Verification of the effectiveness of Ballast Water Treatment Systems

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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 global matter of concern[1,2] – and it still is.[3-5] Aquatic bioinvasion incidents have had large and detrimental ecological and socio-economic consequences and have in some places 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 of 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 of biodiversity and habitats.[8]

To prevent any further stress on fragile ecosystems and economics related to aquatic activities, high awareness and scientific focus on the issue is required. To protect aquatic ecosystem from invasive species, the United Nations IMO ballast water management convention sets up global regulations of ballast water (BW) discharge. The convention was ratified in September 2016 (ratified by Denmark in 2012) and will be enforced in September 2017. It states that all ships discharging BW must first apply a type approved BW management system (BWMS) to meet discharge standards related to numbers of viable organisms in defined size-classes. Similar rules became effective by 2012 in USA.



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The two prevailing treatment principles in type approved BWMS builds on ultraviolet (UV) radiation or electrolytic chlorination, both frequently combined with filtration[12]. The approved methods for determining the effectiveness of the BWMS 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 cells are stained with markers of

plasma membrane damage.[9-11]A special challenge for monitoring the effect of UV based technologies is that UV radiation causes damage to DNA in the cells[13] 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[14, 15]. Effects on DNA cannot be measured by the methods currently prescribed[8]. The problem of identifying false positives and false negatives (recovering organisms) is currently solved by most probable number assays (MPN) for measuring the algal re-growth in treated BW[11], but these assays are time consuming and there is a need for a better characterization of the borderline cases. The U.S. Coast Guard have very recently rejected MPN 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.

The project: Verification of the effectiveness of Ballast Water Treatment Systems/ Verifikation af vandballastanlægs effektivitet (SDU, DHI & the Danish Maritime Fund)

In 2016 the ecotoxicology group at Department of Biology, SDU and DHI, Hoersholm applied the Danish Maritime Fund for funding for a project addressing the topics and issues described above. Fortunately the Danish Maritime Fund found the project interesting and granted the project http://www.dendanskemaritimefond.dk/sdu-oekotoksikologisk-gruppe-verifikation-af-vandballastanlaegs-effektivitet/.

The 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 a near future 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 complies with discharge standards and that performance can be documented.

The project will be divided into two subprojects with a main focus on subproject 1:

Subproject 1:

- Development of automatized counting of CMFDA/FDA marked organisms by use of a High Content Screening platform (HCS). The automatized method will be compared to manual counting on algae monocultures and manual counting of treated ballast water samples from DHI.
- The robustness of the HCS-method to different organism compositions will be investigated by analysis of treated ballast water samples from different seasons.
- The robustness of the HCS-method to changes in physical/chemical parameters will be tested on algae monocultures under controlled laboratory conditions.

The goal for subproject 1 will be a HCS-based method, which is validated against the approved manual CMFDA/FDA-method.

Subproject 2:

- Analysis of multiple markers of cell damage and viability by use of High Content Screening platform (HCS).
- Commercially available fluorescence markers for relevant cell damages will be screened for their ability to predict cell viability or cell death. Test parameters will include uptake in organisms, adhesion to different types of cell walls and sensitivity to specific cell damages.
- The screening will be performed on monocultures of algae grown at SDU and treated ballast water samples from DHI.
- Promising markers for DNA and mitochondria damage will be analysed for their ability to predict cell death in ballast water from treatment systems using UV-light. The selected markers will be tested in combination with CMFDA/FDA staining to compare the robustness to predict cell death/viability.

The goal for subproject 2 will be a multi-marker method for better prediction of viability of organisms treated by a UV-based ballast water treatment system.

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