Proceeding

GEF-UNDP-IMO GloBallast-ROK
The 5th Global R&D Forum & Exhibition on Ballast Water Management

23-25 October 2013
Busan, Korea

MINISTRY OF OCEANS AND FISHERIES

IMO INTERNATIONAL MARITIME ORGANIZATION

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session A1

G9 Related Issues, QA&QC

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Evaluation of Residual Toxicity on Treated Ballast Water Using Bioluminescent Microbe for Developing Technology of Prompt Detection

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ABSTRACT

The bioluminescent microbe test is a well established bioassay tool which is very simple, prompt and easy to apply in laboratory and field testing. It has been used many fields to screen out potentially hazardous waters for further definitive tests, which take high cost and long time. Even though microbe is one of the important component in marine ecosystems, it has not been tested during the approval procedures of most ballast water management systems (BWMS) that make use of active susbtances. In this test, the bioluninescent microbes, Vibrio fischery, were exposed to seawater having various total residual oxidant (TRO) which is the most frequently used active substance, right after spiking and also during the whole storage period. A considerable toxicity of treated water was observed when the microbes were exposed to seawater having 0.01 mg/L TRO as Cl2 , which is lower than the criteria of maximum allowable discharge concentration (MADC) by IMO.

The sensitivity of bioluminescent microbe was also compared with microalgae, which was reported as the most sensitive test organisms in the literatures evaluating residual toxicity of treated ballast water. About 3~10 times lower NOEC and EC50 for all tested DBPs from eco-toxicity testing of bioluminescent microbes have been observed than those of microalgae testings, that the microbes have relevant sensitivity to chemicals produced by the reacting of TRO with various substances in seawater during disinfection.

The result of this research suggested that the toxicity testing with bioluminescent microbe could be applied as an efficient tool for it's simplicity in handling and duration time, potentially for quick monitoring of eco-toxicity on board or for testing of local regulation, which have a relevant sensitivity to residual DBPs as well as the hypochlorite ion or TRO. Further, the toxicity test of bioluminescent microbe would be applied as one of eco-toxicity testing for the approval of BWMS using active susbstances (G9 of IMO).

INTRODUCTION

When deballasting water was treated with active substance(s), a residual toxicity might be shown depending on the type and concentration of active substance(s), and also disinfection byproducts (DBPs) in the discharged water. Therefore ecotoxicity tests are required to be conducted as well as instrumental analyses of some DBPs to evaluate a ballast water management system (BWMS) before operating by the G9 approval procedure.

The G9 procedure asks for estimating the ecological risk of the active substance used in the BWMS for the receiving environment. Toxicity tests, the so called bioassays, need to be conducted to estimate the ecotoxicological impact of the treatment to the environment. A bioassay is a test in which an

organism is exposed to a concentration series of a substance or to whole effluents like discharged ballast water (WETtesting). A batch of these tests including different trophic levels of organisms like microalgae, crustacean and fish are used to assess the risk of treated ballast water in a harbor. However, it is practically difficult to assess discharged water from many of the approved BWMS in time whether they are opperated properly not to pose any ecological risks by dischaging treated waters, because most ecotoxicity tests and DBP ananlyses take days to weeks even by trained staffs of well-controlled laboratories.
Toxicity test in assessing ballast water is compliance monitoring that should allow port authorities to judge the environmental risk at discharge. Using the full battery of testing techniques for each system and at each discharge will be practically impossible. Thus the terms desired for toxicity test would be more simple and instant method to assess the discharged ballast water for compliance monitoring.

A prompt and sensitive assessment using bioluminescent microbe (Vibrio fischeri) are considered as a practical method to evaluate residual toxicity in treated discharge water in the terms of practicability. There, this research would be evaluated the sensitivity of bioluminescent microbe to assess residual ecotoxicity of treated allast water for the purpose of practical application.

MATERIAL AND METHODS

Ecotoxicity of the ballast water treated with hypochlorite (as TRO) was tested using two bioassays for comparing sensitivities to TRO and DBPs: a bacteria test (ISO 11348, 2007) and a microalgae test (OECD 201, 2011/ASTM E1218, 2012). Each bioassay tested with a range of TRO and 6 different DBPs of concern to residual toxicity in ballast water. The samples were diluted in a concentration series according to the test procedures of each bioassay.

Vibrio *fischeri*, bioluminescent microbe is a gram-negative, rod-shaped bacterium that bioluminesces through a population-dependent mechanism called quorum sensing. Colonies of V. fischeri collectively luminesce upon reaching a certain cell density (Stevens and Greenberg, 1997; Hastings and Greenberg, 1971). The bioluminescence intensity reflects the overall health of the organisms and the luminescence reaction, which reflects metabolism, is sensitive to a wide variety of toxic substances. This sensitivity has made them a popular choice for methods to detect environmental pollutants, such as heavy metals and pesticides.

Microalgae are ubiquitous in aquatic ecosystems, because of their ecological importance, sensitivity to many toxicants, ready availability, ease of culture, and fast growth rates (rendering it possible to conduct a multi-generation test in a short period of time), algae are weightily used in toxicity testing.

Experiment 1. Change of ecotoxicity after injection of hypochlorite (as TRO) with aging such as TRO decay

- Concentration gradient of hypochlorite as active substance from 5 to 0.039 mg/L TRO as Cl_2 (nominal concentration)
- Bioassay with bacterial bioluminescence test with elapsed time from 0 to 24 hrs at the proper time
- Determination of TRO concentration with titration method (DPD)

Experiment 2. Comparison of sensitivity of toxicity test between bacteria and microalgae on TRO and 6 DBPs (disinfection byproducts)

- Disinfection byproducts (6 DBPs) with concern and frequent: bromate, chloroform, bromoform, monochloroacetic acid (MCAA), dichloroacetic acid (DCAA), monobromoacetic acid (MBAA)

RESULTS

Bioluminescence inhibition rate of microbe V. fischeri accroding to the decay of TRO was shown 44 % as maximum at elapsed 24 hours when the TRO concentration was 0.01 mg/L TRO as Cl_2 as below MDL (method detection level) of DPD method (figure 1).

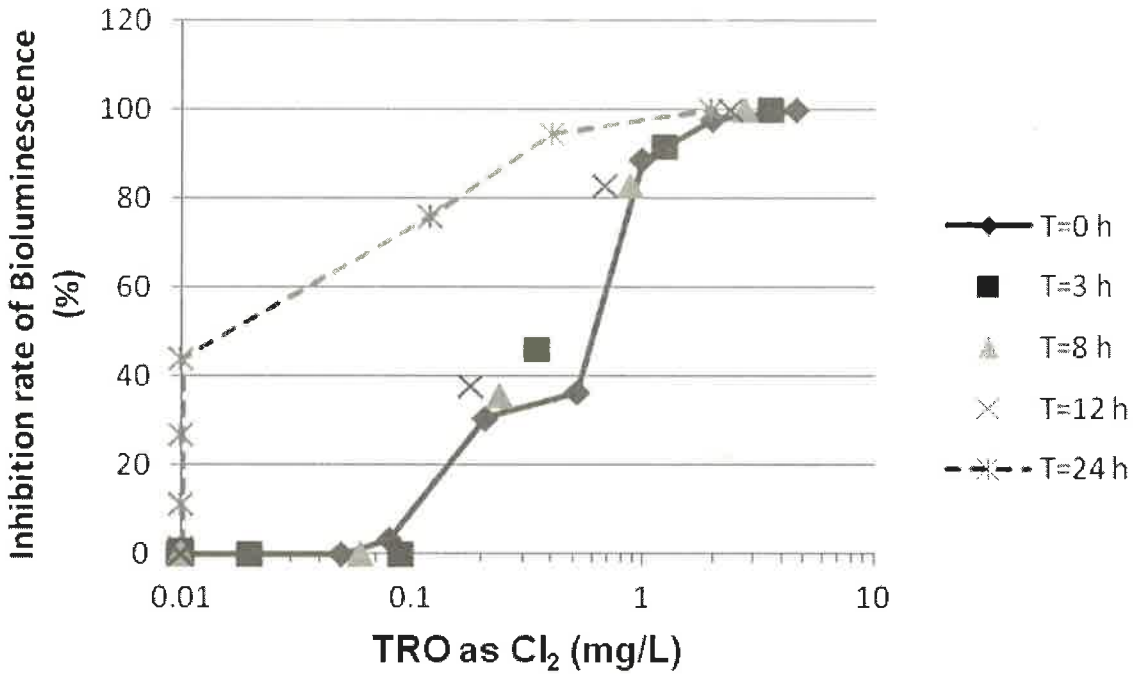

Figure 1. Variation of bioluminescence inhibition rate of microbe, *V. fischeri* with TRO decay till 24 hours

EC50 of inhibition of bioluminescence on TRO was 0.48 mg/L TRO as Cl_2, at which bacteria test was conducted immediately followed by spiking. EC50 of TRO was increased at elapsed 1 hr, slightly decreased with time, finally to 0.05 mg/L TRO as Cl_2 at elapsed 24 hrs (figure 2). The variation of EC50 with aging was shown that toxicity of TRO was increased 10 times at similar concentration. MADC (maximum allowable discharge concentration) regulation of IMO is 0.2 mg/L TRO as Cl_2, in case of TRO concentration of ballast water is below the MADC, ballast water is able to discharge without any neutralization. However, figure 2 was shown that residual toxicity of TRO is potential below the MADC.

