United States Environmental Protection Agency
Office of Wastewater Management
Washington, DC 20460

Options to Curb the Transport of Viral Hemorrhagic Septicemia Virus in Inter-lake Vessel Ballast Water

EPA 841-R-18-001
March 2019

ACKNOWLEDGEMENTS

This report was prepared by the U.S. EPA Office of Water, Water Permits Division with contractor support provided by individuals from Eastern Research Group, Inc. under contract number EP-C-16-003. Special thanks is given to Elizabeth Eddy, Oak Ridge Institute of Science and Education (ORISE) Research Participant, for her contributions supporting this report. This work was funded under the Great Lakes Restoration Initiative (GLRI) in the invasive species focus area as overseen by James (Jamie) Schardt, Jackie Adams, and T. Kevin O'Donnell from the U.S. EPA Great Lakes National Program Office.

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SECTION 1 INTRODUCTION

The Laurentian Great Lakes (Great Lakes) are the largest group of freshwater lakes on the planet. Located on the border of the United States (U.S.) and Canada, the Lakes create an interconnected waterway from the Atlantic Ocean to the Mississippi River. The Saint Lawrence Seaway connects the Atlantic Ocean from the Gulf of St. Lawrence to Lake Ontario. Lakes Ontario, Erie, Huron, Michigan, and Superior are hydraulically connected through a series of rivers and canals, and Lake Michigan is connected to the Mississippi River by the Illinois Waterway. This interconnected waterway presents a major shipping corridor for overseas and coastal commercial vessels to transport goods from global ports to both the U. S. and Canada, as well as a within the Great Lakes using inter-lake vessels.

Commercial vessels require the intake, use, and discharge of large volumes of ballast water to control or maintain vessel draft, buoyancy, and stability. The transport of ballast water and associated sediments from one port to another creates a mechanism of transferring aquatic nuisance species (ANS). These species are introduced to ballast tanks when vessels take on ballast water and are distributed when ballast water is discharged. The transportation of ballast water by commercial vessels has been implicated in the spread of a variety of ANS throughout the Great Lakes (Bain et al., 2010). The spread of ANS by ballast water is of ecological and economic importance in the Great Lakes, and informs regulatory decision making. As of 2016, over 180 ANS have been documented in the Great Lakes (NOAA, 2016). Ricciardi (2006) found that 65 percent of ANS present in the Lakes in 2006 were likely to have been introduced to the Lakes by ballast water.

One particular ANS of interest, Viral Hemorrhagic Septicemia Virus (VHSV; synonym: Egtved virus), caused significant environmental damage to the Great Lakes beginning in 2003 and remains a concern today. VHSV is a deadly infectious fish virus that has affected over 50 species of freshwater and marine fish in various parts of the northern hemisphere (Iowa State University, 2007). In 2005 and 2006, it caused at least seven mortality events (i.e., fish kills) in the Great Lakes (USDA, 2006). Between 2006 and 2009, mortality events continued as the virus spread throughout the Great Lake system. It appears that the quantity of fish kills has declined since the viruses' initial invasion, though concern about the transfer of this virus is warranted, as it has spread to all five Great Lakes and some inland lakes in the states and provinces surrounding the Lakes. While the virus has multiple transport pathways, evidence suggests that ballast water may be a key vector of its range expansion. Therefore, this report examines possible detection techniques and treatment options to curb the spread of VHSV by vessels that operate in the Great Lakes.

1.1 VHSV OVERVIEW

VHSV is a rhabdovirus found in both freshwater and saltwater environments. Rhabdoviruses are bullet-shaped viruses that contain a single-stranded ribonucleic acid (RNA) genome (Bowser, 2009). There are several genetic variations of the virus that exist in global waters. Four genotypes of the virus have been identified in the Northern Hemisphere. Genotypes I, II, and III are most commonly identified in Europe and Japan, while genotype IV has only

been identified in North America, Japan, and Korea (USDA, 2006). The strain endemic to the Great Lakes is genotype IVb, which is the only strain known to infect warm water fish species (e.g., largemouth bass, bluegill) in addition to cold water species (e.g., muskellunge, walleye) (USDA, 2006). Because genotype IVb is a substrain of the genotype found on the Atlantic Coast, it is hypothesized that IVb mutated from VHSV-IV (Pierce et. al, 2013). Research suggests that strain IVb is highly adaptable because it has at least 16 identified variants[1] (Pierce et. al, 2013). Additionally, recent evidence indicates that VHSV has continued to evolve since its introduction to the Great Lakes (Gorgoglione et. al, 2017).

VHSV is "transmitted" when the virus infects a host, and is "spread" when it is relocated, such as by ballast water. The virus is introduced to water when hosts excrete the virus in urine, feces, and reproductive fluids (Sea Grant Michigan Fact Sheet, n.d.). The virus is commonly absorbed by hosts through the gills (USDA, 2006). The virus may also be spread via contact with animate or inanimate objects where the virus is present (USDA, 2006) or by consumption of infected individuals (Iowa State University, 2007). Another vector known to spread the virus is piscivorous birds, as they may ingest infected fish, which may then be relocated through excretion or by dropping the fish prior to consumption (Iowa State University, 2007). While a species of leech (*Myzobdella lugubris*) and the shrimp-like *Diporeia* spp. are known carriers of VHSV, it is unknown if they can transmit the virus between fish (Faisal and Winters, 2011). The transfer of live baits between bodies of water is another potential vector of spread for the virus, as hosts for the virus can be introduced to new bodies of water (USDA, 2006).

Disease induced by VHSV is thought to typically occur at temperatures between 4°C and 14°C (OIE, 2017). Generally considered a cool or cold-water disease, VHSV causes the highest mortality at 9°C to 12°C. Most outbreaks are observed during periods of fluctuating temperature, when stress levels are increased for fish. Outbreaks of the disease have been documented from 2°C to 20°C (OIE, 2017). Once the virus has invaded a host fish, the fish can become a lifelong carrier and shedder of the virus if it survives the disease (USDA, 2006). The percentage of fish that survive infection varies widely based on species and environmental conditions, such as stress. In water, the virus can typically survive for 28-35 days in favorable conditions (Parry and Dixon, 1997).

The impacts to infected fish can be swift and severe. VHSV can cause hemorrhaging of fish tissue, including internal organs. Hemorrhaging of the skin can also occur, ultimately resulting in bulging red eyes and red patches on the body, particularly on the sides and anterior of the head (GLC, 2011). Internally, the virus causes exophthalmia,[2] anemia and vesicles on internal organs (OIE, 2017). VHSV can cause blood vessels to weaken, allowing blood to leak into the surrounding tissue (GLC, 2011). The ultimate cause of death is typically organ failure. Figure 1-1 shows a Yellow Perch and Freshwater Drum infected with VHSV.

[1] Variants are viruses based on an earlier version of the virus with one or more minor changes.
[2] Exophthalmos is the abnormal protrusion of the eyeball or eyeballs.

Figure 1. Yellow perch exhibiting signs of viral hemorrhagic septicemia, including hemorrhages, "pop-eye," and distended abdomen.

Figure 2. Hemorrhages in the liver of a viral hemorrhagic septicemia virus-infected freshwater drum.

Source: USGS, 2010

Figure 1-1. Fish Showing Visible Signs of VHSV Infection

Unfortunately, there is no clear visual diagnostic for fish infected with VHSV, as fish in the chronic state of infection generally do not exhibit any symptoms of the disease (OIE, 2017). Behavioral symptoms may include flashing, lethargy, and nervous behavior (OIE, 2017). Although it may be possible to visually identify infected individuals, identification of VHSV outbreaks in the Great Lakes are typically associated with large scale, observable fish mortality events. Outbreaks can range from a few individuals to several hundred tons of fish (USDA, 2006). For this reason, many smaller outbreaks may occur and go undocumented. In addition, the Great Lakes are large waterbodies with extensive remote areas, so fish kill events may occur and go unobserved. Despite these challenges, the observation of large-scale fish kills is still the de-facto method of identifying VHSV outbreaks in the Great Lakes (Bain et al., 2010).

1.2 Introduction to and Spread of VHSV Throughout the Great Lakes

Until the 1980's, VHSV was considered a pathogen limited to freshwater fish in Western Europe (Bain et al., 2010). Although disease events of rainbow trout in farmed aquacultures in Europe were suspected to have a viral cause, it was not until the 1960's that VHSV was linked to these epizootic events (Bowser, 2009). VHSV was first reported in the U.S. in 1988 in the Pacific Northwest (USDA, 2006). In 2005, the North American genotype IVb was isolated in Lake Ontario following a large fish kill of freshwater drum (*Aplodinotus grunniens*) and round goby (*Neogobius melanostomus*) in Lake Ontario's Bay of Quinte (Bain et al., 2010). Following this event, an archived muskellunge sample from Lake St. Claire (a waterbody that connects Lake Huron to Lake Erie) collected in 2003 tested positive for VHSV. The archived 2003 sample was the earliest identification of the virus in the Great Lakes region. There remains to be a

consensus on the number of species affected by the virus, as new species continue to be discovered, making previous species counts obsolete. As of 2006, the North American VHSV genotype had been documented as potentially infecting over 40 species of fish, including ecologically and recreationally important fish, such as muskellunge (USDA, 2006). At least 18 species of fish in the Great Lakes region have been documented to harbor the virus (Sea Grant Michigan Fact Sheet, n.d).

Source: Faisal et al., 2012

Figure 1-2. Mortality Event in Lake St. Claire in 2006, From Which VHSV was Identified in Infected Fish

It is unclear how long the virus has been present in the Great Lakes, as it may have avoided detection prior to the fish kills that alerted local communities. Estimations indicate that VHSV arrived in the Great Lakes around 2002 (USNPS, 2008). Since that time, the virus has spread to all five Great Lakes. Following the 2005 Bay of Quinte fish kill, several fish mortality events occurred in Lakes Michigan, Erie, and St. Claire, as well as other interconnected waterways. Between 2006 and 2007, VHSV was detected in Lakes Ontario, Erie, Huron, and Michigan (Bain et al., 2010). A 2009 study detected the virus in Lake Superior, marking the virus' identification in all of the Great Lakes (GLC, 2011).

