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Research Article

Potential Threat of Viruses in Ballast Water to Aquaculture

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Abstract

Viruses are among the most abundant species in the biosphere and may pose a significant risk to the ecology, economy and human health with the discharge of ballast water. The viral pathogens in ballast water have the potential to infect and harm various species of fish and shrimp, leading to economic losses and ecological disruptions. Common measures such as ballast water exchange and ballast water management system are unsatisfactory for viral disinfection. In this article, we analyzed the abundance and diversity of viral communities in ballast water as well as their potential threat. The results highlight the need to recognize and address the hidden danger of viruses in ballast water. We also assessed the state of ballast water management, emphasizing the importance of implementing effective ballast water management practices to safeguard the health and sustainability of aquaculture systems and offering several suggestions to enhance viral management in it.

Keywords: Ballast Water; Virus; Aquaculture; Management

Introduction

Ballast water is important for ships during navigation because it can help control the heeling, trim, draught, stability or stress, ensuring the safety and efficiency of the ship's navigation [1]. Nowadays, maritime trade among countries has become increasingly frequent. There are thousands of ships navigating in the sea, resulting in the transfer of seawater between different ports. Previous surveys have shown that approximately 45000 ships engage in shipping operations every year, resulting in the discharge of roughly 14 billion tons of water [2-4]. Referring to the information released by the National Ballast Water Exchange (NBIC), the total discharge of ballast water from ships arriving in the United States in 2018 was 362.5 million tons [5].

Improper discharge of ballast water has detrimental effects on the local ecological environment of the port. When the water is deballasted, some non-indigenous species may enter the environment around the harbor and pose a potential threat of biological invasion. Unknown alien spaces can cause many problems, such as competing for resources with native organisms or introducing serious diseases [6-8]. To curb the spread of harmful microorganisms in ballast water, the International Maritime Organization (IMO) adopted the International Convention on the Control and Management of Ships' Ballast Water and Sediments in 2004 [9]. Implementation of the Convention has effectively strengthened the management of ships' ballast water and sediments.

Viruses are a class of infectious agents characterized by their non-cellular nature and extremely small size. It has so simple structure that cannot live without host support. The virus has seriously impacted on human society. Up to now, the most threatening infectious diseases of human beings or other animals are almost viral diseases. For instance, COVID-19 which has caused huge economic losses to all countries around the world since it has outbroken in 2019, was caused by Severe Acute Respiratory Syndrome Coronavirus 2 that belongs to Coronaviridae [10,11]. Ballast water, as one of the most important carriers of marine biological invasion at present, leads to the transfer of a large number of viral particles, including a variety of viral pathogens [12-16].

Since the development of metagenomic, more advanced technology has been developed, making the detection of viruses more effectively [17-19]. Metagenomic can make the examination of viruses in ballast water a reality and is expected to be applied to strengthen the management of ballast water. In this review, we examine the abundance and diversity of viral communities in ballast water. Moreover, we provide a summary of viral detection technologies and evaluate the effectiveness of ballast water management practices in reducing viral contamination. Our findings emphasize the importance of taking proactive measures to mitigate the risks associated with viruses in ballast water and

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propose the adoption of innovative methods for efficient viral detection.

Material and Methods

The articles included in our review on viral research in ballast water were sourced from reputable databases such as Web of Science and CNKI. To conduct the literature search, key terms such as 'ballast water,' 'virus,' 'pathogen,' and 'diversity' were employed. Recent investigations that are about viral pathogens and their potential risks were collected for analysis. Our review also pays close attention to the latest viral research technologies in ballast water. Additionally, we gathered information on regulations, conventions, and experimental studies related to ballast water management, including the effectiveness of ballast water management systems (BWMS) in treating viruses. Furthermore, we considered the references cited in the aforementioned research to ensure a comprehensive approach to our study.

A comparison of viral abundance in ballast water, sediment, biofilm, and residual water is conducted based on the date collected from recent articles. Additionally, we reanalyzed viral diversity, specifically focusing on viral species, using data from investigations conducted in the Great Lakes, the harbor of Singapore, and Los Angeles and Long Beach (LA / LB). The data was chosen as they employed similar methods in virome research, enabling meaningful comparisons. To provide a comprehensive understanding of viruses in ballast water, our review specifically focuses on viral species at the family level.

Results

Viral community in ballast water

Distribution of virus in ballast tank: The study of viruses in ballast water originated in 2000 when Ruiz investigated seven ships arriving at Chesapeake Bay and found that a large number of virus particles would enter the sea with the unloading of ballast water [19]. Investigation of the ship arriving at the port in Baltimore, the Great Lakes and Japan also showed that the discharge of ballast water would cause numerous foreign viruses to invade the local environment [20-22].

Viruses mainly distribute in the water, biofilms and sediment in the ballast tank (Figure 1). The viral abundance in ballast water, estimated at approximately 10^{10} VLPs/L, is comparable to that found in seawater [23]. While the abundance of viruses in ports may have a minimal impact, unique viruses introduced from other ports may affect indigenous species. After water deballasting, a part of water remains in the tank where viruses are enriched. Drake [24], showed that the abundance of viruses in residual water is 6.2×10^{10} VLPs/L, significantly higher than viruses in ballast water. When fresh water is ballasted, these viruses may quickly rebuild their community so that affects the effectiveness of ballast water exchange or ballast water management system (BWMS).

