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SUMMARY REPORT: AUDITS OF BALLAST WATER TREATMENT SYSTEMS

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AUGUST 2004

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16. Abstract (MAXIMUM 200 WORDS)

The development of ballast water treatment (BWT) technologies is at a very early stage. Many of the proposed BWT technologies have had limited laboratory testing and only a few have been tested aboard ships. Many others are still in the conceptual stage. In order to gain a better understanding of BWT technology development, the U.S. Coast Guard Research and Development Center (USCG RDC) initiated an audit program designed to evaluate the efficacy of promising treatment systems. The program objective was to promote insight into the current status of the scientific and engineering technologies proposed to replace ballast water exchange in reducing introductions of aquatic nuisance species. Vendors of four different BWT systems who were interested in participating in the program invited the USCG to audit their treatment systems and test programs. The audits included observations of the treatment system test operations along with a critical review of the data resulting from the performance evaluation tests conducted by the vendors. Elements assessed as part of the audits included the impacts of the treatment system on marine macro- and micro-biological organisms. This report summarizes the audit program and findings of the four audits.

The audit review team found that weaknesses in the experimental designs and analyses made it difficult to draw definitive conclusions about the treatment performance of the BWT systems. The operational efficiency of these treatment systems had not been tested adequately. The overarching research problem that needs to be addressed by BWT technology testing is how effectively the treatment system inactivates or removes all species present in ballast water. Researchers were either not asking the right questions or not answering the questions asked. Testing should have included species-specific accounting of organism viability/propagation and physical removal of organisms. In addition, the test programs lacked controls, sufficient replication, appropriate test protocols, adequate experimental design, and the level of rigor indicative of a scientifically sound test program. Therefore, a determination that any of the systems qualify as acceptable alternatives to ballast water exchange is premature.

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LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

AHS	AquaHabistat TM
ANS	aquatic nuisance species
ATP	adenosine triphosphate
BTM	Browning Transport Management
BWE	ballast water exchange
BWT	ballast water treatment
DFO	Canada Department of Fisheries and Oceans
DNA	deoxyribonucleic acid
DO	dissolved oxygen
GLBTD	Great Lakes Ballast Technology Demonstration
gpm	gallon per minute
H_2S	hydrogen sulfide
HMI	Hyde Marine, Incorporated
HRSD	Hampton Roads Sanitation District
m^3	cubic meter
MARAD	Maritime Administration
mg/L	milligram per Liter
µm	micrometer (micron)
MSI	Maritime Solutions Incorporated
mW-sec/cm^2	milliwatt-second per square centimeter
NISA	National Invasive Species Act
ODU	Old Dominion University
QA	quality assurance
RDC	Research and Development Center
RNA	ribonucleic acid
RP	Regal Princess
USCG	United States Coast Guard
VTI	Velox Technologies, Incorporated

1.0 INTRODUCTION

An increasing number of nonindigenous organisms are invading the coastal and inland waters of the United States resulting in negative ecological, economic, and health impacts. Defined as any species that enters an ecosystem beyond its historic range, nonindigenous aquatic species include algae, invertebrates, fish, fungi, bacteria, and viruses. While most introduced species do not become established nor create major disturbances within an ecosystem, some have detrimental effects and are considered aquatic nuisance species (ANS). Once an ANS becomes established, elimination is virtually impossible and controlling its spread depletes the environmental quality of affected areas and becomes an endless burden on the economy. The most cost effective way to reduce the impacts of ANS is through the prevention of introductions.

Although ANS may be introduced by other mechanisms (e.g., fouled hulls and aquaculture exchanges), ballast water has been identified as one of the primary vectors through which ANS are transferred to new environments. To prevent such introductions, the National Invasive Species Act (NISA) of 1996 establishes mid-ocean ballast water exchange (BWE) as the standard method of removing unwanted ballast water organisms, against which all other methods of treating ballast water are to be compared. Under NISA, the U.S. Coast Guard (USCG) has the authority to approve ballast water treatment (BWT) technologies to replace BWE, as long as the systems are as effective as BWE in "preventing and controlling infestations of ANS." In order to grant such approval, the USCG must have a thorough understanding of the capabilities and limitations of BWT technologies.

Treating ballast water to remove or inactivate potentially harmful invasive species is a challenging problem. Treatment technologies must address variable water quality parameters (temperature, salinity, nutrients, suspended solids, etc.), high flow-rates, large volumes of water, a diversity of organisms, and ballast water residence times. Effective treatment technology is further complicated by the variability of ships, shipping routes, and ports. The identification of a single treatment technology for all species, ships, and port conditions is unlikely. Rather a suite of treatment technologies will need to be developed to treat ballast water.

The development of BWT technologies is at a very early stage. Many of the BWT technologies currently proposed have had limited laboratory testing and only a few have been tested under real life conditions onboard ships. Many others are still in the conceptual stage. To gain a better understanding of existing BWT technologies, the USCG Research & Development Center (RDC) initiated a program designed to evaluate the efficacy of promising treatment systems. The objective was to promote insight into the current status of the development of ballast water treatment technologies. This report summarizes the audit program and findings of the first four audits.

