, C. Berge, J., Jelmert, A., Olsen S.M., Pellissier, L.,Wisz, M., Kriticos, D., Georgy Semenov G. and Alsos, I.G.

Marine invasive species threats to a warming Arctic (Submittet applied Ecology. Title & MS under revision).

Spain

Submitted By:

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SUMMARY

A study, led by Dr. Eva García-Vázquez (University of Oviedo, Spain) and Dr. Anastasija Zaiko (Klaipeda University, Lithuania) addressed the applicability of metabarcoding methodology for the biosecurity surveillance, and particularly the detection of organisms in ships' ballast waters (Zaiko *et al*., 2015). Opportunities and limitations of the molecular approach were identified from taxonomical datasets rendered by two molecular markers of different degree of universality. The cost-efficacy and possible improvements were discussed for the further successful development and implementation of the approach in ballast water control and NIS surveillance. Apart from this study, no projects specific to shipping as vector of species translocation exist at present in Spain, even though the interest is growing and proposals have been submitted.

Three new ship-mediated species are reported for Spain: i) the marine microalgae *Fibrocapsa japonica* Toriumi & Takano (Raphidophyceae), which was reported offshore in the Eastern Alboran Sea for the first time in autumn of 2006 (Fani *et al.*2014); ii) the marine nemertean *Cephalothrix cf. simula* AM-2013, which was reported for the first time in several locations along the Spanish coasts (i.e. in the North Atlantic coast of Spain in Galicia, Asturias and Cantabria, and in the Mediterranean coast in Catalonia) in a recent survey of nemertean diversity along the Iberian Peninsula coasts (Fernández-Álvarez and Machordom, 2013); and iii) the caprellid amphipod *Caprella mutica* Schurin, 1935, first reported in October 2012 in Illa d'Arousa (42.56135º N 8.95594º W) and then during 2012 and 2013 in other sites of Ria d'Arousa, Galicia, NW Spain, Atlantic coast (Almón *et al*., 2014).

FULL LIST OF PROJECTS:

A TRANSPORT VECTORS

B VECTOR MANAGEMENT

Zaiko, A., Martinez, J.L., Schmidt-Petersen, J., Ribicic, D., Samuiloviene, A., Garcia-Vazquez, E. Metabarcoding approach for the ballast water surveillance – An advantageous solution or an awkward challenge? Mar. Pollut. Bull. (2015), http://dx.doi.org/10.1016/j.marpolbul.2015.01.008

Abstract:

Transfer of organisms with ships' ballast water is recognized as a major pathway of non-indigenous species introduction and addressed in a few recent legislative initiatives. Among other they imply scientific and technical research and monitoring to be conduct-

ed in an efficient and reliable way. The recent development of DNA barcoding and metabarcoding technologies opens new opportunities for biodiversity and biosecurity surveillance. In the current study, the performance of metabarcoding approach was assessed in comparison to the conventional (visual) observations, during the en-route experimental ballast water survey. Opportunities and limitations of the molecular method were identified from taxonomical datasets rendered by two molecular markers of different degree of universality – the universal cytochrome oxydase sub-unit I gene and a fragment of RuBisCO gene. The cost-efficacy and possible improvements of these methods are discussed for the further successful development and implementation of the approach in ballast water control and NIS surveillance.

This study was supported mainly from the Campus of Excellence of the University of Oviedo, with additional funds from the Spanish National Project MINECO CGL2013-42415-R. This is a contribution from the Marine Observatory of Asturias. The contribution from the Klaipeda University was supported by DEVOTES (DEVelopment Of innovative Tools for understanding marine biodiversity and assessing Good Environmental Status) project funded by the European Union under the 7th Framework Programme, 'The Ocean of Tomorrow' Theme (Grant Agreement No. 308392), www.devotes-project.eu.

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C METHODS FOR SAMPLING AND ANALYSIS

D RISK ASSESSMENT

E IMPACTS OF INTRODUCED SPECIES

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

1) *Fibrocapsa japonica* Toriumi & Takano (Raphidophyceae)
Eastern Alboran Sea (SW Mediterranean)
First report from 2006

Fibrocapsa japonica Toriumi & Takano (Raphidophyceae) is a potentially ichthyotoxic marine microalga first reported in Japan (Okaichi, 1972), where it was associated with mass fish mortality events (Toriumi & Takano, 1973; Okaichi, 1989). The exact mechanism of icthyotoxicity in Raphidophyceae is unknown, but it has been linked to several different processes: an abundant production of mucous that clogs fish gills (Fu, 2003), production of reactive oxygen species (ROS) that asphyxiate the fish (Oda *et al*., 1997) and the production of haemolytic compounds (Fu *et al*., 2004) and/or brevetoxins (Khan *et al*., 1996). The presence of brevetoxins in *F. japonica* was recently debated (Guidi-Rontani *et al*., 2010; Pezzolesi *et al*., 2010; Band-Schmidt *et al*., 2012), and thus the overall toxicity of this species is likely due to a combination of the above-mentioned factors acting together (Pezzolesi *et al*., 2010; de Boer *et al*., 2012).

Experiments conducted on *F. japonica* have shown that it has low nutrient uptake efficiency, and its growth should therefore be favoured in high-nutrient conditions, which are frequently encountered in the stratified shallow coastal and brackish waters (Riegman *et al*., 1996; de Boer *et al*., 2004; Handy *et al*., 2005; Cucchiari *et al*., 2008) where Raphidophyceae are typically detected.

F. japonica is known to bloom in temperate and tropical coastal waters worldwide, including the Pacific and Atlantic American coasts (Loeblich & Fine, 1977; Smayda & Villareal, 1989; Tomas, 1998; Del Carmen Cortés *et al*., 2003; Verity, 2010), Korean and Chinese waters (Lee *et al*., 2001), the Arabian Sea (Härnström *et al*., 2009), southern Australia and New Zealand (Rhodes *et al*., 1993). *F. japonica* has been reported in coastal European waters as well: in the French Atlantic (Billard, 1992), in the North Sea (Vrieling *et al*., 1995) and Baltic Sea waters (Vershinin & Orlova, 2008), as well as in the Mediterranean Sea, along both the Tyrrhenian Sea and the Adriatic Sea coasts (Cucchiari *et al*., 2008, and references therein). Kooistra *et al*. (2001) detected a high degree of polymorphisms among the nuclear ribosomal DNA ITS regions from different *F. japonica* strains, likely due to hybridisation mechanisms, and suggested that the exchange of discharged ship ballast waters and/or aquaculture may have induced the expansion of *F. japonica*'s disjointed distribution range.

Kooistra *et al*. (2001) detected a high degree of polymorphisms among the nuclear ribosomal DNA ITS regions from different *F. japonica* strains, likely due to hybridisation mechanisms, and suggested that the exchange of discharged ship ballast waters and/or aquaculture may have induced the expansion of *F. japonica*'s disjointed distribution range.

The presence of *F. japonica* was reported offshore in the Eastern Alboran Sea for the first time in autumn of 2006 (Fani *et al*.2014).