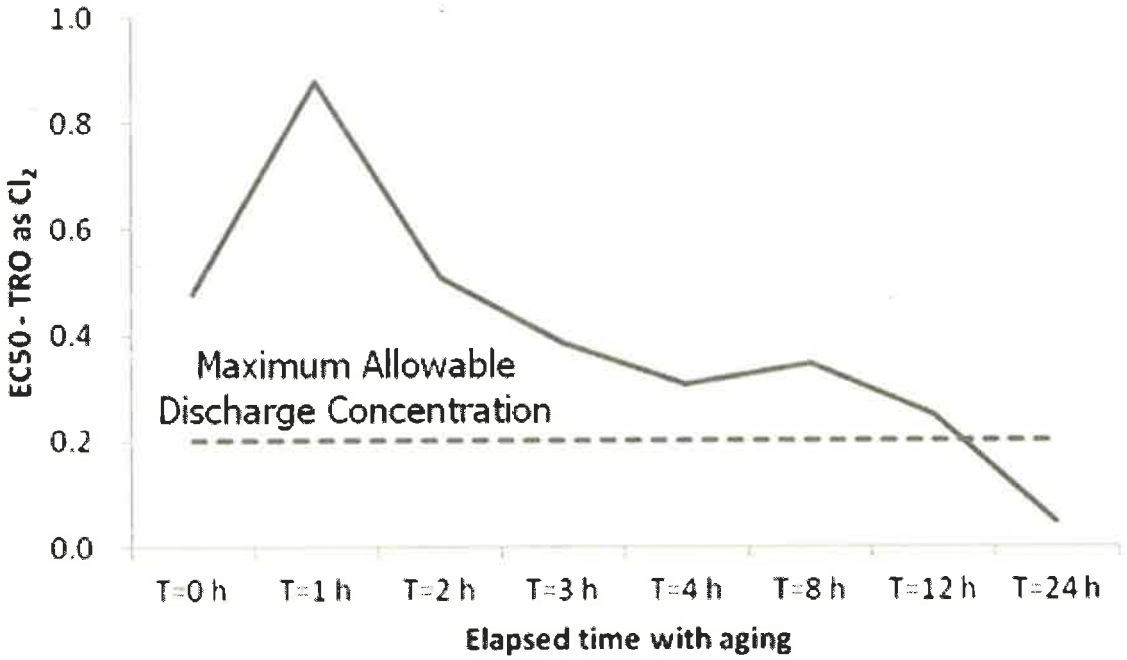

Figure 2. Variation of EC50 with bioluminescence inhibition of microbe *V. fischeri* on TRO with aging

Table 1 shown that the bacteria was more sensitive than microalgae about 3~10 times lower NOEC and EC50 for TRO and all tested DBPs, expecially more sensitive with 9 times based on EC50 of TRO. The bioluminescent microbe, V. fischeri have relevant sensitivity to chemicals produced by the reacting of TRO with various substances in seawater during disinfection.

Table 1. Comparison of toxicological parameters with ecotoxicity on TRO and 6 DBPs between bioluminescent microbe, *V. fischeri* and microalgae, *Skeletonema costatum*

Chemicals (mg/L)	Endpoint for bacteria			Endpoint for microalgae		
	NOEC	LOEC	EC50	NOEC	LOEC	EC50
TRO as Cl_2	0.125 *(0.11)	0.25 *(0.22)	0.18 *(0.13)	1.25 *(1.03)	2.50 *(2.20)	1.59 *(1.33)
Bromate	30.0	60.0	48.1	50	100	178.8
Chloroform	5.0	10.0	32.5	100	200	167.7
Bromoform	0.63	1.25	36.5	100	200	106.8
MCAA	0.63	1.25	14.7	50	100	76.8
DCAA	0.63	1.25	19.8	100	200	159.1
MBAA	0.31	0.63	10.3	50	100	71.7
Method	ISO 11348, Bioluminescence inhibition (30 min) *Measured TRO as Cl_2 (mg/L)			OECD 201/ASTM E1218, Population growth inhibition (72 hrs) *Measured TRO as Cl_2 (mg/L)		

CONCLUSION

The results of this study was shown that bacteria is more sensitive on residual actvie substance(s) and disinfection byproducts (DBPs) in ballast water compared with microalgae, even though microalgae

has been reported as most sensitive species than crustacean and fish in BWMS test report of IMO.

Also, lower concentration of TRO with 20 times below MADC regulation (0.2 mg/L TRO as Cl_2) of IMO was shown notable ecotoxicity on bioluminescent microbs, V. fischeri (figure 2).

Finally, toxicity test with bioluminescent microbe V. fischeri could be applied as an efficient tool for it's simplicity in handling and duration time, especially for quick monitoring of ecotoxicity on board or for testing of local regulation.

Further, the toxicity test of bioluminescent microbe would be applied as one of eco-toxicity testing for application of G9 of IMO.

ACKNOWLEDGEMENTS

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Correlation between disinfection by-products, ecotoxicity and test water quality: update with the new chemicals list from GESAMP

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Abstract

Since 2005, five different BWMS based on chlorination treatment have been tested by Norwegian Institute for Water Research (NIVA) according to the International Maritime Organisation (IMO) guidelines. From these over 50 fullscale test cycles, the dominant DBP were identified and correlated to the toxicity level at discharge. According to the nature of the oxidants and the water quality of the test water, different dominant DBP were detected. Regarding toxicity, 25 % and >50 % of all the tested discharge samples exhibited acute and chronic toxic effects on algae, respectively. In most cases this toxicity was plausibly caused by a high residual oxidant concentration. Of the 22 disinfection by-products (DBPs) that were identified in treated water at discharge, four compounds were at times found at concentrations that may pose a risk to the local aquatic environment. However, there seemed to be no clear indication that the measured DBP concentrations contributed to the observed algal toxicity. These results were reviewed with the additional 17 DBP compounds proposed by IMO in 2013.

Introduction

Of the IMO type approved ballast water management systems (BWMS) availabe on the market today, 45% uses active substance, as chlorine, to inactivate the organsims in the ballast water. Chlorination is either achieved by addition of a chlorine chemical or electrochlorination of saline water. In presence of bromide or iodide as in marine waters, additional disinfectant agents other than chlorine might be produced such as bromine and iodine. By incorporating halogen atoms, as chlorine, bromine and iodine atoms, into organic molecules, halogenated alkanes and other compounds are formed, these compounds are designated as disinfection by-products (DBP). The dominant DBPs generated by chlorinated seawater are bromoform, dibromoacetic acid, bromoacetonitrile and traces of bromophenols. Several DBPs might be harmful for human beings because of their potential carcinogen effect and are regulated in drinking water standards and bathing water standards. Some DBP's may be persistent in the marine environment and bioaccumulate in the food chain. In order to address this DBP challenge, IMO guidelines requires from all BWMS using active substances to document the formation of DBPs due to the treatment and the toxicity of the treated water on algae, crustacean and fish species, during full scale land-based testing. The IMO's joint Group of Experts on the Scientific Aspects of Marine Environmental Protection-Ballast Water Working Group (GESAMP-BWWG) suggested a preliminary list of 18 compounds to be assessed in all BWMS tests before final approval (IMO, 2009a). This list has now been extended in May 2013 to 17 additional DBP compounds. The nature and the abundance of the organic matter might differ and interfere with the formation of DBPs in the test water as in open sea, in coastal, estuarine and river areas due to contribution of terrestrial organic

matter. Since 2005, the Norwegian Institute for Water research has performed over 50 test cycles of 5 different BWMS using active substances at NIVA's land-based full scale testing facility on the Oslofjord. NIVA has evaluated the DBPs and toxicity of the treated water according to IMO guidelines in NIVA's testing water quality. The objective was to study the correlation between DBPs formation and toxicity of the treated water in different test water qualities.

Material and methods

Full scale land-based testing

Between 2005 and 2012, five BWMS were tested by NIVA at its land-based full scale testing facility located 20km south for Oslo. All BWMS were including filtration and chlorination during ballasting. The total residual oxidants (TRO) measured in the ballast water tanks immediately after treatment varied from 1 to 15mg/L as Cl_2 approximately. During discharge, only physical treatment or neutralisation was applied, except for one BWMS that did use active substances, hence increasing the level of TRO to a maximum of 2.0 mg/l at discharge.

All BWMS were tested with high and medium range salinities following the requirements stated in the IMO G8 guidelines (IMO, 2008). To obtain the required salinity range, test water was prepared by mixing high salinity and low salinity water from Oslofjord with freshwater from ground water bore holes or from a local creek. To meet the required contents of dissolved organic carbon (DOC), particulate organic carbon (POC) and total suspended solids (TSS), soluble lignin or methylcellulose, starch and kaolin were added, respectively. A combination of indigenous harvested organisms and cultured surrogate species ($\geq$50 µm: *Artemia franciscana;* $\geq$10-50 µm: *Tetraselmis suecica*) were added to fulfil the biological water quality criteria stated in the IMO G8 guidelines (IMO, 2008).

Each BWMS was subjected to at least 10 test cycles using a minimum of five test cycles for each water quality. Every test cycle includes ballasting operations, 5 days storage period and discharge operations of both treated and control (untreated) waters. 500㎥ test water was prepared in the test water preparation tank. Ballasting operations for treated water consist in pumping and treatment of at least 200㎥ test water over to a storage tank. For control water, at least 200㎥ test water was pumped to another storage tank by-passing the treatment units. After five days storage in the dark, all treated water was subjected to partial treatment or neutralisation during discharge pumping to another storage tank. All control water was pumped in by-pass of the treatment unit to yet another storage tank.

