Shipboard sampling approaches and recommendations by the Great Lakes Ballast Technology Demonstration Project

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Abstract

There are many different types of biological studies on ballast water that could take place on board ships. The best sampling approach depends on specific experimental objectives, combined with cost considerations. This paper details the biological sampling objectives for shipboard studies conducted by the Great Lakes Ballast Technology Project, the sampling methods developed to support them, and the considerations behind these choices. The paper also discusses strengths and limitations of in-line versus in-ballast tank sampling approaches, and their applicability to testing for purposes of ballast water treatment approval and compliance. The paper concludes that in-line sampling offers a simple, thorough, repeatable and accurate option for treatment evaluation and compliance testing, while in-tank sampling may be necessary for more basic biological research.

Introduction

The Great Lakes Ballast Technology Demonstration Project was established in 1996 to accelerate development of practical and effective ballast treatment technologies for ships. It is supported by grants from the Great Lakes Protection Fund and several state and federal agencies.

The Project is co-led by the Northeast-Midwest Institute; a Washington DC based environmental and economic think-tank, and the Lake Carriers' Association, the trade association representing U.S.-Flag vessel operators on the Great Lakes. Together, these two organizations have forged a productive partnership between natural resource protection and maritime industry interests to undertake problem solving work with mutual credibility.

Throughout its seven year history, the Project has carried out extensive and innovative ship-based and barge-based evaluations of flow-through treatment systems; pathogen surveys of vessels entering the Great Lakes; full-scale engineering design studies; an International Ballast Technology Investment Fair; and an economic analysis of global ballast treatment industry prospects. The centerpiece and ongoing emphasis of the Project are its biological and operational field trials at high flow of commercially available ballast treatment equipment.

The biological and operational protocols, including sampling methods developed for the Project's field trials are the result of careful analysis of experimental objectives and the best approaches to achieving them. Here we explore the relationship between sampling approach and shipboard research objectives, describe the Project's experimental objectives and shipboard sampling methods, and identify lessons learnt. The paper concludes with strengths and limitations of sampling methods available, and recommendations.

Relationship between sampling approach and shipboard research objectives

There are many different types of biological studies on ballast water that could take place on board ships. Examples of experimental objectives for shipboard biological studies of ballast water include:

- Surveying ballast tank biota (*What types of organisms live and survive in ballast tanks?*)

- Tracking behaviour and fate of ballast tank biota and changes in community composition over time (*What are the community dynamics of organisms over time in the ballast tank? What is the fate of ballast tank biota after discharge?*)

- Benchmarking treatment performance for purposes of research and development (*How well does a given treatment inactivate specific types of organisms? Is it better than another type of treatment*?)

- Evaluating treatment system function for approval against a regulatory standard (*Is the treatment compliant?*)

- Evaluating treatment function for "spot checks„ (*Is an approved treatment system functioning as expected?*)

Given any one of these objectives, one must evaluate carefully a variety of criteria that may influence decisions on the best sampling approach to use. These include:

- Need for qualitative comprehensiveness (*e.g., in surveys of ballast tank biota, studies of behaviour and fate of ballast tank biota*)

- Degree of focus on biological characteristics of discharge rather than ballast tank contents (*e.g., in system approval, spot checks*)

- Need for quantitativeness (*e.g., in comparisons against a standard, evaluation of treatment effectiveness in general, or comparisons between two treatments*)

- Need for temporal or spatial distribution information during a voyage (*e.g., in measuring changes in community composition*)

- Need for repeatability across voyages and or ships (*e.g. determining if the treatment is as effective on an oil tanker as a bulk cargo carrier, from one use to the next, from one time-period to the next, or from one set of source water conditions to the next*)

The best sampling approach for a given experiment depends upon the research objective. Table 1 illustrates the relationship between biological objectives and sampling considerations.

Table 1. Relationship between biological objectives (vertical column) and sampling considerations (horizontal column)

	Taxonomic comprehensive-ness (qualitativeness)	Focus on biological characteristics of discharge	Quantitativeness	Time-course information	Readily Repeatable
Surveying ballast tank biota	**bb**	**0**	**b**	**bb**	**b**
Behaviour and fate of ballast tank biota/changes in community composition	**bb**	**0**	**b**	**bb**	**b**
Benchmarking treatment performance	**b**	**bb**	**bb**	**b**	**bb**
Evaluating treatment system function for approval against a regulatory standard	**b**	**bb**	**bb**	**0**	**bb**
Evaluating treatment function for "spot checks"	**0**	**bb**	**bb**	**0**	**bb**

bb= High priority   **b**= Medium priority   **0**= Low priority

As Table 1 illustrates, surveys of ballast tank biota and investigations of changes in community composition over a voyage require similar priority sampling considerations, namely taxonomic comprehensiveness and time-course information. Meanwhile, studies to benchmark treatment performance, evaluate treatment function against a standard, or "spot check„ treatment function require a distinct set of sampling priorities, namely direct characterization of discharge quality, quantitativeness, and repeatability.

