BALMAS

Ballast water management system for Adriatic Sea protection

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BALMAS overview

The United Nations had recognized the transfer of harmful organisms and pathogens across natural barriers as one of the four greatest pressures to the world's oceans and seas, causing global environmental changes, and posing threat to human health, property and resources. Ballast water transfer by vessels was recognized as a prominent vector of such species, and was regulated by the International Convention for the Control and Management of Ship´s Ballast Water and Sediments, 2004 (BWM Convention). The BWM Convention sets the global standards on ballast water management (BWM) requirements, while recognizing that regional and local specifics have to be considered for its effective implementation. The Adriatic Sea is a unique and highly sensitive ecosystem. The economic development and social existence of the coastal states strongly depend on the clean and preserved Adriatic Sea. However, the Adriatic Sea is also a seaway mainly used by international shipping transporting goods to or from Europe as hinterland, with also intense local shipping. Increasing, serious concern is the introduction of harmful aquatic organisms and pathogens (HAOP) by ships' ballast water. By developing a joint Adriatic Ballast Water Management Decision Support System, Ballast Water Management Plan and Strategy, BALMAS will ensure uniform BWM requirements to ease shipping and at the same time to maximize environmental and economic protection of all sea users. The general BALMAS objective is to establish a common cross-border system, which will link all researchers, experts and responsible national authorities from Adriatic countries in order to avoid unwanted risks to the environment from the transfer of HAOP. This can be achieved through control and management of ships' ballast waters and sediments. Further, long-term effective ballast water management (BWM) in the Adriatic will be set at the cross-border level utilizing this project's related knowledge and technology.

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1. INTRODUCTION

Since movement of harmful aquatic organisms and pathogens is identified as one of the greatest threats to the marine environment and ballast water is one of the most frequent vectors for its spreading, ports represent extremely sensitive area for HAOP introductions and its further spreading. Ports are often the first places where invasive marine species are introduced and sometimes are maintained and established. Biological invasions in marine habitats change the integrity of native communities, represent threat to the economy and even to human health. Invasive species are believed to accelerate the decline of native populations already under environmental stress, leading to population losses and extinctions on a local scale (Ricciardi, 2004).

Ballast water is an increasing problem in the Adriatic Sea. The amount of ballast water discharged from one average merchant ship per voyage is about 30% to 40% of her deadweight. About 5.6 million tons of ballast waters were discharged in the ports of Adriatic Sea in 2003 and more recently discharge has reached 10 million tons for year

In order to significantly reduce new species introductions by ship ballast water, the International Maritime Organization (IMO) developed the Ballast Water Management Convention (BWMC), which provides a new international legal regime to address this threat. IMO released guidelines that encourage the port states to undertake Port Baseline Surveys (PBS).

PBS are used to develop a baseline list of species including both native and non-indigenous (NIS) species present in ports, to develop the list of Harmful Aquatic Organisms and Pathogens (HAOP) and to ensure qualitative base for tracking the new species introductions. Typically surveys of biota include sampling of several different groups of organisms: hard substrate organisms, soft bottom benthos, plankton and mobile epifauna (*e.g.* fish). All these species groups should be surveyed in a comprehensive sampling protocol.

Suggested sampling protocol is based on CRIMP protocol (Hewitt and Martin, 2001), which is successfully applied in tropic and temperate marine environment, requirements of guidelines for ballast water sampling (IMO, 2004) and requirements of risk assessment (IMO 2007). CRIMP protocol relies on scuba diving sampling technique and may not be applicable in all ports when alternative sampling techniques can be used. The objectives of PBS protocol are: outline the steps that should be taken for baseline survey, specify the abiotic and biotic parameters, which should be analysed, quoted methods and describe the report format.

Suggested sampling protocol in the frame of BALMAS project will be applied in 12 ports of the Adriatic Sea: Bari, Ancona, Venice and Trieste in Italy, Koper in Slovenia, Pula, Rijeka, Šibenik, Split and Ploče in Croatia, Bar in Montenegro and Durrës in Albania.

2. DEFINITION OF THE AREA TO BE SURVEYED

Ports are located in a variety of costal environments ranged in size, pollution, urbanization, configuration of the bottom and in the facilities that are present within them. Non-indigenous species and their different life stages can occupy different habitats and could be dispersed by currents or by their own species movements. They may also occur in natural adjacent environments. First step in developing a design plan is to decide on the extent of survey area. All possible locations where HAOP could be introduced should be taken into account. Questions which should be considered in the definition of an area to be surveyed are:

- Where are the areas in the ports where shipping operations might result in the release of BW? This may include berths were cargo is loaded and unloaded, sea buoys or anchorages where ships wait to enter the port, port marks or points on approach where ballast is discharged.
- Where shipping related activities occurred in the past? This may include wrecks, anchorages for sailing ship.
- Where other vectors for NIS as aquaculture and disposal of material dredged from the port are located?
- How diverse are the habitats nearby?
- How easily and safely those habitats can be sampled?
- What is the rate of water exchange between ports and surrounding areas?
- What resources are available?

Area to be surveyed should be defined by the scientific team in consultation with port authority and preceded by a review of shipping operations and existing information on biological habitats and community composition within the area.

3. SAMPLING SITES

3.1. Distribution of sampling sites

The distribution of sampling sites in the port area should follow a stratified sampling design (Mc Maugh, 2005) in which the sites are divided into logical categories as habitats types and selection is chosen from each category. According to the CRIMP protocol, all the different habitat categories (both soft and hard substrates) should be sampled, as well as all the different habitat types (or structures) of colonization belonging to each category (soft seabed, wharves, breakwaters etc.). Thus, attention should be given to sample all main substrate types available in the port. Special attention and increased sampling efforts should be allocated to the following high priority area types: active berths, inactive/disused wharves, channel markers, tug and pilot vessel berths and slipways; for some specific biological parameters (i.e. NIS seaweed), adjacent areas outside the port should be considered (CRIMP protocol, Hewitt and Martin 2001) (Table 3.1.1.) with high priority. Priority of sampling locations should be defined according to their vulnerability to colonization by the specific biological parameter, so the priority value reported in table 3.1.1. could change depending on the considered NIS. Hydrodynamics condition within port and water exchange between port and surrounding area should be taken into account when selecting sampling sites.

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

Port area	Priority
Commercial shipping facilities	
active berths	1
inactive/ disused wharves	1
channel markers	1
tug and pilot vessel berths	1
Slipways	1
Dredge disposal and spoil ground	2
Breakwaters, groynes etc. for NIS seaweeds priority **1**	3
Adjacent areas outside the port *	
nearby natural habitats**	2
off-shore exposed areas*	2
anchorages	1

3.2. Number of sampling sites

The field sampling will be conducted in a number of sampling sites, or exact locations, within a port. The number of sites required for an adequate survey will depend on the size and type of port and, ideally, on the biological parameter investigated. As a minimum requirement, at least three sampling sites for each biological parameter per port should be selected.

3.3. Abiotic parameters

Minimum requirements for abiotic parameters are temperature and salinity measurements at each sampling site. In addition, water transparency should be measured using a Secchi disc. In order to ensure a better characterization of environmental conditions in ports, nutrients, oxygen and chlorophyll 'a' concentrations could be measured.

Sediment samples could be taken for analysis of grain size and organic content. Sediment samples will allow characterization of the habitats associated with any introduced epifaunal or infaunal species found.