The distribution of the virus suggests a correlation between major shipping ports and other ANS "hotspots," causing researchers to speculate that the virus was introduced by ballast

water transported from the Atlantic Seaboard (GLC, 2011). This theory has been substantiated by the genetic evaluation of the VHSV-IVb, which indicates that this genotype likely originated from the Atlantic coast of North America, specifically the Maritime Provinces of Canada at the entrance to the Saint Lawrence Seaway (GLC, 2011). While many researchers suggest that VHSV was introduced to the Great Lakes via ballast water, consensus has not been reached in the research community. Notably, some researchers cite that there is no clear genetic knowledge of the origin of VHSV-IVb, meaning a clear determination of its source location cannot be considered definitive (Bain et al., 2010). The introduction by other vectors is also a possibility, such as the migration of anadromous or catadromous species from the St. Lawrence River into the Great Lakes (Bain et al., 2010).

1.3 EFFORTS TO CONTROL THE SPREAD OF VHSV

Despite the impacts to the Great Lakes in the early 2000's, federal, state, and local agencies have yet to develop a collaborative management plan to control the spread of VHSV. However, federal and state regulations and best management practices (BMPs) enacted to reduce the transfer of ANS may aid in mitigating the spread of VHSV. Additionally, specific protected areas have implemented their own programs to control the spread of VHSV, as discussed below.

Currently, regulations do not exist that are specific to inactivating VHSV in ballast water for vessels confined to the Great Lakes. While ballast water in the U.S. is typically regulated by the U.S. Coast Guard (USCG), the U.S. Environmental Protection Agency (EPA), and states, these requirements apply generally to all ANS. Furthermore, not all vessels operating in the Great Lakes are required to have Ballast Water Management Systems (BWMS), including bulk carriers confined exclusively to the Great Lakes that discharge the vast majority of all ballast water into the Great Lakes (USEPA, 2013).

While collaborative routine monitoring or treatment programs do not exist, the spread of VHSV has prompted some entities to enact emergency plans. For example, the U.S. National Park Service (NPS) published an emergency prevention and response plan in 2008, which aimed to prevent the spread of VHSV to Lake Superior in the NPS units of Isle Royale National Park, Pictured Rocks National Lakeshore, Grand Portage National Monument, Apostle Islands National Lakeshore, and the Grand Portage Indian Reservation. The emergency plan made recommendations for the parks, including an outreach campaign, recreational boat decontamination, restrictions on use of baits for fishing, and NPS-controlled vessel ballasting practices (USNPS, 2008). The recommendations were coordinated with respective tribal and state regulatory agencies as applicable. In 2006, the U.S. Department of Agriculture's (USDA's) Animal and Plant Health Inspection Services (APHIS) issued an emergency order prohibiting the interstate movement of species of VHSV-susceptible fish around the Great Lakes to prevent the spread of the virus by the aquaculture industry. Certain states have also established provisions to reduce the spread of VHSV by limiting the transportation of live baits.

SECTION 2
STUDY OBJECTIVES AND APPROACH

The objective of this study is to investigate options to prevent the spread of VHSV via ballast water in vessels that traverse the Great Lakes (i.e. inter-lake vessels).[3] Vessels traveling on the Great Lakes include bulkers, tankers, general cargo vessels, barges, tugs, commercial fishing vessels, passenger vessels, and recreational vessels. Of these, bulkers, tankers, and barges rely heavily on ballast water for cargo operations, and are the focus of this report. While the term "Laker" has a very specific meaning according to EPA's Vessel General Permit (VGP)[4] EPA expanded the scope in this document beyond Lakers to include other inter-lake vessels that may also transport ballast water.

EPA conducted a literature review to provide an overview of the virus, including its environmental requirements, its method of transmission and spread, and impacts to fish. This initial literature review aimed to better understand how the virus entered the Great Lakes and how it is spread, as well as examine recent efforts to control the spread.

Additionally, EPA used the U.S Geological Service (USGS) Nonindigenous Aquatic Species (NAS) Database to examine where the virus has occurred in the past and conducted a literature review to determine factors that impact spread. EPA used National Ballast Water Information Clearinghouse (NBIC) data to examine regions of the Great Lakes that may be impacted by ballast water transfer of the virus. To conduct this analysis, EPA used ballast water volume transfer data to determine the most common Great Lakes source and discharge ports, as well as identify the top 25 port pairs based on ballast water volume. The objective of this portion of the study was to examine the highest risk ports for uptake, transfer, and discharge of the virus. These data can be overlaid on past occurrence data to determine whether shipping in the Great Lakes could likely be spreading the virus.

EPA also conducted a literature review to identify potential techniques to detect VHSV in ballast water, although none of the techniques identified appear to be feasible for onboard analysis of VHSV. Because on board detection techniques were not available, a description of potential shore-based laboratory VHSV detection techniques in fish and water is provided.

Finally, EPA conducted a literature review to identify potential techniques to inactivate VHSV in ballast water. EPA identified several BWMS available on the market that have the potential to inactivate VHSV. EPA also explored novel treatments known to be effective at inactivating VHSV, but that have not yet been implemented in a large-scale ballast water application. Additionally, EPA explored nontreatment options, such as ballast water exchange

[3] Note that it is possible that VHSV may continue to be transported into the Lakes by coastal vessels that exit and enter the Great Lakes (coastal vessels that do not cross the Canadian EEZ are not required to manage their ballast water); however, the focus of this study is the spread, rather than the introduction, of VHSV to the Great Lakes.
[4] "Bulk Carrier Vessels that operate exclusively in Lake Ontario, Lake Erie, Lake Huron (including Lake Saint Clair), Lake Michigan, Lake Superior, and the connecting channels (Saint Mary's River, Saint Clair River, Detroit River, Niagara River, and Saint Lawrence River to the Canadian border), including all other bodies of water within the drainage basin of such lakes and connecting channels" (USEPA, 2013).

(BWE) or avoiding ballasting in infected areas, to examine the possibility of reducing the magnitude of VHSV transfer from port to port.

SECTION 3
REGIONS HISTORICALLY IMPACTED BY VHSV AND POSSIBLE FUTURE SPREAD

This section discusses the areas where VHSV has been detected, the factors that influence the spread of the virus, and the areas most likely to be impacted by VHSV in the future. Until the 1980s, the virus was limited to freshwater environments in Western Europe (Bain et al., 2010). According to Faisal et al. (2012), the first description of the disease occurred in Germany in the 1930s where it heavily impacted European rainbow trout farms for five decades. In the 1980s, the virus was detected outside of aquaculture environments in marine and brackish waters of the Pacific Northwest, spurring additional efforts to document the range of the virus. Subsequently, researchers detected VHSV in a variety of wild marine fish in the North Atlantic, Baltic Sea, and parts of the Pacific and Atlantic oceans (Iowa State University, 2007). With increased awareness of the pathogen across the globe, detections of the virus have been made in Scotland, the English Channel, the North Sea, Japan, and Korea (Faisal et al., 2012).

As previously stated, VHSV was initially detected in the Great Lakes system in 2003, and its detection in Lake Superior in 2009 marked the pathogen's presence in each of the Great Lakes (GLC, 2011). Although VHSV has been detected in each of the Lakes, it has not been detected in all areas of each Lake (Bain et al., 2010). The reasons for the erratic distribution of VHSV are unknown, as the processes of the virus' range of expansion are not fully understood (Bain et al., 2010). A key hurdle in understanding how VHSV spreads is the limited knowledge of where the virus currently exists. For these reasons, EPA conducted an extensive literature review and analyzed data to gather information about where the virus was likely to be present based on environmental factors, port locations, associated ballast water transfer and areas where VHSV has been detected in the past.

Ballast water transfer in the Great Lakes is a unique challenge because inter-lake vessels are governed by different requirements than ocean-going vessels. For example, confined Lakers are not subject to the same ballast water discharge limitations as ocean-going vessels. Therefore, the likelihood of spreading the virus by inter-lake transport of ballast water continues to be a viable threat by the current confined Laker fleet. In addition, genetic analysis of the virus strain identified in the Great Lakes has suggested that it may be slightly different from VHSV-IVb isolated in other environments (Faisal et al., 2012), suggesting the virus is changing based on environmental conditions. Recently, many new "quasi-species" of the virus have been identified, illustrating that the virus continues to evolve in the Great Lakes (Gorgolione, et al. 2017). Because the VHSV-IVb strain is capable of mutation when introduced to new environments, relocation of the virus within the Great Lakes system continues to be a threat to local ecosystems and the economies which rely on them.

Furthermore, the distribution of the virus is not fully understood (see Section 6 for further discussion). Historically, large observable fish mortality events have been the de-facto method for detecting the pathogen (Bain et al., 2010). For this reason, the virus may be present in its chronic form in different regions of the Great Lakes, but it has avoided detection because mass die-offs have not occurred or have not been observed. While some efforts have been made to sample the water column for VHSV, researchers speculate that infected fish must be present

locally and in sufficient numbers to produce detectable levels of VHSV in the water column (Bain et al., 2010). In a study where both fish and the water column were tested for the presence of VHSV, fish tested positive for VHSV while the virus was not detected at the same site in the water column, suggesting that testing the water alone may not provide accurate results regarding detection (Bain et al., 2010).

3.1 FACTORS THAT IMPACT VHSV SPREAD

Understanding where the virus may spread in the future requires understanding the factors that enabled VHSV's historical spread. VHSV can persist outside of a host organism for several days under favorable conditions (e.g., optimum temperature, ambient water pH, and the presence of protective coatings on the virus (Parry and Dixon, 1997)). Literature characterizing these factors indicates that the virus could be mobilized from areas even where fish are not present, once it has been shed into the water column (Parry and Dixon, 1997 and Kipp et al., 2018). This is an important factor in evaluating the risk of VHSV spreading to uninfected areas of the Great Lakes. Areas at risk may include locations with optimum temperatures, viable host populations for VHSV, and invasion hotspots in the Lakes. These hotspots generally occur in areas associated with ballast water discharge (Colautti et al., 2003). Evaluating the risk of VHSV spread to new areas requires understanding the interaction of invasion hotspots, shipping ports, discharge and source water areas, and environmental factors.

