ARTÍCULO PRODUCTO DE INVESTIGACIÓN

Perspectivas sobre el agua de lastre: la metagenómica como una prioridad apremiante

Insights to ballast water: metagenomics as a pressing priority

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Resumen

La metagenómica representa un avance fundamental en la genómica, ya que ofrece conocimientos sin precedentes sobre un espectro diverso de microorganismos, incluidos bacterias, virus y hongos, que antes eran difíciles o imposibles de estudiar fuera de los entornos naturales. Sus aplicaciones abarcan un rango notable, desde la exploración de entidades cósmicas hasta las profundidades de nuestros océanos, incorporando numerosos análisis. Sin embargo, su utilización en el contexto del análisis del agua de lastre sigue siendo escasa. Esto destaca el quid de la presente revisión, que tiene como objetivo mostrar la necesidad de la metagenómica en el análisis del agua de lastre. Como es sabido, el agua de lastre es esencial para mantener la estabilidad de los buques en condiciones sin carga o durante condiciones climáticas adversas, con movimientos globales diarios estimados entre 3 y 10 mil millones de toneladas. Un solo buque de carga a granel puede transportar hasta 60.000 toneladas de agua de lastre para una carga de 200.000 toneladas. Se estima que más de 7.000 especies son transportadas diariamente en todo el mundo en el agua de lastre de los barcos, lo

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que plantea importantes riesgos ecológicos cuando estos organismos se descargan en nuevos entornos donde pueden convertirse en especies invasoras, alterando los ecosistemas locales, las economías y la salud humana. A pesar de que la Organización Marítima Internacional estableció directrices estándar D-2 y respaldó varias prácticas de gestión del agua de lastre para su descarga segura, el problema persiste. Esto subraya la necesidad de emplear la metagenómica para mejorar el seguimiento del contenido microbiano en el agua de lastre. La revisión resume algunas investigaciones realizadas en todo el mundo para analizar el agua de lastre mediante metagenómica, y todas exhiben diversos microbios, incluidas potentes formas bacterianas y virales patógenas. Los hallazgos respaldan nuestra opinión y hacen de la metagenómica una herramienta invaluable para monitorear la efectividad del tratamiento del agua de lastre de conformidad con las regulaciones ambientales en evolución. Aunque los desafíos que enfrentan las aplicaciones metagenómicas (es decir, el costo, la complejidad y la necesidad de bases de datos de referencia integrales) son importantes, seguramente serán superables a la luz de los continuos avances tecnológicos y metodológicos. Adoptar la metagenómica ofrece un camino no solo para abordar las amenazas ecológicas que plantean las transferencias de agua de lastre, sino también para salvaguardar el futuro de las industrias navieras mundiales.

Palabras clave: metagenómica, agua de lastre, bioinvasión, Organización Marítima Internacional, ADN ambiental

Abstract

Metagenomics represents a pivotal development in genomics, offering unprecedented insights into a diverse spectrum of microorganisms, including bacteria, viruses, and fungi, that were previously challenging or impossible to study outside natural environments. Its applications span a remarkable range, from exploring cosmic entities to the depths of our oceans, incorporating numerous analyses. Yet, its utilization in the context of ballast water analysis remains scarce. This highlights the crux of the present review, which aims to showcase the need for metagenomics in ballast water analysis. As is known, ballast water is essential for maintaining ship stability under no cargo conditions or during adverse weather, with daily global movements estimated to be between 3 to 10 billion tonnes. A single bulk cargo ship can transport up to 60,000 tonnes of ballast water for a 200,000-tonne load. It is estimated that over 7,000 species are transported globally in ships' ballast water daily, posing significant ecological risks when these organisms are discharged into new environments where they can become invasive species, disrupting local ecosystems, economies, and human health. Despite the International Maritime Organization setting D-2 standard guidelines and endorsing several ballast water management practices for its safe discharge, the issue persists. This underscores the necessity of employing metagenomics to enhance the monitoring of microbial content in ballast water. The review summarizes some research conducted worldwide to analyze ballast water by metagenomics, all exhibiting diverse microbes, including potent pathogenic bacterial and viral forms. The findings support our view, making metagenomics an invaluable tool to monitor ballast water treatment effectiveness in compliance with evolving environmental regulations. Though the challenges facing metagenomic applications—namely cost, complexity, and the need for comprehensive reference databases—are significant, they will surely be surmountable considering continual technological and methodological advancements. Embracing metagenomics offers a pathway to not only address the ecological threats posed by ballast water transfers but also to safeguard the future of global shipping industries.