3.4. Biotic parameters

The following groups of organism should be sampled:

- Human pathogens bacteria (Toxicogenic *Vibrio cholerae* (serotypes 01 and 0139) *Escherichia coli,* Intestinal *Enterococci*)
- Plankton (phytoplankton, zooplankton, ichthyoplankton)
- Dinoflagellate cysts
- Epibenthos and fish community
- Benthic flora and fauna (seaweeds, seagrass, invertebrates)

4. FREQUENCY AND TIMING OF SAMPLING

Due to seasonal distribution of marine organisms and life cycles patterns of different life forms, sampling should be performed at least twice per year. Dinoflagellate cysts, epibenthos and fish community, fouling organisms, seaweeds and benthic infauna should be sampled in spring and autumn period. Plankton communities should be sampled 3-4 times per year (seasonal frequency). Human

pathogens should be sampled at least four times per year in the water and two times per year in sediments (simultaneously to the water sampling). Sampling of zooplankton for the search of toxicogenic *Vibrio cholerae (serotypes O1 and O139)* is optional. If the proper sampling (according to PBS Protocol) has been done in last 3 years, obtained data could be used for PBS.

5. FIELD SAMPLING

Environmental data

GPS location of each of the sampling site should be recorded using the **WGS84** coordinate system. Temperature and salinity at sampling stations should be measured by CTD probe or submersible data logger. Water transparency should be measured using a Secchi disc. Sediment could be collected using dive transects, grabs or cores.

Human pathogens

Water sample of 1000 ml from at approximately 30 cm depth should be taken at each site. Sampling should follow the guidance described in the EU Bathing Water Directive 2006/7/EC. Sediment (surface layer) could be collected using grabs or cores.

Phytoplankton

Samples should be collected using phytoplankton net (mesh size 20 µm) to concentrate sample for qualitative assessment and to estimate the semi quantitative analyses on a scale 1-5 (1=rare, 5=very abundant). One vertical tow should be done at each site. In order to ensure adequate sampling, horizontal tows could be performed. Horizontal tows should be performed at approximately 2 m below the surface and should be conducted at speed of approximately 0.30 m s^{-1}. In order to ensure accurate quantitative analyses one sample per each stations could be sampled by bottle sampler or PVC sampler (hose). Samples should be preserved or kept at low temperature and returned to laboratory for incubation and culturing depends of further analyses. Phytoplankton samples should be analysed according to the Utermöhl method (Utermöhl, 1958).

Zooplankton

Vertical zooplankton net tows with a mesh size appropriate for the area (a standard 200 µm or smaller if applicable) should be used for collecting zooplankton samples. Only one sample at each station should be collected to ensure for adequate sample. Mesh size depends on the size range of zooplankton in the area and needs to be reported with the data. Tow rate should be adjusted to approximately 1 m s^{-1} and the net should be stopped 1 m above the bottom.

Ichthyoplankton

Ichthyoplankton samples should be collected by vertical net tows with 300 µm mesh size. Three vertical tows, 10 to 15 m apart should be conducted to ensure a qualitatively and quantitatively adequate sample size.

Dinoflagellate cysts

Surface sediment for dinoflagellate cysts determination should be collected by gravity corer such as Phleger corer or using diver to collect sediment core by hand. Samples could be collected also using Van Ween grabs from which on the vessel corers are taken. At each station a minimum of two replicates should be taken. Recently dredged areas should be avoided. If the germination of cysts is not to be performed, the raw sample should be fixed as soon as possible to avoid the change of composition (ratio of living and empty cysts) by excystment. Formalin or glutaraldehyde should be used as fixing agent (Matsuoka and Fukuyo, 2000).

Mobile epifauna and fish community

Mobile epifauna, such as crabs, fish and shrimps should be sampled at each site using traps. Traps are selective in nature and therefore provide only relative measures of species abundances. However, methodology for sampling epifauna in the port area is very limited and for example using trawls and gillnets is not possible. Attention should be given to place traps on all available substrates including mud, sand and rocky substrates. Traps should be weighted and signed by surface buoy and could be baited with locally abundant fish. Traps should be tied securely to wharfs or other structures. Three traps should be deployed at each site for at least 48 hours. Catch should be identified and stored in a cooler. Later in the laboratory, species identification should be performed and specimens should be measured, weighed, prepared and preserved. Fish and larger invertebrates can be frozen, smaller invertebrates preserved.

Visual searches, either by divers or using drop-down video equipment, should be conducted at each site. Divers should swim at 10 m transects along the dock/shore at several depths ranging from 0 m to the bottom to record presence and number of any fish species including eventual non-indigenous taxa). Trials will be performed along hard substrates, taking note of the water turbidity and bottom characteristics. Ideally, the sampling design should include three random locations within each harbour and three replicate transects (randomly allocated within each location) for a minimum number of 9 transects/harbour.

Trammel net should be used to sample near-shore fishes. The technical parameters of the net (mesh size, height, etc.) should be free on the basis of the expert judgment on each sampling site.

Flora and fauna along vertical transects

Scuba sampling of flora and fauna should be conducted at each sampling site if possible. Below is the cited sampling procedure according to the CRIMP protocol. Piles or projecting steel facings and dolphins associated with wharves are to be accorded a high priority in sampling. For each berth three transects should be selected to provide a series of vertical samples. The first transect should be located about 10 m from the end of the berth to reduce 'edge effects', and subsequent transects should be at a spacing of 10–15 m. In the case of dolphins that may be separated by more than 10–15 m, samples should conform to the available spacing. Where a wharf or berth has inner and outer rows of piles, the inner piles should be surveyed visually. Prior to sampling, the selected transects have to be marked with paint above the high water mark, their positions recorded and the overall site photographed. For each transect the following, protocols should be followed:

1. Three sampling frames are fixed to the outer surface of the pile if possible at three depths from the surface using bungee cord or some other suitable material. Sampling frames cannot easily be fixed to facings and will need to be held by divers and the outline scraped into the biota.
2. A video transect (optional) of the outer surface of each pile/facing should be made from approximately high water down to the deepest exposed part of the pile/facing using a video camera/recorder in an underwater housing. The camera is maintained at a constant distance (approx. 0.5 m) from the surface of the pile using a distance measuring rod. A scale and depth

meter attached to the rod is positioned so that they fall within the field of view of the camera. Care should be taken to ensure that reflected light does not obscure the readout on the depth meter. The vertical transect should include the three sampling frames and when possible the video camera should be used to record close-ups of the sampling frames by using the zoom capabilities of the camera and scanning the surface within the sampling frame for increased resolution.

3. Photographs should be taken to provide high resolution records of the fouling communities. Photographs of the sampling area within sampling frame should be made prior to destructive sampling. Additional photographs of adjacent area should be made in conjunction with qualitative sampling of fouling communities.
4. Quantitative destructive sampling of the flora and fauna should be made by carefully scraping the organisms inside each sampling frame into a collection bag. These samples are used to provide a detailed analysis of the flora and fauna at specific depths.