Project field trial objectives and biological sampling considerations

The Project's treatment trials have been quantitative studies comparing treatment systems (or levels of treatment) against each other, and assessing overall effectiveness in terms of a range of taxonomic groups and from one voyage to the next. Biological questions of key concern to this sort of research are:

- How effective is the equipment at removing or inactivating zooplankton, phytoplankton, bacteria and viruses from the intake and discharge stream?

- To what extent do organisms regrow, die-off and/or interact with each other following treatment, ballast retention and/or discharge?

- Is treatment effectiveness influenced by variation in physical, chemical, or biological characteristics of source water, and/or attributes of the ship environment?

- How predictive are simulated test scenarios of shipboard treatment outcomes (e.g. for type approval)?

For this work, the Project team therefore sought sampling methods that meet the following criteria:

- Replicable access to sample point (in a given vessel or across vessels)

- Adequate sample volumes relative to total volume of ballast water to achieve statistical power

- Integration of entire ballast tank contents/discharge characteristics

- Applicability to microbial as well as plankton taxa

The Project has also taken into account resource considerations in terms of the Project itself, but also in terms of others who may wish to repeat the procedure. In making decisions on the amount to invest in a given sampling scenario, the Project considered "amortization„ periods, i.e., the extent to which a given sampling infrastructure would be exploited over time. Specifically, where a series of tests comparing a range of treatments is planned for a single vessel, more funds may be efficiently invested in hardware to enhance sample quality than in instances in which a single treatment performance test is to be undertaken on a single ship or tested comparatively across a set of ships.

Specific resource considerations include requirements in terms of:

- Time (*e.g., time required for opening of hatches, setting up sample equipment or preparation of ballast tanks for entry*)

- Personnel (*e.g., number of individuals required to collect a given set of samples safely*)

- Space (*e.g., footprint for any sample collection tubs*)

- Safety (*e.g., concerns over entry into hazardous spaces, sampling during cargo loading/unloading*)

- Installation (*e.g., sample ports, net trolleys or enhanced sounding tube access*)

- Equipment (*e.g., plankton nets, catchment tubs, hoses, plankton pumps*)

Quantitative sampling approaches used in project ship-based tests

The Project has taken two contrasting approaches to biological sampling in each of two shipboard studies. For detailed information about these studies, please see appendix, Cangelosi (2002), and Cangelosi et al (in prep).
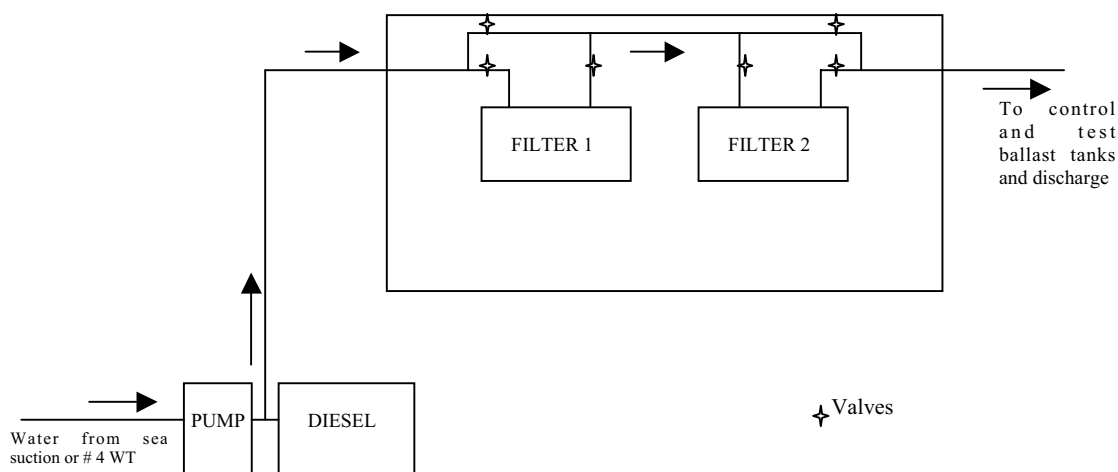
The first sampling approach was designed for extensive comparative analysis of various levels of filtration on a single bulk cargo carrier, the *MV Algonorth.* This quantitative study involved over 17 replications of the experiment on a single vessel, and therefore merited an installation-intensive approach. Plankton net trolleys mounted on transects in matched wing tanks, a sampling platform for the technician to handle the nets, and raised, spring-loaded access hatches to manholes, were all installed. The intent behind these alterations was to facilitate sampling and maximize the comprehensiveness and replicability in the samples over a long series of experimental trials. This approach cost almost $10,000. When averaged over the total number of trials, it cost roughly $600 per trial. It should be noted, however, that this infrastructure remains intact and available for any further testing. (Another example of an installation intensive approach to sampling is currently underway onboard the *ST Tonsina* - see Cooper et al (2002). Installation costs of sampling infrastructure onboard this vessel far exceeds the *MV Algonorth.*)