Keywords: metagenomics, ballast water, bio-invasion, International Maritime Organization, environmental DNA

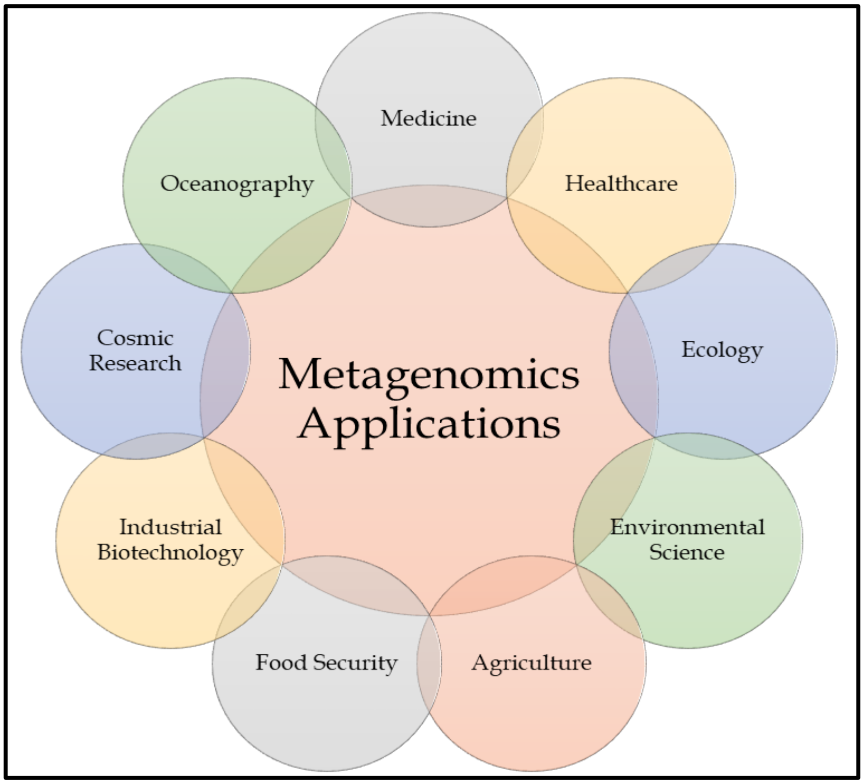
Introduction

The remarkable progress in microbiology and molecular biology has transformed our comprehension of life at a molecular level. Techniques such as CRISPR-Cas9 gene editing, high-throughput sequencing, and single-cell genomics have revolutionized genetic research, enabling precise modifications in DNA for disease research, agriculture, and biotechnology (Thurtle-Schmidt & Lo, 2018). Likewise, advances in genomics have accelerated our understanding of complex biological systems. Genomics, a branch of molecular biology, involves sequencing organisms' genes to gain details about their structure, function, evolution, and mapping of genomes (Solanke et al., n.d.). Within genomics, metagenomics, also known as environmental genomics or eco-genomics, focuses on analyzing genetic materials recovered directly from environmental or clinical samples (Lema et al., 2023).

Owing to its transformative approach, metagenomics circumvents the need to cultivate microbes in the lab, thereby uncovering a wide array of microorganisms, including bacteria, viruses, and fungi, which were otherwise difficult or impossible to study in laboratory settings (Stewart, 2012). The process of metagenomic analysis typically involves the collection of samples from the environment, such as water and soil, complete DNA extraction, sequencing of the DNA, and then computational analysis to identify the genetic material present (Thomas et al., 2012). This culture-independent approach has transformed microbial ecology, revealing vast microbial diversity by directly sequencing the genetic material of environmental samples (Stewart, 2012).