The technology vendors and test teams who have participated in these initial USCG audits are to be congratulated for their pioneering efforts and contributions to the industry's understanding of BWT opportunities and constraints.

2.0 APPROACH

The USCG RDC convened a team of scientists and engineers tasked with conducting scientific audits of four BWT systems and their respective test programs. Four technology vendors interested in participating in the audit program invited the USCG RDC to review their respective technologies and test programs. The audits involved observations of the different BWT system test designs and operations, along with a critical review of the data resulting from the performance evaluation tests conducted by the vendors. The audit team provided a questionnaire seeking non-proprietary information regarding system description, previous test results, experimental design, test plan, protocols, and basic engineering specifications. After reviewing the test plan provided by the vendors, the audit team coordinator visited each test site to develop a data collection plan for the audit. When each vendor was ready to test the treatment system, the audit team visited the test site to observe the tests. Members of the audit team attempted to avoid influencing the conduct of the tests. Once all information was gathered and the tests observed, the audit team evaluated each vendor's full program. Evaluation criteria included the appropriateness and rigor of test protocols, experimental design, and analyses.

Full audits were conducted on the Hyde Marine, Incorporated [HMI] and Browning Transport Management [BTM] systems. A full audit included field observations of prototype systems, as well as detailed assessments of experimental designs, test protocols, and test execution. The scope of the two remaining system audits (Velox Technologies, Inc. [VTI] and Maritime Solutions, Inc. [MSI]), however, was limited to review of system specifications and preliminary test data.

3.0 PARTICPANTS

3.1 USCG AUDIT TEAM

The RDC assembled a multi-disciplinary team of scientists and engineers charged with conducting independent scientific audits of the four BWT systems (Table 1).

Table 1. Members of the USCG audit team.

Team member	Area of Expertise	Organization
Dr. Brian Howes	Marine Biologist	University of Massachusetts
Dr. Craig Taylor	Marine Microbiologist	Woods Hole Oceanographic Institute
Mike Dyer	Team Coordinator	Volpe National Transportation Systems Center
Chris Murray	Marine Engineer	
Ed Conde	Chemical Engineer	
Tom Pedersen	Environmental Statistics	Camp, Dresser, and McGee

3.2 TECHNOLOGY VENDORS AND TEST TEAMS

Each vendor of the different technologies assembled teams to test and evaluate treatment system performance. The test teams designed the experiments, conducted the tests, and analyzed the data. The technology vendors and their test teams are listed in Table 2.

Table 2. Technology vendors and test teams.

Technology Vendor	Test Team
Browning Transport Management (BTM) Norfolk, VA	1) Old *Dominion University* Norfolk, VA 2) *Hampton Roads Sanitation District* Virginia Beach, VA
Hyde Marine, Incorporated (HMI) Cleveland, OH	*Northeast Midwest Institute* Washington, DC
Velox Technologies, Incorporated (VTI) Calgary, Alberta	*Department of Fisheries and Oceans Canada, West Vancouver Laboratory* West Vancouver, British Columbia (BC)
Maritime Solutions, Incorporated (MSI) New York, NY	*University of Maryland, Chesapeake Environmental Laboratory* Solomon's Island, MD

4.0 BALLAST WATER TREATMENT SYSTEMS

Four individual BWT systems were investigated. One was a single-stage deoxygenation treatment system. The remaining three combined physical separation and ultraviolet (UV) radiation into two-stage units.

4.1 DEOXYGENATION SYSTEMS

Deoxygenation BWT systems are designed to extract dissolved oxygen (DO) from ballast water, thereby killing organisms not adapted to low oxygen environments. Deoxygenation can be accomplished by the use of a vacuum chamber over time or by purging the oxygen from the ballast tanks with an inert gas, such as nitrogen.

4.1.1 Browning Transport Management [BTM] System Description

Browning Transport Management developed and patented the AquaHabistat™ (AHS), a single-stage treatment system intended to kill marine organisms in ballast water by removing DO. In this system, ballast water is sprayed into a 3.4 cubic meter (m^3) vacuum chamber with an

absolute pressure of 0.064 atmospheres where the developer asserts DO dissipates from the water leaving it hypoxic (i.e., test level DO = 0.4 mg/L). The treated water purportedly remains hypoxic after being transferred to ballast tanks where most marine organisms will suffocate within two to three days. Upon discharge, the treated ballast water will regain the oxygen lost, leaving no ancillary environmental side effects.

4.1.1.1 BTM's Basic Test Plan

Ambient water was drawn through a 4-inch diameter pipe fitted with a 3/16-inch-mesh basket strainer and pumped into a 17-foot, 4-foot deep, diameter swimming pool designated as the "control" pool (Figure 1). The water was then pumped from the control pool into the vacuum treatment chamber at approximately 300 gallons per minute (gpm). As water was drawn from the control pool, it was replenished with ambient source water. The treated water was then pumped into a second pool designated as the "treatment" pool. The surface of the water in each pool was covered with a plastic tarp to simulate a pressed-up ballast tank and to prevent reoxygenation through diffusion with the atmosphere.

