EPA SAB Ballast Water Advisory Subgroup 3

Draft Outline of Response to Charge Question 4: Limitations of existing studies and reports

I. Introduction

A. Charge from EPA and the Focus of this Document

"What are the principal limitations of the available studies and reports on the status of ballast water treatment technology and system performance and how can these limitations be overcome or corrected in future assessments of the availability of technology for treating ballast water onboard vessels?" Charge question 4 (and the other three specific charge questions) are under the committee's overall charge to "provide advice on technologies and systems to minimize the impacts of invasive species in vessel ballast water discharge" (Feb. 2010 Federal Register notice). While we address Charge Question 4, we also address aspects of the broader charge not covered by any of the four specific charge questions. Specifically, in later sections of this report, we address limitations of technology and systems to enable effective compliance and enforcement, and on-shore treatment systems.

B. Motivation: Standards Drive Technology Development

To be useful, the standards that dischargers are required to meet must be achievable; but to provide an incentive to improve treatment technology, these standards must be more demanding than can be met by the specific treatment systems that are currently in use to treat the discharges.

II. Problems with testing commercial ballast water treatment technology systems: protocols, analysis, and reporting practices that could be improved

A. Major Limitations

Major impediments in evaluating the performance of ballast water treatment technologies include the lack of available data to enable assessment or comparison of most systems; the lack of comprehensive, standardized testing programs; and the lack of standardized, practical protocols for quantifying the removal of some size classes of organisms.

B. Lack of Independent Testing

Testing should be conducted by a party independent from the manufacturer with appropriate, established credentials, approved by EPA/USCG.

C. Need for Experimental Validation of Methods

Experimental validation is needed to demonstrate that testing operations can accomplish separation of organisms with quantified uncertainty (e.g. microbead-based experiments to test filtration)

D. Lack of Standardized Testing Protocols

Testing protocols must be standardized and ideally applied across the full gradient of environmental conditions represented by the earth's ports

1) Test Verification Factors
2) Challenge Conditions -
3) Treatment Facility Experimental Configuration -
4) Verification Testing -

E. Compromises Necessary Because of Practical Constraints

1) Standard Test Organisms (Surrogates) -

2) Other Potential Surrogates Cannot Reliably Quantify Organisms

Algal pigments -

Adenylates

INT - (2-*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride)

3) Sized-Down Treatments-

F. Incomplete Reporting of Results. Complete results, including failures, must be reported.

III. Problems with testing commercial ballast water treatment technology systems: inherent limitations?

A. Statistical Constraints on Protocols and Standards

Tests should be designed and the data analyzed using appropriate volumes and statistical methods, including explicit quantification of statistical power (Lee et al. 2010). To verify reductions to the extremely low concentrations proposed for the largest (>50um) size class requires analysis of impractically(?) large sample volumes.

B. Mismatch Between the "Living" (Viable) Standard and Practical Protocols

Determining whether an organism was "living," "viable" or "infective" at the time of discharge is very expensive in terms of time and effort for all organisms and perhaps impossible for some organisms and live stages. Testing protocols for determining "viability" and/or "living" must be standardized, and the limitations of these protocols must be assessed.

1) Rapid concentration of cells from large volumes kills and/or destroys many organisms –
2) Standardized rigorously tested protocols for evaluating organism viability do not yet exist –
3) Resistant stages present special challenges in attempts to assess viability –
4) Many microbial species are capable of changing their location in counting chambers, which can bias the count
5) Viable when collected (formerly viable) counts should be considered as a practical alternative for quantifying phytoplankton, other protists, and zooplankton –

6) Techniques to detect and quantify harmful bacteria are based upon small volumes relative to ballast tank volumes, and also cannot account for active nonculturable cells –

C. HACCP: an alternative approach to assess treatment capabilities?

An alternative approach is to base the assessment on an analysis of the engineering and operational design of the treatment system and the individual processes used in the treatment system. This is the approach employed when Hazard Analysis and Critical Control Points (HACCP) methods are used to set regulatory and permit requirements.

IV. Relationship of testing protocols to assuring feasibility of voluntary compliance monitoring and enforceability of standards

A. Statement of limitation

Both initial testing of technology protocols (section above) and potential compliance and enforcement monitoring suffer from slow and expensive methods. No information was provided (does any exist?) on whether protocols and systems for compliance monitoring (whether voluntary by ship operator or legally required) and enforcement were being considered alongside the development and testing of technology. The committee feels that it is essential that these be developed in concert with technology testing to avoid a situation where the creation of enforceable policy or rules is difficult or impossible.

B. Practical compliance/enforcement protocols needed.

Full protocols for initial evaluation of a technology (previous slide) are not practical for routine inspections (either self-inspections or regulatory inspections); protocols assessing surrogate parameters (including perhaps chlorophyll, DNA, ATP) for concentration standards should be developed that can be easily and quickly measured on board ship or nearby. If sufficient foundation of rigorous studies demonstrate the relationship between surrogate variables and the numerical standards for living organisms (specified in policy) then such surrogates could be used not only in future compliance and enforcement testing but also in initial testing of technology systems. (see among others, recent paper by Tamburri et al). These protocols also need to work under the full range of environmental conditions relevant to shipping (previous section).

V. Approaches other than shipboard treatment to reduce the risk of invasions from ballast discharges

A. Introduction

Several key approaches other than shipboard treatment of ballast water (including both technologies and operational systems) could help to reduce the risk of biological invasions from ballast water discharges, and contribute to the achievability of discharge standards and permit requirements.

