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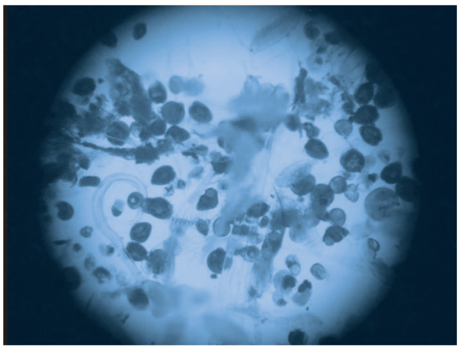
GUIDANCE ON PORT BIOLOGICAL BASELINE SURVEYS (PBBS)

GloBallast Monograph Series No.22



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The GloBallast Partnerships Programme is a co-operative initiative of the Global Environment Facility (GEF), the United Nations Development Programme (UNDP) and the International Maritime Organization (IMO) to assist developing countries to reduce the transfer of harmful aquatic organisms and pathogens in ships' ballast water and sediments and to assist the countries in implementing the International Convention on Ballast Water Management. For more information, please visit http://globallast.imo.org.

The International Ocean Institute (IOI) was founded in 1972 by Professor Elisabeth Mann Borgese as an international knowledge-based institution, devoted to the sustainable governance of the oceans. It operates through a global network of Operational Centers and Focal Points, with its Headquarters hosted by the Government of Malta at the premises of the University of Malta; it is supported by the Ocean Science and Research Foundation (OSRF). Its functions and activities are: capacity development, research, policy analysis, advocacy, dissemination of information, training and education, project implementation and promotion of the peaceful use of the ocean. The IOI centre in South Africa (IOI-SA) coordinates the African region and has been active in marine invasive species management throughout the region, including research and assessment (e.g. conducting port biological baseline surveys), training initiatives and policy development. For more information visit www.ioinst.org and www.ioisa.org.

CSIR-National Institute of Oceanography (NIO) with its headquarters at Dona Paula, Goa, and regional centres at Kochi, Mumbai and Visakhapatnam, is one of the 37 constituent laboratories of the Council of Scientific & Industrial Research (CSIR). CSIR-NIO was established on 1 January 1966 following the International Indian Ocean Expedition (IIOE) that was undertaken from 1962 to 1965. The mission of CSIR-NIO is "To continuously improve our understanding of the seas around us and to translate this knowledge to benefit all". The major research areas include the four traditional branches of oceanography – biology, chemistry, physics and geology & geophysics, besides ocean engineering, marine instrumentation and archaeology. CSIR-NIO provides services to industry and society through projects related to ports & harbours, oil & gas companies, power plants, chemical & pharmaceutical companies that use the water front for their marine facilities, which necessitate systematic study of the coastal environment. For more information visit: www.nio.org, email: ocean@nio.org.

Founded in 1948, IUCN (International Union for Conservation of Nature) brings together States, government agencies and a diverse range of non-governmental organizations in a unique world partnership: over 1,000 members in all, spread across some 160 countries. As a Union, IUCN seeks to influence, encourage and assist societies throughout the world to conserve the integrity and diversity of nature and to ensure that any use of natural resources is equitable and ecologically sustainable. IUCN builds on the strengths of its members, networks and partners to enhance their capacity and to support global alliances to safeguard natural resources at local, regional and global levels. For more information see www.iucn.org.

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Disclaimer

This publication has been prepared by GBP, IOI, CSIR-NIO and IUCN in order to serve as guidance to those who are planning to carry out a port biological baseline survey, in particular in the context of Ballast Water Management. It has been drafted with the specific needs of the countries participating in the GloBallast Partnerships Programme in mind. The publication is not a protocol for surveys; it rather intends to present experiences and lessons learned by the partnering organizations, to help in the planning and execution phases of port biological baseline surveys.

Although all possible efforts have been made to provide a comprehensive and accurate document, its main purpose is to provide a discussion of the relevant concepts and lessons learned, and neither the GEF-UNDP-IMO GloBallast Partnerships Project, the International Maritime Organization (IMO), IOI, CSIR-NIO, nor IUCN take responsibility for the implications of the use of any information or data presented in this publication. Therefore, the publication does not constitute any form of endorsement whatsoever by IMO, GEF-UNDP-IMO GloBallast Partnerships, IOI, CSIR-NIO, nor IUCN, and individuals and organizations that make use of any data or other information contained in the Monograph do so entirely at their own risk.

Executive summary

1. Port Biological Baseline Surveys (PBBS) can form an integral part of Ballast Water Management (BWM), which aims to prevent the transfer and introduction of harmful non-indigenous species carried in ships' ballast water from one marine environment to another. This demands international action and is best accomplished by States through adoption and implementation of the Ballast Water Management Convention 2004 (BWM Convention).

2. The aim of PBBS is to provide inventories of marine life in and around commercial ports frequented by ships carrying ballast water. The underlying reason for these surveys is to determine the presence, abundance and distribution of non-indigenous species (NIS) which may have been introduced by shipping, either in ballast water or attached to hulls, as well as by other vectors. They can also provide a baseline of biological data against which future changes in the structure and function of marine communities can be measured.

3. For countries new to BWM, performing PBBS at selected ports can reveal the current status of NIS in coastal waters, inform decisions on the need for BWM and thus the benefits of adopting the BWM Convention. Following the implementation of BWM measures, PBBS can provide an effective means of evaluating the effectiveness of these measures in preventing NIS introductions. The conduct of PBBS will also prove useful for port surveys carried out under Regulation A-4 of the BWM Convention, based on the IMO guidelines for risk assessment (G7).

4. The success of the BWM Convention depends in part on the ability to assess risks (of species transfer) presented by particular vessels operating between different regions or bio-geographic zones. Biological data are essential to the risk assessment process and there is a need to harmonize the way in which these data are generated. The present Monograph is intended to assist those planning PBBS for the first time by outlining the key elements of survey design, as well as the more important activities and considerations both in the field and the laboratory. This Monograph is designed to be read and used in combination with the Training Course on Port Biological Baseline Surveys (2009) developed by GloBallast and the National Institute of Water & Atmospheric Research (NIWA) based on the Centre for Research on Introduced Marine Pests (CRIMP) protocols for PBBS; and the five previous GloBallast Monographs (17, 18, 19, 20 and 21, which can be downloaded from the GloBallast website http://globallast.imo.org).

5. Chapter 1 explains the various objectives and benefits of PBBS, as well as the role of port surveys in the context of BWM. The introduction also outlines various options for PBBS design, ranging from those of limited scope and relatively low cost to comprehensive surveys of marine biodiversity in the port area that are far more demanding in terms of the resources required. Whereas even the simpler forms of survey can provide information on the occurrence of NIS within a port area, in particular species of macro-invertebrate (some potentially invasive) known to be introduced by shipping, only more complex surveys can provide a baseline against which ecological changes due to NIS introductions may be assessed. It is particularly important to establish specific objectives for each survey, consistent with the time and resources available, as well as the anticipated outcomes.

6. Chapter 2 details some of the more important considerations in the planning and design of PBBS in order to achieve the stated objectives. It stresses the need to fully evaluate, at the start of the process, the resources available - including manpower, expertise, equipment, facilities and, not least, funding. It also describes the advantages of involving stakeholder organizations of which the port authority is by far the most important. The chapter goes on to describe initial site surveys, the selection of sampling sites and equipment, as well as the appointment of survey team leaders and their various functions and responsibilities. Finally, the chapter provides advice on planning for contingencies.

7. Chapter 3 covers practical aspects of PBBS, such as sampling from boats and around the shore and associated topics including communications and safety procedures. The handling and preliminary processing of samples when brought to shore is addressed in some detail. A mobile laboratory or other convenient shore-based

facility is a key requirement of PBBS, enabling samples to be sorted, accurately labelled and preserved prior to more detailed examination at a fully-equipped biological laboratory.

8. Chapter 4 looks at the secondary processing of samples, which may involve microscopic examination, segregation by taxa, further packaging and labelling and dispatch to the relevant specialists for identification, recording and archiving. The point is made that the biological records obtained by PBBS, and in particular records of NIS, are of immense value as part of a global archive that can assist other countries and regions with risk assessments that are central to BWM. The transfer of such records into suitable archives maintained by various institutions (e.g. universities, international institutions, state agencies) is strongly encouraged.

9. Chapter 5 places the guidance in a wider context, with particular emphasis on the needs of the countries participating in the GloBallast Partnerships Programme. It is suggested that PBBS can provide important information that is needed for the implementation of a national BWM strategy, and that ports and port authorities have an important role to play in this context. The importance of data storage and access is emphasized, as is the significance of capacity building.

10. Much of the advice provided in this Monograph is based on experience gained in PBBS workshops and training exercises organized around the world by GBP, in conjunction with a number of specialized agencies and institutions. It is envisaged that the guidance document will add to the documentation available for training purposes, while also being of assistance to those countries embarking on PBBS for the first time. GBP encourages feedback on the use of this Monograph and intends to update it periodically as new information and experience become available.

Glossary & Abbreviations

AIS	Aquatic Invasive Species; any aquatic species that is not native to the ecosystem under consideration and whose introduction or presence may pose threats to human, animal and plant life, economic and cultural activities and the aquatic environment. (In some jurisdictions this may not include a non-indigenous species lawfully or historically introduced for sport fishing.) In many sectors AIS also refers to Alien and Invasive Species.
BWM	Ballast Water Management
BWM Convention	Ballast Water Management Convention; the abbreviated title of the International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004.
CBD	Convention on Biological Diversity
CFU	Colony Forming Units
CRIMP	Centre for Research on Introduced Marine Pests (Australia)
Cryptogenic	Species whose identity as either native or non-indigenous is unclear
CSIR-NIO	Council of Scientific and Industrial Research-National Institute of Oceanography, Goa, India
GEF	Global Environment Facility
GloBallast (GBP)	GEF-UNDP-IMO GloBallast Partnerships Programme; the joint initiative of IMO, UNDP and GEF to address the issue of invasive species in ships' ballast water; GBP's main aim is to assist developing countries to reduce the risk of aquatic bio-invasions mediated by ships' ballast water and sediments.
IMO	International Maritime Organization
Introduction (of species)	The movement, by human agency, of a species, subspecies, or lower taxon (including any part, gametes or propagule that might survive and subsequently reproduce) outside its natural range (past or present). This movement can be either within a country or between countries.
IOI	International Ocean Institute
IUCN	International Union for Conservation of Nature
Native species	Same as *indigenous*; a species, subspecies, or lower taxon occurring within its natural range (past or present) and dispersal potential (i.e. within the range it occupies naturally or could occupy without direct or indirect introduction or care by humans)
Non-Indigenous Species	Same as *alien* and *exotic*; a species, subspecies, or lower taxon occurring (NIS) outside of its natural range (past or present) and dispersal potential (i.e. outside the range it occupies naturally or could not occupy without direct or indirect introduction or care by humans) and includes any part, gametes or propagule of such species that might survive and subsequently reproduce.
PBBS	Port Biological Baseline Survey
UNDP	United Nations Development Programme

1

Introduction & background

1.1 THE PROBLEM OF NON-INDIGENOUS SPECIES (NIS)

The establishment of introduced (non-indigenous) animals, plants and microorganisms in locations outside their native range is one of the most serious threats to the natural ecology of biological systems worldwide (Wilcove et al. 1998; Mack et al. 2000). The growth of international trade during the 20th century, and in particular the development of steel-hull ships and the expansion of shipping, has provided increased opportunities for the transport of species to regions where they did not previously occur. In some cases, successful introduction and establishment of non-indigenous species (NIS) can result in biological invasions, which may adversely affect native biodiversity, industry and human health.

Transport of marine species occurs primarily by shipping activities, through fouling communities attached to hulls and in ships' ballast water and associated sediments, in sea chests and other recesses in the hull structure (Carlton 1985, 1999; AMOG Consulting 2002; Coutts et al. 2003). Historical movements of vessels along coastlines and between continents have facilitated the spread of many hundreds of marine species to new locations, where they have established populations, often in shipping ports and surrounding coastal environments (Cohen and Carlton 1998; Hewitt et al. 1999; Anil et al. 2001; Eldredge and Carlton 2002; Leppäkoski et al. 2002). Consequently, coastal marine environments may be among the most heavily invaded ecosystems worldwide (Carlton and Geller 1993; Grosholz 2002).

Box 1: Comb jelly *(Mnemiopsis leidyi)*

The comb jelly, *Mnemiopsis leidyi*, is endemic to estuaries along the North and South American Atlantic coast. It was first recorded in the Black Sea in 1982, where it became well established, occurring in massive numbers. It also spread rapidly to the Azov, Marmara and Eastern Mediterranean, and towards the end of 1999, was recorded in the Caspian Sea where its biomass eventually exceeded levels recorded in the Black Sea. In 2006 it was recorded in the North and Baltic seas. Since 2009 it expanded its range to the Western Mediterranean. An uncorroborated report of *Mnemiopsis* came from the Indian Ocean in 2010; there is also report from the Australian coast in 2011. Genetic studies indicate that the invasive populations originated from the Northwest Atlantic, the Black/Caspian population(s) from the Caribbean and the Northern European populations(s) from the Northeast coast of the United States.

Mnemiopsis competes for food with commercial fish species and has had a devastating impact on fisheries. The decrease in zooplankton caused by *Mnemiopsis* also had impacts on the food web, causing an increase in phytoplankton, and a decline in predatory fish species and seals. More recently, the accidental introduction into the Black Sea of another comb jelly – *Beroe ovata* – a predator of *Mnemiopsis*, has resulted in a major decline of *Mnemiopsis* there, and a substantial recovery of the ecosystem.

Photo: CSIRO. Sources: GloBallast 2002; Shiganova et al. 2004; Costello et al. 2012.

Today, shipping carries about 90% of world trade in volume and moves an estimated 10 billion tonnes of ballast water globally each year. This water frequently contains a multitude of living organisms – one study estimates that 7,000 species are carried around the world in ballast water every day (USGS 2005). Due to the adverse effects they may have on receiving environments, these 'hitchhiker' species have become a major environmental challenge.

