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GREAT WATERS RESEARCH COLLABORATIVE:

GREAT LAKES SHIP BALLAST MONITORING PROJECT
TECHNICAL REPORT

May 31, 2018

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EXECUTIVE SUMMARY

This Technical Report, developed by the Great Waters Research Collaborative (GWRC), presents methods and findings from the *Great Lakes Ship Ballast Monitoring Project (Project)*, a two-year effort supported by the United States Environmental Protection Agency's (USEPA's) Great Lakes Restoration Initiative via the Maritime Administration. The Lake Carriers' Association requested that the GWRC team conduct this project to help it meet a requirement to execute a study evaluating risk associated with laker ballast water discharge in USEPA Vessel General Permit (VGP) 2013 Part 6.15.5.b., in response to Minnesota's 401 certification of VGP2013. The overarching goal of the *Project* was to characterize aquatic organism densities and community composition in Great Lakes ships' ballast water (uptake and discharge) and analyze target species presence/absence in selected source water and receiving ports. Specifically, the *Project* generated information on Great Lakes vessels' ballast water regarding:

- Densities of a target organism, *Hemimysis anomala*, i.e., the "bloody red shrimp", and other Great Lakes non-indigenous species in ballast uptake and discharge;
- Presence/absence of the *H. anomala* CO1 genetic marker in a subset of source and discharge ports and ballast uptake and discharge;
- Densities and community composition of planktonic organisms (i.e., zooplankton and protists) in ballast uptake and discharge;
- Water quality/chemistry of ballast uptake and discharge; and
- Densities of pathogen indicators *Escherichia coli* and *Enterococcus spp.* in ballast discharge.

Eight Canadian and United States bulk carriers participated in the study. Sampling events occurred during the 2017 calendar year and focused on ballast operations resulting in discharges of water sourced from locations in the lower four Great Lakes to western Lake Superior, including:

- **Fifteen Discharge Sampling Events:** GWRC sampled 15 ship discharges to western Lake Superior loaded from various locations in the lower four lakes.
- **Four Voyage-Wide Sampling Events:** Four of the sampled discharges to western Lake Superior were associated with "voyage-wide" sampling, including associated source harbor water, ballast uptake, and receiving water.
- **One Uptake-Only Sampling Event:** One stand-alone uptake sampling event occurred in central Lake Erie; GWRC was unable to couple with a WLS discharge sampling event.

In summary, this research found laker ballast water from the lower four Great Lakes that was destined to, or directly in, discharge to western Lake Superior ports contained non-indigenous species of aquatic organisms not previously recorded in Lake Superior, and in one case, in the Great Lakes. In voyage-wide sampling events, evidence of *Project*-relevant non-indigenous species were found in the source harbors, the ballast uptake and ballast discharge. The *Project* detected these specimens though it surveyed only a fraction of the ship ballast water destined or discharged to western Lake Superior in 2017, only a small portion of the target ballast uptake/discharge events, and only snapshots in time of the shipping season. Next research steps should focus on practicability and efficacy evaluations of best ballast water management alternatives for the laker ships, as well as further characterization of the risk-release relationship for aquatic invasive species in the Great Lakes.

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ABBREVIATIONS AND ACRONYMS

%T:	Percent Transmittance
ANSI:	American National Standards Institute
BMP:	Best Management Practices
BWMS:	Ballast Water Management System
cfu:	Colony Forming Unit
DNA:	Deoxyribonucleic Acid
DOC:	Dissolved Organic Carbon
eDNA:	Environmental Deoxyribonucleic Acid
Ft:	Feet
GLNPO:	Great Lakes National Program Office
GLSLSS:	Great Lakes and St. Lawrence Seaway System
GPS:	Global Positioning System
GWRC:	Great Waters Research Collaborative
IMO:	International Maritime Organization
LSRI:	Lake Superior Research Institute
MDL:	Method Detection Limits
NBIC:	National Ballast Information Clearinghouse
NOAA:	National Oceanic and Atmospheric Administration
NIS:	Nonindigenous Species
NPOC:	Non-Purgeable Organic Carbon
PI:	Principal Investigator
POM:	Particulate Organic Matter
QAQC:	Quality Assurance and Quality Control
QAPP:	Quality Assurance Project Plan
RL:	Reporting Limit
SOP:	Standard Operating Procedure
SSS:	Shipboard Sampling System
TQAP:	Test/Quality Assurance Plan
TSS:	Total Suspended Solids
USEPA:	United States Environmental Protection Agency
VGP:	Vessel General Permit
WLS:	Western Lake Superior

1. INTRODUCTION

This Great Waters Research Collaborative (GWRC) Technical Report presents methods and findings from the *Great Lakes Ship Ballast Monitoring Project (Project)*, a project funded by the United States Environmental Protection Agency's (USEPA's) Great Lakes Restoration Initiative via the Maritime Administration and carried out in cooperation with several Great Lakes ship owners and operators. The Lake Carriers' Association requested that the GWRC team conduct this project to help it meet a requirement to execute a study evaluating risk associated with laker ballast water discharge in USEPA Vessel General Permit (VGP) 2013 Part 6.15.5.b., in response to Minnesota's 401 certification of VGP2013. The Minnesota Pollution Control Agency approved the GWRC study for that purpose. The overarching goal of the *Project* was to characterize aquatic organism densities and community composition—with particular attention to the presence of non-indigenous species (NIS) not previously reported in Lake Superior—in Great Lakes ships' ballast water (uptake and discharge) and, for a subset of voyages, associated source and receiving port water. Test vessels were eight Canadian and United States lakers. Ballast uptake and source water sampling locations comprised ports in the lower four lakes. Ballast discharge and receiving water sampling locations were ports in western Lake Superior (WLS). For purposes of this research, WLS comprises points west of Silver Bay on the north shore of the western arm of Lake Superior and wraps around to points east of Sand Bay on the south shore of the western arm of Lake Superior (Figure 2). The area includes the active ports of Superior, Wisconsin; Duluth, Minnesota; Two Harbors, Minnesota; and Silver Bay, Minnesota. All other locations in Lake Superior are described simply as Lake Superior sites.

The *Project* defined lakers as vessels that operate exclusively on the Laurentian Great Lakes and are confined to operations upstream of the waters of the St. Lawrence River east of a thumb line drawn from Cap de Rosiers to West Point, Anticosti Island, and west of a line along 63 W. longitude from Anticosti Island to the north shore of the St. Lawrence River. Lakers are distinct from salties, or oceangoing vessels, in that salties are not confined to operations within the Great Lakes and enter/exit the Great Lakes from the Gulf of St. Lawrence. The Gulf of St. Lawrence is the outlet of the Laurentian Great Lakes via the mouth of the St. Lawrence River into the Atlantic Ocean.

The purpose of the *Project*, and sampling exercises associated with it, was to understand characteristics and trends with respect to organism movement into Lake Superior from other locations in the Laurentian Great Lakes. Ship owners agreed to participate in the study, and asked that ship identities and locations be coded. To that end, all results from the monitoring exercises are reported in summary form to assure that individual ships are not identifiable as sources of specific sampled organisms. Dates of sample collection also are reported by month rather than day.

Project objectives were to generate and analyze information regarding:

- Transit and seasonal-related alterations in the presence/absence of a target organism *Hemimysis anomala*, i.e., the "bloody red shrimp", and other Great Lakes NIS, in ballast uptake and discharge water;
- The densities and community composition of planktonic organisms (i.e., zooplankton and protists) and density of the pathogen indicator bacteria *Escherichia coli* and *Enterococcus* spp. in the ballast uptake and/or ballast discharge of Great Lakes vessels; and

- Transit and seasonal-related alterations in water quality/chemistry, and zooplankton and protist densities and communities in Great Lakes ballast uptake versus ballast discharge.

Sampling events took place primarily from July 2017 through December 2017. The concentration of experimental activity in the second half of 2017, and that specific ballast tanks could not be individually sampled during both uptake and discharge (laker ships often load and unload ballast water into two or more ballast tanks simultaneously rather than ballasting/deballasting one tank at a time), made seasonal and transit-related alterations described above impossible to assess. Therefore, rather than assess patterns, this Technical Report provides descriptive characterizations of uptake and discharges *vis a vis* the parameters listed. Specifically, the report presents findings relative to:

- Characteristics of laker ballast water discharged to WLS from non-Lake Superior source ports, including:
    - Vessel and Shipboard Sampling System (SSS) operational information;
    - NIS and target organism (*H. anomala*) detections;
    - Background biological, physical/chemical characteristics.
- Voyage-wide characteristics of laker ballast water, including non-Lake Superior source water, uptake water, discharge water and WLS receiving water; including:
    - Vessel and SSS operational information;
    - NIS and target organism (*H. anomala*) detections; and
    - Background biological, physical/chemical characteristics.

All research activities were consistent with the GWRC's Shipboard Quality Assurance Project Plan (QAPP; LSRI, 2017) and Lake Superior Research Institute (LSRI) standard operating procedures (SOPs). A *Project*-specific GWRC Test/Quality Assurance Plan (TQAP), executed and agreed by all involved parties, guided overall research activities and assured conformance to technical and quality system requirements.

Samples were characterized in terms of general water quality/chemistry and biota, including the presence of organisms in taxa not previously detected and reported in Lake Superior. Genetic detection tools were employed to detect the presence of the target NIS, *H. anomala,* a native of the Ponto-Caspian region of eastern Europe that was first reported in the Great Lakes (Lakes Ontario and Michigan) in 2006 by researchers from the National Oceanic and Atmospheric Administration (NOAA; Kipp *et al*., 2017). At the commencement of the *Project*, this species had been found in samples collected from all of the Great Lakes with the exception of Lake Superior (Kipp *et al*., 2017).[6]

Ballast uptake and/or discharge densities of zooplankton, protists, *E. coli* and *Enterococcus* spp.[7] from laker ships to WLS were calculated. Community composition of organisms entrained in ballast uptake and discharge samples were characterized. Ballast uptake and discharge water physical/chemical characteristics were characterized and compared to that of corresponding ballast source water and receiving ports. Finally, ballast uptake and discharge samples were examined specifically for NIS not yet

[6] After the completion of the *Project*'s sampling events, *H. anomala* was collected in samples from the St. Louis River, near Allouez Bay, Wisconsin. https://nas.er.usgs.gov/queries/CollectionInfo.aspx?SpeciesID=2627&State=WI&HUCNumber=4010301; accessed 26 April 2018.

[7] Though not in the original study plan, analysis of *Enterococcus* spp. was added because as with analysis *E. coli,* it is typical of assessments of ballast discharge and provides useful general information of discharge water quality.

recorded in WLS, and the presence/absence of the CO1 gene of the target organism, *H. anomala*, was evaluated in ballast uptake, source water, ballast discharge and receiving water.

Notably, it was not an objective of this *Project* to determine risk of establishment or invasion associated with NIS detected in laker uptake, or discharge, or associated source and receiving water. Such an assessment, if possible at all, would require a different experimental design. In this study, source and receiving water assessments, which occurred shortly before or after the sampled ballast event, and at varying distances upstream or downstream of the ballasting location, were not designed to deliver direct cause and effect information relevant to that particular ballasting event. Instead they were intended to determine whether there was evidence of an established, breeding population of the *H. anomala* species already in the vicinity of the ship operations. Further, the genetic and microscopic analyses were not designed to conclusively distinguish live/dead status of detected organisms/material in this study. However, detection prevalence in discharge and across harbor sampling sites, and the condition of individual specimens in microscopic analysis, can provide clues as to how recently organisms were vital.

GWRC identified sampling opportunities based on trade route and voyage timing. The goal was to concentrate most of the sampling on ships whose voyages plied from areas in the lower lakes in which *H. anomala* is known to occur, to WLS. Other sampling events were distributed across other ship voyages and vessels of opportunity. Overall the following sampling events took place associated with 16 different ship voyages:

- **Fifteen Discharge Sampling Events:** GWRC sampled 15 ship discharges to WLS loaded from various locations in the lower four lakes.
- **Four Voyage-Wide Sampling Events:** Four of the sampled discharges were associated with "voyage-wide" sampling events. That is, along with the ballast discharge, the associated source harbor water, ballast uptake, and receiving water were sampled. All voyage-wide sampling occurred on ship voyages from southern Lake Michigan to WLS.
- **One Uptake-Only Sampling Event:** One stand-alone uptake sampling event occurred in central Lake Erie; GWRC was unable to couple with a WLS discharge sampling event.

This Technical Report summarizes *Project* methods, including test vessels, and vessel preparation; experimental design and methods; ships, voyages and ballast events sampled; quality assurance and quality control (QAQC) procedures; and *Project* results, discussion, recommendations and conclusions.

2. TEST VESSELS AND VESSEL PREPARATION

In 2016 the *Project* team[8] developed a ship sampling approach and solicited volunteer laker vessels from both United States and Canadian fleets for the study. Participating ship owners volunteered a total of 18 potential test vessels (i.e., ten United States lakers and eight Canadian lakers), all of which were self-unloaders.

GWRC provided technical support to participating ship owners and operators to facilitate their installation of ballast water sample ports. Based on GWRC design recommendations, ship owners installed sample ports on the ballast mains in the best available locations for uptake and discharge sampling. Owing to cargo routes, vessel availability and sampling team logistics, not all of the 18 vessels equipped with sample ports were sampled. In keeping with the *Project*'s goal of focusing on ballast water characteristics rather than individual ships, participating vessels were assigned codes for purposes of data reporting.

All of the volunteer vessels installed a 4 inch steel ANSI flange in a segment of the ship's ballast line which served as many ballast tanks as possible. The sample port flanges were covered with blind flanges when not in use for sampling. Immediately prior to sampling, a pitot-like sample port was installed by ship personnel into the sample flange. Some ships installed an optional return flow port in the ballast main to return filtered sample water back in-line. Engineering best judgement guided identification of the sample point locations; GWRC personnel inspected vessel piping, analyzed fluid dynamics, and recommended the best position for sample uptake and discharge ports. The vessel owners and operators installed the sample points with the blind flanges consistent with the design; GWRC supplied the sample ports. The length of bent elbow pipe varied depending on the diameter of the ballast main. The length was chosen to reach the central third of the ballast main. The sample port was also equipped with a ball valve pipe (Figure 1).

Figure 1. Example Sample Port.

[8] Previously identified as the Great Ships Initiative of the Northeast-Midwest Institute.

3. EXPERIMENTAL DESIGN AND METHODS

3.1. SHIPS/VOYAGES/BALLAST EVENTS SAMPLED

Table 1 summarizes sampling event locations, dates, ballast water source regions, and ballast water hold times. Figure 2 shows the voyage routes subject to sampling. All data are presented in categorical rather than specific terms in keeping with the *Project*'s goal of focusing on the general characteristics of locations and ballast water subject to movement as opposed to specific ships. For these trials, a random subset of the water volume subject to ballasting during port operations was sampled during each sampling event. Consequently, the same water mass was not subject to both uptake and discharge sampling. Further, in some cases, a smaller secondary ballasting event took place from an interim port between the port of uptake and the port of discharge where sampling occurred (Table 1). These secondary uptake volumes ranged from 18.3 to 40.5 percent of the total. Though at times substantial, these secondary ballasting operations did not interfere with the project objectives of assessing NIS movements by laker ships from the lower four Great Lakes to Lake Superior. The *Project* objectives did not include any estimation of a rate of organism transfer from the lower lakes to Lake Superior which might be affected by dilution with interim Lake Superior uptakes. Nor did it include any geographic constraints on the source of water from the lower lakes that was transferred to Lake Superior, which might be affected by mixing of water from different lower lakes locations.

Of the ballast discharges sampled, estimated hold times (from end of initial uptake in the source system until the beginning of discharge in WLS) ranged from 3 to 6 days (Table 1). For the voyage-wide sampling events, the ballast hold times ranged from 3 to 4 days (Table 1).

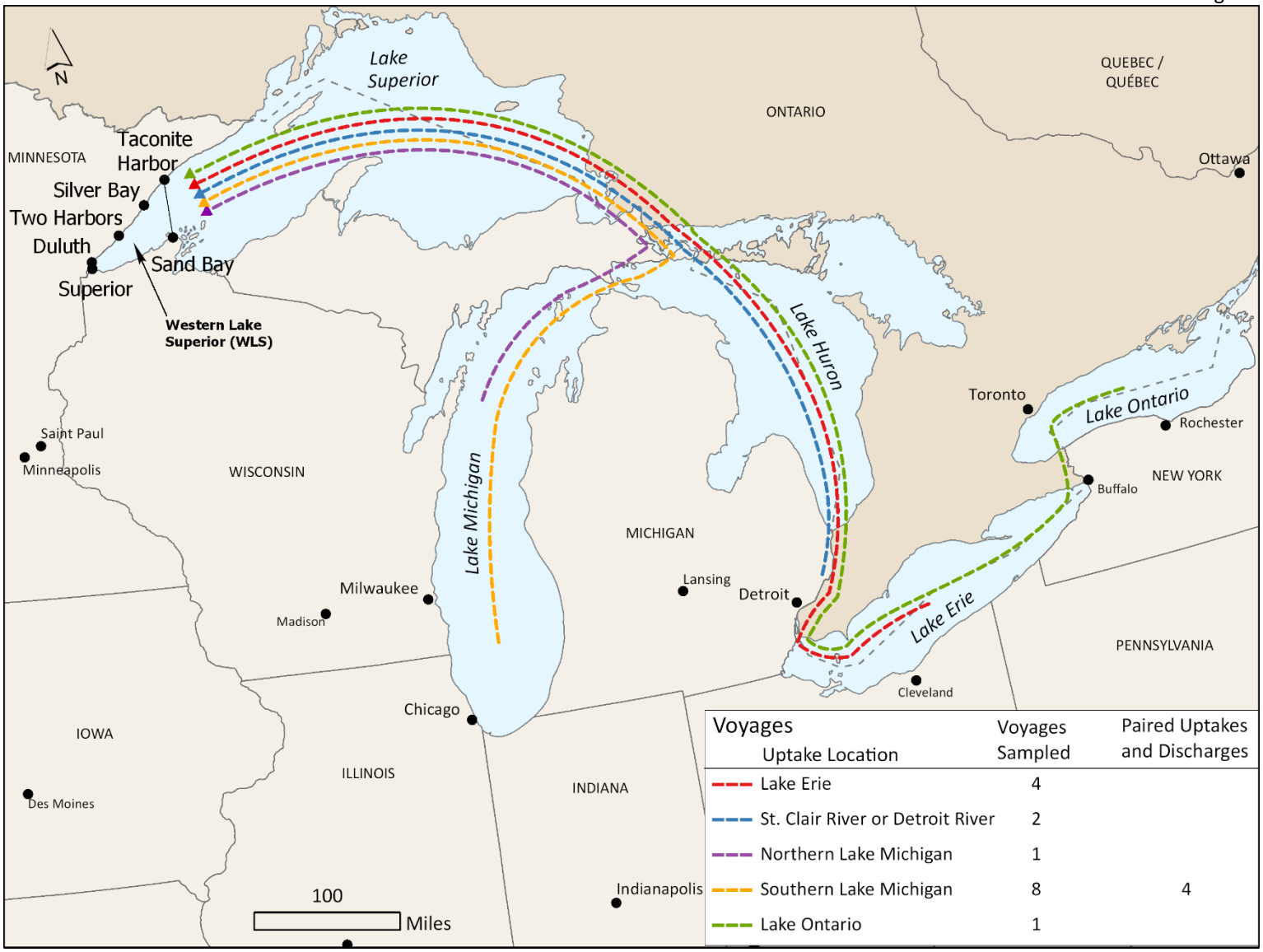


Figure 2. Geographic Overview *Great Lakes Ship Ballast Monitoring Project* Sampling Events.

Table 1. Sampling Event Locations, Dates and Associated Ballast Hold Times. N/A = Not Applicable because Not Sampled. Voyage-Wide = Sampling Occurred at both Uptake and Discharge over the course of the voyage; Ballast Uptake-Only and Ballast Discharge–Only = Sampling occurred only for operation specified over the course of the voyage.

Trial #	Date (Mo/Yr)	Sampling Target	Ballast Uptake/Source Water				Ballast Discharge/Receiving Water		
			Initial Uptake / Source Water Port Location	Locations of Secondary Uptakes into Sampled Tanks[1]	Percentage of Water from Secondary Uptake in Sampled Tanks	Percentage of Water Discharged at Intermediate Locations from Sampled Tanks	Discharge / Receiving Water Location	Estimated Hold Time from Initial Uptake[1]	Estimated Hold Time from Most Recent Uptake[1]
1	Jan-17	Ballast Discharge-Only	Southern Lake Michigan	Eastern Lake Superior	20.2%	N/A	Western Lake Superior	6 Days	4 Days
2	Jul-17	Ballast Discharge-Only	Eastern Lake Erie	N/A	N/A	N/A	Western Lake Superior	4 Days	N/A
3	Aug-17	Ballast Uptake-Only	Central Lake Erie	N/A	N/A	N/A	N/A	N/A	N/A
4	Aug-17	Ballast Discharge-Only	Central Lake Erie	Lake Superior	18.3%	N/A	Western Lake Superior	5 Days	2 Days
5	Aug-17	Ballast Discharge-Only	Southern Lake Michigan	N/A	N/A	N/A	Western Lake Superior	4 Days	N/A
6	Sep-17	Voyage-Wide	Southern Lake Michigan	St. Mary's River, Lake Superior	29.3%	N/A	Western Lake Superior	3 Days	1 Day
7	Sep-17	Ballast Discharge-Only	Southern Lake Michigan	St. Mary's River, Lake Superior	23.0%	N/A	Western Lake Superior	3 Days	1 Day
8	Sep-17	Ballast Discharge-Only	St. Clair River	Eastern Lake Superior, Lake Superior	40.5%	40.5%	Western Lake Superior	3 Days	1 Day
9	Oct-17	Ballast Discharge-Only	Detroit River	N/A	N/A	N/A	Western Lake Superior	4 Days	N/A
10	Oct-17	Ballast Discharge-Only	Southern Lake Michigan[2]	Eastern Lake Superior	21.3%	14.8%	Western Lake Superior	3 Days	2 Days

Trial #	Date (Mo/Yr)	Sampling Target	Ballast Uptake/Source Water				Ballast Discharge/Receiving Water		
			Initial Uptake / Source Water Port Location	Locations of Secondary Uptakes into Sampled Tanks[1]	Percentage of Water from Secondary Uptake in Sampled Tanks	Percentage of Water Discharged at Intermediate Locations from Sampled Tanks	Discharge / Receiving Water Location	Estimated Hold Time from Initial Uptake[1]	Estimated Hold Time from Most Recent Uptake[1]
11	Oct-17	Voyage-Wide	Southern Lake Michigan[2]	St. Mary's River, Eastern Lake Superior	20.5%	16.1%	Western Lake Superior	3 Days	1 Day
12	Oct-17	Voyage-Wide	Southern Lake Michigan[2]	St. Mary's River, Eastern Lake Superior, Lake Superior	19.8%	16.1%	Western Lake Superior	3 Days	<1 Day
13	Nov-17	Voyage-Wide	Southern Lake Michigan	N/A	N/A	N/A	Western Lake Superior	4 Days	N/A
14	Nov-17	Ballast Discharge-Only	Northern Lake Michigan	N/A	N/A	N/A	Western Lake Superior	3 Days	N/A
15	Dec-17	Ballast Discharge-Only	Western Lake Erie	St. Mary's River	24.9%	N/A	Western Lake Superior	3 Days	2 Days
16	Dec-17	Ballast Discharge-Only	Lake Ontario	N/A	N/A	N/A	Western Lake Superior	5 Days*	N/A

[1] Data sourced from National Ballast Information Clearinghouse (NBIC, 2018).
[2] Uptake occurred in two locations in the same harbor area within 1.5 miles. GWRC sampled the first of these uptake operations
* Data provided via personal communication between the *Project* Principal Investigator and the ship captain.

3.2. RESEARCH METHODS

This section summarizes methods for source water, ballast uptake, ballast discharge and receiving water sample/data collection and analysis.