B. Managing ballast uptake

Several studies have recommended various ballasting practices—referred to as ballast micro-management, shipboard management measures, or precautionary management measures—to reduce the number of organisms, or the number of harmful or potentially harmful organisms (such as bloom-forming algae and organisms found in sewage), that are taken up with ballast water.

C. Reducing risk of invasion from ballast discharges

Technologies and practices to reduce the volume of ballast water discharged in US waters could include ballast water exchange; operational adjustments; systems that allow shifting of ballast water between tanks; larger, wider vessels that require less ballast water per unit of cargo; changes in the deballasting practices to minimize reproduction of discharged organisms; and the potential development of ballastless vessels.

VI. Onshore treatment

A. Existing Literature on Onshore Treatment

Onshore treatment includes both treatment facilities built on land and treatment facilities installed on a port-based treatment ship, which will be referred to as "on-land" and "treatment ship" approaches, respectively.

B. Potential Advantages of Onshore Treatment Compared to Shipboard Treatment

Onshore ballast water treatment systems have a greater potential to meet strict standards at a reasonable total cost than do shipboard treatment systems.

C. Potential Operational Issues Restricting the Use of Onshore Treatment

D. Cost of Onshore vs. Shipboard Treatment

E. Potential Capability of Onshore Treatment

VII. Combined approaches and voyage-based risk management

A. Combining previously described approaches

It may be possible to meet more stringent discharge standards, or otherwise reduce the risk of invasions from ballast water discharges, by combining multiple approaches discussed above, including on-shore treatment.

B. Voyage-based risk assessment to prioritize use of treatment technologies, ballasting and deballasting practices, monitoring efforts, and enforcement.

Ship-specific, voyage-based risk assessments have recently been developed for use by ship operators and regulators. Risk assessment protocols consider the origin of a ship with respect to environmental match of port and/or known presence of harmful organisms. These risk

assessments could be useful in compliance and enforcement purposes in addition to risk management purposes.

VIII. References used (incomplete)

Agusti, S. and M.C. Sánchez (2002) Cell viability in natural phytoplankton communities quantified by a membrane permeability probe. *Limnology and Oceanography* 47: 818-828.

Albert, R., R. Everett, J. Lishman, and D. Smith (2010) *Availability and Efficacy of Ballast Water Treatment Technology: Background and Issue Paper*. U.S. EPA and USCG, Washington, DC.

American Public Health Association (APHA), American Water Works Association and Water Environment Federation (2005) *Standard Methods for the Examination of Water and Wastewater*, 21st edition. APHA, Washington, DC.

American Society of Testing and Materials (ASTM) (1996a) Standard Guide for Conducting Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Mollusks. In: *Annual Book of ASTM Standards*, Section 11, Water and Environmental Technology, Volume 11.05 (724-94). ASTM, West Conshohocken, PA.

American Society of Testing and Materials (ASTM) (1996b) Standard Guide for Conducting Acute Toxicity Tests with Echinoid Larvae. In: *Annual Book of ASTM Standards*, Section 11, Water and Environmental Technology, Volume 11.05 (E1563-95). ASTM, West Conshohocken, PA.

Anderson, A., J. Cordell, F. Dobbs, R. Herwig, and A. Rogerson (2008) *Screening of Surrogate Species for Ballast Water Treatment*. Final Report to NSF International, Ann Arbor, MI.

Anderson, A.B., B.O. Johannessen, and E. Dragsund (2004) Ballast water treatment verification protocol – DNV. In: *Proceedings of the 2nd International Ballast Water Treatment R&D Symposium*, by J.T. Matheickal and S. Raaymakers (eds.). IMO, London, UK.

AQIS. 1993a. Ballast Water Treatment for the Removal of Marine Organisms. Prepared by Gutheridge, Haskins and Davey Pty Ltd. Ballast Water Research Series Report No. 1, Australian Quarantine and Inspection Service, Canberra. 106 pages.

AQIS. 1993b. Ballast Water Management Study. Prepared by Thompson Clarke Shipping Pty Ltd. Ballast Water Research Series Report No. 4, Australian Quarantine and Inspection Service, Canberra. 254 pages.

Aquatic Sciences. 1996. Examination of Aquatic Nuisance Species Introductions to the Great Lakes through Commercial Shipping Ballast Water and Assessment of Control Options Phase II. Final Report. A report for the Canadian Coast Guard. Aquatic Sciences Inc., St. Catharines, Ontario. 60 pages.

Avnimelech, Y., B.W. Troeger and L.W. Reed (1982) Mutual flocculation of algae and clay evidence

and implications. *Science* 216: 63-65.

Baldino, R. (1995) *Outline of Standard Protocols for Particulate Organic Carbon (POC) Analyses*. University of Wisconsin - Madison, Madison, WI.

Barcina, I., P. Lebaron, and J. Vives-Rego (1997) Survival of allochthonous bacteria in aquatic systems: a biological approach. *FEMS Microbiology Ecology* 23: 1-9.

Bergh, O., K.Y. Børsheim, G. Bratbak, and M. Heldal (1989) High abundance of viruses found in aquatic environments. *Nature* 340: 467-468.

Binet, M.T. and J.L. Stauber (2006) Rapid flow cytometric method for the assessment of toxic dinoflagellate cyst viability. *Marine Environmental Research* 62: 247-260.

Blenkinsopp, S.A. and M.A. Lock (1990) The measurement of electron transport system activity in river biofilms. *Water Research* 24: 441-445.

Bolch, C.J. and G.M. Hallegraeff (1993) Chemical and physical treatment options to kill toxic dinofla-gellate cysts in ships' ballast water. *Journal of Marine and Environmental Engineering* 1: 23-29.