Moreover, species may be introduced intentionally, such as for aquaculture, or unintentionally through fishing, recreational yachting, etc. However, shipping is responsible for the majority of marine species introductions (Cohen and Carlton 1998; Ruiz et al. 2000; Hewitt et al. 2004).

Determining the impact of NIS identified in ports requires detailed information on the species' local abundance and distribution, seasonality and mechanisms of dispersal, and an evaluation of their interactions with native organisms through space and time (Parker et al. 1999, Mack et al. 2000).

Because containing or eradicating a marine species once it is established is considered to be difficult if not impossible, the management of NIS must focus on precautionary measures (e.g. Thresher and Kuris 2004; Carlton and Ruiz 2005). Under the auspices of the International Maritime Organization (IMO), the international community has been addressing the issue of NIS and ballast water since the late 1980s. The International Convention for the Control and Management of Ships' Ballast Water & Sediments (hereafter referred to as the Ballast Water Management (BWM) Convention) adopted in February 2004 is a key tool to address the issue. The Convention states that ships in international traffic must manage their ballast water to specific standards, ensuring that no, or minimum, harmful organisms are transferred to the next port of call.

1.1.1 The Need for Biological Data

Under Article 6 of the BWM Convention, States are encouraged to undertake scientific and technical research and monitoring including *"observation, measurement, sampling, evaluation and analysis of the effectiveness and adverse impacts of any technology or methodology as well as adverse impacts caused by such organisms that have been identified to have been transferred through ships' ballast water"*.

This clearly recognizes the need for biological information on coastal and inland waters frequented by shipping in order to assess historical exposure to non-indigenous species introductions, to detect impacts on native species and communities exposed to such introductions and to monitor change over time. Where large areas are lacking biological records, it would clearly be appropriate to record the most commonly-occurring plants and animals present in a manner that allows for updating of these records following future monitoring activities.

Whereas few countries have established long-term monitoring programmes specifically to identify NIS and species that may be invasive, such information is a crucial component of risk assessment for ballast water and NIS management. Although port surveys are not a specific requirement under the BWM Convention, risk-based approaches are central to ballast water management. It is therefore appropriate that countries take steps to improve their information base on NIS, and where possible carry out PBBS in their major commercial ports.

For those countries that may be undecided about the benefits of ratifying and implementing the BWM Convention, conducting PBBS can:

a) Reveal the existing state of NIS infestation,

b) Reinforce arguments for preserving the areas' natural biodiversity, and

c) Support granting exemptions under Regulation A-4 of the BWM Convention, based on the IMO guidelines for risk assessment (G7).

1.1.2 The Need for a harmonized approach

The introduction of NIS is a global problem that can be mitigated only through coordinated international action. Inventories of NIS in different regions may form an important part of this international effort, particularly when the NIS datasets are obtained by comparable methodologies. Accordingly, a uniform application of PBBS procedures would help to harmonize approaches to port surveys in different countries and regions, for example, by encouraging similar levels of sampling intensity and thereby facilitate comparisons of NIS occurrence and abundance.

The aim of this document is, therefore, to provide general guidance on the design, planning and execution of PBBS, covering major ports and surrounding areas, as well as to harmonize the data generated by such surveys. It will have particular relevance for those countries with limited experience in conducting such surveys.

1.2 PURPOSE OF PBBS

As conceived in this guidance document, PBBS is a scientific survey of the port's biological communities and ecology, focusing on the identity, distribution and abundance of NIS, some of which may prove invasive and ecologically damaging. Ideally, the survey will provide an inventory of the more readily-observed plants and animals occurring in the various habitats and substrates of the port environment, as well as some of the more cryptic species. This may involve a number of surveys to cover the various taxonomic groups, locations and seasonal conditions. If possible, PBBS should be repeated at intervals of 3 to 5 years, although these follow-up surveys may often be reduced in scope and scale, forming part of on-going environmental monitoring programmes.

Biological and ecological information for port areas is generally scarce and seldom updated. This is due in part to the difficulties associated with scientific sampling in maritime and port areas, especially in light of security measures operating within most major ports. Ports, however, are the most likely places for new marine species to arrive and settle, and therefore a logical place to initiate biological surveys. The type, abundance and distribution of organisms may change considerably due to new species introductions, so even where surveys have previously been carried out, it is important to re-survey periodically.

Environments within port areas are generally distinct from coastal systems due to the large number of artificial structures and the nature of the activities within ports, including but not limited to shipping. But because ports have connectivity to the open coast, any biological changes occurring within the port may have effects on adjacent coastal ecosystems. There is, therefore, an overlap between the areas generally managed by maritime and port authorities with those managed by environmental administrations. This provides an opportunity, as well as a justification, for collaboration and resource-sharing between sectors, important to the success of PBBS.

In the context of BWM, a PBBS has 4 specific purposes:

a) to inform port authorities and lead agencies responsible for BWM about the current position with respect to NIS and cryptogenic species within the port and surrounding areas, including those that may have been introduced by shipping;

b) to prepare an inventory of aquatic plants and animals inhabiting the port and adjacent areas including their distribution and relative abundance;

c) to guide the development of BWM strategies and measures applicable to the port and visiting ships; and

d) to provide a baseline of biological data against which future changes in aquatic communities, including NIS and AIS, can be measured.

There are, of course, other reasons for conducting biological surveys, such as assessments of environmental quality[1] and harvestable marine resources, or studies of the effects of climate change. Whereas it is possible to investigate all these properties simultaneously, it is likely that for many countries this would prove far too demanding in terms of time, human and financial resources. Furthermore, commercial ports are probably not the best areas for the kinds of biological survey that require stable and/or pristine conditions. Ports have numerous man-made structures, are frequently subject to dredging and water quality is often impaired to some degree; there is also much turbulence and redistribution of sediments. Although biological monitoring programmes are routinely carried out in many countries, these rarely include sampling sites in ports.

An important message from this guidance document is that PBBS does not need to be excessively complex or costly and should always be undertaken according to the resources available. It is better to conduct a simple, selective PBBS for a port frequented by ballasted vessels than to avoid the task because it cannot be done comprehensively. On the other hand, a comprehensive survey of the port most at risk from NIS introductions could demonstrate the likely extent of the problem within the region.

The benefits of PBBS for the management of port and coastal environments may include:

i. A survey design that can detect introduced species, paying particular attention to a cross section of marine habitats representative of the region;

ii. A survey team trained in the recognition of introduced species and related laboratory techniques;

iii. Experience in the collection, verification and archiving of taxonomic information essential to the investigation and management of NIS within the region.

Biological information is not only important for protecting the local port and associated environments but also to prevent, avoid, or reduce the potential for species export when ships load ballast water prior to departure. It may also help to reduce the spread of introduced species from the port to adjacent coastal areas. In a broader context, information from port surveys is essential in building regional and global databases on NIS and

[1] Guidance on the measurement of environmental quality parameters is not provided in this Monograph.

ensuring that these databases provide sufficient information for risk assessment. As stated in Article 13(3) of the BWM Convention:

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

It is preferable that PBBS be repeated from time to time so that the biological and ecological conditions of the port can be kept under review. In some cases it may be decided to establish an ongoing monitoring programme, rather than a comprehensive once-off survey; both options will provide data useful in risk assessments and NIS management. PBBS may prove to be an effective catalyst in building support for ratification and implementation of the BWM Convention and is essential for risk assessment and mitigation. In accordance with the Convention, risk assessments based on reliable biological data and on-going monitoring may be used to provide exemptions under Regulation A-4 for specific ships operating between specified ports and locations (e.g. those operating on low-risk routes).

1.3 TYPES OF SURVEYS

1.3.1 Protocols for PBBS

In 1997, IMO adopted the Guidelines for the control and management of ships' ballast water to minimize the transfer of harmful aquatic organisms and pathogens (Resolution A.868(20)) encouraging countries to undertake biological surveys of port environments for potentially harmful non-indigenous aquatic species. PBBS is implicitly encouraged in the requirement for risk assessments (see Guidelines G7).

Several port sampling programmes have developed different sampling protocols, e.g. in USA, Australia/New Zealand (*see box below*) and Europe. Before launching a PBBS programme, it is recommended that the chosen design is compared to existing sampling protocols to ensure that it is appropriate for the area and jurisdiction concerned.

Box 2: CRIMP Port Baseline Survey Protocol

The Australian Centre for Research on Introduced Marine Pests (CRIMP) has developed a technical protocol for carrying out port baseline surveys. This has been used successfully in a number of locations worldwide, including most ports in Australia, all ports in New Zealand and, in a modified format, in the six GloBallast pilot countries (Brazil, China, India, Iran, South Africa and Ukraine), with the result that there is now good knowledge on port biota from a variety of international ports, as well as increased experience with the implementation and adaptation of this protocol.

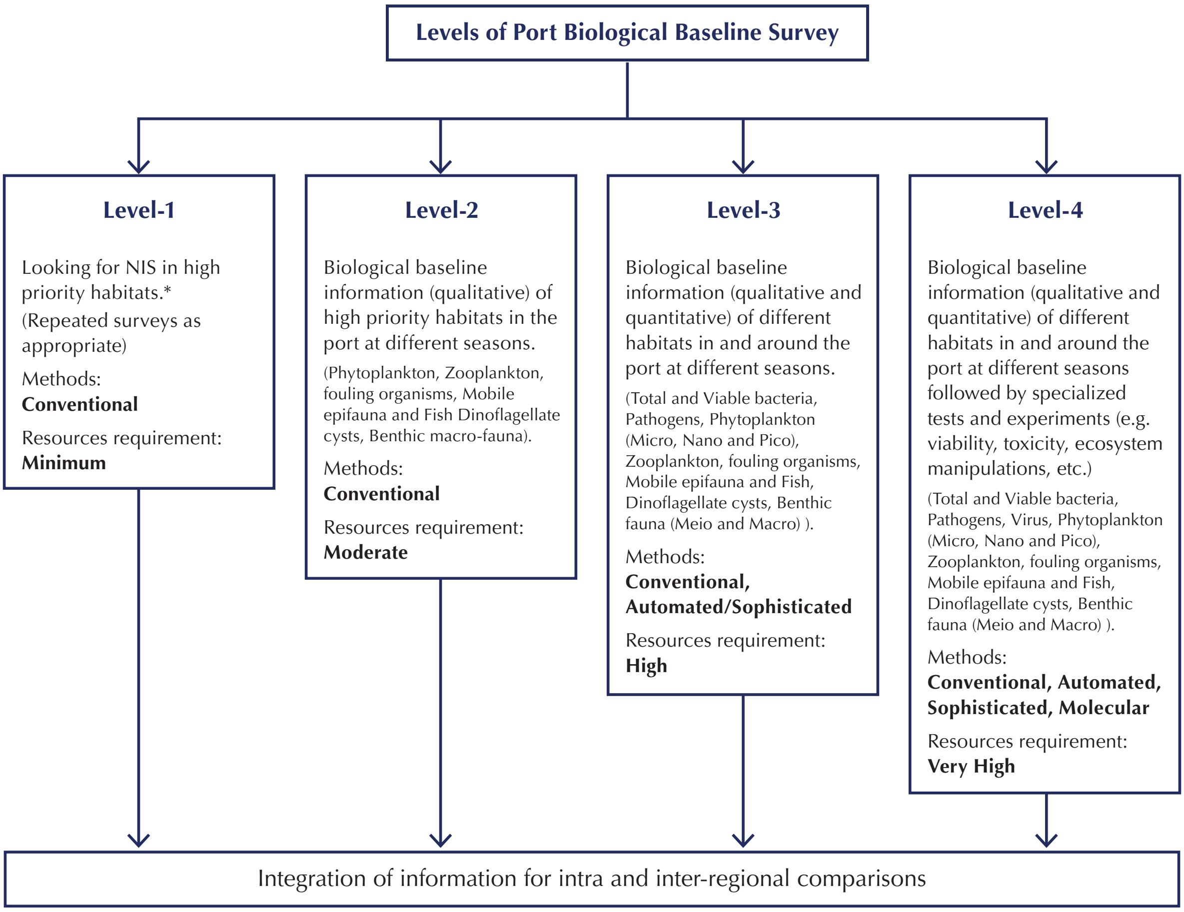
The protocol provides design criteria and methodologies for the collection of baseline data from port areas. It also allows for the inclusion of a targeted approach that gives extra priority to habitats associated with a known group of species. Apart from targeted species, it also helps in determining the distribution and abundance of other introduced species in ports. The protocol recommends the use of a Dive Team for the majority of sample collections.

Source: Hewitt, C.L. and Martin, R.B. (2001).

To date, PBBS have been undertaken (or are under consideration) *inter alia* in Australia, New Zealand, United Kingdom, Brazil, India, Iran, South Africa, Ukraine, China, Ghana, Kenya, Mauritius, Sri Lanka and Vietnam. Surveys are also planned in the Mediterranean region. In 2004, the IUCN Global Marine Program initiated and completed baseline surveys in Mahe Island, Chagos and Aldabra in the Indian Ocean, as part of its marine invasive species management initiatives (Abdulla et al. 2007).

Most of these surveys, to some extent, were designed around the protocols developed by CRIMP (Hewitt and Martin 2001). Very few, however, followed this protocol precisely, as there are always adaptations and compromises to be made, depending on local circumstances, priorities and resources. Although the CRIMP protocol is very comprehensive, in most circumstances it will need some adaption. One of the more significant adaptations is the use of non-diving based sampling methods. Another is a reduction in sampling intensity within particular sites or habitats.

Taking into account the above considerations, a range of examples covering different levels of PBBS, in terms of scope and scale, is shown in Figure 1. These options are categorized into different levels, based on the scope of work, manpower, funding and infrastructure requirements. They are by no means exclusive, but it is clear from the examples that cost and logistics become greater with increasing scope and complexity. Survey designs are flexible and even the simpler examples (Levels 1 and 2), especially when aided by carefully compiled and locally relevant check lists of high-risk species, will yield information of considerable value to the management of NIS introduced by shipping.