The second approach was designed for a once-only study on a ship (the *MV Regal Princess*) with ballast tanks that could not be accessed directly. In this experiment, one of the Project's objectives was to develop a stream-lined but effective approach to shipboard sampling which would be readily repeatable on other vessels. In this case, alterations were limited to the installation of two 1.3 cm sample ports in the ballast piping system, temporary 151 L cone-bottom catchment tubs, and temporary nalgene tubing to connect the two. This assembly cost only $1,000, could be used repeatedly, and was easily removed and available for refit to other vessels. As a result, this system would allow comparative testing across vessels as well as among different treatments on a given vessel. The Project will utilize the same approach in upcoming tests of a UV treatment system on a chemical tanker, the *MT Aspiration.*
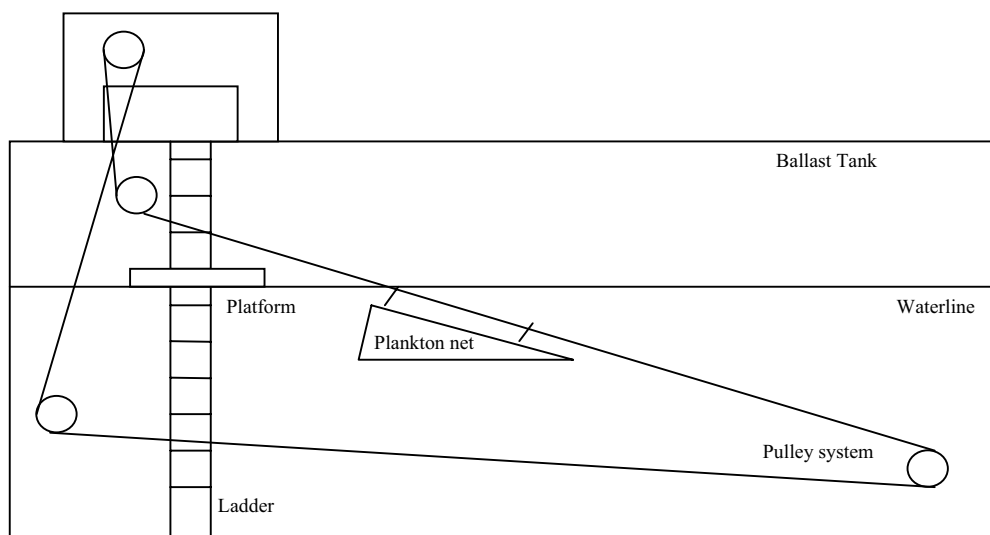
"Low tech,, ballast tank sampling (not supported by installed sampling infrastructure) was rejected as an option for quantitative tests by the Project as too qualitative, uneven, unsafe and disruptive of ship operations.

Description of ballast tank sampling approach - MV Algonorth

The Project undertook comprehensive evaluations of a deck-mounted Automatic Back-Flush Screen Filter in 1997 at a flow rate of 340 m^3/hr onboard an operating commercial bulk cargo vessel (*MV Algonorth*). Experiments took place at various locations in the Great Lakes/St. Lawrence Seaway. Treatments comprised a deck mounted 250 µm pre-filter combined with 25, 50, 100 or 150 µm polishing filter. A deck-mounted diesel pump drew water either from the ship's ballast tanks or the sea. Trials compared water in matched control and treatment upper wing tanks. The tanks were equipped with cable trolleys for identical plankton net transects (running from the bottom to top of the tank along the long dimension). Figure 1 provides a functional representation of the experimental platform used in the experiment. Figure 2 provides a functional representation of the plankton net trolleys mounted on transects and the sampling platform within the confines of the ballast tank.

Figure 1. *Functional representation of the experimental platform, including pump, sample points, filter units, and piping system in relation to ballast tanks for* MV Algonorth b*allast treatment tests*
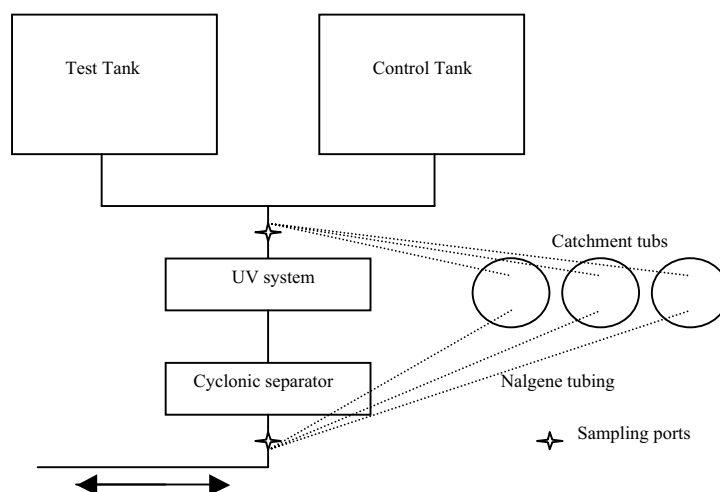

