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## EFFECT OF CELL-SIZE AND TRAVEL TIME ON EFFICACY OF COMPLIANCE MONITORING DEVICES (CMD) IN PREVENTING BIOINVASION BY BALLAST WATER

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### RESUMO

Most recent protocols adopted by Nations to prevent introduction of non-native organisms following the International Maritime Organization guidelines are based on cellular fluorescence due to the presence of pigments like Chlorophyll. Fluorescent organisms (phytoplankton) living in aquatic ecosystems are usually one order of magnitude more abundant than non-fluorescent ones. Therefore, the most widely used Compliance Monitoring Devices (CMDs) estimate the risk of microbiological invasion associated with ballast tanks by relying on the fluorescence property of a ballast water aliquot. We thus addressed the potential effect of cell size and navigation time on the fluorescent signal (peak, width, and area) produced by cells ranging from 10 $\mu$ m to 50 $\mu$ m ESD (equivalent spherical diameter). We found a positive correlation between the cell ESD and the fluorescence signal as measured by width and area. In contrast, the peak of fluorescence did not show any correlation with the cell ESD. The expected relationship between cellular fluorescence and size was evidenced by the width of the signal emitted by cells kept in the dark, but not for the peak of the signal. Large cells take more time than small ones to pass through the PMT detector, thus resulting in a larger width. This is valid for “flowing cytometry” approaches, but not for PAM fluorometry. In contrast, there is little difference between peaks of small and large cells. In this case, cells kept in the dark exhibit similar fluorescence peaks and thus the average fluorescence must represent well the population for purposes of abundance estimative. When analyzing the change in cellular density over 30 hours, that represents a cabotage travel in Brazil, there is a significant reduction in density only after 24 hours in the dark (ballast tank simulation). Similar result was obtained for cellular activity and cells were presumed dead after 24 hours in the dark. The estimative of chlorophyll concentration by PAM fluorometry was unable to detect differences in the population kept in dark condition over time. Thus, the present study demonstrates that cell size and navigation time could affect

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cellular fluorescence and likely lead to a bad estimate of the 10-50µm phytoplankton abundance done by most commercially available CMDs.

**PALAVRAS-CHAVE:** Ballast Water, Bioinvasion, Compliance Monitoring Device, Fluorescence, Phytoplankton

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