Bowles, N.D., H.W. Pearl, and J. Tucker (1985) Effective solvents and extraction periods employed in phytoplankton carotenoid and chlorophyll determination. *Canadian Journal of Fisheries and Aquatic Science* 42: 1127-1131.

Boyer, G.L. (2007) The occurrence of cyanobacterial toxins in New York lakes: Lessons from the MERHAB-Lower Great Lakes Program. *Lake and Reservoir Management* 23:153-60

Boyer, J.N., C.R. Kelbe, P.B. Ortner, and D.T. Rudnick (2009) Phytoplankton bloom status: Chlorophyll *a* biomass as an indicator of water quality condition in the southern estuaries of Florida, USA. *Ecological Indicators* 9s: s56-s67.

Brown & Caldwell. 2007. Port of Milwaukee Onshore Ballast Water Treatment–Feasibility Study Report. Prepared for the Wisconsin Department of Natural Resources. Brown and Caldwell, Milwaukee, WI. 114 pages.

Brown & Caldwell and Bay Engineering. Inc. 2008. Port of Milwaukee Onshore Ballast Water Treatment–Feasibility Study Report. Phase 2. Prepared for the Wisconsin Department of Natural Resources. Brown and Caldwell, Milwaukee, WI. 98 pages.

Brussaard, C.P.D., D. Marie, R. Thyrhaug, and G. Bratbak (2001) Flow cytometric analysis of phytoplankton viability following viral infection. *Aquatic Microbial Ecology* 26: 157-166.

Buchanan, C., R. Lacouture, H.G. Marshall, M. Olson and J. Johnson (2005) Phytoplankton reference communities for Chesapeake Bay and its tidal tributaries. *Estuaries* 28: 138-159.

Bukhari, Z., W.G. Glen, and J.L. Clancy (1999) Effects of pH on a fluorogenic vital dyes assay (4',6'-diamidino-2-phenyl-indole and propidium iodide) for *Cryptosporidium* sp. oocysts. *Water Research* 33: 3037-3039.

Burkholder, J.M. (1992) Phytoplankton and episodic suspended sediment loading: Phosphate partitioning and mechanisms for survival. *Limnology and Oceanography* 37: 974-988.

Burkholder, J.M. (1998) Implications of harmful marine microalgae and heterotrophic dinoflagellates in management of sustainable marine fisheries. *Ecological Applications* 8: S37-S62.

Burkholder, J.M. (2009) Harmful algal blooms, pp. 264-285. In: *Encyclopedia of Inland Waters, Volume 1*, by Likens GE (ed.) Elsevier, Oxford, UK.

Burkholder, J.M. and P.M. Glibert (2006) Intraspecific variability: An important consideration in forming generalizations about toxigenic algal species. *South African Journal of Marine Science* 28: 177-180.

Burkholder, J.M., A.S. Gordon, P.D. Moeller, J.M. Law, K.J. Coyne, A.J. Lewitus, J.S. Ramsdell H.G. Marshall, N.J. Deamer, S.C. Cary, J.W. Kempton, S.L. Morton, and P.A. Rublee (2005) Demonstration of toxicity to fish and to mammalian cells by *Pfiesteria* species: Comparison of assay methods and multiple strains. *Proceedings of the National Academy of Sciences (U.S.A)* 102:3471-3476.

Burkholder, J.M., G.M. Hallegraeff, G. Melia, A. Cohen, H.A. Bowers, D.W. Oldach, M.W. Parrow, M.J. Sullivan, P.V. Zimba, E.H. Allen, and M.A. Mallin (2007) Phytoplankton and bacterial assemblages in ballast water of U.S. military ships as a function of port of origin, voyage time and ocean exchange practices. *Harmful Algae* 6: 486-518.

California SLC. 2009. 2009 Assessment of the Efficacy, Availability and Environmental Impacts of Ballast Water Treatment Systems for Use in California Waters. California State Lands Commission, Sacramento, CA. 180 pages.

California SLC. 2010. Draft 2010 Assessment of the Efficacy, Availability and Environmental Impacts of Ballast Water Treatment Systems for Use in California Waters. California State Lands Commission, Sacramento, CA. 149 pages.

Cangelosi, A.A., N.L. Mays, M.D. Balcer, E.D. Reavie, D.M. Reid, R. Sturtevant, and X. Gao (2007) The response of Zooplankton and phytoplankton from the North American Great Lakes to filtration. *Harmful Algae* 6: 547-566.

CAPA. 2000. Feasibility of Onshore Ballast Water Treatment at California Ports. Prepared by URS Corporation/Dames & Moore. California Association of Port Authorities, Sacramento, CA.

Carlton, J. T., Reid, D. M. and H. van Leeuwen. 1995. Shipping Study. The Role of Shipping in the Introduction of Nonindigenous Aquatic Organisms to the Coastal Waters of the United States (other than the Great Lakes) and an Analysis of Control Options. Report No. CG-D-11-95, U. S. Coast Guard, Groton CT and U. S. Dept. of Transportation, Washington DC.

Choi, K.-H., W. Kimmerer, G. Smith, G.M. Ruiz, and K. Lion (2006) Post-exchange zooplankton in ballast water of ships entering the San Francisco Estuary. *Journal of Plankton Research* 27: 707-714.

Cohen, A.N. 1998. Ships' Ballast Water and the Introduction of Exotic Organisms into the San Francisco Estuary: Current Status of the Problem and Options for Management. A report for CALFED and the California Urban Water Agencies. San Francisco Estuary Institute, Richmond CA.

Cohen, A.N. and B. Foster. 2000. The regulation of biological pollution: Preventing exotic species invasions from ballast water discharged into California coastal waters. Golden Gate University Law Review 30(4): 787-883.