* Areas with increased likelihood of finding NIS

Figure 1: Examples of PBBSs differing in scope, scale and complexity

1.3.2 Adapting protocols to local circumstances

In the case of surveys specifically intended to investigate aquatic biodiversity, the focus is on the variety of organisms at different taxonomic levels and the communities of which they are part. In principle, there is no limit to the taxonomic groups that may be sampled, ranging from viruses to the largest marine mammals. However, where highly specialized equipment is required for sampling, sorting and identification (e.g. micro-benthos), the time, taxonomic expertise and costs involved may increase substantially. For this reason, most biological surveys will be restricted to organisms within a certain size range, for example > 10 µm or > 50 µm, or species identifiable with a good quality binocular microscope (x 10-40 magnification)[2] such as benthic (infauna, epifauna and encrusting) organisms (Hewitt et al. 2004). A significant proportion of the more problematic NIS (i.e. invasive species) so far identified are macro-invertebrates (Hayes et al. 2002). Comprehensive inventories of smaller forms, such as ciliates, bacteria and viruses, may be unrealistic as part of broad-scale surveys; such taxa can be the subject of specialized surveys, should they be warranted and affordable.

[2] Note that information on the biogeographic origin of microscopic organisms is very limited.

The scope of a marine biological survey needs to be defined either in terms of taxa, size-ranges, target species and/or the procedures to be used in sampling, sorting and identification; decisions on scope should take into account the expressed purpose of the survey, the available financial and technical resources, including the requisite taxonomic expertise and capacity for sample throughput.

PBBS in support of BWM may adopt a design strategy that is broad in scope, but should also give particular attention to species and taxonomic groups known to be spread by shipping, as well as their abundance and distribution. Illustrated checklists of the most likely and problematic species transferred between different regions of the world are available from a number of sources (e.g. IUCN 2012; Government of Australia 2012; AquaNIS 2013; Fofonoff et al. 2013; NOAA 2013). Although existing, up-to-date lists of target species are valuable, whenever possible they should be checked against records of potentially high-risk species in other bioregions frequented by incoming vessels. The preparation of target species lists, preferably illustrated, should be considered a preparatory task for the PBBS team. Ideally, the survey should encompass all habitats within and around the port, but it is sometimes more practical and cost-effective to concentrate on the substrates most suitable for the species of concern.

Lists of target species have enabled the use of so-called ‘Rapid Assessments’ of AIS (Ashton et al. 2006; Minchin 2007, 2012) whereby ports, harbours and marinas can be examined for the presence of listed species by smaller survey teams requiring far less time, so that large sections of coastline can be covered with minimum cost. For purposes of BWM, and NIS management generally, such methods are both useful and cost-effective. The implications of selecting a rapid assessment approach should be weighed against a more comprehensive PBBS approach, taking into account the objectives of the survey, and the types of information and data required.

2

Planning & Design

The preceding chapter outlined various reasons for conducting PBBS and clarified the important relationship between *survey purpose* and the various *types of survey*. Although this document is primarily aimed at providing guidance for purposes of BWM, the information generated by PBBS is equally applicable to the management of other NIS vectors, such as biofouling on ships' hulls. At the start of any new survey, it is important to clarify the questions to be answered and how the findings will be used for management purposes.

A typical PBBS is designed to detect introduced species, as well as providing an inventory of species within selected taxonomic groups, and/or of specified size-ranges, to serve as a baseline for future surveys. No single survey will be 100% effective with respect to these aims and results must therefore be treated accordingly, e.g. supplementing them with data from subsequent surveys or monitoring, or focusing on different seasons or taxonomic groups.

2.1 INITIAL STEPS

2.1.1 Assessment of resources

At the outset, it is essential to fully assess the available resources, so that the survey design is realistic, accurately reflecting capacity in terms of manpower, expertise, equipment, facilities and financing. The assessment should also consider the time requirement (including seasonality), access to the survey area and any practical support available from port authorities and other relevant agencies. For each of these factors, the design team should consider possible limitations, the degree of flexibility and potential contingencies.

2.1.2 Stakeholder involvement

Apart from the principal agency responsible for conducting the surveys, other relevant stakeholders should be invited to participate in PBBS activities, starting at the design stage. Table 1 indicates some of the more likely stakeholders that may be involved, or have interest in PBBS, as well as their possible roles and contributions.

Table 1: Potential stakeholder involvement

Stakeholder	Involvement
Maritime authority	Coordination, authorizations, access, vessel communications
Environmental administration	Permits, data, equipment
Port authority	Details of shipping activities, Access to port areas, field laboratory facilities, vessel coordination & communications
Research Institutes in the relevant field	Expertise, equipment, data
Fisheries department/Institute	Expertise, equipment, data
Coast Guard and/or Navy	Boats, divers, safety equipment
Academic institutions	Expertise, equipment, data processing, taxonomic specialists
Tourism board/Department	Awareness raising, community cooperation
Diving & recreation clubs	Assistance with passive monitoring, cooperation
Health department	Bacteria and pathogen analysis, data
Museums	Taxonomic specialists, sample curation
Terminal operators & port users	Cooperation for access and assistance with space, logistics etc.
Parks or reserves	Access to controlled areas, data, collaboration
Community forum (e.g. water quality)	Awareness, cooperation, data
Regional bodies & organizations	Awareness, cooperation, data

Creating awareness and developing collaborative arrangements in advance of the survey can reduce cost and time. It is, however, important to communicate clearly, to define the expectations and roles of all parties involved, and even to use contracts and/or Memoranda of Understanding (MoU) to ensure that involvements are clearly understood. Site visits with key stakeholders can be helpful in survey preparation and design.

One of the principal stakeholders will, of course, be the port authority and it is essential to secure their cooperation and assistance at the earliest stage in PBBS planning. Direct contacts with port officials (e.g. harbour master) are essential for making pre-survey arrangements such as:

- Port familiarization visit
- Obtaining necessary permissions
- Entry passes for personnel and vehicles/mobile laboratory
- Shipping information
- Port resources map
- Electricity and water requirements
- Survey vessel/ boat
- A room or shade for setting up of shore laboratory

2.1.3 Establishing the scope of PBBS

The importance of decisions regarding the type of PBBS was highlighted in Section 1.3. The key considerations regarding the scope of PBBS are illustrated in Figure 2 below:

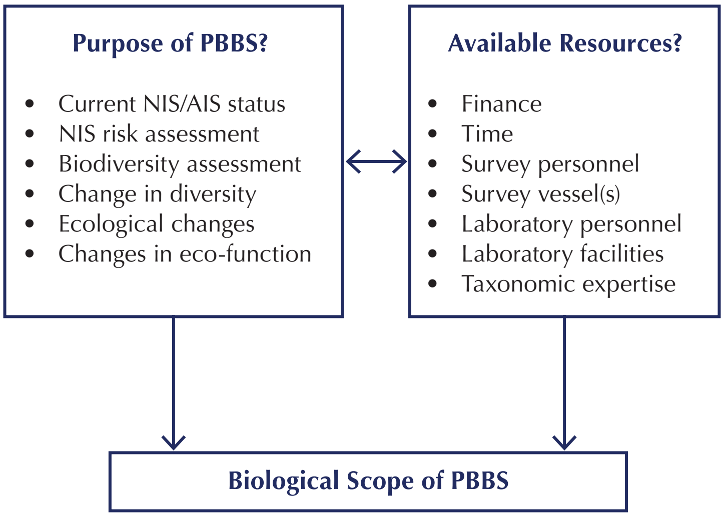


Figure 2: Key considerations in establishing the scope of PBBS

In certain areas it may be appropriate to consider seasonal variation when deciding on the scope and timing of the PBBS. Some organisms may be influenced by the seasonal changes in habitat conditions, which may also affect the likelihood of their detection. In cases where seasonal variation is considered to be a significant factor, the PBBS effort may be split into two field surveys separated by six months, in order to account for such variability and strengthen confidence in the results.

2.1.4 Survey Implementation Plan

The Survey Implementation Plan for PBBS should be clearly documented and sub-divided into two phases: a) field survey (sample collection and sorting) and b) sample analysis. For each phase, the plan should include:

- Schedule of daily activities,
- Roles & responsibilities,
- Contingencies or alternatives,

- Health and safety procedures and concerns,
- Materials, equipment and consumables required.

The plan should be flexible enough to account for any changes in weather, port and shipping operations or personnel availability. It should be realistic with respect to daily work load for individuals, allowing for adequate rest (e.g. for divers operating in strenuous conditions) and sufficient time each day for sample handling (sorting, labelling, preserving, etc.).

2.2 SURVEY DESIGN

2.2.1 Demarcating the survey area

Ports vary greatly in terms of traffic type, position, size, complexity and the types of habitat found in and around them. The survey should maximize the range of habitats sampled and, as far as practical, should include sites representative of areas affected by each of the different port activities. It is also a good idea to include areas outside of the port for comparison. An initial site visit, including a tour of the port by boat, is indispensable for gaining familiarity with the lay-out of the port and to check the suitability of sites for sampling. Photographs of candidate sites can also be taken for future reference.

Pictures 1 and 2: Site visits with key stakeholders can be helpful in survey preparation and design

Factors and features to consider in determining the appropriate survey area, and thus the survey limits, include:

- Operational shipping areas most commonly exposed to introduced species:
  - Cargo (bulk, container and multi-purpose) berths
  - Anchorage areas
  - Navigation buoys
  - Approach channels (where de-ballasting often occurs)
  - Dry docks and cleaning areas
  - Marinas and small craft harbours
- Locations with hard vertical surfaces and areas of relatively undisturbed sediments;
- Areas where dredge spoils are dumped that provide opportunity for invaders to settle and colonize;
- Nearby aquaculture facilities; aquaculture structures provide good substrates for sessile organisms;
- Aerial photographs and water circulation maps providing information on the dynamics of the port and associated areas;
- Accessibility (administrative permissions, security requirements) and ease of sampling, especially in areas of high traffic.

2.2.2 Selecting the sampling sites

Having demarcated the survey area, and having consulted the available charts, maps and photographs, the survey team will already have a good understanding of the potential sampling sites in each of the target habitat/substrate categories. In evaluating sampling sites, accessibility and the safety of survey crews are of high priority.

To get the best results, organisms should ideally be sampled from all port habitats and substrates, including the water column and soft sediments as well as from hard substrates such as coastal defences, dock structures (harbour walls, jetties), navigation buoys, ship wrecks, bridge abutments, etc. The surfaces of drainage culverts, cooling water inlets and power plant outlets should also be examined, as they may provide opportunities for some introduced species to become established.

In summary, apart from the case of selective surveys (*see Section 1.3.1*), the site selection process should ensure:

- A wide range of geographic and habitat coverage
- Optimal opportunity to document species diversity within the scope of the survey
- Maximum likelihood of introduced species detection

A sampling plan should be drawn up detailing the exact number and location of samples to be taken at each of the chosen sites. This will allow for an initial assessment of the scheduling and time requirements to complete the sampling and associated activities. The sampling plan and schedule should be entered into the PBBS implementation plan.

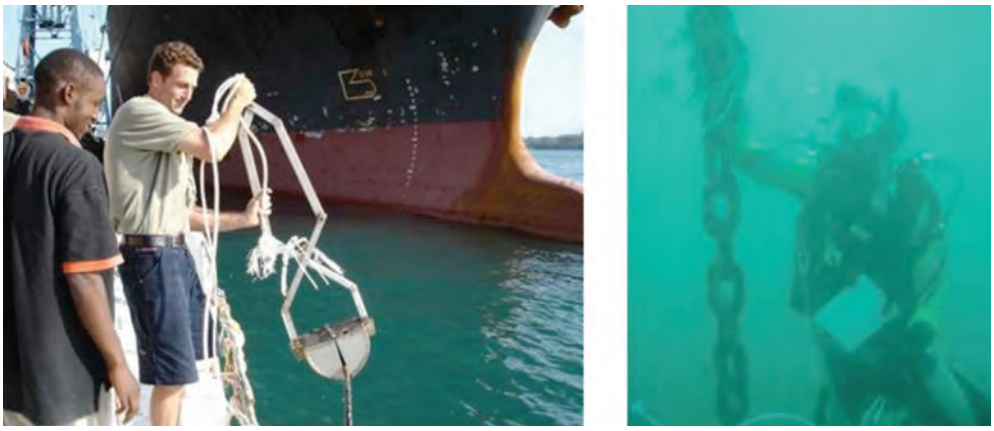
2.2.3 Selecting sampling methods

For each site selected, sampling methods will be dictated largely by the habitat types present. Protocols such as those developed by CRIMP (*see Section 1.3*) can be very specific about the sampling methods to be employed, as well as the numbers of samples to be taken. However, in many instances it would be appropriate to consider alternatives or variations to suit local conditions and capabilities. For example, where diving is hazardous due to the prevalence of predators (e.g. sharks, crocodiles), pollution or venomous jellyfish, etc. surface-operated samplers might be used instead. Also, where the capacity for sample processing is limited, the number of replicate samples taken at each site should not preclude sampling all priority areas and habitats.[3]

While the types of habitat found in the port area will determine to some degree the types of sampling device required, an array of options is available for each organism type, habitat and substrate. The choice of method should be based on likely effectiveness (including local experience), equipment availability, cost and practicality. Table 2 lists some of the more common substrates and a selection of devices used internationally in marine environmental surveys.