Biofilm and sediment are suitable for the virus since there are a large amount of organic matter and a great number of bacteria. Plentiful bacteria provide sufficient hosts and make less distinction between themselves, which is more beneficial for virus infection [25]. The abundance of the virus in biofilm is around 6.33×10^{11} VLPs/L, similar to the viral concentration in the sediment pore water, which is approximately 1.17×10^{12} VLPs/L [24]. The virus in both zones is significantly higher than ballast water, shows that virus will enrich in the biofilm and sediment inside the tank. Significantly, the virus can absorb the solid particle and will not be completely separated by centrifugation, suggesting that there are more viruses in the sediment [26].

Viral species in the ballast water: In 2015, Yiseul [27], firstly used metagenome analysis to study the viral community

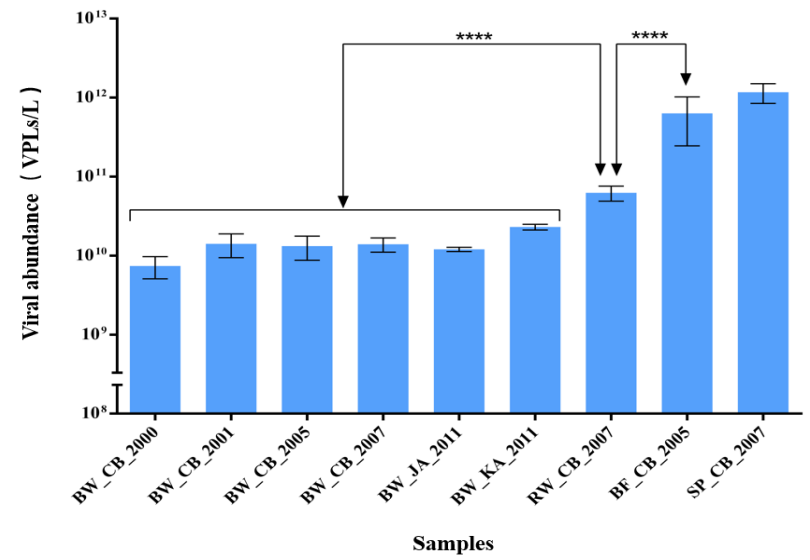

Figure 1 Viral abundance in the ballast tank. BW means ballast water. RW means residual water after water deballasts. BF means biofilm in the ballast tank. SP means sediment pore water in the ballast tank. All data was collected from early research about the virus in ballast tank.

in the ballast water from the ship located in Duluth-Superior Port of the Great Lakes (AB, BB, IB, MB, PB). Through the research of five ship ballast water samples from different lakes, 28 different viral families were detected. In the next few years, metagenome analysis was used to investigate the diversity of viruses in the ballast water of ocean-going ships. The results indicate that there were as various viruses in the ballast water as the samples from the Great Lake [12,14]. In the ship arriving at the ports of Los Angeles / Long Beach (CADO, CASC, CATL, CBAL, CCAR, CCEB, CCOS, CLIB, CNAD, CSAG, CTUL) and Singapore (SCB, SGB, SMB, SQB, SRB), a total of 59 different viral families were detected (Figure 2B).

DNA virus is dominant in the ballast water, which is up to about 99.085% of the virome, including 73.937% of dsDNA and 25.148% of ssDNA virus (Figure 2A). At the order level, Caudovirales is dominant in ballast water, which includes the family of Myoviridae, Podoviridae and Siphoviridae (Figure 2C). Caudovirales is the most widely distributed virus in the global water column [28]. The virus belonging to this order can infect cyanobacteria, heterotrophic bacteria, and archaea, which are ubiquitous in marine [29]. There is a high abundance and diversity of bacteria in ballast water, providing sufficient hosts for these viruses [30]. Thus, it is expected that Myoviridae, Podoviridae and Siphoviridae are dominant in most ballast water.

Next in relative abundance are Microviridae (Figure 2C), the ssDNA virus that infects bacteria [31]. Microviridae are also widely distributed in the world and have been found in the different environment, including marine, fresh water environment, sewage, soil, sediment and human gut [32] . The abundance of Microviridae is distinctive in different ballast water. In some water column, Microviridae is dominant or has high viral concentrate, which shows that there were sufficient bacteria as recent results demonstrated [24,33,19]. On the contrary, the abundance of Microviridae is extremely small in other ballast water columns. The opposite phenomenon may be caused by the lysogenic infection of the phage [34], or the lack of a special host.