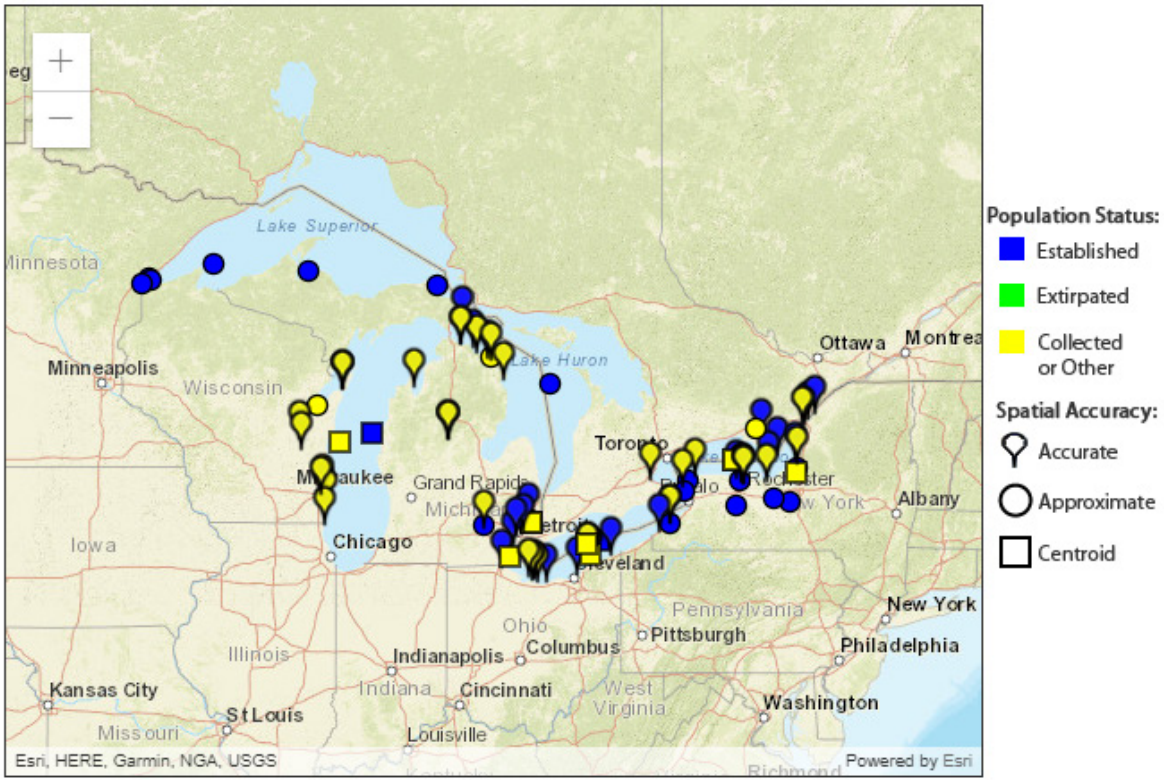
Although VHSV may prefer shallow, colder waters, most of the Great Lakes fall within the suitable temperature ranges for VHSV to thrive (LCA, 2008). Another factor to be considered is the presence of suitable host populations, which allow the virus to reproduce and spread. Since the highest densities of fish populations occur in the littoral zone[5] of the Lakes, these areas may be at higher risk for VHSV outbreaks than open water environments (Vadeboncoeur et al., 2011). Ports exhibiting suitable temperatures and viable populations of host fish are likely at the highest risk of VHSV outbreaks. Ports where environmental conditions are suitable for spawning may increase the risk of VHSV outbreaks, as fish gather in high densities and exhibit social behaviors enabling the virus to spread between individuals (Faisal et al., 2012). In addition, other variables may contribute to the risk of fish infection in certain regions of the Lakes, including introducing the virus to naïve[6] species or the presence of species suspected to be especially susceptible to the virus. It is unclear if individual VHSV-IVb variants would prefer different environments within the Great Lakes system, as there has been limited study on VHSV-IV's environmental preferences.

[5] The littoral zone of the lakes are the areas closest to shore.

[6] Naïve species are species without any exposure history to VHSV and have not developed any resistance to the virus.

3.2 CURRENT VHSV RANGE

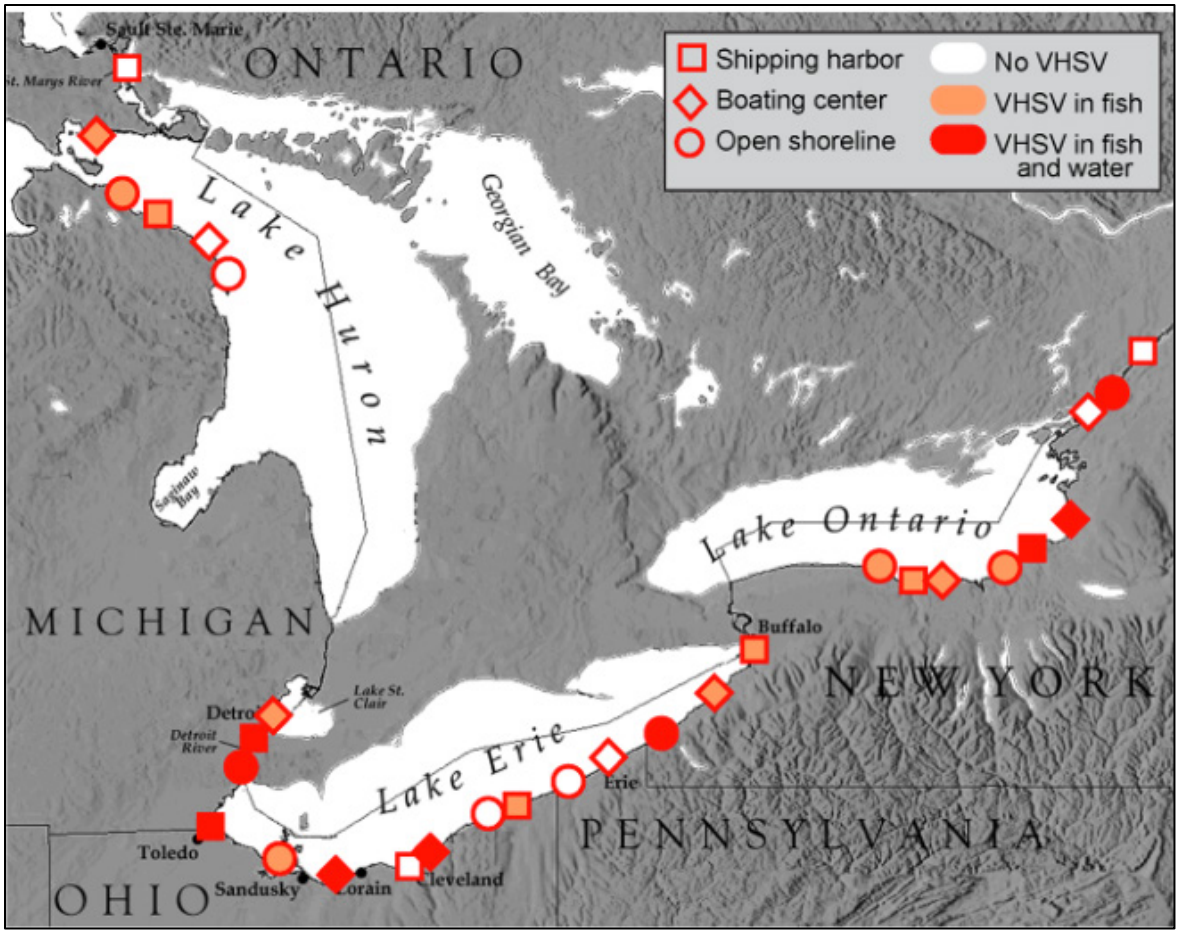
Figure 3-1 illustrates where populations of VHSV-IVb have been documented in the Great Lakes (Kipp et al., 2018).



Source: Kipp et al., 2018

Figure 3-1. Great Lakes Detections of VHSV

Figure 3-2 illustrates a 2010 study of both VHSV presence in fish and the water column at selected locations of the Great Lakes. This map shows a mixed distribution of VHSV at boating centers, shipping harbors, and open shoreline, indicating that VHSV is either not globally present in the Lake Huron, Erie, and Ontario, or is otherwise undetected. In addition, the map provides some context for the types of environments sampled and identifies where VHSV was detected in water, indicating areas where infected host populations shed the virus.



Source: Bain et al., 2010

Figure 3-2. Distribution of VHSV Positive Fish and Water at Sites Classified as Commercial Shipping Harbors, Recreational Boating Centers, and Open Shoreline

Both figures show VHSV's presence at shipping harbors, areas of high shipping activity, and recreational boating centers, supporting the theory that the virus propagated from ballast water and/or recreational boating.

3.3 POTENTIAL VHSV SPREAD

The reviewed literature suggests areas at the highest risk for VHSV invasion via ballast water may be:

- Areas where the virus has not yet been detected;
- Areas where there are naïve populations of fish and suitable environmental conditions;
- Areas where ballast water discharge volumes are largest; and
- Areas where ballast water discharges received have been sourced from locations where VHSV has been previously identified.

To visualize where VHSV outbreaks have the greatest potential to occur as the result of ballast water discharge, four ballast-related factors must be considered: (1) ballast water source location; (2) ballast water discharge location; (3) the volume of ballast water being transferred; and (4) voyage duration. Table 3-1 lists the top 25 Great Lakes port pairs based on the volume of ballast water transferred.