Figure 2. *Functional representation of plankton net, trolley, pulley system and sampling platform in the ballast tank of the* MV Algonorth

Description of in-line sampling approach - MV Regal Princess

The second type of quantitative sampling approach utilized by the Project was in-line sampling through sample ports of ballast intake and discharge. The experiments took place in the summer of 2000, and evaluated cyclonic separation and UV as a treatment combination in an operating passenger vessel (the *MV Regal Princess*). The ballast flow rate was 200 m^3/hr. Sample ports (1.3 cm internal diameter) were installed in the ballast piping system within the engine room of the vessel at the intake and discharge of the combined treatment system. Nalgene tubing channeled sample water from the sample ports to three replicate 151 L catchment tubs, also positioned in the ship's engine room. Sample water was collected throughout the entire duration of the filling and emptying of matched treatment and control ballast tanks through three consecutive fillings of the catchment tubs. Whole water phytoplankton and bacteria samples were drawn directly from the catchment tubs. Zooplankton samples were collected by draining the entire contents of the catchment tubs through plankton nets. Drained water flowed into the ship's bilges. Figure 3 provides a functional representation of the

experimental platform and sampling hardware used in the experiment in relation to the ships' ballast system.

Figure 3. *Functional representation of sample ports, catchment tubs, ballast tanks (control and treatment) and treatment systems (UV and cyclonic separation) for* MV Regal Princess *ballast treatment tests*

Comparison between quantitative sampling approaches

We cannot empirically compare the two quantitative sampling approaches based on the Project studies to date (which evaluated different treatment systems on different vessels). Accordingly, below we describe the shared and unique qualities of each approach, and make recommendations based on our experience. Ultimately, however, a direct comparison of quantitative sampling approaches -- especially these two -- on a single vessel would be of great interest.

Shared Attributes

Perhaps the most important test of a sampling method is whether it is capable of generating statistically powerful data. Fortunately, both the installation-intensive ballast tank sampling, and in-line sampling approaches yielded statistically significant results. They also shared many other positive features. For example, both approaches are:

– Applicable to a wide range of taxa (though both may have quantitative biases relative to the actual suite of ballast tank biota)

– Replicable across source water sites

– Capable of sampling a large volume of water

– Capable of sampling identical volumes of water in each replicate and trial

– Reusable sampling infrastructure

– Capable of sampling the entire contents of the ballast tank (for in-tank, through taking transect tows in ballast tank; for in-line, through tapping entire discharge stream)

– Vulnerable to sampling bias (for in-tank sampling, organisms can avoid capture by plankton nets or pumps; for in-line sampling, some particles may not be captured by pitot tubes as readily as others)

Unique Attributes

Each approach also has unique strengths and limitations. These unique attributes form the basis for judgment as to the relative merits of the two approaches for quantitative studies on ships. The Project has concluded that these attributes argue strongly for further development of the in-line

sampling approach for treatment evaluations and spot checks. In-tank sampling may prove best for research into spatial and temporal dynamics of ballast biota during voyages.

Installation-intensive Ballast Tank Sampling

Strengths (for research on spatial/temporal dynamics during a voyage)
- Organisms in the ballast tank unharmed by passage through a sample port

- Time course and spatially diverse studies of the ballast tank biota possible

Limitations (for treatment evaluations/spot checks)
- Samples reflect midpoint of ballast/deballast sequence (rather than point of discharge conditions)

- Expensive to install hardware infrastructure, such that cannot be readily repeated on another vessel

- Technicians "semi-submerged,,, and exposed to weather and cargo operations

- Technicians not allowed into tanks during certain sea/ship conditions

- Substantial time required to collect complete set of samples (requires two net sizes) leading to longer period between sampling and live analysis.

- Immediate "before and after,, samples not possible

In-line Sampling

Strengths (for treatment evaluations and spot checks)
- Sampling reflects organism condition, concentration and composition upon discharge to the receiving system

- Inexpensive and unobtrusive installation can be readily repeated on other vessels

- Technician gets wet but not "semi-submerged,,, not exposed to weather or cargo loading conditions

- Technician can gain routine access to engine room regardless of ship/weather conditions

- Sampling of ballast stream possible directly before and after treatment

- Organisms cannot avoid sampling equipment

- Infrastructure intact, mobile and available for further tests

- Same infrastructure can be readily installed in other ships to allow comparisons across vessels

Limitations (for research on spatial/temporal dynamics during a voyage)
- Possible greater wear and tear of organisms due to passage through sample port

- Sampling must take place at time of intake and discharge (limiting time-course studies during a voyage)

- Pitot must be designed to minimize bias in capture of entrained particles

- Spatial information of biota within ballast tank is limited

Qualitative sampling approach for ship-based study of pathogens

The Project has conducted only one qualitative study on ballast tank biota, and therefore cannot offer experience in support of comparative analysis of approaches. However, for the benefit of those seeking to make such comparisons, we offer the following description of the novel sampling approach the Project developed for this survey.