Connelly, S.J., E.A. Wolyniak, K.L. Dieter, C.E. Williamson, and K.L. Jellison (2007) Impact of zooplankton grazing on the excystation, viability, and infectivity of the protozoan pathogens *Cryptosporidium parvum* and *Giardia lamblia. Applied and Environmental Microbiology* 73: 7277-7282.

Cohen, A.N. (1998) *Ships' Ballast Water and the Introduction of Exotic Organisms into the San Francisco Estuary: Current Problem Status and Management Options*. Report for California Bay Delta Authority, Sacramento, CA. San Francisco Estuary Institute, Richmond, CA.

Cuker, B.E. and L. Hudson Jr. (1992) Type of suspended clay influences zooplankton response to phosphorus loading. *Limnology and Oceanography* 37: 566-576.

Dames and Moore. 1998. Ballast Water Management Technical Memorandum for the Port of Oakland. Job No. 02801-028-086, November 6, 1998. Dames and Moore, San Francisco, CA.

Dames and Moore. 1999. Phase I Final Report Ballast Water Exchange and Treatment. A Report for the California Association of Port Authorities, Pacific Merchant Shipping Association, Steamship Association of Southern California, and Western States Petroleum Association. Dames and Moore, San Francisco, CA.

Darzynkiewicz, Z., X. Li, and J. Gong (1994) Assays of cell viability: Discrimination of cells dying by apoptosis. In: *Methods in Cell Biology*, by Z. Darzynkiewicz, J.P. Robinson, and H.A. Crissman (eds.). Academic Press, New York.

Dobbs, F.C., and A. Rogerson (2005) Ridding ships' ballast water of microorganisms. *Environmental Science and Technology* 39: 259A-264A.

Doblin, M.A. and F.C. Dobbs (2006) Setting a size-exclusion limit to remove toxic dinoflagellate cysts in ships' ballast water. *Marine Pollution Bulletin* 52: 259-263.

Dobroski, N., C. Scianni, D. Gehringer, and M. Falkner (2009) *2009 Assessment of the Efficacy, Availability and Environmental Impacts of Ballast Water Treatment Systems for Use in California Waters*. Produced for the California State Legislature. Available at: http://www.slc.ca.gov/spec_pub/mfd/ballast_water/documents/2009cslctechreportfinal.pdf

Elliot, J.M. (1971) *Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates.* Scientific Publication No. 25, Freshwater Biological Association, Windermere, UK.

Faruque, S., M.J. Albert, and J.J. Mekalanos (1998) Epidemiology, genetics, and ecology of toxigenic Vibrio cholerae. *Microbiology and Molecular Biology Reviews* 62: 1301-1314.

Fuhrman, J.A. (1999) Marine viruses and their biogeochemical and ecological effects. *Nature* 399: 541-548.

Garvey, M., B. Moriceau, and U. Passow (2007) Applicability of the FDA assay to determine the viability of marine phytoplankton under different environmental conditions. *Marine Ecology Progress Series* 352: 17-26.

Gauthier, D. and D.A. Steel. 1996. A Synopsis of the Situation Regarding the Introduction of Nonindigenous Species by Ship-Transporting Ballast Water in Canada and Selected Countries. Canadian Manuscript Report of Fisheries and Aquatic Sciences No. 2380. Marine Environmental Sciences Division, Department of Fisheries and Oceans, Maurice Lamontagne Institute, Mont-Joli (Quebec), Canada.

Gollasch, S. (2010) *Global Expert Workshop on Harmonization of Methodologies for Test Facilities of Ballast Water Management Systems*. Workshop Report. World Maritime University, Malmö, Sweden, 39 pp.

Greenman, D., K. Mullen and S. Parmar. 1997. Ballast Water Treatment Systems: A Feasibility Study. Produced in cooperation with ENS Chris Freise, U.S. Coast Guard Office of Response. Commissioned by the U.S. Coast Guard and Worcester Polytechnic Institute. Submitted to Professor Matthew Ward, Washington, D.C. Project Center, Worcester Polytechnic Institute. 59 pages.

Gregg, M.D. and G.M. Hallegraeff (2007) Efficacy of three commercially available ballast water biocides against vegetative microalgae, dinoflagellate cysts and bacteria. *Harmful Algae* 6: 567-584.

Hallegraeff, G.M. (1998) Transport of toxic dinoflagellates via ship's ballast water: bioeconomic risk assessment and efficacy of possible ballast water management strategies. *Marine Ecology Progress Series* 168: 297-309.

Hallegraeff, G.M. and C.J. Bolch (1992) Transport of dinoflagellate cysts in ship's ballast water: Implications for plankton biogeography and aquaculture. *Journal of Plankton Research* 14: 1067-1084.

Hallegraeff, G.M., J.P. Valentine, J.-A. Marshall, and C.J. Bolch (1997) Temperature tolerances of toxic dinoflagellate cysts: application to the treatment of ships' ballast water. *Aquatic Ecology* 31: 47-52.

Hampel, M., I. Moreno-Garrido, C. Sobrino, H.L.M. Lubia, and J. Blasco (2001) Acute toxicity of LAS homologues in marine microalgae: Esterase activity and inhibition growth as endpoints of toxicity. *Ecotoxicology and Environmental Safety* 48: 287-292.

Hendry, G.A.F., J.D. Houghton, and S.B. Brown (1987) The degradation of chlorophyll - a biological enigma. *New Phytologist* 107: 255-302.

Holm-Hansen, O. (1973) The use of ATP determinations in ecological studies. *Bulletin of the Ecological Research Commission (Stockholm)* 17: 215-222.

