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Review

Ballast Water Management in Ports: Monitoring, Early Warning and Response Measures to Prevent Biodiversity Loss and Risks to Human Health

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Abstract: Ballast water is recognised as successfully transporting non-native (potentially) invasive alien species and other harmful organisms (human pathogens and toxic phytoplankton) from one region to another. Global warming enables the successful adaptation of non-native species in new areas. The early detection of harmful species increases the likelihood that the response will be effective and cause less damage to biodiversity, ecosystems, economies and human health. Scientific evidence strongly points to the importance of prevention. In this context, this refers to continuous port monitoring, carried out with the aim of detecting harmful species soon after their introduction. The objectives of rapid detection are (a) early warning and prevention of further spread of harmful species through ballast water or natural circulation, and (b) a timely response through eradication or other appropriate strategies to reduce the number or spatial extent of introduced species. This paper provides guidance for the development of ballast water management in ports based on a literature review. Available and new methods for identifying marine species and best practises in port monitoring for the early detection of harmful species, as well as early warning and response measures following the introduction of species in ports, are presented and discussed.

Keywords: non-native species; invasive alien species; ballast water management; port monitoring; early warning; response measures

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1. Introduction

Non-indigenous species (NIS), also referred to as alien, allochthonous, exotic, introduced or non-native species, are organisms that have been intentionally or unintentionally removed from their natural habitat through human activities. Since the 1990s, NIS have been considered as a major threat to native biodiversity and community structures, which can lead to habitat alternations, changes in ecosystem functioning, the introduction of new diseases and parasites, and genetic changes, such as hybridisation with native taxa [1–3]. It has been known for decades that these invasions can negatively impact natural ecosystems, trade and human health [4]. According to a study on terrestrial NIS [5], 1 in 10 imported NIS are introduced to a new location, 1 in 10 introduced NIS become established due to favourable abiotic and/or biotic conditions, and 1 in 10 established NIS become a pest, also known as invasive alien species (IAS) [6].

The first mention of NIS transfer via maritime transport dates from 1854, when Darwin suggested that barnacles were transported on ship hulls from the Pacific to the Mediterranean [7]. Seawater has been regularly used as ballast since the 1880s, replacing dry materials [8]. The idea that ballast water (BW) serves as a vector in the spread of NIS dates back more than a century, when Ostenfeld suggested that the Indo-Pacific diatom *Odontella* (*Biddulphia*) *sinensis* most likely entered European waters with the water or sediments in ships' ballast tanks [9]. However, BW research only began in the 1970s [10] and continued sporadically until the late 1980s [11]. In the early 1990s, an extensive survey of microalgae in BW showed that ships calling at Australian ports contained a wide range of living dinoflagellate and diatom resting stages [12]. Today, we believe that the infamous invasions of the Laurentian Great Lakes by the Eurasian zebra mussel *Dreissena polymorpha* [13], of the Black and Azov Seas by the American ctenophore *Mnemiopsis leidyi* [14] and the transfer of the epidemic cholera *Vibrio cholera* 01 from South America to the United States [15] were triggered by BW.

A study of a 50-year data series (1965–2015) found that an NIS was reported as a first detection somewhere in the world every 8.4 days [16]. The rate increased in the 1990s and 2000s, which can be attributed to the increase in global trade, Ref. [17] or, alternatively, to growing awareness among scientists, governments and the public, and increased funding for surveys, monitoring and other assessments [16].

It is estimated that at least 7000 different marine species are transported in BW around the globe every day [18]. Of the 25 most common NIS reported as new first detections worldwide between 1965 and 2015, BW was suspected to be one of the few potential introduction routes for no less than 22 NIS [16]. In addition, it has been estimated that 10^{20} bacteria and viruses survive annually in a Chesapeake Bay port following BW discharge [11].

The consequences of the introduction of NIS are not easy to identify. A comprehensive review of reported NIS in European seas (here in terms of IAS) identified 86 species with significant impacts on ecosystem services and biodiversity, most of them with both positive and negative impacts (72%), 8% with exclusively positive impacts and about 20% with exclusively negative impacts [19]. Although it is difficult to realistically assess the financial aspect of IAS impacts, annual estimates are at least EUR 12 billion for Europe [20], GBP 1.7 for the UK and USD 137 for the US [21]. These sums have probably been underestimated, as many indirect costs, such as damage to ecosystem services and loss of biodiversity, are not easily quantified or remain undetected [22].

To address the problem of NIS, the European Commission has adopted several regulatory instruments: the Strategy on Invasive Species [23], the Marine Strategy Framework Directive (MSFD) [24], the Biodiversity Strategy [25] and the Regulation on the Prevention and Management of the Introduction and Spread of Invasive Alien Species [26]. For their implementation, they envisage the existence of national and regional lists of NIS [27]. For example, the MSFD proposes that the implementation of the marine ecosystem inventory and monitoring of environmental status should assess the composition of NIS, the number of new introductions and their impact on the community, habitat and ecosystem functionality [24,28].

One of the most specific regulations related to NIS is the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) [29]. The BWM Convention requires that all ships flying the flag of a state that has been ratified by the BWM Convention develop and implement a Ballast Water and Sediment Management Plan [30]. By 8 September 2024, all ships must comply with Regulation D-2, the Ballast Water Performance Standard of the BWM Convention, which includes the installation of a BWM system on board. Regulation D-2 requires that vessels discharge (1) <10 viable organisms per cubic metre for $\geq$ 50 micrometres in minimum dimension; (2) <10 viable organisms per millilitre for < 50 micrometres and $\geq$10 micrometres in minimum dimension; and (3) that the discharge of indicator microbes does not exceed the specified concentrations. Indicator microbes as a human health standard include toxicogenic *Vibrio cholerae* (O1 and O139) with less than 1 colony forming unit (CFU) per 100 millilitres or less than 1 CFU per 1 g (wet weight) of zooplankton samples; *Escherichia coli* less than 250 CFU per 100 millilitres; and intestinal enterococci less than 100 CFU per 100 millilitres.

In addition, the BWM Convention states that parties shall, individually or jointly, endeavour to monitor the effects of BWM in waters under their jurisdiction (Article 6-Scientific and Technical Research and Monitoring). The purpose of the monitoring is to document the presence or absence of harmful aquatic organisms and pathogens (HAOP) in order to inform seafarers of areas where BW should not be taken due to known adverse conditions (Regulation C-2-Warnings Concerning Ballast Water Uptake in Certain Areas and Related Flag State Measures). This is to prevent HAOP from spreading from one port to another and then to a larger area.

The BWM Convention defines HAOP as any aquatic organism "which, if introduced into the sea including estuaries, or into fresh water courses, may create hazards to the environment, human health, property or resources, impair biological diversity or interfere with other legitimate uses of such areas". In line with the definition of the BWM Convention, HAOP is considered to include all potentially harmful species: NIS, cryptogenic species (of unknown origin), native species and pathogens [31,32]. Toxic phytoplankton species and human pathogens, which may be native but not present in the receptor port at the time of their introduction through BW discharge, may pose a risk to human health after discharge, either as a result of the development of harmful toxic bloom (HAB) or through direct exposure during marine activities. The BWM focuses on the timely detection and control of all harmful species observed in the port, regardless of the origin of the species, as long as the species affects or may affect the environment, the economy or human health.

2. Aim

The aim of this review is to provide guidelines for the development of ballast water management in ports. Available and new methods for marine species identification and best practices for port monitoring for early detection of harmful species (NIS, IAS, toxic species and human pathogens), as well as early warning and response measures following an observed intrusion into the port, are presented and discussed. Based on previous knowledge about the possibilities of ballast water treatment and the limitations of modern methodology, the importance of monitoring in ports is highlighted as an optimal and extremely important part of BWM.

3. Ballast Water Management in Ports

Prevention is by far the most important and environmentally desirable strategy to protect against the introduction of harmful species, and is far more cost-effective than any other post-introduction response strategy available [33]. When it comes to protecting the marine environment from the introduction of HAOP using BW, the only option is to follow practices governed by national and international laws and regulations, which requires supporting the rapid implementation of the BWM Convention [34].

