Environmental Research Services

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To Whom It May Concern,

On December 14, 2015 the United States Coast Guard published, its opinion that the Most Probable Number (MPN) method is not considered to be an equivalent alternative to the testing methods prescribed in the Coast Guard regulations on type approval of ballast water management systems (BWMS). In my view this was an ill-advised decision seemingly based on a lack of understanding of this method of determining numbers of live organisms in treated ballast water. The MPN method is already used to count live indicator bacteria in testing to both U.S. Coast Guard and IMO regulatory standards, and has been employed as a means of counting live bacteria for several decades. As such it is regarded as the *definitive* test for determining viable bacteria and this is why it is used to determine live residuals of bacteria in discharged ballast water. There is no reason to assume that it is not valid for live (viable) phytoplankton (protists) in ballast water. In this context, the terms 'live' and 'viable' are interchangeable since the object of ballast water treatment is to stop future colonization of receiving water on release of entrained organisms. As stated by the International Maritime Organization (IMO) Convention, the objective of a successful ballast water management system (BWMS) is

" -- to remove, render harmless, or avoid the uptake or discharge of harmful aquatic organisms" . If organisms cannot reproduce they cannot establish populations in new environments on discharge.

The opinion expressed here stems from a long experience with mitigation of non-indigenous species introductions. My research on this subject goes back to the 1980s/early 1990s when I received U.S. Federal Grant support to investigate the

spread and control of Dreissenid mollusks and other non-indigenous species. At the time I directed the Ambient Toxicity Program for the State of Maryland, testing a broad spectrum of polluted venues for toxic properties. The program involved the culture of many different planktonic organisms and bacteria for toxicity testing and placed my laboratory in a good position to test the efficacy of a large range of treatments to control non-indigenous aquatic species. These included sound energy of different frequencies, UV irradiation, oxidizing and non-oxidizing chemicals (active substances) and a variety of filters and centrifugal separators. These activities over a more than 25 year period led to the development of extensive ballast water testing capability, initially land-based through the (University of Maryland) Baltimore Harbor Ballast Water Demonstration Project and, subsequently with an emphasis on shipboard testing through the University of Maryland and, later, my consulting company, ERS. In all more than 25 different shipboard tests have been completed over a seventeen-year period. While I have maintained an active interest in some of technologies involved with ballast water management, ERS has no financial involvement in the marketing of any current commercial products and technologies employed for ballast water treatment.

This research/testing activity led to the use, and in some cases development of techniques for assessing performance. Throughout this 25 year period, my colleagues and I have been intimately involved with testing techniques, rather than a distant managerial approach. In other words I have personal experience of what works and what doesn't work. For example the FDA/cmFDA fluorescent tagging approach to live/dead assessment of protists remains a highly unreliable means of assessing the live/dead status of entrained phytoplankton. False positives stem from fluorescent signals from dead and abiotic material possibly 'contaminated' by bacteria, and fading of the fluorescent signal can also create uncertainties over the veracity of this method.

An argument used against the MPN method claims that not every taxonomic group will grow under laboratory culture conditions. Of course different species have different nutrient and environmental needs for optimal growth. To think

otherwise would be naïve. In the marine environment it has long been recognized that only a small percentage of heterotrophic bacterial assemblage (often <10%) will grow under lab. incubation conditions. However this doesn't prevent this method (heterotrophic plate counts by MPN) from being employed as an endpoint in U.S. Ballast Water testing. To disqualify this method on the basis that 'some taxa may not grow' is extraordinary. In fact phytoplankton flora perform rather well under laboratory culture conditions. In a comprehensive study of treated vs. untreated ballast water involving meticulous counting and identification of 50 entrained phytoplankton taxa, 66% of the phytoplankton population either maintained numbers or grew over a 24h period, with a mean growth rate of x2.1. Overall the phytoplankton assemblage in untreated samples grew over a 24h period at a mean rate of x1.56. This number represents the mean of all taxonomic groups counted irrespective of their relative abundance in the population as a whole. When adjustment is made for the relative abundance of the individual taxa within the population, the weighted mean growth rate rises to x1.79, or +179%. Treated discharge water showed a >99% decline in overall cell numbers compared with untreated water. Taxonomic groups present in treated water represented a sub-set of (21) taxa recorded from challenge water. Five taxa were recorded from treated samples that were not found in challenge water samples. None of the residual taxa in treated samples were able to grow under fluorescent lights. Collectively a 74% 'negative growth' rate was recorded for all taxa following incubation i.e. -74%. This was unaffected by the relative abundance of the individual taxa within the population, i.e. the weighted mean growth rate did not differ from the average from recorded taxa (Wright and Welschmeyer 2015).

A further 'reason' for disqualifying the Most Probable Number method was a recent statement that MPN was (quote) 'not a real measurement', rather a 'statistical manipulation'. It is difficult to reconcile this statement with an objective appraisal of what this calculation represents. It is no more/no less of a 'manipulation' that (say) an LC_{50} determination of toxicity using a bioassay. To state otherwise is misleading.

In light of this I hope that the U.S. Coast Guard will re-consider their decision to disqualify this commonly used method in determining the live/dead status of phytoplankton, particularly in view of the unreliability of alternative methods. It is true that such a method is not compatible with a relative short appraisal of treatment efficacy. Reaching an endpoint can take several days. However, this type of science does not often provide convenient answers.

Sincerely,

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Reference.

D.A. Wright & N.A. Welschmeyer (2015). *Establishing benchmarks in compliance assessment for the ballast water management convention by Port State Control.* Journal of Marine Engineering & Technology.
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