No 1 December 2014

Project

BALMAS newsletter

Foreword

Dear reader,

welcome to the BALMAS project newsletter! Transnational cooperation in Adriatic region under the BALMAS project: **Ballast Water Management System for Adriatic Sea Protection** became fully operational with the start of the BALMAS project in November 2013. The BALMAS 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.

In November we marked the first birthday of the BALMAS project. We have passed successfully several milestones, some of which are highlighted in this issue. BALMAS project Partners have been fully involved in a number of project activities, such as sampling in selected ports, attendance at numerous workshops and three regular meetings, and appearances in the media where ongoing and future work has been presented. We established also BALMAS Knowledge centre for the purpose of presenting our interim outputs and results to all interested readers.

With all performed activities in the first year, the BALMAS project infrastructure is now completely in place, and we are well positioned to achieve planned objectives within the project time framework.

Leon Gosar*

Leon Gosar

**BALMAS project Coordinator for the Lead Partner, the Institute for Water of the Republic of Slovenia*

BALMAS Roll-up, upper part of the poster (Photo: M. Rogelja)

- In November we marked the first birthday of the BALMAS project
- Implementation of project activities is mainly proceeding according to the work plan
- We tested new quick analyses methods for detection of organisms in ballast waters in comparison with laboratory analyses methods
- Chemical survey of seven Adriatic ports and placement of biomonitoring cages was succesfull

Inside this issue:

Outputs and results of the BALMAS project in the first year

Implementation of the project activities is mainly proceeding according to the work plan from the approved Application Form. Key achieved outputs and results of the BALMAS project are the following:

- 3 Regular BALMAS Meetings on which more than 60 participants were present:
  - Kick-off Meeting in Piran, Slovenia (14. 1. – 16. 1. 2014) with 2 workshops;
  - 2nd Regular Meeting in Split, Croatia (8. 4. – 10. 4. 2014) with 4 workshops;
  - 3rd Regular Meeting in Kotor, Montenegro (7. 10. – 9. 10. 2014) with 5 workshops;
- chemical and/or biological sampling in the 12 selected Adriatic ports (Koper, Trieste, Bari, Ancona, Venice, Pula, Rijeka, Split, Šibenik, Ploče, Bar and Durres);
- 22 reports for the key achieved outputs and results;
- 5 publications about BALMAS project published in daily press or public magazines;
- 9 events organized with public lectures on the issues

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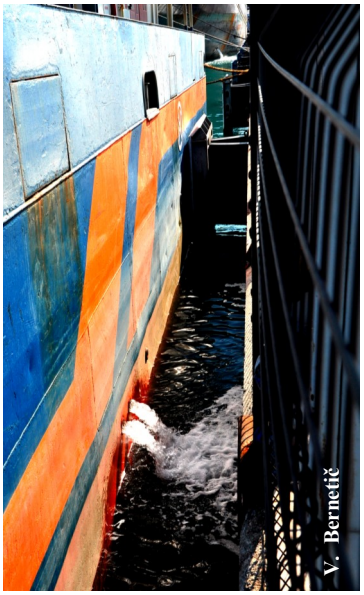
... Outputs and results on the BALMAS project in 12 months



Cargo ship moored in the port area

"Institute for Water of the Republic of Slovenia that coordinates 15 project partners from 5 countries is continuously monitoring the project progress"

BALMAS Roll-up on exhibit for the World Oceans Day at Marine Biology Station Piran (NIB)

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related to the BALMAS project (2 events organized in Slovenia and 7 events in Italy);

♦ more than 40 contributions about BALMAS project and project results published through different media via internet, radio and TV;

♦ 1st BALMAS Press Release launched in May 2014 in English and Slovenian language and later translated in Italian, Montenegrin and Albanian language;

♦ BALMAS Leaflets in English, Slovenian, Croatian, Italian, Montenegrin and Albanian language;

♦ BALMAS Roll-up in English and Slovenian language;

♦ BALMAS promotional material: umbrellas, pencils, writing pads, folders, flasks, pens, notebooks, stickers, bags, calendar and book mark.