3.2.1. BALLAST UPTAKE AND BALLAST DISCHARGE SAMPLE/DATA COLLECTION

The *Project* team collected representative samples of ballast uptake and discharge water masses during routine ship operations. The vessel's crew facilitated these sampling events by ensuring adequate space and power sources and informing GWRC personnel on ballast operational events. Sample types and volumes varied depending upon sampling objectives and comprised:

- Continuous in-line samples filtered through a plankton net with a minimum target volume of 2 m^3 targeting larger organisms, mainly zooplankton (Table 2);
- Continuous in-line whole water samples ("seep samples") of up to 8 L in volume (Table 2) targeting protists and microbes (Table 2); and
- Grab samples of up to 1 L in volume collected at the beginning, middle and end of the ballast sampling operation (Table 2), targeting physical/chemical properties of the water.

The samples were collected using either the active or passive version of the SSS (Figures 3-5; Table 2). The active version pumped sample flow from the ballast main and returned it to the main if a return port was provided. In the passive version, the ballast line pressure powered the sample flow and the filtered ballast water is discharged to the bilge.

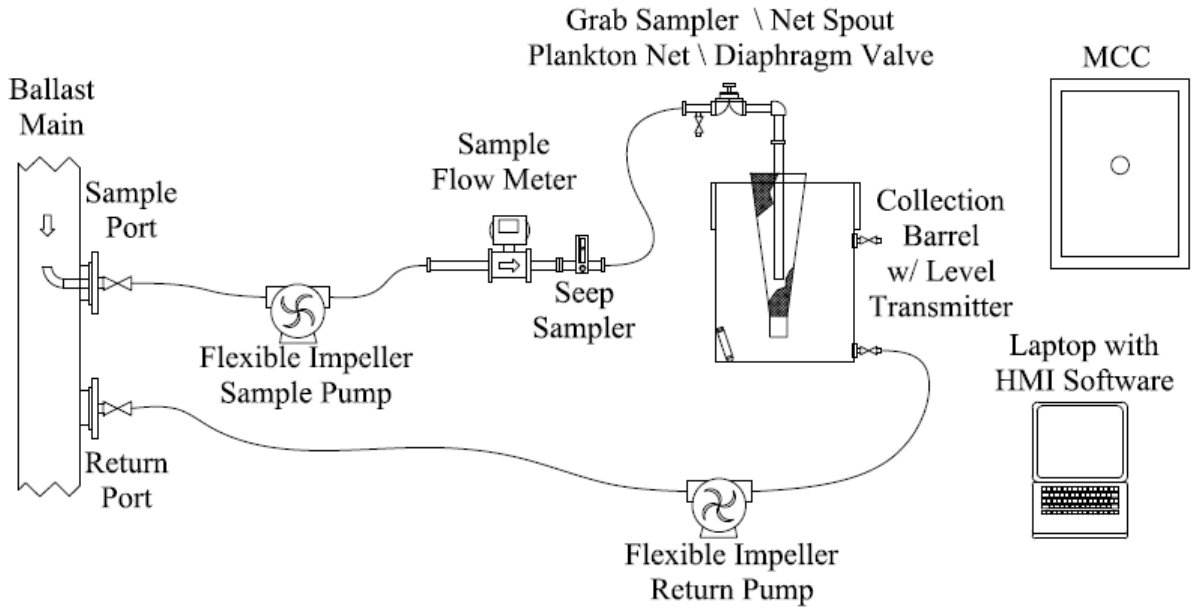
GWRC interviewed the vessel's crew during discharge sampling events to determine the date and port of the last uptake event. GWRC personnel in the control room recorded the water height (soundings) of each ballast tank periodically into the *Great Lakes Ship Ballast Monitoring Project: Ballast Tank Height Measurements* datasheet. The goal was to record a minimum of three soundings for each tank ballasted/deballasted during each sampling event: one at the start, one in the middle, and one at the end of GWRC sampling. Typically, soundings were taken far more often, i.e., every ten to fifteen minutes throughout each event. The times of the first and last soundings did not always line up with the start and end of sampling due to communication lags.

GWRC engineering staff used the soundings recorded during each sampling event to estimate the volume of ballast water subject to sampling. The estimated volume of ballast water sampled was calculated as the volume change of all tanks ballasting/deballasting during the sampling event. When soundings were not recorded at the beginning and end of sampling linear interpolation between available soundings was used to estimate beginning and end volumes. Changes in vessel list and trim were not considered when estimating tank volumes. In some cases, current sounding tables were not available to support conversion of tank heights to volumes, and in these instances volumes were estimated based on ship drawings.

Project personnel collected samples according to *LSRI/SOP/GWRC/12 – Sample Collection Procedures for Ballast Water Monitoring.* The SSS sample pitot delivered a continuous side flow from the ballast main directed into a 35 µm plankton net for sampling of organisms ≥ 50 µm (i.e., zooplankton). GWRC personnel controlled the flow rate to deliver a target minimum sample volume of 2.0 m^3 of water. Beginning with Trial 10, GWRC began collecting an additional larger-volume zooplankton sample using a

plankton net with a larger pore size (400 µm). This larger volume sample was added to the TQAP with the acquisition of a larger pore net, and allowed collection and enumeration of sparser organisms in the size range of the target NIS, *H. anomala*. For these samples, an additional 3.0 m^3 of ballast water was filtered. Collection of this second zooplankton sample was only possible when a return port was installed in the vessel's ballast line, and when sufficient time remained during cargo loading/unloading to allow for another ~60 minutes of sampling. Seep samples were collected into a 19 L carboy from a side-stream of the sample water flow, branching off upstream of the plankton net. Seep sample water was used to assess protist density and taxonomic composition, as well as *E. coli* and *Enterococcus spp*. densities and the presence/absence of *H. anomala* eDNA. Whole water grab samples were collected for characterization of water quality/chemistry via a dedicated side port located off the main sample line.

A multiparameter sonde (YSI EXO2 Multiparameter Instrument and EXO Handheld Display; YSI Incorporated; Yellow Springs, Ohio) was used to measure temperature, conductivity, salinity (via algorithm), turbidity, pH, dissolved oxygen, chlorophyll *a* (green algae) and phycocyanin accessory pigment (blue-green algae). While a sonde measures *in situ* chlorophyll *a* and phycocyanin, it is not as accurate as an extractive technique. Some sources of inaccuracy can be minimized by combining extractive analysis of the samples with the sonde readings of the same samples and applying a correction factor. Due to the constraints of the sampling events, a correction factor was not determined, therefore the uncorrected values obtained can be used for relative comparison purposes only and not actual concentrations. The sonde was calibrated weekly according to *LSRI/SOP/FS/39 – Calibration, Deployment, and Storage of YSI EXO Series Multiparameter Water Quality Sondes*.



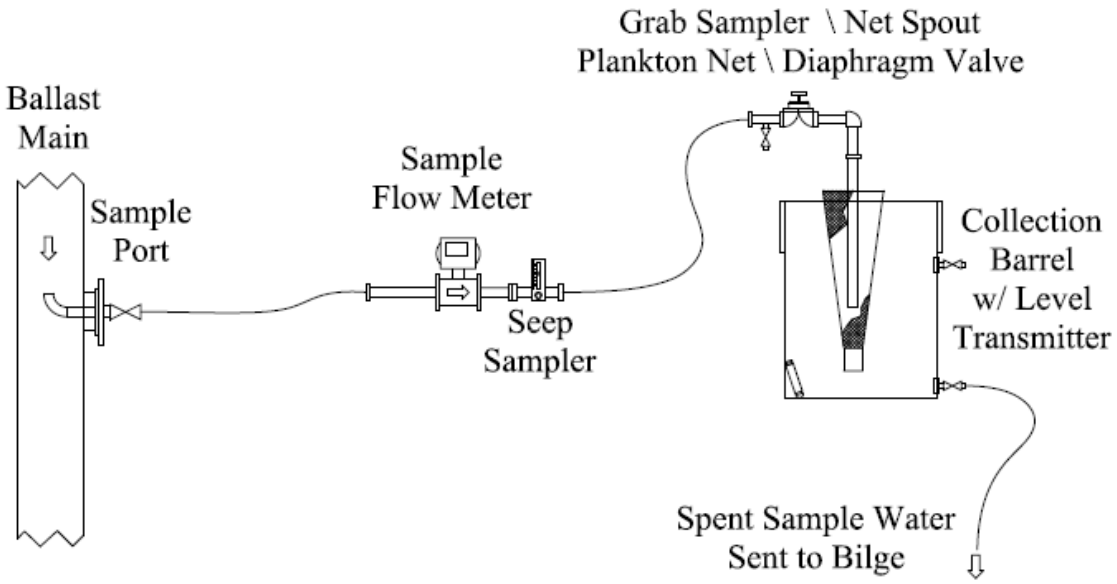


Figure 3. Generalized Schematic of the Shipboard Sampling System (Top Diagram Shows Active Version; Bottom Diagram Shows Passive Version).

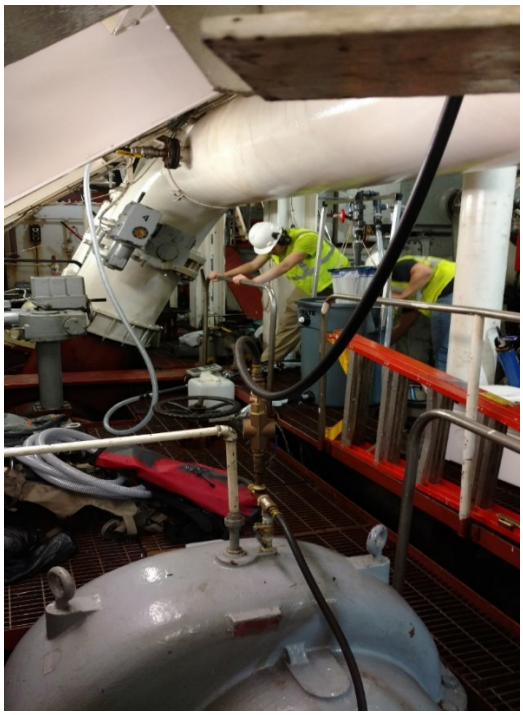


Figure 4. Photo Showing Operation of the Shipboard Sampling System On Board a Test Vessel.

Figure 5. Photo Showing the Shipboard Sampling System's Plankton Net Component On Board a Test Vessel.

Table 2. Operational, Water Quality/Chemistry and Biological Data/Samples and Measurements Collected/Taken During Ballast Uptake and Ballast Discharge Sampling Events. N/A = Not Applicable.

Sampling Event	Category	Parameter	Number of Samples/Measurements Per Sampling Event	Target Sample Volume	Sample Type
Ballast Uptake or Ballast Discharge	Vessel Operations	Ballast Tank Electronic Soundings	Minimum of 3	N/A	Vessel Log
	Shipboard Sampling System Operations	Plankton Net Flow Rate	Continuous	N/A	Plankton Net
		Plankton Net Volume	Continuous	2.0 to 5.0 m^3	Plankton Net
		Seep Sampler Volume	Continuous	5 L (uptake); 8 L (discharge)	Seep Sampler
	Water Quality/ Chemistry	Temperature, Conductivity, Salinity (via algorithm), Turbidity, pH, Dissolved Oxygen, Chlorophyll a (green algae), Phycocyanin Accessory Pigment	3 (Beginning, Middle, End)	500 mL	Grab Sample Line
		Percent Transmittance, Total Suspended Solids, Particulate Organic Matter, Mineral Matter	3 (Beginning, Middle, End)	1 L	Grab Sample Line
		Non-Purgeable Organic Carbon, Dissolved Organic Carbon	3 (Beginning, Middle, End)	125 mL	Grab Sample Line
	Biology	**All Zooplankton:** Total Density and Taxonomic Composition; Live Density for selected discharge samples	1: Uptake; 1: Discharge	2.0 m^3	Plankton Net (35 µm mesh)
		Larger Volume Sample for *Hemimysis anomala:* Total Density (for Trials 10 – 16 and vessels having a return port installed in ballast main)	1: Uptake, 1: Discharge (beginning with Trial 10)	3.0 m^3	Plankton Net (400 µm mesh)
		Environmental DNA: Presence of CO1 Gene of *Hemimysis anomala*	3 (beginning with Trial 6)	1 L	Seep Sampler
		Protists: Total Density and Taxonomic Composition	2: Uptake; 2: Discharge	500 mL	Seep Sampler

3.2.2. SOURCE AND RECEIVING WATER SAMPLE/DATA COLLECTION

Source water and receiving water samples/data were collected in association with the four voyage-wide trials (Table 3), within 20 hours of ballast uptake or discharge. Collection sites included a location in close proximity to the test vessel, and up to three sites within 0.5 mile of the test vessel's docking location, typically within the port facility[9] (Figures 6 - 7).

Longitude/latitude measurements were taken via Global Positioning System (GPS) devices or derived from interpolation on georeferenced aerial photos. The sampling locations are reported relative to location of the test vessel. Water depth was measured using a portable sonar sensor transducer (Venterior VT-FF001 Portable Fish Finder) and weather conditions were qualitatively categorized through observation.

Whole water grab samples for characterization of water quality/chemistry (i.e., percent transmittance, %T; total suspended solids, TSS; particulate organic matter, POM; non-purgeable organic carbon, NPOC; and dissolved organic carbon, DOC) were collected from a depth of approximately 1 meter below the water surface. Temperature, conductivity, salinity (via algorithm), turbidity, pH, dissolved oxygen, chlorophyll *a* (green algae) and phycocyanin accessory pigment (blue-green algae) were measured using the multiparameter sonde. The sonde was calibrated weekly according to *LSRI/SOP/FS/39 – Calibration, Deployment, and Storage of YSI EXO Series Multiparameter Water Quality Sondes*. Finally, three replicate whole water samples were collected in sterile bottles attached to a sampling pole for presence/absence determination of the CO1 gene of *H. anomala* according to *LSRI/SOP/GWRC/13 – Processing and Shipping Samples for Environmental DNA Analysis*.

[9] In one case, Trial 6, source water was collected outside the port facility at a location approximately five miles from the test vessel's docking location.

Table 3. Sample Site Characteristics, Water Quality/Chemistry and Biological Data/Samples and Measurements Collected/Taken During Source Water and/or Receiving Water Sampling Events. N/A = Not Applicable.

Sampling Event	Category	Parameter	Number of Samples/Measurements Per Sampling Event	Target Sample Volume	Sample Location
Source Water or Receiving Water	Location/Sampling Site Characteristics	Longitude/Latitude	Up to 4 sites	N/A	Dock Wall
		Estimated Distance to Test Vessel	Up to 4 sites	N/A	Dock Wall
		Water Depth	Up to 4 sites	N/A	Dock Wall
		Observational Weather Conditions	Up to 4 sites	N/A	Dock Wall
	Water Quality/ Chemistry	Temperature, Conductivity, Salinity (via algorithm), Turbidity, pH, Dissolved Oxygen, Chlorophyll a (green algae), Phycocyanin Accessory Pigment	1 per Site	N/A – In Situ	Dock Wall
		Percent Transmittance, Total Suspended Solids, Particulate Organic Matter, Mineral Matter	1 per Site	1 L	Dock Wall
		Non-Purgeable Organic Carbon, Dissolved Organic Carbon	1 per Site	125 mL	Dock Wall
	Biology	Environmental DNA: Presence of CO1 Gene of *Hemimysis anomala*	3 per Site	1 L	Dock Wall

Figure 6. Generalized Schematic of Ballast Uptake and Source Water Sampling Site Locations for "Voyage-Wide" Sampling Exercises, i.e., Trials 6, 11, 12 and 13. Note: Sites 1 and 2 were not sampled during Trial 6.

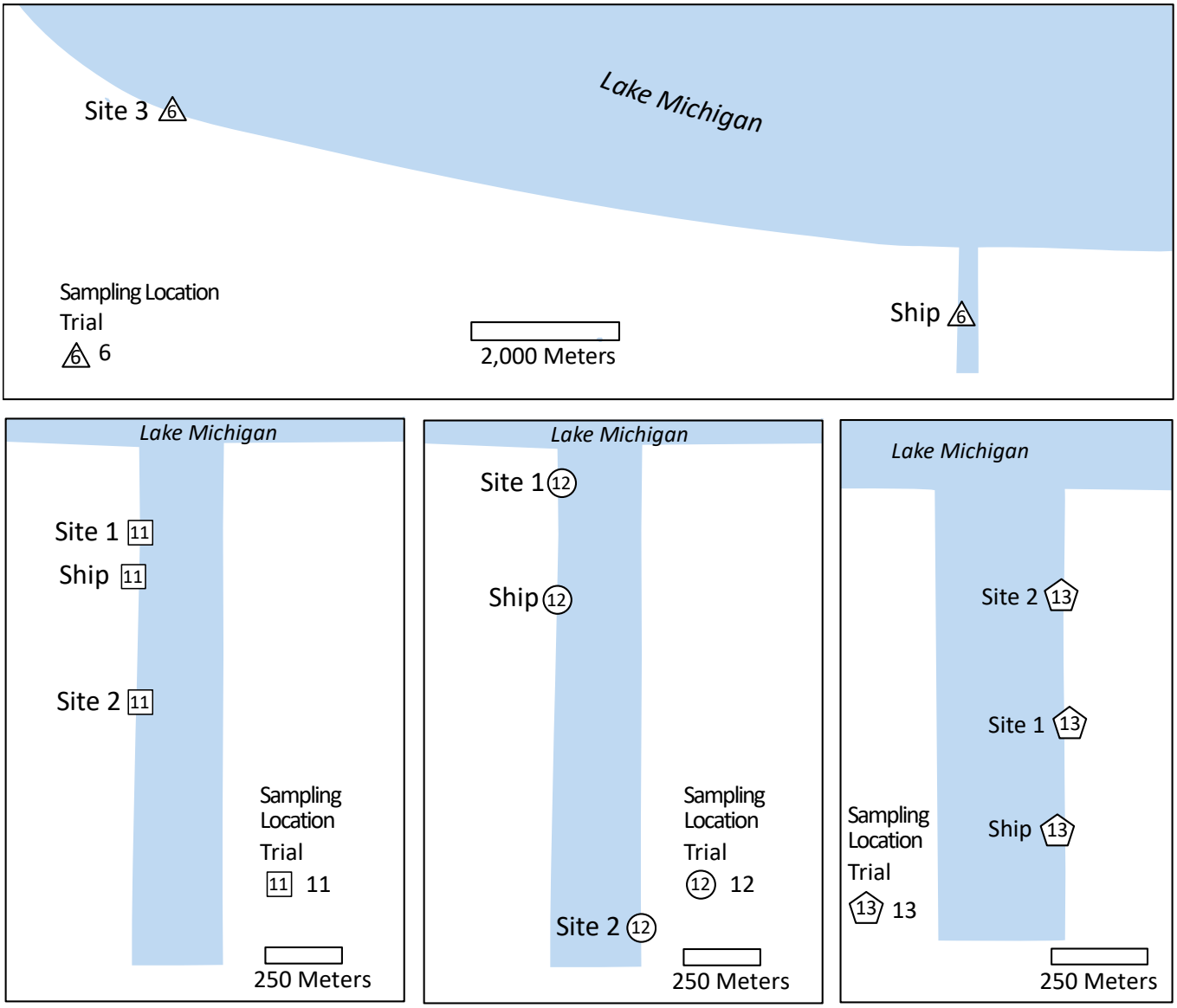
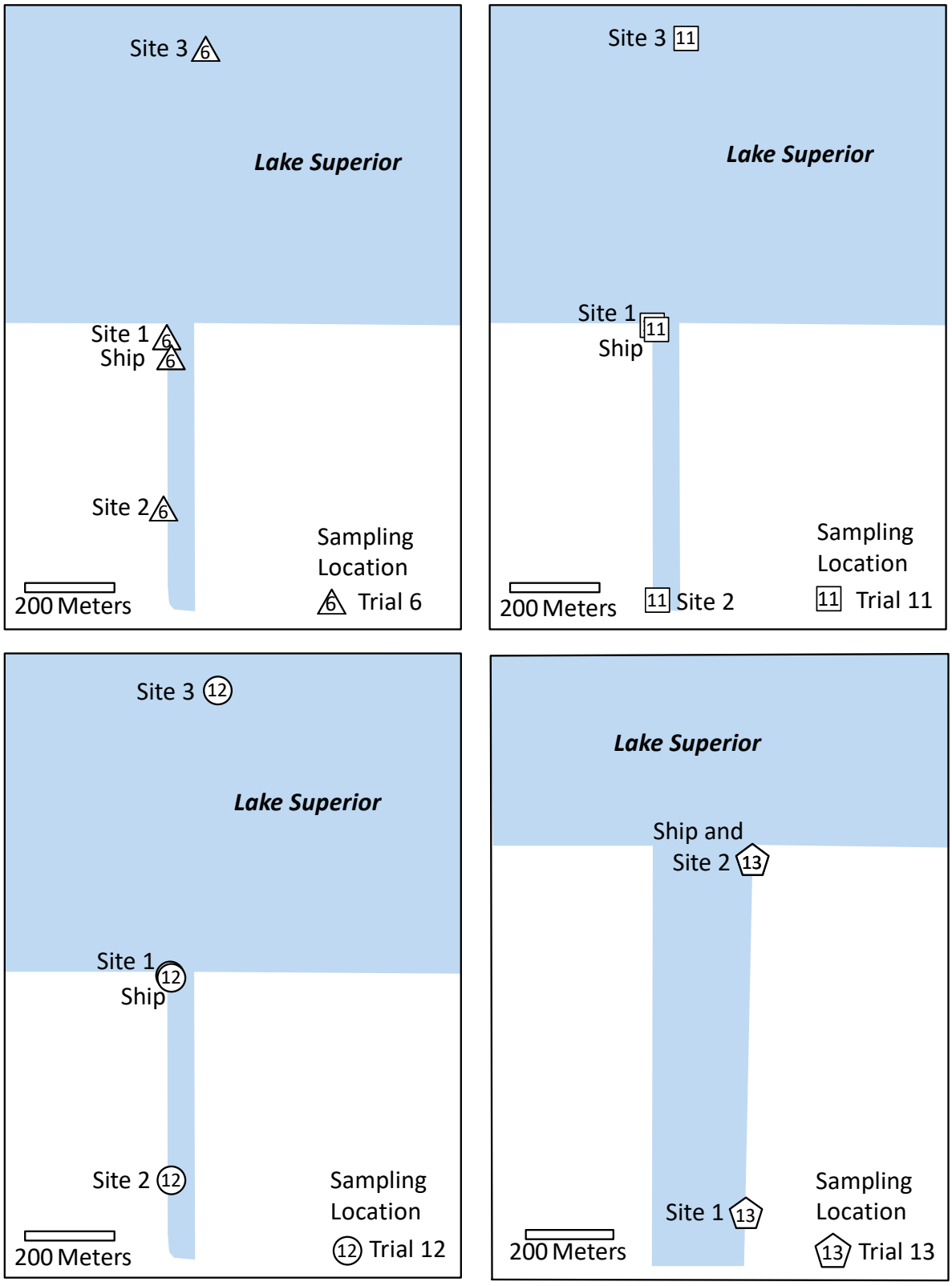


Figure 7. Generalized Schematic of Ballast Discharge and Receiving Water Sampling Site Locations for "Voyage-Wide" Sampling Exercises, i.e., Trials 6, 11, 12 and 13.



3.3. SAMPLE PROCESSING AND ANALYSIS

3.3.1. WATER CHEMISTRY AND WATER QUALITY ANALYSIS

Laboratory-based analysis of %T of ultraviolet light at 254 nm took place following *LSRI/SOP/SA/69 – Laboratory Determination of Percent Transmittance of Light in Water at 254 nm*. %T was measured on both filtered and unfiltered aliquots of each sample collected. Analysis of TSS and POM occurred according to *LSRI/SOP/SA/66 – Analyzing Total Suspended Solids, Particulate Organic Matter, and Mineral Matter (MM).* MM defined as the difference between TSS and POM, was calculated for each sample following analysis of TSS and POM. The reporting limit (RL) for TSS and POM analyses was between 1.25 (800 mL filtered) and 5.00 mg/L (200 mL filtered). Sample analyses for NPOC and DOC were conducted according to *LSRI/SOP/SA/47 – Procedures for Measuring Organic and Inorganic Carbon in Aqueous Samples* (DOC is a proxy for dissolved organic matter). Method detection limits (MDLs) were determined for water quality analyses according to *LSRI/SOP/SA/35 – Procedures for Determination of Method Detection Limit and Limit of Quantification.* Any deviations to these methods were recorded and assessed according to LSRI-GWRC QAQC processes (Section 3.3.3).

