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Review

Biological Risks and Integrated Surveillance of Ships' Ballast Water: Viruses, Bacterial Pathogens, Microalgae, and Ecological Implications

Qihua Wu [1,†], Xingyue Su [2,3,†], Hualiang Zhou [4], Jian'an He [3], Chunlong Jin [3], Lianghui Wei [2,3], Li Zhu [3], Xiaotong Li [3], Ying Ye [3], Chunzhong Zhao [3], Ran Zhang [3,*] and Jie Sun [4,*]

[1] School of Life Sciences and Oceanography, Shenzhen University, Shenzhen 518000, China
[2] College of Animal Science and Technology, Yangtze University, Jingzhou 434025, China;
[3] Shenzhen International Travel Health Care Center (Port Clinic of Shenzhen Customs), Shenzhen 518000, China
[4] Animal and Plant Inspection and Quarantine Technology Centre, Shenzhen Customs, Shenzhen 518000, China
* Correspondence: zhangrank@163.com (R.Z.); sjsophie@sina.com (J.S.)
† These authors contributed equally to this work.

Abstract

This review aims to critically synthesize current evidence on the biological risks associated with ships' ballast water—covering viruses, bacterial pathogens, and microalgae—and to identify the methodological, interpretive, and operational gaps that constrain integrated surveillance from regulatory compliance to risk-informed management. Ships' ballast water is an important vector for transferring microorganisms and aquatic organisms across biogeographic boundaries, creating risks to human and animal health and receiving ecosystems. The International Maritime Organization (IMO) D-2 standard regulates viable organisms and selected microbial indicators, but compliance testing captures only part of the biological hazards associated with ballast-water discharge. This review examines ballast-water surveillance from two complementary perspectives: pathogenic microorganisms of health concern and microalgae, harmful algal taxa, and other non-indigenous organisms of ecological concern. Within the health-related dimension, particular attention is given to viruses because they are not represented by the current D-2 microbial indicators and require distinct approaches for concentration, genome detection, infectivity assessment, and virome characterization. We follow the analytical workflow from sampling and concentration to culture, microscopy, quantitative PCR (qPCR), environmental DNA/RNA (eDNA/eRNA) analysis, viability- and activity-oriented assays, isothermal amplification, biosensors, metabarcoding, and metagenomics. Particular attention is given to the gap between molecular detection and biological viability, because nucleic acids may persist after treatment even when organisms are no longer active or capable of regrowth. For microalgae, ecological relevance also depends on photosynthetic activity, regrowth, and establishment potential. No single method can meet all surveillance needs. Complementary approaches can support screening, biological confirmation, broader hazard discovery, and risk-based ballast-water surveillance while maintaining analytical traceability and regulatory relevance.

Keywords: ballast-water management; environmental DNA/RNA; metagenomics; microalgae and harmful algal species; molecular surveillance; pathogens; shipboard monitoring; viability assessment; viral surveillance; IMO standards

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1. Introduction

Commercial shipping connects aquatic environments that would otherwise remain separated by major geographical and ecological barriers. During ballasting and deballasting, large volumes of water and the organisms they contain may be transferred between ports, creating a recurring pathway for microbial and aquatic-organism dispersal. Studies have shown that ballast tanks contain bacteria, viruses, and other microorganisms, and that these communities can change during transoceanic voyages and ballast-water exchange. Reliable surveillance therefore depends on the entire analytical sequence, from representative sampling and concentration to detection, viability assessment, and interpretation [1–6].

The International Maritime Organization (IMO) Ballast Water Management Convention (adopted in 2004 and entered into force in 2017) provides the main international regulatory framework. Initially adopting a dual-track approach, the Convention used the D-1 ballast water exchange standard as an interim measure before mandating the treatment-based D-2 standard. Regulation D-2 sets discharge limits for viable organisms in defined size classes (≥50 μm and 10–50 μm) and specifies microbiological limits for toxigenic *Vibrio cholerae* O1/O139, *Escherichia coli*, and intestinal enterococci [7]. To support global implementation, the IMO has developed a comprehensive suite of guidelines, including the G2 Guidelines for Ballast Water Sampling, G8 Guidelines for system approval, and G9 Guidelines for system use. These criteria provide clear compliance endpoints for organisms of direct relevance to human health and quantitative performance criteria for broader groups of viable organisms. A critical regulatory milestone was reached in September 2024, when the transition period ended, requiring all ships to fully comply with the D-2 standard. Even so, the regulated indicators represent only a small part of the biological material that ballast water may carry. Molecular studies have detected a much wider range of microorganisms, including potential pathogens, virulence determinants, and antibiotic-resistance genes, revealing a clear gap between compliance testing and broader biological surveillance [8–15].

The biological consequences of ballast-water discharge are not confined to pathogenic microorganisms. Ballast water also transports phytoplankton, microalgae, and other aquatic organisms that may have ecological consequences after discharge. A review of surveys from Chinese ports reported 40 non-indigenous phytoplankton species in ballast water, including red-tide-associated and harmful algal taxa, together with numerous pathogenic bacteria, indicating risks to both ecological systems and human health [16]. Field studies have likewise found substantial viable phytoplankton assemblages in ballast tanks, including potentially harmful taxa, even on vessels that had undergone ocean exchange [17]. Molecular and historical evidence also supports ballast-water transport as a likely introduction pathway for toxic dinoflagellates, linking Australasian populations of *Gymnodinium catenatum* and the *Alexandrium* "*tamarensis complex*" to temperate Asian sources [18]. A recent global review further identified major geographic and taxonomic gaps in ballast-water invasion research, underscoring the need for broader and more balanced biological surveillance [19].

These findings point to two closely related dimensions of ballast-water biological risk. The first concerns human and animal health and includes pathogenic bacteria, viruses, and aquatic-animal pathogens. The second concerns ecological and environmental effects associated with the transport of microalgae, phytoplankton, harmful algal species, and other non-indigenous organisms. The two are not independent. Some microalgae produce toxins that affect humans, aquaculture species, and wildlife, while microbial communities may influence the survival and ecological performance of transported organisms. Research on harmful microalgae has therefore emphasized sensitive detection of toxic or potentially toxic taxa before visible blooms develop [20,21].

