

PROPOSED METHOD FOR VERIFICATION OF D2 BWM STANDARD (IMO, 2004) WHEN THE PRESENCE OF TOXIGENIC *V. cholerae*

Método proposto para verificação do padrão D2 BWM (IMO, 2004) quando da presença de *V. cholerae* toxigênico

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ABSTRACT

*Bioinvasion by ballast water is intensified by the globalization process, which causes an increase in maritime transport. The growing commercial exchange between countries, through maritime transport, determines an increase in the fleet of vessels, increasing the volume of ballast transported. Therefore, verifying the effectiveness of discarded ballast water treatment methodologies becomes a challenge for port authorities. The Ballast Water Management (BWM) Convention (IMO 2004) sets standards for ballast management, specifying the maximum number of microorganisms of health relevance permitted for disposal. Among this *Vibrio cholera* (O1 and 139), which causes cholera, stands out. The study aims to describe and validate a proposal for risk classification and selection of vessels to verify compliance with the D2 standard of the BWM 2004 Convention, thus filling a gap with regard to the problem. 3,350 visits were monitored at the port of the city of Rio de Janeiro (Brazil) between 2016 and 2018. After classifying the vessels, 67 (2%) were described as priority for standard verification. The significant reduction in the number of vessels to be verified, with the proposed methodology, contributes to the feasibility of applying the D2 standard, thus reducing the sampling effort.*

Keywords: Ballast Water; Exotic Species; Cholera; Methodology.

RESUMO

A bioinvasão por água de lastro é intensificada pelo processo de globalização, que ocasiona aumento do transporte marítimo. O crescente intercâmbio comercial entre os países, por meio do modal marítimo, determina um incremento da frota de embarcações, aumentando o volume de lastro transportado. Assim, a verificação da eficácia de metodologias de tratamento de água de lastro descartada torna-se um desafio para as autoridades portuárias. A Convenção de Gerenciamento de Água de Lastro (BWM) (IMO 2004) estabelece padrões para o gerenciamento do lastro, especificando o número máximo de microrganismos de relevância sanitária permitidos no descarte. Dentre estes destaca-se o *Vibrio cholera* (O1 e 139), causador da Cólera. O estudo tem como objetivo descrever e validar uma proposta de classificação de risco e seleção de embarcações para verificação do cumprimento do padrão D2 da Convenção BWM 2004, preenchendo assim uma lacuna no que diz respeito a problemática. Foram monitoradas 3.350 visitas no porto da cidade do Rio de Janeiro (Brasil) entre 2016 e 2018. Após a classificação das embarcações, 67 (2%) foram descritas como prioritárias para a verificação do padrão. A redução significativa no número de embarcações a serem verificadas, com a metodologia proposta, contribui para a viabilidade da aplicação do padrão D2 reduzindo assim, o esforço amostral.

Palavras-chave: Água de Lastro; Espécies Exóticas; Cólera; Metodologia.

INTRODUCTION

One of the characteristics of the globalization process is the reduction of border barriers between countries, especially regarding commercial and cultural exchanges (Wulf, 2021) In this context, navigation was consolidated as the main intercontinental goods transport modal, representing about 90% of it, before the pandemic crisis by COVID, of world commercial exchange (IMO, 2012).

The contemporary globalized scenario is significantly confident for the increase in maritime movement around the world, thus magnifying all port dynamics (Seebens *et al.*, 2013), which, together with the construction of coastal urban structures and trawling activities, interfere directly and significantly across the coastal dynamics of the region (Maldonado *et al.*, 2016).

In this way, port regions end up assuming new and intense demands, becoming links of logistical chains that interact in an intense and rising commercial flow between different regions (UNCTAD, 2020a). It was expected, for the period between 2019 and 2023, an annual growth of 3.8% in tons/year transported by sea worldwide (UNCTAD, 2018). However, due to the global crisis resulting from the COVID-19 pandemic, international investments were reduced by about 40%. This reduction caused socioeconomic turbulence around the world, especially in countries that had structural and financial weaknesses, directly influencing the total cargo transported by the maritime modal (UNCTAD, 2020b).

Since the mid-twentieth century, ocean pollution has been increasing due to the technologies used in vessels (Özdemir *et al.*, 2016). The introduction of cargo residues, including occasional oil spills, the increase of greenhouse gas emissions, the discharge of sewage from ships, and the introduction of invasive species by biofouling on the hulls or by ballast water are factors that aggravate this process of environmental degradation (Tian *et al.*, 2021).

The water presented in Ballast tanks ends up generating one microcosm, and it is discharged into the environment, as the cargo is placed in the ship's holds (Ng *et al.*, 2018),

contributing to the bioinvasion process. The navigation process carries around 12 billion tons of ballast water per year and if the simultaneous movement of the ships is considered, approximately 7,000 to 10,000 aquatic species are carried, per year, inside the ballast tanks (Silva *et al.*, 2019c). A study carried out by Castro *et al.*, 2010, in Brazilian ports estimated that approximately 870 thousand cubic meters of ballast water were discharged at the port of Rio de Janeiro city (Brazil), between 2005 and 2006.

Invasive exotic species directly contribute to the process of native animals and plants extinction, thus creating a cost front for the recovery and prevention of affected environments, impacting the world economy in a significant way (Silva *et al.*, 2019c). Among all these species, those that threaten human health cause great concern (Starliper *et al.*, 2015).

In 2017, the International Convention for the Control and Management of Ship's Ballast Water and Sediments (BWM) of the International Maritime Organization (IMO) (IMO, 2004) came into force. It has as a fundamental role the indication of measures to prevent and safeguard environmental and human health as an attempt to prevent, minimize and, if possible, eliminate the risks of introducing harmful aquatic organisms and pathogens in the ballast water discharged by ships in ports (IMO, 2018a). In Brazil, based on this convention, it was elaborated the Maritime Authority Standard for Ship Ballast Water Management, of the Directorate of Ports and Coast - NORMAM-20 (Brasil, 2019).

The BWM Convention presents in its appendix the regulation D2 (Ballast Water Performance Pattern), which establishes the criteria for the quantity of organisms in the discharged ballast water, establishing specifications related to the presence of relevant microorganisms to human health, specifying that: (i) less than one Colony Forming Unit (CFU) per 100 mL of *Vibrio cholerae* (serogroups O1 and O139) or less than one CFU of this bacterium per g (wet weight) of zooplankton samples; (ii) less than 250 UFC per 100 mL of *Escherichia coli* and (iii) less than 100 UFC per 100 mL of enterococci of intestinal origin (IMO, 2004). Among these health indicators, the present study focused on *V. cholerae*.