3.3.2. BIOLOGICAL SAMPLE ANALYSIS

The taxonomic diversity and total density of zooplankton in ballast water uptake and discharge samples was determined by examination of subsamples from preserved samples using either a compound or dissecting microscope in accordance with the USEPA Great Lakes National Program Office (GLNPO) procedure LG 403 (USEPA, 2016). A minimum of 400 microzooplankton (i.e., rotifers, copepod nauplii, and dreissenid mussel veligers) and 400 to 1,600 macrozooplankton (i.e., cladocerans, and copepod juveniles and adults) were targeted for examination from each sample. Larger organisms, including mysids, amphipods, and the cladocerans *Bythotrephes* and *Cercopagis,* were enumerated from the entire sample. The condition of the specimen was observed and only whole specimens indicating they were alive or recently alive when collected were included in the count. For eight of the samples (i.e., Trial 1, 4, 8, 9, 10 and 15 discharges, and Trial 6 and 8 uptakes), adult harpacticoid copepods were removed from the entire sample in order to increase the detection level of these macrozooplankton taxa. Detection levels for each taxon were calculated as the density of organisms that would be in 1 m^3 of the original water sample if a single specimen was found in the volume of water that was targeted for examination for that particular taxon. The detection levels for microzooplankton, which were examined from relatively small subsamples, are much higher than the detection levels determined for *H. anomala* which were enumerated from the entire sample (Table 5). In some trials, the presence of harpacticoid copepods were noted in the extra portion of the sample that was targeted for examination of larger organisms. In these cases, the presence of the harpacticoids was noted in the sample, but they were not quantitatively enumerated and densities were not calculated.

The density of live zooplankton in ballast discharge samples was determined according to *LSRI/SOP/GWRC/19 – Zooplankton Sample Analysis for Ship Monitoring Projects*. Live analyses were only conducted for samples that could be delivered to the analysts within four hours of sample collection, and were executed on a relatively small volume of sample water to a coarse taxonomic level. When live analyses were possible, live density of major taxonomic groups was determined by counting the number of dead organisms in a subsample and then killing the rest of the organisms and performing a total count of the same subsample. Live density was determined by subtracting the number of dead organisms from the total number of organisms.

Total protist densities and taxonomic diversity analysis of ballast uptake and discharge samples took place following the "Preserved Protist Sample Analysis (Utermöhl, 1958)" method outlined in *LSRI/SOP/GWRC/4 – Site-Specific Validation of CMFDA/FDA Stain and Determination of Protist Concentration in Ballast Water Samples*. In addition, to provide a detailed assessment of diatom assemblages, water samples were digested in strong acid to remove the organic matrix and isolate diatom valves. Diatom remains were then plated on microslides and assessed using oil-immersion light microscopy at 1250 X magnification. This method, which allows for fine taxonomic assessment of diatoms, is detailed in a SOP developed by the USEPA (i.e., SOP LG401, section 6.6; 2010).

Analysis of *E. coli* in ballast discharge followed *LSRI/SOP/SA/56 – Detection and Enumeration of Total Coliforms and E. coli using IDEXX's Colilert™*. Analysis of *Enterococcus spp.* in ballast discharge samples was conducted according to *LSRI/SOP/SA/62 – Detection and Enumeration of* Enterococcus *using Enterolert™*.

Samples for analysis of the CO1 gene of *H. anomala* were collected and processed within 24 hours according to *LSRI/SOP/GWRC/13 – Collection and Processing of Environmental DNA Samples*. Following processing, filters were submerged in Longmire's Buffer and stored in microcentrifuge tubes at -20°C. Preserved filters were held until the end of the 2017 Great Lakes shipping season, and then were shipped overnight on ice to Pennsylvania State University – Behrend for analysis. Analysis was conducted according to Knight *et al*. (2018).

Any deviations to these methods were recorded and assessed according to LSRI-GWRC QAQC processes (Section 3.3.3).

3.3.3. QUALITY ASSURANCE AND QUALITY CONTROL

All sample collection, handling, analysis, and data management activities were conducted according to the LSRI's Quality Management System as outlined in the LSRI Quality Management Plan (2017) and the GWRC Shipboard QAPP (2017). Consistent with the TQAP and the GWRC Shipboard QAPP (LSRI, 2017), any methodological deviations from the planned methods, which occurred during the course of the testing period were recorded and evaluated in deviation forms and are archived at LSRI. All TQAP and SOP deviations were assessed by the *Project's* Principal Investigator. None of the reported deviations are significant enough to render any trial findings reported here invalid. Several deviations required procedural improvements to LSRI-GWRC SOPs for future use. These preventive actions were deemed appropriate by the *Project*'s Principal Investigator.

4. RESULTS

Results are presented here in order of their relevance to the research objective, starting with biological and physical characteristics of laker ship ballast discharges to WLS. Detections of *H. anomala* (by microscope and DNA analyses), and other NIS (by microscope only), are presented along with associated organism community composition and physical/chemical data from the ballast discharges. Next, results of the four voyage-wide sampling events are presented, including measurements of the associated source system, ballast uptake, ballast discharge and the receiving system sampling events (plus one stand-alone ballast uptake sampling event). Detection levels in the tests varied by trial, taxonomic group and sampling approach, and are also reported here.

4.1. CHARACTERISTICS OF LAKER BALLAST WATER DISCHARGED TO WESTERN LAKE SUPERIOR

Fifteen ship ballast discharges to WLS were sampled between January and December 2017. All but one (which occurred in January 2017) took place July through December of 2017 (Table 4). Collectively, we sampled over 78,000 m^3 of the total 586,000 m^3 of ballast that was discharged from the targeted vessels during these sampling events (Table 4).

4.1.1. VESSEL AND SHIPBOARD SAMPLING SYSTEM OPERATIONAL DATA

GWRC never sampled the entire duration of the discharge; each given regular zooplankton sampling event was 24-61 minutes (Table 4). In total, during each sampling event, between 5 and 53% of the ballast water on board the ship was sampled during discharge (Table 4). The number of individual ballast tanks sampled during each event varied from two to sixteen (Table 4). Between 1,200 and 11,300 m^3 of ballast water was subject to sampling during each individual discharge event for regular zooplankton samples (Table 4). GWRC collected sample water at 1.4 to 3.4 m^3/hr (Table 4) to obtain sample sizes ranging from 0.91 to 2.07 m^3 for regular zooplankton samples. For five sampling events, an additional 2,900 to 11,500 m^3 of water was subjected to sampling over a period of 32 to 49 minutes for detection of *H. anomala* (Table 4). The additional sample sizes were 2.07 to 3.08 m^3 (Table 4).

Table 4. Ballast Discharge Trials: Summary of Vessel and Shipboard Sampling System Operational Parameters. Note: Trial 3 was an Uptake-Only Sampling Event and is not Presented in this Table. P= Port, S = Starboard, N/A= Not Applicable (Not Collected).

Parameter	Trial														
	1	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Date (Month, Year)	Jan-2017	Jul-2017	Aug-2017	Aug-2017	Sep-2017	Sep-2017	Sep-2017	Oct-2017	Oct-2017	Oct-2017	Oct-2017	Nov-2017	Nov-2017	Dec-2017	Dec-2017
Ballast Tank(s) Sampled (Tanks were Discharged Simultaneously)	4P, 4S	1P 2P 3P 4P 5P	1P, 1S 2P, 2S 3P, 3S 4P, 4S	1P, 1S 2P, 2S 3P, 3S 4P, 4S 5P, 5S 6P, 6S 7P, 7S 8P, 8S	1P, 1S 2P, 2S 3P, 3S 4P, 4S 5P, 5S 6P, 6S	1P, 1S 2P, 2S 3P, 3S 4P, 4S 5P, 5S 6P, 6S 7P, 7S	2P, 2S 6P, 6S 7P, 7S 8P, 8S	1P, 1S 2P, 2S 3S 4P, 4S 6P, 6S	1P, 1S 2P, 2S 3P, 3S 4P, 4S	All	1P, 1S 2P, 2S 3P, 3S 4P, 4S 5P, 5S 6P, 6S	1P, 1S 2P, 2S 3P, 3S 4P, 4S 5P, 5S 6P, 6S 7P, 7S 8P, 8S	5P, 5S	2S, 2P 6S, 6P 7S, 7P 8S, 8P Forward Draft	1P, 1S 2P, 2S
Shipboard Sampling System Used	Active	Passive	Passive	Passive	Passive	Passive	Passive	Passive	Active	Active	Active	Active	Active	Passive	Active
Shipboard Sampling System Flow Rate (m^3/hour)	2.63	1.91	1.76	2.58	2.03	2.64	1.86	2.02	3.04	2.98	2.66	2.95	1.43	2.93	3.36
Regular Zooplankton Sampling Duration (Hr: min)	0:24	0:59	1:01	0:48	1:00	0:45	0:55	0:51	0:41	0:38	0:45	0:40	0:37	0:40	0:35
Estimated Volume Discharged During Regular Sampling (m^3)	2,665	Not Known[1]	7,420	8,818	7,585	11,308	9,564	1,171	4,318	4,742	7,466	9,565	Not Known[1]	4,216	Not Known[1]
Regular Zooplankton Sample Volume (m^3)	1.04	1.86	1.78	2.04	2.02	1.96	2.00	1.70	2.07	1.97	1.98	2.02	0.91	2.04	1.99
Seep Sample Volume (L)	4	11	7	10.5	10.5	12	9.5	8	8.5	9.5	9.5	9.5	12.5	12	12

Parameter	Trial														
	1	2	4	5	6	7	8	9	10	11	12	13	14	15	16
Larger Volume *Hemimysis* Sample: Ballast Tanks Sampled[2] (Ballast Tanks were Discharged Simultaneously)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	1P, 1S 2P, 2S 3P, 3S 4P, 4S	5S, 5P 6S, 6P	1P, 1S 2P, 2S 3P, 3S 4P, 4S 5P, 5S 6P, 6S	1P, 1S 2P, 2S 3P, 3S 4P, 4S 5P, 5S 6P, 6S 7P, 7S 8P, 8S	N/A	N/A	Not Known
Larger Volume *Hemimysis* Sample: Shipboard Sampling System Flow Rate (m^3/hour)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4.11	3.88	2.66	3.7	N/A	N/A	2.91
Larger Volume *Hemimysis* Sample: Duration (Hr: min)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	0:45	0:32	0:43	0:49	N/A	N/A	0:46
Larger Volume *Hemimysis* Sample: Volume Discharged During Sampling (m^3)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	4,628	2,872	4,974	11,469	N/A	N/A	Not Known[1]
Larger Volume *Hemimysis* Sample Volume (m^3)[2]	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3.08	2.07	3.02	3.03	N/A	N/A	2.24
Total Ballast Volume on Ship (m^3)[3]	53,606	17,655	48,646	39,596	53,606	46,444	40,436	13,656	53,606	53,606	53,606	39,596	15,876	40,378[4]	15,876[5]
Percent of Volume that was Subsampled for Detection of *H. anomala* specimens	4.97%	Not Known[1]	15.2%	22.3%	14.2%	24.4%	23.6%	8.6%	16.7%	12.5%	23.2%	53.1%	Not Known[1]	10.4%	Not Known[1]

[1] Ballast discharge volumes were not calculated because electronic sounding measurements were either insufficient or could not be recorded.

[2] Large zooplankton sample was added to the test plan beginning with Trial 10. Trial 14 did not include this sample because the vessel had completed cargo loading operations during the regular zooplankton sample collection period. Trial 15 did not include this sample because the vessel's ballast main was not equipped with a return port. Trial 16 did include the large zooplankton sample, but ballast volume is not known because soundings were not able to be recorded.

[3] Data sourced from National Ballast Information Clearinghouse (NBIC, 2018).

[4] Forward draft volume not included in NBIC data.

[5] Estimate. The precise volume discharge is not available for this event. Volume listed is from a previous discharge operation in the same port from the same ballast tanks.

4.1.2. NON-INDIGENOUS SPECIES AND TARGET ORGANISM (HEMIMYSIS ANOMALA) RESULTS

The density of *H. anomala* specimens in ballast water discharged to ports in WLS was determined for the 15 separate discharge events between January and December 2017 (Table 4). The Regular Zooplankton Samples and any Larger Volume *Hemimysis* Samples were examined in their entirety for *H. anomala* specimens. The volume of water examined for specimens from each discharge trial (regular sample volume + larger sample volume) ranged widely from 0.91 (Trial 14) to 5.15 m^3 (Trial 10, Table 4), with the largest sample volumes in Trials 10, 11, 12, 13 and 16, when the additional sample was collected with a larger mesh net to increase total sample size for *H. anomala* (Table 4).

This analysis could confirm presence of *H. anomala* in instances in which it was detected; it could not confirm a complete absence of *H. anomala* in samples in which it was not detected. In samples in which there was no detection, any concentrations of these specimens were lower than the reported detection level. The detection limits vary with sample volume analyzed with greater volumes analyzed leading to a lower detection limit (Table 5). Across trials, microscopic analysis detection limits ranged from 0.19 to 1.1 organisms m^{-3} (Table 5).

H. anomala specimens were found in the ballast discharge samples from Trials 10, 11 and 13 which were large volume samples collected during October and November 2017 (Table 6). In all three of these cases, water discharged to WLS had been loaded from ports in southern Lake Michigan where *H. anomala* has been established since 2006 (Table 7). *H. anomala* densities in these samples ranged from 0.2 to 3.3 organisms m^{-3} (Table 6).

Discharge samples from Trials 6 through 16 were also analyzed for the CO1 gene of *H. anomala* (Table 6). *H. anomala* DNA was detected in all of the samples in which specimens were found (i.e., Trials 10, 11 and 13), as well as in samples from Trials 7, 14 and 15, such that six discharge events out of eleven analyzed had detectable *H. anomala* DNA (Table 6). The discharge samples which tested positive for *H. anoma*la DNA were associated with primary and/or secondary uptake events from southern Lake Michigan, northern Lake Michigan, Lake Erie and the St. Mary's River (Table 6). *H. anomala* DNA was not detected in Trials 8, 9 and 16, which were associated with uptake events from the St. Clair River, Detroit River, and Lake Ontario, respectively (Table 6).DNA was also absent from samples from two of the six discharges (Trials 6 and 12) associated with uptakes in southern Lake Michigan.

In addition to *H. anomala,* the *Project* found four additional NIS taxa of zooplankton not previously reported in Lake Superior in samples of ballast water discharged to WLS (Table 6). The benthic harpacticoid copepods *Nitokra hibernica, Heteropsyllus nunni,* and *Schizopera borutzkyi* and the cyclopoid copepod *Thermocyclops crassus* were found in concentrations ranging from 0.5 to 3.0 organisms m^{-3} in samples from nine of the 15 discharge events (i.e., Trials 1, 4, 5, 8, 9, 10, 11, 13, and 15; Tables 6 and 7). Specimens of *Nitrokra hibernica* also were observed in Larger Volume *Hemimysis* Samples from Trials 12 and 16 (Tables 6 and 7). However, because they were not *H. anomala*, the target of the large volume sample analysis, their densities were not calculated (Table 6).

Table 5. Minimum Number of Specimens per Non-Indigenous Species (NIS) Taxon (#/m^3) that would Need to be Present for Detection in Ballast Uptake and Discharge Samples Given Volumes Sampled (i.e., Project Detection Level).

Trial - Event	Microzooplankton NIS Taxon (#/m^3)	Macrozooplankton NIS Taxon (#/m^3)		*Hemimysis anomala*
	Regular Count	Regular Count	Entire Sample	Entire Sample
1 - Discharge	12	1.92	0.96	0.96
2 - Discharge	33	17.16		0.54
3 - Uptake	498	9.50		0.59
4 - Discharge	497	4.50	0.56	0.56
5 - Discharge	308	1.96	0.49	0.49
6 - Uptake	85	13.06		0.41
6 - Discharge	120	7.94		0.50
7 - Discharge	118	8.15		0.51
8 - Discharge	37	1.00	0.50	0.50
9 - Discharge	60	1.18	0.59	0.59
10 - Discharge	74	3.87	0.48	0.19*
11 - Uptake	32	3.54	0.44	0.19*
11- Discharge	30	2.03		0.25*
12 - Uptake	12	2.15		0.20*
12 - Discharge	26	2.02		0.20*
13 - Uptake	7	0.59		0.59
13 - Discharge	4	0.50		0.20*
14 - Discharge	340	1.10		1.10
15 - Discharge	8	1.96	0.49	0.49
16 - Discharge	24	8.02		0.24*

* Indicates larger sample volume collected and analyzed for *Hemimysis anomala.*

Table 6. Summary of Measured Biological Parameters from Ballast Discharge to Western Lake Superior.

DL = Detection Level, see Table 5 for values for each trial; N/A = Not Applicable (Not Collected).

Hn = Heteropsyllus nunni, Nh = Nitokra hibernica, Sb = Schizopera borutzkyi, Tc = Thermocyclops crassus

***** Organism was present in the sample, but not in the portion that was enumerated.

****At least one replicate value was less than the DL of 1 MPN/100 mL. Half of DL was used to calculate the average of the replicates.

	Trial														
Parameter	**1**	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Primary Uptake Location	Southern Lake Michigan	Lake Erie	Lake Erie	Southern Lake Michigan	Southern Lake Michigan	Southern Lake Michigan	St Clair River	Detroit River	Southern Lake Michigan	Southern Lake Michigan	Southern Lake Michigan	Southern Lake Michigan	Northern Lake Michigan	Lake Erie	Lake Ontario
Secondary Uptake Location	Eastern Lake Superior	N/A	Lake Superior	N/A	St. Mary's River, Lake Superior	St. Mary's River, Lake Superior	Eastern Lake Superior, Lake Superior	N/A	Eastern Lake Superior	St. Mary's River, Eastern Lake Superior	St. Mary's River, Eastern Lake Superior, Lake Superior	N/A	N/A	St. Mary's River	N/A
Date (Month, Year)	Jan-2017	Jul-2017	Aug-2017	Aug-2017	Sep-2017	Sep-2017	Sep-2017	Oct-2017	Oct-2017	Oct-2017	Oct-2017	Nov-2017	Nov-2017	Dec-2017	Dec-2017
Zooplankton: Total Density (#/m³)	5,000	49,600	208,000	186,000	83,400	63,900	26,600	37,500	42,800	18,700	14,100	2,600	6,800	8,000	25,700
Zooplankton: Percent Live	58%	72%	74%	NA	NA	53%	NA	72%	NA	66%	NA	63%	NA	83%	NA
***Hemimysis anomala* (#/m³)**	< DL	< DL	< DL	< DL	< DL	< DL	< DL	< DL	3.3	2.7	< DL	0.2	< DL	< DL	< DL
CO1 Gene of *Hemimysis anomala*	N/A	N/A	N/A	N/A	Not Present	Present	Not Present	Not Present	Present	Present	Not Present	Present	Present	Present	Not Present

	Trial														
Parameter	**1**	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Other Nonindigenous Species Not Previously Reported from Lake Superior (#/m³)	*Nh 1*.9	< DL	*Nh* 1.7	*Tc* 2.4	< DL	< DL	*Hn* 0.5 N*h* 3.0	*Nh* 1.8 *Sb* 0.6	*Nh* 1.9	*Sb* 2.0	*Nh**	*Hn* 0.5	< DL	*Hn* 1.5 *Nh* 1.5	*Nh**
Protists: Total Density (Cells/mL)	210	285	1,002	967	1,622	1,248	2,247	368	1,775	1,634	2,084	1,614	22,713	856	1,074
***Escherichia coli:* Density (MPN/100 mL)**	N/A	1.0**	< 1**	< 1	< 1**	7	1.4**	5.6	189.8	3.4	2.4	1.7	51.4	21.2	114.4
***Enterococcus spp.:* Density (MPN/100 mL)**	N/A	4.2	1.2**	2.7	< 1	5.5	1.3	1	133.4	1.0**	2.7	13.6	92.5	404.6	19.7

Table 7. Summary of Information on *Project*-Relevant of Non-Indigenous Species in the Great Lakes.

Taxon	Common Name	Length (mm)	Photo	Native Range	Year and Location of First Record in the Great Lakes	Current Distribution in the Great Lakes	Reference
Hemimysis anomala	Bloody Red Shrimp	6-13 mm		Freshwater margins of Black, Azov,and Ponto-Caspian Seas	2006. Southeastern Lake Ontario and channel from Muskegon Lake to Lake Michigan	Established in Lakes Ontario, Michigan, Erie and Huron. Observed in Superior Harbor of Lake Superior in 2017	Kipp, R.M., A. Ricciardi, J. Larson, A. Fusaro, and T. Makled, 2018
Heteropsyllus nunni	Harpacticoid Copepod	0.5 mm		Atlantic coast of North America	1996. Lake Michigan	Established in Lakes Michigan, Huron, and St. Clair	U.S. Geological Survey, 2018,
Nitokra hibernica	Harpacticoid Copepod	0.5 - 0.75 mm		Black and Caspian Seas, European coast of Atlantic, Arctic and Baltic Seas	1972. Mouth of Niagara River, Lake Ontario	Established in Lakes Erie, Huron, Michigan, and Ontario	Kipp, R.M., A.J. Benson, J. Larson, T.H. Makled, and A. Fusaro, 2018

Paraleptastacus wilsoni	Harpacticoid Copepod	0.45-0.48 mm		Atlantic coast of North America	2017. Southern Lake Michigan	Collected in ballast uptake from Southern Lake Michigan	This Report
Schizopera borutzkyi	Harpacticoid Copepod	0.5-0.6 mm		Black Sea Basin	1988. Lake Michigan	Established in Lakes Erie and Michigan	Kipp, R.M., J. Larson, T.H. Makled, and A. Fusaro, 2018
Thermocyclops crassus	Cyclopoid Copepod	0.7-1.1 mm		Eurasia	2014. Lake Erie	Established in Lake Erie	Sturtevant, R., and P. Alsip, 2018

4.1.3. BACKGROUND BIOLOGICAL, PHYSICAL/CHEMICAL CHARACTERISTICS

Zooplankton: The background densities of zooplankton in ballast water discharged to WLS ranged from 2,600 to 208,000 organisms m^{-3} (Table 6) and included a mixture of rotifers, copepods, cladocerans, dreissenid veligers and a few primarily benthic taxa (Appendix Table 13) during the experimental period, which mainly ranged from July – December, 2017 (Trial 1 took place in January, 2017). Highest densities (> 150,000 m^3) in sampled discharges were observed in August (Trials 4 and 5) when rotifers were at their peak abundance and comprised up to 80% of the zooplankton community (Figure 8). Dreissenid mussel veligers were common in samples collected from August through October irrespective of source water location. The density of cladocerans was greatest in the July discharge sample from Trial 2 which contained water from Lake Erie (Figure 8). Copepod nauplii, juvenile copepodids, and adults were common in all samples analyzed, and dominated the late fall and winter zooplankton community (Figure 8).