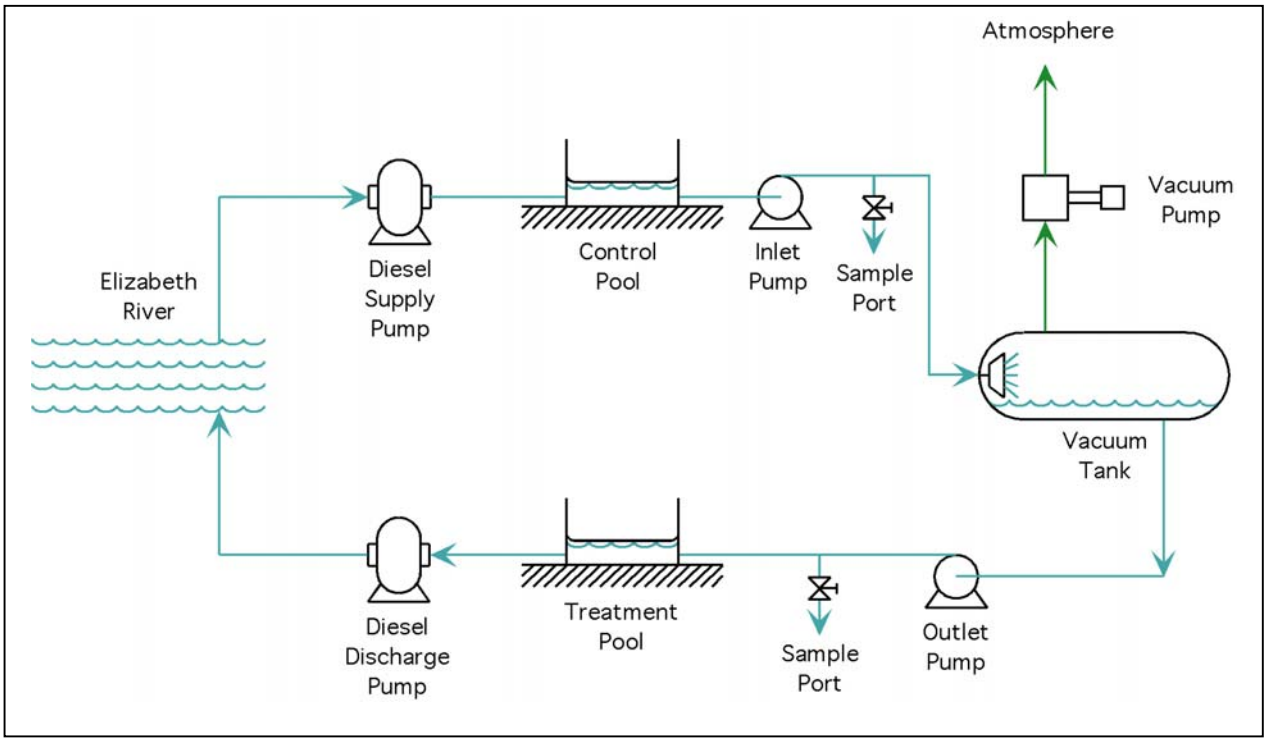

Figure 1. BTM prototype treatment system schematic.

BTM selected two test teams to measure the biological effects of deoxygenation over a period of ten days after treatment. Four net tows for zooplankton were taken every day from each pool. Two tows were taken from the surface and analyzed by Old Dominion University (ODU), and one tow from the surface and one from the bottom were taken and analyzed by Hamptom Roads Sanitation District (HRSD) laboratory.

Three assay approaches were used to evaluate system effectiveness: 1) microscopic examination of screened samples greater than 80 microns (μm) for enumeration, classification, and visual assessment of mortality of zooplankton; 2) enumeration of bacteria that were able to grow and form visible colonies on a nutrient agar medium (viable cell counts); and 3) measurement of adenosine triphosphate (ATP) content of screened samples greater than 80 μm as a proxy for living biomass.

4.1.1.2 BTM Audit Findings

The BTM audit was the most comprehensive of all the systems investigated. Full access to BTM's test plan, raw data, and supporting information enabled a thorough evaluation of this system. On the basis of the review of the test protocol and observations made during experimental tests, the review team identified a number of significant concerns and potential problems with the testing program. Key issues are:

- Adequate Assays: Invasion by foreign species is a problem only if the organisms released in ballast water are alive and able to reproduce. Therefore, the best kind of assays for addressing the effectiveness of a treatment system for preventing an invasion are those that directly assess each organism's viability upon discharge, in as quantitative a fashion as possible. ATP and chlorophyll *a* are quick general measurements but do not permit analysis of variation in response likely to occur among the wide range of taxonomic groups present in ballast water. While ATP may provide a good coarse measure, it does not allow assessment of how each taxonomic group may respond.