The BWM Convention requires BW on all ships to be treated prior to discharge (after 8 September 2024) and calls for (i) continuous port monitoring for the early detection of harmful species to prevent further transfer of BW to other ports and (ii) the prevention of the release of BW suspected of containing HAOP [29,30]. The BWM Convention also proposes measures for two situations: (i) if HAOP is present in the port, BW is received in another area that does not contain HAOP or alternative sources are used such as drinking water or treated water and (ii) if HAOP is present in BW, BW is released to reception facilities in the port. These alternatives are the only real prevention against the introduction of HAOP through BW available today.

As the above measures are largely unacceptable for implementation in ports around the world, following the basic BWM Convention guidelines is the next best option to protect against the introduction of HAOP with BW. BWM in ports can be divided into three activities, each of which contributes to the main problem of reducing and preventing biodiversity loss and risks to human health from the introduction of HAOP with BW from ships: (1) port monitoring, (2) early warning and (3) response measures (Figure 1).

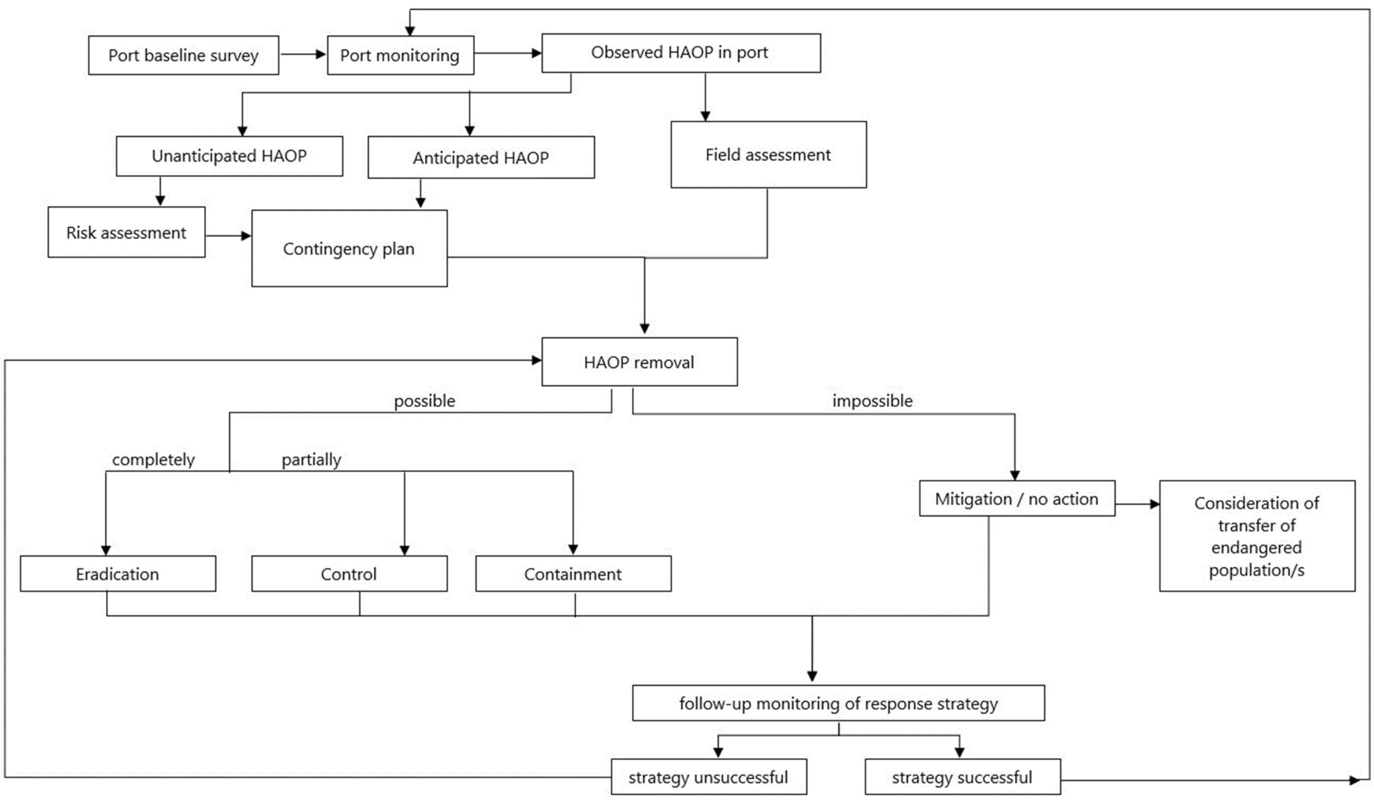

Figure 1. A BWM decision flow diagram (early warning excluded for simplicity).

4. Port Monitoring

4.1. Introduction to Port Monitoring: Port Baseline Survey

The Port Baseline Survey (PBS), a comprehensive baseline survey of the species inventory in commercial ports [35], precedes port monitoring and is a fundamental and integral component of effective BWM. A comprehensive assessment of five major port survey approaches has shown that each is an important tool for determining invasion patterns, surveillance and monitoring programs and responding to introductions, serving to detect over 1185 non-native, 735 cryptogenic and 15,315 native species in 19 countries from different regions [36]. They each have their own specificities and serve particular purposes due to their quantitative, qualitative or mixed approach, their focus on target species or whole communities, and their fieldwork, which is carried out by taxonomic experts as opposed to non-experts.

The five approaches are: (1) the CRIMP (Centre for Research on Introduced Marine Pests) protocols, which are revised protocols for baseline port surveys for introduced marine species including survey design, sampling protocols, and specimen handling protocols; Ref. [37], (2) the rapid assessment survey protocols for introduced, cryptogenic, and native species, Refs. [38–40], (3) the Bernice P. Bishop Museum protocols for the detection and determination of the distribution of introduced, cryptogenic, and native species and for their invasion pathways and vectors, Refs. [41,42], (4) the Chilean aquaculture surveys focusing on a single target species (see [36]), and (5) the passive sampling method employing artificial substrates for the passive collection of fouling epibiotic communities to detect species introduced in ports, determine their distribution patterns and potential threats, monitor vector patterns, and serve as an early warning tool, Refs. [43,44].

4.1.1. The CRIMP Protocols

The CRIMP (Centre for Research on Introduced Marine Pests) protocols consist of revised protocols for baseline port surveys for introduced marine species: survey design, sampling protocols, and specimen handling protocols [37]. They are specifically for port surveys and are designed to maximise the likelihood of HAOP detection. Therefore, sampling strategies focus on habitats and sites that are most likely to be inoculated with species associated with transport vessels (e.g., commercial or recreational vessel hull fouling, ballast water, aquaculture activities). The frequency of sampling is seasonal to cover the entire year and different life stages.

CRIMP protocols are designed to provide a comprehensive baseline which can be followed by repeated surveys at various intervals (from 6 months to several years). They include a combination of quantitative, semi-quantitative and qualitative sampling methods in port or marina areas (high traffic regions), habitats on or around wharf piles and in adjacent soft substrates, marine protected areas and areas frequented by fishing and recreational vessels. As reference sites, pristine areas, i.e., areas which are minimally affected by human activities, and adjacent coastal/island areas are included. Sampling methods are based on best ecological sampling practices and experimental design, and include alternatives in case of various resource constraints [37].

The CRIMP protocols include plankton, fouling organisms and mobile epi-fauna of hard substrates, and epibenthos, mobile epibenthos, cyst-forming species and benthic infauna of soft substrates. Samples are collected or organisms identified in situ via visual survey, photography and videography. Environmental data investigation includes measurements of basic water parameters (temperature, salinity, turbidity) and sediment analysis (grain size and organic content). Non-experts can perform work on the site, as the collected samples and the image material can be processed later. For this reason, these surveys are destructive, but they allow experts who are spatially and temporally dislocated to make an identification.