However, this approach still presents some challenges that need to be addressed. Firstly, the data generated through metagenomic analysis is vast and complex to interpret, requiring sophisticated bioinformatics tools for sequence assembly, annotation, and analysis. Secondly, sample processing is often complicated, and there is a need for more efficient and cost-effective sequencing technologies. Additionally, accessing and sharing genetic resources from different environments may present ethical and legal challenges (Lapidus & Korobeynikov, 2021). Despite these challenges, the applications of metagenomics are wide-ranging and span various fields. Harnessing the power of genetic insights from environmental microbial communities will surely drive innovations in medicine, environmental science, agriculture, biotechnology, and more (Zhang et al., 2021).

Figure 1. Metagenomics application in various fields



Source: Unknown

. In medicine and healthcare, metagenomics has revolutionized our understanding of human health by detailing the composition and function of the human microbiome. It can help determine the abundance and species of gut microbes, which can help monitor human health and wellbeing (W.-L. Wang et al., 2015). It even helps link microbial communities and various diseases such as obesity, diabetes, and certain types of cancer. It also assists in tracking the spread of antibiotic-resistance genes, which is critical for addressing antimicrobial resistance. Moreover, metagenomics facilitates the discovery of novel pathogens, new enzymes and probiotics, improving diagnostic tools and paving the way for personalized medicine (Alves et al., 2018).

. Metagenomics is a powerful tool in environmental sciences and ecology-based research as it can monitor biodiversity and assess ecosystem health (Chiriac & Murariu, 2021). Metagenomics play a crucial role in understanding microbial contributions to biogeochemical cycles and studying pollutants' effects on microbial communities (Kuang et al., 2023). Furthermore, metagenomics aids in bioremediation efforts by identifying microbes and microbial enzymes capable of breaking down environmental pollutants. Notably, metagenomics can help reconstruct the metagenome of an ecosystem using random shotgun sequencing, providing a large amount of data on microbial ecology (Ufarté et al., 2015).

. Metagenomics can significantly contribute to food security by offering insights into the microbiomes associated with animal and plant-based food products. This approach allows for the exploration of genes and transcripts within these microbiomes, which can aid in enhancing the sustainability of food production systems (Billington et al., 2022). Understanding the gastrointestinal microbiome of animals can improve nutrition, health, and growth efficiency, directly influencing meat, dairy, and egg production quality and quantity (Celi et al., 2017).

. In plant agriculture, metagenomics can analyze soil health and the microbial communities crucial for supporting plant growth. This knowledge can lead to more sustainable farming practices by enhancing soil fertility, crop yield, and disease resistance (Nwachukwu & Babalola, 2022). Moreover, metagenomics can assist in the discovery of new biopesticides and biofertilizers, reducing the reliance on chemical inputs (Masenya et al., 2024).

In the sector of industrial biotechnology, metagenomics benefits by identifying novel enzymes and bioactive compounds with industrial applications. These discoveries can develop new drugs, biofuels, and other bioproducts (Xing et al., 2012). For instance, enzymes identified through metagenomic studies can be used in biofuel production, biodegradation, or the synthesis of pharmaceuticals and polymers (Patel et al., 2022).

Apart from the mainline sectors of the economy, metagenomics also holds significance in areas from cosmic heights to ocean depths. Researchers have used metagenomics to search for life in space (Bijlani et al., 2021). Simultaneously, this technology has opened new horizons in oceanography by providing insights into the ocean's microbial life, which plays a vital role in global biogeochemical cycles (Kerkhof & Goodman, 2009). Thus, metagenomics offers a comprehensive approach to exploring microbial worlds, driving innovations across several fields.