Between 2010 and 2018, according to data from the World Health Organization (WHO, 2018), 3.5 billion cases of cholera were registered worldwide, with 34,700 deaths. Of these cases, 45.9% occurred in the Asian continent, 24.1% in the American continent and 17.1% in the African continent. High cholera incidence rates are associated with regions with poor sanitation and socio-environmental problems such as illiteracy and poverty (Bwire *et al.*, 2017; Mukhopadhyay, 2015; Silva *et al.*, 2019a).

V. cholerae has a great capacity for adaptation and survival in the marine environment, having as ideal conditions salinity values between 0.5 and 20, pH between 7.0 and 9.0 and temperatures between 20°C and 30°C (Chowdhury *et al.*, 2017; Kokashvili *et al.*, 2015; Silva *et al.*, 2019b). However, several studies have been demonstrating that these parameters can show greater variations (Ng *et al.*, 2016; Rivera *et al.*, 2013; Silva *et al.*, 2015) and that, under unfavourable environmental conditions, the bacteria can enter a different physiological state called "Viable, but non-culturable" that favours its resilience for years (Ferdous *et al.*, 2018). The presence of this bacterial species, including toxigenic serogroups, has already been described in ballast water samples (Kim *et al.*, 2015; Ng *et al.*, 2018; Rivera *et al.*, 2013).

At the moment there is no monitoring and surveillance standard approved by responsible international bodies as more insurance effectively (Silva *et al.*, 2015), and efficiency of treatment methods becomes a challenge for the competent authorities of countries (Silva *et al.*, 2019c). A lot of methodologies are used in board treatment as chlorination, ozonation, filtration between others (IMO, 2004) nonetheless many studies have shown that combined methods, for example, chlorination or ozonation and chlorination and filtration, are more effective than methodologies with just one process

(Gjornaj *et al.*, 2023). Therefore, the need for constant monitoring of the biota present in the water of ballast tanks is evident, as well as to verify the effectiveness of the treatment methodologies used by the vessels (Satir *et al.*, 2014), thus reducing the risk of introducing organisms potentially harmful to human health (Rivera *et al.*, 2013; Silva, 2015).

The continuous monitoring procedure would bring some benefits to the environmental surveillance provided in the BWM (IMO, 2004) such as: 1) indication of the operation conditions of the water treatment system and 2) verification of that potentially invasive species are not present. In the case of cholera, these advantages will contribute to the prevention of possible outbreaks of the disease due to the introduction of the organism through ballast water, contributing to the process of epidemiological surveillance of the disease worldwide. When analysing coastal countries, for example Brazil (the Brazilian coast is 7,491 km long, making it the 15th longest national coast in the world), this problem requires greater attention since almost 54% of its population lives in these regions (IBGE, 2022).

Although there are several processes and equipment certified for the treatment of ballast water with international approval by the IMO, the monitoring, inspection and verification methodologies do not present clear definitions of sampling standards and neither the selection of ships to be tested. Currently, inspections are rarely carried out when authorizing vessels, with most of the process being based on legal documentation and analysis of compliance with health safety standards (without a focus on ballast) and labour standards. Ballast water sampling is mostly done for scientific research, where each country structures such processes and procedures according to their own understanding and availability of resources (Silva *et al.*, 2022).

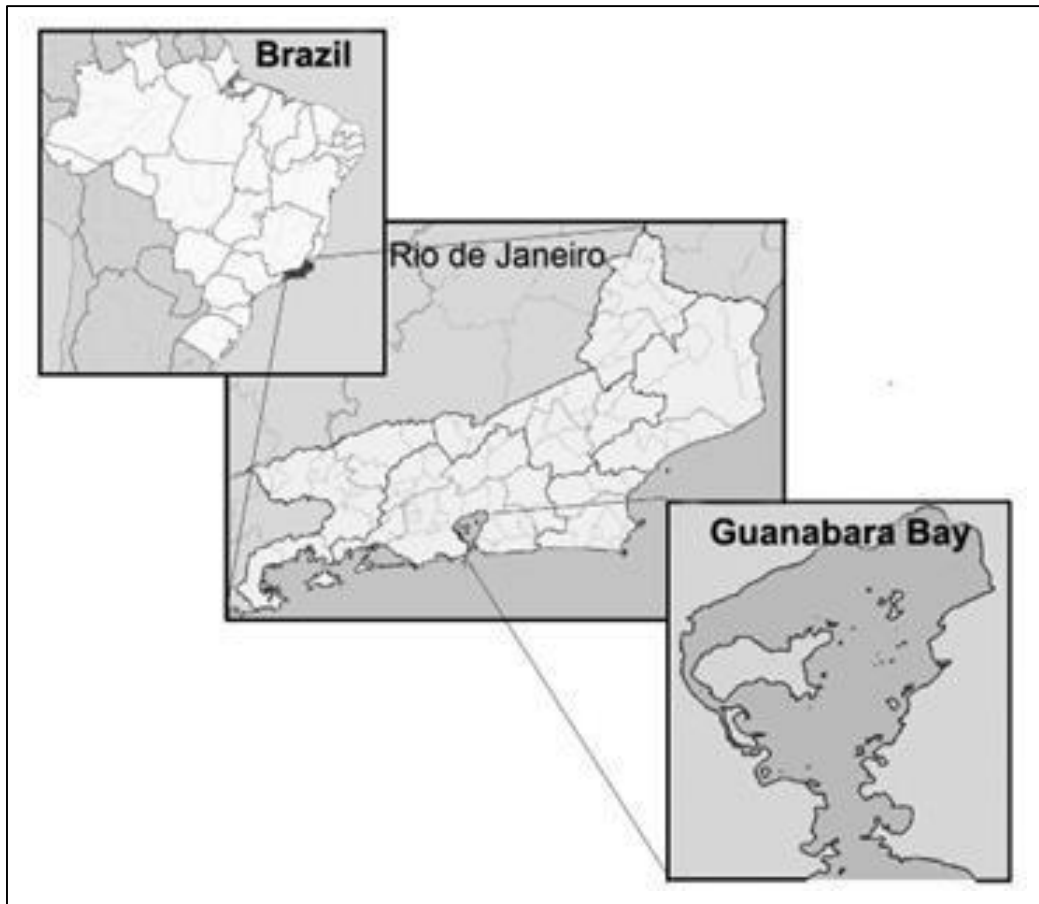
Thus, this work aims to present a methodological proposal for the classification of vessels, based on a risk categorization index associated to their origin - thus contributing for the decrease of the sampling mesh - to evaluate the possibility of the presence of toxigenic *V. cholerae*, as provided in regulation D2 of the BWM. For the application of the proposed methodology, the Port of Rio de Janeiro City (Brazil) was used as a test area.