There were also some viruses having a quite high concentration in the minority ballast water column followed by above phage, including Parvoviridae, Phycodnaviridae and Circoviridae (Figure 2. C). Parvoviridae is a family of ssDNA viruses that infect a wide range of hosts, including humans and animals [35]. The virus belonging to Circoviridae is ssDNA virus. Circoviridae mainly infects the host of birds and mammals, causing fatal diseases [36,37]. Both the Parvoviridae and Circoviridae families are commonly found in ballast water, where they can exhibit high concentrations and even become dominant species within some water columns [15]. The result suggests that the virus in ballast water have a potential impact on the animals if the water is deballasted without any treatment. Phycodnaviridae belongs to an ancient, genetically diverse but morphologically similar family

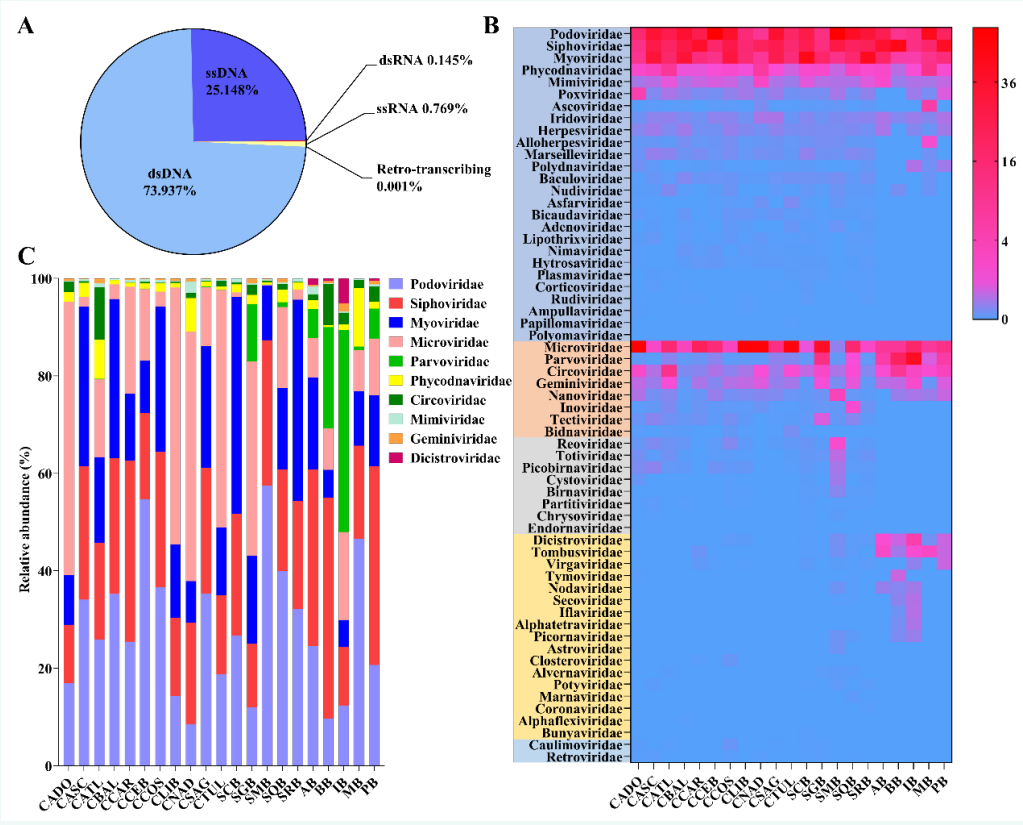

Figure 2 Composition of viral genome in ballast water (A). Heatmap of viral relative abundance in the ballast water (B). Viruses with high abundance in ballast water (C).

of large icosahedral viruses. Phycodnaviridae belongs to the Nucleo-Cytoplasmic Large DNA virus and can infect eukaryotic algae which are ubiquitous in marine and fershwarer [29,38]. Ballast water contains a diverse array of phytoplankton species, serving as hosts for Phycodnaviridae [39,40]. Consequently, it is common to detect the widespread presence of Phycodnaviridae in water samples.

The diversity of the viral community in ballast water: The research on the diversity of the viral community in ballast water is very few. There was only one article involved in it. After comparing with the virome in marine, Antarctic lakes, desert ponds, aquaculture ponds, reclaimed water, potable water, and other temperate lakes by analyzing sequencing similarity with a cross-TBLASTX search, Kim [15] found that viromes in ballast water and harbor water from the Great Lake are distant from other environments except oligomesotrophic lake. The annotation-independent approach was also used to analyze the similarity between ballast water viromes and harbor water viromes. The result indicates that viromes in harbor water have a distance from ballast water viromes. In addition, it also showed that there were distinctions between viromes in ballast water originating from different sources.

Threats of viruses in ballast water: Several viruses in ballast water can pose serious threats as they can affect the ecology, economy and human health. There were 14 pathogens of viral diseases found in the ballast water reported in recent researches, including 4 shrimp viruses, 6 fish viruses, 2 swine viruses, and a human virus [12,14,15]. Among these viruses, 9 pathogens were on the list of studies concerned by the World Organization for Animal Health (OIE) [41,42].