All the organisms should be collected and scraped from the vertical bottom within a frame of at least 20 cm x 20 cm. Such a surface (400 cm^2) is considered to be the minimal sampling area in the case of the Mediterranean communities (Bianchi et al., 2003; Boudouresque and Belsher, 1979). However, according to the CRIM protocol, a larger sampling surface of 0.1m^2 can be considered for collection and scraping field operations and then results will be reported to 400 cm^2 when necessary. Three sampling surfaces have to be scraped on vertical hard bottom, precisely one sampling surface for each selected depth on each transect. The distribution of samples according to depth can be customized according to specific depths of wharf and hydromorphological conditions of the area. For example, the Gulf of Trieste is characterized by the largest tidal differences (semidiurnal amplitudes approach 30 cm) in the Mediterranean Sea. Therefore, at least one sample should be collected in the mediolittoral belt.

Samples should be collected and kept chilled on ice or transported immediately back to the laboratory for processing. If necessary, specific faunal samples should be preserved directly in 90% alcohol or narcotized with isotonic magnesium chloride or menthol for at least one hour prior to formalin preservation, as appropriate within 8 hours of collection.

Scraped samples should be preserved in its entirety (flora and fauna) in a jar and sent to the laboratory for sorting, which will be carried out by taxonomic experts on the animal component before and then passed to the macroalgal experts (or vice versa). In fact, particularly in the presence of encrusting and/or turf NIS seaweeds, it is often very difficult (sometimes impossible) to separate the two components without losing macroalgal species. Once the sorting and estimate of coverage of macroalgal component is being done in the laboratory, the erect/turf species should be pressed in herbarium paper and the fouling encrusting species should be preserved in 4% formalin seawater, in order to create an “archive“ of the seaweeds identified.

In case of very low visibility or other apparent safety issues, other methods can be used. *Rapid assessment sampling* protocol may be a suitable qualitative sampling method for hard substrate organisms at sites of low visibility.

Flora and Fauna along the horizontal transects

Benthic infauna should be sampled along a 50 m transect (10 m optional if necessary) using scuba diver and hand corer. Transect of 50 m should be laid on the bottom perpendicular to the shore starting at sampled vertical transects. Six samples should be taken at each site, along three horizontal transects, located at least 15 m distance from each other. At each transect, two hand corers (by divers) should be taken: one at 50 m from vertical transect and one at 1 m from vertical transect (figure 5, Annex I).

Visual searches to locate and collect macroscopic NIS flora and fauna should be undertaken at along the same transect. If possible, transect can be video recorded and species photographed. A rough estimate of the abundance can be carried out directly in the field by evaluating percentage coverage. If visibility is less than 1m, visual searches and video recordings of transects are unlikely to be a practical option. In these cases, some randomly samples should be taken (i.e. using a van Veen grab) in the port area according to a stratified design, in order to search for a possible colonization of the soft bottom.

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

Table 2- Modified CRIMP sampling methods adopted for BALMAS project purposes

Taxa sampled	Method	Habitat				
		Soft sediment	Hard substrata	Seagrass/ algae	Plankton	Wreck
Dinofl. cysts	Small core (2.5 cm diam.)	X				
Benthic infauna	Large core (18 cm diam) Medium core (4.6 cm diam) for meiofauna	X		X		
Phytoplankton	Plankton net (20 µm)				X	
Zooplankton	Plankton net (200 µm or smaller)				X	
Ichthyoplankton	Plankton net (300 µm)				X	
Mobile epifauna	Traps	X	X	X	X	
Macrobiota	Visual survey	X	X	X		
Sedentary biota	Quadrat scraping		X			
Sedentary biota	Video transect	X	X	X		
Mobile epifauna/fish	Trammel net	X	X	X	X	

In the frame of benthic infauna, meiofauna could be sampled. The locations for the sampling of meiofauna (38 µm -1 cm) are the same as described for the **benthic infauna**.

If possible, meiofauna samples should be taken by SCUBA divers (Fleeger et al., 1988). Divers usually obtain better quality samples because they are able to position the samplers with care and insert the corer slowly (McIntyre, 1971). However, if diving is not possible, a gravity type corer from the boat may be used instead.

The cores have an inner diameter of 4.6 cm, with the length of at least 20 cm. Samples should be taken at least in three replicates, due to patchy distribution of meiofauna (Giere, 2009).

Animals are retrieved from the sediment with centrifugation in Levasil®- distilled water density medium (specific density = 1.17g/cm³). The fauna should be stored in 70% ethanol or in borax buffered 4% formalin.

Public awareness program

Local and overseas experience indicates that conspicuous pest species are rarely first detected by scientists, but more often by fisherman, marine farmers, dive clubs and local communities for sea recreational activities. Thus, it could be very useful to involve local community with data sheet/tracking tabs and with interviews. The initiation of a public awareness program prior to the commencement of a port survey generally provides opportunity for this information to input into design of the survey; however, collecting this kind of information can be very useful during all the different phases of the project.

6. ALTERNATIVE METHODS

Some methods cited in the CRIMP protocol are not suited for all port environments mostly due to poor visibility, hazard of pollution or dangerous marine animals. In these circumstances alternative methods should be used. Alternative methods for PBS according to Inglis and Floerl, 2008 are listed in Table 6.1.

Table 3 - Alternative methods for Port Baseline Survey

Alternative method	Replaces CRIMP method
Gravity corer	Small sediment core
Benthic grab	Large sediment core
Epibenthic sled	Dive transect
Beam trawl	Dive transect, poison station
Box crab trap	Dive transect, poison station
Fish trap	Poison station
Starfish trap	Dive transect

Visual counts (by video recording or by divers) and local ecological knowledge could be used as alternative methods for sampling of mobile epifauna and fish communities.

Soft bottom macrozoobenthos

The macrozoobenthos should be collected in three replicates using a van Veen grab (surface area of 0.1 m^2). Mesh size of 1 mm (or smaller if meiofauna will be analysed) should be used to sieve sediment for soft bottom macrozoobenthos analyses. At least 3 sediment cores should be collected from each site.

Interviews with local fisherman

Local and overseas experience indicates that conspicuous pest species are often detected by fishermen, marine farmers, dive clubs and local communities for sea recreational activities. Thus, it could be very useful to involve local communities in reporting eventual sightings of non-indigenous species. Data collection will be performed trough structured interviews, according to the 'Local Ecological Knowledge' approach (Azzurro et al., 2011).

7. SAMPLE PROCESSING AND ANALYSES

All taxa should be identified and non-indigenous species should be determined to the lowest taxonomic level possible. Species abundance should be reported as number of individuals per volume or weight of sediment. If the abundance data are not available, abundance should be estimated using a scale from 1-5 (1=rare, 5=very abundant) or percentage scale.

Species abundance of native and non-native macroalgal species should be reported in percentage coverage of the 400 cm^2. If very abundant and stratified presence of invasive species (i.e. *Caulerpa racemosa* var. *cylindracea*) is detected (coverage >100%), values of abundance should be expressed as dry weight biomass ($g_{dw}\, m^{-2}$).

Human pathogenic bacteria

Sample analysis and processing should follow the EU Bathing Water Directive 2006/7/EC. Analysis of *Vibrio cholerae* may require specialized laboratory.

Plankton

Sample processing and species identification should be conducted in laboratory according to standard methods and laboratories' best practices. In order to ensure the lowest taxonomic level identification, molecular analyses and electron microscopy are encouraged to be used in addition to conventional analyses by microscope and stereomicroscope.