Hunt, C.D., D.C. Tanis, T.G. Stevens, R.M. Frederick, and R.A. Everett (2005, August 1) Verifying ballast water treatment performance. *Environmental Science and Technology* 321A-328A.

Hurley, W.L. and B.B. Ackers. 2002. Pacific Ballast Water Treatment Pilot Project: Ballast Water Transfer Study: Technical Feasibility with Associated Capital Costs. Prepared for the Port of Seattle, Seattle, WA. File No. 01080. The Glosten Associates, Seattle, WA. 36 pages.

International Maritime Organization (IMO) (2008b) Annex 4 Resolution MEPC.174(58). *Guidelines for Approval of Ballast Water Management Systems (G8).* Available at: http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064807e8904.

International Maritime Organization (IMO) (2008b) Annex 1 Resolution MEPC.169(57). *Procedure for Approval of Ballast Water Management Systems that Make Use of Active Substances (G9).* Available at: http://www.regulations.gov/search/Regs/home.html#documentDetail?R=09000064807e890e.

Inubushi, K., P.C. Brookes, and D.S. Jenkinson (1989) Adenosine 5'-triphosphate and adenylates energy charge in waterlogged soil. *Soil Biology and Biochemistry* 21: 733-739.

Jeffrey, S.W. and M. Vest (1997) Introduction to marine phytoplankton and their pigment signatures, pp. 37-87. In: *Phytoplankton Pigments in Oceanography*, by S.W. Jeffrey, R.F.C. Mantoura, and S.W. Wright (eds.). The United Nations Educational, Scientific, and Cultural Organization (UNESCO), Paris.

Johnson, W.S. and D.M. Allen (2005) *Zooplankton of the Atlantic and Gulf Coasts – A Guide to Their Identification and Ecology*. John Hopkins University Press, Baltimore, MD.

Kaneshiro, E.S., M.A. Wyder, Y.-P. Wu, and M.T. Cushion (1993) Reliability of calcein acetoxy methyl ester and ethidium homodimer or propidium iodide for viability assessment of microbes. *Journal of Microbiological Methods* 17: 1-16.

Kardinaal, W.E.A., L. Tonk, I. Janse, S. Hol, P. Slot, J. Huisman, and P. Visser (2007) Competition for light between toxic and nontoxic strains of the harmful cyanobacterium Microcystis. *Applied and Environmental Microbiology* 73: 2939-2946.

Karl, D.M. (1980) Cellular nucleotide measurements and applications in microbial ecology. *Microbiological Reviews* 44: 739-796.

Kieft, T.L. and L.L. Rosaker (1991) Application of respiration and adenylates-based soil microbiological assays to deep subsurface terrestrial sediments. *Soil Biology and Biochemistry* 23: 563-568.

Klemm, D.J., G.E. Morrison, T.J. Norberg-King, W.H. Peltier, and M.A. Heber (1994) *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*, 2nd edition. Report #EPA/600/4-91/003. U.S. EPA, Washington, DC.

Knoechel, R. and J. Kalff (1978) An *in situ* study of the productivity and population dynamics of five freshwater plankton diatom species. *Limnology and Oceanography* 23: 195-218.

Kokinos, J.K., T.I. Eglington, M.A. Goni, J.J. Boon, P.A. Martoglio, and D.M. Anderson (1998) Characterization of a highly resistant biomacromolecular material in the cell wall of a marine dinoflagellate resting cyst. *Organic Geochemistry* 28: 265-288.

Laabir M, Gentien P. (1999) Survival of toxic dinoflagellates after gut passage in the Pacific oyster *Crassostrea gigas* Thunberg. *Journal of Shellfish Research* 18: 217-222.

Laughton R., T. Moran and G. Brown. 1992. A Review and Evaluation of Ballast Water Management and Treatment Options to Reduce the Potential for the Introduction of Non-native Species to the Great Lakes. Pollutech Environmental, Ltd., Sarnia, Ontario, prepared for the Canadian Coast Guard, Ship Safety Branch, Ottawa.

Lebaron, P., P. Catala, and N. Parthuisot (1998) Effectiveness of SYTOX-Green stain for bacterial viability assessment. *Applied and Environmental Microbiology* 64: 2697-2700.

Lee II, H., D.A. Reusser, M. Frazier, and G. Ruiz (2010) *Density Matters: Review of Approaches to Setting Organism-Based Ballast Water Discharge Standards*. Report # EPA/600/R-10/031. Office of Research and Development, National Health and Environmental Effects Research Laboratory – Western Ecology Division, U.S. EPA

Lemieux, E.J., S. Robbins, K. Burns, S. Ratcliff, and P. Herring (2008) *Evaluation of Representative Sampling for Rare Populations using Microbeads*. Report No. CG-D-03-09, United States Coast Guard Research and Development Center, Groton, CT.

Lund, J.W.G., C. Kipling, and E.D. LeCren (1958) The inverted microscope method for estimating algal numbers and the statistical basis of estimations by counting. *Hydrobiologia* 11: 143-170.

MacIsaac, E.A. and J.G. Stockner (1993) Enumeration of phototrophic picoplankton by autofluorescence microscopy, pp. 187-198. In: *Handbook of Methods in Aquatic Microbial Ecology*, by P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.). Lewis Publishers, CRC Press, Inc., Boca Raton, FL.

MacIsaac, H.J., T.C. Robbins, and M.A. Lewis (2002) Modeling ships' ballast water as invasion threats to the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 59: 1245-1256.

Mackey, M.D., D.J. Mackey, H.W. Higgins, and S.W. Wright (1996) CHEMTAX – A program for estimating class abundances from chemical markers: Application to HPLC measurements of phytoplankton. *Marine Ecology Progress Series* 144: 265-283.