Replicate sampling is carried out when small-scale heterogeneity could affect the detection of target species (e.g., sampling for dinoflagellate cysts). The CRIMP protocols therefore stipulate that most samples are taken at expected introduction sites/habitats and that sampling effort is reduced at non-priority sites. The CRIMP protocols include guidelines for handling and archiving samples, as well as for archiving information and reporting. Although the surveys conducted according to the CRIMP protocols require a lot of time and financial effort, they allow for a high probability of the detection of NIS and the comparison of results between different sites, and thus provide a good cost/outcome ratio.

4.1.2. The RAS Protocols

The rapid assessment survey (RAS) protocols for introduced, cryptogenic, and native species provide a qualitative and non-destructive survey of fouling communities at coastal sites associated with shipping and hull fouling, aquaculture, marinas and commercial and recreational fishing, Refs. [38–40,45,46]. The focus of the RAS protocols is on the early detection of new introductions to facilitate rapid response action to eradicate or control observed species and to provide a list of reference species for future surveys and assessments of the effectiveness of prevention or control measures.

The system relies on taxonomic experts to identify species in situ in a given area and in a period of one hour per location and one week per survey. The strength of the RAS protocols is that the specialists produce a reasonably complete species list in this short period. It is conducted in summer when most species are at their peak in terms of body mass and are therefore most easily detected and identified in port and non-port areas on all available substrates and microhabitats to maximise the number of samples collected. In addition, basic water parameters and a GPS indication of the location are measured.

The assessment includes an evaluation of the likelihood of a species being introduced into a new region based on 10 criteria: (1) the species was previously unknown in the region; (2) the range has expanded following introduction; (3) potential human-caused vectors exist; (4) association with other introduced species; (5) association with artificial structures and environments; (6, 7) discontinuous regional and global distribution; (8, 9) passive life history and global mechanisms for dispersal are absent or insufficient; and (10) exotic evolutionary origin, i.e., the closest relatives are found elsewhere [39].

4.1.3. The BPBM Protocols

The Bernice P. Bishop Museum (BPBM) protocols were developed for detecting and determining the distribution of introduced cryptogenic and native species, as well as their invasion pathways and vectors. Originally, the BPBM protocols were developed to obtain information on NIS and cryptogenic species in Pearl Harbour, Hawaiian Islands, and to compare and complement the historical records and collections available for this port at the museum since 1899 [41].

Consistent with this region, the protocols facilitated the study of NIS in commercial, recreational and historic ports, in areas adjacent to ports and in pristine areas influenced primarily by hull-fouling from military and tourist vessels, fisheries, and mariculture, Refs. [36,41,42]. The BPBM protocols are qualitative and semi-destructive. They require the use of specialised divers who are also taxonomists, collecting samples by hand for species that could not be identified in the field. The protocols are limited to the benthos and mobile fauna. They contain little information on sampling procedures and do not detail the sampling area, reference sites and replicates, which prevents reapplication.

4.1.4. The Chilean Aquaculture Surveys

The Chilean aquaculture surveys, which focus on a single target species, were specifically designed to investigate whether non-native species introduced for aquaculture purposes have escaped from aquaculture facilities [36,47]. Surveys are conducted along transects within 2 km of the facilities, preferably quarterly but at least annually [36]. It is limited to a specific group, originally molluscs, as the target species was an NIS mollusc. Therefore, the visual mollusc survey was conducted with a focus on the target species, all of whose specimens were collected during the dives [36].

4.1.5. The Passive Sampling Method

The passive sampling method uses artificial substrates to passively collect fouling epibiotic communities to detect NIS in ports, determine their distribution patterns and potential threats, monitor vector patterns, and serve as an early warning tool, Refs. [36,44]. The choice of locations for deploying the plates, which serve as artificial substrates, can be based on anthropogenic influences and range from commercial traffic and shipping channels to recreational traffic and a variety of physical environments [44].

Plates can be deployed once at different time periods for monitoring purposes and recovered after a specified period of time (one to several months). In cases where the period for deployment is less than a year, mature communities and seasonal trends may be missed. Species are determined under the microscope within a grid with specific plot dimensions. Species are then assessed based on available published lists and expert opinion as to whether they are native, introduced or cryptogenic.

4.1.6. The PBS Protocol Application

For a detailed and comprehensive PBS, the CRIMP protocols remain the first choice, either in their original version or with some modifications [36], whereas other approaches seem more suitable for monitoring. As PBSs are primarily focused on species detection and identification, they are often referred to as Port Biological Baseline Surveys (PBBS) [35,48,49]. However, the CRIMP protocols, by omitting *biological*, highlight the importance of expanding the survey to include non-biological port features as an important part of BWM.

For example, the CRIMP protocol was used to develop the GloBallast guidelines [35]. In addition, the HELCOM/OSPAR port sampling protocol [45] incorporates the methodology of CRIMP and RAS [38,50] and has been adapted from the HELCOM and OSPAR CEMP marine monitoring protocols [51,52]. The HELCOM/OSPAR protocol and a regional rapid assessment have been applied simultaneously in the assessment of 'hot spots' for potential invasions and in the establishment of a list of macrobenthic baseline species in the coastal Wadden Sea [53]. Based on the evaluation of their effectiveness and scope, the use of the more comprehensive H/O protocol was recommended for future port surveys, complemented by a rapid assessment of natural invasion 'hot spots' such as oyster reefs.

Another example of the use of the CRIMP protocols was the Adriatic PBS Protocol [54], which was developed on the basis of the CRIMP protocols for the joint PBS efforts of the six countries sharing the Adriatic Sea [55]. The main argument for selecting the CRIMP protocols was the comparability of data between the sites studied and the optimal ratio between the resources invested and the potential of species detection [36]. In addition, the CRIMP protocols provide detailed explanations of sampling and measurement procedures in different port habitats. They include plankton and nekton (phytoplankton, zooplankton, mobile fauna and epifauna), hard substrates (mobile epifauna and fouling), and soft substrates (epibenthos, mobile epibenthos, benthic infauna, and dinoflagellate cysts), and seagrass and algal beds, as well as environmental measurements (temperature, salinity, turbidity, and sediment) and meteorological measurements (air temperature, cloud cover, sea state and wind speed/direction).

In addition, the Adriatic PBS guidelines include the strategy for port and area selection, sampling locations and frequency, and parameters. Furthermore, the Adriatic PBS protocol also includes several mandatory (ichthyoplankton, *E. coli* and intestinal enterococci), additional (water transparency, nutrients, oxygen, chlorophyll *a*, meiofauna, and *V. cholerae* serotypes 01 and 0139) and optional parameters (chemical analyses and physical measurements). The inclusion of these measurements allows for a more comprehensive view of BWM and provides an integrated insight into the port features studied and the community living in them.

The Adriatic PBS protocol was optimised for the initial port investigation, which was carried out by institutions with different human and material resources, and therefore led to the provision of recommended and alternative procedures [55,56]. Based on the experience of the Adriatic PBS, it is necessary to highlight an important aspect of the cross-board efforts in the implementation of the PBS. While more detailed guidelines would be very useful to ensure the consistency of data collected across multiple ports, their implementation would require many institutions to completely change or adapt their usual methods, which would ultimately lead to a loss of continuity with previously collected data. However, this problem goes beyond the scope of BWM, and has a broader relevance for scientific and professional studies in a region, and therefore requires a serious and systematic approach to reach an optimal solution [54].

4.2. General Guidelines for Port Monitoring

The PBS species list facilitates the observation of any changes in species composition during port monitoring, including a HAOP introduction, and therefore provides the basis for the EWS as the next step in BWM. In general, databases that are established, updated and maintained on a scientific basis are the most reliable source of information on NIS, population dynamics and ecology, and thus provide a reliable basis for response measures [57,58].