Destroy shrimp aquaculture: The shrimp viruses found in the ballast water include white spot syndrome virus (WSSV), Taura syndrome virus (TSV), infectious myonecrosis virus (IMNV) and Macrobrachium rosenbergii nodavirus (MrNV), which are pathogens on the OIE management list [14,15] . All these viruses have high lethality and strong infectivity.

The contigs that related to WSSV were detected with a considerable number in both ballast water (AB, BB, IB, and PB) and harbor water (IH and MH) from the Great Lake (Figure 3) [15], suggesting that there was a potential risk that ballast water may become the vector of WSSV transmission. WSSV belongs to Nimaviridae and is the pathogen causing white spot syndrome in shrimp. It is the most common and destructive shrimp virus at present [43]. The major sensitive host of WSSV is *Penaeus monodon* and *Penaeus vannamei*, the main species of shrimp cultured artificially [44]. Once the shrimp is infected with WSSV, they will have characterized by anorexia, lethargy, abnormal behavior (such as decreased swimming ability, etc.), swelling of gillies, loose cuticle, enlargement and yellowish discoloration of hepatopancreas, thinning and delayed coagulation of its hemolymph [45]. Besides, there are characteristic white spots with a diameter of 1 mm – 2 mm that will appear on its shell, appendage, or internal surface. WSSV is fatal to the shrimp. The mortality rate of the host infected with the virus is as high as 80% - 100% [45]. WSSV has caused the catastrophe for shrimp aquaculture. For example, the economic loss due to the WSSV infection was estimated at US$ 250 M reported from India during 2006-2008, which consequently result in employment losses of 2.15 M man-day [46] .

TSV was mainly detected in ballast water (IB and PB) from the Great Lakes, whereas only a limited number of contigs were found in harbor water (MH in Figure 3). TSV belongs to Dicistroviridae and is the second most prevalent pathogen of shrimp viral disease after WSSV. The main host of its infection is *P. vannamei* [47]. After acute virus infection, the shrimp will become anorexic, and lethargic with unstable swimming behavior, soft cuticles, and a loose body. It can be observed that multifocal and melanized lesions on the thorax and tail of the shrimp. TSV is also lethal to its host. After being infected with TSV, 60% - 90% of prawns will

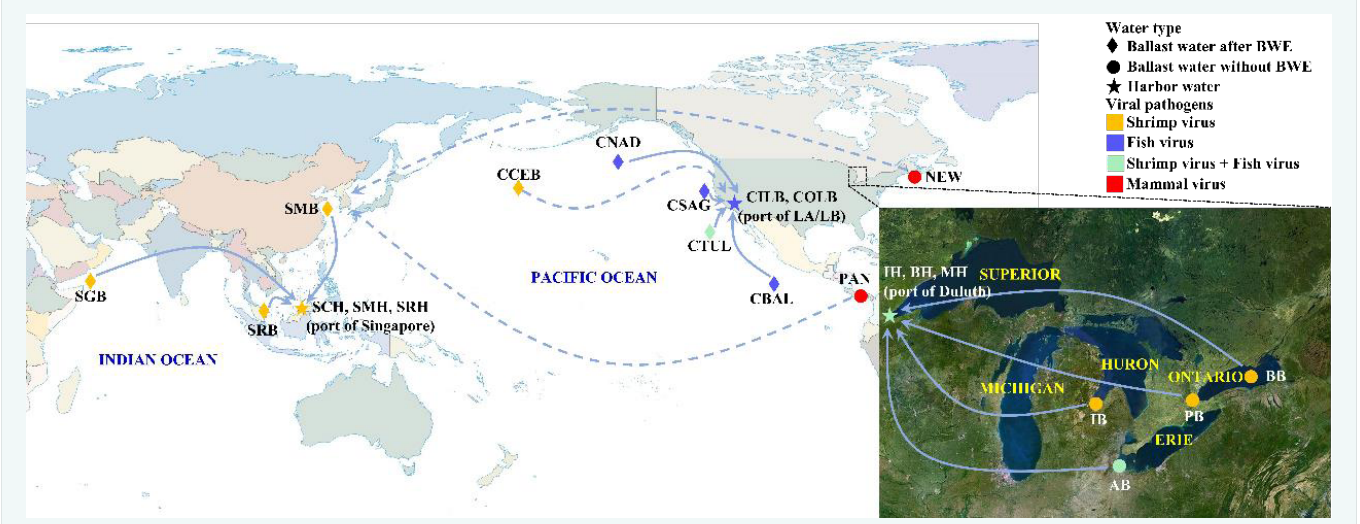

Figure 3 Viral pathogen in ballast water and harbor water. The solid line indicates the presence of viral pathogens in both ballast water and port water. The dashed line indicates that pathogens were only found in the ballast water of arriving ships.

lose their lives [45,48]. When shrimp in the hatchery is infected by TSV, it is commonly treated by replacing TSV-specific pathogen-free or TSV-specific pathogen resistance shrimp [45]. The introduction of the new breed will lead to the invasion of other pathogens without effective regulation. In addition, treatment measures such as drainage will also cause huge economic losses.