MATERIAL AND METHODS

Study site

The Port of Rio de Janeiro is located on the western coast of Guanabara Bay in the city of Rio de Janeiro, Brazil (Figure 1). It has 6,740 meters of continuous quay structures and over 883 meters of facilities, with 10 external warehouses (capacity of 13,100 meters tons of storage), divided in public and private terminals and a turning basin of approximately 11,150 meters in length, from 10 to 73 meters depth (Portorio, 2019). It is bordered by urban settlements with great social and infrastructural deficiencies on access to essential services to human health, which ends up making the area vulnerable to outbreaks of waterborne infectious diseases (Silva *et al.*, 2019b). In 2019, the port of Rio de Janeiro was the fifth Brazilian port complex that received more long-distance vessels, moving around 6.8 million tons of goods with 1,028 berths, which represents approximately 0.7% of all national movement (ANTAQ, 2024).

Figure 1 – Location of the port of Rio de Janeiro City (Brazil)



Source: The Author, 2022.

Cholera Incidence Indicator (CII)

This indicator is intended to suggest the risk of carrying the toxigenic *Vc* in the ballast of visiting ships, focusing on the vessel's origin. Although it is not possible to say with certainty about the occurrence of ballast maneuver in the port of origin, the idea of this indicator is that a ship that received ballast in a region in which there are no reported cases of the disease represents a less important introduction vector than a vessel coming from a location where an incidence/outbreak/epidemic has been reported recently. To this end, a survey of worldwide incidences of cholera was carried out using data from the World Health Organization (WHO, 2018).

The period of survey disease records took place between 2013 and 2017: this interval was selected because it presented more robust and reliable data regarding another indicator used (Basic Sanitation Indicator - BSI), which will be described later. To obtain the CII, only countries that had a case of the disease in the analyzed period were considered. The average of the worldwide incidences (AWI) was calculated among all countries in the years of observation, excluding the countries that obtained, as a sum of the incidences between 2013 and 2017, more than 50% of the total worldwide records in the same period: such procedure was adopted in order to homogenize the indicator categorization intervals. CII =1 was assigned for all countries that had a total incidence above the AWI, CII = 2 for countries that recorded the number of cases between the AWI and its half ($AWI / 2$) and CII =3 for countries that had from 1 to $AWI / 2 - 1$ cases.

Basic Sanitation Indicator (BSI)

Sanitation is the main local vulnerability factor to cholera, besides being a determinant for the introduction of choleric vibrio in the marine environment (which favors the possibility of carrying the bacteria through the collection of ballast water). After an intense search for global data on sanitation indicators, the robustness and reliability of data about the quantity (percentage) of the population with access to sanitation was verified, as available on the World Bank website (World Bank, 2018). Thus, these data were tabulated, between the periods 2013 to 2017, for the construction of the BSI. To calculate the BSI, the measure used was the arithmetical average of the percentage of the population with access to sanitation in the total period (ABS). Countries with ABS up to 50% received BSI=1, 51% to 80% BSI=2 and above 81% BSI=3. BSI=1 was adopted for countries that did not show available data for sanitation access in the consulted platform.

Contracting Country Indicator (CCI)

The indicator suggests that the BWM convention (IMO, 2004) contracting countries should have legal provisions and operational procedures, as a matter of principle, to minimize the risks of introducing species through ballast water. The list of contractors of the Ballast Water Management Convention was obtained by consulting the IMO's official website at [http://www.imo.org/n/About/Conventions/ListOfConventions/Pages/International-Convention-for-the-Control-and-Management-of-Ships'-Ballast-Water-and-Sediments-\(BWM\).aspx](http://www.imo.org/n/About/Conventions/ListOfConventions/Pages/International-Convention-for-the-Control-and-Management-of-Ships'-Ballast-Water-and-Sediments-(BWM).aspx). Started from the point that every contracting country presents some concern (legislation, methodologies, and practices, among others) regarding the length of the BMW IMO (2004). For the Calculation of the CCI, for the calculation of the CCI, non-contracting countries were assigned as CCI = 1 while contracting countries were assigned as CCI=2.

Determining the sampling priority index (SP)

It was obtained by analyzing the sum of the three indicators (CII + BSI + CCI). When the country obtained values between 3 and 4, in its sum of indicators, it was assigned the value 1 for its SP (high risk), values from 4 to 5 SP=2 (moderate risk) and between 7 and 8 SP=3 (low risk). As vessels from locations on the Brazilian coast are possibly in a different situation, since an eventual cholera outbreak would be much easier to be detected than in countries located on other continents, all these vessels received SP=4 (national risk, considered lower).

Despite 18 years with no registered cases, in 2024 was confirmed one autoctone case of cholera in the Salvador city, Brazil, by toxigenic serotype O1 (Ogawa) in one man without a historic of travelling for countries with cholera cases (Martins-Filho *et al.*, 2024). This event encourages reflection on all cholera surveillance in Brazil, a country with continental dimensions and complex social and regional vulnerabilities (Silva *et al.*, 2022).

With regards to the main environmental factor magnificent to the disease, according to data Sanitation Panel of the Brazilian Ministry of Health (Trata Brasil Institute, 2023), fence 15,8% of the Brazilian population don't have access to water and 44,50% have no access to sewage treatment. This situation worsens even more when the analysis is directed to some Brazilian regions, mainly states in the northeast region.

In addition to everything exposed herein, there has been an intense increase in all maritime commercial exchanges in Brazil, with 1,2 mil/ton handled in national ports in 2022 and 1,3 mil/ton in 2023, which further increases the process of global bioinvasion by pathogens (ANTAQ, 2024).