Fani F. *et al*.2014 (http://www.medit-mar-sc.net/index.php/marine/article/view/398)

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2) *Cephalothrix cf. simula* AM-2013

San Vicente do Mar, O Grove, Galicia, NW Spain. 42º 27′ N, 8º 55′ W (Atlantic coast)
Las Represas beach, Tapia de Casariego, Asturias, N Spain. 43º 34′ N, 6º 56′ W (Atlantic coast)
Los Chalanos beach, Muros de Nalón, Asturias, N Spain. 43º 23′ N, 6º 06′ W (Atlantic coast)
Aramar beach, Luanco, Asturias, N Spain. 43º 36′ N, 5º 46′ W (Atlantic coast)
Islares beach, Castro-Urdiales, Cantabria, N Spain. 43º 24′ N, 3º 17′ W (Atlantic coast)
Colera harbor, Cap de Creus, Catalonia, NE Spain. 42º 24′ N, 3º 09′ E (Mediterranean coast)
L'illot del Faradell, Cap de Creus, Catalonia, NE Spain. 42º 20′ 16″ N, 3º 16′ 49″ E (Mediterranean coast)

First report < 2013

In a recent survey of nemertean diversity along the Iberian Peninsula coasts, some morphospecies of *Cephalothrix* that had not been previously reported in this area were found

(Fernández-Álvarez, unpublished data). A case of a marine nemertean alien invasion, which was confirmed by DNA barcoding studies is reported (Fernández-Álvarez and Machordom, 2013). The authors consider *C. simula* to be an alien invader whose larvae could have been introduced to their allochthonous distribution area in the ballast waters of ships. Moreover, it is possible that environmental changes produced by climate change are currently facilitating the settlement of this species. The presence of developed gonads in one specimen and the presence of juvenile individuals reveal that reproduction is occurring in the invaded areas (Fernández-Álvarez and Machordom, 2013).

Species of the genus *Cephalothrix* have predatory habits (Wu and Sun 2006), and their introduction into new environments can affect natural populations by competitive exclusion. The invasion in the Iberian Peninsula has been happening cryptically, and thus, it is possible that it has had several effects on the natural environment (Fernández-Álvarez and Machordom, 2013). The lack of quantitative data along the Iberian coasts for the majority of nemertean species (e.g. García-Pérez and Anadón 2004) makes it impossible to evaluate whether competitive exclusion is operating on autochthonous *Cephalothrix* species (Fernández-Álvarez and Machordom, 2013).

The report of *C. simula* along the Iberian coasts is the first record for this species in this local fauna (Fernández-Álvarez and Machordom, 2013).

Fernández-Álvarez and Machordom, 2013
(http://link.springer.com/article/10.1007%2Fs10152-013-0346-3)

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3) *Caprella mutica* Schurin, 1935
Illa d'Arousa (42.56135º N 8.95594º W) and other sites in Ria d'Arousa, Galicia, NW Spain, Atlantic coast.
First report from 2012-2013

The caprellid amphipod, *Caprella mutica*, is a well-known invasive species, originating in the Sea of Japan, which has been rapidly expanding along the coasts of North America, Europe and Oceania for the last forty years. *Caprella mutica* is frequently associated with man-made structures, especially those dedicated to aquaculture activities, where it can reach high densities of up to 300 000 ind./m^2. In addition, *C. mutica* has a high reproductive capability, which combined with a wide tolerance to salinity and temperature and a great adaptability to food, allows the species to colonize new places efficiently.

In October 2012, *C. mutica* was seen for the first time in Illa de Arousa (42.56135º N 8.95594º W) (Galicia, north-west Spain), established around bateas, floating structures intended for shellfish farming. This was the first record of this species in the Iberian Peninsula, establishing a new southernmost limit of distribution in the European Atlantic waters and confirming the continuity of the colonization southwards. Between 2012 and 2013 a well-established population was found by SCUBA-divers in Galician waters (north-west Spain) at 6 different man-made floating structures along Ría de Arousa (Almón *et al*., 2014).

The vector used by *C.mutica* to colonize new areas is not well understood, although it is suspected that it is more likely to be via commercial and recreational shipping, rather than by the stock movement of cultured species (Cook *et al*., 2007a; Boos, 2009). It has also been suggested that both, artificial buoyant (buoys, ropes and garbage) and natural materials (seaweed) may facilitate the dispersal of the species (Thiel *et al*., 2003).

From a socio-economic point of view, one of the most important issues is the potential impact of this species on native ecosystems and marine aquaculture, limiting the growth of the mussel *Mytilus* spp. on spat collectors (Turcotte, 2010; Daneliya & Laakkonen 2012). The reduced size of these mussels when coexisting with *C. mutica* could be explained by interspecific competition for common food (phytoplankton), in which caprellids benefit from their positioning over mussels; or by the trampling done by caprellids, which would cause the mussels to close, hence interrupting their feeding (Turcotte, 2010). The fact that each structure is an ideal niche for the establishment of new colonies may have dramatic consequences in a region with 3337 mussel bateas and a production of *Mytilus galloprovincialis* of over 200 000 tonnes yearly (Almón *et al*., 2014).

The appearance of this species in Ría de Arousa strongly suggests that it may already be present in other Galician localities, since the environmental conditions are similar as are the type of structures on which they were found. In any case, the fact that *C. mutica* was not present in previous surveys suggests that colonization of the species may be relatively recent (Almón *et al*., 2014).

Almón B. *et al.*2014
(http://journals.cambridge.org/download.php?file=%2FMBD%2FMBD7%2FS1755267214000335a.pdf&code=40a6843044c7428ccc1b3917051f9ad5)

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G OTHER RELEVANT INFORMATION

H REFERENCES

Almón, B., Pérez, J., Bañón, R., Trigo, J. (2014). First record of *Caprella mutica* from the Iberian Peninsula: expansion southwards in European waters. *Marine Biodiversity Records*, 1-4.

Fani F., Nuccio C., Lazzara L., Massi L., Battocchi C., Penna A. (2014). *Fibrocapsa japonica* (Raphidophyceae) occurrence and ecological features within the phytoplankton assemblage of a cyclonic eddy offshore the Eastern Alboran Sea. *Med. Mar. Sci.* 15: 250-262.

Fernández-Álvarez F.A., Machordom A. (2013) DNA barcoding reveals a cryptic nemertean invasion in Atlantic and Mediterranean waters. *Helgoland Mar Res* 67: 599–605

Sweden

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SUMMARY

In Sweden there are projects ongoing about recreational boating and commercial shipping where boat or ship as vector for introduction of non-indigenous are included as parts of the projects. Monitoring of biofouling are conducted with panels deployed both in marinas along the Swedish coasts (salinity gradient from 30 PSU to 3 PSU) and in Gothenburg harbour at the Swedish westcoast (salinity 25 PSU -0 PSU). Currently hull cleaning of commercial ships in ports are getting attention and municipalities together with ports have localized the need of developing a method for use in hull cleaning recommendations in the aspect of non-indigenous species. Sweden is taking part in the HELCOM/OSPAR TG BALLAST where a Joint Harmonized procedure for granting Exemptions from Ballast water treatment is being developed.

Swedish Agency for Marine and Water Management has within EC-Marine Strategy Framework Directive MSFD work for Descriptor 2 (non-indigenous species) proposed a project for monitoring of non-indigenous species in harbours and shipping lanes.

FULL REPORT

A TRANSPORT VECTORS

Invasive species in the Arctic

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In collaboration with the French Research Institute for the Exploration of the Sea (IFREMER)

The main objective of the project is to assess and mitigate the environmental impacts of shipping in the Arctic. The project focus on the introduction of invasive species and pathogens in the Barents Sea. Sampling of Ballast Water and Ship Hull was conducted in September 2014 in Longyearbyen, Svalbard (Spitsbergen). For 2015 sampling with focus on Cruise ships is planned in June. The project will further review international and national legislation regarding ballast water and biofouling management, including existing and future implementation and enforcement procedures.

Website: http://www.wmu.se/project/invasive-species-arctic
Funding: TOTAL corporate foundation

Duration: September 2012-December 2015

Sustainable Shipping and Environment of the Baltic Sea region, SHEBA

Jana Moldanova, Swedish Environmental Research Institute (IVL): jana.moldanova@ivl.se

The objective of the project is to provide an integrated and in-depth analysis of the ecological, economic and social impacts of shipping in the Baltic Sea and to support development of the related policies on EU, Baltic Sea region, national and local levels. Within the project there are two Ballast Water related tasks that will target: nutrient release from untreated and treated ballast water and spread of marine organisms with Ballast Water release. The spread of nutrients and organisms will be modeled and the size categories of organisms used in the model will correspond to the standard given in the BWM Convention (over 50 microns, 50-10 microns and under 10 microns).