The Project designed the sampling approach to support a qualitative survey for the presence of human pathogens in ballast residuals in transoceanic vessels entering the Great Lakes (Knight et al in prep). Sampling took place during the fall of 1997 and summer and spring of 1998. Twenty-eight vessels which entered the Great Lakes reporting "no ballast on board„ to the U.S. Coast Guard were sampled. Sampling was carried out at two locations in the Saint Lawrence Seaway: Montreal, Quebec, Canada and Massena, New York, USA.

The primary constraints on sampling were 1) ship sampling was opportunistic in nature such that pre-installation of sampling infrastructure (such as sample ports) was not possible; and 2) sampling had to take place en-route between locks so direct access to the ballast tanks was not possible. The best solution was to design a device which could sample the tank residuals through a standing aperture like the sounding tube. For effective microbiological sampling from sounding tubes, the equipment had to have the following characteristics:

- Maximum diameter of 4 cm to fit into all sounding tubes which might be encountered

- Capable of retrieving samples from up to 20 m below the deck surface, and if a pumping device is used, capable of pumping water vertically 20 m

- Capable of obtaining sample volumes of between 10 and 100 L within 1 hour

- Able to be disinfected between uses

- Easily carried onto vessels during boarding at locks

- Operated by one or two personnel

In collaboration with Geotech Inc., Denver, CO, Project researchers designed a manually operated inertial pump which met all 5 criteria. The device consisted of various lengths of 1.6 cm diameter rigid polyethylene tubing with a 2.5 cm diameter, 7.2 cm long, cylindrical stainless steel ball-type check valve attached to one end. The device was tested on land using a full-scale model ballast tank sounding tube, and tests predicted the device capable of pumping water 19.2 m vertically with only 15 cm of water in the ballast tank.

Preliminary shipboard tests results were congruent with land-based tests. Additional preliminary tests were conducted to compare deck sampling procedures against samples obtained from inside the ballast tank and to compare numbers of bacteria in samples retrieved via ballast tank sounding tubes with those found in samples collected directly from within the ballast tank. Both tests produced comparative microbiological data indicating that the sampling technology was developed sufficiently for deployment in the pathogens survey.

High volume samples (30 - 40 L) were filtered through a series of four sterile filters: 200 μm plankton mesh, 64 μm plankton mesh, spiral wound protozoan filter, and positively-charged viral filter. Plankton mesh retentates were split and frozen or fixed for analysis of plankton-associated *Vibro cholerae.* Spiral wound protozoan filters were stored at 4 ºC and shipped within 48 hours to the University of Arizona (UAZ) for detection of *Cryptosporidium and Giardia.* Elution of viruses from the viral filter were carried out in the field, with frozen eluates shipped to UAZ for detection of Hepatitis A and members of the enterovirus group.

Photo: Sounding tube sampling device

Low volume samples (1 - 8 L) were split into subsamples, packaged and shipped on ice for overnight delivery to James Madison University and the University of Maryland (UMD) for live analysis of bacterial pathogens and indicator organisms. Two additional subsamples were pumped through high-capacity 0.22 μm pore filters for extraction of total nucleic acids (DNA and RNA).

Another subsample was also pumped through a high-capacity 0.22 μm pore filter to concentrate bacteria for direct viable counting. Initial preparation of this subsample was conducted in the field with fixed samples shipped to UMD for detection of *V. cholerae* and pathogenic *E. coli.* Ten mL of each sample were fixed with formaldehyde for determination of total bacteria using acridine orange direct counting (UMD).

In contrast to the Project's quantitative ballast tank sampling and in-line sampling approaches, this qualitative survey of pathogens is an equipment-intensive ballast tank sampling approach. As with the other two approaches, it has strengths and limitations.

Strengths of this sounding tube sampling approach include:
- Access through sounding tubes

- Sampling of ballast tank residuals

- Can be used across sites, source water conditions, and vessels

- Equipment available for further tests

Limitations of this sounding tube sampling approach include:
- Small percentage of tank volume sampled

- Custom sampling approach cannot be easily replicated without use of same equipment

- Not applicable to plankton

- Could be sampling residual water in sounding pipe

Summary and recommendations

Two fundamental approaches to on-board sampling of ballast water biota are 1) ballast tank sampling (directly sampling water in the ballast tank through a hatch or sounding tube using a plankton pump, net tow, check valve or grab sampler), and 2) in-line sampling (tapping the intake/discharge lines of the ballast system through a sample port). Each can be "low tech„ or "high tech„ and each has strengths and limitations.