All samples were collected directly from the tanks immediately after pumping and mixing operations.

DBPs analyses

Trihalomethanes were analysed by the purge and trap method according to US-EPA 524.2 (1995) with GC-MS detection. Bromate ions were measured by liquid ion chromatography according to NS-EN ISO 10304-1(1992). Haloacetic acids (HAA) were determined by GC-MS after liquid-liquid extraction and derivatisation according to NS-EN ISO 23631 (2006). Acetonitriles were analysed by liquid-liquid extraction and gas chromatography with electron-capture detection according to US EPA 551.1 (1990) method. Bromophenols were quantified by GC-MS after liquid-liquid extraction and derivatisation.

Tribromobenzene, chlorotoluene and halogenated aliphates were analysed by purge and trap GC-MS according to US EPA 524.2 (1995). Whenever an individual DBP was found in the control test water at levels $\geq$10 % of what was measured in the treated test water, the concentration in the treated test water was adjusted by subtracting with the concentration found in the control test water.

Algal toxicity test

Algal growth inhibition tests were done with samples from all test cycles after ballasting and at the time of discharge. The tests were performed according to International Standard ISO 10253 (2006) using the diatom *Skeletonema costatum*, NIVA-strain BAC 1 as test organism. The growth rate of each culture was calculated and expressed as percentage of the growth rate of control cultures in untreated ballast water. If a significant inhibition was observed in the treated ballast water, EC_{50} (50 % growth inhibition) and EC_{10} (10 % growth inhibition) concentrations were attempted estimated based on non-linear regression analysis of the growth rate against the concentration of ballast water. EC_{50} is regarded an acute toxicity test end point, while EC_{10} is regarded a chronic toxicity test end point.

Results and discussion

DBP formation in treated water

Of the close to 100 different potential DBP compounds that have been included by NIVA when analysing samples of treated ballast water, 22 compounds were detected above the detection limit in at least one sample collected at the time of discharge. The majority of the compounds on the DBP list suggested by GESAMP-BWWG for analysis in connection with risk assessment of treated ballast water (IMO, 2009a) are among these detected compounds (monochloroacetic acid was only detected above the detection limit in samples collected directly after treatment and monochloroamine was not analysed for). Notably, chlorate and dibromomethane were not included in the first list from GESAMP-BWWG, but they were among the eight compounds that have been found in 50 % or more of all the treated discharge samples in which they have been analysed. The eight compounds that were detected in over 50% of the total of 9-43 samples analysed were tribromomethane, chlorate, dibromoacteic acid, dibromochloromethane, bromate, dibromomethane, bromochloroacetic acid and dichlorobromomethane. The observed median and maximum concentrations of these eight most often detected DBP compounds were in the range from 2 µg/l to 670 µg/l. In general, the brominated compounds were more predominant than the chlorinated counterparts with some exceptions; chlorate and dichloromethane were found at higher concentrations than bromate and dibromomethane, respectively. Overall, the 5 most often detected compounds exhibited also the highest maximum concentration levels. Notably, tribromoacetic acid which was detected in only 24% of the 34 samples analysed for DBPs, was often detected in relatively high concentrations (53-240 µg/l) as compared to the others DBPs. Nevertheless, some DBP can present high toxicity in low concentration therefore the toxicity effect of some DBP was further studied further.

Oxidants residual concentrations, DBP formation and algal toxicity

All of the samples that exhibited acute or chronic crustacean and/or fish toxicity also exhibited algal toxicity; hence, the chronic toxicity end point of the algal growth inhibition test was the most sensitive of all the applied toxicity tests. Of the 59 samples of treated water collected at the time of discharge,

32 and 15 samples exhibited chronic (EC_{10}) and acute (EC_{50}) algal toxicity, respectively. Though a poor correlation could be observed between toxicity effect and TRO results, especially for brackish water, approximately 75% of the samples in total did show expected results for toxicity effect related to the TRO measurements. However 18 of 59 samples with low TRO values showed toxic effect and five samples with high TRO values showed none toxic effect. This may suggest that there may have been additional factors to residual oxidants that contributed to the observed algal growth inhibition in these samples. To be able to distinguish between the toxic effects caused by DBPs rather than residual oxidants in the water, only samples with TRO values well below the expected no effect concentration (NOEC) of the residual oxidants was used in this assessment. There seemed to be no clear indication that the measured DBP concentrations affected the algal toxicity neither for individual DBPs (tribromoacetic acid, dibromochloromethane, chlorate, monobromoacetic acid and tribromomethane) frequently detected in the discharged water at levels of potential environmental concern nor for the sum of all DBPs. A direct comparison between the chronic toxicity endpoints for the individual DBPs and the highest levels at which they were found in the discharged waters further substantiated this, as their concentrations were at least a factor 25 lower than the predicted no effect concentrations (PNEC) value available in the literature.

Environmental risk from discharged DBPs

A simple environmental risk assessment of the individual DBPs detected in the discharged waters was performed by comparing the predicted environmental concentration (PEC) values determined from the DBPs analyses results during the land-based testing at NIVA and the predicted no effect concentrations (PNEC) value available in the literature. Four of the compounds (tribromoacetic acid, dibromo-chloromethane, chlorate and monobromoacetic acid) were at times found at concentrations that may pose a risk to the local aquatic environment (PEC/PNEC value >1). It should be noted that one of these compounds, chlorate, is not on the DBP list suggested by GESAMP-BWWG for analysis in connection with risk assessment of treated ballast water (IMO, 2009a). We therefore recommend chlorate to be included in the environmental risk assessment. However PEC values are dependant of the technology tested and the PNEC values dependant of the available published data, therefore these results of PEC/PNEC are just an indication from the five BWMS using active substances tested at NIVA and available literature references. All BWMS has to be individually evaluated for each DBP and PEC/PNEC ratio calculation. Further comparison of BWMS testing results from NIVA to DBP results from other test facilities was studied.

Comparison of measured DBP concentrations with results from other test facilities

A comparison between the maximum DBP concentrations observed in the full scale land-based tests done at NIVA's test facility and the maximum DBP concentrations reported from tests done at other test facilities with four BWMS using active substances was performed. The average of the maximum levels found in discharged water from the five different BWMS tested at NIVA were well above the observed median of the maximum values reported by others for bromate (2.2 times higher), for all trihalomethanes (2.1-3.2 times higher), for tri- and di-brominated haloacetic acids (1.3-3.5 times higher) and for dichloroacetic acid (4.8 times higher). These results indicate that the DBP levels found at NIVA were, in general, relatively high. This might be explained by the site-specific test waters that have been used. However, the average of the maximum levels found at NIVA were always within the 90 percentile of the maximum levels reported by others and the overall maximum level of any

of the DBPs found at NIVA were below the highest reported level reported by others. This supports the risk assessment data presented above are relevant for these DBP concentrations measured.

Conclusion

22 of the approximately 100 different potential DBP compounds that were analysed for at the time of discharge were detected above the detection limit. Two of these, chlorate and dibromomethane, weren't on the first DBPs list from GESAMP-BWWG recommended for analysis in connection with risk assessment of treated ballast water. But dibromomethane is now included among the 17 new additional DBP compounds of the new DBPs list from GESAMP. Chlorate was also among the four compounds (tribromoacetic acid, dibromochloromethane, chlorate and monobromoacetic acid) that at times were found at concentrations that may pose a risk to the local aquatic environment, and it is therefore proposed to be included on the list when the list is updated. The precision of the environmental risk assessment was limited by the lack of toxicity data; hence future toxicity tests on these compounds and the so called cocktail effects from the complex mixture in which these compounds are discharged may alter the derived PNEC values considerably. Nevertheless, there seemed to be no clear indication that the measured DBP concentrations affected the algal, crustacean or fish toxicity neither for individual DBPs nor for the sum of all DBPs.

The DBP levels found at NIVA were, in general, relatively high compared to DBP levels found in other land-based full scale BWMS tests. Lignin was identified as an important TRO consumer in the treated ballast water during storage and a possible precursor for the brominated trihalomethanes. As lignin is one of the most common and naturally occurring compounds in the world, its use as a dissolved organic carbon source in the BWMS tests may be regarded as a realistic, but worst case, scenario. Methylcellulose could be a potential replacement for lignin as this compound did not seem to affect neither the TRO consumption nor the formation of trihalomethanes. Interestingly, the presence of lignin appeared to limit the formation of bromate, whereas methylcellulose did not seem to affect the bromate formation potential. Further research should be done with other substantives that are commonly used by test facilities worldwide. Only 5 different BWMS were studied here, all based on chlorination treatment, while IMO reported 34 basic approvals and 20 type approved BWMS using active substances (IMO, 2011e), therefore further study of the correlation between TRO, DBP and toxicity effect with other type of oxidation as ozonation for example should be performed.

Keywords: *ballast water management system testing, chlorination, disinfection by-products, toxicity.*

Ballast Water Treatment by Acoustic Means – Notes on Environmental Acceptablity

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For treatment of ballast water both chemical and physical means have been applied. The physical means consisted predominantly of UV irradiation, but recently acoustic means have been introduced as well. At present the methods use sound of ultrasonic frequencies, but that practice might change over time. Acoustics methods have also been explored as anti-fouling systems, again to prevent transfer of invasive species by ships, with one of the methods explored consisting of sound within the human hearing range. That method was not further developed then, because the loudness prevented crew from going to sleep, yet it had potential and may surface in some form again.