MrNV was only found in the ballast water (AB) of the ship from the port of Toledo (Figure 3). MrNV is one of the family Nodaviridae and mainly infects *Macrobrachium rosenbergii*, whose clinical manifestation is white tail disease [126]. When the shrimp is infected by MrNV, they usually represent lethargy with degeneration of the ends and tail feet. MrNV is extremely harmful to *M. rosenbergii* aquaculture. The larva infected by the pathogen can hardly survive [45]. Besides, MrNV can realize vertical transmission through host egg cells [49]. Although adult shrimp carrying the virus represent low mortality, it is also damaging to the development of the hatchery.

Two researches were showing that IMNV has a high possibility of invading other areas with the discharge of ballast water, since all three ports have detected contigs aligned to the IMNV genome in the ballast water (SGB, SRB, SMB,CCEB, CTUL, and AB) of arrived ship (Figure 3) [14,15]. In the port of Singapore (SCH, SMH, and SRH) and Duluth (BH and MH), IMNV has been found in both ballast water and harbor water while the virus was only identified in ballast water from the ship arriving in the port of LA/LB. IMNV belongs to the Totiviridae and will cause infectious muscle necrosis in shrimp. The main host of it is *P. vannamei* [50]. IMNV can infect shrimp at every stage of its growth cycle, resulting in extensive white necrosis of the host's striated muscles, especially in the abdomen and distal tail. In addition, the virus can also affect the gills and lymph organs of shrimp [45]. IMNV usually breaks out about 9-13 days after infection, killing a great number of sick shrimp [51].

Impacts on Fisheries and fish farming: The fish viruses detected in the ballast water from the early research include red sea break iridovirus (RSIV), infectious spore and kidney necrosis virus (ISKNV), Koi herpesvirus (KHV), viral hemorrhagic septicemia virus (VHSV), striped Jack nervous necrosis virus (SJNNV) and Lymphocystis Disease Virus (LDV). Among these viruses, RSIV, ISKNV, KHV, and VHSV are listed as the research objects by OIE.

Recent research shows that RSIV has existed in the ballast water (CNAD, CSAG, CTUL, and CBAL) among multiple ships arriving at the port of LA/LB. The research also detected RSIV in the harbor water (CILB and COLB) from the port of LA/LB (Figure 3), suggesting that there was a potential threat that RSIV may invade the environment surrounding the port along with the discharge of ballast water. Similar phenomena also occurred in ISKNV that the contigs aligned to the ISKNV genome have identified in the viromes of both ballast water (AB) and harbor water (MH in Figure 3). RSIV and ISKNV belong to Megalocytivirus, which are the main pathogens causing red sea bream iridovirus disease (RSIVD). Both two viruses are highly contagious and can easily spread among fish populations, potentially resulting in large-scale outbreaks and devastating impacts on fish stocks [45]. According to the early investigations, there are more than 30 species affected by RSIVD, including freshwater and marine fish [52,53]. The diseased fish with RSIVD shows an enlargement of some internal organs and cells in the gills. They usually have characterized by lethargy, abnormal behavior, severe anemia, gill ecchymosis, and splenomegaly [52]. The mortality of sick fish with RSIVD is extremely high. In high-temperature water, RSIV-infected hosts have up to 100% mortality [45]. The diseased fish infected by ISKNV also show a similar phenomenon [54].

KHV was found in the ballast water (AB) from Erie and the harbor water (BH, IH and MH) in Duluth (Figure 3), which shows the potential risk for its spread by ship deballasting. KHV is highly contagious and can spread rapidly within aquatic environments, leading to severe disease outbreaks [55]. The virus is now widely distributed in almost all over the world except Australia. KHV primarily affects koi and common carp, causing a condition known as koi herpesvirus disease [56]. Diseased fishes infected with KHV typically exhibit symptoms such as interstitial nephritis, gill necrosis, excessive mucus accumulation leading to impaired respiration, and liver ecchymosis bleeding. Under the condition of acute infection, the inflammation of the gill, intestine, and kidney as well as the serious dysfunction of osmoregulation can lead to the host's death [55]. The presence of KHV can have devastating consequences for the ornamental fish industry, resulting in substantial economic losses due to high fish mortality rates. In affected populations, the mortality rate can range from 80% to 100%, posing a severe threat to the viability of fish farms and businesses involved in the trade of ornamental fish [57].

SJNNV, which belongs to the family Nodaviridae, is one of the main pathogens of fish viral neuro necrosis (VNN) [58]. The fish infected with VNN is usually characterized by explosive acute death. These fish represent cerebral congestion, excessive expansion of the swim bladder, exophthalmos, and eye damage with specific clinical symptoms such as abnormal swimming behavior. The effects of diseases on fish at different growth stages are different. The mortality of diseased fry is as high as 100%, which can cause significant economic losses [59].

Recent research has revealed the transfer of VHSV through ballast water in the Great Lakes [60]. VHSV can infect more than 50 different kinds of marine and freshwater fish [61]. At the early stage of infection, diseased fish usually exhibit non-specific clinical symptoms, including lethargy, skin darkening, exophthalmos, anemia, abdominal edema along with severe behavioral abnormalities. Bleeding can be observed in the fins, gills, mouth, eyes, and bottom of the skin of the fish. Besides, sometimes sick individuals will die quickly after the VHSV infection. Differing from adult fish, the mortality rate of infected fry is as high as 100%, which is unfavorable for economical fish breeding [62].