Viruses deserve specific attention within this framework because they differ fundamentally from cellular microorganisms in abundance, recoverability, host dependence, and the meaning of biological viability. The D-2 standard does not specify a viral indicator, yet ballast-water viromics has revealed diverse viral assemblages and sequences related to pathogens of fish and shrimp, suggesting that viral transport may have implications for aquatic-animal health as well as ecosystem processes [13,22–25].

This broader view changes what ballast-water surveillance needs to answer. The question is not only whether a regulated pathogen is present, but also which organisms are present, whether they remain viable after treatment, whether they retain relevant physiological or functional activity, and whether the detected signal has biological or ecological significance. This distinction matters because DNA may persist after organisms have lost culturability or infectivity. Ballast-water studies have therefore used propidium monoazide (PMA)-based methods and environmental RNA (eRNA) to improve interpretation of post-treatment molecular signals [26,27]. The same concern applies to microalgae: detecting algal DNA confirms the presence of genetic material, but does not show that cells remain photosynthetically active, can recover and reproduce, or are capable of establishing in a receiving ecosystem.

Against this background, this Review extends pathogen-centered monitoring to a broader assessment of ballast-water biological risk. It considers human/animal health and ecological/environmental consequences as two related components and examines how common sampling, molecular, and sequencing approaches can be used to characterize both. This perspective links conventional regulatory monitoring with wider ecological surveillance while retaining the level of interpretation needed for management decisions.

This review addresses three questions. First, which biological hazards beyond the D-2 indicator organisms are relevant to human/animal health and to ecological risk? Second, how should presence, viability, physiological activity, infectivity, and ecological potential be interpreted across bacteria, viruses, and microalgae? Third, which analytical methods can be integrated into a tiered shipboard and port-based surveillance framework, and what evidence conflicts and operational bottlenecks remain?

The innovation of this review lies in three aspects: (i) it treats human/animal-health risk and ecological/environmental risk as related but non-identical surveillance domains; (ii) it explicitly distinguishes organism-specific biological endpoints rather than applying a single "positive/negative" interpretation to all targets; and (iii) it moves from a method inventory toward a critical assessment of evidence conflicts, shipboard feasibility, and research gaps. Practically, this framework is intended to support ballast-water management system verification, port State control, aquaculture and public-health risk prioritization, and the design of baseline and event-based surveillance.

Materials and Methods. This review used a structured, non-systematic literature search. The databases searched were Web of Science Core Collection, Scopus, PubMed, and ScienceDirect, with the search last updated in [October, 2026]. The core search string was: ("ballast water" OR "ships' ballast water" OR "ballast tank") AND (virus* OR virome OR pathogen* OR bacteria* OR microalgae OR phytoplankton OR "harmful algal" OR "non-indigenous species" OR surveillance OR qPCR OR metabarcoding OR metagenomics OR viability OR PMA OR eRNA OR "shipboard detection"). Additional searches combined "ballast water" with "IMO" and "D-2". Records were included if they were peer-reviewed original research or reviews, written in English, and relevant to biological hazards, detection methods, viability/infectivity interpretation, ecological risk, or management of ships' ballast water. Regulatory documents from IMO were included as primary policy sources. Records were excluded if they focused only on treatment engineering without biological endpoints, were conference abstracts, duplicates, or lacked sufficient methodological detail. Screening

proceeded in three stages: title, abstract, and full-text assessment. Because this is a narrative review, the search was designed to identify methodological contrasts and evidence gaps rather than to estimate pooled effect sizes.

2. Biological Consequences of Ballast-Water Discharge

The biological hazards associated with ballast-water discharge can be considered in two broad categories: risks to human and animal health and risks of ecological or environmental change. Pathogen-related concerns have traditionally received greater attention because several microbial indicators are explicitly included in the IMO D-2 framework. Toxigenic *V. cholerae* O1/O139, *E. coli*, and intestinal enterococci provide measurable compliance endpoints, while other bacterial and viral pathogens are increasingly examined using targeted molecular assays and sequencing [7–10,28–30].

The significance of pathogen surveillance goes beyond identifying a particular taxon. Pathogenicity, virulence determinants, antibiotic resistance, abundance, and viability all affect how a positive result should be interpreted. Shotgun metagenomics can reveal functional genes, whereas quantitative polymerase chain reaction (qPCR) can provide sensitive quantitative information for predefined organisms or genes of concern [14,15,31]. A genetic marker alone, however, does not demonstrate infectivity or establish the concentration of viable organisms, particularly after treatment. This remains a major limitation when molecular results are used to inform health-related risk decisions.

Viruses also warrant separate consideration within the health-related dimension because the current D-2 microbial indicators are bacterial and do not directly represent viral hazards. Viral particles can be transported as free virions, in association with particles, or within infected hosts, and the biological meaning of their detection differs from that of culturable bacteria. Ballast-water viromics has shown that bacteriophages dominate many viral assemblages but has also identified sequences related to viruses of fish and shrimp, reinforcing the need to include viral hazards in broader biological surveillance [13,22–25].

The second major concern is ecological. Phytoplankton and microalgae can cross biogeographical barriers in ballast water and may contribute to the introduction of non-indigenous populations. Surveys of U.S. military and commercial vessels found viable phytoplankton in ballast tanks at widely varying concentrations and identified 100 phytoplankton species, including 23 potentially harmful taxa [17]. These findings show that phytoplankton can remain in ballast tanks despite ballast-water exchange, although the quantity transported and the likelihood of establishment differ among voyages and receiving environments.