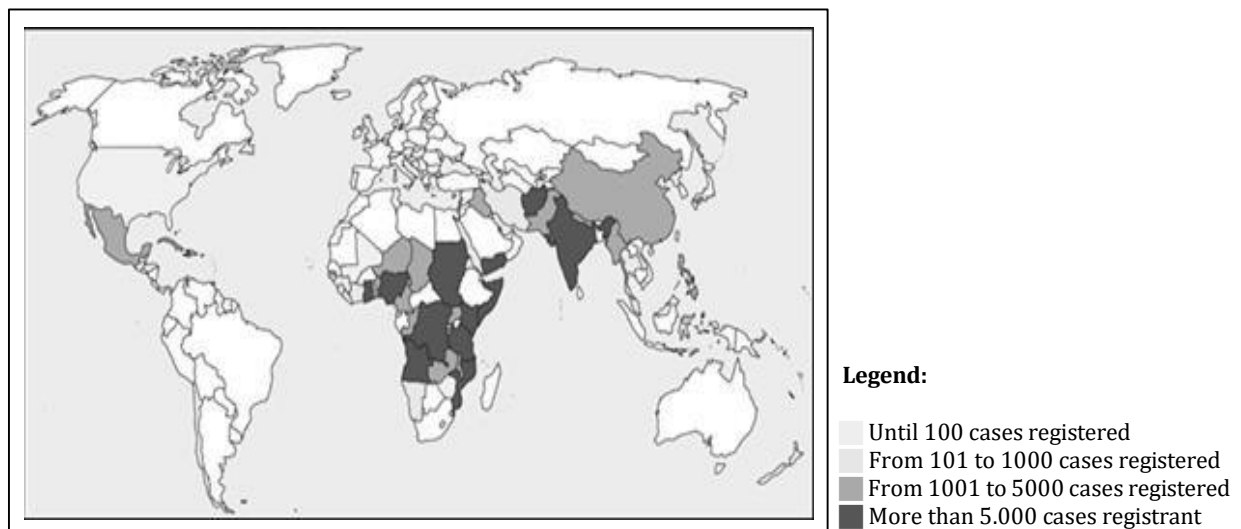
The Methodological application

For validation and testing of the methodology, the monitoring of vessels visiting the study port was performed, concomitantly to the construction of the indicators described, using the virtual tool Marine Traffic ® (<https://www.marinetraffic.com/pt/>). Berthed ships were monitored between January 2016 and January 2018 (25 months), with intervals every three days (This time gap was adopted because it presents, within the work schedule, a feasible interval both for monitoring and for analyzing the data and building the indicators). At the end of the monitoring period, the PA was applied to all the moorings catalogued, and thus, the risk suggested in the methodology was evaluated for each one of them.

RESULTS AND DISCUSSION

The CII indicator revealed the occurrence of about 1.8 billion cases of cholera in 69 countries during the studied period, with emphasis on the Center African, South Asian and Caribbean regions. Even with the high incidences recorded in Asia in 2017 (due to the outbreak in Yemen) and in the Americas (due to the outbreak in Haiti with 177,709 cases between 2013 and 2017), countries on the African continent were the ones with the highest occurrence in the disease curve in the five years period monitored (out of 20 countries with the highest incidences, 13 were African) as illustrated in Figure 2. The only country that was not included in the calculation of the CII was Yemen, which presented approximately 56% of all registered cases in the world over the five years analyzed, and so obtained CII=1. Countries with a total incidence of 1 to 6,406 had CII =3, between 6,407 and 12,813 CII =2 and above 12,814 CII =1 (Table 1).

Figure 2 – Map of incidence of Cholera from 2013 to 2017



Source: Adapted from WHO (2018).

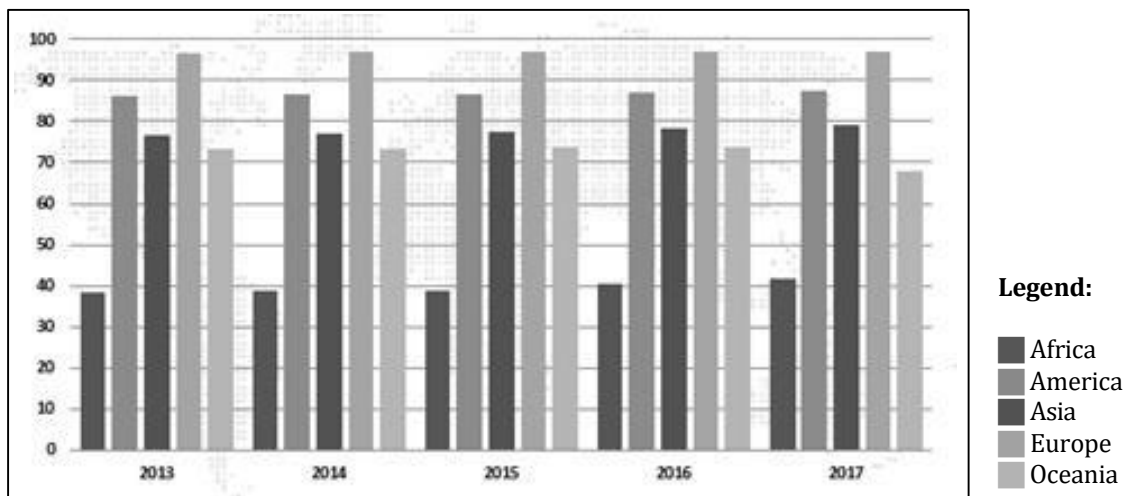
An important reflection on the CII is the underreporting of cases involving erroneous diagnoses by health professionals. As the symptoms of the disease are the same as those presented by most enteritis, it can be diagnosed as another disease with similar symptoms, thus directly influencing this indicator as pointed out by Silva *et al.*, (2015) and investigated by Varallo *et al.*, (2018).

These analyzes indicate that the total sum of global cholera incidence should possibly be greater than that exposed by the WHO, especially in countries that present serious problems of social inequality, diagnosis and communication of the disease (Silva *et al.*, 2019a). This is

especially concerning when it comes to neglected populations as pointed out by Araújo *et al.*, (2013).

Regarding the BSI indicator, although the amount of access to sanitation is still low in the 21st century, a slight evolution was noticed during the years of study. The worst situations were observed in countries on the African continent (Table 1). Of the 69 countries analyzed, 31 had BSI=1 (80.6% of these on the African continent). This situation of sanitation deficit exemplified by African countries is very worrying, because the indicator is based only on the numbers of access to sanitation and not on its quality, which indicates that the situation should be much worse than suggested by the observed percentages, in terms of the sanitary infrastructure of these regions (Figure 3).

Figure 3 – Evolution of sanitation access per continent from 2013 to 2017



Source: Adapted of World Bank (2017).