Marine Environment Protection Committee (2006, 28 Feb.) 54th Session, Agenda item 2 – *Harmful Aquatic Organisms in Ballast Water* – Report of the first meeting of the GESAMP-BWWG. MEPC 54/2/12, IMO.

Marine Environment Protection Committee of Sweden (2006, 21 April) *Harmful Aquatic Organisms in Ballast Water - Basic Approval of Active Substances Used by PureBallast Management System*. International Maritime Organization (IMO). 55th session, Agenda Item 2, MEPC 55/2/5.

Maritime Environmental Resource Center (MERC) (2009a) *Land-Based Evaluations of the Siemens Water Technologies SiCURE™ Ballast Water Management System*. MERC, Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD.

Maritime Environmental Resource Center (MERC) (2009b) *Land-Based Evaluation of the Maritime Solutions, Inc. Ballast Water Treatment System*. MERC, Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD.

Maritime Environmental Resource Center (MERC) (2009c) *Test Plan for the Performance Evaluation of the Severn Trent De Nora BalPure™ BP-2000 Ballast Water Management System*. Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD.

Maritime Environmental Resource Center (MERC) (2009d) M/V *Cape Washington* Ballast Water Treatment Test Facility Validation. Chesapeake Biological Laboratory, University of Maryland Center for Environmental Science, Solomons, MD.

Marret, F. and K.A.F. Zonneveld (2003) Atlas of modern organic-walled dinoflagellate cyst distribution. *Review of Palaeobotany and Palynology* 125: 1-200.

Matsuoka, K. and Y. Fukuyo (2000) *Technical Guide for Modern Dinoflagellate Cyst Study*. Available at: http://dinos.anesc.u-tokyo.ac.jp/technical_guide/main.pdf.

Minton, M.S., Verling, E., Miller, A.W. and Ruiz, G.M. 2005. Reducing propagule supply and coastal invasions via ships: effects of emerging strategies. Frontiers in Ecology and the Environment 3: 304-308.

Montagnes, D.J.S. and D.H. Lynn (1993) A quantitative protargol stain (QPS) for ciliates and other protists, pp. 229-240. In: *Handbook of Methods in Aquatic Microbial Ecology*, by P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.). Lewis Publishers, CRC Press, Inc., Boca Raton, FL.

Montresor, M., L. Nuzzo, and M.G. Mazzocchi (2003) Viability of dinoflagellate cysts after the passage through the copepod gut. *Journal of Experimental Marine Biology and Ecology* 287: 209-221.

Mosher, J.J., B.S. Levison, and C.G. Johnston (2003) A simplified dehydrogenase enzyme assay in contaminated sediment using 2-(p-iodophenyl)-3(p-nitrophenyl)-5-phenyl tetrazolium chloride. *Journal of Microbiological Methods* 53: 411-415.

Murphy, K.R., D. Ritz, and C.L. Hewitt (2002) Heterogeneous zooplankton distribution in a ship's ballast tanks. *Journal of Plankton Research* 24: 729-734.

Myers, Glenford J. (1979) *The Art of Software Testing.* John Wiley & Sons, New York, pp. 12-13,128.

NRC Marine Board. 1996. Stemming the Tide: Controlling Introductions of Nonindigenous Species by Ships' Ballast Water. Committee on Ships' Ballast Operations, Marine Board, Commission on Engineering and Technical Systems, National Research Council. National Academy Press, Washington, D.C.

National Science Foundation (NSF) (2003) *Engineering Controls for Ballast Water Discharge: Developing Research Needs*. Report of a workshop held on April 28-30, Seattle, WA. Bioengineering and Environmental Systems Division of the Engineering Directorate, NSF, Arlington, VA.

Oemke, D. 1999. The Treatment of Ships' Ballast Water. EcoPorts Monograph Series No. 18, Ports Corporation of Queensland, Brisbane, Australia. 102 pages.

Oliver, J.D. (1993) Formation of viable but nonculturable cells, pp. 239-272. In: *Starvation in Bacteria*, by S. Kjelleberg (ed.). Plenum Press, New York.

Parsons, M.G. (1998) Flow-through ballast water exchange. *Society of Naval Architects and Marine Engineers (NAME) Transactions* 106: 485-493.

Parsons, M.G. and M. Kotinis. 2008. Further Development and Optimization of the Ballast-Free Ship Design Concept. Final Report. Great Lakes Maritime Research Institute, University of Wisconsin-Superior and University of Minnesota-Duluth.

Petterson, S.R., R.S. Signor, and N.J. Ashbolt (2007) Incorporating method recovery uncertainties in stochastic estimates of raw water protozoan concentrations for QMRA [quantitative microbial risk assessment]. *Journal of Water and Health* 05. Suppl. 1: 51-65.

Phillips, S. (2006) *Ballast Water Issue Paper*. Report for the Pacific States Marine Fisheries Commission. Portland, OR. 34 pp. (http://www.aquaticnuisance.org/wordpress/wp-content/uploads/2009/01/ballast_water_issue_paper.pdf).

Porra, R.J. (1991) Recent advances and re-assessments in chlorophyll extraction and assay procedures for terrestrial, aquatic, and marine organisms, including recalcitrant algae, pp. 31-57. In: *Chlorophylls*, by H. Scheer (ed.). CRC Press, Boca Raton, FL.

Porter, J., J. Diaper, C. Edwards, and R. Pickup (1995) Direct measurements of natural planktonic bacterial community viability by flow cytometry. *Applied and Environmental Microbiology* 61: 2783-2786.