- Lack of controls: The experiment suffers from both a lack of independence and a lack of control for disturbance, in terms of pumps or other methods of transferring water between the control and treatment pools. The water and associated plankton communities in the AHS treatment pool were derived from the control pool but these were not paired and independent treatments. This design creates the opportunity for the AHS treatment pool to "sample" a portion of the control pool that is not representative. In addition, the "treatment" consists of both the AHS treatment and any associated method of transfer that was not performed on the control tank. For example, water in the treatment pool passed through three pumps and the treatment spray nozzle, whereas water in the control pool passed through only one pump. Thus, the control and treatment were exposed to different amounts of shear stress. This confounds interpretation of results.

- Lack of laboratory inter-calibration: There was no inter-calibration between the two test labs. Zooplankton population values reported by HRSD were between five- and ten-fold higher than values reported by ODU. In spite of these discrepancies between the two labs, no inter-laboratory comparison was made.

- Lack of methods calibration: There was an inconsistent use of a live-dead criterion between the two test teams. "Twitching" animals were scored as inviable by one of the teams and as viable by the other. The audit team noted "twitching" animals later swimming away during the demonstration of the method. Because the treatment system is designed to kill organisms, the "twitching = live" metric would yield a more conservative estimate of the overall effectiveness (lower percentage of organisms killed). Use of different criteria by the two labs in this study highlights deficiencies in the quality assurance (QA) plan for this project and may also contribute to differences in results found by the two laboratories.

- Lack of replicates: The experiments lacked adequate sample replication, which could have been achieved by taking samples from three treatment pools and one control pool that were filled simultaneously.

- Sampling effect: Effects from continuous sampling over time were not taken into consideration by the test teams. The net tows removed approximately 4.5 percent of the standing zooplankton each time a sampling was made (i.e., four tows). Even though a relatively small fraction of the zooplankton population was removed during each sampling, the cumulative effect of sampling was substantial.

- Deoxygenation as a treatment: Deoxygenation will only affect aerobic organisms, such as copepods and is likely to have little effect on organisms that don't require high levels of oxygen (e.g., anaerobes, facultative anaerobes, spores, and cysts).

- Settling and active behavior effects: Clear and indisputable evidence of particulate settling in the pools was observed. What is unclear is the impact (if any) the settling had on the data collected. In the pools, some of the "loss" of organisms may have resulted from settling to the bottom. The issue of zooplankton settling to the bottom of the pools is of concern, as it was not accounted for in the sampling scheme. The test team did not establish to the satisfaction of the audit team that zooplankton did not settle. Also, organisms in the control pool could redistribute in a way that results in a biased sample during transfer into the AHS treatment pool. This bias is not merely a matter of organisms "settling out," but could arise from active behavior of the organisms as well (e.g., phototaxis, avoidance of current, etc.). Thus, it is statistically and biologically incorrect to attribute the differences between treatment and control pools solely to the AHS treatment. Any differences may simply be an artifact of the system design (and independent of the treatment).

4.2 PHYSICAL SEPARATION/ULTRAVIOLET TREATMENT SYSTEMS

Three vendors developed two-stage technologies that relied on physical separation followed by treatment with UV radiation. In both systems, the first stage physical separation is accomplished by generating a strong vortex capable of separating heterogeneous mixtures and suspended solids by differences in specific gravity. Particles and organisms with specific gravities greater than water are forced to the outside of the circulating water to be drained off and discharged back to source waters. Organisms in the remaining water are exposed to high doses of UV radiation.

Mortality from UV radiation results from photochemical damage to the ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contained in the cells of organisms.

Differences in the separation units among the systems evaluated are primarily related to the means of generating the vortex. In the HMI and VTI systems, the hydrocyclone has no moving parts. The vortex is created by the direction and force of the water entering the system. In the MSI system, the "voraxial" separator uses a powered impeller to generate the vortex.

4.2.1 Hyde Marine Incorporated [HMI] System Description

Hyde Marine, Incorporated (HMI) developed a hydrocyclone/UV treatment system from an engineering concept for two-stage BWT. This system was installed and tested aboard a Princess Cruise Line vessel, *MV Regal Princess (RP)*, which docked in, and operated its summer schedule from, the saltwater port of Vancouver, British Columbia, Canada. The RP project was, according to the test team, one of two integral elements of a test and evaluation program; the second was the Great Lakes Ballast Technology Demonstration (GLBTD) project on board a barge located in the freshwater port of Duluth, Minnesota. The test team's characterization of the program was that the GLBTD tests would present more rigorous sampling and analysis, while the RP tests would provide operational experience and more practical lessons.

The audited HMI system flow rate is designed for 880 gpm. The UV chamber provides radiation dosage at 140 milliwatt-seconds per square centimeter (mW-sec/cm^2).