The ecological significance of transported microalgae depends on more than their presence alone. Survival during the voyage, persistence as viable cells or resting stages, abundance at discharge, environmental compatibility, and reproductive capacity all influence whether an introduced population can establish. This process is governed by propagule pressure, survival during transit, and the compatibility of the discharged organisms with the receiving environment. Beyond simple species introduction, transported microalgae can alter primary production, food-web structure, and nutrient cycling, while harmful taxa may produce toxins that affect aquaculture, wildlife, and human health. Detecting an exotic or harmful taxon in ballast water is therefore a transport signal, not proof of establishment or ecological impact. Molecular and historical evidence has linked ballast-water transport to the introduction of toxic dinoflagellates in Australasia, including *G. catenatum* and members of the *Alexandrium "tamarensis complex"* [18]. More broadly, bacteria, viruses, protists, and microalgae are all relevant to ship-mediated bioinvasion because they can occur at high abundance, form persistent stages, and in some cases cause toxicity or disease [16].

Harmful algal taxa are a particularly important part of this ecological risk because their effects are not always proportional to cell abundance. Some microalgae produce toxins that affect humans, farmed fish, shellfish, and marine wildlife, whereas other bloom-forming species can alter food-web structure and ecosystem function [21,32,33]. Molecular methods can provide information on species occurrence and, in some cases, on genes associated with toxin biosynthesis. These signals can support early warning and risk prioritization, but detection of a harmful taxon or toxin-associated gene should not be taken as direct evidence of an active harmful algal bloom or ecological damage [21,32].

Ballast-water monitoring should therefore avoid treating all positive results in the same way. Detection of toxigenic *V. cholerae* O1/O139 may relate directly to an established regulatory threshold, whereas detection of a potentially non-indigenous or harmful microalgal taxon is better treated as an ecological surveillance signal that requires further information on abundance, viability, and environmental relevance. The monitoring objective should therefore guide both the analytical method and the way its result is used in decision-making.

The main biological targets considered in ballast-water surveillance can be grouped according to whether their dominant consequences relate to human/animal health or to ecological change (Table 1).

Table 1. Biological risk dimensions and representative surveillance targets in ships' ballast water.

Risk Domain	Primary Biological Targets	Representative Examples	Potential Consequences	Representative Surveillance Approaches
Human/animal health	Regulated indicator microorganisms	*Vibrio cholerae* O1/O139; *Escherichia coli*; intestinal enterococci	Regulatory non-compliance; potential human-health exposure	Culture/MPN; qPCR; validated confirmatory assays
Human/animal health	Other pathogenic microorganisms	Pathogenic *Vibrio* spp.; opportunistic bacteria; viruses; aquatic-animal pathogens	Infectious disease; aquaculture impacts; emerging health hazards	qPCR/RT-qPCR; culture where applicable; metagenomics
Ecological/environmental	Non-indigenous phytoplankton and microalgae	Diatoms; dinoflagellates; other phytoplankton	Species introduction; community alteration; potential establishment	Microscopy; 18S metabarcoding; species-specific qPCR/eDNA
Ecological/environmental	Harmful algal bloom-associated taxa	*Alexandrium* spp.; *Pseudo-nitzschia* spp.; *Karenia* spp.; other toxigenic taxa	Harmful algal blooms; toxin production; food-web and aquaculture effects	Species-specific qPCR; eDNA/18S metabarcoding; physiological or toxin confirmation

Note. MPN, most probable number. The examples are illustrative rather than exhaustive. Regulatory criteria should be interpreted within the applicable ballast-water management framework.

3. Sampling and Concentration as Components of the Analytical Workflow

Sampling and concentration strongly influence both the sensitivity and representativeness of downstream ballast-water analyses. For low-abundance pathogens, the volume of original water represented in the final extract may matter more than the nominal analytical sensitivity of the downstream assay. Processing larger volumes can improve target recovery, but it can also concentrate suspended solids, salts, disinfectant residues, and extracellular

nucleic acids that inhibit amplification or complicate viability assessment [34,35]. Sampling should also follow the International Maritime Organization's Guidelines for Ballast Water Sampling (G2), which mandate representative sampling typically from the discharge line to ensure legal defensibility during Port State Control inspections with clear records of sampling location, processed volume, holding conditions, and treatment status [36].

The challenge is greater when health-related and ecological targets are assessed from the same sample. Bacteria, viruses, phytoplankton, and microalgae differ in size, surface properties, aggregation behavior, and their association with particles or other plankton. The wide variation in phytoplankton abundance reported among ballast tanks highlights the need for representative sampling rather than reliance on small grab samples [17]. When size fractionation is used, the fraction analyzed should be reported because it can substantially affect the apparent community composition.

Membrane filtration remains a practical concentration method for many microorganisms because relatively large water volumes can be reduced to a small filter area that is compatible with culture, microscopy, nucleic-acid amplification, or sequencing. Recovery, however, depends on organism size and particle association, and performance may be affected by membrane clogging, adsorption, aggregation, and co-concentration of inhibitors. Ultrafiltration can process larger volumes but adds tubing and membrane surfaces, dead volume, cleaning requirements, and the need to account for recovery losses [34].

A well-documented sampling strategy should report the original water volume, processed volume, concentration factor, recovery efficiency, holding time, matrix characteristics, and quality-control results. Where sample volume permits, the concentrate can be divided among complementary analytical streams. The same representative sample can then support culture or microscopy, qPCR or isothermal amplification, viability-oriented analysis, and 16S/18S metabarcoding or metagenomics, allowing molecular results to be compared with independent biological observations.

4. Detection Technologies for Health and Ecological Surveillance

Culture remains an important reference method for D-2 bacterial compliance because it can provide quantitative viable counts that are directly comparable with applicable microbiological thresholds. Its main advantage is biological: growth demonstrates reproductive capacity under the conditions of the assay. Its limitations are equally clear, because environmental microorganisms may enter viable-but-nonculturable states and culture requires incubation time and organism-specific expertise. Culture is therefore best retained as a quantitative biological reference, with molecular methods used to add speed and broader coverage [7,37,38].