Anthropogenic sound, an input of energy into the aquatic environment, has been documented to lead to potentially adverse impact on marine life. The overarching UN Convention of the Law of the Sea (UNCLOS) recognises pollution of the marine environment as the introduction by man, directly or indirectly, of substances or energy into the marine environment. Sound from anthropogenic sources such as Ballast Water (BW) treatment might lead to adverse effects, which is not in agreement with the Ballast Water Management (BWM) Convention (2004), where Article 2 states that strategies for BWM should not cause effects that are worse than the problem to be solved. Increasingly examples have been brought forward of marine life affected by human-generated under-water noise, and not only for cetaceans. Vital behaviours and life strategies in almost all groups of aquatic animals can be affected, with potentially long-lasting effects as will be exemplified below. The effects are often subtle and may affect interactions in the ecosystem that are vital. An example of one such interaction is the migratory strategy of juvenile coral reef fish. Juvenile coral reef fish grow up in open sea well away from the reef. For migrating back they need to recognise the specific sound profile of the parent reef, to orient towards the reef and navigate home succesfully. A human sound source in between the reef and migrating juveniles can greatly hamper homing (ten Hallers & Simpson, 2008, Holles et al., 2013) and other vital functions and behaviours (Simpson at al., in Press).

Ballast water treatment that uses physical instead of chemical means should be evaluated for environmental acceptability according to the BWM Guidelines for approval of ballast water management systems (G8), as are other forms of ballast water treatment by physical means. Although treatment by acoustic means is new, embarking on the route to demonstrate environmental acceptability can draw on a well-documented field. The adverse impact of anthropogenic under-water sound has consistently been exemplified, albeit it to a lesser extent than that of other anthropogenic substances. Underwater noise from maritiem sources in light of the complex acoustic ecology in the aquatic environment was earlier brought forward in light of maritiem policies (ten Hallers-Tjabbes, 2007). As to the supersonic sound of the present BWM developments, it is well known that cetaceans (dolphins, popoises and whales) use high-frequency acoustic signals for orienting, moving and manoeuvring in

their environment. Aditionally some fish (clupeids) species are shown to be able to hear up to 200 kiloHertz (kHz) (Mann et al., 1997; Popper et al., 2004). They might use such capacity to avoid attacks by cetaceans that emit high-frequency sounds for echolocation, as happens in the terrestrial environment where insects avoid predating bats by detecting their echolocation clicks.

In a world that is often dark and turbid, where vision has limited capacity, the acoustic sense is a predominant tool for exploring the surroundings, for finding food, mating partners and shelter areas as well as for avoiding predators. Sound has specific charicteristics in water; it travels fast and far in water (sound velocity in water is five times faster than in air), in particular when lower-frequency sound and has a complex propagation pattern. Specific structures in the aquatic environment (surface and sea floor boundaries and picnoclines – in-water horizontal boundaries between waters of different salinity or temperature) form a layered pattern that can enhance the travelling distance of sound by reflection, reverberation and refraction, to sometimes many times the theoretical distance of the sound waves at the specific frequency itself. Low-frequency sound is also transmitted by water-saturated sediments; sound of higher frequencies bears a high information content. Sound contains high directional information and is therefore highly suitable for guiding long distance navigation of marine animals, such as marine mammals and fish. Sound of low frequencies travels much farther distances than high-frequency sound. Natural sound in the marine environment is generated by abiotic phenomena, such as weather conditions (wind, rain), movement of water and suspended matter (currents, vortexes) and biotic factors, such as moving organisms, vocalisations and other signalling (Urick 1983, 1996).

In marine animals sound reception or acoustic sense is important for communication, orientation and navigation behaviours in support of the animals' biology, its reproduction, migration and survival strategies. Sound mediates recognition of conspecifics, of predators or prey, of suitable habitats for shelter, for feeding, for reproduction or for migration; sound enables recognition of distant targets and is used in intraspecies interactions (Laplanche et al. 2005; Mongomery et al. 2006; Simon et al. 2006). The frequency range that different marine animals can perceive varies greatly between species and animal groups. The important role of underwater sound in marine animal functioning renders them susceptible to disturbance by sound from non-natural sources (Kastelein et al. 2006) the perception of such acoustic stimuli has not been incorporated in the evolutionary processes, where animals could gradually adapt to chainging environmental conditions. When mapping anthropogenic sound sources and animal acoustic windows for the North Sea, many risk areas for acoustic impact were identified (ten Hallers-Tjabbes and Verboom, 1991).

While the impact of underwater noise on cetancean physiology and survival is best known, almost all other aquatic animals recognise sound and respond to it and are hence susceptible to adverse impact by anthropogenic sound. Most human-generated noise can affect animal functioning and behaviour. Acoustic windows of most animals are sensitive to pick up low sound levels and are therefore vulnerable to higher sound levels. It matters whether the frequency range of the acoustic window matches with the antropogenic sound source; outside the most sensitive frequency range of an animal acoustic window, the sensitivity drops to much lower levels or none at all. Fig 1.shows that frequency ranges of different animal acoustic windows and human-generated sound often overlap, while the sound levels of the anthropogenic noise are much higher than those of the animal acoustic windows. Sound levels are presented on a logarithmic scale.

For marine animals sound from human sources can be lethal, cause sub-lethal damage, interfere with

functional behaviour or force animals to avoid favourable locations, such as feeding and breeding grounds. Non-lethal physiological effects include hearing damage and other internal damage, temporary threshold shifts (temporal deafness) (Finneran et al. 2005), masking of sound and changes in behavioural patterns that are based on acoustic sensory stimuli. Sound is a sensory signal, of which disturbance can lead to affected or disrupted behavioural patterns, to the detriment of individual animal functioning and of ecological interactions. Animals may no longer be able to find food, or recognise patterns of survival value, such as migratory routes, sexual partners and offspring or predators.

Which behaviours become affected, and also when, is mainly hypothesised. Avoidance of geographical areas where human noise of high sound levels is present may be the best documented behavioural impact of human noise. Even when avoidance is temporary, it may be of wider ecological consequence, for instance, if a biologically important geographical area is avoided during critical periods (such as breeding grounds at the onset of reproduction). Those animals that cannot avoid areas, either by being sessile or very slow moving or by being in an immobile stage (eggs, moulting etc) are more likely to suffer lasting damage. Animals that do avoid noise, start doing so at a certain range from source, the 'discomfort levels'

When an animal enters the zone of audibility of a human sound source, it will gradually experience more discomfort and eventually starts facing an impact when nearing the source. Different levels of impact of noise can be either seriously damaging or provide no more than an indication that noise may become disturbing or damaging if it were to become louder. When entering such zone of discomfort, an animal is likely to avoid the area, but may do so at the risk to move to less favourable areas. When however the noise has already lead to a deafening effect, the animal may not move away form the source and then runs a risk of additional damage and eventually life-threatening impact.

Adverse impact impact of anthropogenic noise has been documented for several fish species (Popper et al, 2003) and for some invertebrates. Foraging behaviour in stickleback was affected by broadband noise, leading to less foraging effciency (Purser and Radford, 2011). A similar adverse effect on foraging behaviour was demonstrated in shore crabs, while crabs exposed to human-generated noise also were slower in retreating to a shelter when attacked by a predator (Wale et al, 2013). Reef fish are affected by boat noise in many ways, it increaases mortality and the likelihood of predation, while it elevates metabolic rates and affects learning with implications for survivial (Simpson et al., In Press).

Demonstrating environmental acceptability of BWM by acoustic means, according to G8, will pose specific challenges, in particular in applying the requirements in G8 that refer to the relevant provisions in G9. Approval of ballast water management systems that make use of active substances. As such a considerable part of the GESAMP-BWWG Methodology for G9 will apply. Bearing in mind that the GESAMP Methodology has been developed for evaluating systems based on chemical treatment, translating some requirements for physical applications is not straightforward. The practice to evaluate systems that use UV irradiation according to G8 is now well developed; it can be expected that applying a similar format for evaluating systems that are based on acoustic means will pose specific challenges that are related to the character and possible effects of underwater sound. Much of the evaluation framework has ample potential to be easily adapted to refer to noise effects; some more specifically toxicological requirements may need a purpose-oriented translation.