The ultimate goal of port monitoring is the timely detection of HAOP to enable an effective EWS to prevent the loading and transfer of HAOP-containing BW to other ports and to take measures to protect the local environment. Harmful phytoplankton species, human pathogens and faecal indicator bacteria (*E. coli* and intestinal enterococci), which indicate the possible presence of human pathogens in seawater, can have negative impacts on human health through the consummation of marine products and during recreational activities. On the other hand, non-native species, which are even more difficult to detect, pose a significant threat to local biodiversity and the ecological balance of the marine environment.

In typical sampling protocols, the probability of detecting a species is low unless the population density is high [59], so sampling is only reliable for species with medium to high abundance [60,61]. In addition, labour-intensive traditional NIS sampling methods such as traps, grabs, settlement panels [62], observer bias [63] and small or patchy NIS population size, especially in the early stages of invasion, are significant barriers to their early and rapid detection [59]. Consequently, NIS may remain undetected until they form large populations and/or spread [64]. Ideally, a newly introduced species should be detected while it is still rare and geographically limited [65], before its abundance and range increases to the point where any attempt at a response requires intensive effort and large resources, and may become unattainable [66].

No investigation can really prove that something is not there, and the search for rare species is very likely to be unsuccessful. Therefore, "the goal should be reasonable certainty that the effort was sufficient to detect 'rare' species, however that is defined," which is also a challenge in providing funding for this endeavour [67]. To achieve this goal, two approaches need to be combined: the monitoring of target-species and broad- spectrum monitoring, also referred to as active and passive surveillance, respectively [68]. The first approach focuses on previously identified HAOP of concern, including HAB, human pathogens and IAS, and the second on all newly introduced marine species. Established national and/or regional marine inventories for which preventive or response measures have been identified are based on a number of factors such as the potential threat of invasion and the impact on the ecosystem and economy [69]. For example, a preliminary list of native HAOP, including species considered to be globally harmful (potential NIS or IAS) which could be introduced to the Adriatic through BW, has been established specifically for the purposes of port monitoring for BWM in the Adriatic [70].

While the design of monitoring of target species benefits from considerable prior information, Ref. [71], broad-spectrum monitoring should be carried out according to a comprehensive and detailed research plan, as any new introduction will require surveys in all relevant environmental dimensions. The survey design (site selection, frequency and methodology) and its implementation are the essential components of monitoring, as they determine the adequacy and efficiency of the effort, establish interpretive options and support informed policy and management decisions [67]. The main problem is deciding on the overall scale of the effort, as this depends on available staff and financial resources.

4.3. Port Monitoring Methods

Species identification is possible either on the basis of morphological characteristics using the classical method of microscopy or on the basis of DNA using modern laboratory techniques. Port monitoring today mostly depends on a large number of highly qualified taxonomists who can identify native and non-native species, as well as timely laboratory work and expertise in detecting human pathogens. However, conventional methods are characterised by low spatial and temporal resolution, a relatively high workload and limitations in identifying rare and juvenile stages of native species and in distinguishing similar or even the same morphological characteristics (i.e., cryptic species) [72]. As a result, the need for economic methods that are less demanding, especially in terms of the need for human resources and expertise, was recognized. This triggered the development of biodiversity estimation methods based on a molecular approach [73] and autonomous sensor systems that allow for in situ research and data collection over large areas and time spans [74].

4.3.1. Molecular Methodologies

The development of molecular tools has been recognized as a general priority for invasion science [75]. DNA barcoding, the study of a small fragment of the genome (the 'barcode' region [76]), is increasingly used in marine environmental and port biota research [77,78] and in NIS management [79], and could therefore also have applications in BWM. In the following sections, a brief overview of molecular methods is provided, based on a comprehensive review by Zaiko and co-authors of the strengths and weaknesses of environmental DNA/RNA tools for managing and monitoring NIS in marine biosecurity [80].

In addition to DNA barcoding, other molecular-based methods have been developed. They include the traditional end-point Polymerase Chain Reaction (PCR), which is commonly used in marine biosecurity, and a specific end-point PCR assay to confirm certain NIS from environmental samples such as the Atlantic bivalve *Rangia cuneata* [81], the mollusc *Mya arenaria* [82] and the Australian tubeworm *Ficopomatus enigmaticus* [83]. Further progress has been made with quantitative PCR (qPCR, also known as real-time PCR), one of the most promising molecular techniques for the detection of specific and sensitive targets [80]. In particular, assays have been developed for harmful species: dinoflagellates of the genus *Alexandrium* [84,85], sea squirts *Styela clava* [86], and *Didemnum* spp. [68], Amur River clam *Potamocorbula amurensis* [87], and Mediterranean fan worm *Sabella spallanzanii* [88].

Progress has been made in distinguishing between living and non-living cells, usually through the use of intercalating nucleic acid dyes that specifically penetrate, only damaged lipid membranes (i.e., dead cells) [80]. An alternative method for the detection of living organisms is the application of PCR tools to eRNA. This method, although more expensive and challenging, provides more efficient results as RNA degrades faster in the sea (usually in several hours to days) [89,90]. Another method that provides an extremely low detection limit is droplet digital PCR (ddPCR), in which discrete droplets are thermally cycled and then analysed for target DNA using fluorescent sensors [80]. Second-generation DNA sequencing technology, environmental DNA (eDNA) and community DNA made it possible to significantly shorten the time between the first introduction of NIS or secondary spread and observation [91]. While eDNA is ubiquitous in almost all environments and comes from exfoliated cells, faeces, mucus, gametes, cadavers, and countless other sources [92], community DNA is a mixture of whole organisms selected from the ecological matrix [93].

However, a significant advancement in the barcoding approach has been made possible through advances in high-throughput sequencing technology (HTS). The combination of HTS with barcoding is termed 'metabarcoding' and typically involves bulk DNA extraction, PCR amplification and HTS complex species communities to identify multiple taxa sequenced simultaneously from a mixed sample [94,95]. Metabarcoding is highly effective in characterising marine biodiversity and communities, as well as in detecting potentially harmful species [94,96,97]. In addition, the high sensitivity of this method allows for the detection of species with very low biomass [98,99], enabling the sampling of rare taxa that are not detectable using conventional methods. The applicability and value of a metabarcoding approach in the detection of NIS was demonstrated, for example, by a survey of complex zooplankton communities in 16 major Canadian ports that detected 24 NIS out of a total of 379 identified species, some of which were present in very low abundance [94].

The use of qPCR and metabarcoding opened up new possibilities in the study of the biodiversity of microorganisms in the water column and on the seabed, and overcame the limitations of conventional methods, which was particularly important in the identification of human pathogens [72]. In addition, eRNA metabarcoding is increasingly used in ecological research to study the human-induced impacts and resulting changes on marine communities [100], but also for biosecurity [101] and BWM applications [102]. Furthermore, surveillance programmes using eDNA-based tools allow for the observation of the introduction or spread of NIS at an earlier stage than programmes relying solely on classical research methods [94,103,104] and the detection of cryptic species in their initial developmental stages, when their microscopic identification is difficult or impossible [97,105,106].

On the other hand, molecular methods have certain limitations. PCR-based metabarcoding still cannot provide data on the abundance of each species detected [107] nor can it distinguish life stages [72]. Furthermore, results can be false positive, e.g., for non-living or non-target species, accidentally contaminated samples or, as is often the case with HTS metabarcoding, due to biases associated with markers or references [108] or false negatives [109]. In addition, collaboration between molecular ecologists and taxonomists is crucial for the accurate characterisation of species and the provision of quality-assessed barcode sequences in public databases [110]. The availability of taxonomic expertise within the classical microscopic approach to NIS assessment and management has already been highlighted as an important general requirement [111]. The same requirement applies to the development and implementation of molecular tools [112], which is currently limited by the existing barcode sequences in open databases [113].

4.3.2. Non-Molecular Methodologies

The advantages and weaknesses of using methods and technologies developed primarily to improve the assessment of the state of the marine environment under the MSFD are discussed in detail in [72], and some of them that could be applicable in routine BWM monitoring are presented here. For example, microarrays, developed for the in situ identification of harmful algal blooms (HAB) and rapid identification of toxic algae show high sensitivity in some cases, allowing for the identification of some algae down to species level, which is not possible with light microscopy, although in other cases identification of algae remains at genus level.