Table 3-1. Top 25 Great Lakes Port Pairs by Ballast Water Transfer Volume (MT)

Uptake Port	Discharge Port	Ballast Volume (MT)	Voyage Duration (hours)
Gary, IN	Two Harbors, MN	12,647,778	60.9
Burns Harbor, IN+	Duluth-Superior, MN-WI	10,657,497	61.8
Saint Clair, MI	Duluth-Superior, MN-WI	10,136,011	52.2
Monroe, MI	Duluth-Superior, MN-WI	7,880,903	64.1
Indiana Harbor, IN	Duluth-Superior, MN-WI	5,206,177	62.4
Conneaut, OH	Two Harbors, MN	4,991,887	71.4
Sault Ste. Marie (Canada) +	Marquette, MI	4,310,959	15.4
Indiana Harbor, IN	Two Harbors, MN	3,458,004	60.8
Detroit, MI+	Duluth-Superior, MN-WI	3,377,460	64.5
Cleveland, OH+	Silver Bay, MN	3,121,962	67.0
Gary, IN	Duluth-Superior, MN-WI	2,902,093	62.6
Marquette, MI	Duluth-Superior, MN-WI	2,796,616	20.6
Indiana Harbor, IN	Port Inland, MI	2,633,662	24.0
Nanticoke (Canada) +	Duluth-Superior, MN-WI	2,543,977	76.9
Cleveland, OH+	Marblehead, OH	2,354,484	8.0
Detroit, MI+	Two Harbors, MN	2,207,399	62.9
Indiana Harbor, IN	Escanaba, MI	2,171,355	22.4
Ecorse, MI	Two Harbors, MN	2,137,175	62.3
Saint Clair, MI	Two Harbors, MN	1,993,450	50.6
Hamilton (Canada) +	Duluth-Superior, MN-WI	1,798,173	95.8
Duluth-Superior+	Two Harbors, MN	1,720,397	3.9
Toledo, OH+	Duluth-Superior, MN-WI	1,654,050	66.1
Conneaut, OH	Duluth-Superior, MN-WI	1,606,077	73.0
Lat/Lon 47.17, -90.43++	Two Harbors, MN	1,277,180	N/A
Hamilton (Canada) +	Toledo, OH	1,247,343	37.0

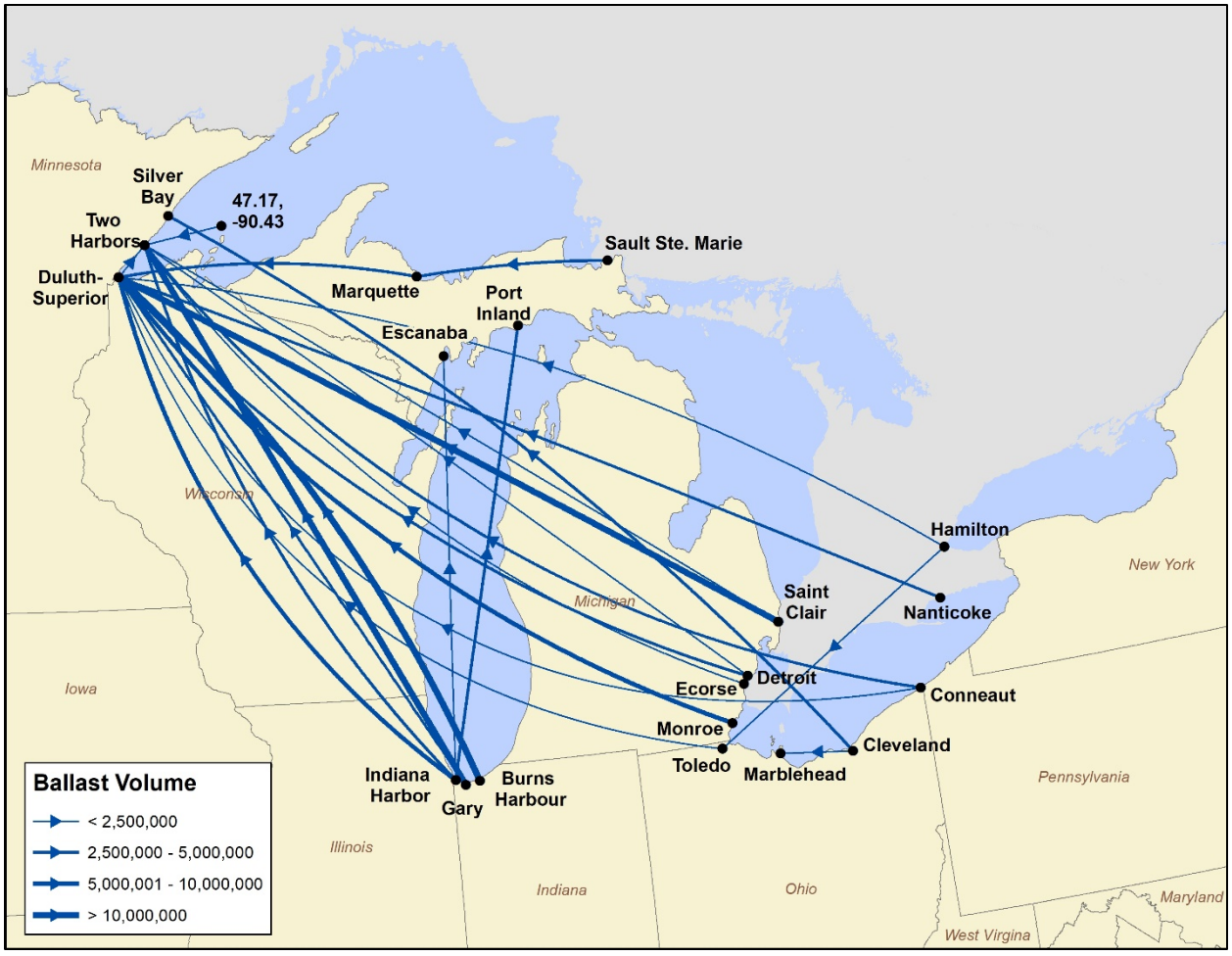
Source: NBIC, 2016 (Years 2011-2014) in USEPA, 2018, and LCA, 2017

+Ports that receive ballast water discharge from overseas sources (USEPA, 2015).

++ This Latitude/Longitude is approximate 7 miles off the coast of the Apostle Islands in Lake Superior.

N/A - No port-to-port voyage duration available due to uptake/discharge location not being located at a port.

Figure 3-3 displays the same information included in Table 3-1 in map format to provide a visual demonstration of vessel routes and the direction the ballast water was moved. Note that uptake and discharge ports are connected by routes depicted by arced lines and not actual vessel routes due to the number of routes included in a single map.



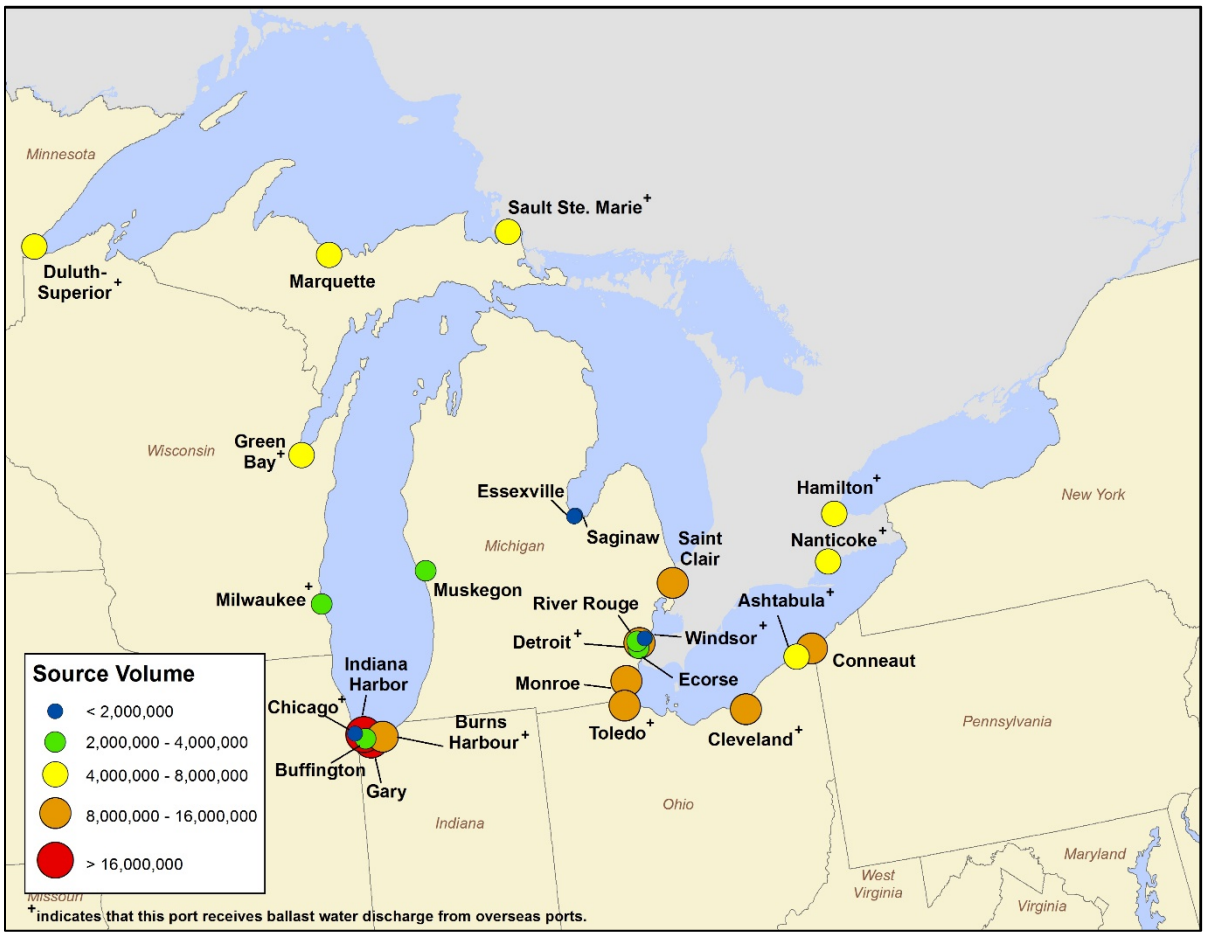
Source: NBIC, 2016 (Years 2011-2014) in USEPA, 2018

Note: This map illustrates one of the major industries that use inter-lake vessels – the transportation of iron ore and coal from the Duluth-Superior area to steel mills and electrical generating plants in Indiana, Ohio and Michigan. When cargo is unloaded in these southern ports, ballast water is taken on and then transferred back to and discharged in the northern ports (USEPA, 2018).