In fact, many pieces of research, especially in ballast water studies, suggest applications of metagenomics as a quality check to determine the presence of microbial load through direct sampling. However, international responses to metagenomic analysis are still limited (Johansson et al., 2017a). This review aims to highlight the need to use metagenomics for ballast water analysis. It provides insights into the ecological, economic, and human health impacts of inadequately discharged ballast water and how metagenomics can help manage ballast water issues.

1. Shipping and ballast water

The shipping industry, often characterized as the cornerstone of global trade, facilitates the transportation of goods across continents. Shipping is crucial, constituting over 80% of the world's economy (Gidwani, 2022). Shipping routes are critical to the global supply chain, with major sea lanes like the Strait of Malacca, Panama Canal, and Suez Canal facilitating the swift movement of goods between key ports (Culpan, 2024). This sector encompasses a vast array of activities, including the operation of merchant ships, shipbuilding, shipping routes management, and logistics services that ensure the efficient delivery of billions of metric tons of cargo annually (Propelling India’s Maritime Vision: Impact of Government Policies, 2023).

In the realm of maritime transport, there are typically four primary types of merchant ships in operation: container ships, bulk carriers, tankers, and general cargo ships. Container ships are primarily responsible for transporting a substantial portion of the world's manufactured goods, employing standardized containers to facilitate efficient handling and transfer. Bulk carriers are specialized vessels focused on transporting loose bulk cargo such as grains, coal, and minerals, while tankers are specifically designed to transport liquid cargo, including oil, chemicals, and liquefied natural gas (LNG) (Reference Manual on Maritime Transport Statistics, 2017). Despite its importance, the shipping industry faces numerous challenges, including environmental impact concerns. Shipping contributes to air and marine pollution, with emissions from ships being a significant source of greenhouse gases. Furthermore, ships carry ballast that contributes to mixing local water from one port to another (Jägerbrand et al., 2019).

Ballast refers to any substance utilized to stabilize an object. In the context of maritime transport, ballast plays a crucial role in preserving equilibrium, steadiness, and the overall structural soundness of a vessel, mainly when it is devoid of cargo. For thousands of years, ships have used ballast in the form of solid materials like rocks, metal chunks, and sandbags. Modern ships use water as ballast due to its easy accessibility and handling ("Ballast Water and Ships," 1996). Annually, around 10-12 billion tonnes of ballast water are used in shipping, with a single bulk cargo ship loading up to 60,000 tonnes of ballast water for a trip. This ballast water filled at the port is typically pumped into ships' ballast tanks and contains aquatic plants, animals, and countless microbes from the water source. It is estimated that over 7,000 species are conveyed worldwide in ships' ballast water on a daily basis ("Ballast Water," 2011).

Often, when a ship reaches a new port and empties its ballast water, the organisms from its initial port face natural barriers like altered climate, salinity, and water temperature, which limit their survival in a new environment (Gollasch, 2007). However, in numerous instances, marine organisms, including bacteria, small invertebrates, and various species' eggs, cysts, and larvae, have shown the ability to adapt to new environments and subsequently establish themselves as invasive or alien species, thereby harming the indigenous ecology of aquatic ecosystems (Bax et al., 2003).

2. Impact of ballast water

The shipping industry is highly regulated to ensure safety, prevent pollution, and promote efficient international trade. Organizations such as the International Maritime Organization set global standards, while national and regional authorities enforce regulations concerning ship design, construction, equipment, operation, and crew qualifications (Karanassos, 2016). Still, the act of taking in and discharging ballast water has been an integral aspect of the shipping industry since its inception. However, it has led to the unintended consequence of transporting aquatic organisms globally.

When vessels take on ballast water in one geographical location and subsequently discharge it in another, there exists the potential for the introduction of non-native aquatic species into new ecosystems (Gollasch & David, 2019). When introduced into ecosystems with no natural predators, these species can become invasive, outcompeting local species, disrupting ecosystems and causing significant damage (Mayfield et al., 2021).

Table 1 highlights some common invasive species that came to different locations via ballast water and how they impacted surrounding environment. Those impacts may be at smaller or bigger scale, however, preliminarily, they can be grouped in three major categories, i.e. ecological, economic and human health.