Lead partner of the BALMAS project – Institute for Water of the Republic of Slovenia that coordinates 15 project partners from 6 countries is continuously monitoring the project progress, resolving the bottlenecks and ensuring that the deadlines are met. Thus, the key outputs and results in the first 12 months were achieved. With such cross-border management and coordination successful implementation of project activities in the remaining 17 months is expected.

Andreja Popit*

**BALMAS project Manager for the Lead Partner, the Institute for Water of the Republic of Slovenia*

BALMAS workshop on organism detection methods and tools for compliance monitoring and enforcement, ballast water sample analysis for indicator microbes

The key objective of the workshop was to practically evaluate the performance of different methods and tools available on the market to identify some of the harmful aquatic organisms and pathogens (HAOP) groups such as indicator microbes and phytoplankton, addressed by the Ballast Water Performance Standard as stipulated in Regulation D-2 of the BWM Convention (International Convention for the Control and Management of Ship´s Ballast Water and Sediments, 2004), *i.e.* to test if the methods can show compliance and non-compliance with the D-2 standard. As described in Article 9 of the BWM Convention, vessels may be inspected to proof compliance with the D-2 standard so that appropriate sampling methods and sample processing methods need to be identified. The samples taken may be analysed indicatively or in detail so that for both approaches suitable sample processing methods need to be determined.

One part of the workshop was dedicated to test the methods and tools to identify colony forming units (CFU) of indicator microbes, such as *Escherichia coli* and intestinal *Enterococci*. A critical point in microbiology is usually the long incubation time for bacteria to grow to get the results as required by the D-2 standard (documented CFU). Having screened suitable bacteria detection methods, we note that the minimum incubation time is 6-8 hours which do not allow a prompt result for an indicative analysis. Furthermore, methods that show

Preparation of samples to determine E. coli with Möller & Schmelz selective media

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... BALMAS workshop on organism detection methods and tools ...

(Continued from page 2)

only the presence/absence of the indicator microbes in the D-2 standard, *i.e.* not a concentration of CFU, need laboratory gear and surroundings to, *e.g.*, avoid contamination. Therefore, it was concluded that none of today's bacteria methods are found appropriate for an indicative analysis.

At the beginning of the workshop the selection of methods was performed and the procedures for sampling and sample processing were discussed. This was followed by the practical part of the workshop, during which different methods and tools to identify indicator microbes were tested on the same set of seawater and freshwater samples, previously prepared using faecal polluted water sample inoculum collected at the at the outlet of the waste water treatment plant, located nearby the Marine Biology Station Piran (MBSS). One set of methods and tools for the sample analysis was selected by Stephan Gollasch and Matej David based upon knowledge and experience gained in different ballast water sample processing studies, which included a comprehensive search of potential methods and tools followed by on board tests of selected methods. The methods selected for this BALMAS workshop were Petrifilm and membrane filtration combined with Dr. Möller & Schmelz media. A second set of analyses was performed by Valentina Turk and Tinkara Tinta (NIB, MBSS) using the membrane filtration method and plating on different agar media (EMB, ENDO and Slanetz Bartley media). In addition, a contact was established to Annie Brooking and Derek Price of Bactest Limited, Cambridge, United Kingdom, *i.e.* the developer and manufacturer of the Speedy Breedy instrument. This instrument was operated at the workshop by Derek Price as additional tool to determine selected *E. coli* and *Enterococci* within the samples.

The membrane filtration method

Christian Moldaenke and Stephan Gollasch operating the BBE 10 cells PAM

"One set of methods and tools for the sample analysis was selected by Stephan Gollasch and Matej David based upon knowledge and experience gained in different ballast water sample processing studies"

Adriatic Sea in the vicinity of the institute. For every series of tests four different algal cell concentrations were prepared, in addition to the natural sample, the sample was once diluted and twice concentrated, to obtain target concentrations of approximately 5, 50, 100 and 200 viable cells/ml.

Participants of the workshop in front of the Marine Biology Station Piran, Slovenia

The second part of the workshop was dedicated to new methods and tools to identify viable phytoplankton cells of the size less than 50 µm in minimum dimension and greater than or equal to 10 µm in minimum dimension (hereafter: 10-50 µm organism). Water samples for analyses were taken from the

The candidate methods and tools for the indicative analysis of phytoplankton were selected based upon knowledge and experience gained in different ballast water sample processing studies. We tested four pulse-amplitude modulated (PAM) fluorometry systems available on the market for indicative

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... BALMAS workshop on organism detection methods and tools ...