4.2.1.1 HMI's Basic Test Plan

The RP test plan called for the hydrocyclone/UV system to be tested as a single unit, therefore no evaluation of the individual components was conducted. The experiments were divided into two types, "in-line and "ballast tank" experiments (Figure 2). Inline experiments were designed to assess the immediate effects of the BWT, whereas the ballast tank experiments assessed changes in the extent of biological inactivation with short hold times (less than 48 hours) within actual ship ballast tanks. The control and treatment samples were collected simultaneously in sixty-gallon tubs. Whole water sub-samples from each tub were taken for phytoplankton and bacteria

analyses. The remaining water was screened through a 20 µm plankton net for zooplankton analysis. A time-series test was conducted by sampling water from designated control and treatment ballast tanks.

Zooplankton were analyzed for viability and enumeration, i.e., one-hundred animals independent of taxa were counted. Whole water samples were analyzed fluorometrically for chlorophyll *a* and phaeophytin. Bacteria mortality was determined by viable cell count.

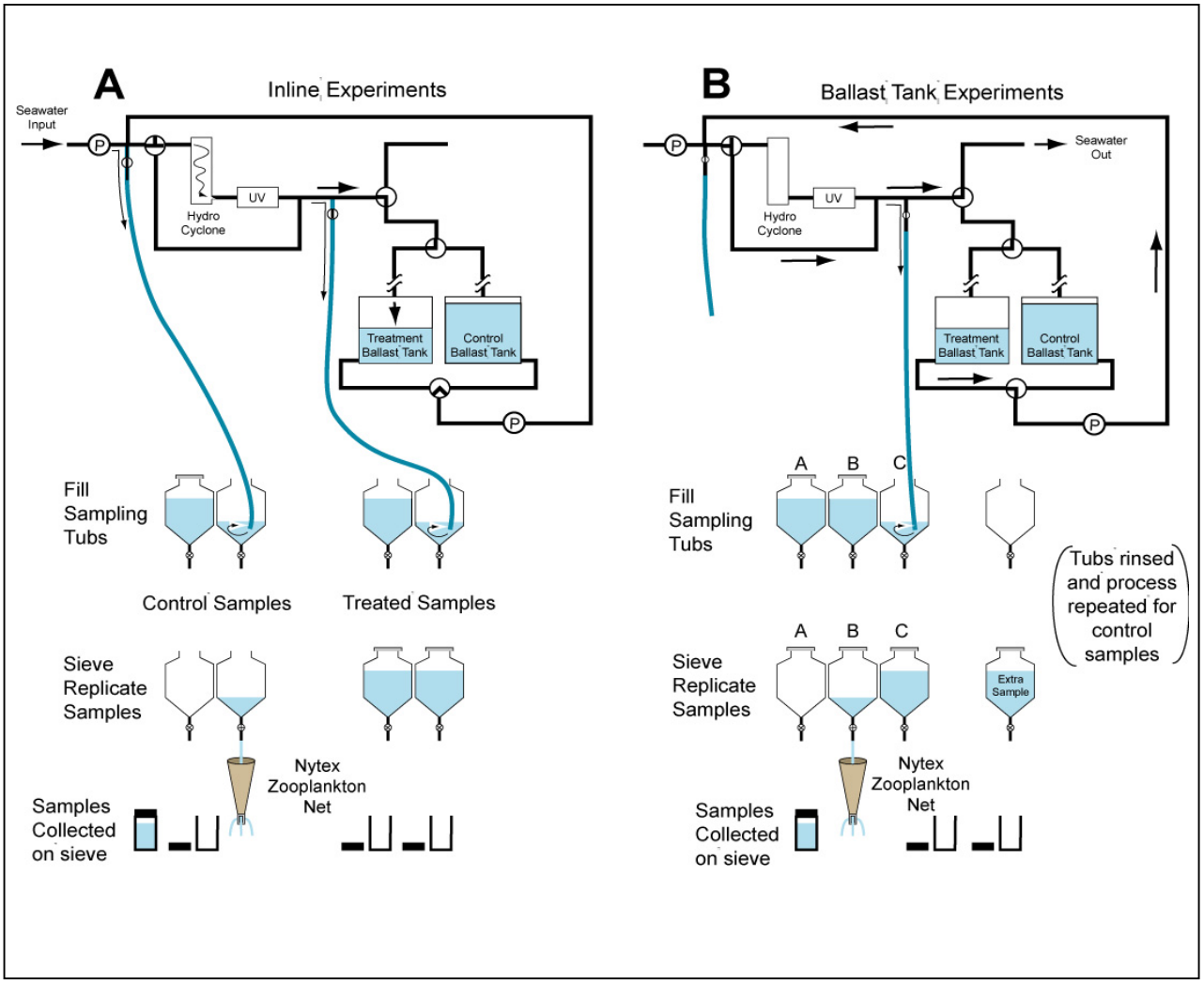

Figure 2. Diagram of ballast water sampling procedure aboard the *MV Regal Princess*.