Flow cytometry and high-performance liquid chromatography (HPLC) are two valuable techniques for obtaining basic data on the taxonomy of phytoplankton based on pigment content. CHEMTAX is a statistical tool used for estimating phytoplankton biodiversity based on the relative contributions of the different taxa to the total concentration of chlorophyll *a* (TChla) in a sample [114]. The disadvantage of this method is that the calculation is based on existing knowledge about the composition of phytoplankton in the area studied [115]. In contrast, the chemical approach to the study of phytoplankton cells allows CHEMTAX to differentiate the proportions of smaller size classes (cryptophytes, prymnesiophytes and prasinophytes) in the TChla concentration, which are collectively classified as indeterminate flagellates with the conventional light microscopic method [116]. In addition, CHEMTAX could provide information that a sample contains a harmful species (e.g., HAB), which is particularly useful when they are known to occur in the study area [117].

With simple activity probes, flow cytometry can indicate the physiological state of the phytoplankton cell [118]. Furthermore, it is possible to automate sample collection and handling, flow cytometry and data processing [119] and use them in environmental monitoring systems. SmartBuoys, for example, are an in situ technology that allows for fully or partially uninterrupted surveys with a high sampling intensity of marine hydrological, physical and chemical parameters (salinity, temperature, turbidity, chlorophyll fluorescence, oxygen saturation and nitrate concentration). In addition, special chemical sensors have been developed to monitor algal toxins, organic pollutants and heavy metal concentrations [120].

Another technique that can be used for the long-term monitoring and assessment of the sea is high-frequency non-invasive (HFNI) valvometers [121], which function like bivalves. The regular closing and opening of the valves is disturbed under the influence of physical or chemical stressors, so these biosensors can be used for the early warning of changing water properties, such as a rise in temperature, pollutant emissions and HAB occurrence.

Underwater video technologies have been developed for the non-destructive study of benthic organisms, especially those found on hard seabed sediments where classical sampling techniques are usually unsuccessful. Video cameras can be attached to a variety of mobile devices such as Remotely Operated Towed Vehicles (ROTVs) or more complex Remotely Operated Vehicles (ROVs) and Autonomous Underwater Vehicles (AUVs). The disadvantage of this technology is the need to use light, which can influence the behaviour of the organisms observed. Another challenge is the storage and processing of the large amount of data generated [122]. Another developed technology based on AUVs is CLEAN SEA (Continuous Long-term Environmental and Asset Integrity monitoring at SEA), which has been upgraded with sensors to measure physico-chemical factors. This technology has made it possible to study the integrity and pollution of the seabed by taking discrete water samples in situ and taking detailed photographic and video recordings, which allow for research into the benthic community.

4.3.3. Remote Sensing

The direct monitoring of biodiversity and observation of patterns on land is made possible through the aerial and satellite remote sensing of optical, thermal and radar images [123,124]. In marine research, remote sensing is mainly used for biomass estimation [125], and more recently for mapping coastal habitats [126]. Although this technology overcomes the spatial and temporal shortcomings of on-site measurements [127], it also has certain limitations. It is limited to cloud-free conditions [128]; the estimates of phytoplankton biomass are based on satellite-derived Chl*a* concentrations [129] and the estimated values need to be confirmed with on-site measurements, which is essential for the quality of the collected information, especially in optically complex coastal and estuarine areas [130].

However, the automatic detection of HABs is also possible with optical satellite imagery that measures water reflectance and inherent properties (IOPS) [131]. The basis of this method is the relationship between the absorption properties of the water and the composition of the pigments, i.e., the backscatter of the water and the size of the phytoplankton cells. It was developed to distinguish between *Karenia mikimotoi* and *Pseudo-nitzschia* sp. in UK coastal waters and phytoplankton blooms of *Phaeocystis globosa* in the southern North Sea. However, this method is limited to algae, which achieve high abundance and cause characteristic water colouration, while many phytoplankton species achieve only low abundance and do not cause colouration.

4.3.4. Innovative Sampling Methodologies

Danovaro et al. [72] mentioned other innovative sampling methods developed to allow for the higher temporal and spatial resolution of sampling. A biodiversity assessment tool, "Autonomous Reef Monitoring Structure" (ARMS), has been developed for a standardised and comparable survey of hardbottom benthic biodiversity. Smaller in size are Artificial Substrate Units (ASUs), nylon pot scrubbers, which are being used to study the recruitment and taxonomy of smaller organisms and species [132]. Advances in robotics have also enabled the high-resolution sampling of samples with irregular species distribution (e.g., zooplankton) using AUVs with mounted bottles for the discrete sampling of seawater and additional sensors to collect environmental data. In addition, to determine the diversity of plankton (from calanoids and copepods to larval invertebrates or specific invasive species, e.g., *Carcinus maenas*), stationary (moored) devices have been developed that can perform in situ molecular assays using 18S ribosomal RNA oligonucleotide probes [133]. In addition, there is a device consisting of a system of pumps installed on the vessel, the Continuous Automated Litter and Plankton Sampler (CALPS), which allows for sampling at a depth along the navigation transect and is suitable for studying the biodiversity and taxonomic composition of zooplankton in a larger research area.

4.4. Concluding Remarks on Port Monitoring

Classical sampling remains the gold standard in the search for potential NIS, which can also serve additional morphological or genetic studies and the monitoring of NIS with data on the developmental stage and condition of the organism, which is completed through DNA studies [67]. The main disadvantages of classical sampling for the purposes of NIS monitoring are the high resource requirements, which include a large amount of specialised equipment due to the poor sampling efficiency of conventional techniques, the low probability of collecting representative samples and trained sampling personnel.

The completion of morphological identification in the laboratory is usually delayed by several months after the collection of samples, and NIS may be completely overlooked due to the impossibility of their identification. On the other hand, the possibilities of identification with DNA-based molecular methods are sometimes hampered by the fact that target markers have not yet been developed for many species, with the insufficient resolution of genetic loci that distinguish the individual species, and with the incomplete reference database of the barcode while metabarcoding is hampered by a complex technical analytical procedure [73,134] which requires specialised equipment and highly skilled personnel, and therefore generally takes no less time than classical microscopic identification [135].

Therefore, a comprehensive biosecurity programme will most likely need to include complementary scientific approaches such as classical research, modelling and risk assessment methods, in addition to molecular methods. The successful protection of marine biodiversity and ecosystems and the associated ecological, economic and socio-cultural values between the experts involved in the various fields of marine conservation and research [80].

5. Early Warning System

The Early Warning System (EWS) for BWM is designed to inform seafarers and other relevant stakeholders of an outbreak, infestation or population of HAOP observed during monitoring in a port, in order to avoid BW uptake and prevent the spread of HAOP from one port to another. The requirements of the BWM Convention must be taken into account when defining what constitutes a warning to ships. According to Regulation C-2 "Warnings Concerning Ballast Water Uptake in Certain Areas and Related Flag State Measures", ships should avoid BW intake in situations where outbreaks, infestations or populations of HAOP are observed in port. This approach refers to the risk assessment criteria of the "Guidelines for Risk Assessment under Regulation A-4 of the BWM Convention (G7)" [136], where the purpose of the warning is to minimise BW intake which may be harmful to the BW reception port [31,137]. Harmful impacts for the port are defined by the Regulation D-2 of the BWM Convention, which stipulates certain requirements as a BW performance standard, as outlined in the Introduction section.