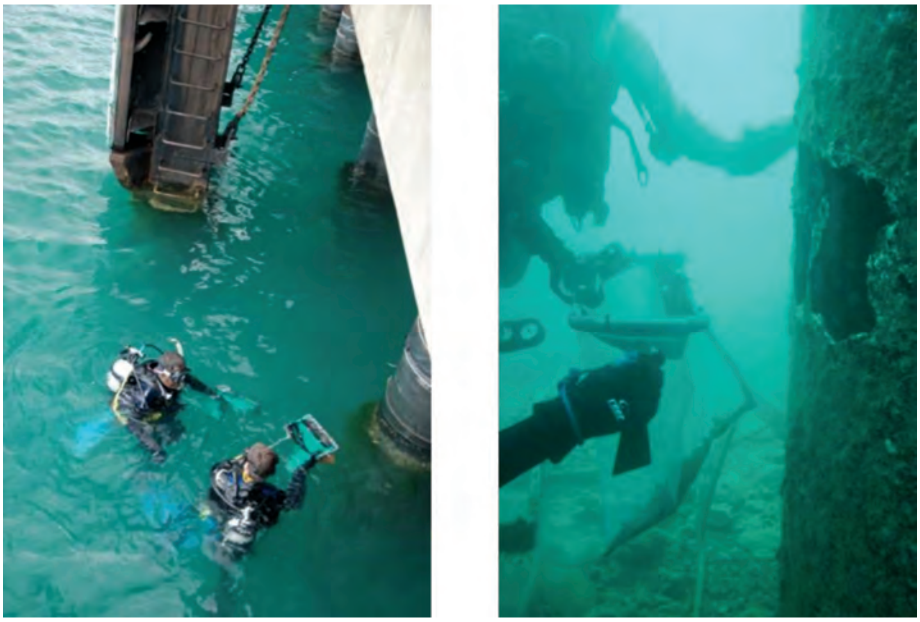
The choice of sampling device will depend on the types of organism to be sampled, as well as the habitats/ substrates in which they live. Table 2 includes examples of different categories of marine organism associated with particular substrates and an estimate of the quantity of material in each sample. However, it is essential to take account of species abundance in selecting the number of samples collected at each site; if a species is not abundant it may not appear in a single sample. Information on abundances obtained from previous surveys or research will help to determine the appropriate number of replicate samples (typically 0-5) needed to represent the biota on or within a particular substrate type.

Pictures 3 to 10 show different kinds of sampling gear.



Pictures 3 and 4: Opportunistic qualitative sampling along buoy chain

[3] Where a new NIS is detected, it would be advisable to extend sampling to establish its abundance and distribution.



Pictures 5 and 6: Field demonstration of quadrat scraping methods

Pictures 7 and 8: Pole scraper used in India

Picture 9: Deploying plankton nets

Picture 10: Beach seines

Further advice on sampling techniques is given in Annex B.

Table 2: Sampling devices for different marine substrates

Substrate types	Relevant Sampling methods	Associated marine organisms
Water column	Niskin/Kemmerer Water Sampler	Zooplankton
	Pump	Phytoplankton/Cysts
	Plankton nets (20µm/100µm) vertical or horizontal haul	Viruses/Bacteria
	Sterile microbiological sampler/syringe/corer/filter	
Hard substrates:		
Concrete walls and facings	Scraper & Quadrat	Fouling organisms
Pilings - concrete and wood	Scraper & Quadrat	Mobile invertebrates and small fish
Breakwaters and rocky barriers	Scraper & Quadrat/Traps	Macroalgae
Buoys and channel markers	Scraper & Quadrat/Visual	
Wrecks and abandoned hulls	Scraper & Quadrat/Visual	
Hulls of vessels incl. yachts	Scraper & Quadrat/Visual	
Rocky/pebble beaches (intertidal)	Transect/Quadrat & scraper/Hand-net	
Rocky/pebble beaches (sub-tidal)	Transect/Quadrat & scraper/Traps	
Rock pools	Scraper, Hand-net	
Reefs - rocky and coral	Transect/Quadrat/Traps	
Soft substrates:		
Non-vegetated sand/mud bottom	Grabs/Cores/Trawl/Benthic sled	Mobile and sessile epifauna
Low-tide mud flat	Grabs/Cores/Benthic sled	Infauna, meiofauna
Sub-tidal mud to sands	Grabs/Cores/Trawl/Benthic sled/Fine-mesh dredge	Dinoflagellate cysts
Sandy beaches	Beach seine/Transect	
Seagrass meadow/algae bed	Transect/Traps	
Mangroves	Grabs/Cores/Traps/Nets	
Saltmarshes	Transect/Traps	
Cyanobacterial algal mats	Transect/Traps	
Animal and plant hosts	Selected tissues and organs	Endo-ecto-parasites, diseases

2.3 THE SURVEY TEAM

PBBS is a significant undertaking, with logistical and technical complexities, and demands meticulous organization and management. The human resources available, and the manner in which these are organized, are integral to the success of the survey. A team approach is essential; there could be one small team of experts, or several sub-teams working together in a coordinated manner and with a common goal. As a rough guide, a team comprising 8-10 individuals divided into two sub-teams in most cases should be sufficient to sample a moderately-sized commercial port in less than a week. The roles of certain key individuals are described below and summarized in Table 3.

2.3.1 Project Leader

The Project Leader should be a senior-level person with a scientific or technical background and should have adequate experience in managing inter-disciplinary environmental projects. He/she will have a major role in designing the survey, as well as selecting the project team and assigning tasks and responsibilities, according to the scope and scale of the project. The Project Leader will also select one or more suitably experienced person(s) as Survey Team Leader(s) who can help in selecting other team members.

2.3.2 Survey Team Leader(s)

Leadership of the field survey will include the supervision of sampling activities, as well as making necessary arrangements with the port authorities. Survey Team Leaders should be well versed in both existing and potential NIS/AIS of the region, as well as with various sampling techniques, and be capable of managing unforeseen difficulties quickly and efficiently. They should list, and ensure the provision of, necessary equipment and organize the survey in accordance with the implementation plan, instructing and assigning tasks to team members, as appropriate. For example, one member of each survey group should be assigned the task of keeping written records of samples, sample locations and all observations regarding species occurrence, abundance and distribution. Survey Team Leaders should also maintain contact with port officials to ensure the team is well informed of port traffic while the survey is in progress.

2.3.3 Other team members

A survey team may vary in size from only a few individuals, each taking on multiple roles and responsibilities, to 15 or more members forming sampling and sorting sub-teams (see Table 3 below for summary of common survey team roles). The members of the field survey team may be drawn from a variety of sources, such as state agencies, port authorities, hydrographic survey units, private companies, diving clubs and university science departments. Ideally, all team members will have experience with some form of sampling and measurement in the aquatic environment, familiarity with small boats and an ability to swim. It is preferable that several members have an appropriate scientific background, be trained in recognizing target NIS/AIS, in operating devices for sampling, and in sample labelling and storage. Such individuals can assist the Survey Team Leader in instructing less experienced staff in the procedures to be used in the field and to allocate tasks accordingly.

Some of the team members may take on additional roles and responsibilities. For example, the survey team should include an appropriately trained person to act as Safety Officer. The Safety Officer has sole responsibility for all aspects of personnel safety, both on land and at sea, covering all field activities for the duration of the project. The brief of the Safety Officer embraces items such as clothing, footwear, life jackets, medical kits, emergency communications, rescue procedures, ensuring equipment operators are properly trained and that survey boats are adequately equipped and seaworthy. In some cases, diving teams may provide their own safety officer and adopt their own safety procedures; this should always be with the knowledge and approval of the team Safety Officer.

Table 3: Summary of common survey team roles

Team member/role	Note
Project Leader	Overall responsibility for and management of the project.
Field Logistics Coordinator	This can be a distinct role, or may be performed by the Project Leader. If separate from Project Leader then this role may be combined with Laboratory Manager.
Field Team Leader(s)	Communicate(s) with Project Leader to implement the survey plan and coordinate the field team members and activities.
Dive Supervisor	Plans and oversees all diving activity. Does not participate as a diver.
Boat Captain	Responsible for boat safety and operations. Should be separate from Dive Supervisor.
Divers/Samplers	In many locations a scientific dive certification may be required. Divers may also take on other sampling activities or team responsibilities.
Sampling Supervisor	Maintains coordination, integrity and logs of all samples as they are generated in the field. Ensures safe transfer of samples to field laboratory.
Laboratory Manager	Establishes and coordinates a safe and efficient field laboratory for sample processing (concurrent to field sampling activities).

2.4 PLANNING FOR CONTINGENCIES

During the implementation of PBBS, it is likely that not everything will go according to plan. Contingency planning, with a view to having back-ups and alternatives in place or on call, is thus advisable. Circumstances in which advance planning is helpful include:

Field operations

It is possible that weather and other field conditions may not allow for the survey implementation plan to unfold as envisioned. For example, port operations may change, sites may prove harder to access than anticipated and boats and other field equipment may malfunction, requiring servicing or replacement. It is a good idea to design a fall-back plan of strategy that allows for such eventualities including "worst-case-scenarios".

Finances

It is possible that the survey expenses will be lower than anticipated and a decision is made to increase the survey intensity along the way. Unfortunately it is more likely that the survey budget will not stretch as far as originally planned and some aspects of the PBBS will need to be foreshortened or abandoned without compromising the main objective. Advance decisions on where and how to exercise such cuts will make for smoother transitions in the field.

Safety/emergencies

Comprehensive health and safety procedures should be part of the implementation plan, including daily briefings from the Safety Officer, inspection and documentation of safety equipment provided and emergency procedures to be followed. Extra support (e.g. phone numbers for emergency services, harbour police, insurance for divers) should be readily available, if and when needed.

Schedule and timing

Any of the above contingencies may lead to a delay in the survey schedule, with consequences for the implementation plan, possibly requiring it to be changed or rescheduled. Advance planning for such contingencies may not be easy but having available alternative dates for the survey, suitable for both port authorities and team members, may help to reduce pressure on Survey Team Leaders should serious difficulties or delays be encountered.

3

Field operations: sampling and sample processing

Efficient fieldwork is the key to successful biological surveys. This chapter gives guidance on the conduct and management of the field component of PBBS projects. It includes considerations relevant to communications and safety, as well as describing the main functions of the survey team and the collection and processing of samples.

PBBS will typically involve one to two weeks of field activities, depending on the size and nature of the port, to collect and process the samples. Several operations will be underway at the same time, so good organization and coordination will be needed to ensure the success of the survey.

Although PBBS can and should include measurements of environmental (e.g. chemical, physical) variables, in addition to biological sampling, these guidelines do not address these non-biological measurements, whether or not these are made directly in the field or involve laboratory testing. In cases where biological and environmental surveys are carried out simultaneously, it is advisable to use separate teams and cruises for these activities. Basic water quality parameters, such as temperature ranges, salinity profiles and oxygen saturation levels, may be captured by the biological field team at each station, and are often the most influential in determining the aquatic communities present within the habitat concerned. Laboratories designed for analysis of other water quality parameters (e.g. nutrients, persistent organic pollutants) require very different facilities to those of biological laboratories.

3.1 COMMUNICATIONS

A team approach to field work will help establish a hierarchy of authority and communication. All team members must know who they should report to (e.g. Project Leader, Survey Team Leaders), as well as how communications will be handled. It is advisable to hold daily briefings both at the start and the end of each day. Both expectations and progress should be discussed in light of any feedback from team members or other stakeholders. All personnel should have direct access to a comprehensive list of contact details, so that anyone can be reached at any time. The most appropriate and sensible means of communication should be used (i.e. mobile phones, VHF radio, etc.).

In addition to internal communications within the project team, it will be necessary for the Project Leader, and occasionally Survey Team Leaders, to be in regular contact with port authorities/officials, and perhaps also with relevant state agencies, sponsors and others, to exchange pertinent information and updates.

3.2 SAFETY

Safety during PBBS is a most important and on-going consideration. **The instructions of the Safety Officer should be observed at all times** ***(see Section 2.3)***. Sampling procedures should not put any survey team member at risk. While working on the decks of survey boats, crowding at one place should be avoided. Cleanliness should be maintained on the deck, with no spillage of water/sediments or oil which could make the surface slippery. The deck also should be cleared of ropes attached to sampling gears and other equipment, as well as plastic ware. Any person operating sampling devices must wear a life jacket or safety belt. In addition, safety shoes, gloves, masks, eye protectors, first-aid kits and clothing appropriate to weather and sea conditions should be available to all field team members.

The complexity and relevance of safety measures to be implemented will be determined by the sample design and implementation plan. Sampling protocols will ideally contain stringent and comprehensive checks and measures for ensuring safety at all stages of PBBS. Attention to these safety measures during the planning stages and in the field will ensure a more relaxed and effective survey operation.

Picture 11: Buddy system being employed by divers taking benthic cores

3.3 FIELD BASE AND LABORATORY

An integral component of the field operation is a fixed or mobile facility that can be used as a base and field laboratory. Having a centralized base for staging and coordination is essential, and if the same location can be used for storage of equipment, consumables and samples, as well as functioning as a sample sorting laboratory, this will make the field work much more efficient. Many types of sample must be processed and preserved within a few hours of their collection and therefore need to be taken to a shore-based laboratory or facility immediately after collection. Some attributes of a suitable field base include:

- Adequate space for tables for sorting, storage of equipment, wet/dry areas, etc.
- Connection to water supply, preferably with washing basins (including drains)
- Electricity
- Ventilation
- Storage and refrigeration facilities
- Locking doors and security
- Easy access to waterside and moorings, and/or sample drop-off location

Alternatively, a containerized mobile laboratory (*see pictures below*), especially designed and equipped for the purpose, and with all the necessary services (electricity, water, drainage, internet connections, etc.) can greatly assist with this kind of work. Such a facility provides a clean space for sample processing that is not normally available on port premises.

The field base should be adequately equipped for sorting samples (rough sorting) and storing them in the appropriate preservatives. Attention to relevant details, such as electricity, water and ventilation (e.g. fans), will help maximize the efficiency of the survey. It should also be stressed that appropriate safety and quality assurance protocols are followed when designing and using a field laboratory.

Pictures 12 to 17 show examples of mobile laboratories.

Pictures 12 and 13: The mobile lab used by the Mauritius Oceanographic Institute in collaboration with the IOI-SA for the PBBS in Port Louis is built into a container that can be moved to the quayside during a survey. It has all the electrical and plumbing adaptations needed and contains adequate storage, microscope, computing and sorting facilities.