Early studies suggested that ballast water may have been one of the pathways facilitating the spread of LCDV into Egypt [42]. LCDV belongs to Iridoviridae and will cause fish lymphocystis disease (LCD) in fish [63]. LCD is rarely fatal to the host, but will form a tumor-like mass on skin and fins which can impact the commercialization of the fish and causing important economic

losses [64]. LCDV has a wide range of hosts. According to the exciting report, there are more than 125 freshwater and seawater species affected by LCDV.

Infringe the life safety of livestock: Swinepox virus (SwPV) and suid herpesvirus (SHV) were detected in the ballast water origin from Panama (PAN) and New York (NEW) respectively (Figure 3). The main host of SwPV infection is piglets less than 4 months old. The incidence rate after infection is as high as 100%, accompanied by serious clinical symptoms [65,66]. The natural host of SHV is pigs, which is related to fatal encephalitis of piglets, male infertility, reproductive disorders of sows, and respiratory diseases of pigs in the growing period [67]. In addition, SHV has a strong interspecific transmission capacity. The virus can also infect other vertebrates such as cattle, sheep, cats, and dogs [68].

Damage to human health: Metagenome analysis of ballast water samples from New York (NEW) found that there were some human endogenous retrovirus (HERV) related gene fragments (Figure 3). HERV can encode reverse transcriptase which can integrate the virus genome into its host genome [69]. In addition, HERV may be associated with autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, psoriasis, breast cancer, and even schizophrenia [70].

Technique for virus detection in ballast water

Epifluorescence microscopy (EFM): Epifluorescence microscopy has been a commom technique to enumerate viruses in sediment [126] and aquatic environments [71-73], for a long time. Since Ruiz [19] firstly found that ballast water probably delivered large numbers of viruses to the port in 2000, EFM has become the most commonly method for detecting the virus in it. The following year, Drake [74] used EFM to study the virus in the ballast water of foreign ships arriving at Chesapeake port, and also pointed out that the abundance of virus in the ballast water was at a high level. Besides, EFM was also used to investigate the impact of ballast water exchange on viral communities and the virus content in other environments in the ballast tank(biofilm, residual water after ballast water discharge, and pore water of ballast tank sediment), further deepening the understanding of the virus community in the ballast tank **[75,24,33,20,123,21].**

EFM uses an epifluorescence microscope to observe and count virus particles after fluorescent staining. EFM dyes mainly compress 6-diamino-2-phenylindole (DAPI), Yo-Pro I, SYBR Green I and SYBR Gold [71,78,79,124]. The scene of EFM intuitively reflects the abundance of viruses in the water column. However, several disadvantages may influence the results. To begin with, the fluorescent will affect the phenomenon of EFM due to the existence of background noises. The early dye, DAPI, has insufficient fluorescence intensity after staining, which may bring the error count due to background noise [72]. The Yo-Pro I overcomes the shortcoming of low fluorescence intensity, but has faultiness that the process of pretreatment is very complex. The stain should be diluted and performed additional operations of desalination. Besides, the time required for staining is up to two days, which may influence the activity of viruses [80,81]. SYBR Green I, the commonly used stain at present, which can observe bright fluorescence after staining and need less time for pretreatment, present a fade of fluorescence within a very short time after staining under some conditions [79]. Another widely used dyes, SYBR Gold, is more stability than SYBR Green I, but has darker fluorescence in the research about the usage of EFM in viral detection in ballast water [71,82]. The abundance of viruses detected by EFM is also biased due to the presence of glowing points that are only virus-like particles without viral activity, which may increase the pressure of viral disinfection. In addition, it is time-consuming to observe and count the virus particles, which may affect the permission of ship docking.

Metagenome: Viral metagenomics is a sequencing technology used to capture the complete genomes of all viruses present in a specific environment. It enables the comprehensive analysis of their genetic diversity and provides valuable insights into their molecular ecology. The analysis of viral metagenome mainly uses second-generation sequencing technology (NGS) or high-throughput sequencing technology (HTS) to sequence the viral nucleic acid sequences of the entire community in the environment, avoiding the limitations of the early research methods based on virus culture. At present, viral metagenome has been proven to be an effective method for environmental monitoring, which mainly include the study of virus diversity and the detection of virus pathogens. The application of metagenome in marine surface [83,84], deep-sea sediments [85,86], hydrothermal vents [87], freshwater lakes and rivers [10,88], desert ponds [89], sewage treatment ponds [90], glaciers [85], and ballast water [12,14,15], has found a large number of unknown virus species, which has promoted the research of global virus diversity. Compared with traditional virus detection methods (such as special indicator species identification, cell culture technology, observation of virus-induced cytopathy, and molecular biological methods), the use of metagenome analysis effectively strengthens the detection of viral pathogens. Kim [14,15], and Hwang [12], carried out the metagenome analysis of ballast water samples and found 14 kinds of viral pathogens, highlighting the risk of ballast water as a means of pathogen transport, because ballast water is widely used in ships of international shipping. Early metagenome research of Class B sewage samples indicated that herpesvirus, papillomavirus and picornavirus had a high incidence [91]. In addition, metagenome has also detected a variety of viral pathogens from reclaimed water, rivers and groundwater [92-94].