Matej David operating the PAM of Turner Designs

"The results of epifluorescence microscopy were comparable among the two phytoplankton experts and were in a majority of tests similar to the BBE 10 cells results. These indicate that staining with FDA and epifluorescence microscopic observations are reliable and efficient methods to evaluate the concentration of viable phytoplankton organisms."

Aleksandra David operating the Walz Water PAM

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analysis: BBE 10 cells (Germany), Hach BW680 (USA), Turner Designs Ballast-Check (USA) and Walz Water PAM (Germany). The first was operated by a representative of the manufacturer Christian Moldaenke, while the others were operated by Aleksandra David and Matej David.

Simultaneously to PAM tests, water samples were examined by the epifluorescence microscopy after being stained with the viability stain fluorescein diacetate (FDA). The epifluorescence microscopy is not suitable for an indicative compliance control check as it requires laboratory surroundings, special equipment and biological expertise to process the samples and it takes at least one hour to generate a result. However, the method is accurate and is therefore considered as detailed sample analysis method for the 10-50 µm organism group. Phytoplankton counts were done in parallel by Patricija Mozetič and Janja France using a Sedgewick-Rafter chamber with a volume of 1 ml.

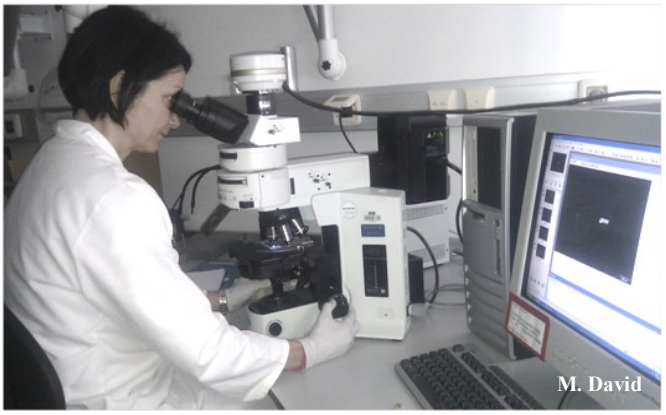
The results of epifluorescence microscopy were comparable among the two phytoplankton experts and were in a majority of tests similar to the BBE 10 cells results. These indicate that staining with FDA and epifluorescence microscopic observations are reliable and efficient methods to evaluate the concentration of viable phytoplankton organisms. This is particularly the case when a detailed analysis of ballast water is needed to check compliance with the D-2 standard. Among the three PAMs considered the Hach PAM gives most consistent risk evaluation results between individual measurements of the same sample and the risk level meets the microscope counts. Considering the results of all four PAMs, it was therefore concluded that the Hach BW680 or the BBE 10 cells are the best PAMs operated during the workshop and should be recommended as a suitable gear for an indicative analysis of ballast water.

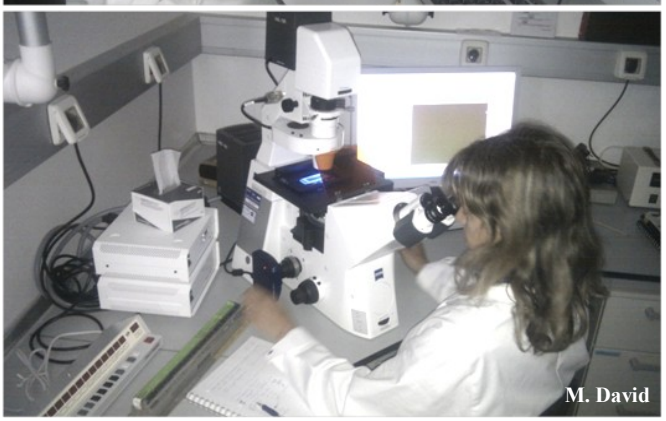
Tinkara Tinta[1], Valentina Turk[1], Janja Francé[1], Patricija Mozetič[1], Matej David[2], Stephan Gollasch[3]

1 – Marine Biology Station Piran, National Institute of Biology, Slovenia
2 – Dr. Matej DAVID Consult, Izola, Slovenia
3 – GoConsult, Hamburg, Germany

Incubator with different media plates

*The workshop was organized at the Marine Biology Station Piran (MBSS), National Institute of Biology (NIB), Slovenia and held from 16th to 19th of June 2014.