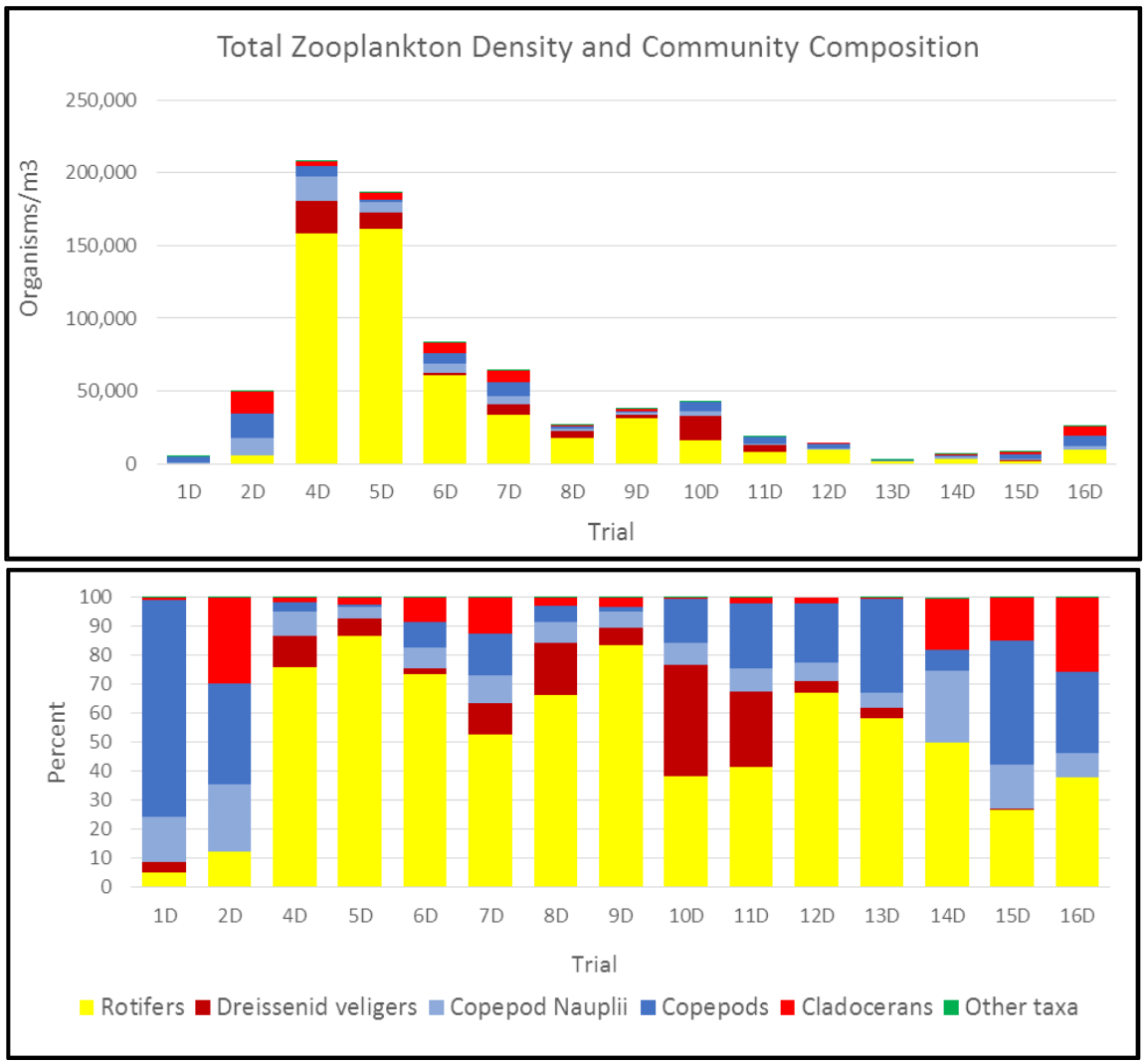


Figure 8. Total Density and Percent Composition of Zooplankton in Ballast Discharge Samples.

The diversity of zooplankton in ballast water discharged to WLS was high, with 139 distinct taxa found during the sampling period (Appendix Table 13). Individual samples contained 23 to 56 taxa, with the greatest diversity among rotifers and copepods (Figure 9). The discharge sample of ballast originating from northern Lake Michigan (Trial 14) was unique in the limited diversity of rotifers in the sample. A number of taxa that are generally found associated with the bottom sediment were collected, including fourteen species of harpacticoid copepods, three of which have not previously been reported from Lake Superior.

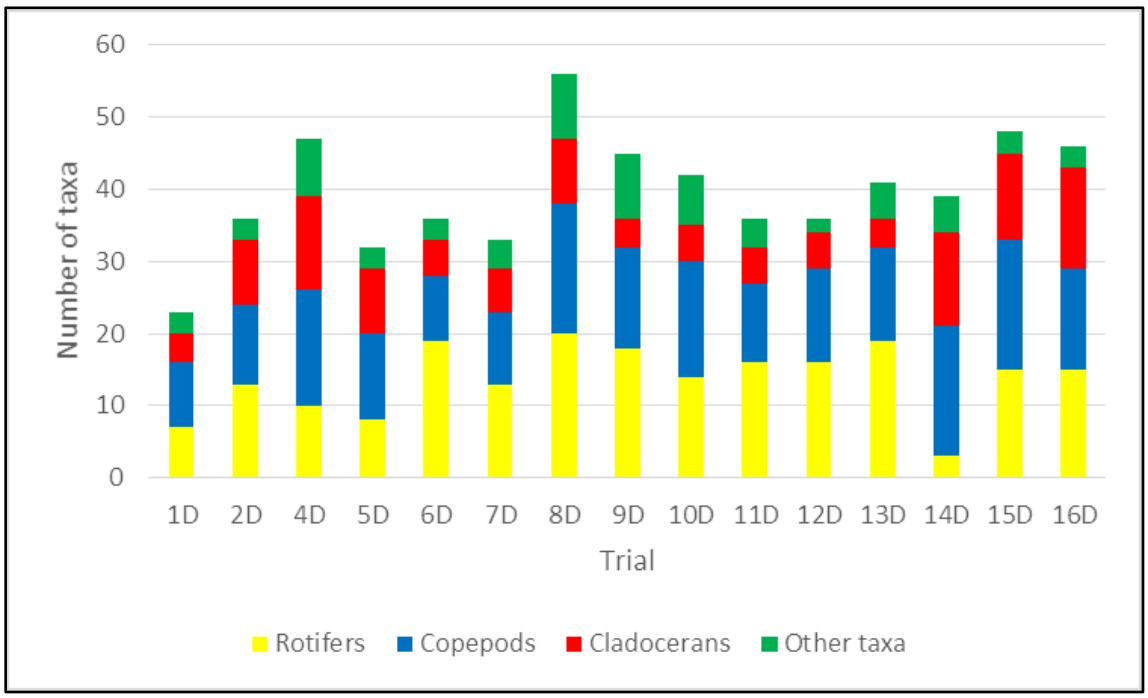


Figure 9. Number of Taxa found in Ballast Discharge (D) Samples.

The density and percentage of live zooplankton in ballast water discharge samples was determined for the eight discharges for which samples were delivered to the analysts within four hours of sample collection (Appendix Table 14). The density of live organisms ranged from 2,200 to 190,000 m^{-3} which was 58 to 84% of the total density observed (Table 6). Mortality was highest for soft-bodied rotifers such as *Polyarthra* which are easily damaged by ballast pumps and sample handling (Appendix Table 14) but overall community composition of live zooplankton (Figure 10) was similar to that of the total zooplankton (Figure 8).

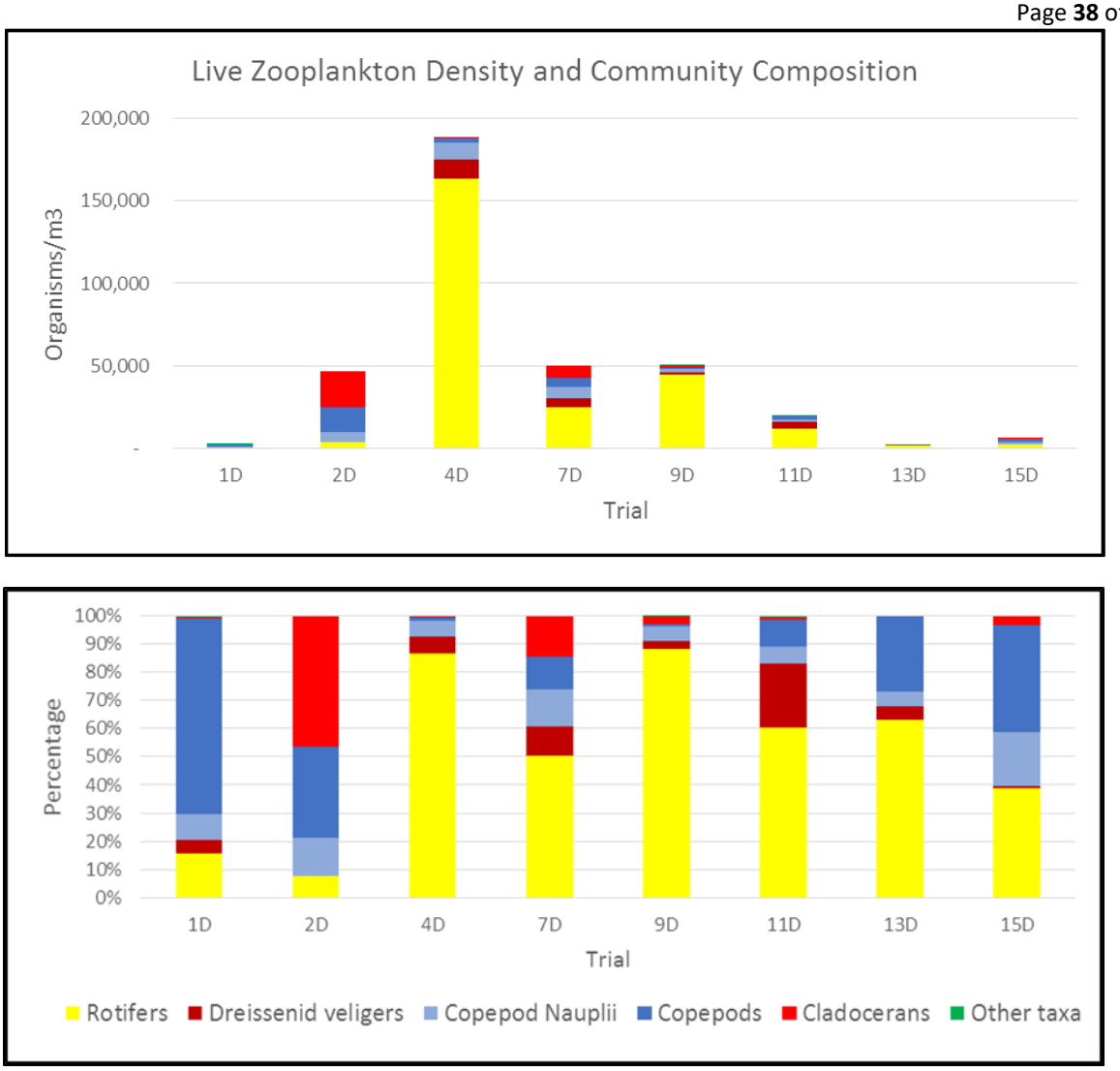


Figure 10. Density and Percent Composition of Live Zooplankton in Ballast Discharge Samples.

Protists: Protist densities in discharges ranged from 210 cells/mL (Trial 1) to 22,713 cells/mL (Trial 14) (Table 6). Assemblages were a mixture of algal groups, though at higher densities the assemblages were dominated by small-celled cyanophytes (Figure 11). Chrysophyte algae also dominated in several samples, followed by diatoms (especially in Trials 1 and 2) and cryptophytes. Green algae were rare and dinoflagellates occurred only occasionally. Two discharges stand out from the rest. Trial 9 had a fairly low density (368 cells/mL) and contained a high proportion of ciliates. Trial 14's discharge contained high concentrations of protists, indicating that the ballast tanks were likely filled during a bloom period in northern Lake Michigan, an observation that is backed by the highest chlorophyll *a* measurement in this study (Water Chemistry/Water Quality, below; Appendix Table 16). High densities in that sample were largely driven by the cyanophytes *Microcystis* and *Aphanocapsa* (Figure 11; Appendix Table 15).

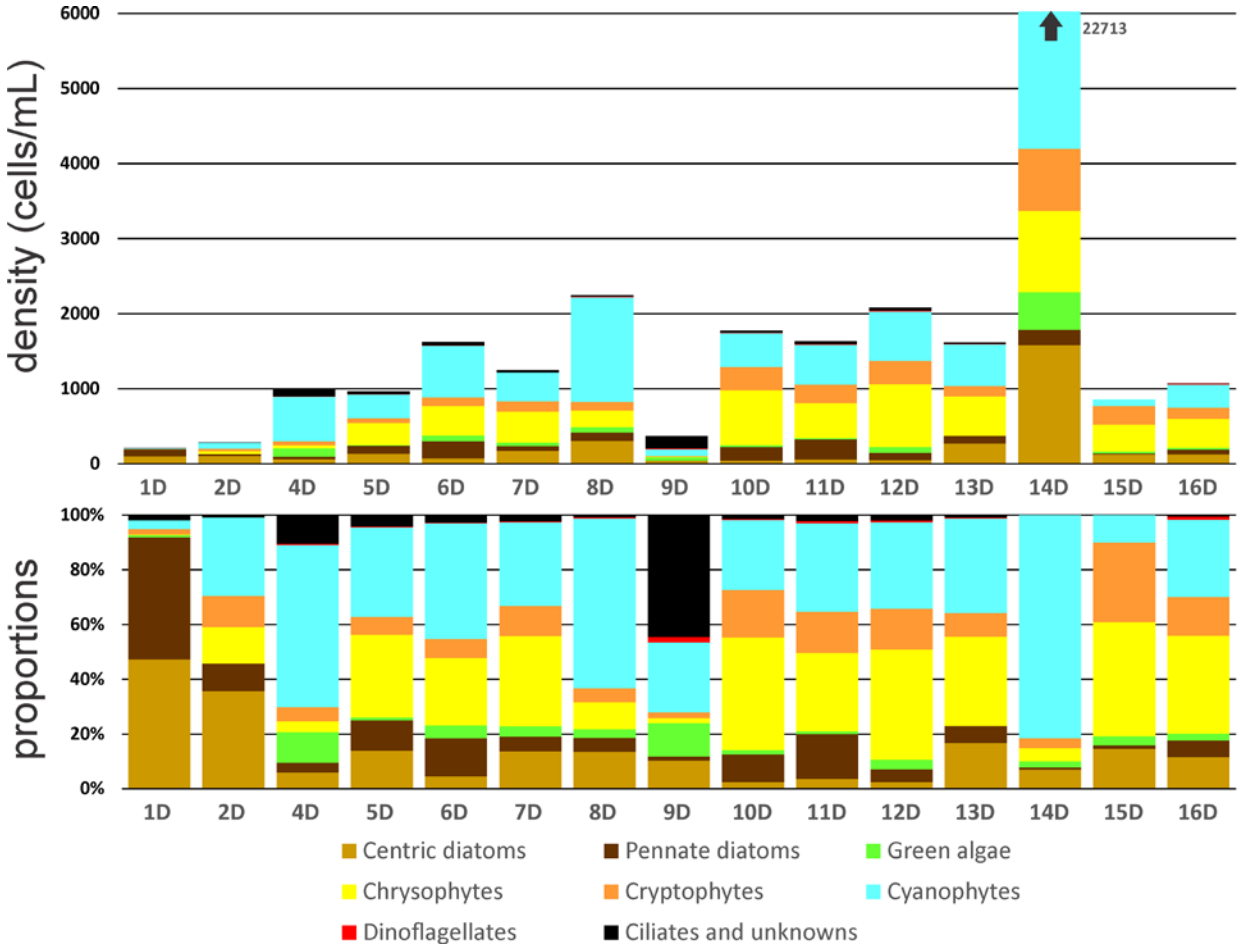


Figure 11. Histograms of Protist Densities (Upper) and Proportions (Lower) in Discharge Samples. Grouping Reflects Major Divisions of the Organisms.

Microbes: The indicator microorganisms (*E. coli* and *Enterococcus* spp.) that were only analyzed in ballast discharge did not vary significantly between source water locations. *E. coli* concentrations in discharge samples ranged from < 1 *E. coli* per 100 mL to 189.8 *E. coli* per 100 mL (Table 6). Values for each trial were below the Ballast Water Discharge Standard of < 250 colony forming units (cfu) per 100 mL set forth in the U.S. Code of Federal Regulations (Title 33; 121.1511.3).

Enterococcus spp. concentrations ranged from < 1 per 100 mL to 404.6 per 100 mL (Table 6). Two of the trials (Trial 10 from southern Lake Michigan and Trial 15 from Lake Erie) had enterococci concentrations over the acceptable Ballast Water Discharge Standard of < 100 cfu per 100 mL set forth in Code of Federal Regulations (Title 33; 121.1511.3). The remaining trials had enterococci concentrations below the required guideline.

Water Chemistry/Water Quality: Table 16 of the Appendix summarizes the discharge water chemistry and water quality parameters captured by both sondes *in situ* and analysis equipment in the laboratory across the 15 ballast discharges to WLS. Values were generally similar, except for Trial 14 discharge of water that was ballasted from northern Lake Michigan which contained distinct water chemistry/quality

values across all parameters except temperature and pH (Appendix Table 16). This trial was responsible for the lowest numbers in the range for %T values and for the highest numbers measured of turbidity, phycocyanin (blue-green) algae pigment, and TSS, among other parameters.

Water temperature varied between 3.4°C and 22.2°C in the discharge samples reflecting regional and seasonal differences. pH was slightly basic and did not vary much with values between 7.64 and 8.18. Dissolved oxygen concentrations were generally near saturation levels and ranged from a low value of 7.57 mg/L to 12.99 mg/L.

Chlorophyll *a* (uncorrected) values were generally quite low (0.06 to 1.95 µg/L) but reached a high of 4.2 µg/L during Trial 14 from northern Lake Michigan. Phycocyanin ranged from 0.06 and 0.59 µg/L (uncorrected) for all samples except Trial 14 which had a value of 2.45 µg/L, likely due to the presence of blue green algae.

Turbidity was generally quite low (1.00 to 3.98 FNU). Higher turbidity levels occurred in Trial 15's discharge from western Lake Erie (9.2 FNU) and Trial 14's discharge from northern Lake Michigan (49.8 FNU). As expected, water transparency was inversely related to turbidity with filtered percent transmittance values ranging from 56.1% to 95.9% and unfiltered portions of the sample displaying values between 31.5% and 94.9%.

TSS values varied from below the detection level to 4.1 mg/L in all samples except those from Trial 14 which had a high of 92.6 mg/L. The concentration of MM correlated well with TSS, with concentrations less than 3.3 mg/L for all samples except Trial 14 with a value of 76.4 mg/L.

POM was below the detection levels for all discharges except Trial 14 which had a POM content of 16.3 mg/L. NPOC and DOC values ranged between 1.9 and 8.7 mg/L with the highest values recorded from Trial 14. NPOC measured was comprised nearly entirely of DOC.

4.2. VOYAGE-WIDE SAMPLING (FOUR VOYAGES)

Four voyage-wide trials took place during the project period. These trials included sampling of 1) one or more southern Lake Michigan harbor water sites associated with a ballast uptake, 2) the uptake itself, 3) ballast discharge into WLS, and 4) two to three sites within the receiving harbor. Sampling took place in keeping with the TQAP, in general, with the following exceptions:

- One uptake sampling event in central Lake Erie in August (Trial 3) could not be paired with planned discharge sampling event in WLS, for logistical reasons. The data are nonetheless included to show background conditions of another port that contributes water to WLS.
- It was not practicable to sample the source water system near to the vessel or ballasting time during the Trial 6 uptake. Samples were collected over a mile from the ship, and 13 hours prior to ballasting (Figure 6, Table 9). Therefore these samples did not represent the water characteristics of the uptake berth or the time of ballasting.
- In three of the four voyage-wide trials, there was a secondary uptake of ballast water *en route* to WLS (Table 1). The interim uptake events which took place in Lake Superior or the St. Mary's River, were not sampled.

4.2.1. VESSEL AND SHIPBOARD SAMPLING SYSTEM OPERATIONAL DATA

Ballast water uptake samples were collected on five dates between August and November 2017 (Table 8). Subsamples of ballast uptake were collected for 0.5 to 1.5 hours during ballast operation at flow rates of 2.5 to 3.1 m^3/hr (Table 8). The ballast volumes subject to regular sampling ranged from >860 m^3 to 4,700 m^3 of uptake water (Table 8). An additional, larger sample volume was also collected targeting *H. anomala* in Trials 11 and 12 (Table 8). Even with this second zooplankton sample, the total amount of water sampled during each of the five uptake events was only a fraction (> 2.1 to > 13.5%) of the total volume ballasted during cargo off-loading operations (i.e., approximately 40,000 m^3 for each uptake, see Table 8).

Table 8. Ballast Uptake Trials: Summary of Vessel and Shipboard Sampling System Operational Parameters. P= Port, S = Starboard, N/A = Not Applicable (Not Collected).

Parameter	Trial				
	3	6	11	12	13
Date (Month, Year)	Aug-2017	Sept-2017	Oct-2017	Oct-2017	Nov-2017
Sampling Location	Lake Erie	Southern Lake Michigan	Southern Lake Michigan	Southern Lake Michigan	Southern Lake Michigan
Ballast Tank(s) Sampled	2S 5S	2P, 2S, 3P, 3S 4P, 4S, 5P, 5S 6P, 6S	3P, 3S 5P, 5S 6P, 6S	3P, 3S 5P, 5S 6P, 6S	5P, 5S
Shipboard Sampling System Used	Passive	Passive	Passive	Active	Active
Shipboard Sampling System Flow Rate (m^3/hour)	2.55	2.50	3.09	2.49	3.00
Sampling Duration (Hr: min)	00:40	00:59	00:40	0:45	0:31
Volume Ballasted During Sampling (m^3)	> 862*	1,459	> 3,223*	4,305	4,689
Regular Zooplankton Sample Volume (m^3)	1.69	2.45	2.26	1.86	1.69
Seep Sample Volume (L)	10.5	6.5	5	6	10
Larger Volume *Hemimysis* Sample: Ballast Tanks Sampled	N/A	N/A	1P, 1S, 3S, 4P, 4S, 5P, 5S	Not Known; No Soundings	N/A
Larger Volume *Hemimysis* Sample: Shipboard Sampling System Flow Rate (m^3/hour)	N/A	N/A	3.77	4.32	N/A
Larger Volume *Hemimysis* Sample: Duration (Hr: min)	N/A	N/A	0:47	0:42	N/A
Larger Volume *Hemimysis* Sample: Volume Ballasted During Sampling (m^3)	N/A	N/A	2,547	Not Known, No Soundings	N/A
Larger Volume *Hemimysis* Sample Volume (m^3)	N/A	N/A	3.02	3.02	N/A

Parameter	Trial				
	3	**6**	**11**	**12**	**13**
Total Volume Ballasted (m^3)[1]	40,630	42,632	42,632	42,632	39,596
Percent of Volume Sampled (Large Zooplankton Sample)	>2.1%**	3.4%	>13.5%**	>10.1%**	11.8%

[1] Data sourced from National Ballast Information Clearinghouse (NBIC, 2018).
*Entire volume not recorded due to operational error.
**Based on recorded volume which is less than total volume.

Table 9. Source and Receiving Water Samples: Summary of Location and Site Characteristics.

Sampling Event	Parameter	Trial											
		6			11			12			13		
Source Water	Sample Collection Time Relative to Ballast Sampling	-13 hours			+2.5 hours			+2.5 hours			-1 hour		
	Location	Southern Lake Michigan			Southern Lake Michigan			Southern Lake Michigan			Southern Lake Michigan		
	Site Designation	Site 3			Site 1	Site 2		Site 1	Site 2		Site 1	Site 2	
	Site Description	West of the slip along the shore			Behind vessel towards lake	In front of vessel, towards shore		Behind vessel, towards lake	From shore at interior of slip		Behind vessel towards lake	Behind vessel towards lake	
	Distance to Ballasting Ship (m)	8,486			40	200		215	950		115	450	
	Water Depth (m)	0.61			9.8	9.3		10.2	8.5		Not Recorded	8.5	
Receiving Water	Sample Collection Time Relative to Ballast Sampling	+7.5 hours			+20 hours			+16.5 hours			-2 hours		
	Ballast Hold Time	3 days			3 days			3 days			4 days		
	Site Designation	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	
	Site Description	Behind vessel, towards lake	In front of vessel, towards shore	Offshore	Behind vessel towards lake	Near shore at interior of slip	Offshore	Behind vessel, towards lake	In front of vessel, towards shore	Offshore	In front of vessel, towards shore	Behind vessel at end of slip	
	Distance to Deballasting Ship (m)	3	3	550	10	140	493	4	29	502	480	50	
	Water Depth (m)	7.5	10	5.5	10.7	3.3	4.5	11.9	8.1	5.5	1	12.8	

4.2.2. NON-INDIGENOUS SPECIES AND TARGET ORGANISM (HEMIMYSIS ANOMALA) RESULTS

Source harbor, ballast uptake, ballast discharge and receiving harbor samples were analyzed for *H. anomala* DNA (i.e., CO1 gene). Ballast uptake and discharge samples also were analyzed microscopically for *H. anomala* and other NIS specimens (Table 10). When the voyage included secondary ballast uptakes only the voyage's initial source harbor and uptake event were sampled. A stand-alone uptake sample event occurred in central Lake Erie (Trial 3) because we were unable to sample the discharge from that trial (Table 10).

Source Harbor Water: Source harbor samples showed positive results for *H. anomala* DNA from at least one sampling site close to the ship's berth (Trials 11, 12 and 13; Table 11) during the voyage-wide trials. Harbor sampling for one voyage-wide trial, Trial 6, could not occur closer than 5 miles away from the ship's berth due to private property and logistical constraints (Table 11), and that sampling event did not yield positive results for *H. anomala* DNA. However, sampling of the same harbor much closer to the ship berth in two subsequent voyage-wide trials did detect *H. anomala* DNA.