There are inherent limitations in metagenomic analysis that can impact the results of viral detection. The reference library plays a crucial role in viral metagenomic analysis. Recently, numerous projects of viromes have been conducted worldwide, exploring the knowledge of viruses in natural and artificial environment [95-101,85,86]. The advancement of viral genetic information has greatly enhanced the accuracy of classification. However, it is important to note that a significant portion of viruses in the world remain unidentified, which poses challenges to our comprehension of viral communities in the environment. While viral metagenomics enables the direct analysis of genetic information derived from the environment, the existence of misclassification and unidentified viruses remains prevalent in

current virome research [102,103]. Despite the development of numerous tools designed to identify the correct viral barcodes, challenges persist in achieving complete accuracy and comprehensiveness. Viruses cannot be completely detected even in an era of terabase-scale metagenomics [104]. Furthermore, due to the limitations of viral isolation and purification techniques, it is difficult to accurately identify the virus that have a low concentration [104].

Management of virus in ballast water

Ballast water convention: Since it was demonstrated that ballast water plays an essential role in biological invasion, significant efforts have been made to manage and control the discharge of ballast water [105]. In 2004, IMO adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) [9]. BWM Convention required ships to meet the standard of ballast water performance (Regulation D-2). Ballast Water Convention has implemented in 2017, and played an essential part in protecting the environment surrounding the harbor. However, regulation D-2 main concerns about concentration of zooplankton, phytoplankton, and bacteria in ballast water (Table 1). Virus, the most abundant species in marine, has been ignored when most countries doing port state control to foreign ships. Nowadays, there were few countries and areas that provide their regulation standard that ships shell control viral concentration before the discharge of ballast water [105,106]. California has developed regulation for ballast water discharge standards having the more stringent requirement that the concentration of virus must lower than 10^4 VLPs/100 ml before deballast after January 1, 2020, and there shall be zero viruses when unloading ballast water after January 1, 2030 [106]. Since viruses have the power for changing the ecology of the environment, impacting the economic and human health, it is extremely important to develop more detail regulations of managing and controlling the virus in ballast water.

Ballast water exchange: Before regulation D-2 was implemented, regulation D-1 about ballast water exchange (BWE) was used to control the organism in ballast water [107]. BWE shows certain effects for some organism such as zooplankton and phytoplankton but hardly influence the abundance of bacteria and virus [107]. For viruses in ballast water, conflicting results have emerged in recent studies. During the navigation from Japan to Australia, the viral-like particles (VLPs) decreased from 10^7 VLPs/ml to 10^6 VLPs/ml after the exchange [21]. However, during another trans-Pacific voyage, the concentration of virus has no significance between the exchange and un-exchange tank [123]. The viral abundance also varied little during the voyage from Israel to the USA [20]. Vanessa et al. [107], indicated that viruses in ballast water may not be influenced by ballast water alone. It may be that BWE change the structure of host community in the ballast water, which affected the structure of viral community and changed its concentration. Kim et al. [14], show the contrary idea that the structure of the viral community have not been influenced after BWE since viral richness in ballast water origin from coastal ocean to open ocean has an insignificant change. It should be noticed that viral pathogens were identified in ballast water weather the ship conduct BWE (Figure 3), showing the limited effectiveness of BWE.

Table 1: Regulation of discharged organisms according to Regulation D-2 Ballast Water Performer standard.

Organism	Regulation D-2
zooplankton ≥ 50 µm	<10 cells/m3
Phytoplankton between 10 - 50 µm	<10 cells/mL
Toxicogenic Vibrio cholera (O1 and O139)	<1 cfu /100 mL
Escherichia coli	<250 cfu / 100 mL
Intestinal enterococc	<100 cfu / 100 mL

Ballast water management system: According to the requirement of the Ballast Water Convention, all ships should install the ballast water management system (BWMS) to meet regulation D-2. The treatment strategies are mainly divided into mechanical, physical, and chemical types [108]. Mechanical separation usually uses filters or hydro cyclones to prevent organisms of large sizes come into the ballast tank. Since viruses are very small, mechanical separation may be useless for virus removal except to prevent the virus exist in the large organism from the ballast tank. Physical disinfection and chemical disinfection are widely used in BWMS. Ultraviolet (UV) radiation was commonly used for disinfection of ballast water because it is low-cost and environment-friendly [109-111].