2.1 Ecological impacts of ballast water

Ecological harm ensues when non-indigenous species disrupt native biodiversity and ecological processes. Altered biodiversity may lead to either extinction of native species or change in their relative abundance (Kurniawan et al., 2022a). Introducing invasive species also disrupts local resources, altering food webs and potentially leading to the extinction of native organisms (Saglam & Duzgunes, 2018). Such ecological imbalances can reduce biodiversity and affect the functioning of ecosystems.

2.2 Economic impacts of ballast water

Often, ecological upheaval affects the functioning of ecosystems and can lead to economic losses, especially in industries reliant on these marine environments, such as fisheries and coastal tourism (Z. Wang et al., 2022). The introduction of exotic species has been recognized as a cause of detriment to the stock of commercial fishing. A common example of such a scenario is the ballast water-based invasion of *Dreissena polymorpha* (Zebra mussel) in the 1980s from Eastern Europe to the Great Lakes, USA (Jr., 1994). They have caused significant ecological and economic damage by clogging water intake pipes and outcompeting native species (Carlton, 2008). Likewise, *Carcinus maenas* (European Green Crab) invaded to the West Coast of the US and negatively impacted local shellfish populations (Tan & Beal, 2015). *Mnemiopsis leidyi* (North American Comb Jelly) was inadvertently introduced to the Black Sea via ballast water (Shiganova, 1998). This inadvertent introduction resulted in disruptions within the local marine ecosystems, as the comb jelly commenced preying on fish eggs and larvae, consequently leading to a significant decline in commercial fish stocks (Knowler, 2005).

2.3 Human health-based impacts of ballast water

Unfortunately, ballast water also serves as a conduit for translocating harmful organisms that not only disrupt marine ecosystems but also pose severe risks to human health (Werschkun et al., 2014). Ballast water carries pathogens and harmful algae that can potentially trigger public health crises (Kraus, 2023). Though directly linking specific outbreaks to ballast water can be challenging, several cases have highlighted the potential for ballast water to transport harmful organisms that have caused health issues. For instance, cholera pathogens (*Vibrio cholerae*) were believed to have been transported through ballast water, contributing to outbreaks in coastal areas. A notable case was the cholera outbreak in Peru in the 1990s, where ballast water was considered a potential vector for the disease (Poirier et al., 2012). In a similar vein, the 1991 South American cholera epidemic stemmed from contaminated oysters and fish in Mobile Bay, Alabama, with one-third of the ships arriving from South America carrying the bacterium (Cohen et al., 2012). Another common set of organisms transported frequently by ballast water worldwide is dinoflagellate species that cause harmful algal blooms, which produce toxins that accumulate in food chains, leading to aquatic and wildlife deaths and seafood contamination that can cause serious illness in humans (Karlson et al., 2021) ("Harmful Algal Blooms," 1999).

Table 1. Various invasive species that got dispersed through ballast water

No.	Invasive Species	Initial Source	Spread to	Impact	Reference
1	*Vibrio cholerae*	Different strains across	America	Cholera Epidemics	(McCarthy & Khambaty, 1994) (Cohen et al., 2012)
2	Zebra mussel	Eastern Europe	North America	Biofouling and infrastructure damage	(Karatayev & Burlakova, 2022) (Hernández Elizárraga et al., 2023)
3	Asian kelp	Northern Asia	Southern Australia, Europe	Displaced native algae causing altered food web	(Peteiro et al., 2016) (Epstein & Smale, 2017)

4	Mitten crab	Northern Asia (China)	Western Europe and North America	Caused soil erosion by burrowing and preyed Native fish populations	(Rudnick et al., 2003)
5	Harmful Algal Blooms	Various sources	Various locations	Toxin release in water bodies and seafood contamination.	(Karlson et al., 2021)
6	North Pacific Seastar	Northern Pacific	Southern Australia	Economic losses by feeding on commercial oyster and clam species.	(Jeff Ross et al., 2003)
7	North American Comb Jelly	America	Caspian Seas	Commerical losses in fishery	(Bilio & Niermann, 2004)
8	Asian carp	Asia	North America	Outcompete native fishes and disrupt food web dynamics	(Weber & Brown, 2011)
9	European green crab	Europe	America	Declined shellfish population	(Tan & Beal, 2015)
10	Cladoceran Water flea	Black and Caspian Seas	Baltic Sea	Clogged fishing nets and trawls	(Dobrzycka-Krahel et al., 2023)

