January 25, 2016

Re: Coast Guard decision on use of Most Probable Number (MPN) method

Open Letter to: Ballast Water Management Stakeholders

I recently became aware of the decision posted in the *Coast Guard Maritime Commons* that the Most Probable Number (MPN) method of quantifying the effects of ballast water treatment have been ruled as unacceptable for testing for living organisms in treated water within the type approval process. Instead, vital stains are the only approved method for validating sterilization technologies under that regulatory process. This letter reflects my professional and research expertise related to the question of ballast water treatment for the control of the spread of non-indigenous species (NIS) through ballast water uptake and discharge.

The Great Lakes Institute for Environmental Research (GLIER, University of Windsor) has over 20 years' experience in the study of NIS, specifically in the Great Lakes, but also globally. Faculty and students at GLIER, U Windsor have worked on the biological factors involved in invasion success, as well as on the implementation of risk assessment and risk minimization as part of the National Research Network "Canadian Aquatic Invasive Species Network" (CAISN). The goal of ballast water treatment is to minimize or eliminate the risk of the introduction and subsequent proliferation of NIS through ballast water discharge. Many published studies from GLIER and CAISN (among others) have formalized the steps necessary for a successful introduction of NIS. The two primary factors driving invasion success are "propagule pressure" (i.e. the number of viable organisms introduced) and NIS performance in the receiving environment. The IMO and USCG regulations on ballast water treatment reflect the need to manage the risk associated with propagule pressure. However, those regulation do not address the technical aspects of quantifying the number of microscopic organisms in large volumes of water, nor do they touch on the methods available for treating the water to reach the acceptable thresholds. In my opinion, those last two questions are critical and can only be reasonable assessed though the best available science and hypothesis-driven experimentation.

Given that MPN is now ruled unacceptable for the quantification of propagule pressure, the remaining allowable technology is fluorescein-based "vital" stains. This approach relies on vital, or living, organisms being identified by fluorescence. However, the resulting fluorescence does not actually detect "life", but rather esterase activity, and that enzymatic activity is not uniquely associated with living cells. Specifically, viable cells may not show fluorescence, while conversely, non-viable cells may still fluoresce. The only biologically justifiable method to

identify “live” organisms is to measure their capacity for growth and reproduction, only living organisms can grow and reproduce. Thus, at a basic level, vital stains are flawed as quantitative measures of living cells, and functional assays, such as MPN, are conceptually better measures of living organisms. A third methodology for quantifying living organisms involves assaying vital cellular function, specifically gene expression levels for genes associated with cellular processes required for cellular growth, replication and ultimately, survival. If those fundamental processes are permanently disrupted, the organism is not viable and poses no risk for establishment or invasion. While genomic technology for quantitatively detecting viable potential invasive species is being developed in my lab and others, it is not yet perfected. However, we have, in collaboration with UV treatment industry, performed genomic and transcriptomic analyses of UV-treated and untreated ballast water cultures, and comparison of those results with MPN measures of organism viability indicated that the two approaches provide equivalent measures of viability. This is in contrast to vital stains which are not capable of detecting the fundamental loss of cellular function associated with UV treatment in certain microorganisms It is my professional opinion that MPN is a reliable and effective methodology for quantifying organismal viability in the context of ballast water sterilization with the goal of controlling invasion risk.

It is clear that the need to exclude NIS introduction via ballast water discharge is high. Regulations designed to ensure proper ballast water sterilization are in place and it is critical that effective sterilization treatments not be excluded based on outdated technology used for quantifying the efficacy of the various treatments. I urge regulators, stakeholders and concerned individuals to use the best available science to evaluate ballast water treatment options, not simply “standard” methods that perhaps are comfortable, but may also be flawed. Biological principles indicate that only viable organisms pose invasion risks, and “viable” means they are capable of growth and replication and hence survival.

Sincerely,

Daniel Heath, Professor & Executive Director,
Great Lakes Institute for Environmental Research
519-253-3000 (EXT 3762); *dheath@uwindsor.ca*