Pictures 14 to 17: External and internal view of a containerized mobile laboratory being used by India for PBBS

In most cases there will be a need to further sort the samples (fine sorting) before final taxonomic analysis can be performed, requiring additional laboratory facilities away from the port area (e.g. associated university or research institute). The extent of fine sorting will depend on the scope of the survey (e.g. sample types, analysis required), as well as the capacities and facilities of the field station. In principle, a well-equipped and reasonably spacious field facility (i.e. serviced building, mobile laboratory) could be used for the fine sorting process; however, in reality there may be time constraints on the survey team presence in the port area and/or facilities. By ensuring that all samples are preserved in sealed containers before leaving the field location, the team can transport the collected samples to more appropriate locations for subsequent sorting and analysis.

3.4 BOATS AND TRANSPORTATION

Every port and every PBBS will differ in terms of the arrangements for access and transportation. In most cases the survey team will be staying in accommodation close to the port area, and arriving by car or boat at the field base each day. Access in and out of the port is likely to be controlled so permits for individuals, as well as all vehicles, may be required. As such trips are likely to be frequent, e.g. for supplies and other purposes (ice, bait, food, etc.), a good relationship with port authorities/officials is essential.

Pictures 18 and 19: Examples of boats used in PBBS

An efficient way to collect the majority of samples is to use a small to medium-sized boat (5-15m) as the main sampling platform. If divers are to be used, a suitable boat will be essential. The boat should be reasonably stable in choppy water, have enough deck or open space for one or two team members to deploy samplers over the side, a dry area for sample labelling and sufficient space for sample and equipment stowage. In deep water, a small, securely-mounted hoist or derrick is useful for deploying heavier devices such as sediment grabs or large plankton nets. In many cases a medium size boat is used as the main dive boat, and a smaller inflatable craft is used for other sampling at the same time (e.g. plankton tows, beach seines). It is, however, possible to conduct PBBS without the use of boats; this may save costs but also limit the areas sampled and involve more complex logistics.

Some sampling sites do not require the use of boats or divers. Most port areas will contain structures such as quaysides, pontoons and stationary barges that afford ideal substrates for a wide variety of marine invertebrates, including various types of NIS. Such substrates can frequently be accessed by land[4] and may usually be sampled by means of long-handled scrapers fitted with nets. Similarly, benthic grab samples may be deployed from the quayside as an alternative to cores taken by divers. In general, however, in-water methods result in better and more consistent quantitative samples than the surface-deployed alternatives.

3.5 COLLECTING SAMPLES

The benefits derived from PBBS will be heavily dependent on the skills of the sampling team, both in sample collection and the recording of information about the different organisms and communities sampled. The team will be guided by the Survey Implementation Plan but should always be prepared to adapt planned sampling strategies, where conditions are found to differ significantly from those that prevailed during the initial port inspection. Sufficient samples should be taken at each site, from within or on the selected substrates, to represent the communities concerned. Ideally, the number of replicate samples (which may or may not be composited) taken from each site/substrate should be sufficient to represent the communities concerned. As a rule, a minimum of 3-5 randomly-distributed replicates will provide a more reliable picture of community structure than a single sample. Observations (especially those of divers) concerning the abundance and distribution of particular species, groups of species and communities, should be recorded continually while sampling. Waterproof notebooks, slates, boards and pencils are most useful in this regard. Such records will help in describing the nature, extent and biodiversity of the communities within the survey area.

The Survey Team Leader will be expected to provide detailed instructions for the collection of samples in different media. PBBS will generate a mixture of sample types, depending on how the samples were taken and brought to shore. For example, some larger attached and free-living organisms may be hand-picked by divers or removed from tow-nets, whereas samples of plankton will be concentrated by filters into small volumes of water, sediments from grabs or cores will be kept intact until wet-filtered and scrapings of sessile communities will typically comprise a wet mass of hard-shelled and soft species.

It is good practice to place all samples into clean and secure containers as soon as possible after collection to protect them against dehydration and significant changes in temperature. Should samples be taken for bacteriological analysis, they will require the use of pre-sterilized tubes or vials (see Annex C). For other samples, there is a variety of suitable containers available, including plastic and glass screw-top jars, food containers with sealed clip-on lids and various sizes of lockable plastic bags. When stored in cooler boxes, these will adequately protect the samples until they can be sorted and preserved.

3.6 SAMPLE HANDLING

The Survey Team Leader should be responsible for all facets of sample handling. Areas used for sample storage should be separate from busy working areas to avoid any damage to samples or their containers. Procedures to be used for sample identification and tracking are discussed in depth in the various available protocols. In essence, the process involves keeping the samples in good condition from the time they are collected until they can be properly sorted and preserved.

3.6.1 Labelling

A labelling system is essential for the organization and archiving of samples and preserved specimens. It will help significantly with the PBBS organization and efficiency. Any established system can be used (an example is given in Figure 3), or even a customized label designed to conform to other data management systems. One team member should be appointed as the keeper of field records (*see Section 2.3.2*); this individual might also help to supervise and document the labelling of samples at the shore station and/or laboratory.

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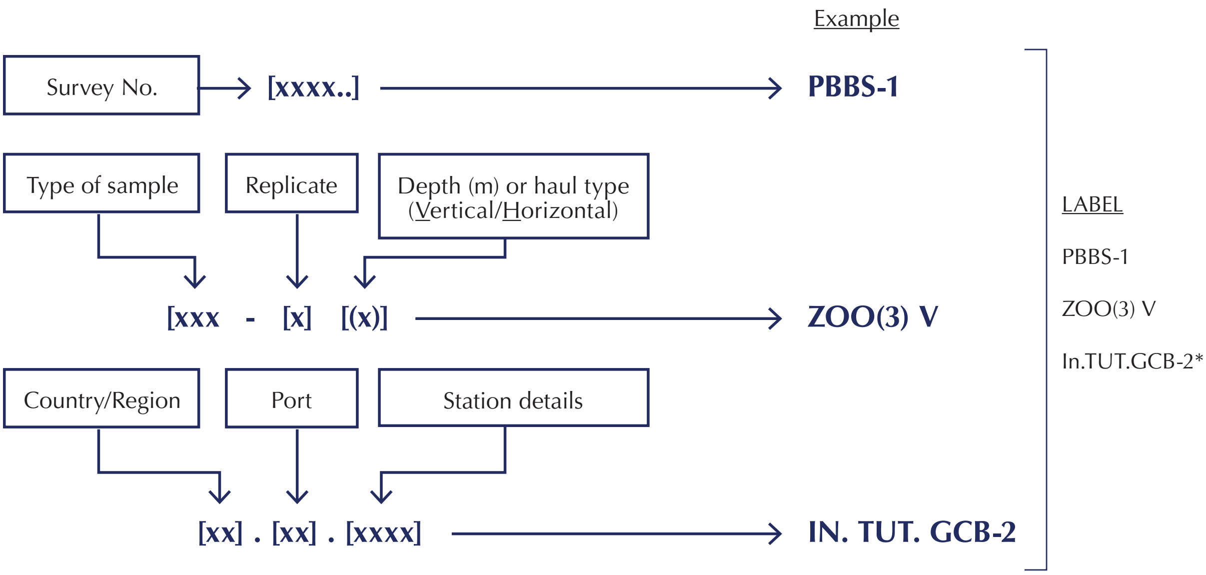
[4] Subject to requirements of the port authority.

Picture 20: Maintenance of consistent field logs and labels is essential for sample tracking and logistics

Some points of information commonly included on sample labels are:

- Location codes – may include country and state, and should certainly include the port code
- Site codes – should reflect the site numeration used in the survey design
- Sample type – an abbreviation should be used for each sampling method being employed
- Replicate number
- Sample detail – indication of depth, distance, etc.
- Labelling of sample with Alpha-numerical code

The labelling system used in India has three lines, comprising three parts for increased clarity. Part one represents the survey number, part two represents sample details and part three deals with other details, as illustrated below:



* This example can be read as General Cargo Berth No. 2. Tuticorin, India

Figure 3: Sample labelling system (CSIR-NIO, India)

Ensuring that adequate supplies of labels and log sheets (preferably waterproof) are prepared in advance will make the field operations more efficient and help avoid interruptions or mistakes. Labels should be completed by the designated field/laboratory record keepers and attached to each container. The lids of containers should also be marked with sample codes for ease of viewing when stacked or stored. It is advisable to have a plentiful supply of labels and sample tracking sheets (field log sheets, rough sorting log sheets, fine sorting log sheets, taxonomy log sheets, etc.) as they will be used and replaced throughout PBBS. The sorting of samples may involve replacing these labels with new ones both inside the preservation containers (written in pencil) and attached to the outside.

3.6.2 Sample sorting

When samples arrive at the field laboratory/facility, they should be checked for proper labelling and recorded appropriately (e.g. in a sample log book), along with details of preservation (*see Section 3.6.3*). Any deficiencies should be referred to the individuals who collected the samples and if they cannot be resolved, the samples should be discarded.

Biological samples that cannot be immediately identified will require preservation and may be arranged either according to taxonomic group or, in the case of mixed samples (e.g. vertical scrapes), by substrate type. Prior to preservation, some larger specimens (e.g. fish) may be photographed along with the scale bar and sample code, and later preserved with appropriate labelling, both outside and inside the container.

The extent of sample sorting conducted in the field will vary considerably. It is preferable to complete as much of the sorting as possible in the field, both to save space in transporting samples and to expedite delivery to the relevant taxonomists. Actual sorting requirements will depend on the nature of the samples which could either be individual (larger) specimens, clumps of sessile organisms (single or multiple species), tubes containing microorganisms or mixed plankton in water, or lumps of muddy or sandy sediment. In the case of larger surveys, it would be useful to have a dedicated team to sort and process samples as they come ashore. Otherwise sampling crews may need to pause periodically to sort and preserve samples quickly after collection.

Clearly, samples that require most effort in sorting will be sediments (to extract the organisms) and mixed samples that need to be divided into taxa appropriate to the experts who will examine them. Subject to the facilities, personnel and time available, sorting may be done in one or two phases (rough and fine sorting). The extent of rough sorting to be conducted in the field will depend on the variety and abundance of organisms present. For example, soft sediments can be wet-sieved to expedite removal of macro-fauna using a variety of mesh sizes; this can sometimes be done at the quayside where water is plentiful and the bulk of the inorganic component can be safely discarded rendering the rough sorting process more manageable. The remaining organisms may then be separated into general categories or taxonomic groups and preserved accordingly. Similarly, clumps of sessile organisms obtained by surface scrapes can be cleaned-up by carefully removing detritus from the living material.

Pictures 22-24: Rough sorting

Should it be necessary, further sorting to facilitate expert identification may be postponed to a later date, following sample preservation. Most taxonomic experts will analyse the specimens of a particular taxonomic group and may discard or leave aside any specimens that fall outside that category. For this reason the fine sorting of the samples must be conducted in accordance with the instructions of the relevant experts involved, so that all samples can be examined and identified efficiently.

Mixtures of small organisms are generally sorted and separated with the aid of binocular microscopes. This is often a tedious and lengthy procedure, and may require considerable effort by several individuals. Students training in the biological sciences can be most helpful in this regard. In some cases, specimens may be sent to taxonomists experienced in identifying a wide range of taxa from the region concerned, and in such cases complete sorting may not be necessary.

Short descriptions of fine sorting procedures, applicable to three of the common faunal size-groups, are given below:

Zooplankton:
The biomass of the sample (preserved in 5% formaldehyde) is estimated as wet weight by using standard protocols. It is then split equally into appropriate parts, for example using a Folsom plankton splitter. One part is taken for the analysis and identification of various taxonomic groups. Numerical abundance is expressed as number per cubic meter.

Macrobenthic fauna:
Preserved samples should be prewashed through 500 µm mesh and sorted under a binocular microscope. When samples are obtained by surface scrapes, numerical abundance of each species can be estimated as numbers per square meter.

Meiobenthic fauna:
Initial treatment and preservation: Samples collected by Van Veen grab are subsampled by means of a 3 cm diameter corer (on site) and then the core is sliced into two equal halves, each being preserved separately. 7% magnesium chloride is added to the sediment sample to relax the organisms and soften the tissue. 5% buffered formalin (Rose Bengal) is added to the sample for preservation and coloration. Buffered formalin is used for preservation to avoid the decalcification of external hard parts of the organisms. Rose Bengal is added to stain the organisms so they may be removed easily while sorting.

Sorting:
The preserved sediment samples are washed in water to remove formalin and passed through a 45 µm sieve to remove sediment and detritus. Then they are sorted using a light microscope and preserved in 5% buffered formalin.

3.6.3 Sample preservation and storage

To avoid the decay of specimens, it is imperative that samples are preserved as quickly as possible, preferably on the same day as collection. Ideally, samples should be kept on ice until they are sorted and preserved. Three steps are involved in the preservation of biological specimens – narcotization, fixation and storage. It is recommended that specimens be grouped according to fixing requirements. Storage of hard and soft organisms together is not recommended, as some fragile specimens may be damaged or destroyed.

Narcotizing agents are used to prevent contraction or flexion of the body and antennae of some types of organism. Either 3% to 5% buffered formalin or ethyl alcohol (70% to 90%) is normally used for fixation purposes. Within a week or two, specimens fixed in formalin should be transferred to ethanol or other long-term storage medium as prolonged storage in formalin, even buffered, will affect morphological features.

Further details of sample preservation for particular groups of organisms are provided in Annex A.

Storage

All samples must be stored under appropriate conditions and their preservatives checked periodically until identification has been completed. Samples for microbiological examination (bacteria, viruses), and those for analysis of phytoplankton, should be refrigerated or frozen and those to be examined for dinoflagellate cysts should be kept cool and in the dark. Following initial identification, samples and/or specimens should whenever possible be lodged with a museum or appropriate institution that can maintain and catalogue the specimens for future reference.

To summarize the processing of samples from collection to identification, a flow diagram is shown in Figure 4 below.