Posch, T., J. Pernthaler, A. Alfreider, and R. Psenner (1997) Cell-specific respiratory activity of aquatic bacteria studied with the tetrazolium reduction method, cyto-clear slides, and image analysis. *Applied and Environmental Microbiology* 63: 867-873.

Reavie, E.D., A.A. Cangelosi, and L.E. Allinger (2010) Assessing ballast water treatments: Evaluation of viability methods for ambient freshwater microplankton assemblages. *Journal of Great Lakes Research* 36: 540-547.

Reeves, E. 1998. Protection of the Great Lakes from Infection by Exotic Organisms in Ballast Water. Report for Ninth US Coast Guard District, Cleveland OH.

Reeves, E. 1999. Exotic Policy: An IJC White Paper On Policies for the Prevention of the Invasion of the Great Lakes by Exotic Organisms. Documents on Water Law, College of Law, University of Nebraska - Lincoln. 132 pages.

Rigby, G. and A. Taylor. 2001. Ballast water management and treatment options. Transactions of the Institute of Marine Engineers 113(3): 79-99.

Romano, G., A. Ianora, L. Santella, and A. Miralto (1996) Respiratory metabolism during embryonic subitaneous and diapause development in *Pontella mediterranea* (Crustacea, Copepoda). *Journal of Comparative Physiology* 166: 157-163.

Rosacker, L.L. and T. L. Kieft (1990) Biomass and adenylate energy charge of a grassland soil during drying. *Soil Biology and Biochemistry* 22: 1121-1127.

Roth, B.L., M. Poot, S.T. Yue, and P.J. Millard (1997) Bacterial viability and antibiotic susceptibility testing with SYTOX-Green nucleic acid stain. *Applied and Environmental Microbiology* 63: 2421-2431.

Ruiz, G.M. and D.F. Reid (2007) Current State of Understanding About the Effectiveness of Ballast Water Exchange (BWE) in Reducing Aquatic Nonindigenous Species (ANS) Introductions to the Great Lakes Basin and Chesapeake Bay, USA. Synthesis and Analysis of Existing Information, NOAA Technical Memorandum GLERL-142.

Ruiz, G.M., G.E. Smith, and M. Sytsma (2006) *Workshop Report on Testing of Ballast Water Treatment Systems: General Guidelines and Step-wise Strategy Toward Shipboard Testing*. Aquatic Bioinvasion Research and Policy Institute, a joint program of the Smithsonian Environmental Research Center (SERC) and Portland State University (PSU). Submitted to the

Pacific States Marine Fisheries Commission, Alaska Department of Fish and Game, and Prince William Sound Regional Citizens Advisory Council.

Sandrin, T.R., D.C. Herman, and R.M. Maier (2009) Physiological methods, pp. 191-223. In: *Environmental Microbiology*, by R.M. Maier, I.L. Pepper, and C.P. Gerba (eds.). Elsevier, New York, NY.

Sarmento, H. and J.-P. Descy (2008) Use of marker pigments and functional groups for assessing the status of phytoplankton assemblages in lakes. Journal of Applied Phycology 20: 1001-1011.

Schlüter, L., T.L. Lauridsen, G. Krogh, and T. Jørgense (2006) Identification and quantification of phytoplankton groups in lakes using new pigment ratios - a comparison between pigment analysis by HPLC and microscopy. *Freshwater Biology* 51: 1474-1485.

Schmid, H., F. Bauer, and H.B. Stich (1998) Determination of algal biomass with HPLC pigment analysis from lakes of different trophic state in comparison to microscopically measured biomass. *Journal of Plankton Research* 20: 1651-1661.

Selvin, R., B. Reguera, I. Bravo, and C.M. Yentsch (1988) Use of fluorescein diacetate (FDA) as a single-cell probe of metabolic activity in dinoflagellates cultures. *Biological Oceanography* 6: 505-511.

Sherr, E.B., D.A. Caron, and B.F. Sherr (1993) Staining of heterotrophic protists for visualization via epifluorescence microscopy, pp. 213-228. In: *Handbook of Methods in Aquatic Microbial Ecology*, by P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.). Lewis Publishers, CRC Press, Inc., Boca Raton, FL.

Sherr, E.B. and B.F. Sherr (1993) Preservation and storage of samples for enumeration of heterotrophic protists, pp. 207-212. In: *Handbook of Methods in Aquatic Microbial Ecology*, by P.F. Kemp, B.F. Sherr, E.B. Sherr, and J.J. Cole (eds.). Lewis Publishers, CRC Press, Inc., Boca Raton, FL.

Songster-Alpin, M.S. and R.L. Klotz (1995) A comparison of electron transport system activity in stream and beaver pond sediments. *Canadian Journal of Fisheries and Aquatic Sciences* 52: 1318-1326.

Steinberg, M.K., S.C. Riley, S.H. Robbins, B.N. Nelson, E.J. Lemieux, and L.A. Drake (2010) Geovalidation of a method to determine the viability of organisms ≥ 10 μm and < 50 μm (nominally protists) in ships' ballast water using two vital, fluorescent stains. *NRL Letter Report* 6130/xxxx, Washington, DC (in press).

Stevens, T.G., R.M. Frederick, R.A. Everett, J.T. Hurley, C.D. Hunt, and D.C. Tanis (2004) Performance verification of ballast water treatment technologies by USEPA/NSF Environmental Technology Verification Program. In: *Proceedings of the 2nd International Ballast Water Treatment R&D Symposium*, by J.T. Matheickal and S. Raaymakers (eds.). IMO, London, UK.

Suttle, C. (2005) Viruses in the sea. *Nature* 437: 356-361

Tagg, R. 1999. Ballast Water Management for Containerships: Implications for the Port of Oakland. Report for the Port of Oakland. Herbert Engineering Corp., San Francisco, CA.