Funding: EU-BONUS (Baltic Sea Region)

Project duration: 2015-2017

Biofouling on ship hulls

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Within this project the problem of biofouling on ship hulls is targeted from a fuel consumption and energy efficiency perspective. The method of hull cleaning is to be optimized considering frequency and force. The required knowledge about the biology present on the ship hull will be reached by characterization of biofouling communities and their attachment strength. The project will also work towards a reduction in impact of hull cleaning on the wear of antifouling paint. Biofouling on ships, including niche areas will be investigated on ships in local, regional and overseas traffic.

Funding: Swedish Energy Agency

Duration: 2015-2017

CHANGE- Changing antifouling practices for leisure boats in the Baltic Sea

Mia Dahlström Sjögren, Swedish Research Institute

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The overall objective of the CHANGE project is to reduce toxic compounds from antifouling paints used on leisure boats in the Baltic Sea. This will be achieved by changing antifouling practices on leisure boats into a sustainable consumption of antifouling products and techniques.

The biofouling pressure within a salinity range is investigated, by deployment of fouling panels (with different paint treatments) on 13 stations along the Swedish west and east coast. The panels will also capture non-indigenous species and in 2013 an 2014 the serpulid polychaete *Ficopomatus enigmaticus* has been recorded on panels at the southernmost station Malmö. (unclear if the species survives winters in Sweden, found around warm water outlets at other places in Europe)

Website: http://www.changeantifouling.com
Funding: BONUS (Baltic Organisations' Network for Funding Science EEIG)
Duration: 2014-2016

Biofouling in Gothenburg harbour

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Fouling organisms, including non-indiginous species are surveyed with PVC-panel deployment in Gothenburg harbour and the archipelago. Panels are in a deployed in a salinity gradient from 0-25 PSU. The importance of different microtopographies of PVC-plates for settlement (number of fouling species/ species richness) is investigated.

Website: http://www.chalmers.se/en/staff/Pages/lena-granhag.aspx
Project duration: 2013-2015

B VECTOR MANAGEMENT

C METHODS FOR SAMPLING AND ANALYSIS

D RISK ASSESSMENT

IRIS –Improving risk assessment of invasive species and cost-efficiency analysis of ballast water treatment methods

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The project is a collaboration between University of Helsinki (Finland), Kotka Marine Research station (Finland) Environmental Development Association (Latvia) and Chalmers University of Technology (Sweden)

The aim of the project is to build a risk assessment tool by combining information about planned route and the key environmental factors in the port, ship type and ballast water treatment methods. By adding likely costs of different treatment methods the tool also allows the search of an optimal route- and ship- specific alternative for ballast water management.

Funding: EU-BSR (Baltic Sea Region)
Duration: 2015

E IMPACTS OF INTRODUCED SPECIES

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

Serpulid polychaete *Ficopomatus enigmaticus*

G OTHER RELEVANT INFORMATION

Swedish Agency for Marine and Water Management has within EC-Marine Strategy Framework Directive MSFD work for Descriptor 2 (non-indigenous species) proposed a project for monitoring of non-indigenous species in harbours and shipping lanes.

Sweden is taking part in the joint HELCOM/OSPAR TG BALLAST where a Joint Harmonized procedure for granting Exemptions from Ballast water treatment is developed. The protocol for port survey of non-indigenous species in port of Gothenburg has been used within the HELCOM/OSPAR TG BALLAST work.

In water hull cleaning of commercial ships in harbours/ports are discussed and municipalities together with ports have localized the need of developing a method/approach for use in hull cleaning recommendations in the aspect of non-indigenous species.

H REFERENCES

United Kingdom

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SUMMARY

The UK continues to participate in discussions at International Maritime Organization regarding the Ballast Water Management Convention. Orkney Islands Council continues to implement the newly approved Ballast Water Management Plan for Scapa Flow. Cefas has co-ordinated the Marine Pathways Project on behalf of Natural Resource Wales and Defra. The project has had contributions from a number of organisations from across the UK and Republic of Ireland. Work conducted by the project has included the assessment of high risk location of introduction, the development of biosecurity advise for stake-holders, the development of monitoring and surveillance programmes and tools, includ-ing assessing the distribution of certain marine non-native species, in addition to examining control measures for certain marine invasive species. The Marine Pathways Project is due to end March 2015, but the group will continue to provide advice to Defra and devolved administrations. The non-native species monitoring phase of Orkney Is-lands Harbour Authority's Ballast Water Management Policy initiated in 2014 and will continue on an annual basis. Completed projects that have been published in 2014 in-clude Marine Scotland's biofouling study on commercial vessels, the Environmental Re-search Institute's rapid assessment of marinas and harbours for marine non-native species and the Biosecurity Plan for the Shetland Islands. New species records for the UK include the Asian shore crab (*Hemigrapsus sanguineus*) at Glamorgan and Kent in May 2014 and Quagga mussel (*Dreissena rostriformis bugensis*) in the River Wraysbury in Octo-ber 2014.

FULL LIST OF PROJECTS

A TRANSPORT

Maritime and Coastguard Agency update
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The UK continues to participate in discussions at IMO regarding the BWM Convention

Native and non-native marine biofouling species present on commercial vessels using Scottish dry docks and harbours

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Biofouling samples from the hulls of commercial vessels using Scottish dry docks and harbours were collected between 2009–2012 to investigate which species are being transported into Scottish waters. The vessels were representative of those servicing the North Sea oil industry e.g. tugs, supply and safety stand by vessels and provided a good indication of the type of fouling found on vessels that typically trade in Scottish coastal waters. The biofouling consisted of typical North Sea species and four established non-native species, *Jassa marmorata, Caprella mutica*, *Austrominius modestus* and *Amphibalanus amphitrite*, were recorded. No new non-native species were recorded during this study. This project has now been published in the Journal *Management of Biological Invasions* (2014) 5: 85-96.

B VECTOR

Ballast Water Management Policy for Scapa Flow, Orkney

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Orkney Islands Council continues to implement the Ballast Water Management Policy for Scapa Flow. In order to maintain pristine environmental status in Scapa Flow the Orkney Islands Harbour Authority has developed an all-encompassing Ballast Water Management Policy that allows for this whilst exceeding international standards. The development of revised Ballast Water Management Policy was started in 2009 and was completed with adoption of the Ballast Water Management Policy of Scapa Flow by Orkney Islands Council on the 10th December 2013.

http://www.orkneyharbours.com/pdfs/bwm/Ballast%20Water%20Management%20Policy%20for%20Scapa%20Flow%2010%20December%202013.pdf

As part of the Ballast Water Management Policy a baseline survey for marine non-native species in Scapa Flow and Loch of Stenness was conducted in 2013. During the baseline survey four non-native species were recorded: Japanese skeleton shrimp (*Caprella mutica*); Jenkin's spire shell (*Potamopyrgus antipodarum*); red seaweed (*Bonnemaisonia hamifera*) and red seaweed (*Heterosiphonia japonica*). These four non-native species have each been recorded in Orkney previously. The baseline survey confirmed their presence and added to our knowledge of their distribution within Scapa Flow and Loch of Stenness.

In 2014 the monitoring phase of the non-native species programme was started. Taxonomic analysis of these samples has not been fully completed yet so we cannot report on the 2014 surveys as yet. However, it is worth noting that a new non-native species was found during the surveys in 2014. A Compass sea squirt (*Asterocarpa humilis*) was found in Kirkwall marina during a rapid assessment survey conducted in September 2014. This

is the first record of it in Orkney and its most northerly record to date. The compass sea squirt was first recorded in UK in 2009 in England and in 2013 in Scotland.

The monitoring programme will continue annually with 2015 surveys starting in June 2015.