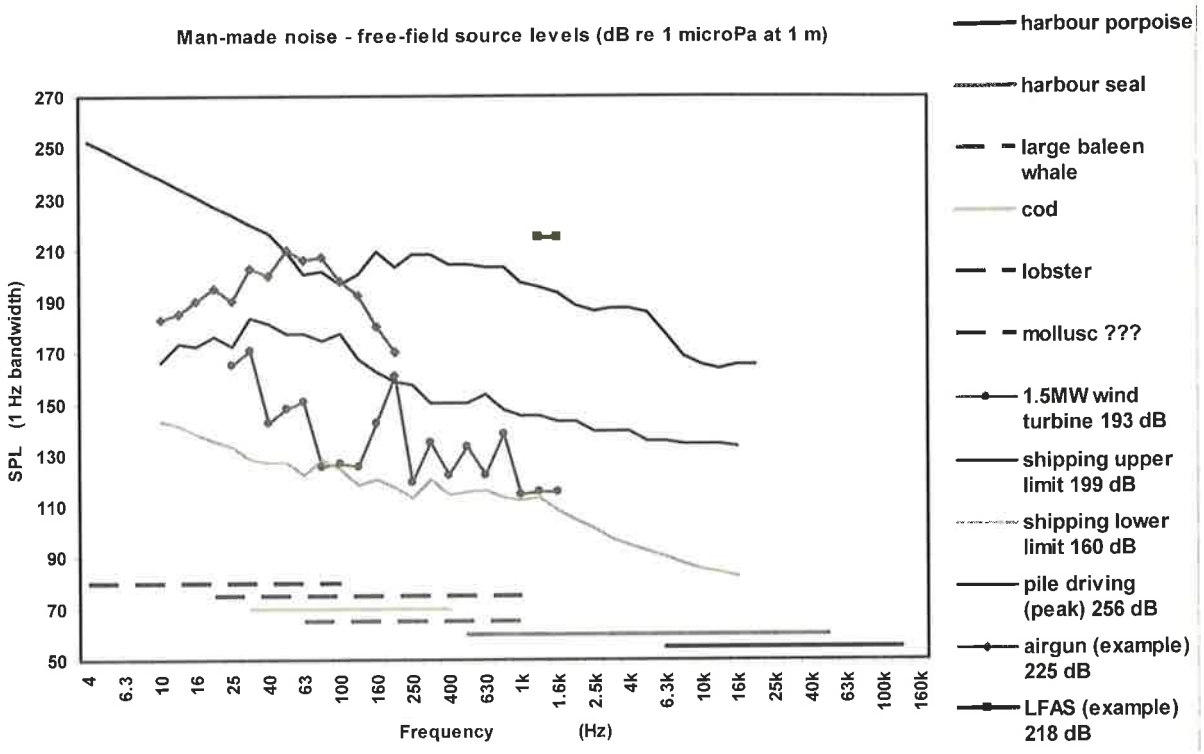

Figure 1. Sound profiles of marine animal acoustic windows (broken lines) and of human-generated noise (solid lines) (Verboom, 2006)

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Keywords: *Anthropogenic noise, ballastwater management, environmental acceptability, underwater sound,*

Ecotoxcity of Treated Ballast WATER by ·OH-Based Process: An Evaluation of Marine Environmental Acceptability

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The establishment of nonindigenous aquatic species (NAS) has been implicated as the causal mechanism for 20% of animal extinctions globally and a contributor to an additional 34% of animal extinctions. Further, NAS are a significant and growing contributor to global impoverishment of "red-listed taxa", and are the dominant driver of species homogenization. More than 80% transported goods worldwide are shipped by sea. Commercial ships with and/or without cargo require ballast water for their stability. It is estimated that 3–10 billion tonnes of ballast water are transported annually worldwide. Ballast water discharges have historically been a major source of NAS introductions to marine ecosystems and are recognized internationally as vectors for the translocation of invasive marine organisms. To reduce the probability of arrival of NAS via ballast water, both Canada and the U.S. have implemented regulations requiring mid-ocean ballast water exchange (BWE) of filled, and saltwater flushing of empty ballast tanks. Recent scientific research shows that BWE and saltwater flushing effectively reduce invasion risk for freshwater systems; however, efficacy is mixed for marine ecosystems. An international Ballast Water Management Convention, when ratified, will require ships to replace BWE with ballast water treatment (BWT). This convention sets a numeric performance standard, which must be met before the release of ballast water into the environment. Since the adoption of the Convention and more particularly Guidelines G8 for approval of ballast water management systems in 2005, a substantial number of treatment systems have been put into development globally. Many systems do not come to public light until they apply for Basic Approval of Guidelines G9 by the Maritime Environmental Protection Committee (MEPC).

The available technologies for BWT apply one or more treatment processes, such as filtration, ultraviolet (UV) radiation, electrolysis, ozonation, etc., and other novel technologies such as ultrasonic, magnetic methods and use of plasma discharge have also been investigated for their treatment efficacy. However, each of method mentioned above has inherent advantages and disadvantages regarding biological efficacy, costs, ship and crew safety, power and space requirements, and environmental soundness. For example, high efficiency UV irradiation depends on low turbidity and high clarity water and unfouled quartz sleeves to achieve good UV transmission through the water. For electrolysis, the efficiency varies according to water conditions (salinity, pH, temperature, etc.), and by-products, especially hydrogen (H_2), have a potential risk of explosion onboard. Ozonation is especially effective at killing micro-organisms, but can produce bromate and other by-products which may cause adverse environmental impacts. Chemical by-products (CBPs) are a mixed chemical group and several of them were found to produce cancer in animals. It is crucial that systems utilizing or potentially releasing toxic substances undergo chemical analysis. Unfortunately, at present complete chemicals prediction is based on experience from fresh water disinfection and several others have not been fully investigated or even chemically identified yet. To overcome the limitation of CBPs evaluation during BWT by lacking completeness and quality of available information, aquatic ecotoxicity tests must be assessed to indicate its comprehensive impact on seawater species based on Guideline G9 of the International Maritime

Organization (IMO).

According to Guideline G9 of the IMO, acute and chronic toxicity test data from freshwater or saltwater representatives of three taxa (algae, crustacea and fish) representing three trophic levels should be performed based on internationally standardized tests (e.g., Economic Cooperation and Development (OECD) guidelines, United States Environmental Protection Agency (US EPA) methods). However, selecting the proper test species would be a problem when carrying out the ecotoxicity tests. For example, specified freshwater species that cannot survive in seawater are used in the OECD guidelines, while the native test species used in the US EPA methods can hardly be obtained and cultured. Therefore, it is necessary to establish ecotoxicity evaluation criteria of treated ballast water in China.

Our objective is to evaluate the potential toxicological impact of ballast water treatment using a combination of filtration at 50 μm with an automatic self-cleaning filter and a killing step using mainly hydroxyl radicals (•OH) based on the strong ionization discharge (SID). During the SID, the high-energy electrons could break the chemical bonds of oxygen and little water at gas phase to form free radicals and/or ions, further producing active oxygen compounds like followed by a series of plasma chemistry reactions, such as O_2^+, O_3, H_2O^+, H_2O_2. The oxygen species were then transferred into the gas/liquid dissolver with a portion of the ballast water to form •OH radicals. Before conducting the ecotoxicity tests, we need to know which samples should be taken. Therefore, chemical analysis is necessary to be performed. Samples for the control water and treated water samples immediately after treatment as well as during the retention period of 5-day storage, were collected and analyzed for 43 CBPs. The results indicated that only 8 kinds of CBPs were detected significantly. Among the measured CBPs, trihalomethanes (THMs) and haloacetic acids (HAAs) concentrations were significant, especially tribromomethane (TBM) and tribromoacetic acid (TBAA). The maximum concentration of TBM was observed at 48 h of storage after treatment, after which it decreased with storage time extension. The maximum concentration of TBAA was observed immediately after treatment, after which it decreased with storage time extension. Other CBPs concentrations such as 2,3,4-trichlorophenol, 2,3,6-trichlorophenol and 2,4,6-trichlorophenol, were quite low and approximately at the level of 0.014–0.503 μg/L. Based on chemical analysis results, the ecotoxicity tests were undertaken on the treated ballast water stored for 2 days.

In this study, three water species common in Chinese coastal zones (i.e., hygrophyte *Skeletonema costatum,* invertebrate *Neomysis awatschensis,* and fish *Ctenogobius gymnauchen*) were selected and used in the ecotoxicity tests. Aquatic toxicity tests, including algal growth inhibition and chronic toxicity for invertebrate and fish, were used to measure the toxicity of treated ballast water discharge. Algal growth inhibition tests were performed in 250-mL glass conical flasks under white-type fluorescent lamps with an automated light/dark cycle of 14 h/10 h in a rotary shaking incubator at 25 ± 2°C. During the test period, the cell density in each flask was measured at 24, 48, 72, and 96 h after the beginning of the exposure period. *N. awatschensis* and *C. gymnauchen* were selected for chronic toxicity tests of invertebrate and fish, respectively. The test chambers were 1-L glass beakers under a photoperiod of 16 h of light and 8 h of darkness in an incubator at 23 ± 2°C. A dilution factor of 0.5 was used and five concentrations of the treated water (6.25, 12.5, 25.0, 50.0 and 100.0%) were selected during the chronic toxicity tests.

Algal growth inhibition tests were evaluated using average specific growth rate. The mean coefficients of variation for section-by-section specific growth rate were 4.50% and the coefficients of variation of average specific growth rates during the whole test period in replicate control waters were 3.11–6.19%,

respectively. These results satisfied the test validity of 35% and 10% described in published OECD 201 Guidelines; therefore, *S. costatum* met the requirement as test species. During the 96-h exposure period, growth inhibition and other toxic signs were not observed in the treated and control groups. The test results indicate that I_rC_{50} (96 h) to algae were equal to or greater than the 100% treated ballast water. There was no difference between the growth in the control water and in the treated ballast water.

For invertebrate toxicity test, the survival rate of *N. awatschensis* was approximately 80–100%, and the average weight of *N. awatschensis* was approximately 0.932–1.255 mg/surviving organism in the controls, respectively. These results satisfied the test validity of 80% and 0.80 mg/surviving organism described in published US EPA methods; therefore, *N. awatschensis* met the requirement as test species. During the 7-day exposure period, mortalities of the tested organisms in both treated and control groups were all <10%. The survival, growth, and reproduction data of *N. awatschensis* in treated water was compared with that in control water followed by a one-sided Dunnett's procedure, and the value of significance were all greater than 0.05, indicating no significant difference between control and treated water.