Figure 3-3. Top 25 Great Lakes Port Pairs by Ballast Water Transfer Volume (MT)

Because of the short voyage durations in the Great Lakes and the ability of VHSV to persist for long periods of time in dead fish, fish parts, and the water column, the risk of transferring the virus from port to port is likely significant. Transit times between ports in the Great Lakes vary from several hours to several days, all of which are significantly shorter than the 28- to 35-day VHSV survival time (Parry and Dixon, 1997). Additionally, larger volumes of ballast water are more likely to carry more individuals (i.e., increasing propagule pressure), suggesting that the virus has a transfer pathway from southern areas of the Great Lakes to Lake Superior.

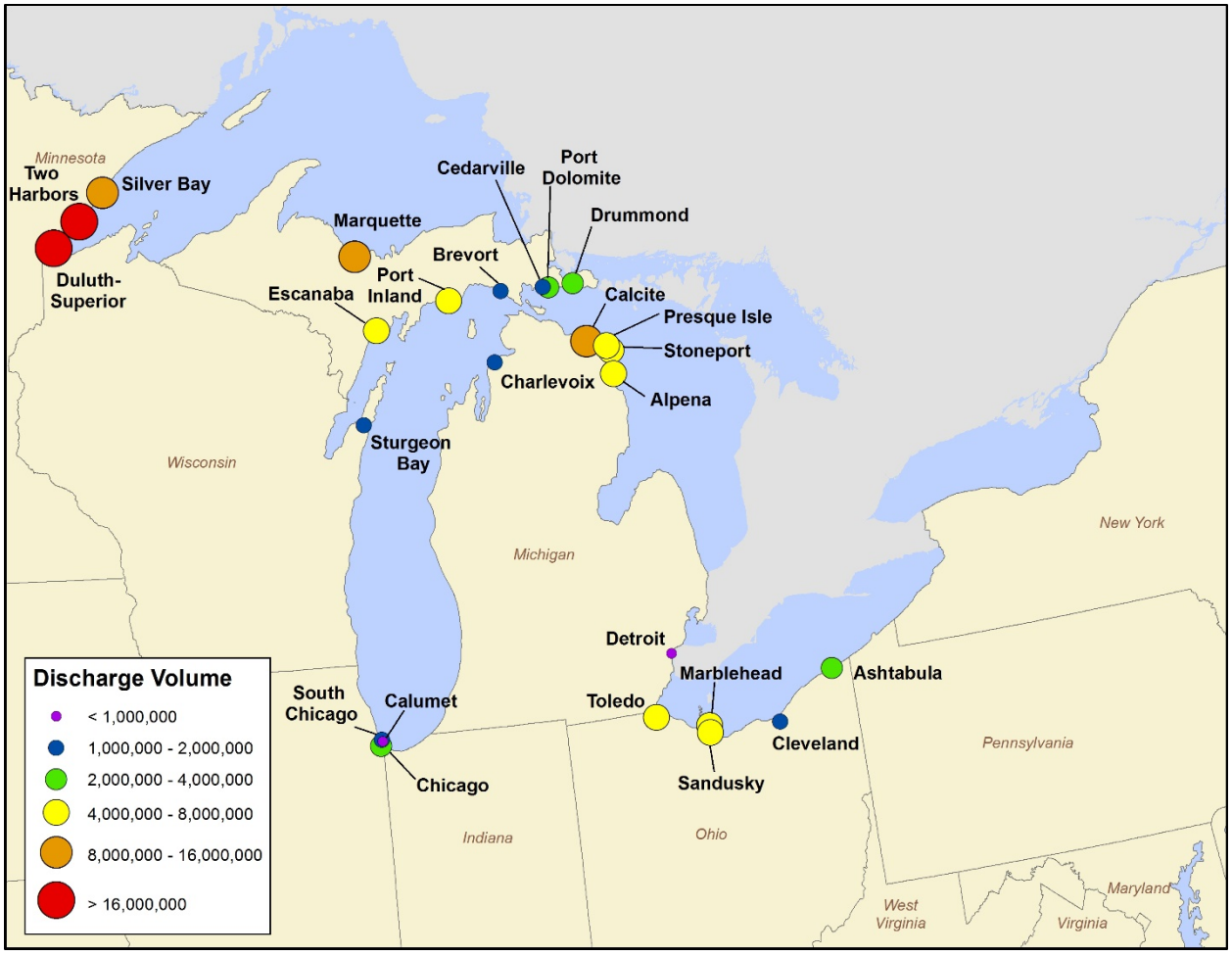
Figure 3-4 illustrates where large volumes of ballast water are sourced. This represents the source areas that may present the greatest risk of catalyzing VHSV invasions elsewhere in the Lakes. Figure 3-1 and Figure 3-2 confirm the occurrence of VHSV in several of the same areas, suggesting these ports are viable for VHSV populations.



Source: NBIC, 2016 (Years 2011-2014) in USEPA, 2018

Figure 3-4. Top 25 Great Lakes Uptake Ports by Ballast Water Volume (MT)

Figure 3-5 indicates where most ballast water in the Great Lakes is discharged, suggesting these areas may be at a higher risk for VHSV invasion than areas that receive little or no ballast water discharges.



Source: NBIC, 2016 (Years 2011-2014) in USEPA, 2018

Figure 3-5. Top 25 Great Lakes Discharge Ports by Ballast Water Volume (MT)

While it is difficult to quantify which areas are at the greatest risk for VHSV outbreaks, a combination of port locations, suitable environments, and ballast water transportation data help identify areas that may be at the highest risk of invasion. Even with a limited understanding of the environmental preferences and range expansion, these factors can inform managers of which areas may be at the highest risk. Comparing Figure 3-3 with Figure 3-1 and Figure 3-2 suggests that VHSV can be transported throughout major port pairs in the Great Lakes, and that ballast water relocation presents a viable mechanism of transportation for the virus. Furthermore, it is reasonable to speculate that VHSV could be transported to all areas of the Lakes where shipping ports are located (Figure 3-6), largely concentrated in U.S. Great Lakes states and the St. Lawrence Seaway. However, limitations of this analysis should be considered, including those posed by the Nonindigenous Aquatic Species (NAS) Database (Kipp et al. 2018), including a lack of comprehensive sampling for VHSV specimens (only reported catches), and by the NBIC (self-reporting of data and lack of data for several categories of excluded vessels (e.g., military).

Source: NBIC, 2016 in USEPA, 2018

Figure 3-6. Great Lakes Ports West of and Including Montreal

SECTION 4
POTENTIAL DETECTION TECHNIQUES FOR VHSV

Interest in detecting VHSV in the Great Lakes developed following fish kills in 2005 and is critical to curbing the spread of VHSV. Detection of VHSV can inform regulators and operators which areas are infected with the virus, allowing vessel operators to implement BMPs, recreational boaters to disinfect their vessels, and port authorities to notify the public. This section focuses on potential detection techniques related to ballast water transfer of VHSV. Ideally, contaminated ballast water could be tested and properly treated to remove or manage the presence of VHSV prior to discharge. However, as discussed below, most available detection techniques involve testing infected or potentially infected fish. Furthermore, it appears the detection techniques currently available for water are not feasible for onboard analysis of VHSV in ballast water due to the technical expertise required, cost of analyzing samples, and the large volumes of water involved. The following subsections detail currently available detection techniques of the virus in fish and water.

4.1 VHSV DETECTION TECHNIQUES IN FISH

Numerous studies have been conducted on VHSV detection and identification in clinical and laboratory settings. Detection of the virus indicates only presence or absence, whereas identification and enumeration can provide quantifiable results. The subsections below describe current detection techniques, including visual observation, clinical methods, and direct detection methods.

4.1.1 Visual Observation

Field diagnostic methods include visually identifying clinical signs and behavioral changes in affected fish. As discussed in Section 1, clinical signs of VHSV-infected fish include abnormal protrusion of the eyeball or eyeballs, pale gills, darkening of the skin, hemorrhages at the base of the fins, gills, eyes and skin, and a distended abdomen; behavioral signs include abnormal swimming behavior, lethargy, and lack of flight response (OIE, 2017).

While this method is inexpensive and does not require specialized equipment or laboratory testing, it is not highly reliable due to several factors. Physical impacts to the fish and associated mortality caused by the virus may vary from a few individuals to several tons of fish. Specifically, impacts to large numbers of fish are more likely to be identified visually, while impacts to only a few individuals may go unnoticed. In addition, these impacts are more likely to be observed in areas with increased recreational and commercial traffic, due to the high frequency of human traffic, whereas unpopulated areas may experience similar impacts to fish that go unnoticed. Furthermore, the absence of these signs does not indicate absence of the virus in fish. Host fish may still carry the virus but may not present any of the symptoms listed above (OIE, 2017). Lastly, observing these physical impacts and/or fish kills does not confirm the presence of VHSV. Additional methods, such as those presented below, should be employed to confirm the impacts are caused by VHSV and not another pathogen.

4.1.2 Clinical Methods

Clinical methods available for detecting VHSV include gross pathology, clinical chemistry, and microscopic pathology. These techniques are typically employed once a fish kill has been identified. Impacted fish are taken into a laboratory and one or more of the following analyses are conducted (OIE, 2017):

- Gross pathology: Gross pathology involves examination of the internal organs, tissues, and body cavities of the fish. Signs of infection include red or purple spots on the skin, muscle tissues, and internal organs, caused by bleeding from broken capillary blood vessels. Indications of infection within the internal organs may include the observation of dark red or necrosis, swollen spleen, a pale and blotched liver, and a pale gastrointestinal tract lacking food material.
- Clinical chemistry: Clinical chemistry involves analyzing the bodily fluids of the fish. Signs of infection include a low red blood cell count. The blood will appear light red and transparent.
- Microscopic pathology: Microscopic pathology involves observing changes in the tissues and cells of the fish. Signs of infection include necrosis and degeneration of cells within the internal organs and vascular system.

While the clinical methods outlined above are more accurate than visual observation, they are not as reliable as direct detection methods. These methods do not provide information on the quantity of the virus present. Clinical methods also require specialized equipment, such as microscopes and dissection tools, and specialized training to operate the equipment and properly identify the clinical signs of VHSV.

4.1.3 Direct Detection Methods

Direct examination involves examining fish for the presence of virus particles, virus antigen or viral nucleic acids.