Dinoflagellate cysts

Cleaning and concentration of cysts from sediment samples should be done by sieving procedure or palynological method (Matsuoka and Fukuyo, 2000). In order to verify the taxonomy identification, the germination of cysts could be performed.

Mobile epifauna

Species identification should be done from the preserved samples and/or photographs.

Hard substrates

Scraped samples and settlement plates should be qualitatively analysed by taxonomic specialist and experts. The list of observed taxa and if possible their coverage and dry biomass per unit of area should be reported.

Seaweeds

In the laboratory the three scraped sampling quadrats have to be reconstructed and species composition and abundance of all macroalgae (native and non-native) shall be determined; abundance must be evaluated as orthogonal projection of each species and expressed as percentage coverage of the 400 cm^2 quadrat surface (Boudouresque, 1971). In the case of species showing percentage coverage < 1%, abundance can be considered negligible and species listed only as a presence. Alternatively, percentage cover of macroalgal taxa can be evaluated by means of analysis of photographic samples using the image processing programs such as "Image J" or "Vision 1.0" (Rende et al. 2009). Nevertheless, due to the low detection power of the visual census technique, (the species level is often not recognizable on the photo samples), this kind of analysis should be always supported by the destructive sample analysis, in order to link the identification of a given species in the destructive sample with the percentage cover observed in the photographic one.

Photographic samples should also be used to give a rough estimate of the abundance of NIS seaweeds found on the soft bottom.

After sorting, the erect/turf species can be preserved in the herbarium paper and the fouling encrusting species in 4% formalin seawater, in order to create an "archive" of the species identified. A general archive of species for the project (so all species, native and NIS) or an archive only for NIS

species can be created. Or even make an archive material (herbarium and samples in formalin) for NIS and a "digital" one for native species.

Soft substrates

Samples should be analysed and processed by taxonomic specialists, scientists or technician experts in laboratory. All non-indigenous species in the samples should be identified. Results should be reported as abundance or estimated scaled abundance 1 – 5 (1=rare, 5=very abundant) or on a percentage scale.

8. REPORTING OF RESULTS

The list of taxa recorded through previously conducting studies in ports and adjacent areas should be included in the results of PBS. Results will be reported in Standard Report Format and Data Sheets. Data sheets should be organized as matrix of list of species and surveyed ports.

Standard Report Format

1. Executive Summary
2. Introduction
3. Objectives
4. Description of the port
    4.1. General features
    4.2. Climate and environmental conditions
    4.3. Hydrodynamic conditions
    4.4. Shipping movement
    4.5. Port development and maintenance
5. Methods
6. Results
    6.1. Port environment
    6.2. Review of existing biological information
    6.3. Native biota
    6.4. Non-indigenous species
    6.5. Cryptogenic species
    6.6. New and unidentified species
7. Translocation risk
8. Recommendations
9. Future surveys and monitoring
10. References

More details concerning the Report format is available in Inglis and Floerl, 2008.

9. CHEMICAL ANALYSES

9.1. Analyses of biocides (organotins) and disinfection by-products from chlorine treatment (Trihalomethanes, haloacetonitriles and haloacetic acids)

9.1.1. General considerations

It has been widely shown that the transport of ballast water (BW) in ships is one of the most widespread mechanisms by which introduction of non indigenous and/or harmful aquatic species occur. The volumes of ballast water taken-up, transferred and discharged into the world's oceans each year far exceed the volumes of any other ship-sourced discharge regulated by IMO, such as Liquid Noxious Substances under Annex II of MARPOL, even if the lowest estimates for ballast water of around 3 billion tonnes per year are accepted (billion = 10^9).

The use of ballast water treatment systems that make use of active substances on-board of ships requires, *inter alia*, a prior assessment of the environmental and health risks which is preformed via a regulatory system for the approval of methods using active substances (GESAMP – Ballast Water Working Group and MECP at IMO). In its document presenting information on the Database developed by the GESAMP - Ballast Water Working Group on chemicals most commonly associated with treated ballast water, the WG noted that many disinfection by-products (DBPs) are commonly found in treated ballast water, irrespective of the technology used in the ballast water management system. In this document, the GESAMP-BWWG prioritized DBPs based on occurrence, frequency and concentrations encountered in its evaluations of ballast water management systems, and selected substances and DBPs in the first phase of the database development among those belonging to the class of haloacetic acids, trihalomethans, haloacetonitriles (IMO, 2013).

As regards to the chemical disinfection options, most of the traditional biocides produce by-products reacting with inorganic and organic fractions in seawater, which are likely to be toxic to the aquatic environment (Gollasch, 1998). Consequently, associated health, safety and environmental considerations need to be taken into account (Müller 1995, Müller & Reynolds 1995).

Additionally, 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 (Ranke, 2001)). The same mechanism might also cause the introduction of chemical pollutants to other regions (Ruiz et al., 2001). Since early 1970s, tributyltin (TBT) has been used as antifouling agent in boat and ship paint applied to the hulls of vessels. In October 2001 the IMO Diplomatic Conference passed a global treaty on the 'Control of Harmful Antifouling Systems on Ships' to ban the application of organotin antifoulants by 2003 (IMO, 2005). Nevertheless, virtually all the regulations on the use of TBT paints have not been applied to the ballast tanks (IMO, 2005).

In fact it was demonstrated that the media 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 (Hua and Liu, 2007).

Already in 2002, GESAMP (IMO, 2002) stated: “In ports frequented by tankers and large freight vessels, huge volumes of BW are regularly discharged within relatively small areas. Thus, if biocide treatments become a regular feature of BW management, there is scope for local marine ecosystems to be constantly exposed to chemicals remaining in the BW when discharged. Even if residual concentrations are undetectable (chemically and/or biologically), or considered acceptably low, the possibility of chronic effects from long-term exposure cannot be discounted. Accordingly, environmental protection authorities of port states should consider periodic monitoring in the vicinity of ports to detect any abnormalities within benthic communities (e.g. loss of biodiversity, reductions in recruitment etc.).”

Considering the semi-enclosed nature of the Adriatic Sea together with the long residence times of its waters compared to the open ocean, problems may arise that are connected to ballasts water contamination.

9.1.2. Field surveys

Chemical baseline activities in ports will comprise the analysis of organotin compounds, brominated and chlorinated by-products (trihalomethanes, haloacetonitriles and haloacetic acids) in selected ports (WP5) and in ballast waters sampled from ships’ tanks (WP7).

All the activities related to the mussel watch materials and chemical analysis will be charged to ISPRA. ISPRA asks project partners collaboration for: 1) sampling activities including logistic support; 2) selection of sampling sites where to locate the mussel cages taking into consideration the specificities of each port. A workshop on ports chemical surveys will be organized during the third meeting of the project (Kotor, October 2014).
Only one chemical survey in ports is scheduled, as a baseline survey.
Surface sediment sampling will be carried out in conjunction with the biological survey (October 2014 campaign).

Mussel cages for mussel watch will be settled during the same survey, in October 2014. FB5 (CNR, Italy) will be responsible for the field campaign in Italy and in the eastern Adriatic countries aimed at settling the mussel cages in October 2014. FB3 (ISPRA, Italy) will be responsible for the general activity of chemical surveys in ports and will take care of the recovery of the mussel cages. The cages will be recovered during a second campaign scheduled 3 months after the settlement (January 2015).
Seawater sampling will be performed in conjunction with mussel watch settlement.
Water and sediments samples from ballast waters in ships’ tanks (WP7) will be taken if possible in correspondence of the biological surveys.