Microscopy is particularly useful for phytoplankton and microalgal surveillance because it provides direct information on cell morphology and abundance. Species-level identification can nevertheless be difficult for morphologically similar taxa and depends heavily on taxonomic expertise. Reviews of harmful-microalgal detection therefore continue to treat microscopy as an important complementary method alongside biochemical, immunological, and nucleic-acid-based approaches [39,40].

qPCR is useful in both health and ecological surveillance. Pathogen assays can target species-, serogroup-, or virulence-associated genes, while species-specific assays for microalgae can detect and quantify harmful taxa; toxin-biosynthesis genes may provide additional information on toxigenic potential. Pearson et al. reviewed qPCR methods for harmful marine microalgae and highlighted their sensitivity, specificity, and usefulness for detection before bloom formation [20]. The main interpretive limitation remains that qPCR measures target nucleic acids rather than viable organisms, so a positive signal should not automatically be taken as evidence of viable cells or ongoing biological activity.

For viral targets, the amplification workflow also depends on genome type. DNA viruses can be measured directly by qPCR or digital PCR, whereas RNA viruses require reverse transcription before amplification (RT-qPCR or RT-dPCR). These assays can provide sensitive detection of predefined viruses, but viral genome copies should not be interpreted as equivalent to infectious virus [41,42].

Environmental DNA (eDNA) offers broader surveillance when the organisms of interest are not fully defined in advance. eDNA-based approaches can include species-specific qPCR as well as high-throughput sequencing. Feist and Lance reviewed genetic methods for harmful-algal-bloom monitoring and highlighted the value of eDNA for sensitive detection and early warning [21]. Ballast-water metabarcoding studies have similarly shown that DNA sequencing can reveal exotic and potentially harmful algae that may be missed by conventional methods. In ships entering the Bay of Biscay, 72 algal species were detected, of which 22.2% were classified as exotic to the region and 11.1% as harmful, illustrating the value of DNA-based surveillance for broad screening [39].

The main limitation of DNA-based surveillance is interpretation after treatment, because nucleic acids can persist after cells have been inactivated. A 2024 ballast-water study comparing eDNA and eRNA metabarcoding reported that 42% of amplicon sequence variants (ASVs) occurred only in DNA libraries and estimated an approximately 23% false-positive contribution from DNA under the study conditions. eRNA recovered a comparable community profile with fewer apparent false-positive signals, particularly in treated samples containing more dead cells [26]. eRNA is therefore a useful complement to eDNA, but it should not be treated as absolute proof of viability because RNA abundance also depends on transcriptional activity, degradation, extraction efficiency, and reverse-transcription bias.

Rapid nucleic-acid amplification methods, including loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA), offer a route toward field and shipboard testing. Reviews of harmful-algal-bloom diagnostics have considered PCR and isothermal amplification alongside microarrays, biosensors, and lateral-flow formats, while consistently noting that sample concentration, extraction, and portability are critical parts of the overall workflow [32,33]. The same applies to ballast-water pathogen monitoring, where RPA-lateral flow assays, LAMP, and portable biosensors have been evaluated for rapid screening [43–45]. Immunoassays have also been explored; for example, the SMART II assay has been assessed for toxigenic *V. cholerae* O1/O139 in ship ballast water [46]. In practice, the important endpoint is a reliable sample-to-answer workflow rather than amplification time alone.

5. From Molecular Detection to Viability and Physiological Status

Distinguishing molecular presence from biological viability remains a persistent problem in ballast-water monitoring. Ultraviolet (UV), electrochlorination, ozonation, and other treatments may damage cells or prevent reproduction while leaving DNA that can still be amplified. A positive post-treatment qPCR result may therefore originate from an intact viable cell, a damaged but nonculturable cell, a dead cell with preserved DNA, or extracellular DNA. These states have very different implications for health and ecological risk [26,27,35].

PMA- or ethidium monoazide (EMA)-assisted qPCR can reduce amplification from DNA associated with cells that have lost membrane integrity. Membrane integrity, however, is not equivalent to reproductive capacity or infectivity, and PMA performance can vary with turbidity, cell aggregation, light exposure, salinity, and inhibitors. Recent reviews of PMA-qPCR therefore recommend treating it as a viability-enrichment method that requires matrix- and organism-specific validation rather than as a universal measure of viability [27,47].

For viruses, the corresponding biological endpoint is infectivity rather than cellular viability. A viral genome may remain detectable after treatment even when the particle can no longer initiate infection. Virus-specific interpretation should therefore distinguish genome detection from evidence of particle integrity or infectivity, rather than applying cellular viability criteria directly to viral targets [41,42].

For microalgae, biological status can also be assessed through physiological measurements such as chlorophyll fluorescence, photosynthetic efficiency, pigment composition, metabolic activity, and recovery growth. These measurements help distinguish genetically detectable cells from populations that retain physiological competence. This distinction is directly relevant to ecological risk, because detection of a microalgal species becomes more meaningful when cells can survive, recover, reproduce, and respond to conditions in the receiving environment.

These considerations suggest a practical sequence of interpretation: presence, viability, physiological activity, and functional or ecological potential. For pathogens, this means moving from DNA positivity toward evidence of viable and potentially infectious organisms. For microalgae, it means moving from species detection toward evidence that populations remain physiologically competent and capable of establishment or harmful activity. Combining molecular measurements with independent biological or physiological endpoints can therefore provide a more informative assessment of treatment performance than any single assay. These organism-specific differences in biological endpoints are summarized in Figure 1.