The example of the EWS developed for the Adriatic Sea can serve as a model for the establishment of similar systems elsewhere [70]. According to the EWS for the Adriatic Sea, the efficiency of the EWS depends on the predefined criteria for issuing warnings to ships. This includes a list of HAOP that have been identified as significant for a given geographic region and a categorisation of their impact. The preliminary HAOP inventory, impact assessment and subsequent categorisation of HAOP in the Adriatic were compiled based on available national and regional HAOP information, the results of international programmes such as the IOC Harmful Algal Bloom Programme (IOC-UNESCO taxonomic reference list of harmful microalgae) and the scientific literature [138–142]. Each species has been assigned an impact type based on the demonstrated impacts. These include impacts on human health (toxic, venomous and poisonous species, parasites and pathogens), the economy (losses and damage to fisheries, aquaculture, infrastructure and tourism) and the environment at the level of species (from population displacement to extinction), habitats (habitat modification, reduction or loss) and ecosystem functions (from minor to severe impairment) [141]. For each species, the level of impact was assessed (strong, medium, low, zero or positive or unknown) based on documented impacts in the Adriatic Sea or elsewhere, which should be updated regularly based on new knowledge [70]. In cases where NIS have been observed which are not on the HAOP list, it is necessary to decide quickly whether the species observed is a high-risk species in order to facilitate EWS and response measures. (Decision making is explained in the following section of this paper.)

In the case of HAO detection, it is proposed that a warning is issued if two conditions are fully met: (1) the species has a strong impact on human health, the environment and/or the economy, and (2) the species is present in an abundance which can be defined as an outbreak or bloom, i.e., the abundance of the species exceeds half of the total abundance of the ecological group to which it belongs (i.e., phytoplankton, macroalgae, zoobenthos or fish) [141]. It is not easy to define a threshold value for HAB bloom because species reach different values. Harmful species of the genus *Dinophysis*, for example, rarely exceed 10^4–10^5 cellsL^{-1}, while *Cochlodinium polykrikoides* even exceeds 10^{10} cellsL^{-1} and highly toxic species such as *Pyrodinium bahamense* var. *compressum* and *Alexandrium catenella* can reach 10^6–10^7 cellsL^{-1}, although they can have dramatic effects even at barely detectable concentrations (10^2–10^3 cellsL^{-1}) [142]. As far as pathogens are concerned, given that their levels are often above these thresholds, it is suggested that a warning is only issued when *V. cholerae* is present [70]. On the other hand, it is considered that the list of human pathogens relevant for issuing warnings should be extended [143,144].

Furthermore, according to the EWS for the Adriatic Sea, three types of bodies should be formally established within the system based on their specific function and given specific tasks and responsibilities to address environmental and human health concerns. First, national and local environmental and health authorities are responsible for categorising HAOP impacts, establishing monitoring specifications and available response measures and, respectively, for notifying states potentially at risk from identified HAOP (national) and issuing warnings to the public and relevant stakeholders in the affected area (local). Second, academic and/or research institutions and environmental protection agencies are responsible for port monitoring, notifying national environmental and health authorities when HAOP is detected, and assisting in categorising HAOP impacts, establishing monitoring requirements and possible response actions. Thirdly, local and national maritime authorities are responsible for issuing warnings to ships in situations where the necessary criteria for a warning are met and for informing the IMO and other maritime authorities of the situation. In addition, it was pointed out that the establishment of national HAOP focal points would facilitate the exchange of information between the EWS bodies responsible for the Adriatic Sea in decision-making processes.

Finally, according to the Adriatic EWS, the information flow for BWM would be as follows. If the port monitoring authorities determine the need for a warning, the information should be passed on to the maritime, environmental and health authorities. Maritime authorities should issue a warning to ships, i.e., inform seafarers and the IMO of the positions affected by HAOP and that BW intake should take place at an alternative location. States that may be at risk must be warned of the situation by national environmental and health authorities. The warning should include the coordinates of the area(s) affected by the HAOP, which will be communicated to the maritime authorities by the institutions carrying out the monitoring of the port. In addition, the warning should include the coordinates of an alternative location for BW intake or an alternative water supply. The same procedure should be followed to inform all alerted parties of the termination of the warning.

6. Response Measures

When a HAOP or potentially harmful species is identified, a decision must be made whether or not to initiate a response, and one of the most important questions is whether or not the species poses a risk. For species that are considered high priority, it would be useful to prepare contingency plan in advance so that action can be taken quickly once they are detected. Unless otherwise stated, the response structure is based on: "Invasive Alien Species: A Toolkit of Best Prevention and Management Practices" [34] and "European strategy on invasive alien species" [145].

A HAOP contingency plan is a carefully considered plan of actions to be taken in the event of the detection of a harmful species or a suspected invasion. Optimally, a general plan should be prepared that sets out the broad principles, responsibilities and likely stakeholders that should come together to develop a detailed action plan for a particular event. This plan should include a list of institutions with defined roles and responsibilities and elaborate procedures. These should describe: appropriate responses and possible measures to prevent further spread, rules for obtaining additional government funding, measures to ensure the necessary training and equipment, permits to carry out necessary measures such as the administration of poison and established funds or access to an accelerated funding procedure for initial operations. In addition, as there is a large number of harmful species and a variety of measures available for each, a set of species-specific contingency plans should be developed for specific species identified as high priority in compiled regional HAOP lists.

If a contingency plan is in place, it is possible to prepare and implement a rapid response plan before an emergency occurs, but it is necessary to decide how to respond to the observed harmful species. To make the most appropriate decision, the following is required:

(I) General factors

1. The accuracy of the species identification has been confirmed and it was detected early in the port.
2. The necessary preparations for a rapid response and the allocation of resources to cover initial management costs, permitting and logistics have been made in advance.
3. Legal mechanisms are in place to isolate the species in quarantine to contain the spread and allow time for countermeasures to be implemented.
4. Monitoring to verify the confinement of species to quarantine is possible.
5. A pre-established support network of technical, practical, administrative, financial and legal contacts to implement the response strategy.
6. Resources are available to monitor progress and modify or discontinue the response based on the results achieved.

(II) Specific, species-related factors

7. Information on developmental stages, physiological characteristics and ecological tolerance of species.
8. Insights into the results of applied response methods for the species or closely related taxa in other regions.

However, most introductions will most likely be by species for which no risk assessment has been prepared in advance, so that a risk assessment must be carried out immediately after discovery. According to the precautionary principle, it is best to assume that any new introduction is harmful until it is concluded that it is "safe". Conducting a thorough and rapid risk assessment is challenging, as various facts need to be taken into account, including numerous technical possibilities, in which many interested parties should be involved. However, a timely assessment in case of the early detection of NIS, before they increase in number and/or cover a large area, offers the possibility of eradication, which is the most desirable strategy.

In practise, the potential impacts of NIS are usually difficult to determine because biological invasions entail complex ecological interactions with mostly uncertain outcomes [4,146]. Assessing the impacts and risks of NIS is prone to inaccuracies due to a lack of data on ecosystem response modes, interactions with other species and the spatial resolution of data [147]. However, even with incomplete data, a preliminary, quick estimate must to be made, which can later be modified with more information [148].

At the same time as the risk assessment, further details on introductions need to be collected (field assessment), including estimates of the number of introductions and the range and the likely speed of spread. In addition, the assessment should include information on the latitude/longitude, biological characteristics of the species, confirmation of the identity of the suspected species and samples taken, conclusions on the potential impact, spread or management options and accessibility of the area. This rapid assessment will estimate the need for response and the choice of strategy. However, the decision will depend on the availability of resources, the feasibility of the response method and the conclusion of the cost–benefit assessment.

The structure of the response should be based on existing institutional arrangements and include an institution which could take the lead and provide the backbone for all activities (leadership team) and other institutions which would be involved depending on the type and area at risk. Ideally, there should also be a 'standing team', which is put together prior to an intrusion and enables a rapid response (response team). The members of this team can be selected according to the circumstances of the situation, being closest to and most familiar with the area being invaded, but roles and responsibilities must be agreed in advance.

Additional experts from the country or even abroad may be invited because of their particular expertise. Procedures need to be standardised so that staff from different institutions can be easily included in the team. Scientific, technical, regulatory, policy, operational, legal, financial and communication expertise should be included in the team as needed. Training for the implementation of the measures must be conducted in advance as there is rarely enough time to train the team after the detection of an intruder [149].