Direct sampling of ballast tank contents offers the opportunity to apply several types of sampling methods, including plankton nets and direct grab samples of ballast sediments. It also allows repeated sampling of the water within a tank over the course of a voyage to detect and determine causes of changes in ballast tank biota. These strengths lend themselves to detailed studies of biological processes in the ballast water over the course of a voyage, and surveys of ballast tank biota.

Direct sampling of ballast tanks has limitations, however, for quantitative studies such as treatment evaluations. Access to tanks for such sampling is often uneven, unsafe, and crew-time intensive. As a result, sampling often must be opportunistic rather than adhering to a strict experimental regime. It is also very difficult to achieve spatially comprehensive samples using direct tank approaches without expensive installation of sampling infrastructure. Even if such installation can be invested in a given test, such infrastructure requirements will hamper the replicability of the experiment on another ship.

Most importantly, in-tank sampling approaches are not a good fit for treatment evaluations because the ship's ballast pump and piping can affect ballast biota between the ballast tank and discharge. In addition, some treatment systems may be activated on intake and/or discharge. For these studies, the composition and condition of ballast-entrained biota at the point of discharge is most important.

In-line sampling, on the other hand, may not be sufficient for in depth qualitative research. It cannot provide information on the specific part of the ballast tank environment that a given organism may inhabit during a voyage, only the fact that it may occur in the ballast stream at a certain time in the

discharge process. If there are organisms or life stages that never leave the ballast tank, in line sampling will not detect them.

In-line sampling is, however, readily repeatable from one ship to the next or one trial to the next on a given ship. If it is undertaken continuously or periodically throughout the filling and emptying of the ballast tank, samples over time will capture any stratification that may exist in entrained organisms in the ballast stream from the top, middle and bottom of the ballast tank.

Based on the quantitative experiments the Project has undertaken, the criteria influencing decisions on sampling approaches, and resource considerations, we believe that in-line sampling is a more promising approach than direct ballast tank sampling -- even that involving expensive installations of sampling infrastructure -- for ballast treatment evaluations. This approach is particularly compelling for experiments involving benchmarking of treatment performance, evaluation of treatment function against a standard, and evaluation of treatment function for "spot checks„. Though it is not possible to directly sample ballast tank residuals in this manner, it can be argued that these residuals are only relevant to treatment evaluations if they produce a signal in the discharge entering a receiving system.

In theory, in-line sampling should be equally applicable to studies involving ballast water exchange as ballast treatment, though this has never been tested. To apply in-line sampling to a BWE study, one would utilize the same analytical methods as are used in studies involving direct ballast tank samples. The numbers and types of organisms present in the near coastal source water (sampled through in-line sample ports upon ballast intake) would be evaluated in in-line samples of ballast discharge with and without exchange. Again, if the near coastal organisms are less concentrated or less viable in the discharge than in the ballast tank, the approach would yield more informative results (i.e., relevant to impacts on the receiving system) than direct tank sampling. Moreover, while direct ballast tank sampling is more suitable for qualitative surveys of ballast tank biota and changes in community composition within a given tank over time, we believe in-line sampling also should be undertaken in these experiments if the condition and composition of the ballast tank biota that are ultimately discharged from the ship into the receiving system are relevant.

From an efficiency standpoint, the installation of sample ports for in-line tests is consistent with the need for on-going monitoring and spot-checks by researchers and regulatory agencies. At a very low investment entire fleets could install similar sample ports allowing agency officials access to rapid, representative and comparable samples of ballast intake and discharge. These sample ports can also be useful in comprehensive pathogen surveys of visiting ships. Finally, in-line sampling is easily emulated in shore-based evaluations of treatments, allowing for greater comparability between shore-based and shipboard studies.

As with all sampling approaches in developmental stages, many questions need to be answered before we can wholeheartedly accept or reject a given approach. In the case of in-line sampling, additional research questions include:

- What is the nature of in-line sampling biases, if they exist, and how might they differ from biases associated with in-tank sampling?

- How can biases be minimized?

- If biases must exist, do they interfere with meeting experimental objectives?

The Project will be continuing to refine and trial the in-line sampling approach for ballast treatment evaluations and spot-checks in upcoming field trials of a UV treatment system onboard a chemical tanker, the *MT Aspiration*. Biological and operational effectiveness testing onboard this vessel will begin mid-2003. If possible, the Project would like to explore using this approach to compare the effects of BWE and treatment on the vessel. In the meantime, the Project highly recommends careful comparative analysis of the potential benefits of in-line quantitative sampling approaches prior to any recommendation for the adoption of a standard international shipboard sampling approach for treatment evaluation involving direct access to ballast tanks.

In conclusion, in-line sampling is an important option for quantitative treatment evaluations, and compliance testing because it offers a simple and replicable approach to sampling ballast water that can be consistent across ships and voyages. Such sampling also allows research to focus on the discharge itself, and can take account of any heterogeneity within the ballast tank by making in-line sampling continuous throughout the filling or emptying of the tank.