C METHODS FOR SAMPLING AND ANALYSIS

D RISK ASSESSMENT

Marine Pathways: Managing marine non-native species

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Hannah Tidbury Hannah.tidbury@cefas.co.uk

The Marine Pathways Project, which began in 2013, aims to reduce the risk associated with pathways by which marine invasive non-native species may be introduced into the British Isles. The main objectives of the project are the:

- Assessment of the presence and distrbution of existing marine INNS.
- Development of monitoring programmes to detect the introduction of invasive non-native species.
- Assessment of high risk regions/pathways for marine invasive non-native species introduction
- Raising awareness of marine INNS with stakeholders and developing codes of practise to reduce the risk of introduction and spread.
- Research and trialling of strategies for the control and eradication of marine INNS to increase preparedness in the event of their introduction.

The project is a collaborative programme of work including input from Department of Environment, Food and Rural Affairs (Defra), Natural England, Natural Resources Wales - Cyfoeth Naturiol Cymru, Scottish Natural Heritage, Marine Scotland, Irish Sea Fisheries Board - Bord Iascaigh Mhara, Centre for Environment, Fisheries and Aquaculture Science (Cefas), Bangor University, Marine Biological Association and Cornish Wildlife Trust. The project is being co-ordinated by Cefas and funded by Defra and Natural Resource Wales. Much of the work conducted will assist in the implementation of the Marine Strategy Framework Directive. There have been a number of outputs from the project, some of which are still in the process of being finalised. The project is due to end in March 2015, but the group will continue to function and provide advice to Defra and devolved administrations. Published reports, papers and other output are currently available, along with additional information on the project can be found at the projects website, further outputs will be placed on the website as they become available www.nonnativespecies.org/index.cfm?sectionid=105

Rapid assessment of marine non-native species in northern Scotland and a synthesis of existing Scottish records

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A comprehensive survey of marine non-native species was undertaken across a number of harbours/ marinas in northern Scotland, July/ August 2012 by the Environmental Research Institute. Large-scale development of wave and tidal energy farms is planned in the Pentland Firth and Orkney waters, northern Scotland. This survey provided the first dataset of presence and distribution of non-native species in the area, and can be used as a baseline to monitor the potential for this development to facilitate the introduction and spread of non-native species. The surveys provided sixty new locality records and extended the northward national range for most of the non-native species found. The number of non-native species found in the surveyed harbours/ marinas was positively associated with the presence of floating structure and the vessel activity indices: number of vessels and perimeter of quayside. This project has now been published in the journal *Aquatic Invasions* (2015) 10: 107-121.

Biosecurity Plan for the Shetland Islands

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Non-native species monitoring, as part of Shetland's Biosecurity Plan developed by the NAFC Marine Centre, has now been completed. New records for the Shetland Islands include *Corella eumyota*, *Dasysiphonia japonica* and *Bugula simplex*. The Plan has been approved and forms supplementary information to the Shetland Islands' Marine Spatial Plan. It is available at www.nafc.uhi.ac.uk/departments/marine-science-and-technology/biosecurity-planning

E IMPACTS OF INTRODUCED SPECIES

F NEW RECORDS OF SHIP-MEDIATED SPECIES (report to AQUANIS when applicable)

Hemigrapsus sanguineus (Asian shore crab)
Aberthaw, Vale of Glamorgan, Wales (First report)
Herne Bay, Kent, England (First report)
Reported in May 2014
Paul Stebbing paul.stebbing@cefas.co.uk

Dreissena rostriformis bugensis (Quagga mussel)
River Wraysbury and Wraysbury reservoir, SE England (First report)
Reported by the Environment Agency in October 2014 and identification confirmed by Dr David Aldridge, Cambridge University.
Paul Stebbing paul.stebbing@cefas.co.uk

G OTHER RELEVANT INFORMATION

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United States

Submitted By:

Lisa Drake, lisa.drake@nrl.navy.mil

SUMMARY

In the past year, research on ballast water as a vector of aquatic nuisance species (ANS) focused on the transport of microorganisms, while research on biofouling focused on ships' available surfaces for the transfer of organisms. Work on the treatment of ballast water proceeded in several areas: use of potable water generators, quantification of total residual oxidants (TRO), efficacy of filter systems, effects of ballast water management systems (BWMS) on ballast tanks and systems, and effects of additives used in land-based verification testing of BWMS. Compliance testing of BWMS was addressed by developing a framework for validating and verifying compliance tools as well as initiating a verification study of tools using variable fluorescence and adenosine triphosphate (ATP) to determine the number of living organisms in discharged ballast water. Regarding risk assessment, a study is underway to characterize the risk-release relationship of invasive species in the Great Lakes using meso-scale experiments and field surveys. Finally, two new species of algae were reported in the Northwest Atlantic: a green alga *Ulva laetevirens*, from New Zealand was found in Connecticut and a red alga, *Laurencia caduciramulosa,* has invaded Key Biscayne, Florida. Several species of crustaceans, fish, and bryozoans have expanded their range in the East coast.

FULL LIST OF PROJECTS

A TRANSPORT VECTORS

Microorganisms in Ballast Water

Maritime Environmental Resource Center (MERC) and the Smithsonian Environmental Research Center (SERC)

Mario Tamburri: tamburri@umces.edu

MERC initiated a pilot study employing metagenomics techniques to explore microorganisms in ballast water and on vessel hulls, as well as their implications for ship-mediated dispersal of potentially harmful bacteria.

Microorganisms in Ballast Water

Old Dominion University (ODU)

Fred Dobbs: fdobbs@odu.edu

Researchers at ODU gave a presentation on the metagenomics of bacteria in ballast water at the 2014 meeting of the International Society for Microbial Ecology.

Biofouling Research

MERC

Mario Tamburri: tamburri@umces.edu
MERC continued evaluations of ship biofouling as a source of invasive species, including (1) holding a workshop on methods for surveying vessels, (2) conducting assessments of wetted surface area of ships (Davidson *et al.*, in prep.), and (3) carrying out laboratory studies on physiological tolerances of common fouling organisms.

MERC researchers published a report on the status of vessel biofouling regulations and compliance technologies (Hagan *et al.* 2014; www.maritime-enviro.org/Reports.php).

Total Wetted Surface Area and Total Niche Areas in the Global Fleet: First Steps in Determining the Potential Extent of Ships' Biofouling
U.S. Naval Research Laboratory (NRL), MERC, and SERC
Lisa Drake: lisa.drake@nrl.navy.mil

At present, there is little quantitative information about the magnitude of vessels' biofouling on a global scale. Estimates of the total wetted surface area (WSA) and the total niche area are essential to 1) determine the potential scope of biofouling and to 2) inform management strategies to reduce the transfer and effects of ANS. Using individual ship characteristics and multiple statistical models from ~191,000 commercial ships in the world fleet, the total WSA and niche area were estimated. A manuscript on the WSA has been submitted for publication (Moser *et al.*, submitted), and a manuscript on the niche area is in preparation (Moser *et al.*, in prep.)

B VECTOR MANAGEMENT

Land-Based Testing of a Potable Water Generator
MERC
Mario Tamburri: tamburri@umces.edu

Land-based testing of a potable water generator (for use as ballast water on non-cargo carrying vessels) was completed onboard the MERC Mobile Test Platform. The final report is available at www.maritime-enviro.org/Reports.php

Total Residual Oxidants (TRO)
Maritime Environmental Resource Center (MERC)
Mario Tamburri: tamburri@umces.edu

MERC and colleagues published a paper describing approaches to quantify TRO (Zimmer-Faust *et al.* 2014) and continued investigations of discharge toxicity associated with the neutralization of chlorine treated ballast water.