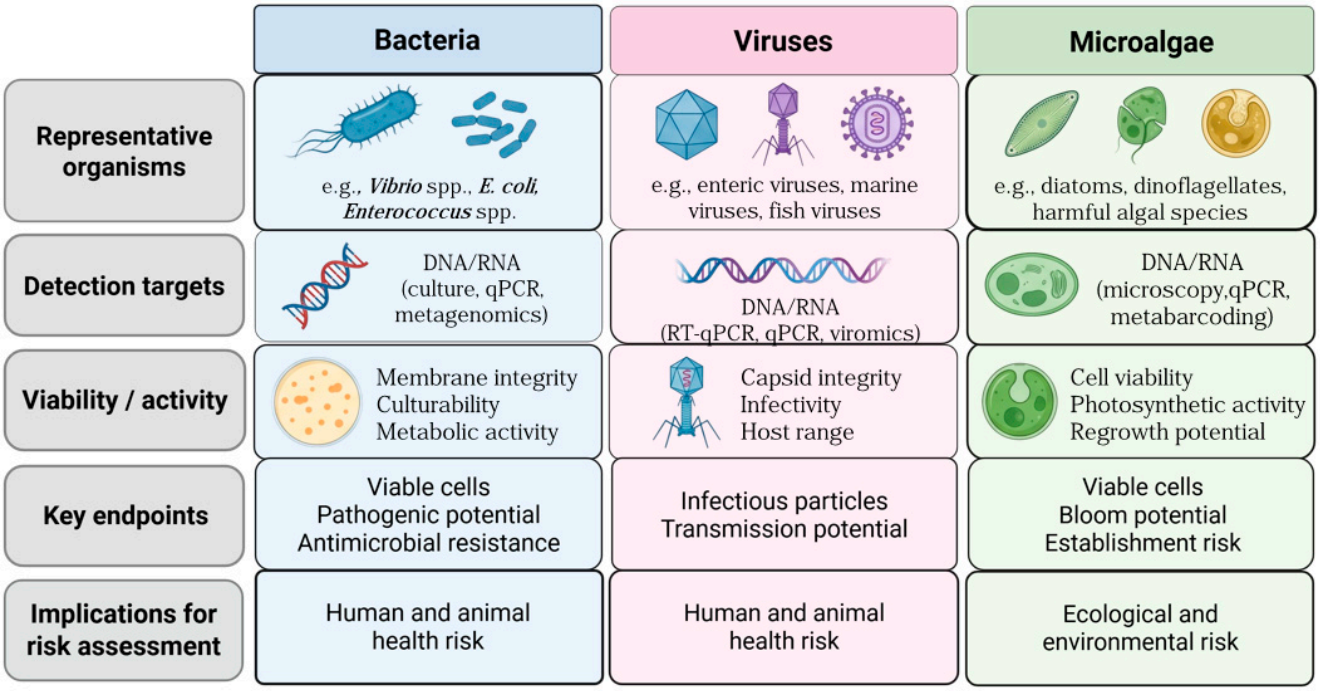


Figure 1. Different biological endpoints for bacteria, viruses, and microalgae in ballast-water surveillance. Molecular detection has different biological meanings across target groups: interpretation progresses from cellular presence to membrane integrity and culturability for bacteria, from genome detection to capsid integrity and infectivity for viruses, and from molecular detection to viability, physiological activity, and establishment or bloom potential for microalgae.

6. Metabarcoding, Metagenomics, and Community-Level Surveillance

High-throughput sequencing has broadened ballast-water surveillance from predefined targets to community-level characterization. Studies using 16S amplicon sequencing, full-length 16S rRNA gene sequencing, shotgun metagenomics, and viromics have consis-

tently shown that ballast water contains diverse microbial communities and that sequencing can identify potential pathogens and functional genes that are poorly represented by culture-based surveys [11–15,31]. A recent study at Qingdao Port that combined metagenomic sequencing with cultivation likewise detected a wider range of potential pathogens and resistance- and virulence-associated genes than culture alone, showing the value of pairing discovery-oriented sequencing with independent confirmation [48].

Virus-focused sequencing requires additional methodological consideration because viruses lack a universal phylogenetic marker equivalent to the 16S or 18S rRNA genes. Viral metagenomics therefore depends more strongly on particle enrichment, nuclease treatment, amplification strategy, sequencing depth, and reference-database coverage. A substantial fraction of ballast-water virome sequences may remain taxonomically unassigned, which should be considered when interpreting apparent viral diversity and pathogen-related signals [13,22,23].

The ecological component of surveillance requires a complementary marker system. Whereas 16S rRNA sequencing is mainly used for bacterial community profiling, 18S rRNA metabarcoding can characterize eukaryotic microorganisms, including phytoplankton. A large-scale eDNA survey in the southwestern South China Sea used the 18S V4 and V9 regions to characterize micro-eukaryotic communities and detected 29 molecular harmful-algal taxa, including toxigenic *Alexandrium* and *Pseudo-nitzschia* as well as the fish-killing species *Margalefidinium polykrikoides* and *Karlodinium veneficum* [49]. This illustrates how 18S metabarcoding can extend molecular surveillance beyond bacterial pathogens to ecologically relevant phytoplankton.

Metabarcoding is well suited to broad taxonomic screening, whereas shotgun metagenomics adds information on functional genes and genomic context. In ballast-water studies, metagenomic binning has been used to link antibiotic-resistance genes to potential bacterial hosts, showing the value of connecting taxonomic and functional information [31]. A similar principle applies to ecological hazards: detection of an algal taxon can be supplemented with information on toxin-associated genes or other functional features, while qPCR or digital PCR can provide quantitative confirmation for priority targets.

Sequencing is nevertheless primarily a discovery tool rather than a direct measure of viability. DNA from inactivated cells may remain detectable after treatment, and taxonomic assignments depend on database completeness and marker resolution. For this reason, discovery and confirmation should be treated as separate steps: sequencing can identify candidate biological hazards, while quantitative and viability-oriented assays can determine whether those signals are sufficiently robust to justify operational follow-up [26,27].

Table 2 summarizes the main analytical approaches and the roles they can play in integrated ballast-water surveillance.

Table 2. Comparative roles of major analytical approaches for integrated ballast-water biological surveillance.

