

To: Stakeholders and others interested in Ballast Water Management  
Re: Most Probable Number method

Recently, the U.S. Coast Guard rejected the Most Probable Number, or MPN, method for enumerating viable microorganisms in ballast water discharge as an equivalent alternative to the method prescribed in the Coast Guard's regulations, which classifies organisms as live or dead based on enzymatic activity and membrane integrity as assessed with vital stains, and motility. The Coast Guard decision can be appealed.

With support from industry and the Natural Sciences and Engineering Research Council of Canada, Dr. Hugh MacIntyre and I have been studying ballast water treatment issues since late 2012. In particular, we have been comparing different methods that are used to assess the effectiveness of ballast water treatment, including the MPN method and the vital stains method prescribed by the Coast Guard. Our focus is on transparent, peer-reviewed research for publication in the open literature, and we apply to the research more than 70 years of combined experience studying the physiology and ecology of phytoplankton.

The first of a series of papers was published in 2015 ([link](#)). To the best of our knowledge it is the first and only comprehensive discussion of MPN as it is being used for the testing of ballast water management systems. Our findings, also described in a scientific commentary ([link](#)), put past criticisms of the MPN method in a new light. Importantly, the problem of so-called unculturable species, highlighted in criticisms of MPN, is shown to be much less serious than was commonly thought in 2012, when the Coast Guard Final Rule was published. Focusing on scientific arguments that were not tied to any particular treatment technology, we concluded that with careful evaluation, MPN could be an effective method for assessing the viability of phytoplankton after ballast water treatment.

Our research suggests that uncertainties in MPN can be quantified and compared to those in the prescribed protocol, which include some significant and unavoidable errors that Dr. MacIntyre discusses in an overview of scientific results that are currently in review ([link](#)).

An up-to-date comparison of the uncertainties of MPN vs the prescribed method would help in assessment of the relative protection of the environment from each testing method. No such comparison is in the public domain, but relevant evidence from our laboratory and elsewhere is accumulating. This evidence supports adoption of MPN as an approved method to determine the efficacy of ballast water management systems, regardless of treatment technology.

Sincerely,

John J. Cullen, Ph.D., FRSC  
Professor Emeritus