Invasions are often highly unpredictable, so the availability of accessible, flexible and substantial resources from the onset of an incursion is critical to an effective, timely and sustainable response [149]. Funding can span several financial years and may need to be shared between different jurisdictions to avoid disruption or unfinished actions. In many countries, the only way to obtain funding is after an intrusion has been detected. This makes it difficult to respond quickly and effectively to such emergencies and to implement long-term programmes, likely leading to delays until funds are allocated. However, financial support for planning is also important as developing responses to invasion can take a long time and may require a large commitment.

6.1. Response Strategies

Four main strategies have been identified in tackling the problematic NIS that has spread in a particular region: eradication, containment, control, and mitigation. The most difficult and preferred approach is eradication. In cases where a risk assessment has shown that an introduced species will have serious consequences, eradication is the best strategy. However, the eradication strategy should only be used if there is a high probability that the species will be eradicated. If it is concluded that the condition is irreversible and that eradication is unlikely, further strategies are possible: containment, i.e., maintaining the species within regional barriers, and control, i.e., maintaining the population size below the tolerable threshold. Although it is not straightforward, the threshold needs to be determined before control begins. If eradication is feasible, it should always be the first choice, rather than containment or control, as it is cheaper and has less long-term impact on the environment.

The final strategy, mitigation/no action, is about finding the best way to "live with" an introduced species and trying to mitigate the impact of the NIS on native species. However, if they are financially and technically feasible, reasonably humane and safe for people and native species, it is better to opt for eradication, containment or control [33,34,145]. Containment or control seem to be more economical than eradication and are therefore often preferred, although they are cheaper only in the short term. Another reason is that the resources and commitment may not be as high as for an eradication programme, and funding may fluctuate from year to year depending on the perceived importance of the problem, political pressure, and public awareness.

6.1.1. Eradication

Eradication is a strategy that involves the elimination of the entire population of a given species, at all life stages and resting stages [34]. From an environmental point of view, eradication is the most efficient strategy [33,145]. The best chances for successful eradication are in the early stages of invasion, when the species is present in low abundance and/or confined to a limited area [34,145]. The probability of successful eradication increases if it is carried out during the period of greatest susceptibility of the species [34]. However, the period during which eradication is possible is short and lasts until the species reaches a certain abundance and/or spreads to a certain extent in the area. It is very difficult to predict with certainty the duration of this critical period and therefore rapid eradication is extremely important [33,34]. Furthermore, it should be emphasised that eradication in the marine environment is only possible in exceptional circumstances, i.e., in a fairly closed area such as geographical or ecological islands isolated by physical and/or ecological barriers [34,145].

During the eradication itself, possible routes of spread of the species between the infected and the managed area must be monitored to prevent re-invasion [34]. The eradication programme should include a monitoring period to ensure success, reduce the possibility of reinvasion and allow for the early detection of the eradicated species if it re-establishes [34]. Nevertheless, the risk of reinvasion is very high and its prevention requires careful and long-term management.

Successful eradication requires support and good coordination between all institutions involved [145]. This ensures that no other measures are carried out in the same area that conflict with the objectives of eradication. Eradication should only be carried out if the resources and commitment of all stakeholders are secured [34]. Resources should be made available for a longer period than the estimated time for eradication so that unexpected problems that may arise during the programme can be resolved and sufficient resources are available for subsequent monitoring. However, eradication has been and remains impossible in most cases [34].

6.1.2. Containment

The aim of containment is to limit the spread of species within certain geographical boundaries, to prevent their spread to neighbouring states, to remote and/or ecologically important areas, or to delay the increase in species abundance in order to carry out eradication more efficiently [33,34,145]. Methods of containment should be selected according to their effectiveness, selectivity and the undesirable effects they may cause [33]. Priorities should be set in the selection of containment areas, based on the classification of natural value, the degree of disturbance, the importance of invasion pathways and the achievability of success [33]. Follow-up monitoring is crucial and must be linked to a rapid response system to eliminate any future intrusions [33,34].

6.1.3. Control

The aim of control is to limit the abundance and cover density of species in order to limit their impact to a pre-determined level that is acceptable in the long-term [33,34,145]. Before starting the control programme, it is necessary to carry out a cost–benefit analysis, clearly define the desired objectives and plan follow-up monitoring [33].

6.1.4. Mitigation

A final option is to do nothing. In cases where eradication, containment and control are deemed unfeasible for technical or financial reasons, or because they are considered socially or politically inappropriate [145], or after previous failures to control an invader, the last resort is to "live with" the species as best as possible and mitigate impacts on biodiversity and endangered species [34]. The difference between mitigation and other strategies is that they focus on endangered native species and are most often applied in the context of conservation programmes [34]. In its simplest and possibly most extreme form, this means translocating a viable endangered native species to areas where the invader is absent or, in the case of a rehabilitated system, no longer exists [34].

6.2. Response Methods

Regardless of the choice of strategy, it is of great importance to choose the most appropriate method for its implementation and the period when the species is most at risk. However, methods cannot be applied at all times of the year, as they may be completely ineffective at certain times of the year. Therefore, choosing the timing is not an easy task. When choosing a method, it is necessary to consider: (a) the practicality of the method and the likelihood of a positive outcome, (b) the estimated duration of the response, (c) the cost–benefit ratio, and (d) the potential impact on the human population, the marine ecosystem or the economy. Although each individual intrusion case must be evaluated as unique, it is advisable to plan and consider all possible methods used to date to find the best method for this particular case.

6.2.1. Mechanical

Mechanical methods can be carried out by directly taking specimens of a selected species by hand or using tools. Filters, pumps and barriers such as curtains or floating poles can be used to completely eradicate HAB, reduce abundance or range and prevent further spread of the species [150]. There are a number of effective methods to control population size and reduce recruitment of invasive fish populations, such as electrofishing and the use of nets (e.g., fish traps, seines, gill nets) [151,152].

Jellyfish can be prevented from reaching the beach by using Pelican boats, which remove unwanted floating debris from the water [153]. For particularly harmful species (especially *Physalia physalis*, which floats on the surface of the sea), trials have been conducted with the direct removal of individuals from the sea and beaches. Another technique is the bubble curtain [153–155], which is essentially a series of air bubbles that rise from the bottom and burst at the water surface. Bubble curtains are not a true physical barrier and have no moving or electrical parts in the water, but it is known that marine animals do not like to swim through bubbles, so swimmers and wildlife are safe with this concept.

Mechanical machines have also been developed such as the "Jellyfish Terminator" robot, which chases swarms of jellyfish along the coast to destroy them [154]. There is also the Jellyfish Elimination Robotic Swarm (JEROS), which consists of an autonomous surface vehicle (ASV), nets to remove jellyfish, and an autonomous navigation system that floats on the surface and uses submerged nets to suck up jellyfish as they move, which are then crushed by a propeller.

6.2.2. Physical and Chemical

Physical and chemical methods to inhibit or remove HAB from seawater include the application of chemicals such as copper compounds (e.g., copper sulphate, barley straw and chemical oxidants [156]) or mineral compounds, clays and other flocculants. However, the production of a new chemical compound requires considerable financial resources, so it is not often that a product is developed precisely to solve an environmental problem.

Protocols for eradicating invasive fish usually involve chemical treatments with rotenone or are administered by other means. It is common to administer a lethal dose in bait, although this non-specific method can cause collateral losses of non-target fish [157].

Unlike chemicals, which can have undesirable consequences due to their potentially lethal effects on other organisms [158], flocculation is considered the most cost-effective and appropriate method for the rapid removal of HAB [159]. A variety of flocculants, especially clay, have been used and researched since the late 1990s [160], but also with uncertain effects on the environment, especially on the benthos. Nevertheless, clay as a method is among those expected to give the best results due to abundant clay sources, low cost and good flocculation properties. Therefore, the species-specific applicability of different types of clay is one of the most important research goals in this field [161].

Among chemical methods, research on pheromones has shown potential in controlling invasive fish, as most fish use pheromones for social communication. There are anti-predator, social and reproductive cues [151,162], which produce "primer" effects that manifest as developmental and/or endocrinological changes, and/or "releasing" effects that cause significant behavioural changes [163].