4.2.1.2 HMI Audit Findings

The installation of the HMI system aboard a ship made the tests and audits more difficult procedures than they needed to be. Because the ship could only ballast and deballast at sea, the audit team was unable to observe the actual system tests. Instead, the team was given a quick demonstration of how the tests were conducted. It was not possible, based on this demonstration, to evaluate whether the test plan protocols were properly executed during the actual tests.

A significant problem noted by the audit team was contamination. For example, some samples were contaminated, as evidenced by the formation of hydrogen sulfide (H_2S), although the source itself could not be isolated. The possibility of contamination was also suggested by the high counts of "new" taxa (i.e., taxa not present in the influent samples) in the samples collected from the ballast tanks.

The test protocol lacked clarity and appropriate scientific detail as to sampling procedures, experimental methods, and overall experimental design and methodology. Important details were left to the inference of the reader, and it was difficult to conceptually reproduce the experiments from the information in the protocol provided. The test plan did not address the effects of the 90-degree, half-inch sample ports and the shear forces they created. The test team presented little or no results from the *MV Regal Princess* tests and instead relied on results from the GLBTD project. However, since there was no inter-calibration of methods or tests used in the two studies, it was difficult to compare experiments and results or to place credence in the claims as made.

Although the overall approach for zooplankton enumeration and assessment of mortality is inherently adequate (e.g., direct counts for enumeration, visualization of structural integrity, response to stimuli, heartbeat for mortality assessment), the methods used do not permit accurate assessment of less abundant yet important members of the population, especially given their doubtful statistical validity. Mortality was determined by counting a total of 100 organisms generally within fewer than four groupings. For the dominant groupings, statistical validity was adequate given that generally more than a 30-count was obtained. However, for groups that were commonly present, but in lower numbers, the method did not provide sufficient information to

determine removal efficiency, due to the variability between samples. Collectively, these lower frequency groups cause concern as they may represent between five and ten percent of the total zooplankton population at all sites and may include major taxonomic groups documented to contain ANS.

Bulk measurements of chlorophyll *a* and phaeophytin were used to assess phytoplankton viability. Chlorophyll *a* is not an appropriate viability indicator as it remains intact for indeterminate periods after cell death and varies by species and initial physiological state. No assessment was made on the effectiveness of the treatment system on key phytoplankton transport stages (e.g., cysts). Phytoplankton cysts are important to assess as are an important life history stage of toxic dinoflagellates, which are responsible for many types of harmful algal blooms.

4.2.2 Velox Technologies, Incorporated [VTI] System Description

Velox Technologies, Incorporated (VTI) developed an engineering concept similar to the HMI system, i.e., centrifugal hydrocylone separator/UV treatment system. The VTI system underwent shore-side testing at the Department of Fisheries and Oceans Canada Laboratory in West Vancouver, BC, before the audit program was initiated. The audit team obtained data about this testing during an interview with the principal investigator.

Delays with VTI's test bed and scheduling precluded further testing, and an audit of the VTI system was not conducted. This was unfortunate because the experimental approach used in the preliminary shore-side testing offered a rigorous experimental design and execution, with superior dosage and mortality data for specific indicator species.

4.2.2.1 VTI's Basic Test Plan

Due to delays early in VTI's test program, no test plan or other technical information was provided regarding the system and test program.

4.2.2.2 VTI Audit Findings

Because no information was supplied, an audit could not conducted on the VTI treatment system.

4.2.3 Maritime Solutions, Incorporated [MSI] System Description

Maritime Solutions, Incorporated, developed a two-phase BWT system. The primary treatment component was a centrifugal voraxial separator. For secondary treatment, MSI planned to test and compare treatment effectiveness of two alternatives, UV radiation and a non-oxidizing biocide, SEAKLEEN®.

Due to various equipment and schedule delays, an audit could not be conducted. Replies to the questionnaire, however, provided some technical information regarding the system and test program. The MSI system was to be installed aboard the United States Maritime Administration (MARAD) ship SS CAPE MAY, a former Lykes Lines Seabee vessel. The designed flow rate of the treatment system was 1500 gpm and the UV output was 100 mW-sec/cm^2. The biocide was to be metered into the flow at a final concentration of 1 milligram per liter (mg/L).

4.2.3.1 MSI's Basic Test Plan

The test plan called for samples to be taken at input and output of the voraxial separator and after the secondary stage (UV or biocide). The intent of this design was to quantify the treatment performance of the individual system components and compare them to the results of testing the system as a single unit. Samples would be analyzed immediately after collection and after an exposure time of 12 – 48 hours.

Phytoplankton would be assayed using in vivo and extractable chlorophyll *a* fluorescence measurements. Bacteria would be enumerated using total plate counts. Supporting methods include direct counting of total ambient bacteria and viable ambient bacteria using sole carbon substrate utilization profiles. Zooplankton survivorship would be determined for the dominant taxa only.

