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LYLES SCHOOL OF CIVIL ENGINEERING

27 December 2015

Department of Homeland Security
United States Coast Guard

Dear USCG:

I read with interest the posting on 14 December 2015 in the *Coast Guard Maritime Commons* entitled "Coast Guard decision on use of Most Probable Number method." The posting indicates that MPN-based methods will not be viewed as equivalent to so-called "live/dead" methods that have been approved for use in the Type Approval process. I find this approach to be at odds with the goal of ballast water management and the scientific and engineering principles that govern the behavior of water treatment processes.

The IMO's Ballast Water Management Convention was established "… to prevent the spread of harmful aquatic organisms from one region to another, by establishing standards and procedures for the management and control of ships' ballast water and sediments." By extension, this implies that ballast water treatment systems should be designed and implemented to minimize the likelihood of invasion by non-indigenous aquatic organisms.

In a very real sense, this is analogous to the one of the central goals of drinking water treatment, which is to minimize the likelihood of transmission of waterborne, communicable diseases. This water treatment objective implies that treatment systems should be designed and implemented to minimize the likelihood of human infection by aquatic pathogens.

Although important differences between invasion by non-indigenous aquatic organisms and human infection by aquatic pathogens can be identified, there are also important similarities. Both involve an "invasion" of a system (a port or a human) by a non-indigenous aquatic organism that has the potential to harm the system. In both cases, a successful invasion will require that a sufficient number of non-indigenous organisms are introduced to the system, and conditions within the system must be sufficient to support their replication. If both conditions are met, an invasion (infection) is possible.

The similarities between these processes are important in the context of this letter, primarily because many important lessons have been learned in the drinking water arena that translate directly to other water treatment applications. Of particular importance is the translation of these lessons to ballast water treatment.

A clear example of a "lesson learned" relates to the selection of analytical methods for measurement of the response of a potential invading population to a treatment intervention. Until the late 1990's the consensus in the engineering and scientific communities was that UV-based methods were minimally-effective for control of protozoan parasites, such as *Cryptosporidium parvum* or *Giardia lamblia*. This incorrect conclusion was based on work that had been conducted to that time that was based on the use of "vital dyes" to quantify the responses of these parasites to UV irradiation. It was not until assays based on actual infectivity (*i.e.*, completion of the life cycle of the parasite,

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including reproduction) were included that the same communities discovered that, in fact, these protozoan parasites are remarkably sensitive to UV radiation. Since the first papers were published to demonstrate the *true* sensitivity of protozoan parasites to UV irradiation, UV irradiation has emerged as the process of choice for treatment of water in circumstances where contamination by protozoan parasites represents a potential problem. This has had a tremendous, positive impact on potable water production in the United States and elsewhere. Drinking water in the U.S. (and other developed countries) is now safer because UV disinfection processes have been implemented as part of a strategy to control protozoan parasites. This is a critical issue in the water treatment arena because protozoa have been identified as being responsible for a large fraction of infections that have been associated with drinking water and recreational water.

In my opinion, the choice of analytical endpoint(s) for definition of ballast water treatment methods should be influenced by this important historical lesson. In particular, I believe that the analytical methods to be applied to define conformance with ballast water treatment standards should be based on the ability of aquatic organisms to invade. As described above, a "successful" invasion will require that a threshold number of organisms be introduced to the system (*e.g.*, a port or harbor) and that the organisms are able to replicate. If these conditions are met, then an invasion is possible.

With these facts in mind, the use of analytical methods that demonstrate the ability of an organism to reproduce would appear to be valid. Such methods are available. With bacteria, common examples include plate-counting and most probable number (MPN) methods in which samples containing a target organism are allowed to incubate under conditions that favor reproduction of the target. A positive signal in these methods is attributable to actual reproduction of the target organism. The inclusion of these methods for quantification of the indictor bacteria that are included in the ballast water standards is a logical choice. In a very real sense, these methods provide the truest measure of the potential for invasion. Like all analytical methods, they are imperfect; however, they appear to be less likely to be influenced by error (positive or negative) than other, indirect methods, such as those than involve "vital dyes."

I believe that to the extent possible, analogous methods should be applied for quantification of the responses of other organism types in ballast water management. My research group at Purdue University is completing a study regarding the use of an MPN method for assessment of the ability of UV-based processes to control the algal species *Tetraselmis*. This organism has been suggested as a potentially relevant indicator species for aquatic organisms in the $10\ \mu m \leq d_m < 50\ \mu m$ size class, in that it is ubiquitous and resistant to exposure to UVC radiation. We have developed an MPN-based method for *Tetraselmis* that is repeatable and that can be conducted using commonly available laboratory equipment. I am aware that several other groups have developed similar methods.

With these facts in mind, it appears that MPN-based assays are available for use with ballast water treatment systems with the $10\ \mu m \leq d_m < 50\ \mu m$ size class. I believe these methods are appropriate for use with UV-based systems, in that they are consistent with the well-known mechanisms of inactivation that govern the behavior of these systems. Moreover, I believe these methods may be valid for use with other (non-UV-based) treatment processes.

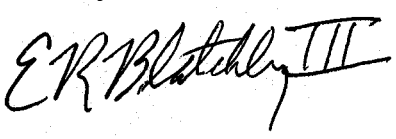
The inclusion of these methods would represent an appropriate and scientifically-defensible approach to management of ballast water treatment systems. Moreover, this approach will facilitate the use of UV-based methods for management of ballast water. Not only are UV-based systems effective for limiting reproduction of aquatic organisms in treated water, they offer the potential for meeting the goals of ballast water management in an environmentally-responsible manner.

UV-based processes do not represent a panacea. As in the case of drinking water production, UV-based processes are likely to be most effective when they are incorporated as part of a system that

includes other, upstream processes (*e.g.*, physical separation, such as a filter and/or membrane-based system). Moreover, it is likely that in some situations, UV-based processes will not be the process of choice. However, UV-based processes represent an important part of the "palette" of treatment processes that is available for use in ballast water management. Their inclusion as a part of any water treatment process should be quantified and regulated by analytical methods that are consistent with the known mechanisms by which they function.

With these facts in mind, I encourage you to reconsider your decision to exclude MPN-based methods as an acceptable assay for measurement of the performance of ballast water treatment processes. I believe that the use of these methods is justifiable based on rigorous scientific and engineering principles, and that its use will allow for the most effective control of invasions associated with ballast water discharge. Please feel free to contact me if you have questions or comments.

Sincerely,



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