Land-based Performance Evaluation in Ambient and Augmented Duluth-Superior Harbor Water of Eight Commercially Available Ballast Water Treatment System Filter Units

Great Ships Initiative (GSI), Northeast-Midwest Institute (NEMWI), University of Wisconsin Superior (UWS), University of Minnesota Duluth (UMD), and AMI Consulting Engineers
Allegra Cangelosi: acangelo@nemw.org

This study consisted of controlled freshwater operational and biological evaluations of the performance of eight commercially available BWMS filter systems (FSs). Tests occurred at the GSI Land Based Test Facility in Superior, Wisconsin, USA in Sept. and Oct. 2013. Eight commercially available FS units spanned a range of FS technologies and nominal pore sizes. Tests took place over a five-week period, at a rate of 2 FS per week, with each FS unit subjected to 4 test cycles of 3-4 hours each, at a rate of one test cycle per day. Each test cycle duration was based on a target volume of water processed, with the volume processed by a FS within each test cycle equivalent to 3 times the FS design flow rate (designated by the developer) per hour of operation, The intake water for two-thirds of the test cycle flow was ambient DSH water, while the final third was amended with ISO 12103-1, A2 Arizona Fine Test Dust (Powder Technology, Inc.; Burnsville, Minnesota, USA) to >24 mg L^{-1} TSS in the intake water. Biological efficacy performance endpoints were percent reduction and absolute density on discharge of zooplankton (total and live ≥50 μm in minimum dimension), and total protists (≥10 μm and <50 μm size class). Operational performance endpoints were the difference between pre- and post-FS flow rates (allowing calculation of backflush volume), pre- and post-FS differential pressure. GSI analyzed biological and physical/chemical parameters of each test cycle's intake water. GSI also monitored and documented operational parameters during the test cycles. Variations in biological and physical/chemical intake conditions were analysed for their influence on FS performance, and controlled for statistically in performance comparisons. Reductions in total zooplankton ≥50 μm ranged from 31.2 - 99.9%; FS performance was clearly challenged by the numerous smaller-sized soft-bodied organisms in this regulated size class in the DSH. FS removal of protists ranged from 22 - 89%. There was a statistically significant and large magnitude negative relationship between FS nominal pore size and percent reduction for organisms in both size classes. Operationally, each FS performed without significant mechanical failure or servicing for the duration of testing. Pressure differential and percent flow lost to backflush as a percent of total water processed ranged from <2 - 12.8%. Operational performance parameters measured did not strongly correlate (positively or negatively) with biological performance such that clear and necessary "trade-offs" could be asserted. In particular, based on GSI findings, volume lost to backflush is not necessarily greater with higher organism removal, though unmeasured operational parameters, such as energy consumption may be. The report was published December 4, 2014, and is available for download on the greatshipsinitiative.org website.

Impacts on Ballast Water Management Systems (BWMS) on Ballast Tanks and Systems

MERC and NRL
Mario Tamburri: tamburri@umces.edu

An expert workshop was convened to assess the effects of BWMS on ballast water tanks and systems, with a focus on corrosion. The overarching conclusions from the workshop were:

1. A flow-chart approach to assessing the effects of BWMSs on ballast systems is recommended. Here, a literature search would be conducted to determine if existing studies indicate that laboratory assessments of ballast water treatments should be conducted. If so, the NACE International Protocol should be followed, with additional parameters measured (as above).
2. No shipboard corrosion experiments carried out in concert with US Environmental Testing Verification (ETV) Protocol shipboard testing are recommended. Instead, the value of shipboard testing will be to determine the variability in the BWMS operation (e.g., real-world dose and variations in dose), and conditions (temperature, etc.); in land-based testing, that data would also be desirable.
3. In the laboratory, a dose response curve should be conducted for each BWMS treatment against the coated materials, uncoated materials, and non–metallic materials.

Framework for the Validation and Verification of Compliance Tools
NRL and MERC
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To assess, validate, and select compliance tools, a framework—consisting of three parts—was presented: proof-of-concept, validation and verification, and final selection stages (Drake *et al.* 2014). Next, a case study describing the proof-of-concept stage was discussed. Specifically, variable fluorescence was evaluated as an approach for determining compliance with the discharge standard for living organisms ≥10 μm and <50 μm (typically protists). Preliminary laboratory experiments were conducted, which were followed by an expert workshop to gauge the feasibility of this approach and propose hypothetical thresholds indicating when the discharge standard is undoubtedly exceeded. Subsequently, field trials were conducted to assess this approach and recommended thresholds. All results were favorable, indicating the validation and verification stages are merited to further evaluate fluorometers as compliance monitoring tools.

Verification of Compliance Tools
NRL, MERC, and GSI
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A verification study of compliance tools using variable fluorescence and adenosine triphosphate (ATP) has been initiated.

C METHODS FOR SAMPLING AND ANALYSIS

Examination of additives used to augment "challenge water" used in verification testing of ballast water management systems: Mass yields and biological impacts
NRL
Lisa Drake: lisa.drake@nrl.navy.mil

In this study, materials used to supplement dissolved organic matter (DOM), particulate organic matter (POM), and mineral matter (MM) used to achieve challenge water criteria were evaluated. To determine the additives' contributions to DOM and POM pools, the mass yields of *Camellia sinesis* (decaffeinated iced tea) extract and humic matter were calculated at different temperature and salinities. Additionally, the response of ambient organisms to these additives was measured in mesocosm experiments, in which changes in organism concentrations were measured after a 5-d holding time. Living organisms were grouped into three size classes: ≥50 μm (nominally zooplankton), ≥10 to <50 μm (nominally protists), and <10 μm (measured as culturable, aerobic, heterotrophic bacteria). Significant differences in concentrations between control and treatment mesocosms after 5 d were not detected for organisms in the ≥10 to <50 μm or the ≥50 μm size classes. However, bacterial concentrations increased significantly in mesocosms augmented with exogenous materials. Thus, direct impacts (or indirect impacts through increased bacterial concentrations) were not apparent among organisms in the two largest size classes. Finally, a literature review of DOM, POM, and total suspended solids concentrations in coastal waters was conducted. It revealed that the challenge water concentrations outlined in the ETV protocol are at the middle to upper range of concentrations observed in coastal and estuarine water. The mean DOM and POM concentrations in this data set typically fell short of the ETV minimum requirements, and more data are needed to fully assess the suitability of these requirements.

D RISK ASSESSMENT

Characterizing the Risk-Release Relationship for Aquatic Invasive Species in the Great Lakes

NEMWI, in collaboration with UWS, and UMD
Allegra Cangelosi:acangelo@nemw.org

The overarching objective of the Risk-Release project is to define and implement experimental methods for parallel (1) mesocosm and (2) field survey approaches to characterizing the risk-release relationship for aquatic invasive species establishment in the Great Lakes. The mesocosm experiments (4 sequential experiments at 4 inoculation levels x 5 replicates) assess cause and effect of establishment probability vs. inoculation densities. The experiments entail simultaneous filling of 20 one cubic meter tubs with ambient water from a typical receiving system in the Great Lakes, inoculation in sets of five of varying numbers of a sentinel invader, the spiny water flea, *Bythotrephes,* holding the water for 2 weeks, draining the entire volume, counting individuals and durable parts of individuals to reconstruct organism growth and reproduction activity across replicates (this is possible with *Bythotrephes*), assigning probability of growth and reproduction to each inoculation level based on findings, and assessing, post hoc, any correlations across tests and replicates, of ambient condition parameters and establishment success. The objective of the field survey experimental approach is to determine the real-world relationship of propagule discharge concentrations and establishment of a second sentinel invader (the bloody-red mysid, *Hemimysis*) in specified harbors, using targeted field sampling rooted in opportunity mapping models, eDNA detection tools, and repeated measures analysis.

The two experimental approaches can support each other over time. In particular, mesocosm experiments' results can inform how to best target field survey effort and build effective models for predicting establishment success across conditions.

E IMPACTS OF INTRODUCED SPECIES

None to report.