Patricija Mozetič (top photo) and Janja Francé (bottom photo) using two epifluorescence microscopes counting viable algae in parallel

Port baseline survey – Analyses of biocides (organotins) and disinfection by-products from chlorine treatment (trihalomethanes, haloacetonitriles and haloacetic acids)

Ballast water management systems make use of active substances on-board in order to minimize the transfer of harmful aquatic organism and pathogens. The majority of the traditional biocides are oxidant agents, that reacting with the organic matter in seawater, produce disinfection by-products which are likely to be toxic to the aquatic environment. In the Database developed by the GESAMP - Ballast Water Working Group, chemicals most commonly associated with the treated ballast water and prioritized based on occurrence, frequency and concentrations are those belonging to the class of haloacetic acids, trihalomethans, haloacetonitriles (IMO, 2013).

Moreover, there is a clear potential to discharge through ballast waters other chemical products, including organotins compounds, into the sea. Such discharges could be at levels well in excess of what is released by current anti-fouling paints (estimated for TBT at 1900 tonnes/year, for copper at 27000 tonnes/year). The same mechanism might also cause the introduction of chemical pollutants to other regions.

In fact it was demonstrated that the vector of worldwide transportation of TBT is not limited to hull and other exterior portion of ships, but also ballast water may act as a vector of much greater capacity and contribute to the re-buildup of TBT contamination in estuaries, international seaports, and coastal regions.

For these reasons, in order to assess chemical contamination in selected ports of the Adriatic sea, a baseline survey based on the Active Mussel Watch (AMW) activity, supported by water and sediment analysis, has been proposed and is currently carried out by ISPRA, the Italian National Institute for Environmental Protection and Research. The purpose of the baseline survey is to assess chemical contamination of the seaport in the frame of the implementation of Ballast Water Convention in the Adriatic sea.

The AMW methodology is based on mussels transplantation from unpolluted areas to selected zones to be investigated and has been frequently applied to monitoring programs for assessing chemical contamination of seawater.

Mussels, as filtering organisms, are able to bioaccumulate contaminants in their flesh according to their bioavailability in the environmental surroundings.

Cages containing the bivalves have been prepared from a mussel farm in an unpolluted area of the Tyrrhenian sea (Anzio). Seven ports have been chosen for the investigation (Bari, Ancona, Rijeka, Durres, Koper, Split and Bar) and in each port three monitoring sites have been selected.

The mussels translocation has been carried out by the Dallaporta R/V with the support of CNR ISMAR. The campaign started from Ancona on 26 October 2014 and then the R/V reached the ports of Split, Koper, Bar, and Bari. In the harbors of Rijeka and Durres, mussel cages were placed directly by the local project Partners.

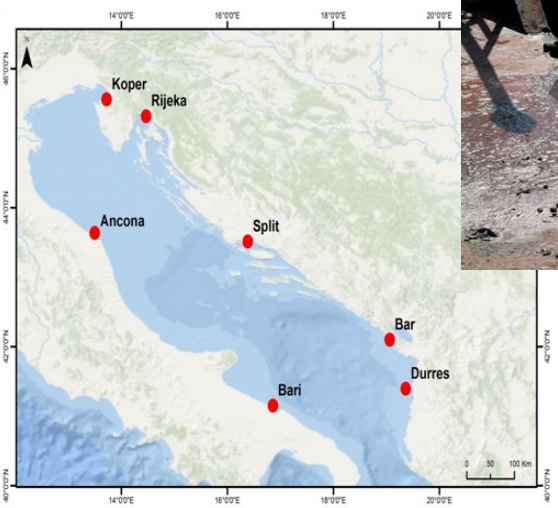
The exposure period will last 12 weeks, then the recovery of the cages will take place on January by divers or with the support of small boats.