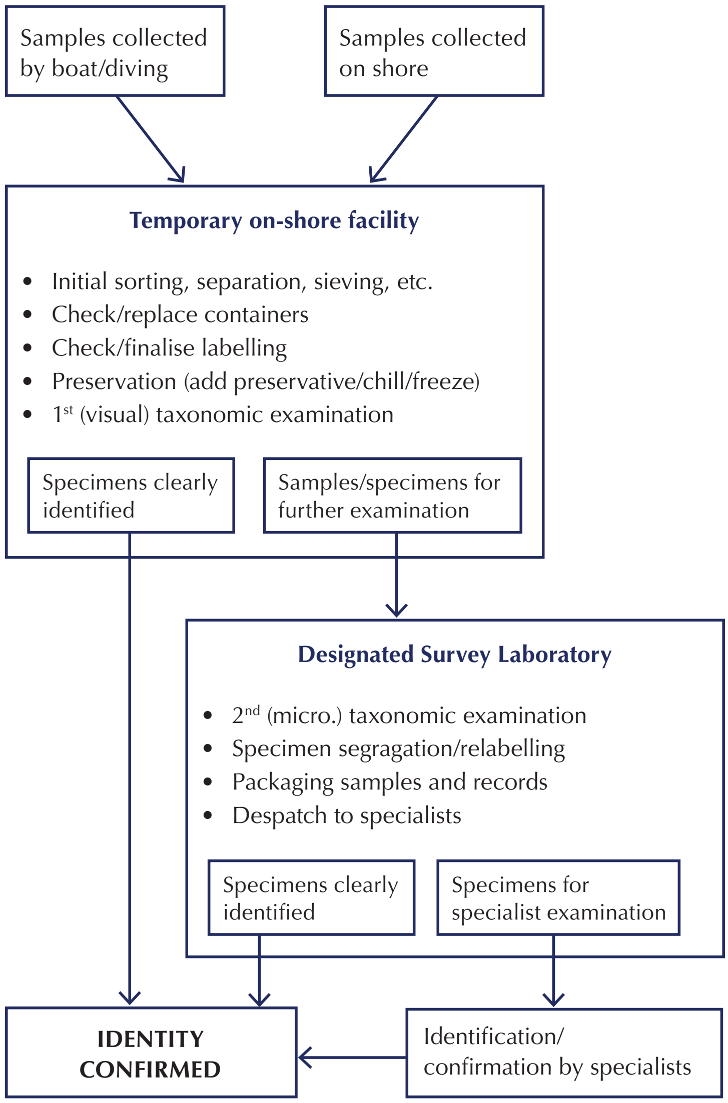


Figure 4: The process of sample handling, from field to identification

4

Biological recording

This chapter gives an overview of biological information derived from PBBS that is relevant to BWM and the control of NIS generally. It identifies the principal data required and the means of obtaining them.

4.1 CATEGORIES OF OUTPUT

Chapter 1 listed the various aims of PBBS and their implications for survey design. In general terms, the surveys can generate 3 principal categories of biological information regarding the port and adjacent coasts:

a) Basic: The current status of NIS,

b) Intermediate: NIS abundance and distribution,

c) Complex: Overall biodiversity to provide a baseline for future surveys.

Each of these has specific data requirements. Taking into account all three categories, there is an increasing amount of data required from a) to c), and concomitant increases in time and cost.

a) Basic – Current status of NIS

The simplest and quickest form of NIS survey, often referred to as a 'rapid assessment', involves the use of a pre-prepared list of target species, selected on the basis of one or more of the following criteria:

- Known to have been introduced into this (or an adjacent) region;
- Considered likely to be introduced by human activities and potentially invasive;
- Could possibly be introduced, known to be invasive and ecologically damaging.

Several coastal States have already compiled short-lists of species that meet one or other of these criteria. A significant proportion of candidate species may be macro-invertebrates that can easily be photographed and described so as to assist surveyors in recognizing them. Ideally, observers trained to recognize target species would be included in port survey teams.

Lists of target species, including species that are either too small, or too difficult to locate and recognize, will nevertheless be useful to biologists and taxonomists examining samples in the laboratory.

b) Intermediate – NIS abundance and distribution

The occurrence of NIS that is new to the area, when confirmed, is clearly important to those responsible for NIS prevention and management. However, it is equally important to establish its abundance and distribution. Key questions to be answered are whether or not the species has become established, has developed a breeding population and is gradually extending its range.

The need for information on abundance and distribution enforces the value of field identification of target species. For example, if a single specimen or isolated colony of a target species is identified during the sampling phase, the Survey Team Leader may decide to increase the number and density of sampling at comparable sites to further explore its distribution. This will greatly increase the value of the survey results and could save time, as otherwise it may be necessary to revisit the site at a later date.

A guide to rating Abundance and Distribution Ranges is shown below.

Table 4: Abundance and Distribution Ranges (ADR, after Olenin et al., 2007)

ADR Class	Description
A	Low numbers in one or several localities
B	Low numbers in many localities, or moderate numbers in one or several localities, or high numbers in one locality
C	Low numbers in all localities, or moderate numbers in many localities, or high numbers in several localities
D	Moderate numbers in all localities, or high numbers in many localities
E	High numbers in all localities

c) Complex – Overall biodiversity

An obvious limitation of surveys focusing on target species is that they do not describe the indigenous fauna and flora and therefore cannot be used to detect impacts on native biodiversity, or future changes in communities or species associations. A full-scale PBBS, aiming to provide a baseline account of aquatic life in the area, might be used to detect such impacts and changes over time.

The analysis and reporting of full-scale surveys can be a time-consuming exercise. Thus, as part of such surveys, it is important to identify and immediately report any suspected or identified NIS, so that managers can consider options for mitigation and prevention without waiting for the final PBBS report. To this end, the survey team should include members who can recognize the species concerned; any such observations should be reported promptly to the Project Leader.

4.2 FACILITIES

The previous chapter recommended the use of a temporary field laboratory, close to the port, for use as a survey team headquarters as well as for labelling, sorting and preserving samples, and for storage of samples and equipment. This could be either a mobile laboratory unit, or a fixed facility modified for the purpose. Depending on the survey design, and the range of biological material collected, some species may be identified either in the field or at the field laboratory, whereas others would be sent to one or more centres of taxonomic expertise. Such facilities often exist in university science departments, museums, agencies responsible for agriculture, water management, geology and health, and in most institutions engaged in environmental research.

In practice, the range of activities to be undertaken in either a temporary or permanent laboratory will be dictated by the scope of the survey, the expertise within the survey team, the availability of specialists and where they are located. If a survey focuses on a list of high-risk species, easily visible and readily identified by trained field surveyors (e.g. sessile macro-invertebrates), much of the identification work will be done either at the point of sampling, or at the field laboratory. For more general and full-scale collections, not all of the expertise required to identify organisms to species level is likely to be available locally and it is normal for samples to be sent to specialized taxonomists, sometimes overseas, by prior arrangement. For more comprehensive surveys, such as those covering micro-flora and micro-fauna in water and sediments, tasks including separation, sorting and culture will require more specialized facilities.

There may be further considerations in choosing the location for PBBS activities. It would certainly be convenient if a single facility were to be designated as the project headquarters, and especially if individuals engaged in reporting were housed on the same premises.

4.3 TAXONOMIC ANALYSIS

Taxonomy is the formal classification of organisms. There are different methods for identifying aquatic organisms ranging from simple visual examination through basic light microscopy, microscopy, scanning electron microscopy, allozyme electrophoresis, DNA sequencing and, recently, genomics. Identification keys for particular groups of organisms are available in the scientific literature and there are also web-based interactive identification keys and global biodiversity mapping techniques (Godfray 2002). A recent innovation is the development of a web-based inventory of AIS and related data, structured by region and aimed at global coverage (AquaNIS 2013).

Identification of specimens contained in the samples collected during PBBS should be done by biologists with experience of the biota of the area and/or with recognized taxonomic specialists. The taxonomists required for the identification of survey specimens should be contacted and engaged well before the survey is implemented. Apart from the specimens to be identified, each taxonomist should receive:

- Information on how the samples are labelled, coded and preserved; and
- Excel templates for reporting of results.

In addition, taxonomists may also require:

- Herbarium sheets for macro-algae; and
- Prepared slides for bacteria, phytoplankton, zooplankton, etc.

The taxonomists should be requested to report results to the Project Leader and, where applicable, to the person responsible for preparing the survey report. They should also provide archive specimens (e.g. voucher specimens for museums or biological data centres), whenever possible.

Determining if a species is native or non-indigenous

The species collected during PBBS may be native to the region or non-indigenous, i.e. the port area is located outside the species' native biogeographical range. Where the origin of a species is unknown or uncertain, it is termed *cryptogenic* (Carlton 1996). A list of categories used for biogeographic designation is shown in Table 5.

Table 5: Approach to categorization of species/specimens

Species/Specimens	Categorization
Native species	Native species are those that occurred within the biogeographical region historically and have not been introduced by human mediated transport.
Non-indigenous species (NIS)	Non-indigenous species (NIS) are those known or suspected to have been introduced as a result of human activities. A series of questions posed by Chapman and Carlton (1991) can be used to guide decisions about whether a species is non-indigenous; as exemplified by Cranfield et al. (1998). These are as follows: i. Has the species suddenly appeared locally where it has not been found before? ii. Has the species spread subsequently? iii. Is the species' distribution associated with human mechanisms of dispersal? iv. Is the species associated with, or dependent on, other non-indigenous species? v. Is the species prevalent in, or restricted to, new or artificial environments? vi. Is the species' distribution restricted compared to natives? vii. Does the species have a disjunctive worldwide distribution? viii. Are dispersal mechanisms of the species inadequate to reach the region, and is passive dispersal in ocean currents unlikely to bridge ocean gaps to reach the region? ix. Is the species isolated from the genetically and morphologically most similar species elsewhere in the world?
Cryptogenic species	Cryptogenic species are those whose identity (native or non-indigenous) is unclear. In certain cases they may have been spread around the world in the era of sailing vessels prior to scientific survey (Carlton 1996; Chapman and Carlton 1991), such that it is no longer possible to determine their original native distribution.
Species new to science	This category includes species previously undescribed in the scientific literature.
Indeterminate species (IS)	Specimens that cannot be identified to species cannot usually be ascribed a biogeographic origin. This group includes: (1) organisms that are damaged or juvenile and lack morphological characteristics necessary for identification, and (2) taxa for which there is not sufficient taxonomic or systematic information available.

In cases where survey team members have been involved in preparatory work to develop a list of target NIS species relevant to the area, and have been trained to recognize them,[5] the team itself will identify many of the more visible, listed species (e.g. invasive macro-invertebrates) that appear in the samples collected. Other NIS organisms may be identified by the specialists (i.e. consultant taxonomists) engaged for the project and familiar with the biota of the region. In cases of doubt, the identification of a suspected NIS should be confirmed by taxonomic experts in its native region.

The above table includes nine questions (Chapman and Carlton 1991) that will help in differentiating any species suspected not to be native to a region. To ensure consistency in reporting, it is important that the taxonomists categorize NIS in accordance with these criteria when they return their identifications. The list of information on NIS to be provided by taxonomists is as follows:

- The authority and location of type specimen;
- Whether the identification represents a new record for the region (i.e. whether the species is already known to be present in the region or whether it has not previously been recorded in the region);
- If the species is known to be present in the region, whether its collection from the surveyed port indicates a regional range extension;
- Native and non-indigenous global distribution of the species (if known); and
- Materials (e.g. literature, museum collections) consulted for the purpose of the identification.



Pictures 25-27: Regional taxonomy workshops may be held to expose participants to field collections and laboratory activities associated with PBBS and specimen identifications

4.4 THE SURVEY REPORT

When preparing a PBBS report, it is important to keep in mind the various audiences for the report, e.g. government agency, national task force, various stakeholders, etc. Comprehensive PBBS reports can be extremely detailed and lengthy. However, for many purposes, reports can be selective and therefore more concise.

In some cases several different versions may be required. In addition to a report that meets the needs of the principal sponsors, shorter versions can be prepared in which the scope, presentation, and in particular the scientific content, have been adapted to the needs of particular audiences. In order to be read and understood by those with little technical knowledge, reports should be written in a clear, narrative style, making full use of carefully selected pictures and graphics.

Where a series of surveys and reports is anticipated, standardization of the reporting format will facilitate the comparison of findings from different areas.

A suggested minimum content for a PBBS report is as follows:

Executive summary

Aims and scope of the survey

Description of the area studied

- – Port operation and shipping movements
- – Physical environment
- – Existing biological information

[5] Regional taxonomy workshops can be a useful way of exposing participants to field collections and laboratory activities associated with PBBS and specimen identification.

Survey methods

Overview of results

- NIS species identified, abundance and distribution
- Synthesis of indigenous biodiversity

Location and availability of records, archives, specimens

Conclusions

Recommendations

Bibliography (including References) Appendices

A more comprehensive list of the topics that might be covered by PBBS reports, including a wider range of management considerations, is given in Annex D.

5

Optimizing the benefits of PBBS

5.1 PBBS IN A WIDER CONTEXT

As stressed in this document, PBBS may involve substantial investments in terms of time, human resources and funding, depending on their scope. However, the benefits can be optimized by ensuring that the results are utilized as much, and as widely, as possible, both within the overall national strategy for BWM and NIS, but also by contributing to knowledge of the marine environment. In many countries, a shortage of information on biological communities is a weakness that is hampering management of the marine environment. The data gained from PBBS can help to reverse this situation.

Within the GloBallast Partnerships Programme, a series of guidance documents has been produced to assist countries in their implementation of the BWM Convention, not least through the development and enforcement of a National BWM Strategy (see GloBallast Monograph No. 18). The PBBS is also linked to the other tools and guidance suggested by the GloBallast Partnerships Programme: the development of a national ballast water status assessment (GloBallast Monograph Series No. 17); the development of an economic assessment for BWM (GloBallast Monograph Series No. 19); and the identification and management of risks from organisms carried in ships' ballast water (GloBallast Monograph Series No. 21).

Following publication of survey results, it is important to ensure that the port authority and other stakeholders remain involved in addressing any concerns identified and opportunities for their mitigation. Although port authorities remain an important catalyst and potential coordinator for these initiatives, there is often a need for strategic and policy-level developments to encourage more proactive management of species of concern. Integrated management across the entire logistics chain is the only way to ensure a comprehensive, participatory and standardized approach to the reduction of alien species introduced through shipping vectors. Accordingly, the BWM Convention encourages scientific baseline assessments such as PBBS as a component of BWM activities, and as a means of catalysing multi-sectorial engagement.