F NEW RECORDS OF SHIP-MEDIATED SPECIES

(report to AQUANIS when applicable)

Ulva laetevirens (Areschoug 1854) (Chlorophyta)
Holly Pond, Stamford, Connecticut, Long Island Sound (6/21/2011); 41°2′57.87′′N, 73°29′55.66′′
First Report
This green alga was described from Australia, and later found in New Zealand and the Mediterranean Sea. This is the second record from North America. It was previously collected in Kouchibouguac National Park, New Brunswick, on the Gulf of St. Lawrence (Kirkendale *et al.* 2013). Possible vectors include hull fouling and ballast water. Populations in all these locations show little genetic divergence (Kirkendale *et al.* 2013; Mao *et al.* 2014).
Judy Pederson: jpederso@mit.edu

Laurencia caduciramulosa (Masuda & Kawaguchi 1997) (Rhodophyta)
Key Biscayne, Florida, Atlantic Ocean (8/13/13, 25°43′29.18″N, 80°08′41.78″ W, growing on *Thalassia testudinum* leaves; Collado-Vides *et al.* 2013)
First Report
This red alga was described from Vietnam and later found in the Mediterranean, the Canary Islands, Brazil, and Cuba. This red seaweed is expanding its range around in tropical and subtropical world, probably due to transport by shipping.
Judy Pederson: jpederso@mit.edu

Hediste* (=*Nereis*) *diversicolor (O. F. Műller 1776)
Previous Sighting
Established polychaete in the northwest Atlantic, in the Gulfs of Maine and St. Lawrence, but recent molecular analysis indicates that it is a historical introduction to the East Coast of North America (Einfeldt *et al.* 2014). The nominal species is actually a complex of many cryptic species distributed from the Baltic to Morocco, and in the Mediterranean and Black Seas, frequently in brackish estuaries. Northwest Atlantic populations represent three of these cryptic species, but their genetic diversity is greatly reduced, relative to European populations (Einfeldt *et al.* 2014). The earliest record, of which we are aware, in North American waters, is from the Shubencadie River, Nova Scotia, in the Bay of Fundy (1922, Berkeley and Berkeley 1956). The earliest US record is from Durham, New Hampshire, on the Great Bay (5/7/1953, MCZ IZ 35699, Museum of Comparative Zoology 2014). The known range in the Gulf of Maine is from Scituate, Massachusetts, north to Kingsport, Nova Scotia on the Minas Basin (Pettibone 1963). Populations in the Gulf of

St. Lawrence are genetically distinct and represent a separate introduction from different European populations (Breton *et al.* 2003; Audzijonyte *et al.* 2008; Einfeldt *et al.* 2014). This polychaete has direct development, and lacks a planktonic larvae or pelagic spawning forms. Early introductions by solid ballast are the most probable vector (Einfeldt *et al.* 2014), although juveniles swim to a limited extent (Scaps 2002).
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***Megabalanus coccopoma* (Titan Acorn Barnacle)**
Range Extension
This tropical Eastern Pacific barnacle was first reported in US waters as dead specimens collected in 2001 in Texas, Florida, and Louisiana, but was found to be established in Florida by 2005. Established populations were collected as far north as Rodanthe, North Carolina, just north of Cape Hatteras. In experiments, the animals ceased responding at 4.7 ◦C, and died at 2.3 ◦C. Larval development was completed to settlement at temperatures as low as 16 ◦C. Crickenberger (2014) predicted that reproducing populations could occur as far north as South Bristol, Maine (43.85 °N, 69.54 °W). However, the larvae of the existing populations near Cape Hatteras had sufficient metabolic reserves for natural dispersal only as far as Virginia Beach VA. During the severe winter of 2010, the population retracted southward to just north of Cape Canaveral, and then recolonized its earlier range by 2012. Adult functional tolerance appears to be the major factor limiting poleward range extension in this barnacle (Crickenberger 2014).
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***Palaemon elegans* (Rock Shrimp) and *P. macrodactylus* (Oriental Shrimp)**
Range Extension
Two shrimp species were first reported in 2010 in the East Coast of the U.S.; Palaemon *macrodactylus* was first reported in New York City area and is moving northward whereas *P. elegans* was first observed in Salem, Massachusetts. Ongoing studies to determine the extent of the species distribution and abundance was initiated last summer (J. Carlton, pers. comm.).
Judy Pederson: jpederso@mit.edu

***Eriocheir sinensis* (Chinese Mitten Crab)**
Range Extension
Only one report of a Chinese Mitten Crab was received in 2014. This crab was an adult female (55 mm carapace width), collected on October 20, 2014 from the Mianus Pond fishway in Greenwich, Connecticut, on the Mianus River, a Long Island Sound tributary. A juvenile crab was collected here in 2012 (Matthew Goclowski, Darrick Sparks, personal communications, USGS Nonindigenous Aquatic Species Program 2014). We have received no other reports of mitten crabs form the East Coast of the United States.

Although the European green crab, *Carcinus maenas* has been in North America for over 200 years, the recent invasion (reported in 2007) of a northern haplotype was anecdotally correlated with increased populations to the south. The increased crab populations are a concern for shellfishermen. A recent unpublished study by Larissa Williams from Bates College and colleagues suggests that the northern haplotype is restricted to Penobscot Bay, Maine and north. The increased crab populations reported in areas south of Penobscot, Maine may be due to warming sea temperatures, but even in cold winters, the

population sizes appear to increase in many areas. It is unclear if this is a climate change phenomenon or the result of other factors that are favorable for green crabs.
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Tricellaria inopinata
Range Extension
As previously reported, this Pacific Ocean bryozoan was first reported from Woods Hole, Massachusetts, in 2010 (Johnson *et al.*, 2012), and later expanded its range south to Newport, Rhode Island, and north to Hampton New Hampshire (Wells *et al.* 2013). A genetic examination of 4 populations (Woods Hole, Boston, Marblehead, and Gloucester, Massachusetts) found that the Marblehead and Gloucester populations had a separate origin from the Woods Hole population, indicating at least two separate introductions (Johnson and Woolacott 2014).
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***Pterois miles/volitans* spp. (Lionfish)**
Range Extension
Lionfish (probably mostly *P. volitans*, Red Lionfish) are now extensively distributed from Cape Hatteras to the Texas-Mexico border (USGS Nonindigenous Aquatic Species Program 2015). Within that range, Lionfish have been found using the Loxahatchee River estuary in Florida, and can be expected to be found in other southeastern US estuaries. Lionfish were found to survive and feed at salinities as low as 5 PSU in aquaria, and in the field, entered lower salinities briefly while pursuing prey. Fish, caged in a downstream location in the estuary, tolerated tidal salinity fluctuations between 7 and 35 PSU, but fish caged at locations further upstream did not survive when salinities dropped below 5 PSU (Jud *et al.* 2014). Predation by these caged lionfish had significant effects on benthic organisms, reducing numbers of Grass Shrimps (*Palaemon* spp.) by ~90% (Layman *et al.* 2014). Moving offshore, the Gulf of Mexico, a Remotely Operated Vehicle (ROV) survey found numerous lionfishes in deep mesophotic continental shelf communities, at 50-175 m depth, with an apparent increase in abundance from 2011 to 2013. These communities, dominated by coralline algae, sponges, and soft corals, are considered ecologically sensitive habitats (Nuttall *et al.* 2014). A genetic study of lionfish in Puerto Rico found that only *P. volitans* was present in Puerto Rico, and that there was an apparent decrease in genetic diversity, from North Carolina to Puerto Rico and Colombia, suggestive of a founder effect, resulting from gradual dispersal from source populations in Florida (Toledo-Hernandez *et al.* 2014).
Judy Pederson: jpederso@mit.edu

***Acanthurus pyroferus* (Chocolate Surgeonfish)**
Range Extension
In 2014, a single specimen was caught near Palm Beach, Florida. This was the only non-established, exotic marine fish reported in US waters in 2014, according to the USGS Nonindigenous Aquatic Species Database. At least 32 species of tropical marine fishes, mostly single specimens of Indo-Pacific species have been released in Florida waters since 1990 (USGS Nonindigenous Aquatic Species Program 2015).
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G OTHER RELEVANT INFORMATION

Ninth International Conference on Marine Bioinvasions, to be held in Sydney, Australia, January 2016

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Annex 4: Annotated Eutrophication Monitoring Guidelines

JAMP Eutrophication Monitoring Guidelines:

Phytoplankton Species Composition

1. Introduction

Phytoplankton species composition serves as an indicator of the effects of eutrophication. Nutrient enrichment/eutrophication may give rise to shifts in phytoplankton species composition (*e.g.* from diatoms to flagellates, some of which are nuisance or toxic) and an increase in the frequency and/or magnitude and/or duration of phytoplankton blooms and/or of nuisance/potentially toxic blooms. Invasive non-indigenous species of phytoplankton also have the potential to cause shifts in local species compositions through competition, and/or bloom formation and/or toxic events. These guidelines are intended to support the minimum monitoring requirements of the Nutrient Monitoring Programme[1].