Water samples in the ports of Koper, Bar, Bari and Split were taken in correspondence of the sites selected for AMW activity. It was possible to take sediment samples only in Split, whereas for the other ports the project Partners will be involved.

Biomonitoring cages containing mussels

"There is a clear potential to discharge through ballast waters other chemical products, including organotins compounds, into the sea."

Sediment sampling with Van Veen grab

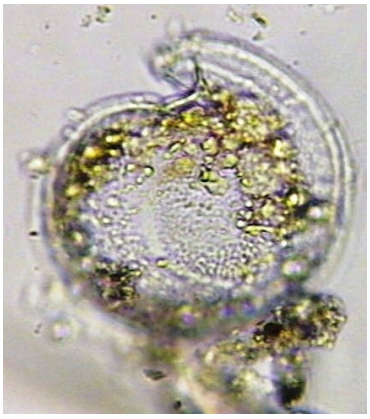


Sampling sites in the Adriatic area

Alfonso Scarpato, Giulia Romanelli, Daniela Berto*

**Italian National Institute for Environmental Protection and Research (ISPRA), Rome, Italy*

BALMAS project presented at the XVII plenary meeting of EU National Reference Laboratories for Marine Biotoxins



Gambierdiscus recognized as the main source of toxins responsible for ciguatera (Copyright by Marine Research Centre Foundation)

"The project is perceived as a tool to investigate the possible introduction or geographic spread of certain marine algae which are able to produce toxins .."

The 2014 plenary meeting of EU National Reference Laboratories for Marine Biotoxins was held on October 23rd and 24th in Lisbon, Portugal. The meeting was attended by 33 representatives from 23 different European countries (22 EU Member States + 1 EFTA country). On that occasion the Marine Research Centre Foundation of Cesenatico, as National Reference Laboratory for Italy, presented an outline of BALMAS project and its main goals. Particular attention was paid to activities concerned with Port Baseline Surveys and monitoring of Harmful Aquatic Organisms and Pathogens, which could provide information about the presence and possible negative effects of unwanted species in Adriatic Sea ports and surrounding areas as hot spots of ballast water discharges. The project is perceived as a tool to investigate the possible introduction or geographic spread of certain marine algae which are able to produce toxins responsible for an array of human illnesses associated with consumption of seafood and, in some cases, respiratory exposure to aerosolized toxins. Results gathered from project activities are of interest to a wide range of stakeholders, including the Emerging Risks Exchange Network (EREN) established by the European Food Safety Authority (EFSA) in 2010. The network already recognized, for example, that the geographic expansion of the distribution of the algae (*Gambierdiscus* spp.) capable of producing ciguatoxins is a concern at EU level and particularly for the Mediterranean and that Ciguatera Fish Poisoning is likely to become more frequent in Europe through new and increased exposure to ciguatoxins accumulated in fish contaminated in local waters.

Silvia Pigozzi*

**Marine Research Centre Foundation, Cesenatico, Italy*

'PORTO APERTO' conference, Ancona 24-25 October

Research vessel G. Dallaporta CNR Italy

In the framework of the European project **Intermodadria**, the Autority Port of Ancona organized a Conference at the Muse theatre, in order to present to the general public, in particular to the students, all the activities linked to the Ancona port and the future job opportunities. Seminars were carried out by many specialists with different port professions, such as: Maritime Agency, Customs Agency, Finance Police, Agency for Maritime Transports and Maritime Health Agency.
As a part of this event the CNR was involved to present the BALMAS project, as an important European project addressing an ecological problem connected to the ports: the ballast waters risks and management. During the event a permanent stand was set up with BALMAS banner, brochure and gadgets to inform people about the project and main activities. More than 100 people took part at the Conference and visited our stand: high school students, Coast Guard, navy representatives, the Mayor, the council members for port and environment, other stakeholders and citizens.
In particular, the curiosity of students and professors was stimulated and many questions arose regarding not only the mission of the project, but also on the meaning of ballast waters and non-indigenous species.

Elisa Baldrighi*

**Institute of Marine Science (CNR-ISMAR), Ancona, Italy*

Compiled and edited at MBS Piran - NIB by dr. Vesna Flander Putrle, dr. Mateja Grego, and Vlado Bernetič

More about:
http://www.balmas.eu/
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