Source: Own elaboration

3. International responses for ballast water

In 2004, the International Maritime Organization ratified the International Convention for the Control and Management of Ships' Ballast Water and Sediments to address the environmental risks associated with ballast water transfers. As a specialized agency of the United Nations, the International Maritime Organization is tasked with developing and upholding a comprehensive regulatory framework for shipping. This encompasses aspects such as safety, environmental considerations, legal affairs, technical collaboration, and maritime security.

3.1 D-2 Standard Guidelines

The Ballast Water Management Convention, initiated by the International Maritime Organization and enacted in September 2017, endeavours to alleviate the ecological, economic, and public health repercussions associated with transporting aquatic organisms and pathogens (David et al., 2015). Within this framework, the International Maritime Organization has instituted the D-2 standard, which comprises a series of directives governing the release of ballast water and sediments by vessels (Outinen et al., 2021). Notable directives may encompass the following (Duc Bui et al., 2021):

. Ships must ensure that ballast water contains fewer than ten viable organisms of a size greater than or equal to 50 micrometres in minimum dimension per cubic meter.

. Ships must also ensure that ballast water contains fewer than ten viable organisms of sizes between 10 micrometres and 50 micrometres in minimum dimension per millilitre.

. Human pathogens must be less than set specific limits. For example, *Vibrio cholerae* must be less than one cfu per 100 millilitres. *Escherichia coli* and intestinal Enterococci should be less than 250 cfu and 100cfu per 100 millilitres, respectively.

. Ships must maintain a Ballast Water Record Book and carry an International Ballast Water Management Certificate, which validates that the vessel complies with the ballast water management requirements specified by the convention.

. Ships must employ approved ballast water treatment systems to effectively remove or deactivate the organisms to meet the specified standards before the ballast water is discharged into new environments.

Despite the challenges and expenses associated with implementing ballast water management practices, the advantages of mitigating bioinvasions—such as safeguarding biodiversity, fisheries, and economies from the disruptive impacts of invasive species—significantly outweigh the costs (Nie et al., 2023). As such, the shipping industry and regulatory bodies worldwide continue to work towards more efficient and effective ballast water management solutions to safeguard marine environments while facilitating global trade. Overall, the global efforts to manage and treat ballast water represent a critical step toward protecting marine ecosystems and human health from the potential negative impacts of the shipping industry's operational practices. By adhering to the D-2 standard, the shipping industry takes a significant step forward in mitigating the risks associated with transporting invasive aquatic species across the globe (David & Gollasch, 2015).

3.2 Ballast water management approaches

The ballast water management convention necessitates the adoption of ballast water management systems to treat water using mechanical, physical, chemical, or biological methods before discharge into new environments (Lakshmi et al., 2021). Effectively managing and treating ballast water is essential in mitigating adverse environmental impacts and addressing the spread of invasive species, leading to establishing international regulations and advancing innovative technologies (Jing et al., 2012). Some major sustainable approaches and technologies for ballast water management include:

. Ballast Water Treatment Systems: Ships are increasingly equipped with onboard treatment systems that sterilize or remove organisms from ballast water before it's discharged. Standard technologies include UV radiation, filtration, electro-chlorination, ozonation etc (Kurniawan et al., 2022b). Figure 2 provides insights into different approaches for ballast water treatment systems.

. Ballast Water Exchange: A method to reduce the risk of invasive species transfer involves exchanging coastal ballast water with open ocean water, where fewer organisms are likely to survive or establish populations if discharged (Bradie et al., 2021).