4.2.3.2 MSI Audit Findings

Not enough information was provided in the questionnaire to fully evaluate MSI's test plan. Delays in the vendor's scheduling precluded a site visit and therefore, an audit of this system was not conducted.

5.0 SUMMARY OF FINDINGS

The audit review team found that in general, weaknesses in the experimental designs and analyses of the two fully audited BWT systems made it difficult to draw definitive conclusions about treatment performance. The operational efficiency of these treatment systems had not been tested adequately. The overarching research problem that needs to be addressed by BWT technology testing is how effectively the treatment system inactivates or removes all species present in ballast water. In the opinion of the audit team, researchers were either not asking the right questions or not answering the questions asked. Many of the approaches used did not assess mortality directly. For example, proxy measures, such as ATP, do not fully address treatment effects on viability. It was the audit team's opinion that testing should have included species-specific accounting of organism viability/propagation and physical removal of organisms. To varying degrees, the test programs lacked sufficient controls and replication, appropriate test protocols, adequate experimental design, and the level of rigor indicative of scientifically sound test programs. Therefore, a determination that the audited BWT systems qualify as acceptable alternatives to ballast water exchange is premature.

The test programs observed have been managed as engineering development and prototyping projects, when they should be first and foremost applied biological research programs. While test team efforts were, in many cases, adequate within individual disciplines, the overall test team programs were not indicative of the carefully stepped approach required for biological research and development.

The observations summarized below apply generally to all test program phases. The recommended actions or program modifications are based on the deficiencies observed during audits of the test programs.

5.1 Experimental Design

Experimental design issues can lead to serious problems in the interpretation of results, particularly in regards to the application of the treatment system as a whole or its component parts elsewhere (i.e., in a different environment at a different scale of treatment). Problems were identified in some instances in the areas of management and articulation of program goals, establishment of a firm basis in biological science, QA planning resulting in poor execution of protocols, and a general lack of method validation. In more than one audit, improper selection of measurement methodologies, lack of monitoring of key chemical and biological indicators, and inadequate sampling schema resulted in a poor understanding of a system's performance. Good experimental designs should consider the following factors:

- ***Hypothesis*** – The scientific goals of a test program must be carefully articulated in an experimental hypothesis. In several instances, the product vendors and test teams failed to provide a clearly defined hypothesis. Experimental hypotheses should include target taxa - biotic level, species types (e.g., fish, invertebrates, protozoa, zooplankton, phytoplankton, bacteria), and life stage (e.g., cysts, spores, eggs, larvae, juvenile, adult) - and a clear, scientific rationale for their selection. These target taxa should be logically linked to a discussion of ANS assemblages and population densities, test locations, source waters, and environmental factors. Finally, the kill method and mortality targets should be explained.

- ***Quality Assurance*** – Vendors and test teams should prepare QA project plans for their complicated and multidisciplinary experimental programs. Such plans should include the experimental design and all the particulars of biological assays (personnel, QA, sensitivity, methods checks, detection limits, references to previous work) and should be subject to peer review.

- ***Experimental Hardware*** – The selection and installation of test equipment should be undertaken to minimize the introduction of uncertainties into the experiments and the resulting data. Care should be exercised to avoid the use of inadequately mixed holding tanks. Treatment components should be oriented according to design, and not constrained by

space limitations. Sampling pumps and sample ports should be designed to minimize effects on mortality. Shipboard equipment and ballast tank effects on the treatment and testing processes should be fully explored and understood. The experimental design should address the selection and effects of each piece of equipment used.

- ***Shipboard Environment*** – Test system experimental designs should address the operational environment within which the tests take place and take into account the associated experimental risks. These risks include the health and safety issues for technical staff, and the potential effects of shipboard piping, tanks, and mechanical components (i.e., shear-induced mortality, cross contamination, and other water quality inputs) that may affect data and results. While shipboard testing is important to evaluate the treatment systems in "real world" applications, the results are only valid if the conditions of the tests are rigorously quantified and controlled.

- ***Water Quality*** **–** System tests should include adequate real-time data on water characteristics, including turbidity, conductivity, temperature, salinity, DO, organic content, and inorganic content. These covariates add meaning to the tests, because the researchers can track and understand external inputs. These measurements during the tests also allow for better assessment of treatment effectiveness and provide the vendor and audit teams with needed information on the range of environmental conditions within which the system is effective.

- ***Indicator (Target) Species*** – Test programs should select appropriate target indicator taxa that allow for the quantification of the mortality of any individual species. It is essential that, in selecting indicator taxa, consideration be given to the wider range of potential ANS, such as bacteria, viruses, phytoplankton, dinoflagellates or their cysts, and algae. Experimental logic should be developed that will identify the target species to be assayed, and the appropriate sampling, culturing, and data acquisition methods to be used.

- ***Isolation of the "Active Kill Components"*** – Adequate numbers and placement of sampling ports in the treatment system are required to allow for the segregation of mortality effects due to individual treatment components (e.g., separation and UV) and the shear effects of ballast

water passing through one or more pumps or other piping and mechanical components. Control and treated samples should be collected whenever water passes through different pumps for example.