Takano, C.T., C.E. Folsome, and D.M. Karl (1983) ATP as a biomass indicator for closed ecosystems. *BioSystems* 44: 739-796.

Turner, R. E. (1978) Variability of the reverse-flow concentration technique of measuring plankton respiration. *Estuaries* 1: 65-68.

United States Coast Guard (USCG) (2001) Report to Congress on the Voluntary National Guidelines for Ballast Water Management. Washington D.C.

US Coast Guard. 2008. Ballast Water Discharge Standard - Draft Programmatic Environmental Impact Statement. U. S. Coast Guard, Washington, D.C. 218 pages.

United States Environmental Protection Agency (U.S. EPA) (1993) *Methods for the Determination of Inorganic Substances in Environmental Samples*. Report 600/R-93-100. Office of Research and Development, U.S. EPA, Washington, DC.

United States Environmental Protection Agency (U.S. EPA) (1997) *Method 445.0: In vitro Determination of Chlorophyll* a *and Pheophytin* a *in Marine and Freshwater Algae by Fluorescence*. U.S. EPA, Cincinnati, OH.

United States Environmental Protection Agency (1999) *Criteria for Evaluation of Proposed Protozoan Detection Methods*. Report #EPA 815-K-99-02. Office of Research and Development, U.S. EPA, Cincinnati, OH. Available at: http://www.epa.gov/microbes/critprot.pdf.

US EPA. 2001. Aquatic Nuisance Species In Ballast Water Discharges: Issues and Options. Draft Report for Public Comment – September 10, 2001. U.S. Environmental Protection Agency Office of Water, Office of Wetlands, Oceans and Watersheds, Office of Wastewater Management, Washington, D.C.

United States Environmental Protection Agency (U.S. EPA) (2002a) *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms*, 5th Edition. Report # EPA 821-R-02-012. Office of Water, U.S. EPA, Washington, DC.

United States Environmental Protection Agency (U.S. EPA) (2002b) *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, 4th Edition. Report # EPA 821-R-02-013. Office of Water, U.S. EPA, Washington, DC.

United States Environmental Protection Agency (U.S. EPA) (2002c) *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms*, 3rd edition. Report # EPA 821-R-02-014. Office of Water, U.S. EPA, Washington, DC.

United States Environmental Protection Agency (U.S. EPA) (2003) *Ambient Water Quality Criteria for Dissolved Oxygen, Water Clarity and Chlorophyll a for the Chesapeake Bay and Its Tidal Tributaries*. Report EPA 903-R-03-002. Office of Water, U.S. EPA, Washington, DC.

United States Environmental Protection Agency (U.S. EPA) (2010, July) *Environmental Technology Verification Program (ETV) Draft Generic Protocol for the Verification of Ballast Water Treatment Technologies,* Version 5.0. U.S. EPA ETV in cooperation with the U.S. Coast Guard Environmental Standards Division (CG-5224) and the U.S. Naval Research Laboratory. National Sanitation Foundation International (NSF), Ann Arbor, MI, 62 pp. + appendices.

Veldhuis, M.J.W., T.L. Cucci, and M.E. Sieracki (1997) Cellular DNA content of marine phytoplankton using two new fluorochromes: taxonomic and ecological implications. *Journal of Phycology* 33: 527-541.

Veldhuis, M.J.W., G.W. Kraaij, and K.R. Timmermans (2001) Cell death in phytoplankton: correlation between changes in membrane permeability, photosynthetic activity, pigmentation and growth. *European Journal of Phycology* 36:1-13.

Vézie, C., J. Rapala, J. Vaitomaa, J. Seitsonen, and K. Sivonen (2007) Effect of nitrogen and phosphorus on growth of toxic and nontoxic *Microcystis* strains and on intracellular microcystin concentrations. *Microbial Ecology* 43: 443-454.

Victoria ENRC. 1997. Report on Ballast Water and Hull Fouling in Victoria. Parliament of Victoria, Environmental and Natural Resources Committee. Victorian Government Printer, Melbourne, Australia.

Vollenweider, R.A. (ed.) (1974) *A Manual on Methods for Measuring Primary Production in Aquatic Environments*, 2nd edition. International Biological Program Handbook No. 12, Blackwell Scientific, Oxford, U.K.

Wetzel, R.G. (2001) *Limnology*, 3rd edition. Academic Press, New York, NY.

Wetzel, R.G. and G.E. Likens (2000) *Limnological Analyses*, 3rd edition. Springer-Verlag, New York.

Williams, S.C., Y. Hong, D.C.A. Danavall, M.H. Howard-Jones, D. Gibson, M.E. Frischer, and P.G. Verity (1998) Distinguishing between living and nonliving bacteria: Evaluation of the vital stain propidium iodide and its combined use with molecular probes in aquatic samples. *Journal of Microbiological Methods* 32: 225-236.

Wonham, M.J., W.C. Walton, G.M. Ruiz, A.M. Frese, and B.S. Galil (2001) Going to the source: role of the invasion pathway in determining potential invaders. *Marine Ecology Progress Series* 215: 1-12.

Zhang, F. and M. Dickman (1999) Mid-ocean exchange of container vessel ballast water. 1. Seasonal factors affecting the transport of harmful diatoms and dinoflagellates. *Marine Ecology Progress Series* 176: 243-251.

Zurawell, R.W., H. Chen, J.M. Burke, and E.E. Prepas (2005) Hepatotoxic cyanobacteria: A review of the biological importance of microcystins in freshwater environments. *Journal of Toxicology and Environmental Health, Part B,* 8: 1-37.