Method Category	Representative Approaches	Primary Targets	Information Obtained	Key Strengths	Major Limitations
Culture/microscopy	Culture; MPN; light microscopy	Culturable bacteria; phytoplankton and microalgae	Culturability; morphology; abundance	Direct biological interpretation; taxonomic observation	Slow; expertise-dependent; incomplete coverage
Targeted molecular detection	qPCR; dPCR; LAMP; RPA	Known pathogens or priority algal taxa	Target-specific presence and/or abundance	High sensitivity and specificity; potentially rapid; quantitative	Requires prior target selection; nucleic-acid copies do not establish viability

Table 2. *Cont.*

Method Category	Representative Approaches	Primary Targets	Information Obtained	Key Strengths	Major Limitations
Viability- or activity-oriented molecular methods	PMA/EMA-qPCR; RT-qPCR; eRNA	Membrane-intact cells; RNA-associated biological activity	Viability-enriched or activity-associated molecular signal	Improved interpretation of residual nucleic acids	Matrix- and assay-dependent; not an absolute viability endpoint
Community profiling	16S/18S metabarcoding	Bacterial communities; microalgae and other eukaryotic microorganisms	Community composition and diversity	Broad taxonomic coverage; discovery-oriented; scalable	Marker and database bias; limited direct viability inference
Shotgun metagenomics	Shotgun DNA sequencing; metagenomic binning	Microbial communities	Taxonomic composition; functional genes; genome context	Untargeted hazard and functional discovery	Cost; bioinformatic burden; reference gaps; limited viability inference
Environmental nucleic-acid surveillance	eDNA/eRNA sampling and sequencing	Aquatic organisms and microbial communities	Environmental DNA/RNA signatures; activity-associated RNA signal	Broad detection; non-invasive sampling; early-warning potential	Nucleic-acid persistence/degradation; extraction and matrix biases

Note. Method performance and interpretation depend on the matrix, target, and assay. qPCR, quantitative polymerase chain reaction; dPCR, digital polymerase chain reaction; PMA/EMA, propidium monoazide/ethidium monoazide; RT-qPCR, reverse-transcription quantitative polymerase chain reaction; eRNA, environmental RNA. These measurements should be treated as viability- or activity-associated evidence rather than universal proof of viability.

7. Interpreting Human-Health and Ecological Risks

In our view, three levels of evidence should be distinguished before a viral detection is translated into risk: (i) detection of viral nucleic acid in ballast water; (ii) evidence of intact, infectious particles after treatment; and (iii) epidemiological or quantitative evidence linking discharge to infection in exposed hosts. Most published ballast-water virome studies provide only level (i), and quantitative microbial risk assessment (QMRA) is currently the only route by which such detections can be converted into a dose–response-based risk estimate.

Separating human-health and ecological risk is useful when analytical results are translated into management decisions. Pathogen surveillance is mainly concerned with infection potential, virulence, viable concentration, and regulatory thresholds. Microalgal surveillance is more often concerned with transport of non-indigenous species, ecological establishment, harmful algal blooms, and toxin production. The two areas share several analytical tools, but they do not require identical biological endpoints and should not be reduced to the same positive/negative interpretation.

For health-related hazards, interpretation may depend on organism identity, virulence determinants, viable concentration, and infectivity. For ecological hazards, additional considerations include species origin, environmental compatibility, viability, reproductive capacity, persistence, and bloom-forming potential. The Bay of Biscay DNA-metabarcoding study illustrates this difference: detection of exotic or potentially harmful algae demonstrates a transport signal, but it does not by itself show that those organisms will establish successfully after discharge [39].

A practical way to interpret these results is to move from presence to abundance, viability, physiological activity, and finally functional or ecological potential. For pathogens, the final question may be whether a detection has infectious or public-health relevance; for microalgae, it may be whether cells can establish, form blooms, or produce toxin-

related ecological or health effects. This approach allows molecular and sequencing data to complement, rather than replace, the regulatory information provided by D-2.

The same caution applies to functional genes. Detection of an antibiotic-resistance gene, toxin-biosynthesis gene, or virulence determinant indicates biological potential, but the signal is more informative when it can be linked to a viable host, a mobile genetic element, or a confirmed high-priority organism. Ballast-water metagenomic studies of antibiotic-resistance and virulence genes have already demonstrated the value of making this connection [31]. The relationship between biological consequences, analytical surveillance, risk interpretation, and management responses is summarized in Figure 2.

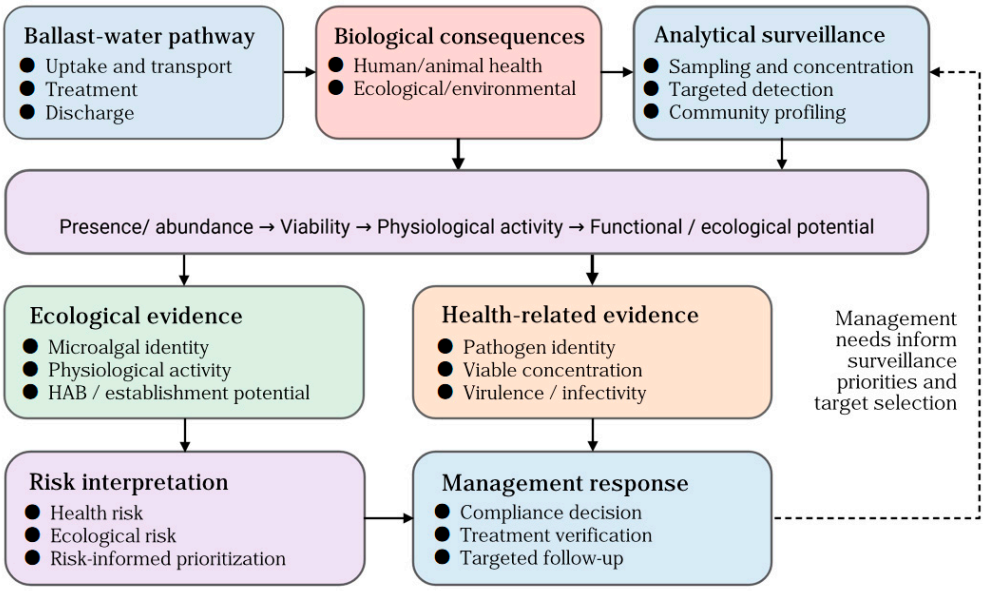


Figure 2. Integrated framework for ballast-water biological surveillance and risk interpretation. Ballast-water uptake, transport, treatment, and discharge are linked to human/animal-health and ecological/environmental consequences, while analytical surveillance and biological-status interpretation provide evidence for risk-informed management and targeted follow-up.