Ballast Uptake: Both *H. anomala* specimens and *H. anomala* DNA were detected in all four uptake event samples, with specimen densities ranging from 0.2 to 2.4 organisms m^{-3} (Table 10). In addition, specimens of other NIS, previously unreported in Lake Superior (Table 7), also were present in all four voyage-wide sampling events' uptake samples (Table 10). Specifically, the harpacticoid copepod *Schizopera borutzkyi* was present in all four uptakes from Lake Michigan; and *Heteropsyllus nunni* was found in three of the four Lake Michigan-based uptake samples (Table 10). *Nitokra hibernica* was found in Lake Michigan uptake water in Trials 6, 11 and 13, as well as in uptake water from the central basin of Lake Erie (Trial 3; Table 10). *Paraleptastacus wilsoni* was found in two of the Lake Michigan uptake samples; there are no previous records for this estuarine/marine species in the Great Lakes (Table 10).

Ballast Discharge to WLS: There were detections of NIS in three out of the four ballast discharges to WLS from the voyage-wide trials (Table 10). Discharge samples from two trials (i.e., Trials 11 and 13) contained both *H. anomala* specimens and DNA (Table 10). Trial 11 discharge samples also contained *Schizopera borutzkyi* specimens at a concentration of 2.0 organisms per m^{-3}; *Nitokra hibernica* was present in the discharge from Trial 12 (specimens were noted but not enumerated because the volume in which the detections occurred targeted larger macroplankton); and Trial 13 also contained *Heteropsyllus nunni* at a concentration of 0.5 organisms m^{-3} (Table 10).

Receiving Harbor: *H. anomala* DNA was detected in receiving water samples in three out of four voyage-wide receiving harbor sampling events, specifically, Trials 6, 11, and 12, but not 13 (Table 11). The detections were associated with sampling sites located within 30m of the discharge site; receiving harbor samples taken at the sampling locations furthest from the ship did not show signs of *H. anomala* DNA for any trials (Table 11). The receiving harbor samples from Trial 13, the sole receiving system sampling event in which there were no detections of *H. anomala* DNA across sampling sites, were also the only receiving system samples collected prior to the ship discharge sampling event as opposed to within one day afterward.

Table 10. Summary of Biological Parameters for Voyage-Wide Trials.
P = Present but Not Enumerated, DL = Detection Level, N/A = Not Applicable (Not Collected).

		Trial				
Parameter	**Sampling Event**	**3**	**6**	**11**	**12**	**13**
		Lake Erie	**Southern Lake Michigan**	**Southern Lake Michigan**	**Southern Lake Michigan**	**Southern Lake Michigan**
Zooplankton: Total Density (#/m³)	Uptake	250,000	93,000	22,000	11,100	1,700
	Discharge	N/A	83,400	18,700	14,100	2,600
***Hemimysis anomala* (#/m³)**	Uptake	< DL	0.4	0.4	0.2	2.4
	Discharge	N A	< DL	2.7	< DL	0.2
Other Introduced Taxa Not Previously Reported from Lake Superior (#/m³)	Uptake	19.0 *Nitokra hibernica*	1.2 *Heteropsyllus nunni* 22.9 *Nitokra hibernica* 0.8 *Paraleptastacus wilsoni* 29.0 *Schizopera borutzkyi*	0.4 *Heteropsyllus nunni* 0.9 *Nitokra hibernica* 3.1 *Schizopera borutzkyi*	P *Schizopera borutzkyi*	1.2 *Heteropsyllus nunni* 5.3 *Nitokra hibernica* 1.8 *Paraleptastacus wilsoni* 4.7 *Schizopera borutzkyi*
	Discharge	N A	< DL	2.0 *Schizopera borutzkyi*	P *Nitokra hibernica*	0.5 *Heteropsyllus nunni*
Protists: Total Density (Cells/mL)	Uptake	210	285	1,002	967	1,623
	Discharge	N/A	1,622	1,634	2,084	1,614

Table 11. Occurrence of *Hemimysis anomala* DNA and Specimens in Samples Across Voyage-Wide Trial Sampling Events. DL = Detection Level.

		Trial											
		6			11			12			13		
Source Water Samples	**Source Site**	Site 3			Site 1	Site 2		Site 1	Site 2		Site 1	Site 2	
	Source Site DNA Result	Not Detected			Present	Present		Not Detected	Present		Present	Present	
Uptake Samples	**Uptake DNA Result**	Present			Present			Present			Present		
	Uptake Density of Specimens (#/m³)	0.4			0.4			0.2			2.4		
Discharge Samples	**Discharge DNA Result**	Not Detected			Present			Not Detected			Present		
	Discharge Density of Specimens (#/m³)	< DL*			2.7			< DL*			0.2		
Receiving Water Samples	**Receiving Site**	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	
	Receiving Site DNA Result	Present	Not Detected	Not Detected	Present	Not Detected	Not Detected	Present	Present	Not Detected	Not Detected	Not Detected	

*See Table 5 for detection levels for *Hemimysis anomala* specimens for each trial.

4.2.3. BACKGROUND BIOLOGICAL, PHYSICAL/CHEMICAL CHARACTERISTICS

Zooplankton: Table 10; Appendix Table 13; and Figure 12 summarize zooplankton data from the paired uptake and discharge events during Trials 6, 11, 12, and 13 from Lake Michigan, as well as the single uptake from Lake Erie (Trial 3). Total zooplankton densities were generally similar for each of the paired uptake and discharge events (Appendix Table 13) although the percentage of rotifers in the samples was often higher in the discharge samples than in uptake samples (Figure 12). The disparate rotifer numbers may have resulted from sampling differing portions of the ballast water mass on uptake versus discharge, or rotifer reproduction during the three-day period between ballast uptake and discharge.

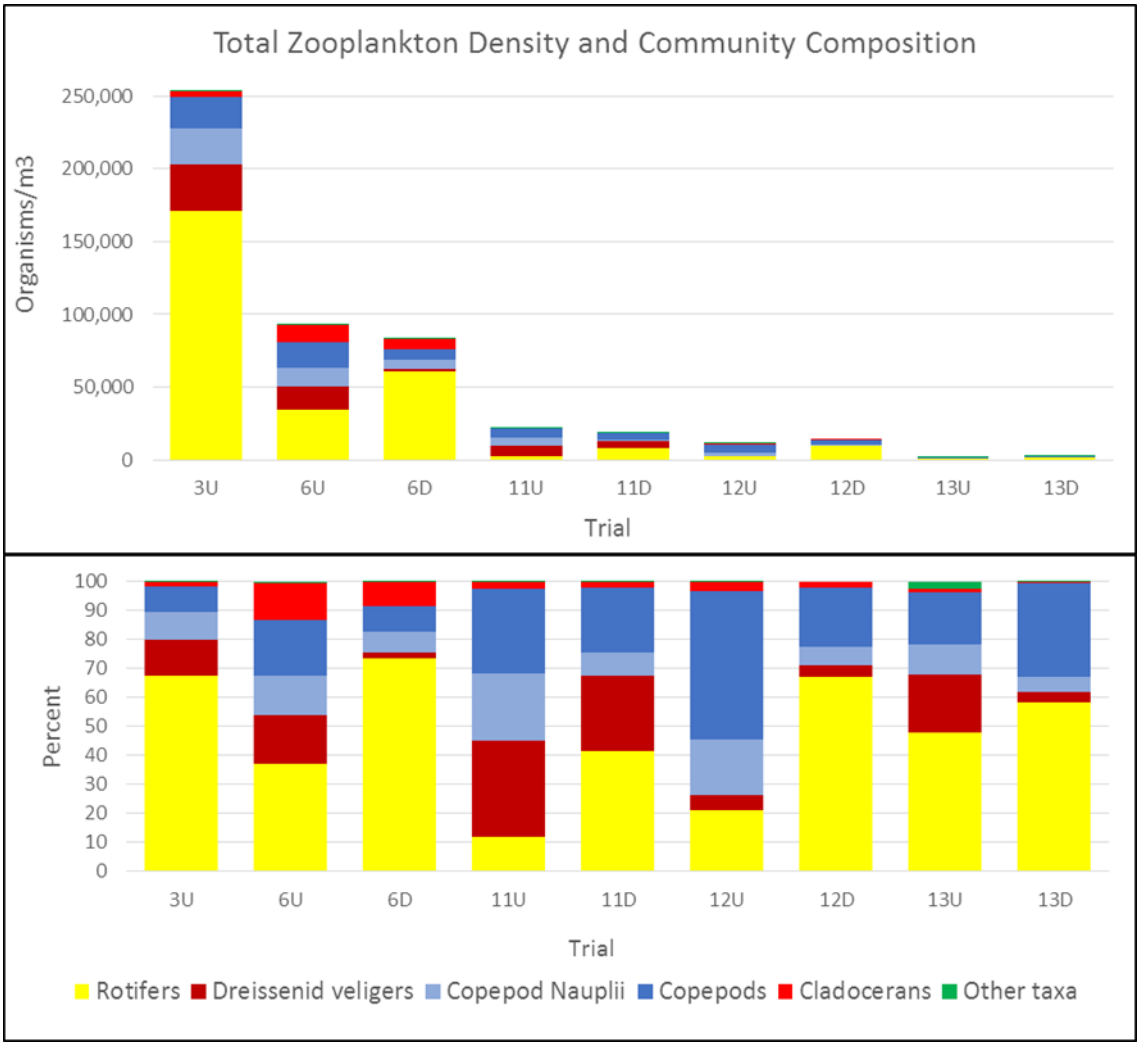


Figure 12. Density and Percent Composition of Zooplankton in Paired Ballast Uptake and Discharge Samples.

Protists: Protist densities varied widely across locations, generally increasing over the course of the year (Table 15), a trend that is largely attributed to an increase in cyanophytes such as *Microcystis* and *Aphanocapsa*. Although the earliest densities (Trials 3 and 6) were low, they were dominated by diatoms which is typical of spring assemblages in the Great Lakes. [Trials 11 and 12 had comparative uptake and discharge samples. Those densities declined by about half upon discharge due mostly to the loss of cyanophytes. The other comparative set (Trial 13) indicated no notable difference between uptake and discharge samples.] Although only a few uptake samples (Trials 3, 6, 11, 12, 13) were analyzed for protists, samples contained a mixture of taxa similar to discharge samples. Uptakes for Trials 11 and 12 had densities higher than 1,000 cells/mL and were dominated by cyanophytes (mostly *Aphanocapsa*). Trials 11 and 12 densities declined by about half upon discharge due mostly to the loss of cyanophytes (Table 10). The other comparative set (Trial 13) indicated no notable difference between uptake and discharge samples (Table 10).

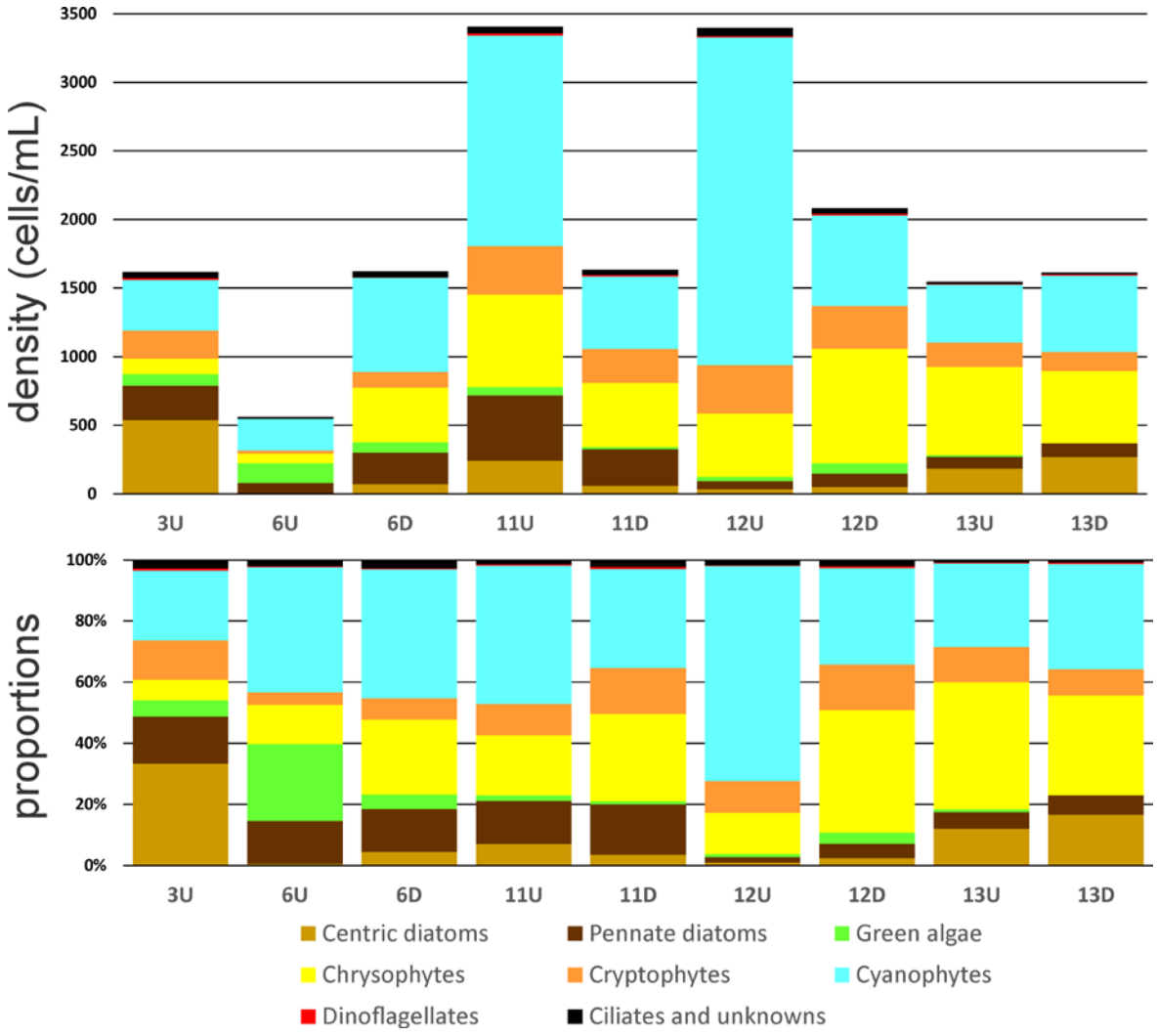


Figure 13. Histograms of Protist Assemblage Composition by Major Divisions of Organisms Showing Densities (Upper) and Proportions (Lower) in Voyage-Wide Uptake Samples. For Comparison, Voyages with Paired Uptake and Discharge Samples also have Discharge Samples Shown.

Water Chemistry/Water Quality: Water chemistry and water quality measurements were determined for samples from source harbors in southern Lake Michigan, ballast uptakes, ballast discharges, and from receiving harbors in WLS in the four voyage-wide trials (Table 12; Appendix Tables 16-19). An uptake sample also was collected from central Lake Erie (Trial 3), but no paired harbor or discharge samples were collected for this trial. During Trials 6, 11, and 12, additional water was ballasted from sites in Lake Superior between initial uptake and discharge, potentially influencing water quality in the ballast tanks during transit.

Turbidity, TSS and MM measurements were generally higher in ballast uptake and the nearby source water than in the harbor water some distance from the ship (Table 12), possibly as a result of bottom sediment being suspended during docking and ballasting operations. Levels of all three parameters dropped during transit, presumably associated with material settling in the ballast tanks, or the influence of interim uptake operations. Resulting concentrations in ballast discharges were similar to, or less than, those of the receiving waters.

The temperature of the source harbor and ballast uptake samples were quite similar to each other in a given voyage, and showed seasonal variation from 24.3°C in August (Appendix Tables 17 and 18) to 11.5°C in November. Ballast water temperatures generally cooled approximately 3-5°C during transit through Lake Superior, but were still 2- 9°C warmer than the receiving harbors where they were discharged (Table 12).

The sondes recorded uncorrected chlorophyll *a* levels of 0.19 to 1.96 µg/L and uncorrected phycocyanin levels of 0.07 to 0.53 µg/L in the paired ballast uptake and discharge samples (Table 12). Chlorophyll *a* levels dropped by approximately 0.8 µg/L between uptake and discharge for Trials 6 and 11, which may have been due to algae mortality or settling during the three-day transit, or dilution with interim ballast uptake water from Lake Superior. The low chlorophyll *a* and phycocyanin levels generally in the samples indicated that algal blooms were not occurring in the source harbors during these paired trials between September and November. A late season diatom bloom in the receiving waters of WLS during Trial 13 in November likely contributed to high chlorophyll *a* levels, reaching 8.47 µg/L, in the receiving harbor in this trial.

The transparency of both filtered and unfiltered water samples from source harbors, uptake and discharge samples and receiving waters was generally quite high with 84.5 to 97.0% transmittance at 254 nm (Table 12). The only exception was the receiving water of WLS during Trial 13 which had very low transparency (7.9 - 10.8%T).

Organic carbon, measured as NPOC and DOC, was fairly low and did not fluctuate much between source harbor, uptake, discharge and receiving water samples, ranging from 1.9 to 2.9 mg/L (Table 12). The only exception was the receiving water from Trial 13 which had high organic carbon levels (17.0-17.7 mg/L as NPOC).

Most of the other water quality parameters were similar between source harbor, uptake, discharge, and receiving water sampling events, including pH, and dissolved oxygen (Table 12). The pH was slightly basic with uptake values ranging from 7.82 to 8.18. Dissolved oxygen levels remained near saturation (Appendix Tables 16-19).

Table 12. Voyage-Wide Trials: Summary of Chemistry and Water Quality Parameters (Average ± Standard Deviation). N/A = Not Applicable (Not Collected).

Parameter	Trial 6					
	Source: Site 3	**Uptake**	**Discharge**	**Receiving: Site 1**	**Receiving: Site 2**	**Receiving: Site 3**
Temperature (°C)	21.84	21.21± 0.18	18.11 ± 0.40	16.50	16.40	16.60
Specific Conductivity (µS/cm)	363.3	303.7 ± 1.4	200.0± 14.8	102.8	108.6	103.9
Turbidity (FNU)	1.57	4.89 ± 0.85	2.37 ± 1.79	3.62	2.31	1.41
pH	8.30	8.18± 08	7.91 ± 0.23	7.87	7.97	7.98
Dissolved Oxygen (mg/L)	8.86	8.44 ± 0.02	8.91 ± 0.16	9.75	9.84	9.83
Chlorophyll *a* (µg/L)*	0.03	1.51 ± 0.33	0.69 ± 0.06	0.47	0.85	0.37
Phycocyanin Accessory Pigment (µg/L)*	0.39	0.27 ± 0.26	0.07 ± 0.02	0.19	0.18	0.18
Percent Transmittance Filtered (at 254 nm)	N/A	97.0 ± 2.0	95.9 ± 0.31	94.4	94.4	94.8
Percent Transmittance Unfiltered (at 254 nm)	N/A	95.3 ± 1.9	94.9 ± 0.12	92.5	93.8	93.4
Total Suspended Solids (mg/L)	N/A	7.9 ± 0.8	< 1.43 ± 0.0	1.9	< 1.43	< 1.43
Particulate Organic Matter (mg/L)	N/A	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.43	< 1.43	< 1.43
Mineral Matter (mg/L)	N/A	6.7 ± 0.7	< 1.43 ± 0.0	1.6**	< 1.43	< 1.43
Non-Purgeable Organic Carbon (mg/L)	N/A	2.4 ± 0.25	2.4 ± 0.24	2.1	1.8	1.7
Dissolved Organic Carbon (mg/L)	N/A	2.3 ± 0.18	2.1 ± 0.11	2.1	1.7	1.7

Parameter	Trial 11						
	Source: Site 1	**Source: Site 2**	**Uptake**	**Discharge**	**Receiving: Site 1**	**Receiving: Site 2**	**Receiving: Site 3**
Temperature (°C)	14.86	15.14	17.32 ± 0.17	13.27 ± 0.51	4.749	4.941	4.669
Specific Conductivity (µS/cm)	306.7	312.6	292.5 ± 4.3	241.9 ± 3.76	103.7	107.3	103.2
Turbidity (FNU)	2.82	1.2	5.96 ± 0.23	2.12 ± 0.27	1.35	2.22	1.29
pH	8.21	8.23	8.13 ± 0.03	7.9 ± 0.12	7.9	7.92	7.93
Dissolved Oxygen (mg/L)	10.05	9.97	9.92 ± 0.02	10.43 ± 0.18	12.82	13.09	13.02
Chlorophyll *a* (µg/L)*	1.42	1.51	1.96 ± 0.32	1.10 ± 0.22	0.28	0.35	0.23
Phycocyanin Accessory Pigment (µg/L)*	0.22	0.22	0.20 ± 0.01	0.41 ± 0.03	0.2	0.3	0.34
Percent Transmittance Filtered (at 254 nm)	93.6	95.0	93.3 ± 0.90	94.8 ± 0.11	96.7	96.4	96.4
Percent Transmittance Unfiltered (at 254 nm)	92.7	94.2	89.9 ± 0.16	93.7 ± 0.25	96.2	95.9	96.4
Total Suspended Solids (mg/L)	5.0	< 1.25	6.0 ± 0.5	< 1.25 ± 0.0	< 1.25	< 1.25	< 1.25
Particulate Organic Matter (mg/L)	< 1.25	< 1.25	< 1.25± 0.0	< 1.25 ± 0.0	< 1.25	< 1.25	< 1.25
Mineral Matter (mg/L)	4.3	< 1.25	5.0 ± 0.5	< 1.25 ± 0.0	< 1.25	< 1.25	< 1.25
Non-Purgeable Organic Carbon (mg/L)	2.4	2.3	2.5 ± 0.26	2.6 ± 0.37	1.8	1.5	1.6
Dissolved Organic Carbon (mg/L)	2.2	2.1	2.3 ± 0.06	2.3 ± 0.17	1.5	1.5	1.5

Parameter	Trial 12						
	Source: Site 1	**Source: Site 2**	**Uptake**	**Discharge**	**Receiving: Site 1**	**Receiving: Site 2**	**Receiving: Site 3**
Temperature (°C)	13.26	13.39	15.48 ± 0.33	11.40 ± 0.18	4.62	4.53	4.58
Specific Conductivity (µS/cm)	297.6	310.7	278.7 ± 3.2	249.0 ± 2.5	104.7	106	100.4
Turbidity (FNU)	2.56	1.87	6.33 ± 0.39	1.99 ± 0.08	8.23	8.85	4.53
pH	8.17	8.17	7.97 ± 0.11	7.94 ± 0.07	7.79	7.59	7.6
Dissolved Oxygen (mg/L)	10.55	9.96	10.07 ± 0.03	10.00 ± 0.04	12.42	12.5	12.77
Chlorophyll *a* (µg/L)*	0.12	1.85	1.32 ± 0.18	1.41 ± 0.33	0.67	0.82	0.81
Phycocyanin Accessory Pigment (µg/L)*	0.43	0.48	0.32 ± 0.02	0.23 ± 0.01	0.34	0.37	0.39
Percent Transmittance Filtered (at 254 nm)	94.6	94.1	93.8± 0.00	94.2 ± 0.35	90.3	89.2	94.0
Percent Transmittance Unfiltered (at 254 nm)	93.4	93.3	89.1 ± 0.76	92.5 ± 0.50	86.2	84.5	92.0
Total Suspended Solids (mg/L)	5.3	2.7	4.6 ± 1.1	< 1.25 ± 0.0	3.5	3.1	1.8
Particulate Organic Matter (mg/L)	< 1.25	< 1.25	< 1.25 ± 0.0	< 1.25 ± 0.0	< 1.25	< 1.25	< 1.25
Mineral Matter (mg/L)	4.4	1.8	3.7 ± 1.0	< 1.25 ± 0.0	3.1	2.7	1.4
Non-Purgeable Organic Carbon (mg/L)	2.5	2.5	2.2 ± 0.11	2.0 ± 0.12	2.2	2.0	1.7
Dissolved Organic Carbon (mg/L)	2.5	2.6	2.1 ± 0.13	1.9 ± 0.11	1.9	2.0	1.6

Parameter	Trial 13					
	Source: Site 1	**Source: Site 2**	**Uptake**	**Discharge**	**Receiving: Site 1**	**Receiving: Site 2**
Temperature (°C)	11.53	11.74	13.04 ± 0.08	8.15 ± 0.32	2.968	1.737
Specific Conductivity (µS/cm)	312.3	310.4	311.0 ± 1.7	246.4 ± 7.5	205	190.4
Turbidity (FNU)	4.46	3.7	6.34 ± 4.92	3.98 ± 2.26	30.95	37.68
pH	8.15	8.07	8.18 ± 0.05	8.04 ± 0.06	7.8	7.91
Dissolved Oxygen (mg/L)	10.53	10.33	10.37 ± 0.02	11.03 ± 0.05	12.49	13.09
Chlorophyll *a* (µg/L)*	0.91	1.34	0.19 ± 0.00	0.28 ± 0.04	8.47	7.9
Phycocyanin Accessory Pigment (µg/L)*	0.26	0.23	0.53 ± 0.22	0.26 ± 0.01	0.54	0.61
Percent Transmittance Filtered (at 254 nm)	92.8	92.9	93.2 ± 0.04	91.4 ± 0.04	10.8	9.5
Percent Transmittance Unfiltered (at 254 nm)	90.7	91.0	91.3 ± 0.11	90.2 ± 0.27	8.3	7.9
Total Suspended Solids (mg/L)	3.7	4.2	3.4 ± 0.3	< 1.25 ± 0.39	26.8	7.7
Particulate Organic Matter (mg/L)	< 1.67	< 1.67	< 1.25 ± 0.0	< 1.25 ± 0.0	2.7	< 1.67
Mineral Matter (mg/L)	3.2	3.4	2.9 ± 0.4	< 1.25 ± 0.0	24.1	7.0**
Non-Purgeable Organic Carbon (mg/L)	2.2	2.5	2.6 ± 0.08	2.9 ± 1.1	17.0	17.7
Dissolved Organic Carbon (mg/L)	2.1	2.2	2.5 ± 0.13	2.6 ± 0.51	17.2	17.4

*Values are for relative comparison only, values are not calculated with a correction factor.
**POM was less than the reporting limit but was measurable. Actual measured value was used in MM calculation.