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Appendix:
Details of project sampling trials and approaches

Ship Trial 1: *MV Algonorth*

The Project undertook the first comprehensive evaluations of filtration as a possible ballast treatment system in 1997 onboard an operating commercial bulk cargo vessel (*MV Algonorth*) at various locations in the Great Lakes/St. Lawrence Seaway System.

For the purposes of this study, the ship's port and starboard #3 wing tanks were physically divided by a horizontal bulkhead into lower and upper wing tanks. The matched #3 port and starboard 220 m^3 upper wing tanks were used as the experimental tanks. Duplicate manual trolley systems were installed in each of the experimental tanks to allow the

Photo: *MV Algonorth*

sampling of water by diagonal plankton net trawls. Steel platforms, or sampling stages, were also installed below the access hatches for the operator to stand or kneel on while running the trolley or collecting the nets.

Water was pumped at a nominal 340 m^3/hour by a diesel-driven self-priming centrifugal pump mounted on deck above the starboard #3 upper wing tank. An extensive piping system, 20 cm diameter pump suction piping and 15 cm diameter pump discharge piping, was installed to allow for experiments to be conducted independently from most vessel operations, and raised, spring-loaded access hatches were installed over the existing manholes to allow easy entry to the experimental tanks. Experimental ballast water was drawn from either the starboard #4 wing tank (1,000 m^3) if vessel operations allowed the filling of this tank, or directly from a dedicated sea suction.

The matched control and test tanks were filled during vessel transits specifically for experimental purposes. Control water was pumped directly to the control tank, bypassing the treatment system, while test water was routed through the treatment equipment into the test tank. The treatment system tested was an Automatic Backwash Screen Filter (ABSF), which was installed in a purpose-built container mounted on deck above the port #3 upper wing tank. The ABSF system consisted of two filter units in series; a pre-filter unit equipped with a 250 µm mesh filter screen followed by a polishing unit equipped with one of a series of smaller interchangeable polishing filter screens.

Four different polishing filter screen mesh sizes were tested for their effectiveness at reducing zooplankton and phytoplankton abundance and diversity in ballast water; 25 µm, 50 µm, 100 µm and 150 µm. In order to avoid sample distortions resulting from test tank contamination by previous tests, screen mesh sizes were tested in cycles from smallest to largest, and the tanks were cleaned with high pressure water before the ascending order of tests was repeated

Before each test, both control and test tanks were filled to one-third capacity in sequence and then topped up to two-thirds capacity. This allowed room (ullage) in the upper part of the tank for the sampling operator. This filling scenario was also especially important to help assure homogeneity between the test and control source when the tanks were being filled from the sea suction, and the ship was moving in transit during ballasting.

When the #4 starboard tank was used as a source reservoir, the flow was diverted over the side of the vessel for at least 5 minutes prior to filling the test and control tanks to eliminate settled materials which could be picked up by the initial flow. The time required to fill the two tanks was approximately 1.5 hours.

The diagonal plankton net trawls were hand-drawn over 10 m at a rate of approximately 0.5 m/sec. Each 0.3 m diameter plankton net trawl filtered approximately 0.64 m^3 of water. Sets of 4 replicate samples were collected first with 80 µm mesh nets, followed by 4 replicate samples collected with 20 µm mesh nets. Three of the replicates from each set were preserved in 10 % Lugol's solution; the remaining replicate was used for live analysis.

The preserved plankton samples were sorted and counted at a shoreside laboratory. Sizing involved measuring total body length with an ocular micrometer. Live analyses were conducted in the shipboard laboratory, located in what had been the ship's conference room and owner's quarters. Live samples were observed through a Leica dissecting microscope, and data recorded on prepared forms.

Plankton tows were conducted at least 5 minutes apart to allow the water column to return to relative equilibrium following the disturbance created by each net tow. It also took approximately 5 minutes to carry out a tow, remove

Photo: Diagonal plankton net trawl

the net from the trolley, rinse the net, remove the cod end, put on a new cod end, put the net back on the trolley, and run out the trolley for the next tow. The smaller, 20 µm plankton net samples were collected after the 80 µm net samples, since the smaller mesh nets produced a stronger wave front that could have elicited avoidance response from the more mobile plankton. If the larger mesh nets were used last, the numbers of those more active species could have been reduced or absent from the net path.

Seventeen trials were undertaken in total, of which 13 yielded usable results, including – 4 tests of the 25 µm screen; 3 tests of the 50 µm screen; 4 tests of the 100 µm screen; and 2 tests of the 150 µm screen.

Physical/chemical source water information was collected regularly using Hydrolab's Datasonde 4. Data included measurements of turbidity, salinity, temperature, pH, and dissolved oxygen. Measurements were collected from ballast tanks and sometimes, overside, but only when the vessel was in port, or in a lock.