Regulations including the Water Framework Directive (WFD), Marine Strategy Framework Directive (MSFD) and EU Regulation on the prevention and management of introduction and spread of invasive alien species (1143/2014) require assessment, monitoring and recording of invasive alien species by Member States. As such, these phytoplankton species should also be recorded during routine phytoplankton analysis.

2. Purposes

The measurement of phytoplankton species composition is carried out for, *inter alia*, the following purposes:

1. to establish the spatial distribution and frequency of phytoplankton blooms;
2. to establish temporal trends, over periods of several years, in phytoplankton species composition and their relative abundance;
3. to identify key phytoplankton species.
4. to identify non-indigenous phytoplankton species.

3. Quantitative objectives

[Secretariat note: in addition to the general purpose of the programme, an explicit quantified statistically formulated objective for temporal trend and spatial distribution monitoring requires development.]

[1] The Nutrient Monitoring Programme as adopted by OSPAR 1995 (OSPAR 95/15/1, Annex 12).

4. Sampling strategy

An understanding of the complexity of the hydrography of estuarine or coastal seas is necessary before starting to survey or sample the phytoplankton. Thus, there is a need for routine hydrographic observations at the same time as the surveys/sampling. Apart from the influence of water column structure on phytoplankton dynamics there is a need to consider horizontal (spatial) and temporal variability in order to establish the frequency and location of sampling. Sample sites should be further apart than the horizontal tidal amplitude but sufficiently close to resolve the presence of strong gradients. Sampling frequency should take account of seasonal variability in the relative abundance of the species of interest.

The abundance of "key species" should be examined. The "key species" are dominant and/or nuisance and/or potentially toxic and/or non-indigenous species. Examples of tax~~a~~species which are dominant and/or nuisance and/or potentially toxic and/or non-indigenous are: *Alexandrium* spp. (*Gonyaulax*), *Ceratium* spp., *Chrysochromulina polylepis*, *Corymbellus aureus*, *Coscindiscus wailesii*, *Dinophysis acuminata*, *Gymnodinium catenatum*, *Gyrodinium aureolum*, *Lepidodinium viride*, *Noctiluca scintillans*, *Phaeocystis* spp., *Prorocentrum balticum*, *Prorocentrum minimum*, *Prymnesium parvum*, *Pseudochattonella verruculosa* and *Pseudonitzschia* spp. ~~Attempts should be made to establish the overall species composition.~~ An overall species composition should be established. The presence of non-indigenous species should be recorded as part of a full community analysis. The IOC-UNESCO Taxonomic Reference List of Harmful Micro Algae (http://www.marinespecies.org/hab/index.php) contains up-to-date information on the status of harmful species.

Particulate organic carbon, total organic carbon, particulate organic nitrogen, light (PAR/Secchi depth), chlorophyll fluorescence, temperature and salinity should be measured as supporting/interpretation parameters.

Aerial surveillance (for example under the Bonn Agreement) will help to identify annual and interannual variability in phytoplankton bloom development and will also help target specific sampling in relation to phytoplankton bloom events. Short synoptic surveys may be useful for following the dynamics of phytoplankton blooms within a growth season, *e.g.* by helicopter. Complementary crowdsourcing of phytoplankton bloom reports eg. NOAA's Phytoplankton Monitoring Network or www.phenomer.org may also extend the areas under observation for phytoplankton blooms.

5. Sampling equipment

Techniques for sampling are various. Nets are limited in that they do not retain all phytoplankton but can concentrate from a large volume and are helpful for determining the species composition. Nets are semi-quantitative if used with a flow meter attached. Water bottles sample discrete and smaller volumes but retain all organisms and are thus necessary for quantitative studies. A brief outline of various sampling devices can be found in Karlson *et al.* (2010) and Franks and Keafer (2004) provide a detailed overview on the different types of sampling strategies that can be incorporated into a phytoplankton monitoring programme.

6. Storage and pre-treatment of samples

In general, samples are preserved with a suitable fixative such as lugol or formalin[2]. Many small, naked flagellates are destroyed by fixatives and can only be identified live. In the event of an algal bloom a live sample should be collected from the area. For preservation of microflagellates, 20 % paraformaleyhde (final volume 2%) can be added to samples which should then be snap-frozen and stored at -80 ºC until analysis.

7. Analytical procedures

Microscopic analysis allows direct identification of phytoplankton species and quantification in terms of cell numbers. Many small (<5 µm) cells will be very difficult to identify by light microscopy (LM) and may have to be recorded as unidentified. ~~The counting procedure should be based on the proposals of ICES (1996).~~ The latest IOC guidance for quantitative phytoplankton analysis should be referred to (Karlson *et al.*, 2010), as should Standards EN 15204: 2006 and EN 15972: 2011. Given the rapid development of flow cytometry and the large number of small fluorescent cells present in samples, these are best determined by fluorescence microscopy or flow cytometry. A combination of flow cytometry and immuno-labelling may in the near future enable the rapid and conclusive identification and counting of toxic species if sufficient immuno-labels are available. New methods may offer the opportunity for fast-automated analysis of phytoplankton species samples. In order to record non-indigenous phytoplankton speices that may be present, a full LM phytoplankton community analysis should be performed. The presence of a non-indigenous species may be missed if only analysing for toxic and/or harmful species.

Molecular tools can be more sensitive for the early detection of non-indigenous and/or small (<5 µm) phytoplankton species, and are also useful for the identification of early and/or cyst stages likely to be introduced via ballast water, rare taxa or badly conserved specimens. Molecular methods do not need strong taxonomic expertise and follow standard procedures. New techniques are now emerging for global assessments such as e-DNA and metabarcoding. While more effort is needed to build up reference databases, these are very promising analytical tools.

8. Analytical quality assurance[3]

The quality assurance programme should ensure that the data are fit for the purpose for which they have been collected, *i.e.* that they satisfy the objectives of the monitoring programme. Emphasis should be placed on the intercalibration of species identification on a regular basis. A phytoplankton checklist must be compiled during intercalibration exer-

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2 Recommendation on the fixative to be used should be updated on the basis of the outcome of a suitable calibration exercise.

3 A joint ICES/OSPAR Steering Group on Quality Assurance of Biological Measurements related to eutrophication parameters was established in 1997 in order to coordinate the development of quality assurance procedures, the implementation of quality assurance activities (*e.g.*, the conduct of workshops and intercomparison exercises) and the preparation of appropriate taxonomic lists of species. This work will cover phytoplankton species and is a fairly long-term programme of about five years. Good cooperation will be ensured with the ICES/HELCOM steering group on Quality Assurance of Biological Measurements in the Baltic Sea.

cises. Participation in quality assurance/quality control (QA/QC) schemes such as the annual BEQUALM phytoplaknton ring test, run under the auspcies of the National Marine Biological Analytical Quality Control (NMBAQC) scheme BEQUALM is required for ensuring data quality. Organisations can also aquire certification through national, European or international accreditation schemes eg. Good Laboratory Practice (GLP) and the United Kingdom Accreditation Service (UKAS).