As far as CCI is concerned, of the 174 Member States registered with the IMO by the year 2018, 81 (46.5%) are signatories to the BWM Convention IMO 2004, as shown in Appendix B. However, despite the low number of signatories, they controlled about 80% of all the world's merchant maritime fleet in operation by 2018. As for the SP, 12 countries obtained SP =1: of which 8 are African, 3 Asian and 1 American; 27 with SP =2: 20 Africans, 6 Asians and 1 European; and 30 with SP =3: 10 are Asians, 10 Europeans, 7 Americans, 2 Africans and 1 Oceanic (Table 1).

Table 1 - Values of the constituent Indicators for the construction of the Sampling Priority (SP) by country

| N° | Country/Continent | CI 2013 | CI 2014 | CI 2015 | CI 2016 | CI 2017 | TI | CII | SA 2013 | SA 2014 | SA 2015 | SA 2016 | SA 2017 | ASA | BSI | CCI | SP | AT |
|----|-------------------|---------|---------|---------|---------|---------|---------|-----|---------|---------|---------|---------|---------|-------|-----|-----|----|----|
| 1 | Afghanistan | 3.957 | 45.481 | 58.064 | 677 | 33 | 108.212 | 1 | 31,1 | 31,8 | 31,9 | 34,7 | 37,5 | 33,4 | 1 | 1 | 1 | 0 |
| 2 | Angola | 6.655 | 213 | 0 | 78 | 828 | 7.774 | 2 | 50,6 | 51,1 | 51,6 | 51,8 | 52,0 | 51,4 | 2 | 1 | 2 | 11 |
| 3 | Australia | 3 | 4 | 2 | 1 | 3 | 13 | 3 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 3 | 2 | 3 | 3 |
| 4 | Benin | 528 | 832 | 0 | 761 | 11 | 2.132 | 3 | 19,0 | 19,6 | 19,7 | 22,6 | 25,5 | 21,3 | 1 | 1 | 2 | 5 |
| 5 | Burundi | 1.557 | 582 | 442 | 434 | 399 | 3.414 | 3 | 47,8 | 48,0 | 48,0 | 48,1 | 48,2 | 48,0 | 1 | 1 | 2 | 0 |
| 6 | Cameroon | 26 | 3.355 | 124 | 0 | 0 | 3.505 | 3 | 45,4 | 45,6 | 45,8 | 45,9 | 46,0 | 45,7 | 1 | 1 | 2 | 18 |
| 7 | Canada | 1 | 4 | 3 | 1 | 4 | 13 | 3 | 99,8 | 99,8 | 99,8 | 99,9 | 99,9 | 99,8 | 3 | 2 | 3 | 14 |
| 8 | Chad | 0 | 0 | 0 | 0 | 1.266 | 1.266 | 3 | 12,0 | 12,0 | 12,1 | 15,8 | 19,5 | 14,3 | 1 | 1 | 2 | 0 |
| 9 | Chile | 2 | 24 | 0 | 0 | 0 | 26 | 3 | 99,0 | 99,0 | 99,1 | 99,2 | 99,3 | 99,1 | 3 | 1 | 3 | 41 |
| 10 | China | 53 | 24 | 13 | 0 | 14 | 104 | 3 | 74,2 | 75,4 | 76,5 | 76,7 | 76,9 | 75,9 | 2 | 1 | 2 | 32 |
| 11 | Congo | 1.624 | 0 | 0 | 15 | 0 | 1.639 | 3 | 27,9 | 28,3 | 28,7 | 28,9 | 29,1 | 28,6 | 1 | 2 | 2 | 1 |
| 12 | Côte d'Ivoire | 56 | 235 | 199 | 0 | 0 | 490 | 3 | 22,0 | 22,3 | 22,5 | 28,8 | 35,0 | 26,1 | 1 | 1 | 2 | 9 |
| 13 | Cuba | 181 | 76 | 0 | 0 | 0 | 257 | 3 | 93,1 | 93,2 | 93,2 | 93,4 | 93,6 | 93,3 | 3 | 1 | 3 | 3 |
| 14 | Denmark | 0 | 0 | 0 | 1 | 0 | 1 | 3 | 99,6 | 99,6 | 99,6 | 99,7 | 99,8 | 99,7 | 3 | 2 | 3 | 2 |
| 15 | Dominican Rep. | 1.954 | 603 | 546 | 1.159 | 122 | 4.384 | 3 | 83,3 | 83,6 | 84,0 | 84,3 | 84,6 | 84,0 | 3 | 1 | 3 | 0 |
| 16 | Dem. Rep. Congo | 26.944 | 22.230 | 19.182 | 28.093 | 56.190 | 152.639 | 1 | 27,9 | 28,3 | 28,7 | 29,0 | 29,2 | 28,6 | 1 | 1 | 1 | 2 |
| 17 | England | 6 | 14 | 15 | 19 | 0 | 54 | 3 | 99,2 | 99,2 | 99,2 | 99,4 | 99,6 | 99,3 | 3 | 2 | 3 | 6 |
| 18 | France | 0 | 0 | 2 | 0 | 0 | 2 | 3 | 98,7 | 98,7 | 98,7 | 99,0 | 99,2 | 98,9 | 3 | 2 | 3 | 9 |
| 19 | Germany | 0 | 0 | 0 | 1 | 0 | 1 | 3 | 99,2 | 99,2 | 99,2 | 99,4 | 99,5 | 99,3 | 3 | 2 | 3 | 7 |
| 20 | Ghana | 50 | 28.944 | 692 | 175 | 0 | 29.861 | 1 | 14,7 | 14,8 | 14,9 | 15,2 | 15,5 | 15,0 | 1 | 2 | 1 | 9 |
| 21 | Guinea | 319 | 1 | 0 | 0 | 0 | 320 | 3 | 19,4 | 20,0 | 20,1 | 22,3 | 24,5 | 21,3 | 1 | 1 | 2 | 2 |
| 22 | Guinea Bissau | 969 | 11 | 0 | 0 | 0 | 980 | 3 | 20,5 | 20,7 | 20,8 | 22,4 | 24,0 | 21,7 | 1 | 1 | 2 | 1 |
| 23 | Haiti | 58.809 | 27.753 | 36.045 | 41.421 | 13.681 | 177.709 | 1 | 27,1 | 27,4 | 27,6 | 27,7 | 27,8 | 27,5 | 1 | 1 | 1 | 1 |
| 24 | India | 6.008 | 4.031 | 889 | 841 | 385 | 12.154 | 2 | 38,5 | 39,5 | 39,6 | 40,4 | 41,1 | 39,8 | 1 | 1 | 1 | 48 |
| 25 | Iran | 256 | 0 | 86 | 0 | 634 | 976 | 3 | 89,9 | 89,9 | 90,0 | 90,4 | 90,8 | 90,2 | 3 | 2 | 3 | 2 |
| 26 | Iraq | 1 | 0 | 4.965 | 3 | 0 | 4.969 | 3 | 85,6 | 85,6 | 85,6 | 88,6 | 91,5 | 87,4 | 3 | 1 | 3 | 1 |
| 27 | Israel | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 3 | 1 | 3 | 0 |
| 28 | Italy | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 99,5 | 99,5 | 99,5 | 99,7 | 99,9 | 99,6 | 3 | 1 | 3 | 10 |
| 29 | Japan | 8 | 10 | 7 | 0 | 7 | 32 | 3 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 3 | 2 | 3 | 7 |
| 30 | Kenya | 0 | 35 | 13.291 | 5.866 | 4.288 | 23.480 | 1 | 29,9 | 30,1 | 30,1 | 28,8 | 27,5 | 29,3 | 1 | 2 | 1 | 3 |
| 31 | Liberia | 92 | 44 | 0 | 0 | 0 | 136 | 3 | 16,4 | 16,6 | 16,9 | 17,1 | 17,2 | 16,8 | 1 | 2 | 2 | 3 |
| 32 | Malawi | 0 | 0 | 693 | 1.792 | 344 | 2.829 | 3 | 40,1 | 40,6 | 41,0 | 42,3 | 43,5 | 41,5 | 1 | 1 | 2 | 0 |
| 33 | Malaysia | 171 | 189 | 244 | 0 | 3 | 607 | 3 | 96,0 | 96,0 | 96,0 | 96,6 | 97,1 | 96,3 | 3 | 2 | 3 | 10 |
| 34 | Mali | 23 | 0 | 0 | 0 | 0 | 23 | 3 | 23,8 | 24,2 | 24,7 | 27,4 | 30,0 | 26,0 | 1 | 1 | 2 | 0 |
| 35 | Mexico | 187 | 14 | 1 | 0 | 0 | 202 | 3 | 84,4 | 85,1 | 85,2 | 86,2 | 87,2 | 85,6 | 3 | 2 | 3 | 12 |
| 36 | Mozambique | 1.869 | 480 | 8.739 | 883 | 5.892 | 17.863 | 1 | 20,3 | 20,4 | 20,5 | 31,5 | 42,5 | 27,0 | 1 | 1 | 1 | 2 |
| 37 | Myanmar | 33 | 400 | 103 | 782 | 0 | 1.318 | 3 | 79,5 | 79,5 | 79,6 | 79,6 | 80,2 | 79,7 | 2 | 1 | 2 | 0 |
| 38 | Namibia | 3 | 485 | 0 | 0 | 0 | 488 | 3 | 33,6 | 34,0 | 34,4 | 34,7 | 34,9 | 34,3 | 1 | 1 | 2 | 6 |