5. DISCUSSION AND CONCLUSION

This *Project* sampled a small fraction of the United States and Canadian laker vessel ballast water discharges to WLS originating from outside Lake Superior during 2017. For perspective, over the course of 939 vessel visits to WLS ports in 2017, ships discharged 27.2 million cubic meters of water from non-WLS Great Lakes navigation system source harbors (NBIC, 2018). Appendix Table 20 lists the volumes associated with the top twenty sources of non-WLS ballast water to WLS ports. We sampled just 15 discharges or 2.2 percent of the 2017 total. Our sampling occurred within just a portion of the shipping season (mostly July – December). Further, our sampling events were relatively short (i.e., 0.5 to 1.5 hours) compared to the duration of overall ballast operations. Volumes sampled per target discharge event were just 1,200 to 21,000 m^3, or 5 to 53 percent, of the volume deballasted.

It is notable that despite these limitations, the research presented here was nonetheless sufficient to address the fundamental *Project* research question as to presence of *Project*-relevant NIS (i.e., NIS not previously recorded in Lake Superior) in laker ballast uptake in the lower four Great Lakes and discharge to WLS. Specifically, we detected our target invader, *Hemimysis anomala*, a species unreported in Lake Superior at the time of this research, in multiple sampling events. We detected the non-indigenous cyclopoid copepod *Thermocyclops crassus,* not previously recorded in WLS, in the ballast discharge of one trial (Trial 5) which had taken up water in southern Lake Michigan. In ballast uptake and/or discharge samples we found three non-indigenous harpacticoid copepod species (*Heteropsyllus nunni, Nitokra hibernica,* and *Schizopera borutzkyi*) not previously reported in WLS, though they have been previously reported in the Great Lakes (Table 7). We also detected in one ballast uptake the harpacticoid *Paraleptastacus wilsoni*, an NIS never before reported in the Great Lakes; ours is the first record. The condition of the specimens met the requirements of the *Project* methods of inclusion, indicating that the organisms were alive or recently alive upon collection.

Whether, and for how long, any NIS species detected in this *Project* already may have been in WLS harbors is an important question, and the answer is more certain for some taxa than others. Our target NIS, *Hemimysis anomala*, is readily captured with conventional sampling methods, so its distribution in the Great Lakes is fairly well documented (first detection, 2006; established in southeastern Lake Ontario and channel from Muskegon Lake to Lake Michigan, Table 7). The only known detection in Lake Superior was reported recently from an independent U.S. Fish and Wildlife Service study which took place contemporaneous with our work: a single specimen (live/dead status unknown) of *H. anomala* was collected at a site near one of our WLS receiving ports during the summer of 2017 (Kipp *et al*., 2017). We detected *H. anomala* specimens in uptake water from southern Lake Michigan (Trials 6, 11, 12, and 13) and in discharge water to WLS (Trials 10, 11, and 13). *H. anomala* DNA was also present in water samples from all of the trials in which *H. anomala* specimens were detected, as well as in additional ballast water discharge samples from Trials 7 (uptake from southern Lake Michigan), 14 (uptake from northern Lake Michigan), and 15 (uptake from Lakes Erie and/or St. Mary's River). The greater prevalence of *H. anomala* DNA than specimens in our samples is consistent with the greater sensitivity of genetic environmental indicators than microscopic analysis in the context of relatively small volumes (< 6 m^3) of ballast water.

H. anomala DNA also was detected in samples of the source water adjacent to vessel ballasting and deballasting activities, suggesting that these source harbors were a likely origin of the genetic material in the ballast water, as opposed to it being residual from some other ballast operation. The *H. anomala*

DNA we detected in the receiving water of WLS was in samples collected near in time and location to the ballast discharge site. The receiving water sites that we sampled at some distance from the subject ballast discharge did not show the presence of *H. anomala* DNA.

The cyclopoid copepod NIS, *Thermocyclops crassus,* which we detected in our samples is also planktonic in nature and would likely have been captured, if present in large enough numbers, in prior harbor monitoring exercises. This species, first detected in 2014 and now established in Lake Erie (Connolly *et al*., 2017), has not yet been detected in Lake Superior. It's presence in ballast water that was taken up in Lake Michigan and discharged in WLS (Trial 5) suggests that its range in the Great Lakes may have expanded and that laker ballast water transport is an active vector.

In contrast to these planktonic NIS species, the duration of occurrence in the Great Lakes and WLS is less certain for the benthic harpacticoid copepod NIS we detected, i.e., *Heteropsyllus nunni, Nitokra hibernica, Schizopera borutzkyi, and Paraleptastacus wilsoni*. Not surprisingly, the highest density of harpacticoid copepods, including the harpacticoid copepods NIS, that we detected were in an uptake sample (Trial 6 in southern Lake Michigan) in which a lot of debris was present, suggesting that the harbor bottom sediment had been disturbed prior to or during the ballasting operation. The timing of first introduction or establishment of these harpacticoid copepod NIS in Great Lakes harbors is difficult to discern. Benthic zooplankton often are not targeted in routine harbor zooplankton surveys. Plankton samples supporting existing literature on NIS presence in WLS (and elsewhere) are generally collected with fine mesh nets (63 to 153 µm) that are towed from 1 or 2 m above the bottom to the water surface. These samples retain small planktonic organisms while minimizing disturbance of the bottom sediments where benthic species reside. Meanwhile, benthic sampling methods—grab samples often sieved through 250 to 500 µm mesh—target larger bottom dwelling organisms, such as insect larvae and amphipods. Thus, neither of these sampling regimens is optimized to routinely or quantitatively capture the small harpacticoid copepods associated with bottom sediments which we found entrained in ballast water samples.

Of the harpacticoid copepod NIS we detected, *Heteropsyllus nunni, Nitokra hibernica,* and *Schizopera borutzkyi* have been recorded in the Great Lakes, though not in WLS. First detections in one or more of the lower four Great Lakes of these species were recorded in 1996, 1972, and 1998, respectively (Table 7). The harpacticoid copepod NIS *Paraleptastacus wilsoni* which we detected in southern Lake Michigan uptake water, has never before been reported in the Great Lakes; ours is the first record. Examination of harbor sediments in more detail may reveal wider distribution of the harpacticoid copepod NIS we found, or specimens of additional harpacticoid copepod NIS for which laker ballast operations are a ready vector. Examination of previously collected harbor samples could help to establish a timeline for the appearance of these species.

The *Project* experimental design, including all biological and physical/chemical assessments of ballast water *vis a vis* harbor water, did not set out to—and should not be used to—inform estimates of the rate of survival of detected NIS zooplankton species upon discharge to, or risk of establishment in, a receiving system over time. Such an assessment, if possible at all, would require a substantially different experimental design and set of methods.

With respect to other categories of organisms analyzed in this study, our protist analyses did not set out to detect, and did not incidentally detect, non-indigenous protist taxa; speciation and confirmation of historical presence of these tiny organisms is quite difficult. Accordingly, non-detection through this

study is not the same as nonexistence. The USCG Ballast Water Discharge Standard set forth in Code of Federal Regulations (Title 33; 121.1511.3) limits the discharge of these species to < 250 cfu per 100 mL for *E. coli* and < 100 cfu per 100 mL for *Enterococcus* spp. All discharges were consistent with the Ballast Water Discharge Standard limits for these indicator organisms, except two of the trials, Trial 10 from Southern Lake Michigan and Trial 15 from Lake Erie, which had *Enterococci* spp. concentrations which exceeded the acceptable limit at 133 and 405 MPN per 100 mL, respectively. With respect to physical/chemical conditions, the project data reflect expected Great Lakes water quality including the instances of sediment disturbance or algal bloom conditions during uptake.

Several research priorities directly follow from our findings; further monitoring solely to determine if laker ships are an active vector for NIS movement from the lower four Great Lakes to Lake Superior is not one of them. The vector has been adequately demonstrated by this and previous studies (e.g., Adebayo et al, 2014). Future research should focus on identification of best management practices (BMPs)/ballast water management systems (BWMSs) with strong applicability to, and practicability within, the special case of lakers. The BWMS/BMP research scope should include examination of any feasible alternatives for lakers that may significantly reduce live organisms in discharge, even if effectiveness may be incomplete relative to the USCG/IMO discharge standard, or is limited to a subset of target taxonomic categories. This research will clearly be productive as effective and practicable BWMS/BMP alternatives for lakers have not yet been identified or broadly accepted due to several unknowns. Further, the research value will be durable over time. That is, even if transoceanic organism transfers by saltie ships into the Great Lakes were attenuated by policy and regulatory advances, multiple vectors of NIS to the Great Lakes and changing climatic conditions will perpetuate the potential for laker involvement in unwanted NIS spread for the foreseeable future. An example of a non-ship-mediated harmful organism that led to urgent concerns over potential spread by laker ships was the emergence of the rhabodvirus VHS virus, a virulent fish pathogen with an earliest known occurrence in Great Lakes fish tissue of 2003 (Bain et al, 2010). Research priority also should be placed on developing reliable and cost-effective approaches to monitoring harbors and ship ballast water for specific new unwanted NIS species. This capacity will enable any emergency responses to newly-identified unwanted NIS to be more effective and efficient for industry and the environment. Finally, there should be on-going research on the rates and patterns of laker NIS movements within the Great Lakes, and ways to characterize the relationship between organism drop-off rates and patterns, and organism establishment, i.e., the risk-release relationship. This research could help elucidate the associated value of BMPs/BWMSs implementation in the special case of US and Canadian laker fleets voyage patterns, or a particular unwanted NIS species. However, risk-release research is far more long-term in nature, and the value of any findings more ephemeral than the other research priorities stated, as each species has unique requirements for establishment, and organism communities and receiving systems constantly adapt and change over time.

In summary, this research detected NIS of aquatic organisms which were not previously recorded in Lake Superior (and in one case, the Great Lakes), including the target NIS *Hemimysis anomala*, in laker ballast water discharged in WLS. In voyage-wide sampling, evidence of project-relevant NIS were found in the source harbors, the ballast uptake and ballast discharge to WLS. The *Project* detected these species though it surveyed only a fraction of the ship discharges to WLS in 2017, only a small portion of the target discharge events, and only snapshots of the shipping season. Next research steps should focus on practicability and efficacy assessments of best BMPs/BWMSs alternatives for the laker fleets of ships, harbor and ballast water surveillance for unwanted NIS, and further characterization of the risk-release relationship for laker-mediated NIS movements in the Great Lakes.

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APPENDIX

Tables 13 – 20 below provide *Project* measurement values and data referenced in this report. Tables include:

- Table 13. Density of Zooplankton (#/m^3) in Shipboard Ballast Uptake (U) and Discharge (D) Samples.
- Table 14. Density of Live Zooplankton (#/m^3) in Shipboard Discharge Samples. D = Discharge.
- Table 15. Density of Protists (cells/mL) in Shipboard Ballast uptake (U) and Discharge (D) Samples.
- Table 16. Ballast Discharge Trials: Summary of Water Chemistry and Water Quality Parameters (Average ± Standard Deviation).
- Table 17. Ballast Uptake Trials: Summary of Water Chemistry and Water Quality Parameters (Average ± Standard Deviation). NM = Not Measured.
- Table 18. Source Water Trials: Summary of Chemistry and Water Quality Parameters. NC = Not Collected.
- Table 19. Receiving Water Trials: Summary of Chemistry and Water Quality Parameters.
- Table 20. Major Sources and Volumes of Ballast Water Discharged to Western Lake Superior from Other Great Lakes Ports in 2017.

Table 13. Density of Zooplankton (#/m^3) in Shipboard Ballast Uptake (U) and Discharge (D) Samples.

*****Taxa introduced into the Great Lakes and not previously reported from Lake Superior. [a]Taxa were examined in the entire preserved 35 µm mesh zooplankton sample. [b]Value includes results from an additional 400 µm mesh zooplankton sample. **P**-Taxa was present but not enumerated.

	Trial																		
Organism	**1_D**	**2_D**	**3_U**	**4_D**	**5_D**	**6_U**	**6_D**	**7_D**	**8_D**	**9_D**	**10_D**	**11_U**	**11_D**	**12_U**	**12_D**	**13_U**	**13_D**	**14_D**	**15_D**	**16_D**
Rotifers																				
Ascomorpha ecaudis									74											
Ascomorpha ovalis						85	120		148	715	74	32								
Asplanchna priodonta			995						111											
Bdelloidea												32		12			8.2		7.8	
Brachionus havanaensis				497																
Cephalodella gibba						85											4.1			
Cephalodella macrodactyla																33				
Cephalodella spp.		33								P	74			P						
Collotheca spp.		66	995		4,614	256	3,118	945	371	715	891	443	1,342	206	1,252	86	198		7.8	24
Conochilus unicornis			498			426	2,159	1,300	1,856		817	32	244	P	678	33	62			71
Dicranophoridae									37											
Filinia terminalis																				24
Gastropus stylifer	12					1,789	600	2,008	854	417	1,114	32	579	97	704	46	334		481	306
Kellicottia bostoniensis												32								47
Kellicottia longispina		398		497			480	1,181	1,411	P	594		579	24	496		140		85	71
Keratella cochlearis	94	2,121	26,372	56,106	308		120	709	1,373	13,529	1,411	316	579	109	652		288	2,378	1,242	1,767
Keratella crassa	23	1,226	995	49,154	615	511	240	236	1,002	2,682	74	32	P		P			679	31	24
Keratella earlinae		331	3,483	23,832	923		P	591	74	1,073			P	P		6.6	4.1	340	47	118
Keratella hiemalis																	4.1		16	
Keratella quadrata		66																		24
Keratella spp.										P										
Keratella tecta						85				60		32	P			13	4.1		39	
Lecane flexilis									520	60	P		30	12	P		4.1			

	Trial																		
Organism	**1_D**	**2_D**	**3_U**	**4_D**	**5_D**	**6_U**	**6_D**	**7_D**	**8_D**	**9_D**	**10_D**	**11_U**	**11_D**	**12_U**	**12_D**	**13_U**	**13_D**	**14_D**	**15_D**	**16_D**
Lecane inermis																			7.8	
Lecane luna		33																		
Lecane tenuiseta																	4.1			
Lecane ungulata																6.6				
Monostyla closterocerca						256	P	118	223	60	520	32	P	P	26	13	16			
Monostyla copeis							P										4.1			
Monostyla lunaris							P		111				P	P					7.8	
Monostyla spp.		66																		
Notholca acuminata																13			7.8	
Notholca labis															P					
Ploesoma hudsoni						85														
Ploesoma truncatum			1,493	993	1,846	1,192	1,919	945	148	238	223									
Polyarthra major		33	4,478	497	308		120			119		32	61		P		37			
Polyarthra remata	12	66	41,797	497	129,508	14,906	37,176	13,114	3,675	7,569	6,165	949	1,768	279	1,799	60	29			94
Polyarthra vulgaris	35	1,657	77,126	25,322	23,072	11,243	13,072	11,460	1,039	3,218	1,857	348	1,677	121	704		91		132	5,255
Stephanoceros fimbriatus						341	120													
Synchaeta spp.	70		12,937			3,151	1,799	827	4,380	775	2,525	316	762	1,480	3,156	497	288		39	1,815
Trichocerca bicristata							P													
T. multicrinis		33																		
T. porcellus															P					94
T. rousseleti							120	354	186	119			152		26		16		7.8	
Trichocerca similis				497		85			37											
Trichocerca spp.						85								P						
Trichocerca tigris																6.6				
Trichotria pocillum												P		P	P	6.6				
Trichotria spp.	12																			
Trichotria tetractis														12		6.6				
Unknown rotifer																6.6				24

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Mollusca																				
Dreissena veligers	176		31,845	22,343	11,074	15,502	1,679	6,734	4,789	2,146	16,564	7,279	4,817	582	548	352	87		31	
Copepods																				
Copepod nauplii	774	11,468	24,382	17,378	7,383	12,861	6,236	6,262	1,893	2,205	3,120	5,032	1,555	2,123	887	179	140	1,699	1,218	2,097
Cyclopoids																				
Acanthocyclops spp.											P	P	P	P	P					
Acanthocyclops brevispinosus	P	17			23	65	7.9	16	2.0	2.4	7.7	81		97	2.0	33	1.0	16	2.0	
Acanthocyclops robustus		34	28	31					1.0	2.4								23	14	P
Diacyclops thomasi	54	566		58	14	196	40	81	66	3.5	12	11	12	19	12	20	1.5	22	20	497
Eucyclops agilis						P														
Eucyclops elegans												P						1.1		
Eucyclops prionophorus																		2.2		
Eucyclops spp.				4.5					1.0									6.6	P	
Macrocyclops albidus																				P
Mesocyclops edax	P	120	2,051	324	12	26	16	8.1	1.0	3.5	P	P		2.2	2.0	0.6		12	P	40
Microcyclops rubellus																		1.1		
Paracyclops chiltoni									3.0											
***Thermocyclops crassus**					2.4[a]															
Tropocyclops prasinus m.		120	513	971	3.9	118	79	81	20	37	108	92	73	65	10	3.6	1.0	4.4	43	8.0
Tropocyclops spp.																0.6				
Cyclops copepodites	952	5,010	1,595	220	376	5,383	2,668	1,792	261	108	542	835	716	860	478	100	313	285	822	5,168
Mesocyclops copepodites		51	3,077	827	25			41	24	5.9	3.9		2.0	6.5	2.0			4.4	20	136
Tropocyclops copepodites			304	504	2.0	144	16	16	9.0	24	66	28	16	4.3	8.1	1.8	1.0		14	

	Trial																		
Organism	**1_D**	**2_D**	**3_U**	**4_D**	**5_D**	**6_U**	**6_D**	**7_D**	**8_D**	**9_D**	**10_D**	**11_U**	**11_D**	**12_U**	**12_D**	**13_U**	**13_D**	**14_D**	**15_D**	**16_D**
Calanoids																				
Epischura lacustris			85	P	9.8	P	32	P	3.0	15	54	177	122	62	51	2.4	2.5		18	
Eurytemora affinis		34	57	54	22	P	16	16		42	P	18		P		1.2	0.5	60	556	249
Leptodiaptomus ashlandi	146	292			446	196	95	57			108	85	59	82	109	1.8	4.0		5.9	16
Leptodiaptomus minutus	1,205	103		P	51	261	167	244	7.0	13	46	42	26	8.6	12	2.4	2.0	1.1	37	P
Leptodiaptomus sicilis	322			P					2.0		3.9	3.5	12	P	36	0.6	167	1.1	266	128
Leptodiaptomus siciloides		17			2.0													3.3	55	48
Limnocalanus macrurus	3.8										P				10		62		2.0	P
Skistodiaptomus oregonensis	184	2,677	1,139	450	5.9	13	7.9	33	4.0	1.2	15	P	12	34	26	2.4	3.0	2.2	603	48
Skistodiaptomus reighardi		51		P		39													7.8	
Diaptomid copepodites	829	8,167	12,534	3,022	313	10,244	3,557	6,485	950	111	5,449	4,968	3,101	4,413	2,130	121	276	3.3	368	514
Epischura copepodites			123	207	49	39	143	81	83	18	77	18	6.1	6.5	18		1.0		3.9	
Eurytemora copepodites			408	117	211	915	238	220	1.0	87	139	53	4.1	2.2	2.0	4.1	1.0	9.9	595	369
Senecella copepodites											P						8.9			
Harpacticoids																				
Attheyella illinoisensis									1[a]					P						
Canthocamptus robertcokeri				1.1[a]					0.5[a]	0.6[a]				P				7.7		
Canthocamptus sp.									0.5[a]								0.5	9.9		P
Canthocamptus staphylinoides												0.4[a]						1.1		
Canthocamptus vagus																		1.1		
Epactophanes richardi				0.6[a]						12[a]										
Heteropsyllus nunni						1.2[a]			0.5[a]			0.4[a]				1.2	0.5		1.5[a]	

	Trial																		
Organism	**1_D**	**2_D**	**3_U**	**4_D**	**5_D**	**6_U**	**6_D**	**7_D**	**8_D**	**9_D**	**10_D**	**11_U**	**11_D**	**12_U**	**12_D**	**13_U**	**13_D**	**14_D**	**15_D**	**16_D**
Mesochra alaskana						1.2[a]					0.5[a]			P						
Moraria spp.				0.6[a]																
Nitokra hibernica	1.9[a]		19	1.7[a]		23[a]			3[a]	1.8[a]	1.9[a]	0.9[a]			P	5.3			1.5[a]	P
Nitokra lacustris									1[a]											
Nitokra spinipes										0.6[a]										
Paraleptastacus wilsoni						0.8[a]										1.8				
Schizopera borutzkyi						29[a]				0.6[a]	1[a]	3.1[a]	2.0	P		4.7				
Harpacticoid copepodites			9.5	2.2[a]		105			2.0	7.1	12	0.9[a]		4.3		8.3	1.5	9.9		
Other Copepods																				
Ergasilus spp.				P															2.0	
Cladocerans																				
Alona affinis				P																
Alona guttata						13														
Alona quadrangularis				P												4.7	0.5	5.5	2.0	
Alona spp.			9.5	4.5												0.6		723		64
Bosmina longirostris (*Bosmina* spp.)	46	13,521	1,329	274	4,632	11,603	6,829	7,527	685	1,267	112	502	326	344	263	6.5	8.4	5.5	168	1,348
Bosmina spp. (*Eubosmina* spp.)	P	17		P	2.0	39	262	196	40	13	23	32	26	11	20		2.0	2.2	211	128
Bythotrephes longimanus				5.1[a]	0.5[a]	71[a]	14[a]	9.2[a]	1[a]	1.2[a]	4.7[b]	7[b]	22[b]	0.8[b]	3[b]					
Cercopagis pengoi		0.5[a]				20[a]														
Ceriodaphnia lacustris		17	19						6.0	11										
Ceriodaphnia spp.																0.6			P	8.0
Chydoridae												3.5		2.2				8.8	P	P
Chydorus gibbus	1.9			P																

	Trial																		
Organism	**1_D**	**2_D**	**3_U**	**4_D**	**5_D**	**6_U**	**6_D**	**7_D**	**8_D**	**9_D**	**10_D**	**11_U**	**11_D**	**12_U**	**12_D**	**13_U**	**13_D**	**14_D**	**15_D**	**16_D**
Chydorus sphaericus		17	9.5	9.0	2.0				1.0									138	3.9	40
Daphnia ambigua			9.5																	
Daphnia galeata mendotae	P	909	665	94	2.0	65		P	13		P	P	4.1		4.0		5.0		571	249
Daphnia longiremis		17																		
Daphnia retrocurva		172	646	45	7.8	13			5.0										196	P
Daphnia spp.																		3.3		
Diaphanosoma birgei			1,519	3,184	7.8	P			1.0										2.0	
Disparalona/Alonella												P				0.6		11	2.0	
Eubosmina coregoni		86			2.0	39	16	114								0.6		8.8	23	4,686
Holopedium gibberum				45	9.8	P	71	73	9.0		3.9		2.0		2.0					P
Ilyocryptus acutifrons																				8.0
Ilyocryptus spinifer												P								
Ilyocryptus spp.																		1.1		
Latona setifera																		1.1		
Leptodora kindtii			28	P		P														
Leydigia leydigi			19													1.2		9.9	2.0	8.0
Macrothrix laticornis																6.5		294		48
Monospilus dispar												11		2.2		0.6				
Sida crystallina			P																	
Simocephalus spp.																				P
Mysids																				
Hemimysis anomala						0.4[a]					3.3[b]	0.4[b]	2.7[b]	0.2[b]		2.4[a]	0.2[b]			
Other Organisms																				
Echinogammarus ischnus						1.6[a]														

	Trial																		
Organism	**1_D**	**2_D**	**3_U**	**4_D**	**5_D**	**6_U**	**6_D**	**7_D**	**8_D**	**9_D**	**10_D**	**11_U**	**11_D**	**12_U**	**12_D**	**13_U**	**13_D**	**14_D**	**15_D**	**16_D**
Gammaridae		0.5[a]	0.6[a]	0.6[a]					1[a]	0.6[a]	0.5[a]									
Gammarus fasciatus												0.4[a]								
Gammarus spp.																				0.5[a]
Water Mite	1.9			P		P			1.0	2.4	P	3.5						1.1		
Chironomidae	3.8	P	28	9.0	2.0	13	P	8.1	1.0	3.5	3.9	P				1.2	0.5		3.9	P
Nematoda			19	4.5		P		8.1	4.0	3.5		14		11		7.7	0.5	2.2		P
Oligochaeta			19	P	2.0	157			18	3.5	3.9	P		2.2		25		8.8		
Ostracoda				P		13	7.9		7.0		7.7	P				1.2		12		
Caddisfly larvae						13			1.0											
Chaoborus			P																	
Hydra			28	P		P				1.2		3.5	P		P	1.8				
Mollusca													P							
Tardigrade						444				1.2		11		P		4.7	1.0			
Planaria		P	9.5					8.1	3.0	1.2		3.5						4.4	2.0	
Total Density	4,958	49,616	253,699	208,070	185,884	93,102	83,345	63,893	26,550	37,487	42,825	21,964	18,670	11,097	14,126	1,746	2,629	6,824	8,047	25,663

Table 14. Density of Live Zooplankton (#/m3) in Shipboard Discharge Samples. D = Discharge.