For fish toxicity test, the survival rate of *C. gymnauchen* was approximately 100%, and average dry weight per surviving larvae of *C. gymnauchen* was 0.39–0.45 mg in the controls, respectively. These results satisfied the test validity of 80% and 0.35 mg/surviving larvae described in published US EPA methods; therefore, *C. gymnauchen* met the requirement as test species. During the 7-day exposure period, no dead *C. gymnauchen* were observed in 100% (i.e., undiluted) treated water. The weighted analysis of variance (ANOVA) was also assessed with Dunnett's test and the results indicated no significant difference in survival, growth, and/or reproduction for *C. gymnauchen* at any test concentration.

Overall, aquatic toxicity tests of three trophic levels for treated ballast water were performed in this study. The results indicated that no visibly toxic signs or mortality of treated ballast water on day 2 of storage after treatment were observed. Therefore, the toxicological risk of discharged ballast water after •OH radicals treatment to the receiving environment is of no concern.

Keywords: *ballast water treatment, ecotoxicity, hydroxyl radicals, strong ionization discharge*

Effects of Neutralizer and Organic Matter in Freshwater and Salt Water Treated with Chlorine on Algal Growth

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Abstract

The algal specific growth rate (SGR) of salt water species, *Isochrysis galbana* and fresh water species, *Selenastrum capricornutum* are not significantly different when the algal culture was added with 10mg/L of starch without TRO and neutralization, except SGRs of 72-96h and 96-144h. The mean SGR of early culture (48-72h) of *S. capricornutum* is higher than that of *I. galbana* but lower at late culture (96-144h) at both cultures with or without starch.

The potential toxic effect of Disinfection Byproducts (DBPs) after reaction of TRO with starch or organic compounds after degradation of algal cell was observed at both cultures of freshwater and salt water. When the algal culture with additional starch was neutralized just after treatment of 10 mg/L TRO as Cl2, the adverse effect on the growth of *S. capricornutum* appeared at 96h after inoculation, while that of *I. galbana* was appeared at 144h after inoculation. When the algae was inoculated to the culture with starch and neutralized after treatment of 10 mg/L TRO as Cl_2 at 1 to 5 days after treatment, *S. capricornutum* appeared recover the growth at 96h after inoculation, while that of *I. galbana* was not to 144h after inoculation. *S. capricornutum* is not too sensitive on algal growth to the addition of starch while that of *I. galbana* is sensitive to algal growth when the TRO was neutralized with sodiumthiosulfate. The vitality of algal cell from each group was observed by FACs, and the results are not different to that of cell growth.

1. INTRODUCTION

The toxicity of treated ballastwater comes from both total residual oxidant (TRO) and their disinfection byproducts (DBPs). The biodegradation of organic pollutants in water also produces soluble microbial products, and it may become new DBPs (Liu and Li, 2010). As an active substance of ballastwater treatment, TRO is easily neutralized with sodiumthiosulfate, while DBPs are not. The majority of DBPs in treated ballast water are formed from the reaction of oxydants with organic compounds in water (Huang and Yeh, 1997). It is reported that some test facilities add starch to satisfy the test condition under G8 at Land-based Test for IMO and/or Type approval. The addition of starch may lead some un-considered factors for risk assessment due to potential reaction with Active substance and/or over-growth of bacteria may lead to increase of carbon content as an organic matter to potential reactants for the production DBPs in test water.

It is recommended by IMO that TRO in discharged ballastwater should be lower than 0.2mg/L as Cl_2 by IMO. But some algal toxicities of neutralized treated water has been reported, even the TRO in discharged treated ballastwater is neutralized to 0.2mg/L with neutralizer. It is not clarified that those algal toxicity comes from TRO, DBPs, or the combined effects from mixture of chemicals in neutralized water (Krasner, 1989). Also, it is important view about algal toxicity from chemicals at fresh water with similar conditions like marine water. There can be potential over usage of neutralizer to certain aquatic system such as harbor. It is needed to elucidate the properties of neutralizer in

aquatic ecosystem if the neutralizer has the adverse effect on algal growth at impact zone of chemical application. The effect of neutralizer on aquatic environment may different depending water quality, such as salinity, organic contents etc.

The impact of chemicals in both environment of fresh water and marine water supposed to be evaluated for their safe use (Zhang, 2009). The neutralizer, sodium thiosulfate to reduce the oxidant in treated water with oxidation radicals such as TRO was studied in connection with algal growth of *Selenastrum capricornutum* for fresh water and *Isochrysis galbana* for marine water. The effects of neutralizer in both waters have been compared with the addition of starch as organic content in water.

2. MATERIALS AND METHODS

Isochrysis galbana from UTEX was cultured in the medium (F/2 medium 33psu of salinity), and *Selenastrum capricornutum* from UTEX was cultured in the medium (Bristol 0.1psu of salinity), with or without treatment combinations of NaOCl, starch or neutralizer.

Each algal culture was treated with or without NaOCl for final concentration of 10mg/L TRO as Cl_2 after addition with or without 10mg/L of Starch as the additional organic compounds. Each species of algae was inoculated to the treated culture with or without neutralization of 1.0mg/L of sodium thiosulfate. TRO was neutralized by addition of 20ul sodium thiosulfate for each flask as designed for treated and neutralized group.

The inoculation concentration for *I. galbana* and *S. capricornutum* were 10.0 and 7.7 $x10^4$ cells/mL, respectively, to the culture at 0, 1 or 5 days after setting of treatment combination. The algal growth was measured from each algal culture with variables of cell number, viability by FDA with FACs at every 24h after inoculation.

3. RESULTS

3.1 Comparison of Specific Growth Rate of *Selenastrum capricornutum* and *Isochrysis galbana* with additional Starch.

The mean Specific Growth Rates (SGR) of both salt water species, *Isochrysis galbana* and fresh water species, *Selenastrum capricornutum* are not significantly different whether the algal culture was added with 10mg/L of starch or not. But when the SGRs of both species at 72-96hrs and 96-144hrs were compared between cultures with or without starch, there was significant difference between the means of each species (Table 1) between them. The mean SGR of early culture (48-72h) of *S. capricornutum* is higher than that of *I. galbana* but lower at late culture (96-144h) at both cultures with or without starch. The other comparison does not show any significant differences between them. The statistical differences are not observed in both cultures with or without starch in TR0 of both culture of starch or salinity.

Table 1. Comparison of Specific Growth Rates (SGRs) in Isochrysis galbana (salt water) and Selenastrum capricornutum (freshwater) with starch in the culture

Starch in Algal Culture(mg/L)	Duration (h)	SGR (Mean+SD) /species		t	p
		Isochrysis galbana	*Selenastrum capricornutum*		
0	48-72	1.160+0.242	1.650+0.301	-3.82	0.001
	96-144	0.762+0.064	0.536+0.210	2.58	0.020
10	48-72	1.115+0.293	1.704+0.294	-4.27	0.001
	96-144	0.749+0.162	0.446+0.106	4.70	0.001

3.2 The effect of TRO, Starch and/or Neutralizer on Fresh Water

The mean cell numbers among the group of the culture with starch and TRO (ST), Starch only (CS), TRO only (CT) and control without starch and TRO (CC) after 72h inoculation were significantly different, when the culture of *Selenastrum capricornutum* was inoculated at day 0 of TRO Treatment (Table 2). The mean cell number of algal culture with starch and TRO (ST) was decreased along with the extension of culture time. The potential toxic effect of Disinfection Byproduct after reaction of TRO with starch or organic compounds after degradation of algal cell was observed from the culture. The algal growth with starch and neutralizer was not decreased. The growth of algae with TRO only after neutralization was recovered 144h after treatment.

Table 2. Effect of neutralizer and/or starch on the growth of Selenastrum capricornutum in mean cell number with (SD) (10E4/mL) inoculated at Day 0 of TRO treatment with 10 mg/L as Cl_2 (CC, without starch and TRO; CT, TRO only; CS, Starch only; ST, starch and TRO; HAI, hours after inoculation).

Group/HAI	24	48	72	96	144
ST	5.3(1.5)^{a*}	10.3(2.5)^{a}	88.3(17.9)^{a}	293.0(57.4)^{b}	727.3(60.1)^{c}
SC	5.0(1.732)^{a}	10.3(1.1)^{a}	91.3(6.4)^{a}	390.6(45.0)^{a}	1196.6(94.6)^{a}
CT	4.6(1.5)^{a}	6.0(2.6)^{a}	36.6(8.6)^{b}	194.6(20.5)^{c}	887.6(96.6) ^{b}
CC	4.6(1.5)^{a}	10.0(2.6)^{a}	82.0(13.5)^{a}	426.3(16.5)^{a}	912.3(9.4)^{b}

*Means with the same letter in same column are not significantly different

When the algae was inoculated 1 day after TRO treatment, where the initial TRO was 4.0 mg/L, the mean cell numbers among the group of culture after 48h inoculation were significantly different (Table 3). The inhibitory effect of TRO (CT) and TRO with Starch (ST) after neutralization on algal growth has been observed, like the group comparison with initial TRO treatment of 10.0 mg/L at day 0. But the growth of algae in the culture of TRO with starch was recovered at 144h after inoculation.