Legend: CI (Cholera's indicator); TI (Total incidence); CII (Indicator of Cholera's incidence); SA (Population percentage with sanitation access); ASA (Average of SA); BSI (Indicator of basic sanitation); CII (Indicator of contracting country); SP (Sampling Priority); AT (Total monitored vessels).

Table 1 - Values of the constituent Indicators for the construction of the Sampling Priority (SP) by country (cont.)

| N° | Country/Continent | CI | | CI | CI | CI | TI | CII | SA | SA | SA | SA | SA | ASA | BSI | CCI | SP | AT |
|----|---------------------|-------|--------|--------|--------|-----------|-----------|-----|-------|-------|-------|-------|-------|-------|-----|-----|----|-----|
| | | 2013 | 2014 | | | | | | | | | | | | | | | |
| 39 | Nepal | 0 | 993 | 80 | 169 | 7 | 1,249 | 3 | 42,6 | 44,2 | 45,8 | 48,4 | 51,0 | 46,4 | 1 | 1 | 2 | 0 |
| 40 | Netherlands | 0 | 0 | 0 | 1 | 0 | 1 | 3 | 97,8 | 97,7 | 97,7 | 98,0 | 98,3 | 97,9 | 3 | 1 | 3 | 0 |
| 41 | Niger | 585 | 2.059 | 51 | 38 | 0 | 2.733 | 3 | 10,5 | 10,8 | 10,9 | 14,7 | 18,5 | 13,1 | 1 | 1 | 2 | 0 |
| 42 | Nigeria | 6.600 | 35.996 | 5.290 | 768 | 12.174 | 60.828 | 1 | 29,6 | 29,3 | 29,0 | 31,0 | 33,0 | 30,4 | 1 | 2 | 1 | 13 |
| 43 | Norway | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 98,1 | 98,1 | 98,1 | 98,7 | 99,3 | 98,5 | 3 | 2 | 3 | 8 |
| 44 | Oman | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 96,7 | 96,7 | 96,7 | 96,8 | 96,9 | 96,8 | 3 | 1 | 3 | 2 |
| 45 | Paquistan | 1.069 | 1.218 | 0 | 0 | 0 | 2.287 | 3 | 60,0 | 61,8 | 63,5 | 67,8 | 72,0 | 65,0 | 2 | 1 | 2 | 1 |
| 46 | Philippinas | 6 | 4.547 | 0 | 0 | 134 | 4.687 | 3 | 72,5 | 73,2 | 73,9 | 74,1 | 74,2 | 73,6 | 2 | 1 | 2 | 4 |
| 47 | Katar | 0 | 0 | 0 | 0 | 5 | 5 | 3 | 98,0 | 98,0 | 98,0 | 98,3 | 98,5 | 98,2 | 3 | 2 | 3 | 0 |
| 48 | República of Coreia | 3 | 0 | 0 | 0 | 5 | 8 | 3 | 14,8 | 14,9 | 15,0 | 15,4 | 15,7 | 15,2 | 1 | 2 | 2 | 4 |
| 49 | Russia | 0 | 2 | 0 | 0 | 0 | 2 | 3 | 72,2 | 72,2 | 72,2 | 72,8 | 73,3 | 72,5 | 2 | 1 | 2 | 0 |
| 50 | Rwanda | 0 | 0 | 0 | 355 | 0 | 355 | 3 | 60,0 | 60,8 | 61,6 | 61,8 | 61,9 | 61,2 | 2 | 1 | 2 | 0 |
| 51 | Saudi Arabia | 0 | 0 | 0 | 0 | 5 | 5 | 3 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 3 | 2 | 3 | 3 |
| 52 | Sierra Leone | 377 | 0 | 0 | 0 | 0 | 377 | 3 | 13,0 | 13,1 | 13,3 | 15,4 | 17,5 | 14,5 | 1 | 1 | 2 | 14 |
| 53 | Singapore | 2 | 4 | 0 | 0 | 3 | 9 | 3 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 3 | 2 | 3 | 3 |
| 54 | Somalia | 6.864 | 28.020 | 7.536 | 15.619 | 75.414 | 133.453 | 1 | 23,8 | 23,9 | 24,1 | 24,1 | 24,2 | 24,0 | 1 | 2 | 1 | 0 |
| 55 | South Africa | 1 | 0 | 0 | 0 | 0 | 1 | 3 | 65,3 | 65,8 | 66,4 | 66,5 | 66,6 | 66,1 | 2 | 2 | 3 | 20 |
| 56 | Southern Sudan | 0 | 6.421 | 1.818 | 4.295 | 16.088 | 28.622 | 1 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 6,7 | 1 | 1 | 1 | 0 |