9.1.3. Site selection

Chemical baseline surveys will be carried out by ISPRA in seven ports: two Italian ports (Ancona and Bari), two Croatian ports (Rijeka and Split) and one port each in Slovenia (Koper), Montenegro (Bar) and Albania (Dürres).
The number of stations (a maximum of three per port) and their location will be chosen based on the results of a preliminary study of the ports’ characteristics (maritime traffic, logistic area) and indications by local maritime authority.

9.1.4. Field sampling

Sediment

The chemical contamination baseline of organotin compounds and brominated and chlorinated by-products will be evaluated in selected Adriatic ports, through analysis of surface sediments.
Surface sediments will be sampled in selected sampling sites (in duplicate) for each analyses. The upper 2 cm will be recovered and placed in polyethylene falcon tubes (50 ml) and stored in the dark at -20 °C until analysis for TBT and haloacetic acids. Thrihalomethanes and haloacetonitriles: the upper layer of sediment sampled, rapidly collected and inserted in an apposite 40 ml vial for volatiles analysis, will be stored at +°4C until the analysis that will be performed no later than 15 days from the sampling date.

Mussel watch methodology

As filter feeder organisms, mussels are able to bioaccumulate chemical contaminants, with an accumulation degree proportional to the relative occurrence of these substances in the surrounding environment.

In the last two decades, the use of indigenous populations of wild or cultivated mussels in monitoring programs to assess levels and trends in the chemical contamination of coastal waters has been successfully applied. More recently, “active bio-monitoring strategy” has been adopted, based on mussels transplantation from unpolluted or not impacted areas to selected coastal zones, characterized by potential environmental impact due human activities (Romeo et al., 2003, Scarpato et al. 2010). The advantages of adopting this technique are mainly to be referred to 1) the often scarce natural mussel stocks in several coastal areas, 2) the control of factors like: exposure times, depth of caging, age, size, stage of sexual maturity (immersion should preferably take place during the period of sexual dormancy), and other physiological factors that can interfere and affect the accumulation mechanisms of contaminating substances. Bioaccumulation phenomenon is influenced by environmental conditions, such the trophic state of the water in which mussels are immersed. Data standardization procedures are applied, based on the use of the Condition Index (CI) that is a biometric variable closely related to tissue concentrations of most contaminants (Andral, 2004).

Mussels to be transplanted come from an aquaculture farm. The batch is made up with adult mussels 18–24 months old, of standardized shell size (50 ± 5 mm). Before transplant, mussels (an amount of approximately 3 kg), are collected and stored in polyethylene bags, then re-immersed in situ for ten days to permit them to re-cluster; this practice aims to reduce the mortality risk during transplantation.

Mussel cages are then transported from the farm to the oceanographic vessel and maintained on board in a tub. Finally, the cages are immersed in selected sites in the ports.

During recovery, mortality of the mussels and other biometric parameters are recorded, in particular: length, width, and height of the shells. Tissues of an adequate number of mussels are separated from the shells, divided in shares, weighed and then frozen at -20°C in polyethylene falcon tubes (50 ml).

In order to determine the Condition Index (C.I.), 15 mussels are chosen and the height of each shell is measured. Shells are cleaned up by any remaining flesh and limestone, then dried at 60°C for 48 hours, and weighed. The ratio between the dry weight of the flesh and the dried weight of the shells represents the Condition Index.

Seawater samples

Water samples (2 l) will be collected in selected ports next to the mussel cages and for each station in duplicate for each analyses. Samples will be acidified (HCl, pH ~ 2) for organotins analysis and stored at -20°C until the analysis in the dark.

Quantity of seawater sample to be collected for analysis of haloacetic acids is 1 liter in duplicates, in a dark glass bottle. NH_4Cl must be added to the sample as preservative and stored at a temperature of +4C until analysis.

Seawater for analysis of trihalomethanes and haloacetonitriles must be collected in appropriate 40 ml vials for analysis of volatiles compounds; NH_4Cl must be added to the sample as preservative and pH adjusted to 4.5-5.0 by adding HCl. Samples must be stored at +4°C until analysis.

9.1.5. Analytical methods

Chemical determination of organotin compounds in sediments, biota and seawaters will be performed through a modified version of the method reported in Morabito et al., (1995) and Binato et al., (2007) (cfr. Boscolo et al., 2004, Berto et al., 2007; Appendix 1, Appendix 2).

Chemical analysis of haloacetic acids in seawater will be performed according to a modified EPA method 552 (*Determination of haloacetic acids and dalapon in drinking water by liquid-liquid microextraction, derivatization, and gas chromatography with electron capture detector)*.

Trihalomethanes and haloacetonitriles in seawater will be performed according to EPA Method 5030 (*Purge-and-Trap for Aqueous Samples*) and EPA method 8260b (*Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry*).

Chemical analysis of haloacetic acids in sediment will be performed as described in Scott B.F. et al, 2005 for extraction from sediment, and then according to EPA method 552 for instrumental analysis.

Chemical analysis of trihalomethanes and haloacetonitriles in sediment will be performed according to EPA Method 5035 (*Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples)*, and EPA method 8260b (*Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry)*.

Chemical analysis of haloacetic acids in mussel tissue will be performed by acid digestion, followed by solvent extraction and derivatization (EPA method 552 modified).

Chemical analysis of trihalomethanes and haloacetonitriles in mussels tissue will be performed by static headspace technique coupled with GC/MS detector (according to a modified version of EPA method 5021, EPA method 8270) or alternatively will be performed by Purge-and-Trap coupled with GC/MS detector (Roose et al., 1998, Appendix 3).

9.1.6. Expected results and proposal

The planned activities will provide the first data-set on concentration and distribution of biocides (organotins) and disinfection by-products (bromoderivatives and chloroderivatives) in Adriatic ports, which could be used as a chemical baseline for subsequent studies/monitoring activities.

Another result would be the capacity building within the Adriatic area for these type of surveys: ISPRA proposes to transfer to the other partners involved in the project the sampling and chemical methodology used through a parallel workshop during the second meeting of the project (Split, April 2014).

10. PHYSICAL ANALYSES – optional parameter

10.1. General considerations

Release of ballast waters represents a potential danger of allochthonous and/or toxic phytoplankton species input into the natural ambient. The intense dynamics of the Kvarner Bay increases the possible risk of dispersion of the potentially introduced species from the sources of BW release and/or species accumulation also to the wider area of the Adriatic. Hence at the location of BW release, as well as at the potential accumulation location, e.g. stagnant sea gyres, are important to be detected and intensity of water exchange in their vicinity estimated. In that sense we plan to make 3D high resolution numerical model (based on ROMS; e.g. Janeković et al., 2010) for the Kvarner Bay, nested into Adriatic Sea mode. Those models will be forced with realistic atmospheric model fields, all in order to gain as good as possible dynamical response in the project targeted regional sea. In addition, we will setup model to compute residence (renewal) time for the whole region during different seasons, identifying regions with the low exchange rate (as well as those with fast renewal dynamics), which should serve as guidance for optimal regions where the impact will persist for longer time and have semi permanent ecological impact on the system. In order to verify and test our model results, we will setup observation network using ADCP (Acoustic Current Doppler Profilers) with CTD at the bottom mounted current meter station for one year period. Those stations will be at the entrances into the Kvarner Bay giving unique opportunity to close the Bay synchronous, i.e. at the same time.