PBBS are effective catalysts for the development or implementation of comprehensive ballast water or marine invasive species management frameworks. They assist ports in preparing their management plans and facilitate collaboration between the scientific and maritime communities.

Data from PBBS are necessary for risk assessments, either in the context of Compliance Monitoring and Enforcement (CME, i.e. understanding what routes/vessels may pose a high risk to the local marine environment – see Box X below), or for the assessment of possible exemptions granted to some ships under the BWM Convention (Regulation A-4). The results are also useful for identifying target or risk species that require on-going monitoring, or any further management or control measures.

In this context, the Scientific and Technological Research Council of Turkey (TÜBITAK) has developed a computerized risk assessment tool based on the GloBallast BWRA methodology, described in the GloBallast BWRA User Guide (available to governments on request to IMO-GloBallast, see GloBallast Monograph Series No. 21).

5.2 THE ROLE OF THE PORT AUTHORITY

From a port management perspective, PBBS may form part of the port's overall environmental management programme and is consistent with best practices for the sustainable development of trade facilities, transport chains and the local economy. PBBS can assist in identifying marine environmental problems and opportunities for management intervention. In particular, it can help to catalyze buy-in and engagement of agencies and other stakeholders having diverse roles in alien species management.

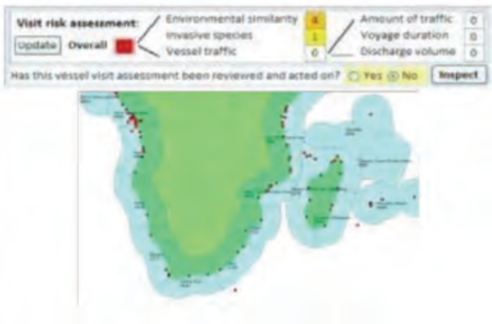
Box X: IOI-SA BWRADS System Applied in Port Louis, Mauritius

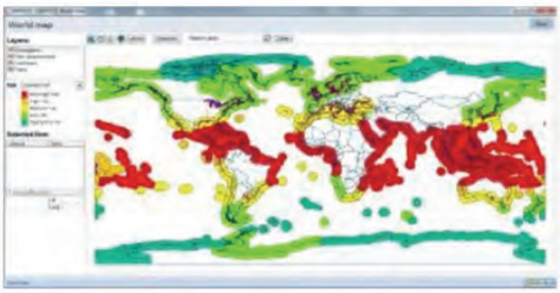
The International Ocean Institute-Southern Africa (IOI-SA) has developed a Ballast Water Risk Assessment and Decision Support (BWRADS) system for use in management applications such as Port State Control inspections. The pilot initiative was customized for the government of Mauritius, and installed at Port Louis to assist ballast water control measures. The Mauritius Oceanographic Institute assisted with the system development as a follow-up to the comprehensive PBBS and BWM efforts being supported by the Shipping Administration of Mauritius. The system is designed to support the Compliance Monitoring and Enforcement (CME) efforts associated with the implementation of the IMO BWM Convention. Although Mauritius has not yet ratified the Convention, the management framework is being put in place to prepare for possible ratification in the near future.

The BWRADS system is designed to provide an assessment of vessel or ballast tank-specific risk associated with invasive species to help target compliance control efforts (e.g. ship inspection) towards the highest risk vessels entering the port. It then provides guidance for the type of inspection to be conducted, while archiving the data provided. The system is based on the information supplied by a vessel in the Ballast Water Reporting Form (BWRF). When the key information is entered by the user, an assessment of the relative risk for invasive species introduction is produced, along with an interpretation of the risk and associated decisions to be made.

The risk assessment has three basic components: Environmental similarity, voyage-specific risk, and presence of known invasive species in source waters. Global records of invasive species presence and distribution were incorporated into the system. The addition of local species and ecosystem data gathered during the PBBS is essential to the overall strengthening of the assessment.

This system is designed specifically for use by port, maritime or environmental authorities to assist in CME and BWM generally. It is not intended to be used as a basis for exemptions under the IMO BWM Convention. The system is however adaptable to any port or region of the world, and its potential for further development and broader application is currently being assessed.





In summary, from the standpoint of managers within port authorities, PBBS can yield the following benefits:

- Provide baseline data on species and habitats to support environmental monitoring programmes;
- Detect introduced/alien species as a basis for targeted monitoring or control programmes;
- Facilitate risk assessment regarding the potential for further introductions, the spread of existing introduced species or the exportation of particular threat species to other areas;
- Provide information on ballast water operations of ships;

- Improve the reputation of the port authority (and other decision-makers and role players) as proactive and progressive in terms of best practices and sustainable development. This may provide opportunities to secure external support (i.e. funding, volunteers, public involvement, government support, corporate funding, etc.) for the management and control of alien species;
- Safeguard port and related infrastructure from the damage and costs associated with alien species impacts (this has implications for port development/construction, as well as maintenance requirements);
- Help to achieve alignment with international best practices for shipping;
- Inform the development and implementation of port environmental and ballast water/biofouling management plans; and
- Provide a focal point for collaborative engagement of port community stakeholders and the various relevant management sectors.

Ports may adopt a number of practices that will help to improve their programmes in environmental management, including BWM:

a) Policy statement

Port authorities may develop and display a policy statement relating to the port's mission, responsibilities and strategic objectives regarding sustainable practices, including biodiversity management. Another supporting policy document could outline the problems, trends, challenges and opportunities in relation to particular issues (in this case, alien species management); this could include specific activities and initiatives aimed at collecting data, managing and mitigating impacts and sharing information with stakeholders. The policy statement and supporting document would constitute a commitment that informs business planning, port and infrastructure development. Ideally, these documents would be updated at regular intervals to ensure they remain relevant and effective.

b) Environmental Management Systems (EMS)

Various Environmental Management Systems and tools exist to assist organizations in managing their environmental risks and opportunities, and to streamline management initiatives from a business planning perspective. The international ISO 14001 standard, however, is widely recognized across the globe and popular with a variety of organizations within the marine transport industry (i.e. port and logistics chain). This standard outlines requirements associated with the development and implementation of policy, objectives and plans for environmental compliance and best practice management.

c) Training and skills development

Any successful PBBS and accompanying management initiatives cannot work in isolation of training and capacity building activities. Training and awareness raising should not just be limited to practitioners in port surveys and alien species management, but should also include port management, port stakeholders and port users. Tailor-made training and awareness sessions can be developed for specific groups.

5.3 DATA STORAGE AND ACCESS

An integral part of PBBS is the systematic archiving of biological records, particularly of NIS, not only for future reference, but also for the benefit of the international community engaged in preventative NIS programmes. Such programmes are heavily dependent on reliable, up-to-date information on the status of NIS in different regions, in order to assess the risks associated with different routes and vectors, to develop suitable management measures and to identify priorities for risk mitigation.

Suitable data archives should be created at national level and it is strongly recommended that national databases be made available for inclusion in archives at regional and international levels. One archive system presently under development that aims to record data on NIS by region, and ultimately worldwide, is the AquaNis system at the Klaipeda University, Lithuania. This is already a valuable source of information for those engaged in NIS risk assessment and, if adequately supported, will continue to grow in importance.

5.4 IMPROVING CAPACITY FOR PBBS

Despite recent efforts by several countries to increase their support for PBBS and alien species surveys generally, there is still a fundamental lack of information regarding marine NIS in most areas of the world.

Until recently, most PBBS initiatives had been carried out either in more developed parts of the world, or were funded by external resources (GloBallast, NGOs/IGOs, etc.). However, there has been a recent and very encouraging shift towards funding being allocated locally (e.g. Ghana, Mauritius, India), which could be seen as an enhanced appreciation of the crucial need for improving the information base, as well as building capacity locally, rather than bringing in outside experts (see Box Y).

Box Y: ASEAN-India Cooperative Project

This is a cooperative project, involving ten countries (Brunei Darussalam, Cambodia, India, Indonesia, Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam), primarily aimed at awareness, developing cooperation and regional networking of scientists and experts to problems associated with marine invasive organisms. The objectives of the project are to and extent of transfer of organisms through shipping, to gather data from ship inspections Surveys and to identify important pest organisms and vulnerable areas in ships and ports. In connection, data on hard substratum communities is collected passively through deployment of panels in all the participating countries simultaneously. The data obtained on fouling can be uploaded to a web page and analyses are carried out through image processing software specifically designed for the purpose. This platform is of immense value in exchanging data between different ports/countries/regions. The project is also aimed at undertaking a regional study of known invasive organisms and to develop an understanding of the pathways of invasion so that methods for mitigation can be developed. This cooperative project will help in developing necessary expertise and enable development of region specific mechanisms for addressing the issue of marine bio-invasions and facilitate implementation of the requirements of international conventions. For more information, visit: www.bwmindia.com.

This capacity building element to PBBS is most important, and should be seen in the context of regional and national BWM strategies being developed around the world. A continuing capacity (locally, nationally, regionally) for biological monitoring and risk assessment will be required to support CME of BWM measures and thus implementation of the BWM Convention.


Pictures 28 and 29: Participants at a regional PBBS training workshop in Mombasa, Kenya


Pictures 30 and 31: Participants at a regional PBBS training workshop in Batumi, Georgia

6

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ANNEX A

Preservation schemes for different taxonomic groups

Anemones	Initially transfer the specimens to seawater to allow expansion of body/organs and then freeze or add menthol or magnesium chloride and leave overnight. Fix in formalin by adding an appropriate amount (10% of formalin) to the frozen specimens making sure it mixes as it defrosts and then store in formalin.
Aplacophora	Relax specimen using menthol, magnesium chloride or iced water and then fix in formalin. Rinse in water and store in 70% alcohol.
Asteroids	Fix in concentrated formalin with seawater (1:5) overnight. Remove the specimen from fixative and dry under the sun to remove moisture. Store dry. Alternatively, fix in formalin for 24-48 hrs and transfer to 70% alcohol for long term storage.
Brachiopods	Fix in formalin and store in formalin.
Cephalopods	Kill specimen by freezing, chilling or suffocation. Thaw and then fix in formalin. Finally, store in formalin or 70% alcohol.
Corals (Soft)	Allow the specimen to fully expand in seawater and then narcotize by freezing or adding menthol or magnesium chloride. Fix in formalin for 2-4 hrs. Remove formalin by rinsing with water and then store in 70% alcohol.
Crinoids	Fix in formalin for 2-3 days. Store in 70% alcohol.
Crustaceans	Specimen to be fixed in formalin and finally stored in formalin or 70% alcohol.
Ctenophores (comb jellies)	Fix in formalin and store in formalin or 70% alcohol.
Ectoprocts	**Hard species:** Fix in formalin and then dry. Store in dried condition. **Soft and lightly calcified species:** Fix in formalin for 4-12 hrs and then store in 70% alcohol.
Holothurians	Fix in formalin overnight and rinse thoroughly in water, or fix in 100% alcohol. Store in 70% alcohol.
Hydroids and hard corals	First narcotize in menthol or magnesium chloride overnight then fix in formalin. Finally, store in formalin or 70% alcohol.
Leeches	Narcotize specimen using menthol or iced seawater. Fix in formalin and store in formalin.
Molluscs (general)	Fix in formalin and store in formalin. Small specimens may be stored in 70% alcohol.
Oligochaete worms	Relax in menthol or magnesium chloride, fix in formalin and store in formalin or 70% alcohol.
Ophiuroids and Echinoids	Large and solid specimens may be treated as for asteroids above. Others may be fixed in formalin and stored in 70% alcohol.
Opisthobranchs	Should be relaxed before fixing. Allow specimens to crawl in seawater and then freeze overnight. Add formalin to frozen container or use menthol, magnesium chloride in seawater or iced seawater for relaxing purpose. Fix in formalin and finally store in 70% alcohol.
Platyhelminths	Use menthol or magnesium chloride for relaxation of specimens. Alternatively, place the specimen directly on the frozen formalin in a container to relax and then fix in formalin on ice.

Polychaete worms	Large specimens should be narcotized using menthol or magnesium chloride prior to fixing. Fix in formalin and finally store in formalin or 70% alcohol.
Polyplacophora	Flatten, fix in formalin and then store in formalin. Small specimens may be stored in 70% alcohol.
Sipunculan, Nemertean and Echiuran worms	Relax overnight using menthol or magnesium chloride in seawater followed by fixing in formalin and finally store in formalin.
Sponges	Fix overnight in 100% alcohol or in well-buffered formalin. Specimens can be preserved either in formalin or 70% alcohol (after thorough rinsing in water to remove the formalin).
Urochordates	For large solitary ascidians, narcotization overnight is recommended prior to fixing. Use menthol or magnesium chloride in seawater for narcotizing. Fix in formalin and finally store in formalin or 70% alcohol.

ANNEX B

Some common procedures used in sampling marine communities

Section 2.2.3 of these guidelines listed various devices used in sampling different marine substrates. Sampling methods, including the number of replicates and depths at which samples are collected, can vary widely depending on the target biota (i.e. survey objectives) and the characteristics of the site, as well as available time, funds and resources in terms of taxonomy, equipment and facilities. Descriptions of some of the more common methods are given below.

Hard substratum organisms:

Hard substratum organisms can be collected by active or passive sampling methods.

Active sampling involves scraping within a quadrat an area of 0.1 m^2 whereby the detached material is captured in a mesh or plastic bag. The scraper generally consists of a sharp blade mounted on either a long or short handle. Scrapers can be operated either by divers, or deployed on a pole from the surface. Where the total depth exceeds 6 m, samples are typically collected from three depth intervals: below high water mark (inter-tidal), mid-depth and near bottom. Where the water depth is less than 6m, one or two samples may suffice. As soon as possible after collection, samples should be rough sorted and preserved as appropriate (see Annex A).