4.1.3.1 Microscopic Methods

Histological sections from the tissues of the fish are prepared on slides and observed under a microscope. Histological sections from diseased fish show degeneration and necrosis of the kidney, spleen, and liver. Sections of the skeletal muscle may show many groups of red blood cells, while the muscle fibers remain undamaged (OIE, 2017).

Preparing histological sections and observing them under a microscope is time consuming, requiring days or weeks. Examining histological sections of fish tissue also requires specialized equipment, which not all entities have and can be expensive to purchase. Specialized training is also required to operate equipment and properly identify prepared slides of fish tissue under a microscope.

4.1.3.2 Cell Culture

The culturing of VHSV in cells involves development of viral cytopathic effect (CPE)[7] in cell culture, then identifying the virus using either antibody-based tests or molecular techniques described below (OIE, 2017). Cell culture is the USDA-APHIS approved diagnostic method for VHSV; however, it only indicates presence of the active or inactive virus, as opposed to the number of viruses. Furthermore, cell culture takes a month or more to identify VHSV and is not as sensitive as the molecular techniques listed below (Pierce, et al. 2013).

4.1.3.3 Antibody-based Antigen Detection Methods

Antibody-based antigen detection methods for the detection of VHSV, such as indirect fluorescent antibody test (IFAT) and enzyme-linked immunosorbent assay (ELISA), were developed in the early 1990s. Antibody-based antigen detection methods are based upon the binding of antibodies and antigens and using those reactions to detect the presence over the virus. These methods have continuously improved over the past decades.

These techniques can provide detection and identification relatively quickly (e.g., results can be provided within a few days, typically) compared with virus isolation in cell culture. However, various parameters, such as antibody sensitivity and specificity and sample preparation, can influence the results and ultimately lead to false-negatives (OIE, 2017).

4.1.3.4 Molecular Techniques

The following molecular techniques are currently available for detecting VHSV in fish and are listed in order of increasing accuracy:

- Conventional reverse transcriptase-polymerase chain reaction;
- Real-time reverse transcriptase-polymerase chain reaction; and
- Quantitative real-time reverse transcriptase-polymerase chain reaction (qRT-PCR).

Generally, they require less time than both cell culture and antibody-based antigen detection methods, providing results in a matter of hours, and are more accurate than cell culture (Pierce, et al. 2013). More rapid and accurate molecular techniques are continually being developed and may be applied in detecting VHSV in fish in the future.

4.2 VHSV Detection Techniques in The Water Column

EPA identified one study that tested for VHSV in the water column. The study, conducted by Bain et al. (2010), collected 10-liter water samples at various locations in Lake Ontario, Lake Erie, and Lake Huron. The water samples were then pressure filtered to remove water and concentrate them into 300 milliliter containers. The concentrated samples were then analyzed using qRT-PCR. Due to the large flow rates involved in inter-lake ballast water transfer (e.g., flow rates range from 9,080 m^3/hr to 18,120 m^3/hr for the 1,000-foot U.S. flagged vessels), it is unlikely that all ballast water could be tested for VHSV using this method; however, a

[7] Cytopathic effect is structural changes in host cells that are caused by viral invasion.

subsample could be collected from a ship's ballast tank and tested for VHSV. Results from the qRT-PCR test could be available the same day. It does not appear that qRT-PCR testing for VHSV in water is commercially available yet, and EPA did not identify any other studies testing for VHSV in water.

4.3 SUMMARY

Detection of the virus is limited due to high laboratory costs and few laboratories providing the service (Greene, 2018).[8] In addition, infected individuals must be present in sufficient quantities to produce detectable levels of VHSV in water samples (Bain et al., 2010). For these reasons, the primary method of VHSV detection may continue to be the observation of fish kills and detection techniques for the virus in fish, barring future advancement of detection technologies in water.

[8] Numerous attempts were made to contact university researchers to discuss the costs of various VHSV detection methods; however, these attempts were unsuccessful.

SECTION 5
POSSIBLE VHSV TREATMENT OPTIONS

Several contextual elements must be considered to determine which treatment techniques are relevant to VHSV inactivation in ballast water. First, an overview of regulations that pertain to BWMS are considered to determine if they have the potential to require treatments that are applicable to VHSV (Section 5.1). Second, review of treatment technologies currently used by the industry and their potential to inactivate[9] VHSV are explored, as these techniques are already being employed by the industry (Section 5.2). Third, the potential for novel treatments to inactivate VHSV in the context of ballast water are discussed, as lessons learned by the aquaculture industry, bench scale studies, and pilot scale projects may provide innovative options for the shipping industry (Section 5.3). In addition, nontreatment options are briefly discussed to evaluate whether they could mitigate the spread of the virus (Section 5.4).

5.1 BALLAST WATER MANAGEMENT REGULATIONS

Although the transfer of ballast water by ships has long been a recognized mechanism for transportation for ANS, it was not until recently that regulations were promulgated to address this concern. The issue was formally brought forth by the International Maritime Organization (IMO) to the international community in 1990 and resulted in the establishment of voluntary guidelines. These guidelines were adopted by the IMO in 1993 and were bolstered by additional measures in 1997 (Kelly and Kazumi, 2007). The IMO adopted the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWM Convention) in 2004, establishing standards and procedures for the management and control of ships' ballast water (IMO, 2004). The BWM Convention specifies ships are to implement a Ballast Water Management Plan and includes guidelines for testing, approving, and documenting ballast water management, including a ballast water records book. In addition, the BWM Convention is written to ensure that ballast water management practices do not cause greater harm than they present to the environment (IMO, 2004) and establishes limits on the number of organisms discharged in ballast water (Kelly and Kazumi, 2007). The BWM Convention entered into force on September 8, 2017; 69 countries representing over 75 percent of the world merchant shipping tonnage have signed onto the convention as of April 1, 2018; though, the U.S. is not a party to the Convention. Despite the U.S. not signing onto the convention, the USCG and EPA have adopted similar requirements (USCG, 2012 and USEPA, 2013). Regulatory aspects provide important perspective as to why certain treatment systems are implemented by the industry, while others go largely unused; this informs which systems have the potential to treat for VHSV.

International and U.S. ballast water requirements have been supplemented by a voluntary ballast water management plan specific to the aquatic ecosystem of the Great Lakes (LCA, 2008). The Lake Carriers Association (LCA) recommended the implementation of additional BMPs specific to the spread of VHSV in the Great Lakes in 2008. These BMPs outline two main voluntary actions based on recommendations provided by the USCG. Because VHSV is known to replicate in broad temperature ranges that encompass most temperatures found in the Great

[9] Inactivated viruses are considered incapable of infection, but are still present. When inactivated, VHSV cannot infect host organisms.

Lakes, these supplemental BMPs are recommended regardless of water temperature (LCA, 2008). One of these BMPs recommends minimizing BWE in near-shore environments where larger fish populations may be higher than in off-shore locations (LCA, 2008). Further, this provision directs members to conduct BWE in the warmest and deepest[10] water possible prior to entering an environment where VHSV has not been detected. Additionally, the provision identifies certain areas as having a higher risk for VHSV, including Lake St. Clair and the western basin of Lake Erie (LCA, 2008). It should be noted that these BMPs were targeted at reducing the risk of VHSV's spread to Lake Superior, where the virus has now been identified. Based on a recent survey of U.S. Laker companies (LCA, 2018), safety concerns associated with travelling without ballast water and costs associated with additional ballasting time present barriers to industry implementation of these practices. For example, LCA indicated that none of their vessels have performed BWE due to stability issues, additional time required, ballast system configuration, and structural limitations of some vessels. However, vessels do attempt to comply with other BMPs. For example, in some cases, ballasting is delayed as long as possible after the start of cargo off-loading to allow maximum clearance between the bottom of the vessel and the channel or dock bottom; then a minimum of ballasting is done to allow safe maneuvering. Additionally, ballasting may be augmented away from the dock following cessation of cargo off-loading (LCA, 2018).

Additionally, EPA's 2013 VGP (USEPA, 2013) imposes several mandatory ballast water management requirements for Lakers in Part 2.2.3.4 of the permit, and shares many similarities to IMO, USCG, and LCA recommendations. Operators must perform annual inspections to assess sediment accumulation of their vessels, which may lead to removal of sediment. In addition, vessels must minimize the quantity of ballast water taken up at dockside. This requirement typically means limiting uptake of ballast water required to safely depart the dock and completing ballasting in deeper water (USEPA, 2013). It is unclear how often this practice is implemented, as it may not be safe for the vessel to depart the dock to complete ballasting and may also cause structural concerns for the vessel while loading and offloading cargo. Lastly, Part 2.2.3.4 of the permit requires annual inspections of sea chest screens to ensure they have not been damaged and are operating properly (USEPA, 2013).

The 2013 VGP also requires any new confined Lakers built after 2009[11] to operate a BWMS to meet numeric limits for their ballast water discharge that, in some cases, would reduce the virus's ability to spread by reducing the transportation of live fish or fish parts. However, the numeric ballast water discharge limits are specific to bacteria and larger organisms and not to viruses.

The remainder of this section discusses BWMS of interest, as well as the feasibility of other options to eliminate the transport of VHSV between ports.

5.2 BWMS OF INTEREST

While BWMS are not currently installed on U.S. flagged Lakers that ply the Great Lakes, there are systems on the market that have the potential to treat for VHSV. This section discusses

[10] Note that warm water in the Lakes is typically associated with shallower environments, making this aspect of the provision difficult to follow.
[11] As of as March 2018, no U.S. flagged Lakers exist on the Lakes that were built prior to 2009.

which of these systems have the potential to treat for VHSV in the Great Lakes and explores the feasibility of these systems. There is an assortment of BWMS that apply physical and chemical processes to reduce organisms in ballast water, often utilizing a combination of technologies to meet requirements.