8. Toward Integrated Shipboard Biosurveillance

No single analytical method is likely to meet all ballast-water monitoring needs. Rapid qPCR, RPA, LAMP, and biosensors can support pre-discharge screening, while culture, targeted molecular confirmation, and viability-oriented assays can provide stronger evidence on biological status. Metabarcoding and metagenomics are better suited to periodic baseline surveys, unexplained events, emerging hazards, and source attribution. Used in this way, the methods form a tiered monitoring strategy that extends pathogen-focused surveillance to include ecological hazards [43–45].

A representative concentrate can be divided among several analytical streams: microscopy or culture for direct biological observation, qPCR or isothermal amplification for targeted screening, PMA/eRNA or physiological assays for biological-status assessment, and 16S/18S sequencing or metagenomics for broader discovery. Using the same starting sample improves comparability among methods and allows signals of concern to be checked using independent analytical principles.

For shipboard use, however, amplification chemistry is only one part of the problem. Filtration, extraction, inhibitor removal, closed-cartridge operation, contamination control, reagent stability, and tolerance to changes in salinity and suspended solids may determine whether a rapid assay performs reliably outside the laboratory. The full sample-to-answer workflow should therefore be validated under realistic vessel, route, seasonal, and treat-

ment conditions, with recovery efficiency, invalid-test rates, operator requirements, and total turnaround time reported alongside analytical sensitivity and specificity [37,38,43–45]. The transition to the D-2 performance standard entered a key implementation phase in September 2024, and global enforcement by Port State Control is now actively scrutinizing treatment efficacy, further increasing the need for standardized and operationally interpretable monitoring procedures [50].

Viral Surveillance: Specific Targets, Methods, and Interpretation

Viruses represent a distinct surveillance problem because the viral fraction of ballast water includes bacteriophages, viruses of algae and invertebrates, and viruses associated with vertebrate hosts. Metagenomic studies have shown that viromes can differ among source waters and geographic regions, and that ballast-water exchange and voyage duration may influence viral richness and composition [13,22]. A separate survey of ships arriving in Busan documented high DNA-virus diversity and identified viruses associated with invertebrates, algae, and animals, supporting the potential translocation of geographically structured viral communities through ballast water [23].

Recent studies have extended this evidence to ballast-tank residues and route-specific comparisons. At Shanghai Port, virome profiles differed between ballast water, residual water, and sediment, and shrimp hemocyte iridescent virus, a strain of decapod iridescent virus 1, was detected across these compartments [24]. A subsequent analysis of international shipping routes found significant differences in DNA-virus diversity and community composition among routes and reported route-specific distributions of virus-associated antibiotic-resistance genes and virulence factors [25]. These observations suggest that residual water and sediment should not be ignored as potential viral reservoirs and that voyage history may help prioritize viral surveillance. The major pathways of viral transport and the potential reservoirs encountered during ballast-water uptake, voyage, treatment, and discharge are illustrated in Figure 3.

Virus monitoring also requires sample-processing strategies that differ from those used for bacteria or microalgae. Free virions are generally smaller than cellular targets and may occur at low concentrations, whereas a proportion can adsorb to suspended particles or accumulate in tank residues. Large-volume concentration, including ultrafiltration or adsorption–elution approaches, may therefore be required before nucleic-acid extraction. Process controls are particularly important because losses can occur during concentration, extraction, and reverse transcription. Experience from environmental-water virology shows that large-volume ultrafiltration can improve viral recovery and detection and that recovery efficiency should be reported alongside analytical sensitivity [41,42].

For targeted surveillance, DNA viruses can be quantified by qPCR or dPCR, whereas RNA viruses require reverse transcription before amplification. Target selection should follow the surveillance objective. Human-health surveillance may prioritize enteric or fecal-associated viruses when fecal contamination is suspected, whereas aquaculture and ecological surveillance may focus on viruses of fish, crustaceans, mollusks, or algae that are relevant to the receiving environment. By contrast, viromics is useful when the viral community is not known in advance or when emerging viruses are of concern. Because viruses lack a universal marker gene, virome results are more sensitive than 16S/18S surveys to sample preparation, sequencing depth, database completeness, and bioinformatic classification [13,22–25,41,42].

The interpretation of a positive viral molecular signal also differs from cellular viability assessment. For viruses, the biologically relevant endpoint is infectivity rather than membrane integrity or reproductive growth. Viral genomes can remain detectable after ultraviolet or oxidative treatment even when infectivity has been reduced, and different

virus groups can vary substantially in treatment susceptibility. Molecular measurements should therefore distinguish genome detection from evidence of intact particles or infectivity. Depending on the target, this may involve cell-culture or host-based infectivity assays, bacteriophage surrogates, or capsid-integrity and enzymatic pretreatment approaches coupled with qPCR or RT-qPCR [41,42]. A virus-specific analytical workflow linking genome detection with structural integrity, infectivity assessment, and risk interpretation is presented in Figure 4.