| 57 | Spain | 0 | 0 | 2 | 0 | 0 | 2 | 3 | 99,9 | 99,9 | 99,9 | 99,9 | 99,9 | 99,9 | 3 | 2 | 3 | 82 |
| 58 | Sweden | 0 | 0 | 1 | 0 | 0 | 1 | 3 | 99,3 | 99,3 | 99,3 | 99,6 | 99,8 | 99,5 | 3 | 1 | 3 | 0 |
| 59 | Switzerland | 0 | 0 | 2 | 0 | 0 | 2 | 3 | 99,9 | 99,9 | 99,9 | 100,0 | 100,0 | 99,9 | 3 | 2 | 3 | 0 |
| 60 | Tanzania | 270 | 0 | 11.563 | 11.360 | 4.895 | 28.088 | 1 | 14,5 | 15,0 | 15,6 | 23,8 | 32,0 | 20,2 | 1 | 1 | 1 | 4 |
| 61 | Thailand | 8 | 12 | 125 | 52 | 8 | 205 | 3 | 93,1 | 93,0 | 93,0 | 95,0 | 97,0 | 94,2 | 3 | 1 | 3 | 1 |
| 62 | Togo | 166 | 262 | 35 | 0 | 0 | 463 | 3 | 16,1 | 16,4 | 16,5 | 16,7 | 16,8 | 16,5 | 1 | 1 | 2 | 2 |
| 63 | UAE | 0 | 0 | 0 | 0 | 12 | 12 | 3 | 97,5 | 97,5 | 97,6 | 97,9 | 98,1 | 97,7 | 3 | 2 | 3 | 8 |
| 64 | United States | 14 | 14 | 4 | 14 | 11 | 57 | 3 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 100,0 | 3 | 1 | 3 | 153 |
| 65 | Uganda | 748 | 309 | 1.461 | 516 | 252 | 3.286 | 3 | 18,8 | 19,0 | 19,1 | 19,8 | 20,5 | 19,4 | 1 | 1 | 2 | 0 |
| 66 | Venezuela | 4 | 0 | 0 | 0 | 0 | 4 | 3 | 94,4 | 94,4 | 94,4 | 94,6 | 94,8 | 94,5 | 3 | 1 | 3 | 17 |
| 67 | Yemem | 0 | 0 | 0 | 15.751 | 1.032.481 | 1.048.232 | 1 | 53,1 | 53,5 | 53,7 | 53,9 | 60,0 | 54,8 | 2 | 1 | 1 | 0 |
| 68 | Zambia | 0 | 0 | 0 | 0 | 1.794 | 1.794 | 3 | 43,5 | 43,7 | 43,9 | 44,2 | 44,4 | 43,9 | 1 | 1 | 2 | 0 |
| 69 | Zimbabwe | 0 | 0 | 60 | 10 | 0 | 70 | 3 | 37,2 | 37,0 | 36,8 | 37,2 | 37,5 | 37,1 | 1 | 1 | 2 | 0 |

Legend: CI (Cholera's indicator); TI (Total incidence); CII (Indicator of Cholera's incidence); SA (Population percentage with sanitation access); ASA (Average of SA); BSI (Indicator of basic sanitation); CII (Indicator of contracting country); SP (Sampling Priority); AT (Total monitored vessels).

About the vessels monitoring to validate the methodology, 3,350 berthings were observed, 1,687 from national ports (SP=4). Regarding the origins of monitored vessels, the ten with the greatest quantitative representation in the period were: United States (SP=3) with 153 berths, Spain (SP =3) with 82 berths, India (SP=1) with 48 berths, Chile (SP=3) with 41 berths, China (SP=2) with 41 berths, South Africa (SP =3) with 20 berths, Cameroon (SP=2) with 18 berths, Venezuela (SP=3) with 17 berths, Sierra Leone (SP=2) and Canada (SP=3), both with 14 berths (Table 1).

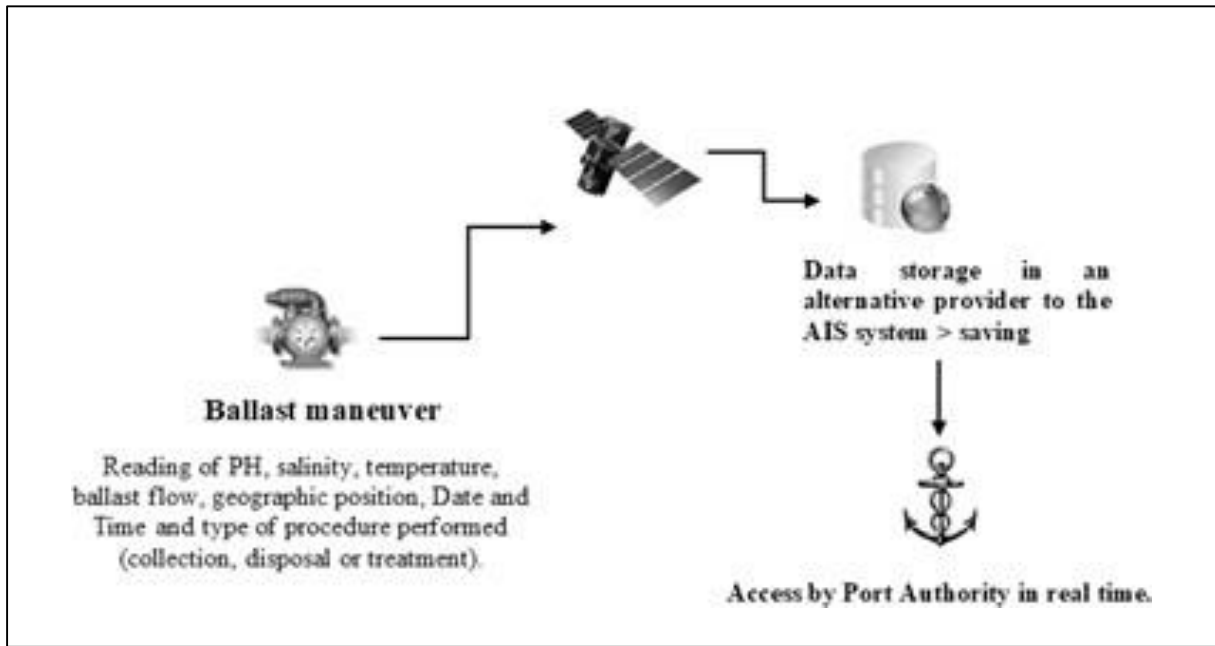
Another point of attention is the geographical issue between countries that are low risk but are located in regions neighboring other countries with moderate or high risk that have a maritime connection or migratory flow. In this situation, warning signs for this location must be analyzed as well as a periodic (annual) review and construction of new values for the proposed indicators.