The selection of the BWMS for a vessel is based on several vessel-specific and environmental factors. Cost, available space, electricity needs, fresh or saltwater application, and ability to meet regulations all play a role in the selection of a BWMS. Adoption of technologies by the industry is largely based on limiting commercial liability, as operators need to maintain compliance at all times. In addition, service characteristics such as cargo handling, ballast capacity, pump capacity, available space, average travel time, vessel type, power requirements, and ease of operation are all additional considerations (USEPA, 2018). The unique attributes presented by the Great Lakes environment include significantly shorter voyage times than ocean-going vessels and a cold, freshwater environment. Table 3-1 includes the voyage duration of the top 25 port pairs ranked by ballast water transfer volume. Type approval certificates for BWMS often dictate ballast water hold times where appropriate to ensure that their technologies are effective (i.e., ballast water is treated consistent with the system design and operation as specified in the type approval certificate). BWMS type approval is often subject to operational limitations. For example, many of the ultraviolet (UV) based BWMS require ballast water hold times equal to or greater than 72 hours. As shown in Table 3-1, only three of the top 25 port pairs have voyage durations over 72 hours.

The following subsections describe common BWMS technologies that may have the capacity to inactivate VHSV in the Great Lakes. This includes technologies used by BWMS that have received USCG type approval,[12] and others that have not. Note that detailed information on cost, systems, and performance can be found in the EPA's Technical Development Document (TDD) describing the current state of ballast water management (USEPA, 2018).

5.2.1 UV Disinfection

As of mid-2018, the largest group of type approved BWMS employed by the industry was UV light disinfection technologies (USEPA, 2018). First, these technologies use filters to remove larger organisms and sediment from source water, increasing the efficiency of treatment. Source water then passes through the UV disinfection chamber where UV light is used to kill or inactivate organisms. During discharge, ballast water is generally routed back through the UV chamber for additional treatment (USEPA, 2018). UV systems are type approved to treat flowrates up to 6,000 m^3/hr (USEPA, 2018) and the range of temperatures expected to be encountered throughout the Great Lakes.

Among pathogens of interest, viruses are most resistant to UV disinfection followed by bacteria and parasites (USEPA, 2006). The efficacy of UV systems to inactivate VHSV is unknown, though VHSV is highly sensitive to certain frequencies of UV light, most notably

[12] Manufacturers' BWMS approved by the USGC are considered "type approved." The status of USCG review and approval of BWMS are presented on the USCG Marine Safety Center (MSC) website, currently accessible at https://www.dco.uscg.mil/Our-Organization/Assistant-Commandant-for-Prevention-Policy-CG-5P/Commercial-Regulations-standards-CG-5PS/Marine-Safety-Center-MSC/Ballast-Water/. As of July 31, 2018, nine BWMS have received type approval (USCG, 2018).

"UVC" which has a wavelength between 200-280 nanometers (Iowa State University, 2007). Although UVC light has proven to effectively inactivate viral microorganisms at a laboratory level, as of 2012, it had yet to be demonstrated whether large scale commercial applications could effectively treat for VHSV (Afonso, 2012). While UVC has been effectively inactivating VHSV in fish processing plant effluent and on bench scale levels (Afonso, 2012), it remains unknown if successful results are transferrable to BWMS.

Additionally, the majority of the USCG type-approved UV systems[13] require a 72-hour hold time. Because many of the major shipping corridors identified in the Great Lakes have a voyage duration time of less than 72 hours (Table 3-1), vessels would need to delay cargo loading until the hold time is achieved (USEPA, 2018). However, the USCG type approved a UV system in July 2018 with no hold time for freshwater. While the hold time barrier is eliminated for this system, additional research is needed to determine the transferability of UV treatment inactivating VHSV in fish processing and/or laboratory settings to BWMS with different source water characteristics (e.g., turbidity and temperature).

5.2.2 Electrochlorination

The second largest group of BWMS employed by ocean-going vessels is electrochlorination treatment to reduce living organisms in the water (USEPA, 2018). Like UV technologies, the majority of electrochlorination systems first use filters to remove gross or solid material and increase disinfection effectiveness. The technology works by using electricity to generate chlorine- and bromide-containing oxidizing compounds from seawater (USEPA, 2018). These compounds are then dosed into ballast tanks to achieve sufficient disinfection. Because this technology requires available salt water, it would require inter-lake vessels to prepare a synthetic seawater solution (USEPA, 2018), which is inefficient and time consuming. In addition, U.S.-flagged inter-lake vessels do not have coated ballast tanks, so introducing chlorine into the ballast water would result in increased corrosion rates within ballast tanks. While electrochlorination may be a more viable option for ocean-going vessels because they have the option of bunkering seawater, it seems highly unlikely that the technology would be efficient for inter-lake vessels.

The efficacy of electrochlorination to inactivate VHSV is expected to be similar to that of chlorine-based chemical disinfection treatments discussed in Section 5.2.3. While chlorine shows potential as an inactivator for VHSV, more studies on its efficacy in ballast water are needed. Additional factors that also warrant study include organic content in ballast water, sediment in ballast water, and if sufficient mixing is achieved (i.e., in the ballast water tank where treatment occurs).

Regarding feasibility, the USCG has approved several electrochlorination systems, many of which have no minimum hold time. While that aspect is promising for inter-lake vessels with short voyages, the requirement for vessels to prepare synthetic seawater and coat their tanks, as mentioned above, relegates this technology infeasible for inter-lake vessels at this time. Furthermore, cold ambient water conditions could limit the geographic and temporal effectiveness of this technology, as USCG type approval certificates for electrochlorination-

[13] As of July 31, 2018.

based BWMS specify minimum temperatures of water and/or electrolyte feed ranging from -2°C to 17°C, and Great Lakes surface water temperatures vary seasonally from 0°C to 25°C (typically ≤ 15°C, approaching near 0°C at the end of the season).

5.2.3 Chemical Addition Disinfection

The next most popular BWMS treatment group is chemical addition disinfection. Like electrochlorination, chemicals are added to ballast water to reduce the presence of living organisms. However, unlike electrochlorination, chemical disinfection requires the storage of chemicals on the ship. Chemicals are added to incoming ballast water and may include chlorine, chlorine dioxide, peracetic acid, or other biocides (USEPA, 2018). Because treated ballast water may contain residual chemical concentrations higher than discharge limits, discharging ballast water passes through sensors to determine the concentrations of chemicals, including disinfection byproducts, in the discharge. If needed, a reducing agent such as sodium bisulfate can be added to further treat the ballast water down to the applicable discharge limits (USEPA, 2018). Many systems also use filtration to remove larger organisms in the ambient water to reduce the chemical demand for disinfection. The most commonly used chemical is chlorine, which is paired with the neutralizing chemical sodium sulfite during discharge (USEPA, 2018).

As previously noted, VHSV is sensitive to many common disinfectants (Iowa State University, 2007). The only identified overlap with BWMS chemicals and disinfectants known to inactivate VHSV is chlorine, suggesting chlorine may have a potential to be used in treating VHSV in ballast water (USEPA, 2018; Torgersen and Hastein,1995). Chlorine is considered to inactivate VHSV, although EPA was unable to identify an accepted concentration to do so. Type-approved systems that use chorine target concentrations of 2-10 mg/L (USEPA, 2018). While chlorine addition systems may be a viable technology to reduce the spread of VHSV in ballast water, additional studies on the reaction of VHSV to chlorine are needed.

Regarding feasibility, VHSV has been shown to be inactivated by 540 mg/L of chlorine in 20 minutes (Kelly and Kazumi, 2007). It is unclear if the lower concentrations of chorine used by BWMS would effectively inactivate VHSV. In addition, if organic material is present, significantly higher concentrations of chlorine may be needed to treat the ballast water (Torgersen and Hastein, 1995). Further, inter-lake vessels typically lack the coated ballast tanks of ocean-going vessels, meaning the tanks will corrode and may be compromised when exposed to chemicals (USEPA, 2018.). None of the USCG type approved BWMS are chlorine chemical addition systems. However, in 2017, the USCG type approved a BWMS using chlorine dioxide with a hold time of 24 hours. That technology option improves the possibility of a viable option for a majority of the port pairs identified in Table 3-1; although, ballast tanks would have to be coated and additional studies would be needed to demonstrate the effectiveness of chlorine dioxide to inactivate VHSV.

5.2.4 Ozone Disinfection

Though ozone disinfection is not widely used, it is included here due to its possible effectiveness in treating VHSV. This technology is implemented by injecting ozone gas into ballast water to reduce the presence of living organisms. In commercial vessels, ozone is

produced onboard the vessel using an ozone generator. Depending on the system, a neutralizing chemical may be used prior to discharge to meet discharge requirements.

EPA did not identify any studies on the efficacy of ozone treatment related to VHSV inactivation.

Regarding feasibility, ozone disinfection requires hold times of less than 72 hours, suggesting it may be feasible for inter-lake vessels. However, ozonation of bromide containing water can create bromate, which is a carcinogen (Gunten and Holgne, 1994). It is unclear the amount of bromate that would be produced by these systems and how it may affect wildlife in the Lakes. Based on these factors, additional research should be conducted on the efficacy of inactivating VHSV with ozone disinfection.

5.2.5 Temperature Treatment

This type of BWMS involves applying heat generated from the vessel's engines to heat ballast water to kill aquatic organisms. Temperature treatments are typically used on ocean-going vessels that consistently operate long voyages.

Remington (2014) demonstrated that VHSV can be inactivated in temperatures over 20 degrees Celsius (Remington, 2014), which is within the operating capacity of temperature treatment systems.

While temperature is a well-documented mechanism to inactivate the virus, it is likely that the large volumes of very cold ballast water carried by inter-lake vessels would eliminate its applicability to the Great Lakes. At these volumes and temperatures (e.g., 16 million gallons at 2°C), waste heat is not a feasible option for heating the vessel's ballast water to pasteurization temperatures to kill living organisms including VHSV. Therefore, an additional heat source would be required. If a vessel used diesel fuel to achieve a pasteurization temperature of 72°C of approximately 16 million gallons of ballast water at 2°C, the vessel would need over 125,000 gallons of diesel per de-ballasting event (assuming 100% heat transfer from the diesel fuel to the ballast water), thus rendering this technology as unrealistic for these types of vessels (ERG, 2018).

5.3 Novel VHSV Treatment Options

In addition to examining currently employed BWMS for their efficacy in inactivating VHSV and feasibility for inter-lake vessels, EPA also explored other options known to be effective in treating VHSV but have not yet been implemented in ballast water management. As discussed above, VHSV is inactivated by chlorine, but also by a host of other chemicals including formalin, iodophor disinfectants, sodium hydroxide and sodium hypochlorite (Iowa State University, 2007).