Table 3. Effect of neutralizer and/or starch on the growth of Selenastrum capricornutum in mean cell number with (SD) (10E4/mL) inoculated at Day 1 of TRO treatment with 10 mg/L as Cl_2 (CC, without starch and TRO; CT, TRO only; CS, Starch only; ST, starch and TRO; HAI, hours after inoculation).

Group/HAIr	24	48	72	96	144
ST	6.0(3.4)^{a*}	12.6(7.3)^{b}	43.0(30.0)^{c}	183.3(35.7)^{b}	1815.6(177.9)^{b}
SC	7.6(0.5)^{a}	49.0(8.0)^{a}	226.3(45.7)^{b}	539.3(54.0)^{a}	1919.3(22.3)^{ab}
CT	5.0(1.0)^{a}	7.6(3.7)^{b}	15.0(19.9)^{c}	95.6(74.9)^{b}	1168.3(328.8)^{c}
CC	6.3(1.5)^{a}	53.0(7.8)^{a}	418.3(29.9)^{a}	621.3(29.6)^{a}	2227.0(104.9)^{a}

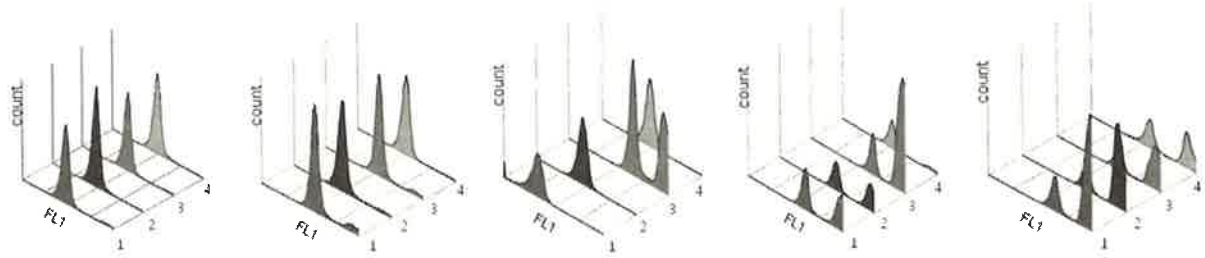
*Means with the same letter in same column are not significantly different

When the algae was inoculated 5 days after TRO treatment, where the initial TRO was 2.0 mg/L, the mean cell numbers among the group of culture after 24h inoculation were significantly different (Table 4 and Fig. 1). The inhibitory effect of TRO (CT) and TRO with Starch (ST) after neutralization on algal growth is quite similar as the results of 4.0mg/L TRO, but the difference in cell number among group was observer at 24h after inoculation.

Table 4. Effect of neutralizer and/or starch on the growth of Selenastrum capricornutum in mean cell number with (SD) (10E4/mL) inoculated at Day 5 of TRO treatment with 10 mg/L as Cl_2 (CC, without starch and TRO; CT, TRO only; CS, Starch only; ST, starch and TRO; HAI, hours after inoculation).

Group/HAI	24	48	72	96	144
ST	6.6(1.5)^{bc}	17.3(2.0)^{c}	50.3(15.9)^{b}	194.6(22.0)^{c}	1068.3(141.0)^{bc}
SC	12.0(2.6)^{a}	3.3(2.3)^{a}	97.0(11.1)^{a}	572.3(64.5)^{b}	1356.6(81.2)^{b}
CT	3.3(1.1)^{c}	8.0(2.0)^{d}	39.6(8.0)^{b}	183.0(84.5)^{c}	922.6(307.0)^{c}
CC	7.6(2.5)^{b}	48.3(5.7)^{b}	342.6(66.1)^{a}	741.3(70.9)^{a}	1755.3(101.6)^{a}

*Means with the same letter in same column are not significantly different



24(HAI) 48(HAI) 72(HAI) 96(HAI) 144(HAI)

Figure 1. Comparison of the effect of neutralizer with starch in the culture (10mg/L) on growth of Selenastrum capricornutum inoculated at Day 5 of TRO treatment with 10 mg/L as Cl_2. (1, CC-without starch and TRO; 2, CT-TRO only; 3, CS-Starch only; 4, ST-starch and TRO; HAI, hours after inoculation)

3.3 The effect of TRO, Starch and/or Neutralizer on Salt Water

There was no difference in mean cell numbers among the group of the culture with starch and TRO (ST), Starch only (CS), TRO only (CT) and control without starch and TRO (CC) until 96h of growth, when *Isochrysis galbana* was inoculated at day 0 of TRO Treatment (Table 5). Both mean cell number of algal culture with starch and TRO (ST) and TRO only (CT) were decreased along with the extension of culture time to 144h, while that of algae without TRO was increased.

Table 5. Effect of neutralizer and/or starch on the growth of Isochrysis galbana in mean cell number with (SD) (10E4/mL) inoculated at Day 0 of TRO treatment with 10 mg/L as Cl_2 (CC, without starch and TRO; CT, TRO only; CS, Starch only; ST, starch and TRO; HAI, hours after inoculation).

Group/HAI	24	48	72	96	144
ST	6.3 (1.1)^{ab}	9.0(3.6)^{a}	36.3(3.7)^{a}	53.0(5.2)^{b}	59.6(2.3)^{c}
SC	8.3(0.5)^{a}	9.0(6.2)^{a}	33.3(2.3)^{a}	109.6(12.7)^{a}	403.6(2.0)^{a}
CT	1.0(1.7)^{c}	7.0(1.0)^{a}	33.0(7.0)^{a}	101.0(14.0)^{a}	74.3(11.5)^{c}
CC	5.3(1.5)^{b}	7.0(1.0)^{a}	27.3(3.5)^{a}	72.3(6.6)^{b}	348.6(13.6)^{b}

*Means with the same letter in same column are not significantly different

When the algae was inoculated 1 day after TRO treatment, where the initial TRO was 4.0 mg/L, the mean cell numbers among the group were significantly different through all duration of culture (Table 6). The inhibitory effect of TRO (CT) and TRO with Starch (ST) after neutralization on algal growth has been observed, like the group comparison with initial TRO treatment of 10.0 mg/L at day 0.

Table 6. Effect of neutralizer and/or starch on the growth of Isochrysis galbana in mean cell number with (SD) (10E4/mL) inoculated at Day 1 of TRO treatment with 10 mg/L as Cl_2 (CC, without starch and TRO; CT, TRO only; CS, Starch only; ST, starch and TRO; HAI, hours after inoculation).

Group/HAI	24	48	72	96	144
ST	6.0(1.7)^{b}	10.0(1.7)^{c}	33.0(2.6)^{c}	51.3(3.0)^{b}	112.6(20.5)^{c}
SC	6.0(2.0)^{b}	25.0(4.3)^{a}	59.3(8.1)^{ab}	206.0(16.7)^{a}	505.6(45.9)^{b}
CT	4.3(1.5)^{b}	19.0(3.0)^{b}	42.0(19.4)^{bc}	63.6(22.0)^{b}	85.6(10.7)^{c}
CC	10.3(1.5)^{a}	24.3(0.5)^{a}	73.3(8.5)^{a}	182.3(21.5)^{a}	651.6(29.2)^{a}

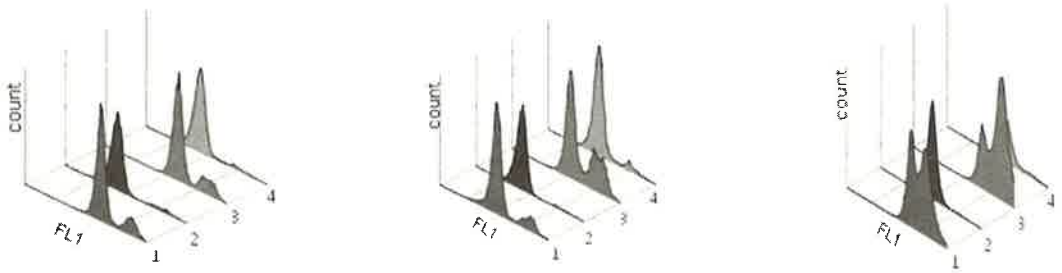
*Means with the same letter in same column are not significantly different

When the algae was inoculated 5 days after TRO treatment, where the initial TRO was 2.0 mg/L, the mean cell numbers among the group of culture after 72h of inoculation were significantly different (Table 7 and Fig. 2). The inhibitory effect of TRO (CT) and TRO with Starch (ST) after neutralization on algal growth is quite similar as the results of 4.0mg/L TRO of Freshwater algal culture.

Table 7. Effect of neutralizer and/or starch on the growth of Isochrysis galbana in mean cell number with (SD) (10E4/mL) inoculated at Day 5 of TRO treatment with 10 mg/L as Cl_2 (CC, without starch and TRO; CT, TRO only; CS, Starch only; ST, starch and TRO; HAI, hours after inoculation)

Group/HAI	24	48	72	96	144
ST	5.3(2.0)^{a}	18.3(3.7)^{a}	22.3(1.5)^{b}	30.6(6.6)^{c}	49.0 (1.0)^{c}
SC	11.6(2.0)^{a}	25.0(2.0)^{a}	68.0(8.8)^{a}	158.6(6.6)^{a}	598.6(40.0)^{a}
CT	7.3(4.1)^{a}	16.6(3.7)^{a}	21.3(1.5)^{b}	30.6(8.1)^{c}	46.3(1.5)^{c}
CC	5.6(5.5)^{a}	24.6(7.7)^{a}	60.0(3.0)^{a}	100.0(24.9)^{b}	444.0(59.7)^{b}

*Means with the same letter in same column are not significantly different



72(HAI) 96(HAI) 144(HAI)

Figure 2. Comparison of cellular fraction among treated group of Isochrysis galbana with time by FACs. The algae were inoculated to the culture after neutralization with sodiumthiosulfate at Day 5 of TRO treatment with 10 mg/L as Cl_2 (1, CC-without starch and TRO; 2, CT-TRO only; 3, CS-Starch only; 4, ST-starch and TRO; HAI, hours after inoculation)

4. Conclusion and Discussion

The sensitivity of salt water species, *Isochrysis galbana* and fresh water species, *Selenastrum capricornutum* against the combination of starch with TRO after neutralization is different, while that of both algae without starch, TRO and neutralizer was not. The SGR of early culture of *S. capricornutum* is higher than that of *I. galbana* but lower at late culture at both cultures with or without starch. The other comparison does not show any significant differences between them.