There were some studies that tested the effectiveness of UV in viral disinfection. Kim chose four phage to test if the UV radiation is useful in deceasing the abundance of virus [13]. And the results showed that all the phage were killed by UV radiation at low does, indicating that the UV radiation may have its power to control viruses in ballast water. Each tested the efficacy of ballast water UV-radiation treatment on virus infectivity, indicating that a higher does (400 mJ/cm^{-2}) compared commonly used dose (300 mJ/L) is needed in order to get batter effectiveness of viral disinfection [112].

Discussion

Assessment of the viromes in ballast water

Bacteriophages are the major component of viruses in ballast water, followed by animal and algae viruses. In the water originating from the Great Lake, the virus that infects animals has a higher concentration. This may due to two points. On the one hand, the ships frequently travel between the Great Lake and ports all over the world, making the basin more vulnerable to invasive species [15]. On the other hand, as the shipping nodes and important industrial area, there is a dense crowd living between the Great Lake. Human activities and sewage discharge may induce additional vital pathogens into the basin [113,114].

Researches also showed that the viromes in ballast water are similar but have own characteristic. The dominant species in ballast water is usually Caudovirales (mainly include Myoviridae, Podoviridae, and Siphoviridae). However, the viral pathogens in ballast water are significantly different [12,14,15]. These viral pathogens may origin from the harbor where the water was

ballasted. Besides, viruses can be suspended in the water when the sediment was disturbed [117-125]. The virus in sediment and biofilm which are amassed in the ballast tank for a long time may also be resuspend when it was disturbed during the discharge of ballast water.

Limited by the incomplete library of marine virus and technology of virus concentration, there were still a large number of viruses that cannot be identified, which may impede the analysis of the structure of the viral community in ballast water. The impact that BWE made on the virus in ballast water are still unknown. Researches of viral communities in ballast water and sediment should be studied by using more perfect technologies. The traditional methods such as PCR and viral isolation are also recommended to understand the potential risks of ballast water discharging caused by viral pathogens.

Development of viral management in ballast water

EFM is a main method for viral detection in ballast water and widely used in viral abundance research. However, the deficiencies of EFM, such as the influence of background noise, bias in actively virus detection and high time consumption, may affect the effectiveness of ship management. With the increasing global cooperation among nations, there is a growing need for the development of more precise and efficient methods for the viral investigation in ballast water. The utilization of flow cytometry in viral enumeration has demonstrated distinct advantages, offering valuable insights for the development of innovative approaches [85,119]. Adopting new technologies may improve management efficiency.

With the development of sequencing technology and bioinformatics, metagenomic technology has shown increasingly vigorous vitality in the study of virus communities [101,119,120]. The application of metagenome in the study of viruses in ballast water has revealed a variety of virus communities and provided a new tool for the detection and management of viral pathogens in ballast water. With the development of various new virus classification and identification tools and the further improvement of the reference database, it is gradually becoming possible to accurately identify and monitor threatening viruses from environmental DNA [121,122]. Advances in technology and a better understanding of viruses can also greatly facilitate the control and management of viruses in ballast water.

Ballast water convention requires ships to take necessary measures to avoid or minimize the introduction of organisms when loading and unloading ballast water. Regulation D-2, which is one of the core requirements of the convention, will apply to all the ships in 2024 [9]. The regulation requires ships to control the organic concentration before the water was deballasted. However, the regulation misses the risks that caused by viruses. And there are some suggestions that may help to strengthen the management of ballast water. The standard made by California provide a reference of viral management [13]. And the viral concentration that lower than 10^4 VPLs per 100 mL can be required to reduce the risks of viral invasion.

However, there still are some limitations to using UV for managing the virus in ballast water. For one thing, different viral species show different resistance to UV radiation [112]. For another thing, the effectiveness of UV is significantly influenced by the turbidity of ballast water [13,112-126]. Moreover, recent research only used special viruses for testing the function of BWMS. More real ship inspections are needed to further develop the device of viral disinfection.

Conclusion

Epifluorescence microscopy and viral metagenome are both common tools for viral research. It was found that there were abundant and diverse viruses in ballast water. The viruses were mainly distributed in the water, sediment, and biofilm in the ballast tanks. When ships deballast water or clean up the ballast tank, the virus will come into the new environment unless they receive sufficient attention during the discharge process. The viral species in ballast water mainly include bacteriophage, eukaryotic algal viruses and animal viruses whose hosts are nearly all the species in the world. Following the deballasting process, viruses have the potential to invade the surrounding environment of the port, posing risks to the ecology, economy, and human health. There is still a gap in the knowledge of potential viral pathogen threat. We suggest that viruses, especially viral pathogens, should be further studied by using various ways such as PCR and viral isolation to understanding their risks for the nature and human society. In addition, the regulations of ballast water management are mainly concerned about the zooplankton, phytoplankton, and bacteria nowadays, neglecting the threat of virus in ballast. Ballast water exchange and ballast water management systems are both methods used for management and control of ballast water, but are still imperfect for viral disinfection. And more effectiveness verification such as real-ship experiment of ballast water management systems (BWMS) should be conducted to ensure that the virus in the discharged ballast water is harmless to the environment. In order to avoid the destruction caused by the virus in ballast water, more attention should be paid to the virus in ballast and more efficient measures should be developed.

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