5.3.1 Chemical Addition of Iodophors

Iodophors have been used in aquaculture settings to disinfect the surface of eggs to prevent the spread of the disease. Recreational disinfection with iodophors is encouraged to boaters to clean surfaces exposed to fish and fish parts. While disinfection processes have been

proven to be effective in the scenarios, there are many unknowns related to their scalability to BWMS. While currently employed BWMSs do not use these chemicals, the application could be considered as an option for inter-lake vessels.

The efficacy of disinfection processes in ballast water is highly speculative. Unknowns include the concentrations needed to induce inactivation, the effects of these chemicals on ballast water tank corrosion, the safety of discharging treated water back into the Lakes, the hold time required to induce inactivation, and whether these chemicals would meet type-approval requirements.

It is also unclear how the efficacy of iodophors for disinfection purposes would be affected by ballast water that may contain sediment and organic content. Whether a BWMS could be designed to incorporate these iodophors, and whether they would present advantages over the use of chlorine, warrants additional study.

5.3.2 Sodium Hydroxide Addition

Pilot studies are currently underway to determine the efficacy of adding sodium hydroxide (NaOH) to ballast water to increase the pH of the water and inactive VHSV. Perhaps most notably, Cangelosi et. al (2013) developed a pilot system for the M/V *Indiana Harbor*, a large inter-lake vessel.

Sodium hydroxide systems have the potential to raise pH in ballast water to greater than 11.5 standard units (s.u.). The pH of the ballast water can then be reduced by sparging with wet-scrubbed diesel exhaust from the vessels engines prior to discharge, and is reduced to less than 9.0 s.u. VHSV has been shown to inactivate in pH levels less than 2.5 s.u. and greater than 12.2 s.u. (Iowa State University, 2007).

Sodium hydroxide addition has the potential to be advantageous for application in inter-lake vessels because it can be used with uncoated ballast water tanks. However, increasing pH levels over 12 s.u. may require a significant volume of sodium hydroxide. While the exact additional chemical quantity to meet these pH requirements is unknown, and is likely affected by a variety of site specific factors, this increase may prohibit the implementation of the technology by causing vessels to transport large quantities of chemicals on board. Overall, the technology warrants additional testing at the land and vessel scale (Cangelosi et al., 2013).

5.4 NONTREATMENT OPTIONS TO MITIGATE THE SPREAD OF VHSV

While BWMS appear to be the most likely option for inactivating VHSV, nontreatment options may be a viable mechanism to reduce the number of viruses transferred from port to port. BWE is used by ocean-going vessels and reduces the spread of aquatic organisms between similar habitats by exchanging ballast water in deep-water environments. BWE could reduce the transfer of VHSV infected water and organisms by discharging ballast water in environments that are less suitable for the virus. Another nontreatment option is abstaining from ballasting in ports where VHSV has been identified to limit the intake and distribution of the virus.

5.4.1 Mid-Lake BWE

Identifying areas in the Lakes that are less likely to harbor the virus could inform the identification of lower risk areas for BWE. If VHSV was detected at a specific port, a vessel may be able to travel to a lower risk area and perform BWE. Identification of lower risk areas would require additional testing for the virus in open areas of the Lakes. While VHSV prefers certain temperatures and areas where susceptible species are present, it is unknown the degree to which the virus is present in deep-water off-shore environments. While it is likely that lower densities of fish (i.e., required hosts) are present outside the littoral zones of the Lakes, it is unknown whether a risk to these populations is significant. In addition, it is unknown how BWE would affect virus concentrations in these areas, and if multiple vessels performing BWE in the same areas could create an additional mechanism of spreading the virus.

5.4.2 Avoidance of Ballasting In-port

As previously stated, an alternative to performing BWE could be avoiding ballasting in ports where VHSV has been identified. However, due to previously stated safety concerns, it is unknown whether vessels could feasibly leave ports with little or no ballast water. It is also unknown how areas outside of the ports would be monitored and deemed safe for ballasting.

SECTION 6
DATA QUALITY AND LIMITATIONS

As noted throughout this report, a full evaluation of VHSV spread, detection, and treatment in ballast water is limited by available information. Although VHSV has been studied since its discovery, the recent emergence of the VHSV-IVb substrain suggests that it has been a lesser subject of study when compared to other strains. Much of the study of VHSV-IVb was directed at (unsuccessfully) stopping the virus' spread into Lake Superior, and there is some indication that research has slowed after the virus was considered to have colonized all of the Great Lakes. Additionally, much of the information identified during the literature search applied to the occurrence and treatment of VHSV in aquaculture settings. While a portion of that literature is relevant in any setting (e.g., virus characteristics, impacts to fish), much of it cannot be applied to the ballast water setting (e.g., detection and treatment).

While disinfection for VHSV has been studied in a recreational boating context, ballast water applications have been less studied. VHSV is known to be deactivated by a number of cleaning solutions, but it is unknown how applicable these biocides would be on a ballast water tank scale. Furthermore, it is unknown how chemicals would affect ballast tanks, if these chemicals could meet regulations to treat for other invasive species, and if they could be safely discharged back into the Lakes.

It also remains largely unknown if currently available BWMS could have the capacity to treat for VHSV. As previously stated, hold times for certain technologies are too long for major shipping routes in the Lakes, and it is unknown if these treatment methods could be effective with shorter hold times. In addition, it is unknown how certain technologies such as ozone disinfection would affect VHSV.

Additionally, the environmental preferences of the virus, as well as the distribution, both geographical and within the water column, are not fully understood. As previously noted, VHSV prefers a certain temperature range and areas where available host species are present. However, it is not definitively known if VHSV is present in off-shore, deep-water environments. In general, very little is known about where the virus is located for a number of reasons. First, infected fish need to be present in adequate numbers to detect the virus in water (Bain et. al, 2010). Additionally, occurrences are not usually recorded unless impacted fish (physical symptoms or mortality) are noticed. Due to the enormity of the Lakes, it is likely occurrences go unrecorded because no one was present to observe them. It is therefore important when examining occurrence maps not to assume areas without identifications indicates the virus is not present, but just that it has not been identified in those locations to date. As previously noted, detection techniques are costly, time consuming, and are not always available. Therefore, it remains largely unknown how the virus is distributed in the Lakes. Overall, it would be extremely beneficial to have more data available regarding the distribution of the virus.

Another unknown factor at this time is the threshold concentration of the virus needed to viably infect host organisms. It is unknown how the concentrations of the virus introduced by discharged ballast water would affect species of fish present at the discharge location. In

addition, how the proximity of infected individuals to ballast water intake would enable the virus to be distributed by ballast water discharge has not been studied.

SECTION 7 CONCLUSION

VHSV's spread throughout the Great Lakes threatens local ecosystems and the local economies that depend on them. While it appears that the level of research on VHSV has decreased since the virus reached all five Great Lakes in 2009, recent research has demonstrated that the virus is adaptable and new quasi-species may pose an additional threat when relocated to new environments in the Lakes. Further, naïve species that have not had previous exposure to VHSV are threatened by the relocation of the virus. For these reasons, the spread of VHSV by ballast water remains relevant and consequential. Due to the significant volume of ballast water transferred between ports within the Lakes, and the likely transport of the virus via this vector, future outbreaks of VHSV are possible and may be catalyzed by the relocation of ballast water. Therefore, it is important to examine possible detection and treatment techniques.

As noted previously in this report, incomplete understanding of the environmental preferences, current distribution, and spread of the virus limits the ability to designate which areas are at the most risk. Review of applicable literature suggests that all areas of the Great Lakes are at risk of outbreaks, particularly the port pairs identified in Table 3-1. Comparison between previous occurrences and the highest volume ballasting/deballasting activity suggest these areas may be at a significant risk for future outbreaks.

Regarding possible detection techniques related to ballast water, there are currently no viable options for on board testing or rapid water testing of the source port. Even if the surveillance of source water were considered by visual observation of infected fish, confirmation testing of VHSV is impeded by high laboratory costs and few laboratories providing the service. In addition, infected individuals must be present in sufficient quantities for laboratory methods to detect the virus in the water column (Bain et al., 2010). For these reasons, the primary method of detection may continue to be the observation of fish kills and observable signs of the virus.

Regarding possible treatment options to inactivate VHSV in ballast water, implementing existing or designing new BWMS that have the capacity to inactivate VHSV may be a viable option in the future. Research is needed to assess whether successful inactivation of VHSV using UV in fish processing plants and in the laboratory are transferrable to treatment of ballast water. Similarly, further research is required to determine if inactivation of VHSV in bench-scale tests and aquaculture applications using chlorine dioxide addition are transferrable to BWMS. This technology poses additional challenges because of the corrosivity of chlorine dioxide on the uncoated steel ballast tanks on confined U.S. flagged inter-lakes vessels. Regarding nontreatment options such as BWE and avoiding ballasting in ports known to have VHSV, research indicates these options may reduce the uptake of VHSV; however, it is unknown whether these options are safe and viable for inter-lake vessels.

While the identified detection techniques and applicable treatment options to impede the spread of VHSV is limited by our understanding of the virus, emerging technologies may be able to address the spread by ballast water transfer in the future, provided additional research demonstrates these technologies are effective in inactivating VHSV. However, some technologies face additional barriers, such as chlorine dioxide treatment and corrosion of

uncoated tanks, and hold time requirements of certain USCG type approved UV-based BWMS. Currently, the unique environment of the Great Lakes poses a difficult hurdle for BWMS by challenging manufacturers to develop treatment systems that can operate with shorter voyage times and in cold, extremely low salinity freshwater environments on vessels with uncoated ballast tanks. Additionally, these vessels would need to make space for the BWMS, and likely additional power generation and supply services, imposing significant costs for these vessel operators. While the result of VHSV's presence in the Great Lakes is likely to unfold in the coming decades, researchers and regulators continue to explore whether the spread of VHSV by ballast water can be controlled. Ongoing research of the virus will continue to inform the discussion of how we can protect a multi-billion-dollar fishery, the ecosystems that support it, and the economies which rely on it.

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