Ballast water and invasive species: 
Development of new methodologies for efficient assessment of organism viability in treated ballast water

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Description of topic and related issues
The introduction of invasive species from ships’ ballast water discharge has for many years been a serious global matter1, 2 – and it still is.3-5 Aquatic bioinvasion incidents have had large and detrimental consequences ecologically, socio-economically and in some places even caused near-extinction of endemic species.6, 7 The spreading of invasive species through ballast water causes tremendous economic losses around the world. A WWF report from 2009 estimates that aquatic invasive species annually are responsible for global environmental damage and economic losses for more than 7 billion US dollars.8 The costs include damage and repairs to fisheries, aquaculture, water supply systems, industrial infrastructure and harbours and do not include all the indirect losses caused by changes to biodiversity and habitats.8

To prevent any further stress on fragile ecosystems and economics related to aquatic activities, high awareness and scientific focus of the issue is required. To protect aquatic ecosystem from invasive species, the United Nations IMO ballast water management convention (ratified by Denmark in 2012) sets up global regulations of ballast water (BW) discharge. All ships discharging BW must first apply a type approved BW management system (BWMS) to meet discharge standards related to number of viable organisms in defined size-classes. Similar rules became effective by 2012 in USA.

The recognized methods for determining viable cells are based on labour intensive direct microscopic counting of the number of live organisms. To facilitate counting, living cells are stained by fluorescent markers of esterase- or P450 activity and/or dead cells are stained with markers of plasma membrane damage.9-11 Ultraviolet (UV) radiation and electrolytic chlorination, frequently combined with filtration, represent the two prevailing treatment principles in type approved BWMS.12 A special challenge for monitoring the effect of UV based technologies is that UV radiation causes damage to DNA in the cells13, which can either result in later death or in
survival due to DNA repair. Current staining methods may therefore produce false positives because dead or dying organisms are recorded as viable. Effects on DNA cannot be measured by the methods currently prescribed. The problem of identifying false positives and false negatives (recovering organisms) is currently solved by most probable number assays for measuring the algal re-growth in treated BW, but these assays are time consuming and there is a need for a better characterization of the borderline cases. US Coast Guard have very recently rejected most probable number assays because the regulations specifically require ballast water treatment systems to be evaluated based on their ability to kill certain organisms. Further improvement of the current methods used for assessment of BWMS is therefore of high environmental as well as economic importance.

**Ongoing PhD-project (2016-2019)**

The PhD-project plans to test, develop and establish new standard methodologies and know-how for high quality assessments of planktonic organism viability in BWMS under different biotic and abiotic water conditions. The aim is that these analytical methods in the longer run can be applied and used as standard monitoring procedures for validation of implemented type approved BWMS. The applied methods and the science behind will support the development of cleaner and more efficient BWMS. They will help to ensure that treatment performance comply with discharge standards and the detection of insufficient BWMS and/or undetected BWMS failures.

The project will be divided into three sub-projects with a main focus on sub-project (1):

1. **Testing of novel staining methods:** Flow cytometry for counting viable organisms works well for monocultures, but is limited when counting samples containing multiple species and colony forming/filamentous plankton. Comparative studies will be used to evaluate a number of novel markers and staining methods for practical viability assessment of planktonic organisms/cells from treated BWMS. Likewise to relate their resource requirements and quality. Results will be compared with existing methods. Besides high quality validation of the tested methods other characteristics such as cost, simplicity, resource and time demands will be considered. The project will investigate a number of live stains based on the presence of metabolic activity and also test efficiency of multi-labelling. Furthermore, the potential complementary use of advanced microscopy systems and image analysis techniques for a more automated and robust quantitative determination of live/dead organisms and species identification will be explored.

2. **Staining challenges of phytoplankton:** Algal cell structure challenges current staining methods as they are developed for cytotoxicity measurements in mammalian cells. Algal cells walls might prevent the entry of dyes into the cell or bind dyes non-specifically. The project will focus on if existing staining methods and flow cytometry analysis are also applicable on a variety of algal species.

3. **Identification of robust key species:** Some species tend to resist ballast water treatment. The sub-project will focus on the identification of robust key species and their attributes and on establishing dose-response relationships to different treatment processes with the aim to determine the best approach for their efficient removal from the discharged ballast water.
**References**


10. MEPC (2008). *Guidelines for approval of ballast water management systems (G8).* Marine Environment Protection Committee - IMO.