5.2 Protocol and Sampling Issues

The audit team found that incorrect or inadequate protocols were used and that adequate protocols were not properly executed. Factors that should be considered include:

- ***Replication*** – Replication of samples is required, particularly where large volumes of water are involved in experiments. The design should provide for adequate equipment types and numbers for the replicates needed, and address the effects of time, tide, water chemistry, temperature, etc., on replicate samples.

- ***Sub-discipline Standard Protocols*** – A number of marine biology sub-disciplines should be involved in any test program (e.g., invertebrate biology and microbiology) and the proper application of their associated standard protocols is essential. One example is the use of multiple rather than single media for proper assays of bacteria. Another is understanding the limits of aggregate chlorophyll assays in the determination of phytoplankton mortality. Extractable chlorophyll content is inadequate for this purpose, as chlorophyll remains intact for significant periods of time and varies among different species and even within species for different physiological and environmental conditions.

- ***Live Versus Dead Metrics –*** Criteria for determining viability, at a minimum, should be consistent within a single test program. "Twitching" animals were scored as inviable by some laboratories and as viable by others, even within the same test program. The audit team noted "twitching" animals later swimming away during the demonstration of the method by the laboratory personnel. The lack of standard criteria for viability within the general ballast water research community also makes it difficult to conduct comparative analyses between different programs conducted nationally.

- ***Potential Contamination*** – Good laboratory practices must be followed to ensure the test teams introduce no potential contamination. If contamination is suspected, its source and impact to the test results must be investigated thoroughly. The audit team observed in one case that the nets were not cleaned between tows during the audit. This omission opens the possibility for cross contamination of samples between treatment and controls. In another instance, contamination was revealed by the presence of H_2S and "new" taxa, although the source could not be identified.

- ***Quality Assurance –*** In addition to samples for quantifying the effectiveness of the treatment systems, samples should be collected for verification of the assay methods as applied and in the environment being tested. This additional sampling is particularly important when subjective protocols are being used, such as mortality of zooplankton. It is critical that these data be included in any discussion of the test results. For example, in one of the cases where two laboratories evaluated the same samples, very large quantitative differences were found in the resultant counts. While these data were used to evaluate the treatment system, they were not used to evaluate the protocols and the results remain open to criticism.

- ***Other Sampling Issues*** – Other sampling errors such as improper splitting of sub-samples (e.g., due to failure to properly mix and homogenize the parent sample) are a matter of proper execution. The researcher must be aware of other sampling-induced errors such as sample removal (for example, the case observed where repeated plankton net tows were each removing the standing zooplankton in about 4.5 percent of the sampled water's volume). In other cases, failure to randomize sampling order had the potential to bias results, and use of an open petri dish without grids for enumeration of zooplankton had potential for inaccuracy. Good experimental procedure would require randomized sampling and a grid to guide the analyst and prevent missing areas or double counting.

5.3 Data Management

The audit team recognized the desire for protection of data by those who invested in the development of new technology applications. However, in the case of BWT systems, scientific

rigor and public interest require full access to test data. It is not possible to fully evaluate the validity of test programs or treatment systems, or to make sound policy decisions that protect the environment without sufficiently assuring the quality and integrity of test data submitted.

6.0 RECOMMENDATIONS

It should be noted that the results of the audits conducted represent a snapshot in time in 2000. It is recommended that the USCG continue to monitor the state of development of BWT technology as the industry matures. It is also recommended that the USCG provide researchers who plan to test the performance of BWT systems with general guidelines for developing a scientifically sound test program. Included in the guidelines should be a discussion about the inappropriate use of proxy measures, such as ATP and chlorophyll *a*, in determining organism viability and the necessity to evaluate system effectiveness at the species level.

Recommendations for technology developers concern properly developed test programs. The test programs should include scientific identification of target taxa and development of bench-scale treatment units to establish dose-response correlations for those taxa. Subsequent steps should include the construction and testing of a shore-side prototype treatment unit (pilot scale) and, finally, a prototype shipboard unit for full-scale testing. The question that needs to be addressed during shipboard testing is not the level of treatment required to achieve the desired kill/removal, but rather whether the treatment can be consistently delivered under the challenges of the shipboard environment. The former question of the relationship between treatment level and efficacy must be established during earlier stages of development where conditions can be better controlled. Even in the controlled laboratory setting, biological tests are logistically complicated, time consuming, and expensive to conduct.

The selection of indicator taxa used in treatment test evaluations should be justified, as should the choice of approaches for assessing mortality of the selected taxa. Research is needed in this area as no standardization exists at this time.

Proper management of these multi-disciplinary programs is a critical aspect; all participants must have clearly defined roles and the test program director should maintain the team's focus on the scientific goals articulated.

Finally, the technology vendors and test teams who have participated in these initial USCG audits should be encouraged to continue with further development and testing of these promising BWT technologies.