10.2. Analyses of hydrographic and dynamic conditions

Hydrographic condition and geostrophic currents – sampling strategy

For physical baseline survey obligatory abiotic parameters (temperature and salinity measurements) will be used. Frequency and timing of field sampling will be performed during each field sampling of the obligatory parameters (most probably with the seasonal frequency, i.e. 4 times per year). The number of stations and their location will be chosen based on the results of a preliminary study of the ports' characteristics (maritime traffic, logistic area) and indications by local maritime authority (as determined for abiotic parameters sampling). Dynamic depths of isobaric surfaces, as well as geostrophic currents distribution analysis will be carried out as an additional parameter solely by CMR-RBI in only one of the Croatian ports: Rijeka.

Hydrographic condition and geostrophic currents – data analysis

Based on density distribution in the water column the intensity of vertical convective motions will be estimated. For assessments of the geostrophic currents distribution dynamic depths of isobaric surfaces will be estimated based on density data, following the standard methods (e. g. Supić et al., 2000). Density will be calculated from temperature and salinity data.

Sea current and CTD measurements – sampling strategy

Sea currents and CTD measurements will be carried out as additional parameters solely by CMR-RBI at the main entrances of Kvarner Bay synchronically bringing unique possibility to calculate flux inside/outside during one-year period. Those valuable data will provide framework for estimate of the residence time as well as backmark for numerical model. In the case of winter strong bora outbreaks important for generation of dense water and ventilation (dense water sinking on the bottom rich with O_2) of deep parts of the Kvarner Bay we will be able to capture jets at the bottom mounted CTD probes.

Measurements will be deployed in the September/October 2014 for one year, until September/October 2015. During the period sea current will be sampled every 15 minutes with vertical resolution of 1 or 2 meters, depending on the station depth. CTD instruments will sample at the same temporal frequency as current meters capturing bottom density via temperature and salinity.

Sea Current and CTD – data analysis

After recovering of the instruments (planned during the last year of the project – Sep/Oct 2015) temporal and spatial (fine depth resolution) of current meter data will be performed. As measuring at the same time temperature and salinity we will analyze density flux in/out of the Kvarner Bay related to the currents.

Models and geostrophic currents

A comparison between geostrophic estimation and modelled currents will be provided by a descriptive method. We will compare geostrophic estimated current field with observations, during corresponding CTD casts encompassing current meter stations.

10.3. Reporting of results

Results of the PHYSICAL ANALYSES will be available in different periods of the project due to differences in the sampling frequency. Results concerning the hydrographic condition and geostrophic currents will be delivered until the end of April 2015 and will be completely included in the joined Report of the Port Baseline Study of all partners for the WP5, act 5.1. Joined report will include partial results concerning the Parameter Ivica, which will be completely delivered until the end of February 2016, in the report of the Port monitoring activities in the WP5, act 5.2.

In the first part of the project we will setup nested numerical model for the Kvarner Bay using high resolution both in vertical and horizontal direction. Some preliminary results will be reported within the Report.

Results of the PHYSICAL ANALYSES will contribute in the following parts of the joined Report: 4.3., 7. and 8. addressing the problem of the hydrodynamic conditions within port and water exchange between port and surrounding area, which should be taken into account in RA and DSS of the BALMAS project.

Literature

Andral, B., Stanisiere, JY., Sauzade, D., Damier, E., Thebault, H., Galgani, F., Boissery, P., 2004. Monitoring chemical contamination levels in the Mediterranean based on the use of mussel caging, Marine Pollution Bulletin 49, 704–712.

Azzurro E., Moschella P., Maynou F., 2011.Tracking signals of change in Mediterranean fish diversity based on Local Ecological Knowledge. Plos ONE 6(9) e24885. doi:10.1371/journal.pone.0024885.

Berto, D., Giani, M., Boscolo, R.; Covelli, S., Giovanardi, O., Massironi, M., Grassia, L. Organotin (TBT and DBT) in water, sediment and gastropods of the southern Venice lagoon (Italy). Mar.Pollut. Bull. 2007, 55, 425−435.

Bianchi, C.N., Pronzato, R., Cattaneo-Vietti, R., Benedetti Cecchi, L., Morri, C., Pansini, M., Chemello, R., Milazzo, M., Fraschetta, S., Terlizzi, A., Peirano, A., Salvati, E., Benzoni, F., Calcinai, B., Cerrano, C., Bavestrello, G., 2003. I fondi duri, in: Gambi, M.C., Dappiano, M. (Eds.), Manuale di metodologie di campionamento e studio del benthos marino mediterraneo, pp. 199-222.

Binato G., Biancotto G., Piro R., Angeletti R., 1998. Atomic adsorption spectrometric screening and gas chromatographic-mass spectrometric determination of organotin compounds in marine mussels: an application in samples from the Venetian Lagoon. Fresenius Journal of Analytical Chemistry, 361, 333-337.

Boscolo, R., Cacciatore, F.; Berto, D.; Marin, M. G., Giani, M. Contamination of natural and cultured mussels (Mytilus galloprovincialis) from the northern Adriatic Sea by tributyltin and dibutyltin compounds. Appl. Organomet. Chem. 2004, 18, 614−618.

Boudouresque, C.F. (1971). Méthodes d'étude qualitative et quantitative du benthos (en particulier du phytobenthos). *Tethys*, **3**: 79-104.

Boudouresque, C.F., Belsher, T.-. 1979. Le peuplement algal du port de Port-Vendres: Recherches sur l'aire minimale qualitative. Cah. Biol. mar. 20, 259-269.

Fleeger, J. W., Thistle, D., and Thiel, H.: Sampling equipment, in: Introduction to the study of meiofauna, edited by: Higgins, R. P., and Thiel, H., Smithsonian Institution Press, Washington DC, London, 115-125, 1988.

Giere, O.: Meiobenthology:the Microscopic Motile Fauna of Aquatic Sediments, 2nd. rev. & extended ed. Berlin ed., Springer, 2009.

Gollasch, Stephan. "Removal of barriers to the effective implementation of ballast water control and management measures in developing countries." Report prepared for the IMO MEPC Ballast Water Working Group. Short version (1998).

Hewitt, C.L., Martin, R.B. (2001) Revised protocols for baseline port surveys for introduced marine species: survey design, sampling protocols and specimen handling. Centre for Reasearch on Introduced Marine Pests. Technical Report No. 22. CSIRO Marine Researh, Hobart, 46pp.

Hua J., Liu S.M., Butyltin in ballast water of merchant ships. Ocean Engin. 2007, 34, 1901–1907

IMO (2004) International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004. International Maritime Organization, 13 February 2004. London: IMO.

IMO (2007) Guidelines for Risk Assessment under Regulation A-4 of the BWM Convention (G7). International Maritime Organization, Marine Environment Protection Committee, Resolution MEPC. 162(56), 13 July 2007. London: IMO.

IMO Assembly Resolution 868 (2002) Guidelines for the Control and Management of Ship's Ballast Water to Minimize the Transfer of Harmful Aquatic Organisms and Pathogens.

IMO, 2005. Anti-fouling systems. /http://www.imo.org/Environment/mainframe.aspS.