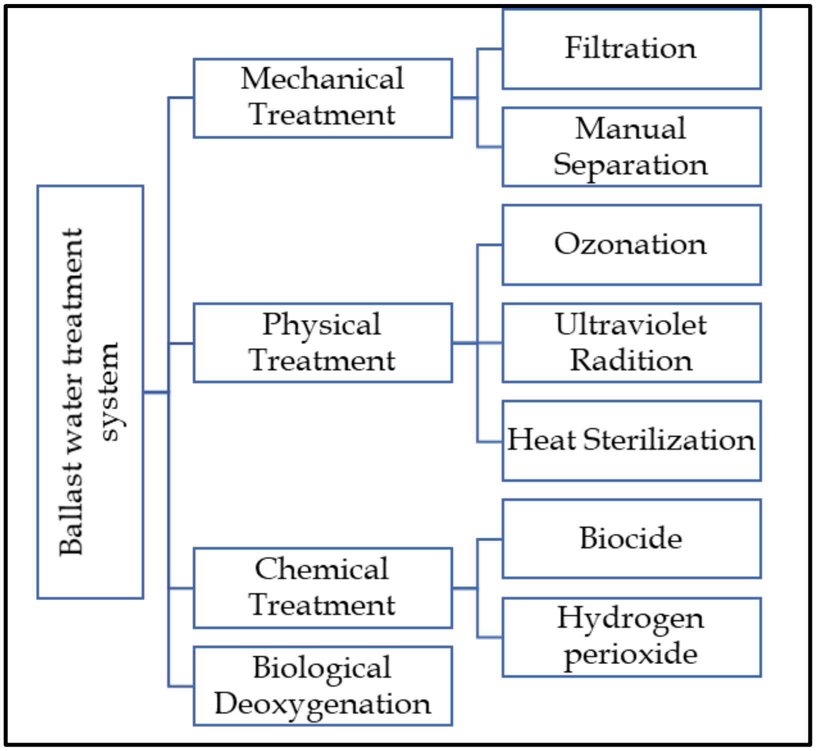
. Port Reception Facilities: Developing and enhancing port reception facilities to treat or safely contain ballast water, preventing untreated water's discharge into local ecosystems.

. Regulatory Compliance and Enforcement: Implementing strict regulations and enforcement mechanisms is fundamental to ensuring compliance with ballast water management standards, including regular inspections and certification of treatment systems (Maglić et al., 2019).

. International Collaboration: Given the international nature of shipping, sustainable ballast water management requires global cooperation. Sharing best practices, technologies, and monitoring techniques is vital for addressing this challenge collectively (Bilawal Khaskheli et al., 2023).

. Environmental DNA (eDNA) Monitoring: Using eDNA to monitor and assess the biodiversity of ballast water provides a non-invasive, accurate, and relatively quick means to detect the presence of invasive species, allowing for more responsive management practices (Fonseca et al., 2023). This wades back to the applications of metagenomics for ballast water analysis; such practices are crucial steps towards the global effort to protect marine ecosystems and human health from the unintended consequences of the shipping industry's operations.

Figure 2. Various approaches for ballast water treatment



Source: Own elaboration

4. Metagenomics for Ballast Water Analysis

Metagenomics plays a critical role in the analysis of ballast water, offering significant advances in monitoring and managing the ecological impacts of shipping activities. It is a way better approach than traditional methods for analyzing ballast water, which often rely on culturing and microscopic identification of organisms and are limited in detecting a broad range of species, especially those at early life stages or in low abundance (Zhang et al., 2021b). Metagenomics involves the direct genetic analysis of genomes within an environmental sample and offers a comprehensive and efficient alternative (Ruppert et al., 2019).

Metagenomics plays a critical role in the analysis of ballast water due to its capacity to offer a comprehensive overview of the biodiversity transported in ships' ballast water. This data is essential for conducting risk assessments and formulating management strategies to mitigate the introduction of invasive species. By detecting a wide spectrum of DNA, metagenomics facilitates the identification of present species as well as their potential pathogenicity and resistance genes, thereby giving valuable insights about any potent microbe into new environments (Darling et al., 2017).