Passive sampling methods involve the use of artificial substrates (e.g. settlement plates, panels, stone-filled wire-mesh baskets, etc. of known area/volume) suspended under water for different exposure periods and collection of settled assemblages by scraping or rinsing, as appropriate.

Mobile epifauna and fish:

Beach seines may be used to sample near shore juvenile fishes over sandy or muddy substrates. A 25 m seine with 15 mm mesh can be used. Gill nets and casting nets may also be effective in the collection of fishes within the confines of the port. Baited fish traps may also prove effective, as well as traps designed for shrimp and crabs. Locally-used trap designs and methods are recommended. Some specific trap designs are detailed in the CRIMP protocol.

Benthic infauna:

Benthic infauna can be sampled either by divers (e.g. corer) or using an appropriate grab (e.g. Van Veen) operated from a boat or quayside.

Divers may insert a tubular hand corer (0.025 m^2) into the sediment (approx. 250 mm deep) and seal the upper hole with a rubber bung or screw cap before withdrawing the corer from the sediment. On surfacing, it is emptied into a 0.5 mm mesh bag and washed underwater by agitation.

Samples collected using box corer or a Van Veen grab with known grabbing area (0.04 m^2) may be transferred into a 0.5 mm mesh bag with a drawstring mouth and a tapering bottom and washed underwater by agitation to remove fine sediment. The retained material can then be washed into a plastic bag and preserved in a 10% Rose Bengal and sea water formalin mixture. Subsequently, the sample is preserved in 5% formalin. Numerical abundance may be estimated using a stereo zoom microscope. Population density is expressed as numbers/m^2 and biomass as wet weight mg/m^2 (after removing the hard parts).

Benthic meiofauna:

Benthic meiofauna can be sampled by means of sediment cores either taken by divers using Acrylic or PVC tube of 10-15cm length (2.5 cm dia.) or collected from a Van Veen grab with a window. The sediment cores are transferred into plastic containers and preserved in 10% Rose Bengal and sea water formalin mixture. On shore, samples are washed gently through a 63-500 micron mesh. The retained material is preserved in 5% formalin in $MgCl_2$.

Sampling in rocky inter-tidal pools:

Samples of sedentary fauna and flora are collected from a known area (0.1 m^2). Scraped material should be rough sorted and preserved. The rough sorted samples should then be subjected to fine sorting and photographed where appropriate. Macro-algae should be photographed and dry-pressed for future reference.

Zooplankton:

Zooplankton may be collected either with a suitable plankton tow-net (e.g. Heron-Tranter) or by pumping water through an appropriately-sized (e.g. 100 µm) plankton mesh attached to the outside of the boat. Tow-net hauls may be vertical or oblique, as deemed appropriate, and at a low speed. The net is rinsed with water and the zooplankton collected in a plastic bottle, preserved with 5% formalin in seawater. In the laboratory, the sample may be split appropriately, if required, using a Folsom plankton splitter.

Phytoplankton:

There are 3 size-classes of marine phytoplankton: 1) Pico-phytoplankton (0.2 to 2.0µm); 2) Nano-phytoplankton (2.0 to 20µm) and 3) Micro-phytoplankton (20 to 200µm). To sample the entire range, several different methods may be required. As noted in the introduction to this Annex, the number of replicate samples, and the depths at which they are taken, will depend on the objectives of the survey, the characteristics of the site and the overall resources available.

For the investigation of pico-phytoplankton (0.2-2.0µm), small quantities of seawater (e.g. 1.8ml), collected from the surface and bottom using a Niskin or Van Dorn sampler, are placed in cryovials (2ml) and preserved with 0.2% paraformaldehyde (see CRIMP protocol). Subsequently, these vials should be transferred to liquid nitrogen for storage and transportation.

For nano-phytoplankton (2-20µm) and dominant micro-phytoplankton a known quantity of seawater (1 litre) may be collected from surface and bottom, using a Niskin or Van Dorn sampler, and preserved with a few drops of Lugol's iodine solution in plastic bottles. After transportation to the shore laboratory, the phytoplankton cells are concentrated by allowing the sample to settle for 48 hrs and subsequently removing the supernatant and making it up to a known volume.

Supernatant water may be removed by siphoning with a plastic tube with a piece of 10µm mesh covering the dipped end.

For the larger micro-phytoplankton (20-200µm), vertical or horizontal hauls can be made with a fine-meshed plankton net (20µm). With horizontal hauls, the net should be maintained approximately 2m below the surface and towed at a speed of 0.3m s^{-1}. Subsequently, the collected plankton cells should be washed off the net using seawater and transferred into appropriately labelled containers. Samples for incubation and culturing purposes should be kept in cool conditions; otherwise they can be preserved with a few drops of Lugol's iodine solution.

As an alternative to the above, a large volume (10-20 litres) of water may be passed through 20µm mesh and the retained cells re-suspended with a known volume of seawater from the same depth.

The sample is then preserved as above.

Dinoflagellate cysts:

Sediment cores (acrylic or PVC tube of 10-15cm length) can be collected either with the help of divers or using a Van Veen grab with windows, as appropriate. A Van Veen grab has many advantages; it is efficient, easy and safe to operate, quick and, most importantly, cost effective. The cores should be kept on ice and transported to a shore laboratory for analysis or culture studies as appropriate. If culturing of cysts is not desired, the raw sample should be fixed as soon as possible. For long term preservation, neutralized formalin or glutaraldehyde can be used as fixing agent. There are two different processing methods for cleaning and concentrating cysts from sediment: sieving without chemicals and a palynological technique using several chemicals (see flowchart below in Figure 5 and Matsuoka and Fukuyo 2000; Hyeon Ho Shin et. al. 2013). The choice of method is dependent on the purpose of the study. For cyst assemblage analysis, the palynological method is recommended. For culturing (establishing clone culture and cyst-motile form relationship and toxin production), sieving is the method of choice.

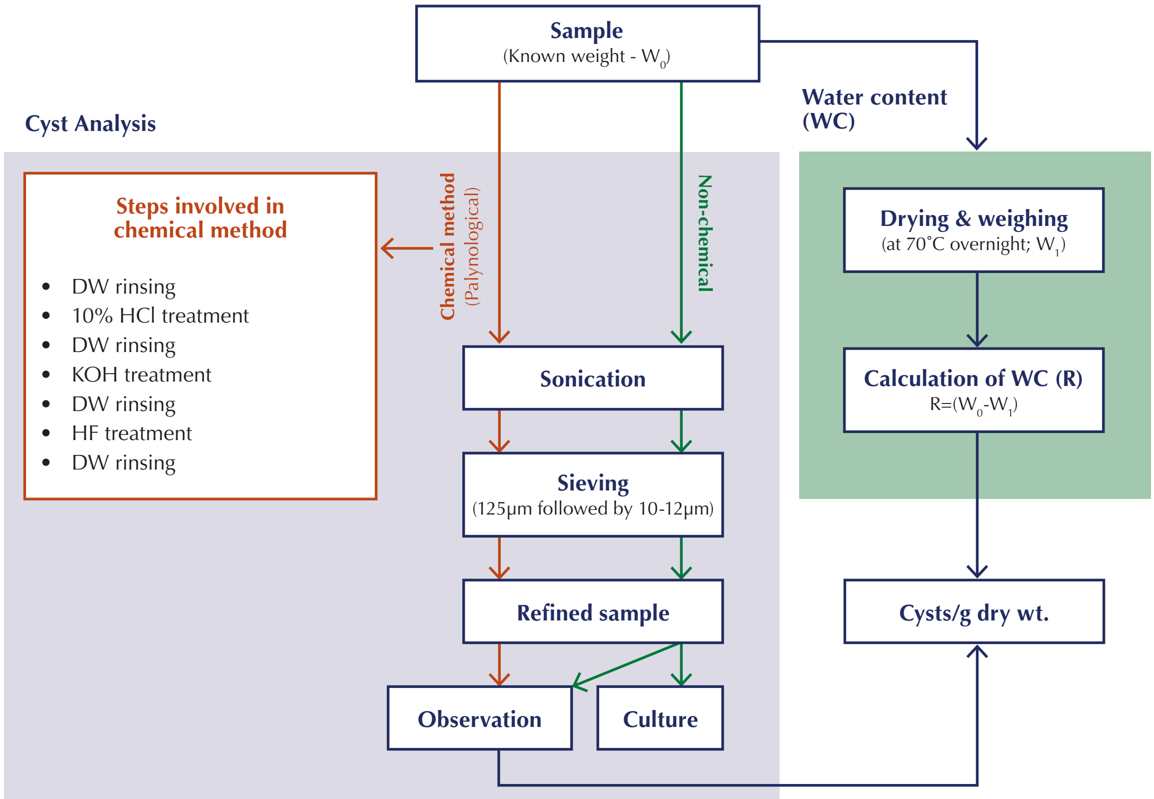

Figure 5: Techniques used to prepare sediment samples for cyst studies

DW – Distilled water, HCl – Hydrochloric acid, KOH – Potassium hydroxide, HF – Hydrofluoric acid

ANNEX C

Common techniques for bacteriological investigations

Bacteria can be classified using 3 different methodologies:

1. **Phenotypic Classification**

   Microscopic morphology

   Macroscopic morphology

   Biotyping

   Serotyping

   Antibiogram patterns

   Phage typing

2. **Analytic Classification**

   Whole cell lipid analysis

   Whole cell protein analysis

   Multifocus locus enzyme electrophoresis

   Cell wall fatty acid analysis

3. **Genotypic Classification**

   Guanine and cytosine ratio

   DNA hybridization

   Nucleic acid sequence analysis

   Plasmid analysis

   Ribotyping

   Chromosomal DNA fragment

Standard spread-plating method used for viable bacteria quantification

Culturable bacteria may be quantified using Zobell Marine Agar 2216. Pathogenic forms are quantified using specific media (Hi-media) following the manufacturer's instructions. For this, the sample is diluted and spread plated on Thiosulphate-Citrate-Bile Salts (TCBS) for Vibrios, MacConkey agar for coliforms, Enterococcus Confirmatory agar for Streptococcus and HiCrome EC0157:H7 Selective Agar Base to which HiCrome EC0157:H7 Selective Supplement (FD187) is added aseptically for *E. coli*. All the plates of specific media are incubated at 37°C for 24 hrs and colonies are counted.

To reduce uncertainties associated with counting of the pathogenic bacteria, *V. cholerae*, *S. faecalis* and *E. coli* are randomly picked from the selective agar and are confirmed by using a series of appropriate biochemical tests like gram staining, string test, oxidase, catalase, motility, indole, gas from glucose, Methyl Red, Voges Proskaeur and citrate utilization.

The culturable bacterial abundance (Viable Bacterial Count, VC) is expressed as Colony Forming Units (CFU) ml^{-1} for water samples or CFU g^{-1} for sediment and fouling samples. The sample to be analysed for Total Bacterial Count (TBC), that includes culturable and non-culturable bacteria, is fixed with formaldehyde (final concentration 1 to 2%; v/v).

Total Bacterial Count (TBC) using epifluorescence microscopy

The quantification of bacteria is done through the use of acridine orange and epifluorescence microscopy (Daley and Hobbie 1975) and the values are expressed as CFU ml^{-1} for water samples or CFU g^{-1} for sediment and fouling samples.

Other dyes such as SYBR Greens I and II, SYTOX Green, and the SYTO family are less dependent on medium composition and can be used for enumerating bacteria in marine environments (Marie et al. 1997; Lebaron et al. 1998). Because SYBR Green I (SYBR-I) has a very high fluorescence yield, it is strongly recommended to use this dye to enumerate bacteria in marine samples.

Flow cytometry (FCM) and enumeration of bacteria

Flow cytometry (FCM) is a useful tool for enumerating and characterizing microorganisms. It is used extensively for assessing the viability of microorganisms. It also offers the ability to physically separate the selected cells of interest by cell sorting for further molecular and physiological analysis. Bacterial samples stored in liquid nitrogen are stained or labelled with fluorescent tags that enable them to be identified electronically when passing through a beam of laser light. The advantage of associating FCM with the fluorescent molecular probes for differentiating viable and active or dead cells is noteworthy. Conventional methods for bacteriological tests of seawater quality take a long time to complete and the same can be achieved quickly and accurately using FCM which is capable of counting more than 1000 cells s^{-1}. This is particularly useful for assessing compliance with ballast water discharge standards.

Flow cytometry combined with Fluorescence In Situ Hybridization (FISH) is an increasingly popular method of enumerating cells in environmental samples. The advantages of FCM over conventional microbiological techniques are the speed and accuracy of analysis. When compared to conventional culturing techniques there is also the advantage of being able to detect viable but non-culturable cells in seawater sample which represent a major fraction of marine bacterial species. The main concern is whether or not harmful organisms are being discharged into port waters and, if so, in what quantities. Flow cytometry analysis of ballast water may be important for control and optimization of different technologies used in ballast water treatment.

Microorganism Identification and Classification based on MALDI-TOF MS

MALDI-TOF MS is a reliable, high throughput method for the classification and identification of microorganisms. Starting from a single colony or other biological material, sample deposition and preparation with MALDI matrix is performed within a few minutes. After sample drying and loading the instrument spectra acquisition is completed rapidly. A prerequisite is the establishment of high quality spectra libraries for the area under investigation.

ANNEX D

Possible lay-out and content of PBBS reports of extended scope

Executive summary

Glossary

Introduction

- Description of ports in the region/area
- Description of the port to be studied
- Port operation and shipping movements
- Physical environment
- Existing biological information

Survey methods

- Physical and chemical measurements (as appropriate)
- Sample collection
- Survey of fouling and benthic organisms
- Underwater camera surveys
- Sediment sampling for cyst-forming species
- Plankton sampling and identification
- Survey of fish species
- Laboratory sorting and identification of organisms

Results

- Water and sediment characteristics
- Fouling and benthic organisms
- Plankton sampling and identification
- Phytoplankton
- Zooplankton
- Survey of fish species
- Assessment of risk of new introductions to the port

Management of existing non-indigenous species

Prevention of new introductions

Conclusions

Recommendations

References

Appendices

More Information?

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