IMO, MEPC 65/INF.14 (2013) Information on the GESAMP-BWWG Database of chemicals most commonly associated with treated ballast water.Morabito R., Chiavarini S., Cremisini C., 1995. Speciation of organotin compounds in environmental samples by GC-MS. In: Quevauviller, P., Maier, E.A., Griepink, B. (Eds.), Quality Assurance for Environmental Analysis, cap. 17, pp. 435-464. Elsevier, Amsterdam.

Inglis, G., Floerl, O. 2008. Training Course on Port Biological Baseline Survey. Gebze-Kocaeli, Turkey, 21-24 October, 2008. 90pp.

Janeković, I., Dutour Sikirić, M.; Tomažić, I.; Kuzmić, M. 2010. Hindcasting the Adriatic Sea surface temperature and salinity: A recent modeling experience. Geofizika 27, 2, 85-100.

Matsuoka K. Fukuyo, Y. 2000. Technical guide for modern dinoflagellate cyst study (ver. 1.0 / Jan.1, 2002), Asian natural environmental science center, University of Tokyo. (http://dinos.anesc.u-tokyo.ac.jp/).

McIntyre, A. D.: Deficiency of gravity corers for sampling meiobenthos and sediments, Nature, 231, 260, 1971.

McMaugh, T. (2005). Guidelines for surveillance for plant pests in Asia and the Pacific. ACIAR

Monograph No. 119. Australian Centre for International Agricultural Research, 192 p.

Müller, K. & Reynolds, G. L. (1995): Disinfection of Ballast Water. A Review of Potential Options. Lloyd's Register, Rep. Summary, 38 pp. + appendices

Müller, K. (1995):, Disinfection of Ballast Water. A Review of Potential Options. Lloyd'scRegister, 29 pp. + appendix

Ranke, J. & B. Jastorff, 2000. Multidimensional risk analysis of antifouling biocides. ESPR – Environmental Science & Pollution Research, 7 (2): 105-114.

Ricciardi, A., 2004. Assessing species invasions as a cause of extinction. *Trends in Ecology & Evolution*, 19 (12): 619.

Rende S.F., Frangella S., Polifrone M., Stroobant M., Burgassi M., Cinelli F. (2009) - Vision 1.0: experimental software for a rapid evaluation of the macrophytobenthonic cover. *Biologia Marina Mediterranea*, 16 (1): 296-297 ;

Romeo, M., Hoarau, P., Garello, G., Gnassia-Barelli, M., Girard, JP., 2003. Mussel transplantation and biomarkers as useful tools for assessing water quality in the NW Mediterranean. Environ. Pollut. 122, 369-378.

Roose, P., Udo, Th., Brinkman, Improved determination of VOCs in marine biota by using on-line purge and trap-gas chromatography-mass spectrometry. The Analyst, 1998 (123), pp. 2167-2173.

Ruiz, G., M., Miller, A.W., Lion, K., Steves, B., Arnwine, A., Collinetti, E., Wells, E., 2001. Status and trends of BW management in the United States: first biennial report of the National Ballast Information Clearinghouse 2001.

Scarpato A., Romanelli G., Galgani F. , Giovanardi F., Giordano P. , Calvo M. , Caixap J., BenBrahim S., Sammari C., Deudero S., Boulahdid M. & Andral B.. 2010. Western Mediterranean coastal waters - Monitoring PCBs and Pesticides accumulation in *Mytilus galloprovincialis* by active mussel watching: the Mytilos project. J. Environ. Monit. 12 : 924–935

Scott B.F., Spenser C., Martin J.W., Barba R., Bootsma H.A., Jones K.C., Johnston A.E. and Muir D.C.G., 2005. Comparison of Haloacetic Acids in the Environment of Northern and Southern Hemispheres.Environmental Scence and Technology, 39, pp.8664-8670.

Supić, N., Orlić, M., Degobbis, D., 2000. Istrian Coastal Countercurrent and its Year-to-Year Variability. Estuar Coast Shelf S 51 (3), 385-397.

U.S. Environmental Protection Agency, 1996. EPA Method 5021Volatile Organic Compounds in Soils and Other Solid Matrices Using Equilibrium Headspace Analysis, Dec. 1996, siteweb: www.epa.gov.

U.S. Environmental Protection Agency, 1996. EPA Method 5030 Purge-and-Trap for Aqueous Samples and EPA method 8260b Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS).

U.S. Environmental Protection Agency, 1996. EPA method 5035 *Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples*,and EPA method 8260b *Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)*.

U.S. Environmental Protection Agency, 1996. EPA method 8260b *Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)*

U.S. Environmental Protection Agency, 2003. EPA Method 552 Determination of haloacetic acids and dalapon in drinking water by liquid-liquid microextraction, derivatization, and gas chromatography with electron capture detector, Revision 3. July 2003, siteweb: www.epa.gov.

U.S. Environmental Protection Agency, 2003. EPA Method 8270, Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS), Revision4, February 2007, siteweb: www.epa.gov.

Utermöhl H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton - Methodik. Mitt. Int. Ver. Theor. Angew. Limnol. 9: 1–38.

Annex

Example of modified CRIMP sampling protocol for benthos applied by Institute of Oceanography and Fisheries in harbor Split in Kaštela Bay

For each harbor, minimum three sampling sites have to be inspected (Figure 1). For each sampling site, three transects are selected to provide a series of vertical (hard bottom) and horizontal (probably sediment bottom) samples (Figure 2). So, **three transects per sampling site!**

The first transect should be located at least 10 m from the end of the berth, and subsequent transects at a spacing of 10–15 m (Figure 6.1.1). In the case of piles and dolphins that may be separated by more than 10–15 m, samples should conform to the available spacing (Figure 2).

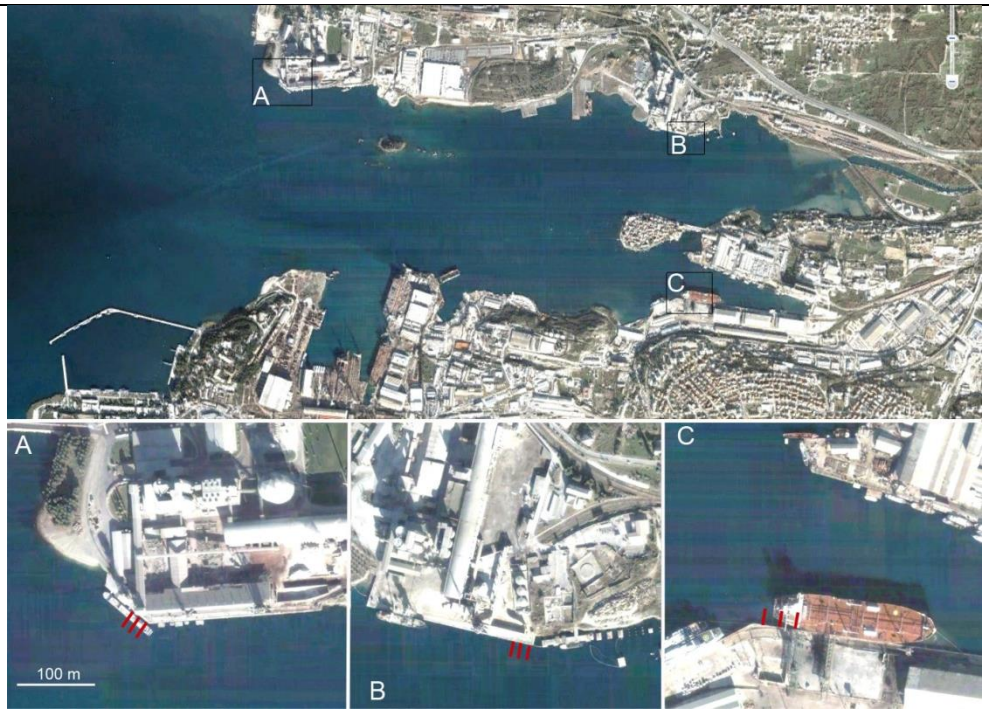

Figure 1- Harbor Split with three sampling sites (A, B, C). On each sampling sites, three transects were selected on 10-15 m distance