Of the total berths monitored, the methodology indicated a total of 39 vessels most likely to be carrying the toxigenic *Vibrio cholerae* in their ballasts and being more suitable targets for checking the efficiency of the D2 standard (SPI=1 and SPI=2), which effectively represents a reduction of 98.8% in the sampling effort, representing an advance in the ability to detect a possible presence of vibrio in their tanks. These ships came from Afghanistan, Benin, Burundi, Cameroon, Chad, Democratic Republic of Congo, Ghana, Guinea, Guinea Bissau, Haiti, India, Kenya, Malawi, Mozambique, Niger, Nigeria, Sierra Leone, Somalia, Southern Sudan, Tanzania, Togo, Uganda, Zambia and Yemen. These places should be considered, according to the methodology, the primary targets for microbiological analysis.

Considering that each ship has several segregated ballast water tanks, the question still remains as to the selection of specific ballast water tanks in each ship to be sampled, as the number of samples is limited. The most promising approach to solve this problem is the ship's ballast water operation log, which is mandatory by the BWM Convention. The specific tank should be selected using the log and the last port in which ballast water is pumped in, in those countries that are most risky, according to the method. However, it should be pointed out that currently, each port State has access only to the operation certificate of the ballast water treatment plant operation in each ship, given at six-month intervals, for the D2 standard, or the ballast operation log, for the D1 standard. This state of affairs has no redundancy and no way to provide an independent inspection of the quality of operation of each ships ballast water treatment plant.

Due to the numerous variables involved, whether in relation to the ballast water tanks used and the region of origin of the captured water, or in relation to the best choice of tank water to be sampled for analysis, it would be recommended to develop a system for sending data on water and the geographic position of its catchment, via a satellite communication channel with a database. This would make it possible to record information, access it prior to berthing and choose the tanks to be sampled, according to suggestion in Figure 4.

Figure 4 – Evolution of sanitation access per continent from 2013 to 2017



Source: The Authors (2021).

CONCLUSIONS

Despite the importance of the ballast water issue, especially about the bioinvasion process of harmful species and the existence of specific global legislation, monitoring procedures regarding the efficiency of the D-2 standard of visiting vessels to world ports present themselves as a great challenge, mainly due to the large movement of ships around the world. In some countries, including Brazil, there is a slight movement towards the building of procedures and methodologies for control and monitoring of ballast water management.

The BWM (IMO 2004, 2018) have normative character and this way each country establishes their surveillance, inspection and control methodologies. Quantitative data about this dynamic are not disclosed by the signatory states of the convention (number of ships sampled, positive and negative samples of pathogens regulated by the standard, for example) and are rarely found in scientific literature in the area. Besides this, there is no specific methodology for sampling and analyzing samples concerning the D2 standard authorized by the IMO. This is exactly the reason why this work becomes so innovative.

At the moment, the verification of the effectiveness of on-board ballast treatment systems is done only by some nationally certified laboratories for this microbiological analysis, every six months. As far as we know, there is no installed capacity in countries like Brazil that allows a independent verification of the effectiveness of the ballast treatment methodologies on board.

The gradual increase in the number of vessels in operation, caused by the globalization process, ends up causing complex and varied navigation routes around the world. This scenario puts pressure on the naval industry to manufacture more vessels, which are faster and larger, contributing to an increase in the volume of ballast operations carried between ports. With specific regard to Cholera, it presented a high worldwide incidence in the analyzed period (which is probably even higher than what is reported), especially in countries with a maritime coast that present major socio-environmental problems. In this way, ballast water management becomes an important point in the process of global surveillance in environmental and human health.

Therefore, methodologies that aim to select ships to be sampled by the performance of the

D1 standard and the effectiveness of the D2 standard are necessary in an attempt to minimize the bioinvasion process of pathogens, since, in addition to all the problems already mentioned, the number of personnel involved in the inspection process is very small in most countries. In addition to the methods of selecting ships for inspection, there is a demand for the development and application of simple and consistent microbiological methods, liable to intercalibration, so that each country can effectively monitor the ballast treatment systems' performance on its own or visiting vessels, without relying only on certificates issued outside its effective control.

The methodology presented in this work proved to be efficient in reducing the huge sampling effort in order to achieve a viable sample in technical, economic and personnel terms, and its main objective is to motivate decision makers to improve the entire process described here and directly create or improve the related public policies. To this end, the incorporation of interdisciplinary debates about the regularity and procedures of monitoring and control routines, the inspection processes and the use of more complete ship monitoring tools (as suggested in Figure 4, are important points for the improvement of the whole methodological process suggested in the work. Every preliminary proposal has as main goal to start a construction process focused on solving a problem, as efficient as possible.

What is intended by this proposal is to create a conceptual and methodological basis to provide a direction to accomplish this task and create mechanisms for continuous improvement and implementation of measures to reduce the introduction of harmful species by ballast water worldwide and thus contribute with the creation of debates and public politics that decrease all problem that involve the bioinvasion problematic specifically with regard the routines of verification of the efficiency of the treatment of ballast water on board vessels. This way it can contribute to the process of mitigation of impacts on all coastal dynamics and eventual problems of public health caused by the ballast process of vessels around the world.

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