Sample	1_D		2_D		4_D		7_D		9_D		11_D		13_D		15_D	
Taxa	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³
Rotifers																
Cephalodella															27	27
Collotheca					519	519	1,722	1,722	1,985	1,985	1,253	1,253	67	67		
Conochilus			110	220			344	1,722	165	165	74	516	111	178		
Gastropus	56	56					344	1,377	993	993	811	958	311	334	214	268
Kellicottia			0	330	519	519	0	1,033			442	590	156	267	80	161
Keratella	70	98	3,190	3,410	121,005	128,795	1,722	1,722	13,071	13,733	811	811	178	267	1,552	1,606
Monostyla											0	74				
Ploesoma					519	1,558			165	662						
Polyarthra	56	70	330	4,510	39,469	78,939	19,970	56,468	25,149	41,198	6,487	13,195	22	512	107	294
Synchaeta	225	281			0	1,039	1,033	1,722	2,647	2,647	1,695	2,359	534	756	268	294
Trichocerca					1,558	3,635	0	344	165	165	74	147	22	67	27	27
Cladocerans																
Bosmina	14	14	21,708	25,081	1,039	1,039	7,231	8,608	1,324	1,655	147	295			187	214
Ceriodaphnia			147	147												
Chydoridae															54	54
Daphnia			733	1,027	519	519									321	348
Other Cladocerans			147	147												
Sidid					0	3,116										
Copepods																
Calanoids	1,292	2,486	7,480	12,614	519	1,558	3,787	5,165	165	165	1,622	2,506	467	667	1,472	1,847
Cyclopoids	506	773	7,627	8,360	2,077	2,597	2,066	2,066	165	331	295	295	133	200	749	1,017
Nauplii	239	506	6,380	10,560	10,387	13,503	6,542	7,231	2,647	3,640	1,106	1,769	111	111	1,124	1,204

Sample	1_D		2_D		4_D		7_D		9_D		11_D		13_D		15_D	
Taxa	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³	Live, #/m³	Total, #/m³
Other Organisms																
Dreissenid	126	169			11,425	17,657	5,165	5,165	1,324	1,655	4,349	4,423	111	111	27	54
Nematodes	14	28														
Planaria											74	74				
Protista >50									165	165					0	27
Total Organisms	2,599	4,481	47,852	66,406	189,557	254,994	49,926	94,342	50,132	69,159	19,240	29,265	2,225	3,537	6,209	7,440
Percent Live		58%		72%		74%		53%		72%		66%		63%		83%

Table 15. Density of Protists (cells/mL) in Shipboard Ballast uptake (U) and Discharge (D) Samples.

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Achnanthes sp.		1.8	17.5																	
Ankistrodesmus fulcatus									4.1	5.8										
Ankistrodesmus gracilis			2.2	1.7	1.7									4.2						
Aphanizomenon flos-aquae					126.1										103.6					
Aphanocapsa sp.			43.8	533.5	124.5	229.5		270.0	82.2	47.8		1,128.0	29.0	2,317.4	124.3	169.3	168.5	8,527.8		
Aphanothece sp.					20.2		129.8		1,232.3											
Asterionella formosa	24.0		0.1	0.2	0.4	0.1	4.3	1.0	0.9	0.0	0.3	0.7	9.4	0.3	0.5	0.2	10.7	8.8	0.1	1.2
Aulacoseira granulata	0.6		3.0		0.3				2.3				0.2					694.0	1.1	13.3
Aulacoseira sp.	54.2	3.5		6.6					4.1	8.7							16.9			
Bitrichia sp.					1.7	3.3	4.1		4.1								4.2			
Centric diatom		47.5	269.5	23.2			36.5	85.9	147.9	10.1	22.4	121.2	29.0	16.9	24.9	93.1	118.0	291.2	62.3	53.5
Chlamydomonas sp.			13.1	19.9			4.1		4.1	2.9				4.2					5.7	
Chrysochromulina sp.			4.4			9.8	44.6	81.8	37.0			8.4	18.1	16.9						
ciliates	1.4		15.3	39.8		1.6	8.1	4.1	4.1	10.1	4.5	8.4		8.4	16.6		4.2			3.0
Cocconeis sp.				6.6		1.6							3.9	4.2		4.2				
Cosmarium sp.					3.4		16.2	8.2						8.4						
Crucigenia quadrata												16.7								
Crucigenia rectangularis				26.5																
Crucigenia tetrapedia						13.1														
Cryptomonas erosa			32.9	1.7	1.7	1.6		4.1	8.2			4.2	3.6	12.6	20.7	4.2	4.2	41.6	5.7	20.8
Cryptomonas reflexa		0.9	26.3	6.6	6.7		8.1	20.5	12.3			25.1	10.9	25.3	12.4	12.7	4.2			3.0
Cryptomonas rostriformes			4.4																	
Cryptomonas sp.	4.1																			

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Cyclotella sp.					67.3	1.6														
Dictyosphaerium sp.						82.0														
Dinobryon attenuatum						1.6														
Dinobryon bavaricum													3.6		4.1	4.2				
Dinobryon cylindricum																	16.9			
Dinobryon divergens					3.4															
Dinobryon sertularia								12.3			9.0				12.4				5.7	
Dinobryon sp.									4.1											
Elakatothrix sp.											9.0		7.2							
Fragilaria crotonensis	36.3	0.1	8.2	0.4	40.5	13.9	99.8	12.6	2.9	0.1	14.7	34.4	33.4	3.8	5.2	4.3	11.8	16.7	0.2	7.6
Fragilaria sp.																				17.8
Gleocystis sp.			35.1					20.5												
Gloeocystis sp.				19.9						8.7								332.8		
Golenkinia sp.				1.7											4.1					
Gymnodinium sp.			4.4	1.7	1.7			4.1	8.2	2.9		12.5	10.9	8.4	8.3	4.2				3.0
Gyrosigma sp.						1.6														
Haptophytes		8.0	13.1	8.3	163.1	4.9	105.5	94.1	37.0	7.2	107.6	121.2	105.1	88.5	111.8	76.2	84.3			32.7
Kephyrion sp.							4.1		12.3			4.2	10.9		4.1					
Kirchneriella lunaris						6.6														
Lyngbya sp.									8.2				163.0		290.0				85.0	208.2
Mallomonas sp.							4.1				4.5	4.2			4.1					
Merismopedia glauca												50.1								
Merismopedia sp.		1.8		59.7				24.5					14.5							
Merismopedia tenuissima			114.0							46.3										
Micratinium pusillium														12.6						

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Microcystis sp.																		9,983.8		
Monoraphidium contortum				1.7		1.6									4.1	4.2				3.0
Monoraphidium minutum				6.6	5.0		8.1					4.2								
Monoraphidium setiforme						1.6								4.2	4.1					
Navicula sp.						4.9		12.3	4.1				3.6							
Nitzschia acicularis	0.1		4.8	1.4	0.4			0.2		0.0	4.8	2.4	5.6	0.9	0.8	0.9	1.6	3.5	0.1	0.1
Nitzschia sp.								4.1											5.7	
Ochromonas sp.					11.8															
Oocystis borgei							16.2		28.8		17.9		7.2							
Oocystis sp.			4.4	1.7																
Oscillatoria limnetica	6.8	68.3	181.9		16.8		555.8	32.7	8.2		381.0	254.8	137.7			135.4				95.2
Pediastrum duplex						23.0				15.9										
Peridinium sp.			8.8	3.3	1.7	1.6	4.1		4.1	4.3	4.5	4.2			4.1		8.4			8.9
Phacus sp.													3.6							
Pinnularia sp.											4.5									
Pseudanabaena limnetica																	252.8			
Pseudokephyrion sp.					1.7				4.1						4.1					
Rhodomonas lens		1.8	52.6	41.4	5.0	4.9	8.1	24.5			40.3	87.7	3.6	54.8	20.7	4.2	16.9	41.6	11.3	26.8
Rhodomonas minuta		29.7	92.0	3.3	50.5	16.4	97.4	90.0	94.5	7.2	269.0	233.9	228.2	261.2	256.8	156.6	113.8	748.8	232.3	101.1
Scenedesmus bicaudatus																				17.8
Scenedesmus bijuga				3.3		9.8	16.2	12.3	24.6			25.1								
Scenedesmus quadricauda			26.3	19.9		1.6	16.2			11.6		16.7			62.1	8.5		166.4		
Scenedesmus sp.	1.8																		22.7	
Snowella/Gomphosphaerium sp.													144.9							

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Spiniferomonas sp			2.2			1.6	4.1		16.4				14.5							
Staurastrum sp.			4.4																	
Synechococcus sp.		12.0	28.5		26.9			53.2	61.6		71.7	104.4	39.8	71.6	140.8	118.5	134.8			
Synedra sp.		8.0	70.1																	
Synedra/Nitzschia		4.4	37.3	11.6	30.3	23.0	20.3	12.3	28.8	2.9	71.7	217.2	94.2	16.9	33.1	12.7	8.4			
Tabellaria fenestrata																				
Tabellaria flocculosa	1.2		4.3	0.2		1.8	1.2	0.2			0.1	1.0	1.0	0.1	0.3		0.3	0.9	0.1	0.3
Tetraedron minimum				1.7		1.6		8.2	4.1											3.0
Tetraedron sp.				6.6																
Tetraedron trigonium																				3.0
Trachelomonas sp.									4.1											
unid chryso ovoid flagellates	0.5	30.2	89.8	11.6	111.0	34.4	231.2	208.6	94.5		609.7	513.8	315.2	353.9	695.9	562.8	421.3	1,081.6	351.3	351.0
unidentified flagellate fusiform		2.2	21.9		3.4		36.5	16.4	12.3	29.0	22.4	33.4	29.0	50.6	24.9	12.7	8.4			
unidentified flagellate ovoid			2.2	63.0	37.0	8.2				124.5		4.2								3.0
unknown round	2.7		4.4	1.7		1.6		8.2					7.2							
Uroglenopsis/Uroglena sp.				19.9		16.4		12.3	12.3			20.9								
Urosolenia (Rhizosolenia) sp.	2.7				8.4	1.6	44.6	4.1	24.6		9.0	20.9	10.9	8.4	16.6	25.4	33.7			14.9
Diatoms																				
Achnanthidium cf. caledonicum					0.7															
Achnanthidium exiguum		0.0			0.4															
Achnanthidium minutissimum	3.2	1.1	20.5	3.0	2.0	2.4	6.2	2.5	10.0	0.6	7.2	9.7	14.8	2.1	6.9	2.9	2.2	3.5	1.0	4.5
Actinocyclus normanii		2.7							0.9	0.1										0.6
Adlafia minuscula		0.0																		

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Amphora alpestris										0.0	0.4	1.4	1.0	0.1	0.3		0.5			
Amphora inariensis	1.8	1.6	1.9	0.3	0.9	1.9	1.6	0.6	2.3	0.1	3.3	8.7	4.6	1.7	3.1	3.5	1.9	5.3	0.1	0.5
Amphora ovalis	0.1	0.1			0.2	0.1		0.3		0.0	1.1	0.7		0.2	0.2	0.3	0.3	2.6		0.1
Amphora pediculus		0.6	0.3			0.1			0.3	0.0	0.1	2.4	0.7	0.1	0.1				0.0	
Amphora cf. exima										0.0										
Amphora exima											0.1								0.0	
Amphipleura pellucida									0.3											
Aulacoseira ambigua			29.2	1.1	0.5	0.0			0.5	0.1	0.1		0.4	0.1	0.1	0.5		68.6	1.6	13.6
Aulacoseira distans		0.5		0.1					0.9	0.2							0.5	5.5		0.3
Aulacoseira granulata var. *angustissima*				0.2						0.1		0.6	0.2					8.2	0.2	
Aulacoseira islandica	0.3		2.2	0.1							0.1							16.5	0.4	0.2
Aulacoseira italica				0.1								0.3								
Aulacoseira pusilla						0.0		0.3				1.2	0.1			0.9	0.5	2.7	0.4	0.9
Aulacoseira subarctica																		5.5		
Bacillaria paxilifera										0.0	0.1	0.3								
Brachysira vitrea	0.3					0.2		0.1	0.3	0.0	0.3	0.3	0.2	0.1	0.6		0.2		0.1	0.3
Caloneis bacillum	0.2	0.0	1.9	0.1	0.2										0.2			1.8		
Cavinula cf. cocconeiformis		0.0																		
Cavinula cf. jaernefeltii																				0.1
Cocconeis disculus										0.0	0.1	0.3			0.1					
Cocconeis neothumensis		0.1								0.0		0.3	0.5	0.1						0.2
Cocconeis pediculus	0.3	0.1	0.3	0.1			0.4			0.0		0.3	0.7						0.0	0.1
Cocconeis placentula	0.3	0.1	0.7			0.1	0.4		0.3	0.0				0.2	0.2	0.1		3.5	0.0	0.2
Cocconeis placentula var. *lineata*				0.1																

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Ctenophora pulchella				0.1																
Cyclotella atomus (fine form)	8.5	3.3	62.1	3.1	0.3	0.1	1.2	1.7	5.1	2.8	2.3	30.4	5.1	4.4	7.6	37.1	40.8	22.0	13.8	4.4
Cyclotella sp. *with auxospore*	0.8	0.3		0.1								0.3								
Cyclotella bodanica	0.8			0.2	1.2		0.3	1.7	0.9	0.1			0.2	0.0	0.1		2.4			
Cyclotella comensis var. *1*	5.6	1.6		2.6	11.0	0.3	8.3	6.7	19.4	0.8	2.7	16.7	4.1	2.1	1.3	6.6	3.3		2.3	0.8
Cyclotella comensis	17.8	24.4	16.5	7.1	42.7	1.1	20.2	47.3	73.5	8.3	11.5	50.7	12.1	7.5	9.5	22.1	57.4	5.5	12.6	11.0
Cyclotella comensis rough center w/ process	1.1			1.7	5.1	0.0	3.7	14.3	10.2	1.5	0.3		1.0	0.1	0.8		3.3		0.4	0.8
Cyclotella meneghiniana	0.8		13.5	1.2	0.5	0.0	0.1	0.2	2.8	0.6	0.1	2.4	0.7	0.0	0.2	0.9		65.9	3.2	2.2
Cyclotella michiganiana	2.8	0.3		0.2	2.7	0.1	0.8	3.6	0.5		1.3	4.5	1.4	0.4	1.2	3.8	4.3		0.7	1.7
Cyclotella ocellata	2.8	10.9	15.7	3.8	0.5	0.0	0.1	1.5	5.1	1.9	0.1	0.6	0.2	0.2	0.5	0.5	2.8		3.2	0.3
Cyclotella operculata	0.3																			
Cyclotella tripartita	0.3							0.2									0.9			
Cymbella cf. lange-bertalottii																		1.8		
Cymbella cymbiformis																				0.1
Cymbella mexicana												0.3								0.1
Cymatopleura solea								0.1												
Cymbella tumida			0.7																	0.1
Cymbopleura naviculiformis											0.1		0.2	0.1			0.2		0.0	
Cyclostephanos dubius			2.2	0.1	0.2			0.2		0.1		0.3	0.1	0.0		0.9	0.9		2.1	0.6
Cyclostephanos invisitatus	0.3	0.5	24.7	0.9		0.0	0.1	0.2	0.5	0.7	0.1	0.9	0.6	0.3	0.6	5.2	2.4	41.2	9.1	1.3
Cyclostephanos tholiformis			2.2	0.2																0.5
Denticula tenuis				0.1			0.8	0.2	0.3	0.1					0.2			1.8		1.1
Diatoma ehrenbergii		0.0					0.4		0.3			0.7	0.2		0.1			0.9		
Diatoma tenuis	0.2							0.1		0.0	0.3		0.5	0.2	0.2	0.3	0.2			

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Diatoma vulgaris			0.3								0.1	0.7	0.5				0.3			0.1
Diploneis elliptica											0.1				0.1	0.1				
Diploneis oculata	2.0	0.1	0.1	0.2	4.6	7.0	13.7	3.3	0.6	0.1	9.6	17.7	6.8	3.6	4.0	6.0	4.3			0.1
Diploneis parma	0.1										0.1	0.7								
Diploneis pseudovalis										0.0		0.3			0.2		0.2			
Diploneis puella						0.1														
Diadesmis contenta			0.1															0.9	0.0	
Discostella pseudostelligera	1.1	2.7	74.9	5.2	1.8	0.0	1.7	7.4	29.1	0.6	3.6	9.5	2.2	1.6	2.7	9.4	10.0	2.7	3.5	2.2
Encyonema ventricosum			0.3																	
Encyonema caespitosum			0.1			0.1				0.0		0.3								
Encyonema leibleinii			0.1										0.2			0.1		0.9		
Encyonema reichardtii		0.0																		
Encyonema silesiacum			0.4	0.1										0.1						0.2
Encyonema triangulum											0.1									
Encyonopsis cesatii									0.6	0.0			1.0		0.1					0.1
Encyonopsis microcephala	0.4	0.2	0.1	0.1		0.2	0.8	0.2	1.2	0.1	0.4	3.1	1.0	0.2	1.1	0.3			0.0	0.1
Entomoneis ornata																		0.9		
Epithemia sorex																		2.6		
Eucocconeis cf. flexella						0.1														
Eucocconeis flexella	0.2			0.1			0.4								0.2					
Eucocconeis laevis	0.2					0.1					0.1	0.7			0.2	0.1	0.2		0.1	0.1
Eunotia cf. incisa	0.2																			
Eunotia curvata		0.0	0.1									0.3								
Eunotia incisa										0.0										
Fallacia cf. lenzii		0.1	0.6																	

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Fallacia pygmaea					0.4															
Fallacia tenera			0.1																0.0	
Fragilaria capucina							0.8	0.1	0.6	0.0			0.5	0.1	0.1	0.4	0.5	1.8		
Fragilaria demerarae	0.1					0.1											0.2		0.0	
Fragilaria mesolepta		0.1	0.4	0.3		1.1			0.6	0.0								0.9		6.7
Fragilaria sinuata										0.0										
Fragilaria vaucheriae	1.6	0.5	3.4	0.2	1.1	1.3	1.2	0.6	0.3	0.1	1.3	7.0	2.2	1.1	1.8	1.1	0.3	10.6		2.8
Geissleria decussis																	0.3			
Gomphonema augur			0.1																	
Gomphonema innocens																		3.5		
Gomphonema minutum			0.1			0.1		0.3	0.6	0.1			0.5		0.1	0.2	0.3		0.2	
Gomphonema minusculum																			0.1	0.1
Gomphonema olivaceum	0.1		0.4															2.6		0.1
Gomphonema parvulum			0.1	0.1										0.1			0.2		0.1	
Gomphonema sp.					0.4				0.9								0.2		0.1	
Gomphonema truncatum																				0.1
Gomphonema vibrio								0.1											0.1	0.1
Gyrosigma acuminatum	0.1	0.0															0.2			
Halamphora cf. montana																			0.0	
Halamphora oligotraphenta													0.2							
Halamphora normanii			0.1								0.4								0.0	
Halamphora thumensis										0.0				0.1						
Halamphora veneta						0.1														
Hippodonta capitata	0.2																			
Hippodonta hungarica	0.2	0.1							0.3		0.6		0.5							

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Hippodonta luenebergensis						0.9	2.0	0.3		0.0	1.7	4.2	3.6	0.2	0.9	0.9	0.8		0.0	
Hippodonta sp.				0.1																
Karayevia clevei	0.1	0.4		0.1		0.3			0.3			1.4	0.2		0.1					
Karayevia laterostata	0.3	0.1				0.2	0.4	0.1		0.0	0.3	1.7	1.5	0.2	0.8				0.0	0.1
Kobayasiella cf. subtilissima																			0.0	
Kolbeia ploenensis											0.3									
Lemnicola hungarica																		2.6		
Luticola mutica				0.2										0.1						
Mastogloia baltica																				0.1
Melosira varians				0.2					0.5	0.1	0.1					0.5				
Meridion circulare			0.3																	
Navicula antonii		0.2	1.6	0.1		0.1		0.2				0.3	0.2		0.2				0.0	0.1
Navicula atomus																		1.8		
Navicula capitatoradiata	0.2	0.1	0.7		0.2	0.1		0.1	0.3	0.0	1.0	1.0	0.5		0.2			1.8		
Navicula cincta															0.1					
Navicula cryptocephala	0.3	0.2	0.9	0.1		0.5	0.4	0.1		0.0		1.4	1.2	0.2	0.1	0.1		2.6		0.1
Navicula cryptotenella		1.0	3.4	0.9		0.5	0.8	0.2	1.5	0.2	2.4	4.9	2.2	0.5	1.1	0.2	0.6	1.8	0.1	0.2
Navicula gregaria		0.1	4.9	0.6							0.3	0.7		0.2			0.3	6.2	0.4	0.1
Navicula menisculus	0.3						0.4													
Navicula radiosa	0.6	0.2	4.3	0.1		0.5	0.4	0.3		0.0	0.1		0.5		0.4	0.2	0.3	4.4	0.0	0.5
Navicula reinhardtii														0.1				0.9		
Navicula reichardtiana		0.8	0.4	0.2		0.1						1.4	0.2		0.2	0.2				0.1
Navicula rhynchocephala			0.3	0.1		0.1	0.4		0.6	0.0			0.5		0.1	0.1			0.0	
Navicula salinarum																		1.8		
Navicula trivialis									0.3	0.0		1.7	0.2						0.0	0.1