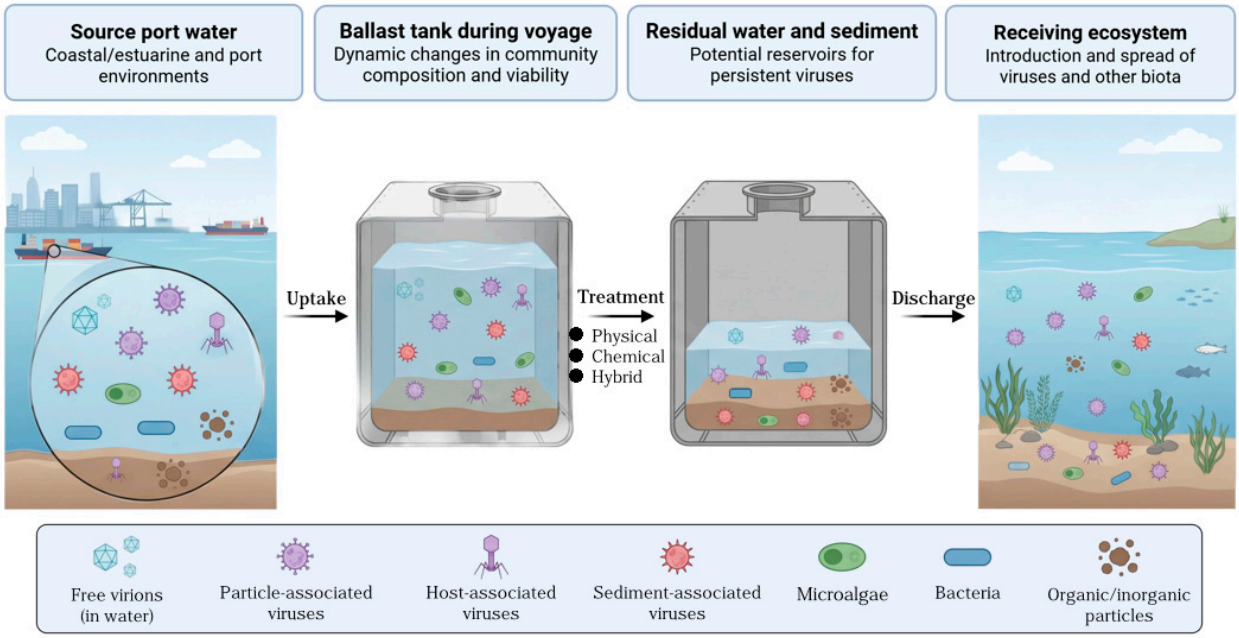


Figure 3. Viral transport and reservoirs in ships' ballast water. Viruses may enter with source-port water, persist during voyages as free virions or in association with hosts, particles, residual water, and sediments, and subsequently be discharged into receiving ecosystems.

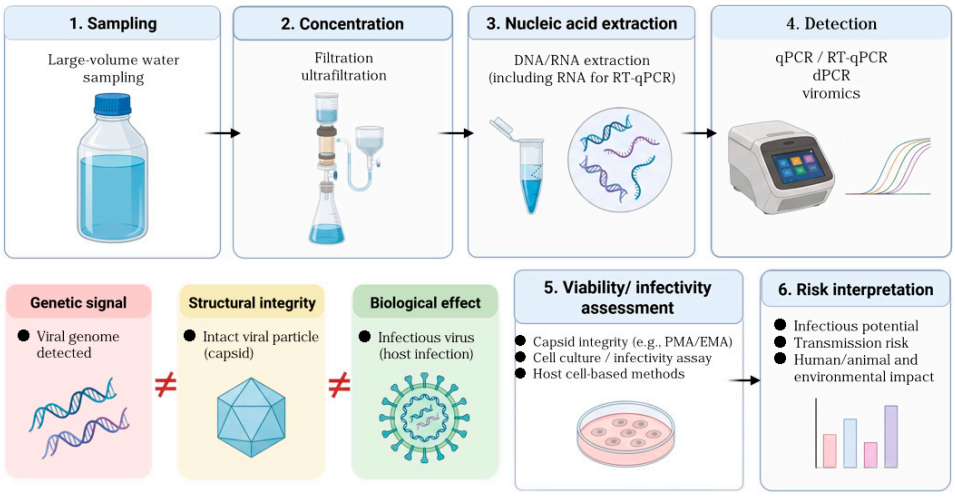


Figure 4. From viral genome detection to infectivity assessment in ballast-water surveillance. A positive viral genome signal does not necessarily indicate an intact viral particle or an infectious virus; concentration, nucleic-acid detection, structural-integrity or infectivity assessment, and contextual risk interpretation should therefore be considered as complementary steps.

For shipboard use, a practical viral surveillance model is likely to be tiered. Rapid qPCR or RT-qPCR could be applied to predefined high-priority viruses after an appropriate concentration step, while shore-based viromics could provide periodic broad surveillance,

route-level baselines, and investigation of unexpected events. A key research need is to establish which viral targets and infectivity-related endpoints provide information that is sufficiently reproducible and biologically meaningful for ballast-water management, particularly because no viral indicator is currently included in the D-2 microbiological criteria [22–25,41,42].

9. Research Priorities and Conclusions

Several practical priorities follow from this review. First, sampling and concentration should be standardized in terms of effective analyzed volume and organism-specific recovery. Second, viability should be treated as an inference that depends on the assay and target rather than as a universal binary property. Third, molecular surveillance should extend beyond regulated bacterial indicators to include both health-relevant pathogens and ecologically relevant organisms such as microalgae and harmful phytoplankton. Fourth, broad sequencing results should be linked to quantitative and viability-oriented measurements. Finally, field validation should assess the complete sample-to-decision pathway under realistic shipboard conditions [37,38].

Virus-specific priorities include defining surveillance panels for human and aquatic-animal health, validating concentration and process-control procedures in saline and particle-rich ballast-water matrices, and determining which molecular or structural markers best predict infectivity after treatment. Route-specific virome baselines and systematic evaluation of residual water and sediments also deserve attention because recent evidence indicates that viral assemblages vary among shipping routes and ballast-tank compartments [22–25,41,42].

Including microalgae in ballast-water surveillance does not mean that all transported phytoplankton should be regarded as an environmental threat, nor does it imply that molecular methods should replace current regulatory testing. Rather, microalgae represent an important ecological component of ballast-water biological risk that is not fully captured by pathogen-centered compliance monitoring. Ballast-water surveys, DNA metabarcoding studies, and harmful-algal research show that phytoplankton and microalgae can be transported by ships and that potentially harmful taxa can be detected molecularly, while their actual ecological significance depends on viability and conditions in the receiving environment [16–18,20,21,32,33,39,40].

Future ballast-water surveillance would benefit from linking these two major dimensions of biological risk. Human and animal health can be addressed through surveillance of pathogenic bacteria, viruses, and relevant functional determinants, while ecological and environmental risk requires attention to microalgae, phytoplankton, harmful algal taxa, and other non-indigenous organisms. Both can be examined through a common analytical sequence that includes standardized sampling, rapid targeted detection, viability- or activity-oriented confirmation, sequencing-based discovery, and risk interpretation. This combined approach can broaden ballast-water management beyond endpoint compliance while retaining the analytical traceability and regulatory clarity needed for practical decision-making.

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