Ship Trial 2: MV Regal Princess

In the summer of 2000, the Project conducted biological experiments evaluating cyclonic separation and UV as a possible ballast treatment combination at full-scale. The evaluation took place onboard the *MV Regal Princess*, a commercial cruise liner, which operated between Vancouver, BC and various locations within Alaska during the period of testing.

The treatment combination was installed in the engine room of the ship. The cyclonic separator was designed to remove particles based on specific gravity while the UV chamber provided secondary biocidal treatment.

Photo: *MV Regal Princess*

This experiment offered a unique opportunity to measure the influence of the shipboard environment on treatment performance. For each taxonomic grouping (zooplankton, phytoplankton and bacteria), the *MV Regal Princess* tests comprised:

5. In-line tests, in which the biological characteristics of the ballast stream were compared immediately pre- and post-treatment

6. Short-term exposure tests, which measured the effects of treatment versus no treatment on water pumped into and immediately removed from the ballast tank (to detect effects of physical exposure to the ballast system)

7. Long-term exposure tests, in which the effects of treatment versus no treatment on water held in the ballast tank for 18-24 hours was measured (to detect the cumulative effects of retention time in a ballast tank on treatment effectiveness)

For the purposes of this study, matched #10 port and starboard 90.3 m^3 ballast tanks were utilized as control and test tanks. These tanks were connected to a single 200 mm suction/discharge main line via branch lines controlled by valves. An electrically powered, vertical, self-priming centrifugal ballast pump operating at approximately 200 m^3/hour was used to fill and empty the ballast tanks. Actual ballast pump rate varied by 10 to 15 percent from the nominal pump rate, with the ballasting flow rate found to be consistently higher than the deballasting rate.

The ship's overall ballast infrastructure also handled other ship waste water, including connections to two laundry water tanks, and was also capable of taking suction from the bilge. This resulted in overlapping between the ballast water, grey water and bilge water operations, occasionally resulting in some mixing of the various waste waters.

Both control and test ballast water was pumped through the cyclonic separation and UV treatment combination during ballasting and deballasting. The system was inactive while control water was being pumped. This experimental design allowed for differences between control and test to be attributable to biological factors of the treatment combination rather than a physical component of the ballast distribution system. All ballast tank exposure tests involved a dual pass through the treatment system.

Sample ports of 1.3 cm diameter were installed in the system piping upstream and downstream of the combined treatment system to facilitate in-line sampling of water en-route to and from the control and test ballast tanks. Samples for zooplankton, phytoplankton and bacterial analysis could be drawn upstream and/or downstream of the treatment combination during ballasting or deballasting operations through these sample ports. These sample ports did not interfere with the collection of adequate concentrations of live zooplankton samples.

The sample ports were fitted with 1.4 cm internal diameter nalgene tubing to transfer sample water to three 227 L polyethylene cone bottom catchment tubs that were installed in the ship's engine room near the treatment combination. These catchment tubs were gravity-drained through 5.1 cm bottom valves and hoses. Whole water phytoplankton and bacteria samples were collected from the catchment tubs during filling using 1 L nalgene bottles. Zooplankton samples were collected by filtering the catchment tub's draining contents through 30 cm diameter 20 μm mesh plankton nets held in cushioning 19 L bucket reservoirs.

Photo: Catchment tubs and nets

Each in-line test consisted of three pre-treatment (control) and three post-treatment (test) replicate paired samples collected sequentially via the three catchment tubs. A total of three independent in-line trials were carried out for zooplankton; four for phytoplankton and five for bacteria.

Short- and long-term ballast tank exposure tests differed among taxonomic groups. Zooplankton analysis involved collection of three replicate pre-treatment samples on entrance to the ballast tank, and following ballast tank exposure, three replicate pre-treatment and three replicate post-treatment samples on exit. In contrast to in-line tests, the catchment tubs were filled to 151 L for zooplankton

analysis. Phytoplankton were only analyzed during long-term exposure tests, with three replicate pre-treatment samples collected inbound to the ballast tank, and following ballast tank exposure, three replicate post-treatment samples collected outbound. Bacteria analysis involved the collection of three replicate pre- and post-treatment samples inbound to the ballast tank, and following ballast tank exposure, three replicate pre- and post-treatment samples collected outbound. For zooplankton and phytoplankton, 3 corresponding control samples were taken inbound from the upstream sampling ports, and three outbound from the matched-pair ballast tank using the downstream sampling port. When taking bacteria samples, pre-treatment samples taken inbound to the ballast tank were used as control samples.

A total of three independent trials evaluating short-term exposure to the ballast system were carried out for both zooplankton and bacteria. Long-term exposure studies involved three independent tests for zooplankton, phytoplankton and bacteria. A preliminary investigation comparing the viability of zooplankton in pump versus gravity-fed ballasting operations was also undertaken.

Physical/chemical source water information was collected regularly using Hydrolab's Datasonde 4. Data included measurements of turbidity, salinity, temperature, pH, and dissolved oxygen. Measurements were collected from inside the catchment tubs, and directly from the source water while in port.