Furthermore, metagenomics serves as a valuable tool for monitoring the efficacy of ballast water treatment systems, a vital aspect in ensuring compliance with the escalating regulations aimed at safeguarding marine environments. Metagenomic analysis offers a swift and precise method for evaluating the biological content of treated ballast water, thus ensuring compliance with environmental standards (Darling et al., 2017).

Despite the significant advantages of metagenomics in analyzing ballast water, its application is limited. Some possible reasons could be its complexity, the need for high-quality reference databases and the cost involved (Lapidus & Korobeynikov, 2021). Nevertheless, as mentioned in Table 2, many pieces of research have proved that metagenomics can be an excellent choice for analyzing unnoticed organisms and viruses with greater efficacy. Further, advancements in technology and methods will likely mitigate some of these challenges, enhancing the utility of metagenomics in environmental monitoring and protection efforts, especially regarding ballast water analysis.

Table 2. Overview to research on metagenomics-based analysis of ballast water

No	Sampling site	Type of organism	Quantification	Major Species identified	Reference
1	Jiangyin port in Jiangsu, China	Bacteria and Archea	103 species	*Proteobacteria, Bacteriodota and Actinobacteriota*	(Xue, Han, et al., 2023)
2	Port of Duluth	Viruses	550 million sequences	*Double stranded DNA phages Siphoviridae, Podoviridae,*	(Kim et al., 2015)

				Myoviridae, Microviridae etc.	
3.	Jiangyin port in Jiangsu, China	bacteria eukaroytes, archea and viruses	Total 14,403 species including 422 pathogens	*Proteobacteria, Bacteriodota Actinobacteriota, Escherichia coli, intestinal Enterococci*	(Xue, Tian, et al., 2023)
4.	Archieved sediments from North American Great Lakes or the Chesapeake Bay, USA.	Dinoflagellates	Total 73 species with 36 harmful algal bloom forming	*Apocalathium malmogiense, Margalefidinium polykrikoides, Polykrikos geminatum, Scrippsiella acuminata*	(Shang et al., 2019)
5.	Busan Port, Korea	DNA viruses	Isolated viruses belonged to 22 viral families	*Myoviridae, Podoviridae, Siphoviridae, Swinepox virus, Raccoonpox virus, Suid herpesvirus, and Human endogenous retrovirus*	(Hwang et al., 2018)
6.	Yangshan Port (Shanghai, China)	Bacteria	Includes 16 potential pathogens	*Proteobacteria, Bacteroidetes Firmicutes, Gammaproteobacteria Aeromonas, Enterobacter*	(L. Wang et al., 2020)

7.	Samples collected over the course of M/V Arctic (bulk carrier) from Quebec City, Canada till Deception Bay, Australia	Bacteria	6.6 million sequence reads	*Actinobacter* *Trabulsiella* *Enterobacter,* *Acidovorax,* *Curvibacter* *Bordetella* *Sphingobacteria,* *Flavobacteria, Vibrio,* *Mycoplasma*	(Johansson et al., 2017b)

Source: Own elaboration

Conclusions

The shipping industry's practice of exchanging ballast water for its operations poses significant environmental challenges by facilitating the global transport of non-native aquatic species. Once introduced into new ecosystems, these species can become invasive, causing widespread ecological disruption, economic losses, and even impacts on human health. The application of metagenomics to ballast water analysis presents a groundbreaking solution, offering an excellent approach to detect microbes, including those at early life stages or in minimal abundance. This technique not only aids in the crucial tasks of risk assessment and management strategy development but also serves as an invaluable tool to monitor ballast water treatment effectiveness in compliance with evolving environmental regulations.

While the implementation of metagenomics is currently faced with challenges such as cost, complexity, and the need for robust reference databases, ongoing advancements in technology and methodology promise to overcome these barriers. Ultimately, the continued refinement and adoption of metagenomics in the analysis of ballast water are essential for safeguarding marine ecosystems, preserving biodiversity, and ensuring the sustainable future of global shipping.

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