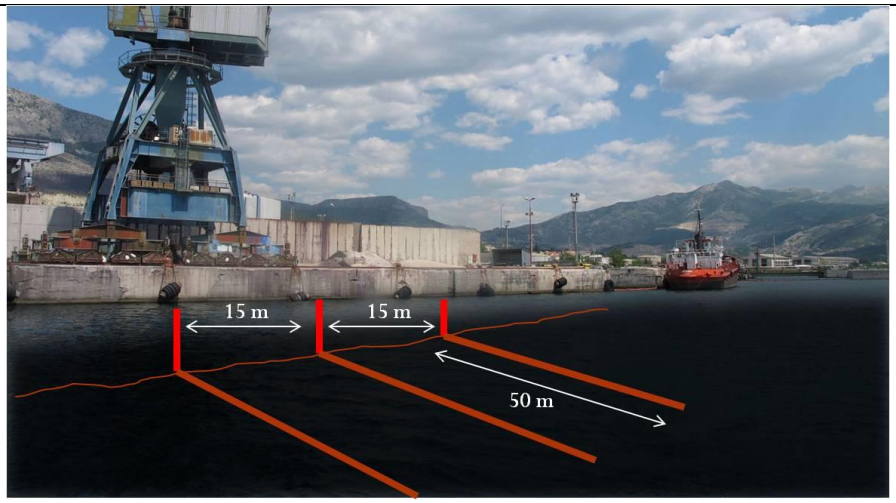

Figure 2- Three transects per each sampling site. Transects have to be on 10-15 m distance. They consists of vertical hard bottom part and 50 m long horizontal part, usually on sediment bottom.

For optimum procedure, two divers and one helper on small supporting boat are needed.

Procedure for each transect

Each transect consists of vertical hard bottom and horizontal usually soft bottom.

Vertical hard bottom

1. Three 0, 10 m^2 (0, 25 m x 0, 4 m) sampling plots have to be collected on vertical hard bottom. One sampling plot for each depths (0,5 m, 3,0 m, and 7,0 m) (Figure 6.1.2). Distribution of sampling depth can be customized depending on specific depth of wharf.

Sampling rectangle can be easily made of commercial plastic water pipes and net with 1, 0 mm mesh (optional 0, 5 mm mesh) (Figure 3). Diver holds a sampling rectangle with one hand and scraps biota with the other hand using brick hammer with flat head (Figure 4).

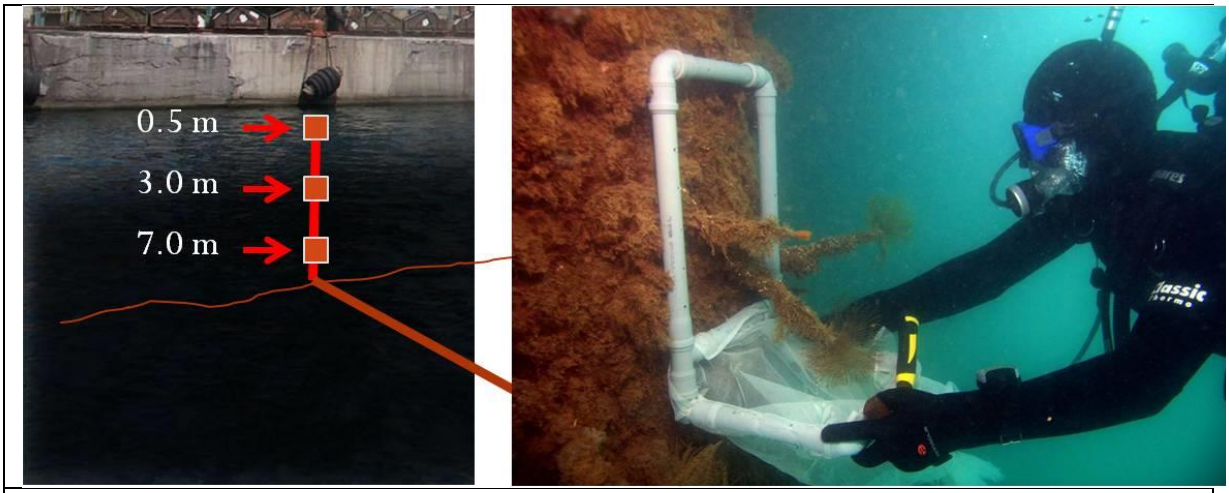

Figure 3- On each depth (0.5, 3 and 7m), one sampling plot has to be collected using sampling rectangle (0,25 m x 0,4 m) with net bag (1,0 mm mesh).

Figure 4- Sampling on sampling plot using brick hammer and sampling rectangle (0,25 m x 0,4 m) with net bag (1,0 mm mesh).

After sampling on quadrat, additional random samples have to be collected around the sampling depth. All additional samples can be collected into sampling quadrat net if statistical analysis is not a priority. Otherwise, additional samples have to be collected in separate net-bags.

Three sampling rectangles can hang on a rope lowered from boat on different depths (better option) or a diver can deliver full sampling quadrat net to helper on boat (possible health problems due to jo-jo diving). The helper than unloads the sample in a bucket and returns sampling rectangle to the diver. Prior to destructive a sampling, sampling plot has to be photographed. Additional photos have to be made around the sampling plot to cover as much species and fouling communities as possible. For high quality photos, a digital camera has to be equipped with separate strobes.

Horizontal soft bottom

Sampling and inspection has to be done on 50 m long transects. One diver takes tubular hand corer (Ø =18 cm, h = 30cm) and two net bags (1 mm mesh) and pulls a 50 m long transect rope (meter) perpendicular to the wharf. Second diver follows him, makes photos of bottom/species, and collects samples if necessary.

On 50 m from vertical transect (Figure 5), one core sample has to be collected and transferred to net-bag (1mm mesh).

Figure 5- Infauna sampling on 50 m long transect using hand corer (Ø = 18 cm, h = 30 cm).

Net-bag has to be shacked periodically to remove sediment. Divers are following the transect and dive towards the beginning of the transect. On 1 m, additional core sample has to be collected and transferred to second net-bag (Figure 5). Divers *ascend* and deliver samples to the helper on boat.

Divers and boat change transect.

Total sample collection for one sampling site (that includes three transects in total): 9 sampling plots: 3 x on 1,5m depth, 3 x on 3 m depth, 3 x on 7 m depth.

6 core samples: 3 inner (1 m on transect) and 3 outer cores (50 m on transect).

Additional random samples

Photo documentation of sampled plots and additional photos

Estimated time for three transects (one sampling site): 1h – 1h 30 min.