Summary of Ballast Check 2 as an Assessment Tool for Monitoring Ballast Water Discharge Compliance in Hawai`i

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I. Introduction

One of the top vectors of aquatic non-indigenous species introductions into the State of Hawai'i is ballast water (BW) discharge. As a result, monitoring for BW discharge compliance may aid in minimizing further introductions into and across the State through early detection and concomitant action by the State or USCG. However, currently established procedures for measuring BW discharge compliance of living organisms, following USCG regulations, are unconducive for routine assessments, especially for the 10-50 μm size category of organisms which mostly include autotrophic protists and some heterotrophs.

Currently, the 10-50 μm size class of organisms are enumerated using epifluorescence microscopy to determine compliance; this method requires technically trained staff, expensive-bulky instrumentation, and it is labor intensive, even for experts. Due to these reasons, the epifluorescence microscopy method for enumerating the 10-50 μm size class is unrealistic for performing routine BW compliance checks. Therefore, an investigation into simpler tools, that provided rapid-reliable results or at least a proxy of the biosecurity risks, was conducted. One of the technologies that was tested was the Turner Designs, the Ballast Check 2 (BC2). BC2 is described to simplify BW discharge compliance monitoring for the 10-50 μm size class, by utilizing phytoplankton fluorescence as a proxy for cell viability and cell concentration. Results from our BC2 investigations are provided in this summary.

II. Methods

Between November and December 2017, duplicate surface water samples were acquired weekly from Honolulu Harbor, Kewalo Basin Harbor, and Pearl Harbor to acquire a variation of protist assemblages. In addition, duplicate surface samples were collected opportunistically from Ma`alaea Harbor, Maui. All samples were dark-adapted and kept on ice before being processed. Samples were concentrated from approximately 500 mL to 14 mL in an attempt to increase statistical significance. Each concentrated sample was analyzed in triplicate under the BC2. Similarly, triplicate subsamples were analyzed in a Sedgewick rafter cell using an epifluorescence microscope equipped with a dichroic filter, specific for phytoplankton analysis. In addition, green 10 μm microbeads were used to size organisms. Statistical analyses followed methodology described by Bland & Altman, 1999. Triplicates were averaged and log transformed to ensure a normal distribution of the data via SPSS statistical analysis program. In addition, a Bland-Altman Plot was created to visualize our results (Figure 1). Values for the mean difference and limits of agreement (LoAs) were back calculated using the antilog to obtain a value to be interpreted in relation to the original data.

III. Results

The mean difference between the two methods was 1.16 organisms/mL (confidence interval 0.85-1.59). The upper limit of agreement was 4.67 organisms/mL (confidence interval 2.73-7.99). The lower limit of

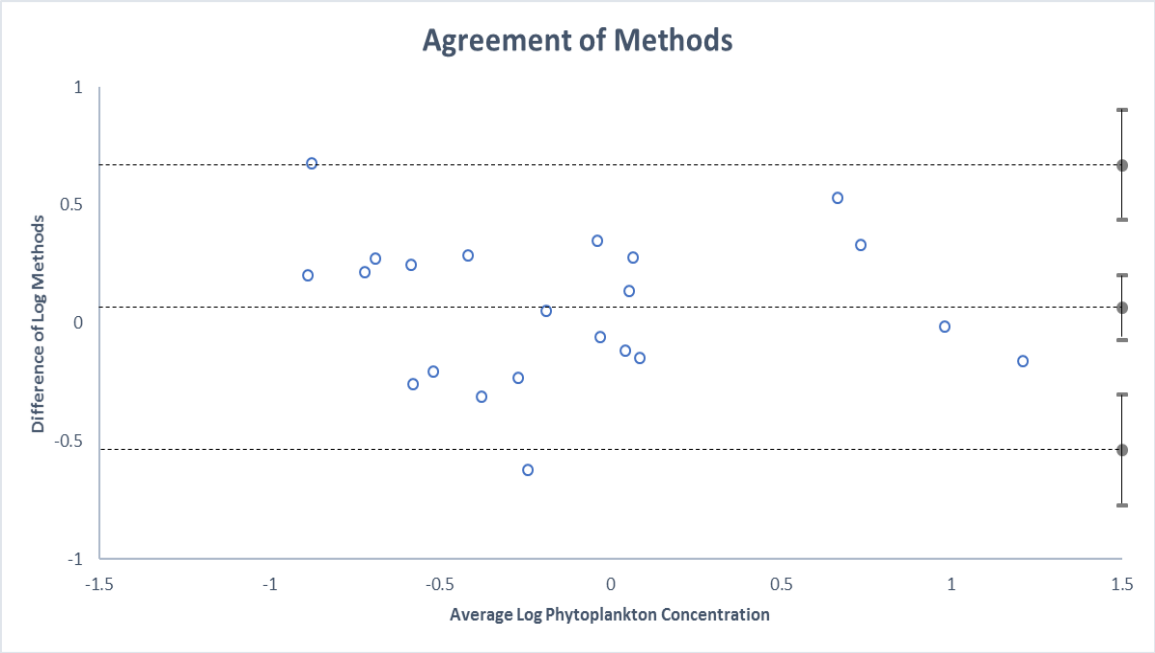

Figure 1. Agreement of the Ballast Check 2 abundance values and microscope counts. Each data point represents an average of triplicates for each methodology, and all measurements were log transformed (n=22). The x-axis is the average of the two values for each sample, and the y-axis is the difference between the pair. The mean difference (1.16 organisms/mL, CI 0.85-1.59) is represented by the middle dotted line. The lower and upper limits of agreement (0.29 and 4.67 organisms /mL, CI 0.17-0.50, and 2.73-7.99, respectively) are the top and bottom dotted lines. Confidence intervals are indicated by error bars.

agreement was 0.29 organisms/mL (confidence interval 0.17-0.5).

IV. Discussion/Conclusion

The average difference of the BC2 to microscopy counts of phytoplankton was 1.16 organisms/mL, indicating that the BC2 was able to provide results comparable to microscopy counts. However, it is important to recognize the LoA range which indicates that 95% of the time, the two methods varied anywhere between 0.29 - 4.67 organisms/mL from the average.

In general, the instrument appears to be optimized for lower phytoplankton concentrations that typically occur in managed BW, where phytoplankton values are right on the cusp of BW discharge compliance values for the 10-50 µm size class. While the instrument cannot provide a risk assessment of the 10-50 µm heterotrophs, it can act as a fairly reliable proxy for the living 10-50 µm phytoplankton concentration thereby aiding authorities in the decision to allocate more resources towards further investigation or alternatively, moving onto the next vessel.

Regarding the user-interface, the BC2 was easy to learn and created minimal plastic waste. All the equipment and materials were included in a padded brief case that created peace-of-mind during travels on/off ships and as carry-on luggage on an airplane. However, the most compelling difference between the microscope counts and the BC2 was the rapidity in which the results were provided. The BC2 delivered results within 5 minutes for a single sample ran in triplicate, whereas a sample processed in triplicate under the epifluorescence microscope took 15- to 17-times longer.

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