9. Reporting requirements

~~[Secretariat note: reporting procedures require development. As a component of the 1997 ICES Work Programme, the Oslo and Paris Commissions have formally requested ICES to establish a databank for phytoplankton species. The work will include the development of a reporting format and a species code list. The reporting procedures should include a national report containing information on methods used and any other comments or information relevant to an ultimate assessment of the data. In order to establish the acceptability of the data, they should be reported together with the dates and results of participation in intercalibration exercises.]~~ [This is now HAEDAT – so this note can be deleted].

All harmful algae events are recorded onto the Harmful Algae Events Database (HAEDAT). Non-indigenous phytoplankton species identified during routine phytoplankton community analyses should be reported to national authorities. Publically available database(s), such as AquaNIS (http://www.corpi.ku.lt/databases/index.php/aquanis) should be used for reporting and data storage for non-indigenous species. A Harmful Algal Information System (HAIS) is being developed in cooperation with WoRMS, ICES, PICES and ISSHA and can be accessed here http://haedat.iode.org//

10. References

~~ICES (1996). Report of the ICES/HELCOM Second workshop on quality assurance of biological measurements in the Baltic Sea, Warnemünde, Germany, 16-20 September 1995. ICES CM 1996/E:1.~~ [should be deleted as out of date]

Franks, P.J.S. and Keafer, B.A. (2004). Sampling techniques and startegies for coastal phytoplankton blooms. In: G.M. Hallegraeff., D.M. Anderson and A.D. Cembella (eds), *Manual on Harmful Marine Microalgae*, UNESCO Publishing. Pp 51-76.

Karlson, B., Cusack, C. & Bresnan, E. (Eds) (2010). Microscopic and molecular methods for quantitative phytoplankton analysis. Intergovernmental Oceanographic Commission of UNESCO, Paris. IOC Manuals and Guides, no. 55. IOC/2010/MG/55, 110 pp.

Annex 5: Draft multi-annual Terms of Reference for 2016–2018

The **Working Group on Ballast and Other Ship Vectors** (WGBSOV), chaired by Sarah Bailey, Canada, will work on ToRs and generate deliverables as listed in the Table below.

	Meeting dates	Venue	Reporting details	Comments (change in Chair, etc.)
Year 2016	14-16 March	Olbia, Italy	Interim report by DATE to SSGEPI	
Year 2017	xx-xx March	TBD	Interim report by DATE to SSGEPI	
Year 2018	xx-xx March	TBD	Final report by DATE to SSGEPI, SCICOM	

ToR descriptors

ToR	Description	Background	Science Plan topics addressed	Duration	Expected Deliverables
a	Conduct strategic planning (identify and develop collaborative activities, advance and standardize methods, etc.) to advance research and address knowledge gaps through review of national activities and to respond to new requests for advice.	ICES strategic plan Goal 2: understand the relationship between human activities (e.g., shipping) and marine ecosystems to estimate pressures and impacts, and develop science-based sustainable pathways; and Goal 3: Evaluate and advise on options for the sustainable use and protection of marine ecosystems. Potential advice requests from agencies such as OSPAR.	17, 25, 27	3 years	Report to ICES. Respond to advice requests, as applicable.
b	Evaluate methods for collection and analysis of ballast water samples to inform national and/or international procedures for compliance testing of ballast water management systems	The Convention for the Control and Management of Ships' Ballast Water and Sediments, (2004) (BWMC) aims to minimize the transfer of harmful aquatic organisms with the ballast water from ships. It is imperative that the BWMC is implemented in a scientifically valid and standardized way	17, 27, 31	3 years	Comparative methods paper submitted to scientific journal

		globally. There are science and advisory requirements related to validated methods and procedures.			
c	Evaluate methods for, and outcomes of, type approval and operational testing of ballast water management systems to inform national and/or international procedures for type approval of such systems	As previous	17, 27, 31	3 years	Input on the general applicability or otherwise of such methods to IMO or national regulators through meeting participation, correspondence group and/or technical paper
d	Investigate and evaluate climate change impacts on the establishment and spread of ship-mediated nonindigenous species, particularly with respect to the Arctic	This work will be carried out jointly with WGITMO. Contributes to SICCME and ICES high-priority action area 'Arctic research'	3, 10, 13, 17	3 years	At least one scientific journal paper evaluating risk of ship-mediated invasions to the Arctic
e	Investigate and evaluate methods/technologies to assess risks of, to minimize extent of, and to respond to vessel biofouling to inform national and/or international policies or guidelines	Ships' biofouling is, with ballast water, a primay bioinvasion vector. As management of invasion vectors is the only effective way to reduce risks of new invasions, addressing biofouling issues is of high priority in bioinvasions management.	11, 13, 17	3 years	Input on the general applicability or otherwise of such methods/technologies to IMO or national regulators through meeting participation, correspondence group and/or technical paper
f	Evaluate the current role/importance of shipping in relation to other invasion vectors/pathways globally	This work will be carried out jointly with WGITMO. As invasion of non-indigenous species is truly of global nature, such a review should have global coverage. Although shipping has been claimed as the most important invasion vector, there are regional specificities and also temporal considerations.	17, 27	3 years	Review Paper in scientific journal

Summary of the Work Plan

Year 1	**Working on all ToRs, but with special focus on ToRs a, c, and d.**

Year 2	Working on all ToRs, but with special focus on ToRs b, e, and f.
Year 3	Report on all ToRs

Supporting information

Priority	The work of the Group forms the scientific basis for essential advice related to the movement of harmful aquatic organisms and pathogens via ballast water and other shipping vectors. As a joint working group it also follows and supports related work within the IMO and IOC.
Resource requirements	The research programmes which provide the main input to this group are already underway, with resources provided by national governments and scientific funding agencies. The additional resources required to undertake activities in the framework of this group are negligible.
Participants	The Group is normally attended by some 25-35 members and guests, but has more than 65 members in total.
Secretariat facilities	None.
Financial	No financial implications.
Linkages to ACOM and groups under ACOM	The group will serve as primary respondent to incoming advice requests on various issues related to ship-mediated introductions.
Linkages to other committee or groups	There is a very close working relationship with WGITMO. Potential or occasional linkage with WGBIODIV, WGHABD, WGIMT, WGMABS, WGPME and WGZE.
Linkages to other organizations	International Oceanographic Commission (IOC), International Maritime Organization (IMO), North Pacific Marine Science Organization (PICES). In addition, the outcomes are relevant to other national and international organizations involved in the development of regulatory policies.

Annex 6: Technical minutes from RGJAMP

Review of ICES Working Group on Ballast and Other Ship Vectors (WGBOSV), 16–18 March 2015, regarding Annex 4 (Annotated Eutrophication Monitoring Guidelines – JAMP Eutrophication Guidelines: Phytoplankton Species Composition)

28 May 2015

Reviewers: Harri Kuosa, Finland (chair) and Donald Boesch, USA

WGBOSV Chair: Sarah Bailey, Canada

ICES Secretariat: Sebastian Valanko

WGBOSV has added/modified the text directly to JAMP Report Draft to clarify the role of phytoplankton monitoring of non-indigenous species.

1) Introduction. The additions are relevant if they fit to the format of JAMP Report.

2) Purposes. Also relevant addition.

3) Quantitative objectives. No modifications.

4) Sampling strategy. The full 'key species' list, updated regularly is probably required in case it directs sampling effort and analysis. In case of non-indigenous species sampling in harbours can be crucial for early warning signs.

5) Sampling equipment. Modifications relevant.

6) Storage and pre-treatment of samples. Modifications relevant.

7) Analytical procedures. Modifications relevant. Molecular tools are useful, but their utilization should be carefully planned along with sampling strategy. Hot spots and relevant seasons should be emphasized, if the goal is to find out if a new species has arrived. If it is possible to identify in LM, its distribution can be assessed, but in case it is difficult to identify alternative methods have to be considered.

8) Analytical quality assurance. Modifications relevant.

9) Reporting requirements. Modifications relevant.

10) References. Modifications relevant.