6.2.3. Biological

The biological method involves the planned use of naturally occurring substances, or large numbers of specimens of a species that is at a higher trophic level and is a natural enemy, against a particular invader.

- The basic classical biological method involves the introduction of natural enemies from the home region of the observed invader into the region where the invader is harmful. This is because, although invaders are often fought by their natural enemies in their home environment, they usually enter new environments without them. The aim is to weaken the invader as a competitor to the native community, thereby reducing its abundance and environmental impact, rather than eradicating it completely.

- If the enemy of the invader can reproduce in a new environment, the enemy augmentation method can be used, in which the species is additionally bred, usually in large quantities, and introduced into the environment.
- There is also the method of habitat management, where the population of species that have a predatory and parasitic relationship with the invader is promoted and alternative hosts and food sources are introduced into the environment.

However, despite its great potential, the use of marine biological control raises significant logistical, Ref. [164], and environmental concerns, Ref. [165], due to unknown risks that can potentially cause irreversible damage. The biological control of HAB involves the application of species or pathogens such as viruses, bacteria, parasites, zooplankton or bivalves that can lyse HAB cells or eliminate them through ingestion or filtration.

It is possible that viruses are highly specific and effective control agents, meaning that a particular HAB could be controlled while other species remain intact. In reality, however, viruses are often so species-specific that they are unable to infect different genetic strains specific to the same host species due to their high host specificity. Small organisms (zooplankton and ciliates) that feed on algae can be bred and used against HAB, but it is logistically extremely challenging to breed the necessary number of specimens of a given species that would truly successfully control an HAB bloom [164].

Some other methods studied include algicidal biosurfactants, complex molecules produced by various microorganisms such as *Pseudomonas aeruginosa* [166], biologically derived substances such as plant extracts or natural chemicals from plants and microorganisms [167], or a combination of two or more methods (e.g., biosurfactants with clay) [168]. Studies conducted under laboratory or field conditions resulted in a reduction in photosynthetic efficiency and cell viability, leading to high removal efficiency. However, the effects on other species need to be further investigated to rule out possible toxic effects.

On the other hand, in situ measurements demonstrated that microalgae develop lower abundances when macrophytes are present [169], suggesting that algae and seagrasses synthesise and secrete allelochemicals, which are indeed a natural biological method of mitigation. Furthermore, some macrophytes caused a decrease in HAB abundance with relatively little negative environmental impact [170].

When successful, classical biological control is very cost-effective compared to other methods and achieves durable, self-sustaining results. It is generally considered safe for the environment because highly specific active ingredients are used. The main weaknesses are that it is not possible to estimate the level of control achieved and the time taken for the agents to be fully effective. Biological control is considered successful when the abundance of the invader decreases to a satisfactory level and the relationship between prey (host) and predator (parasitoid) reaches a dynamic equilibrium.

6.3. Alternative Approach to NIS Management

A recent review of the results of efforts to control invasive marine species around the world found that nearly 40% have failed [171]. The number of attempts reported in the literature barely reached 40, suggesting that they are being undertaken reluctantly. Even more discouraging is the fact that only six successful eradication attempts have been reported in the global literature [172]. Given these data and the fact that actual prevention of the introduction of HAOP by BW, as mentioned above, is generally not possible, other options need to be explored.

Bioprospecting is about the identification and extraction of new bioactive compounds with various potential applications, e.g., in biomedicine, human health, food supply, food supplements, cosmetics and the search for antifouling and antimicrobial agents. Dealing with invasive species can turn a threat into a resource, as shown by the increasing research on invasive species such as the jellyfish *Cassiopea andromeda* [173] or the seaweed *Asparagopsis armata* [174]. Research on this seaweed, which has spread to several coasts around the world, has shown that it is an important source of antimicrobial compounds with a broad spectrum of activity. The use of invasive species to obtain natural bioactive compounds thus offers a dual opportunity: the high availability of the biological material for the purpose at hand and the mitigation of the negative impacts of invasive species through the collection of specimens, which contributes to ecosystem integrity and sustainability.

The commercial use of invasive species could also be a unique opportunity to simultaneously mitigate negative impacts and turn the costs of mitigation into benefits for local communities [175]. The use of invasive species as a culinary resource, such as lionfish [176], offers additional benefits, such as raising public awareness of invasive species and encouraging citizen participation in identifying new populations and other control measures [177]. However, promoting the commercial use of IAS carries risks and such initiatives should be carefully evaluated as they could lead to counter-effects [171,177]. Risks could include illegal attempts to spread IAS to new areas, as pressure could be created to conserve the species for its sustainable use, ultimately exacerbating its invasive potential [175,177], but also posing a threat to biodiversity and ecosystem integrity through fishing activities such as dredging, as in the case of *Rapana venosa* in the Black Sea [178].

6.4. Public Outreach

Finally, one of the most important elements of the response is to inform the authorities of its implementation. It is of great importance to share the knowledge gathered and the results of the implemented measures with the relevant authorities, as well as with the local population and other countries [145]. This approach enables the further development and successful application of response methods.

In general, an important aspect of preventing the introduction of NIS is to raise awareness among politicians, authorities, companies and scientists, as well as the public, about non-native, invasive and generally harmful species, their risks and the possibilities to prevent further introduction [179].

Targeted campaigns about the dangers of bringing alien species from holiday or releasing (alien) pets or aquariums into the wild could be relatively effective. In addition, scientific and public presentations and web-based information platforms provide an excellent opportunity to raise awareness of the NIS and IAS problem [145,179] or, e.g., http://www.iucngisd.org/gisd/ (accessed on 23 September 2023); https://www.gbif.org/ (accessed on 23 September 2023); https://giasipartnership.myspecies.info/en (accessed on 23 September 2023); https://www.nobanis.org (accessed on 23 September 2023); https://www.neobiota.info (accessed on 23 September 2023).

For implementation to be successful, it is extremely important that the public supports and understands the expected economic and environmental impacts of the applied response to the detected invader. Therefore, coordinated public outreach efforts should be an integral part of rapid response efforts [149], and for BWM in general.

7. Final Remarks

Apparently, global warming and marine transport have allowed NIS to survive and proliferate in areas where this was not previously possible [16,180,181]. Although BWMS will become mandatory in all states that have ratified the BWM Convention in September 2024, scientific research has already highlighted some serious weaknesses in the D-2 discharge standard that applies to BW treatment systems.

Namely, the standard does not require the identification of individual species, although numerous harmful species could survive ballast water treatment and are also discharged with BW in accordance with the BWM Convention [182]. In addition, the standard does not address organisms less than 10 μm in size, which represents the potential for the release of a significant number of harmful species, including bacteria and viruses, pathogenic and toxic protists such as HAB, which fall into this category [182,183]. Furthermore, even if the discharge standard supports a significant reduction in cell number, a defined threshold may still allow the release of an inoculum of harmful species, and this sufficient number of cells (even if difficult to estimate) may pose a risk of invasion [184].

The spread of organisms from the port to the surrounding area by currents and the circulation of water masses has already been documented [185–187]. People eating seafood could come into direct contact with human pathogens or toxic species, especially during the bathing season, as organisms can spread from the port to nearby beaches [188]. The early detection of the harmful species reduces the possibility of the failure of the applied measures and potential harmful effects [65].

The harmful effects of IAS are becoming an increasing problem in coastal areas, and this is increasingly being recognised by managers and decision makers [189]. In many countries, the response to the problem of invasive species is a dual approach of preventing new introductions and controlling the spread and abundance increase in species already introduced [190,191]. Scientific evidence points to the importance of adopting a preventive approach to stop the deliberate and accidental introduction of invasive species via human activities or their spread to new regions, thereby reducing the risks of direct adverse impacts on the economy, health or general human welfare [192].

As a final suggestion for the implementation of BWM in ports, one can only use the quote: "Risks of invasions may be very low, but the potential damages are high" [181].

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