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Navicula veneta	0.6			0.1																
Navicula viridula	0.2					0.1							0.2						0.0	
Neidium ampliatum	0.1																			
Neidium binodeformis	0.1												0.2		0.1					
Neidium dubium																0.1				
Neidium iridis													0.5							
Nitzschia amphibia				0.1	0.2			0.1		0.0	0.3		0.5	0.2	0.2	0.4	0.3	3.5		0.1
Nitzschia dissipata	0.2	0.1	3.6	0.4	0.2	0.4	0.4	0.3	0.9	0.0	1.0	2.1	3.1	0.1	0.4	0.2	0.5	2.6	0.1	0.3
Nitzschia fonticola	2.7	0.4	1.2	0.6	0.7	1.2	2.0	0.2	5.3	0.0	2.4	12.9	4.4	1.3	1.9	2.2	2.1	10.6	0.2	0.3
Nitzschia frustulum		0.1	0.1							0.0										
Nitzschia gracilis																0.1				
Nitzschia lauenburgiana										0.0	0.6	0.3		0.2			0.2	1.8		0.1
Nitzschia linearis	0.5	0.1	1.2	0.3	0.2			0.1		0.0	1.4	2.8	2.9	0.3	0.9	0.1	1.3	4.4	0.0	0.2
Nitzschia palea	5.6	1.6	18.0	4.1	2.6	2.3	2.3	2.1	4.1	0.2	7.5	20.9	16.7	3.2	5.3	5.0	5.3	38.8	0.5	0.6
Nitzschia recta								0.2		0.0	0.7	1.0	0.5		0.2					0.1
Nitzschia sinuata var. *tabellaria*			0.3																	
Nitzschia subacicularis	0.8	0.0		0.1	0.4	0.2	1.2	0.2	0.9		0.1	0.7	0.2				0.3			
Parlibellus crucicula								0.1												
Parlibellus protracta													0.2							
Pinnularia lundii										0.0										
Pinnularia microstauron																		1.8		
Pinnularia viridis												0.7								
Planothidium biporomum											0.1						0.3	1.8		0.1
Planothidium delicatula																		0.9	0.0	

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Planothidium frequentissimum			0.9							0.0								2.6		
Planothidium lanceolatum	0.2	0.0																		
Planothidium minutissimum		0.6	0.3		0.4	0.2			1.5	0.0		0.7	0.2	0.2	0.2		0.2	1.8	0.0	0.3
Planothidium rostratum		0.1					0.4												0.0	
Planothidium sp.																				0.1
Placoneis cf. amphibola											0.1									
Placoneis cf. elginensis					0.2															
Placoneis clementis	0.6	0.1											0.2		0.1	0.1				
Placoneis elginensis	0.1		0.1	0.1	0.2	0.3	1.2	0.6		0.0	0.7	1.4	0.5	0.2	1.0	0.8	0.3		0.0	
Placoneis gastrum		0.1																		
Pseudostaurosira brevistriata	0.2	0.5		0.1		0.7	1.2	0.1	4.4	0.3	2.0	7.0	1.5	0.2	0.1	0.3	0.6	7.1	0.3	0.6
Pseudostaurosira parasitica	0.2			0.1		0.2					0.3	2.8		0.1	0.2	0.1				0.1
Pseudostaurosira parasitica var. *subconstricta*																		2.7		
Psammothidium helveticum					0.2				0.6		0.1									
Psammothidium ventrale									0.3											
Reimeria sinuata		0.0	1.8	0.2							0.3			0.1						
Rhoicosphenia abbreviata		0.1						0.1		0.0		0.3		0.1	0.2	0.1		7.1	0.0	0.3
Rhopalodia gibba															0.1					
Rossithidium linearis			1.2	0.8	0.2	0.1	0.4			0.0	0.1						0.2		0.1	0.3
Sellaphora bacillum											0.3				0.1	0.1				
Sellaphora cf. bacillum	0.1												0.2							
Sellaphora laevissima		0.0	0.1							0.0	0.1					0.2			0.0	0.1
Sellaphora pupula	0.2	0.4	0.7	0.1		0.4	0.8	0.4		0.0	0.7	1.0	1.0		0.1	0.2		1.8	0.1	0.1

Organism	Trial																			
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Staurosirella leptostauron						0.1		0.1				0.3							0.0	
Staurosirella pinnata	2.4	1.2	0.3	0.1	2.2	4.2	10.1	2.3	5.6	0.2	13.3	57.0	13.1	4.8	4.4	7.3	4.1	10.6	0.6	0.2
Staurosira binodis							0.8				0.4	0.7				0.4				
Staurosira construens		0.1								0.0	0.8	0.3	0.2	0.2			0.3	4.4	0.0	0.3
Staurosira construens var. *venter*	0.1	0.0							1.8	0.1		0.7			0.1			5.3	0.1	0.5
Stephanodiscus alpinus Type I			10.5	0.4		0.0		0.2			0.2			0.0		1.4		13.7	0.2	
Stephanodiscus alpinus Type II/III			9.7	0.9	0.2	0.0				0.4	0.1				0.1	0.9	0.5		1.6	0.3
Stephanodiscus binderanus		0.3			0.2													93.3		4.9
Stephanodiscus cf. hantzschii f. tenuis															0.1					
Stephanodiscus hantzschii f. hantzschii	0.6	0.5											0.1				0.5	32.9		1.7
Stephanodiscus hantzschii f. tenuis										0.1		0.3	0.1			0.5	0.5	19.2	0.4	0.3
Stephanodiscus medius																	0.5			0.5
Stephanodiscus niagarae		0.3															0.5	19.2	0.2	1.6
Stephanodiscus parvus	0.3	1.4	0.7	0.3	0.2	0.0		0.2				0.6	0.2	0.1	0.1	1.9	1.9	126.2	5.3	5.3
Stephanodiscus subtransylvanicus																			0.2	0.2
Stephanodiscus sp. *#10*	0.3	1.4	2.2	0.1	0.2		0.1	0.2		0.2		1.8	0.2				0.5	43.9	0.4	2.0
Stephanodiscus sp. *#51*															0.1		0.5			
Stauroneis anceps							0.4	0.1												
Surirella bifrons										0.0										
Surirella brebissonii																0.1				
Surirella cf. acuminata										0.0										

Organism	Trial																		
	1_D	2_D	3_U	4_D	5_D	6_U	6_D	7_D	8_D	9_D	10_D	11_U	11_D	12_U	12_D	13_U	13_D	14_D	15_D	16_D
Surirella minuta			0.7	0.1														0.9	0.0	
Surirella ovalis			0.3																	
Synedra filiformis	0.5	0.1	18.6	1.0	7.9	0.9	3.5	1.0	5.0	0.0	6.9	5.2	9.9	1.3	2.7	0.5	3.5	0.9	0.0	0.3
Synedra filiformis var. *exilis*			3.3		0.2														0.0	
Synedra ostenfeldii	0.1			0.1															0.0	
Tryblionella angustata		0.2				0.5	0.4				0.4	3.1	0.5	0.4	0.2	0.4				
Tryblionella angustatula	0.2		3.6	1.0	0.2	0.3	0.8	0.4	0.3	0.0	0.6	1.7	1.2	0.3		0.3				
Tryblionella hungarica											0.1					0.2				
Tryblionella levidensis			0.1																	
Tryblionella salinarum																				0.1
Ulnaria acus												0.3								
Ulnaria delicatissima var. *angutissima*									0.6				0.5	0.2	0.3		0.6			
Ulnaria ulna				0.2		0.1		0.1			0.1									
Total Density	210	285	1,617	1,002	967	561	1,623	1,248	2,247	368	1,775	3,405	1,634	3,396	2,084	1,545	1,614	22,713	856	1,074

Table 16. Ballast Discharge Trials: Summary of Water Chemistry and Water Quality Parameters (Average ± Standard Deviation).

Parameter	Trial														
	1	2	4	5	6	7	8	9	10	11*	12	13	14	15	16
Temperature (°C)	3.42 ± 0.27	15.83 ± 0.25	22.22 ± 0.12	19.65 ± 0.13	18.11 ± 0.40	17.79 ± 0.09	18.03 ± 0.30	18.41 ± 0.14	15.60 ± 0.11	13.27 ± 0.51	11.40 ± 0.18	8.15 ± 0.32	6.86 ± 0.40	6.36 ± 0.27	6.93 ± 0.35
Specific Conductivity (µS/cm)	276.9 ± 0.4	284.0 ± 0.9	237.9 ± 83.2	237.6 ± 14.9	200.0 ± 14.8	226.3 ± 3.9	176.1 ± 4.6	213.8 ± 3.0	265.1 ± 3.4	241.9 ± 3.76	249.0 ± 2.5	246.4 ± 7.5	493.5 ± 11.6	248.8 ± 15.0	376.4 ± 4.0
Salinity (PSU)	0.13 ± 0.00	0.14 ± 0.00	0.12 ± 0.04	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.00	0.08 ± 0.01	0.10 ± 0.00	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.00	0.12 ± 0.01	0.24 ± 0.01	0.12 ± 0.01	0.18 ± 0.00
Turbidity (FNU)	4.69 ± 2.58	2.08 ± 0.44	3.03 ± 1.34	1.00± 0.13	2.37 ± 1.79	2.45 ± 1.85	2.17 ± 1.33	2.80 ± 0.88	8.17± 1.85	2.12 ± 0.27	1.99 ± 0.08	3.98 ± 2.26	49.8 ± 12.6	9.25 ± 0.74	4.13 ± 1.05
pH	7.64 ± 0.02	8.13 ± 0.01	7.90 ± 0.02	8.12 ± 0.05	7.91 ± 0.23	8.10 ± 0.05	8.07 ± 0.1	8.10 ± 0.05	8.18 ± 0.02	7.90 ± 0.12	7.94 ± 0.07	8.04 ± 0.06	7.97 ± 0.04	8.07 ± 0.08	8.13 ± 0.18
Dissolved Oxygen (% Saturation)	97.9 ± 0.9	92.0 ± 0.7	86.7 ± 1.6	91.4 ± 0.9	94.2 ± 1.0	95.4 ± 0.2	94.8 ± 5.6	87.4 ± 0.6	97.9 ± 0.4	99.6 ± 1.2	91.6 ± 0.7	94.1 ± 0.5	99.4 ± 0.4	93.8 ± 1.3	90.7 ± 1.2
Dissolved Oxygen (mg/L)	12.99 ± 0.02	9.05 ± 0.10	7.57 ± 0.20	8.35 ± 0.10	8.91 ± 0.16	9.05 ± 0.02	8.96 ± 0.48	8.20 ± 0.07	9.75 ± 0.04	10.43 ± 0.18	10.00 ± 0.04	11.03 ± 0.05	12.05 ± 0.14	11.55 ± 0.19	10.97 ± 0.25
Chlorophyll *a* (RFU)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.07	0.00 ± 0.00	1.08 ± 0.36	0.04 ± 0.06	0.01 ± 0.01
Chlorophyll *a* (µg/L)**	1.18 ± 0.27	0.27 ± 0.03	0.06 ± 0.02	0.37 ± 0.05	0.69 ± 0.06	0.54 ± 0.08	0.88 ± 0.23	0.29 ± 0.10	0.73 ± 0.07	1.10 ± 0.22	1.41 ± 0.33	0.28 ± 0.04	4.23 ± 0.55	1.95± 0.26	1.44 ± 0.47
Phycocyanin Accessory Pigment (RFU)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.17 ± 0.50	0.00 ± 0.00	0.00 ± 0.00
Phycocyanin Accessory Pigment (µg/L)**	0.56 ± 0.03	0.12 ± 0.01	0.06 ± 0.01	0.15 ± 0.02	0.07 ± 0.02	0.15 ± 0.01	0.18 ± 0.04	0.10 ± 0.01	0.22 ± 0.03	0.41 ± 0.03	0.23 ± 0.01	0.26 ± 0.01	2.45 ± 1.17	0.59 ± 0.06	0.50 ± 0.04
Percent Transmittance - Filtered (254 nm)	94.2 ± 0.06	94.6 ± 0.61	93.5 ± 1.2	93.0 ± 0.44	95.9 ± 0.31	92.3 ± 2.9	86.5 ± 4.5	92.5 ± 0.10	95.0 ± 0.07	94.8 ± 0.11	94.2 ± 0.35	91.4 ± 0.04	56.1 ± 0.84	74.4 ± 2.8	90.6 ± 0.2
Percent Transmittance - Unfiltered (254 nm)	91.1 ± 0.17	93.0 ± 0.87	90.4 ± 1.2	92.2 ± 0.52	94.9 ± 0.12	89.6± 0.18	84.8 ± 4.8	90.6 ± 0.20	91.4 ± 0.45	93.7 ± 0.25	92.5 ± 0.50	90.2 ± 0.27	31.5 ± 5.1	69.9 ± 3.1	86.8 ± 0.4
Total Suspended Solids (mg/L)	2.2 ± 0.06	< 1.25 ± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	2.0 ± 2.3	< 1.43 ± 0.51	3.9 ± 0.8	< 1.25 ± 0.0	< 1.25 ± 0.0	< 1.25 ± 0.39	92.6 ± 54.4	2.3 ± 0.1	4.1 ± 2.4

Parameter	Trial														
	1	2	4	5	6	7	8	9	10	11*	12	13	14	15	16
Particulate Organic Matter (mg/L)	< 1.67 ± 0.0	< 1.25 ± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.43± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.25 ± 0.0	< 1.25 ± 0.0	< 1.25 ± 0.0	16.3 ± 8.6	< 1.25 ± 0.0	< 1.25 ± 0.0
Mineral Matter (mg/L)	1.7 ± 0.17	< 1.25 ± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.43 ± 0.0	1.8 ± 1.9	< 1.43 ± 0.0	2.8 ± 0.7	< 1.25 ± 0.0	< 1.25 ± 0.0	< 1.25 ± 0.0	76.4 ± 45.8	1.8 ± 0.1	3.3 ± 2.0
Non-Purgeable Organic Carbon (mg/L)	2.4 ± 0.06	2.6 ± 0.34	2.9 ± 0.12	2.4 ± 0.16	2.4 ± 0.24	2.2 ± 0.23	2.8 ± 0.57	2.4 ± 0.17	2.2 ± 0.18	2.6 ± 0.37	2.0 ± 0.12	2.9 ± 1.1	8.7 ± 0.44	3.7 ± 0.8	2.7 ± 0.2
Dissolved Organic Carbon (mg/L)	2.1 ± 0.05	2.5 ± 0.30	2.6 ± 0.11	2.3 ± 0.17	2.1 ± 0.11	2.1 ± 0.09	2.9 ± 0.54	2.4 ± 0.10	2.0 ± 0.15	2.3 ± 0.17	1.9 ± 0.11	2.6 ± 0.51	8.3 ± 0.04	3.6 ± 0.4	2.5 ± 0.1

* N=2; **Values are for comparison only, values are not calculated with a correction factor.

Table 17. Ballast Uptake Trials: Summary of Water Chemistry and Water Quality Parameters (Average ± Standard Deviation). NM = Not Measured.

Parameter	Trial				
	3	6	11	12	13
	Central Lake Erie	Southern Lake Michigan	Southern Lake Michigan	Southern Lake Michigan	Southern Lake Michigan
Temperature (°C)	24.30 ± 0.45	21.21± 0.18	17.32 ± 0.17	15.48 ± 0.33	13.04 ± 0.08
Specific Conductivity (µS/cm)	289.4 ± 0.8	303.7 ± 1.4	292.5 ± 4.3	278.7 ± 3.2	311.0 ± 1.7
Salinity (PSU)	NM	0.14 ± 0.01	0.14 ± 0.00	0.13 ± 0.00	0.15 ± 0.00
Turbidity (FNU)	6.81 ± 1.54	4.89 ± 0.85	5.96 ± 0.23	6.33 ± 0.39	6.34 ± 4.92
pH	7.82 ± 0.18	8.18 ± 08	8.13 ± 0.03	7.97 ± 0.11	8.18 ± 0.05
Dissolved Oxygen (% Saturation)	83.1 ± 2.0	95.2 ± 0.1	103.5 ± 0.3	101.0 ± 0.5	98.6 ± 0.00
Dissolved Oxygen (mg/L)	6.95 ± 0.21	8.44 ± 0.02	9.92 ± 0.02	10.07 ± 0.03	10.37 ± 0.02
Chlorophyll *a* (RFU)	0.33 ± 0.13	0.13 ± 0.05	0.24 ± 0.03	1.32 ± 0.18	0.00 ± 0.00
Chlorophyll *a* (µg/L)*	2.10 ± 0.39	1.51 ± 0.33	1.96 ± 0.32	1.32 ± 0.18	0.19 ± 0.00
Phycocyanin Accessory Pigment (RFU)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Phycocyanin Accessory Pigment (µg/L)*	0.14 ± 0.01	0.27 ± 0.26	0.20 ± 0.01	0.32 ± 0.02	0.53 ± 0.22
Percent Transmittance -Filtered (at 254 nm)	92.1 ± 0.57	97.0 ± 2.0	93.3 ± 0.90	93.8± 0.00	93.2 ± 0.04
Percent Transmittance -- Unfiltered (at 254 nm)	85.9 ± 0.96	95.3 ± 1.9	89.9 ± 0.16	89.1 ± 0.76	91.3 ± 0.11
Total Suspended Solids (mg/L)	8.6 ± 2.4	7.9 ± 0.8	6.0 ± 0.5	4.6 ± 1.1	3.4 ± 0.3
Particulate Organic Matter (mg/L)	< 1.43 ± 0.0	< 1.43 ± 0.0	< 1.25± 0.0	< 1.25 ± 0.0	< 1.25 ± 0.0
Mineral Matter (mg/L)	8.6 ± 2.4	6.7 ± 0.7	5.0 ± 0.5	3.7 ± 1.0	2.9 ± 0.4
Non-Purgeable Organic Carbon (mg/L)	2.8 ± 0.27	2.4 ± 0.25	2.5 ± 0.26	2.2 ± 0.11	2.6 ± 0.08
Dissolved Organic Carbon (mg/L)	2.6 ± 0.19	2.3 ± 0.18	2.3 ± 0.06	2.1 ± 0.13	2.5 ± 0.13

*Values are for comparison only, values are not calculated with a correction factor.

Table 18. Source Water Trials: Summary of Chemistry and Water Quality Parameters. NC = Not Collected.

Parameter	Trial						
	6	11		12		13	
	Site 3	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Temperature (°C)	21.84	14.86	15.14	13.26	13.39	11.53	11.74
Specific Conductivity (µS/cm)	363.3	306.7	312.6	297.6	310.7	312.3	310.4
Salinity (PSU)	0.17	0.15	0.15	0.14	0.15	0.15	0.15
Turbidity (FNU)	1.57	2.82	1.20	2.56	1.87	4.46	3.70
pH	8.3	8.21	8.23	8.17	8.17	8.15	8.07
Dissolved Oxygen (mg/L)	8.86	10.05	9.97	10.55	9.96	10.53	10.33
Dissolved Oxygen (% Saturation)	101	99.4	99.2	100.9	95.4	96.9	95.4
Chlorophyll *a* (RFU)	0.00	0.13	0.18	0.13	0.02	0.08	0.13
Chlorophyll *a* (µg/L)*	0.03	1.42	1.51	0.12	1.85	0.91	1.34
Phycocyanin Accessory Pigment (RFU)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phycocyanin Accessory Pigment (µg/L)*	0.39	0.22	0.22	0.43	0.48	0.26	0.23
Percent Transmittance-Filtered (at 254 nm)	NC	93.6	95.0	94.6	94.1	92.8	92.9
Percent Transmittance Unfiltered at 254 nm)	NC	92.7	94.2	93.4	93.3	90.7	91.0
Total Suspended Solids (mg/L)	NC	5.0	< 1.25	5.3	2.7	3.7	4.2
Particulate Organic Matter (mg/L)	NC	< 1.25	< 1.25	< 1.25	< 1.25	< 1.67	< 1.67
Mineral Matter (mg/L)	NC	4.3	< 1.25	4.4	1.8	3.2	3.4
Non-Purgeable Organic Carbon (mg/L)	NC	2.4	2.3	2.5	2.5	2.2	2.5
Dissolved Organic Carbon (mg/L)	NC	2.2	2.1	2.5	2.6	2.1	2.2

*Values are for comparison only, values are not calculated with a correction factor.

Table 19. Receiving Water Trials: Summary of Chemistry and Water Quality Parameters.

Parameter	Trial										
	6			11			12			13	
	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2
Temperature (°C)	16.5	16.4	16.6	4.75	4.94	4.67	4.62	4.53	4.58	2.97	1.74
Specific Conductivity (µS/cm)	102.8	108.6	103.9	103.7	107.3	103.2	104.7	106.0	100.4	205.0	190.4
Salinity (PSU)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.10	0.09
Turbidity (FNU)	3.62	2.31	1.41	1.35	2.22	1.29	8.23	8.85	4.53	30.95	37.68
pH	7.87	7.97	7.98	7.90	7.92	7.93	7.79	7.59	7.60	7.80	7.91
Dissolved Oxygen (mg/L)	9.75	9.84	9.83	12.82	13.09	13.02	12.42	12.50	12.77	12.49	13.09
Dissolved Oxygen (% Saturation)	99.4	100.6	100.5	99.5	101.0	100.8	96.1	96.6	99.2	93.1	93.2
Chlorophyll *a* (RFU)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	1.86	1.55
Chlorophyll *a* (µg/L)*	0.47	0.85	0.37	0.28	0.35	0.23	0.67	0.82	0.81	8.47	7.9
Phycocyanin Accessory Pigment (RFU)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phycocyanin Accessory Pigment (µg/L)*	0.19	0.18	0.18	0.20	0.30	0.34	0.34	0.37	0.39	0.54	0.61
Percent Transmittance-Filtered (254 nm)	94.4	94.4	94.8	96.7	96.4	96.4	90.3	89.2	94.0	10.8	9.5
Percent Transmittance-Unfiltered (254 nm)	92.5	93.8	93.4	96.2	95.9	96.4	86.2	84.5	92.0	8.3	7.9
Total Suspended Solids (mg/L)	1.9	< 1.43	< 1.43	< 1.25	< 1.25	< 1.25	3.5	3.1	1.8	26.8	7.7
Particulate Organic Matter (mg/L)	< 1.43	< 1.43	< 1.43	< 1.25	< 1.25	< 1.25	< 1.25	< 1.25	< 1.25	2.7	< 1.67
Mineral Matter (mg/L)	1.6**	< 1.43	< 1.43	< 1.25	< 1.25	< 1.25	3.1	2.7	1.4	24.1	7.0*
Non-Purgeable Organic Carbon (mg/L)	2.1	1.8	1.7	1.8	1.5	1.6	2.2	2.0	1.7	17.0	17.7
Dissolved Organic Carbon (mg/L)	2.1	1.7	1.7	1.5	1.5	1.5	1.9	2.0	1.6	17.2	17.4

*Values are for comparison only, values are not calculated with a correction factor.**POM was less than the reporting limit but was measurable. Actual measured value was used in MM calculation.

Table 20. Top Twenty Sources and Volumes of Ballast Water Discharged to Western Lake Superior from Other Great Lakes Ports in 2017.

Ballast Uptake Location	Volume of Ballast Discharged (m^3)	Percentage of Total Volume (%)
Gary, Indiana (USA)	4,378,118	16.09
Indiana Harbor, Indiana (USA)	3,160,299	11.61
Burns Harbor, Indiana (USA)	2,674,673	9.83
Conneaut, Ohio (USA)	2,052,410	7.54
Saint Clair, Michigan (USA)	2,006,685	7.37
Detroit, Michigan (USA)	1,843,434	6.77
Monroe, Michigan (USA)	1,841,978	6.77
Cleveland, Ohio (USA)	1,109,803	4.08
Hamilton, Ontario (Canada)	1,080,973	3.97
Toledo, Ohio (USA)	850,356	3.12
Essexville, Michigan (USA)	789,347	2.90
Marquette, Michigan (USA)	758,243	2.79
Ashtabula, Ohio (USA)	690,520	2.54
Quebec City, Quebec (Canada)	664,483	2.44
Nanticoke, Ontario (Canada)	628,384	2.31
Sault Ste. Marie, Ontario (Canada)	382,251	1.40
Windsor, Ontario (Canada)	210,962	0.78
Sturgeon Bay, Wisconsin (USA)	193,451	0.71
Presque Isle, Michigan (USA)	176,590	0.65
Montreal, Quebec (Canada)	170,034	0.62