The potential toxic effect of Disinfection Byproducts after reaction of TRO with starch or organic compounds after degradation of algal cell was observed at both cultures of freshwater and salt water. When the algal culture with additional starch was neutralized just after treatment of 10 mg/L TRO as Cl2, the adverse effect on the growth of *S. capricornutum* appeared at 96h after inoculation, while that of *I. galbana* was appeared at 144h after inoculation. When the algae was inoculated to the culture with starch and neutralized after treatment of 10 mg/L TRO as Cl2 at 1 to 5 days after treatment, *S. capricornutum* appeared recover the growth at 96h after inoculation, while that of *I. galbana* was not to 144h after inoculation. *S. capricornutum* is not too sensitive on algal growth to the addition of starch while that of *I. galbana* is sensitive to algal growth when the TRO was neutralized with sodiumthiosulfate.

It is considered that there is no significant difference of SGR between *S. capricornutum* and *I. galbana* in control with given media with inoculation concentration. However the response sensitivity of two species against potential DBPs from starch and other organic matter from organisms, which comes from the reaction with TRO, is different when the testing algae was cultured longer than 96h. It is not recommended that use starch as an additive of artificial organic content to satisfy water quality condition of certain guidelines include G8 testing of BWMC.

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Keywords: *Algae, Freshwater, Salt Water, Selenastrum capricornutum, Isochrysis galbana, Neutralizer, TRO, DBPs, Ballast Water Discharge.*

session A2

Methods, Sampling & Analysis

Comparison of ballast management options on a vessel with uptake in freshwater – ballast water exchange in combination with and without a ballast water management system

Stephan Gollasch | GoConsult

Mining Knowledge from Testing Treatment Systems: Understanding of Plankton Dynamics During Ballast Water Tests

Matthieu Bernard Duchemin | DHI Water & Environment, Ballast Water Technology Innovation Centre

Special Emphasis on the Need for Monitoring of Dinoflagellate Cysts in Treated Ballast Water and Remaining Sediments

Hyunjin Cho | International Maritime Organization

A Monitoring System for the Performance of Ballast Water Management Systems

Frank Stuer-Lauridsen | LITEHAUZ Maritime Environmental Consultancy

Comparison of ballast management options on a vessel with uptake in freshwater - ballast water exchange in combination with and without a ballast water management system

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The International Ballast Management Convention includes provisions for two ballast water management options: ballast water exchange (BWE) and ballast water management systems (BWMS). While BWMS are expected to remove or exterminate most taxa from ballast water, BWE is particularly protective for freshwater ports by introducing a salinity barrier that reduces survival of freshwater taxa. As a result, a combination strategy using both BWE and BWMS might provide best available protection for freshwater ports. The main objective of this study is to evaluate the efficacy of the combined strategy through shipboard trials with freshwater ballast. Four treatment scenarios were selected for the test: 1) control (no treatment), this tank was filled in the freshwater Port of Hamburg; 2) BWE alone, this tank was filled in Hamburg, and exchanged in the Bay of Biscay >50 nautical miles from nearest shore in waters >200 metres depth; 3) BWMS alone; this tank was filled and treated on uptake using filtration and electrochlorination in Hamburg; and 4) BWE plus BWMS, this tank was filled and treated on uptake in Hamburg, and exchanged in the Bay of Biscay, with the incoming exchanged water again treated. All four tanks were discharged before arriving in Algeciras (Spain). Preliminary results from the first voyage indicate plankton (>50µm in minimum dimension) density decreased in all cases: BWMS alone (99.8%), BWE+BWTS (99.3%), control (90.3%) and BWE alone (89.8%). Additional work is underway to determine if taxa present after BWE are expected to have low survival if introduced to a freshwater port.

Keywords: *ballast water exchange, ballast water management,ballast water treatment, freshwater*

Mining Knowledge from Testing Treatment Systems: Understanding of Plankton Dynamics During Ballast Water Tests

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Experiments were carried out to understand the effects of ballast water tanks environmental conditions (several days of holding time in the dark) on the mortality of plankton present during the tests. The mortality rates of some key reported taxa were calculated assuming an exponential mortality and ranged from 0.75 to 2.09 %.d-1 with the highest mortality rates observed for Artemia and the lowest for Rotifers. The mortality rates of the different taxa were not driven by the size class from which they originated. Additionally, laboratory experiments to determine whether the use of Artemia salina as a surrogate organism was a driving force for the mortality of plankton (grazing mediated) during tests were also carried. The survival rate of plankton when A. salina was added was only 4% lower than in the controls and therefore other causes than grazing by A. salina should be considered to understand the mortality rates observed during the tests.

Finally, because the assessment of resting stages viability is difficult, a last experiment on the hatching success of A. salina cysts during a 5 days test cycle was carried out. After 48 hours of hatching under optimal conditions, non-hatched cysts were capable of surviving 5 days holding times but only 4.5 % of the remaining cysts could hatch under optimal conditions over a period of one month after the tests ended.

Keywords: *Cysts, Natural decay, Testing methods*

Special Emphasis on the Need for Monitoring of Dinoflagellate Cysts in Treated Ballast Water and Remaining Sediments

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The increasing interest in the harmful aquatic organisms and pathogens carried by the ships' ballast water is reflected in the adoption of the BWM Convention by the member states of the IMO in February 2004. Since then, the IMO has been developing a number of Guidelines for uniform implementation of the Convention. Nevertheless, one of the most controversial issues still is how the ballast water is sampled and analysed without any unnecessary detention of the ships in ports. Regulation D-2 of the BWM Convention stipulates ballast water performance standard where viable organisms of certain minimum dimension should not be discharged into the sea in concentrations above certain limit. The current practices of ballast water sampling include the use of plankton nets and/or equipment that uses fluorescence for size-fraction of viable organisms. However many microorganisms have spines or flagella and sometimes they form chains, which means it would be difficult to enumerate organisms of certain target-sizes as per D-2 standard. Detection and enumeration of dinoflagellate cysts is another challenge in ballast water management, although dinoflgellate cysts was one of the main concerns that brought ballast water issues to the attention of international community. The risk of dinoflagellate cysts in the ships' ballast water, being discharged into uncontaminated ports leading to potential red-tide algal blooms by the successful germination of the cysts is high, however there have been few discussions on how to detect dinoflagellate cysts in the ballast water for compliance with D-2. It is important that the treated ballast water is monitored at regular intervals for viable dinoflagellate cysts in addition to the checking of proper operation of the Ballast Water Management System (BWMS) and record books by the Port State Control Officers (PSCOs), while we need to strike a balance between the need for uninterrupted operation of ships in ports and to protect the marine environment from the invasive species.

Keywords: *ballast water and its sediment, detention of ship, dinoflagellate cysts, minimum dimension, monitoring*

Introduction

Recently, invasive species which are carried from place to place by maritime transportation have disturbed natural environment where there are no particular predators. A good example of such invasive species transfer (GEF-UNDP-IMO GloBallast Partnerships and IOI (2009)) is the introduction of European shore crab (*Carcinus maenas*) which is native to Europe and Northern Africa, and when introduced to the USA, Australia and South Africa, caused the decline of other crab and bivalve species. Another famous

example is the zebra mussel (*Dreissena polymorpha*), native to Europe but spreading rapidly throughout the waterways of North America, having been introduced to the US in ballast water (GEF-UNDP-IMO GloBallast Partnerships Programme and IUCN, 2010). In response, US Congress passed the Non-indigenous Aquatic Nuisance Prevention and Control Act of 1990 (USCG website, 2013).

The public concern about invasive species was initially associated with dinoflagellate cysts carried by ships' ballast water. In the Australian region, toxic dinoflagellate blooms were unknown until the late 1980s, until such blooms were caused by *Gymnodinium catenatum* in the ports of Hobart (Hallegraeff et al. 1988). Fossil cyst records of this species are absent from the whole Australian region, recent cyst beds are confined to Southeast Tasmania. 210Pb-dated sediment cores from the Hobart region unambiguously demonstrate its sudden appearance around 1972 coinciding with the commencement of bulk woodchip export from Southern Tasmania via Japanese cargo vessels (Hallegraeff, 1998). It was suggested a plausible scenario for their successful introduction and establishment in Australian water is 1) ballast water intake during seasonal plankton blooms and to a lesser extent via resuspended cysts in sediments from Japanese or Korean ports; 2) survival of resistant resting cysts during the ballasting process, the voyage in a dark ballast tank, and subsequent ballast water discharge; 3) successful germination of cysts, sustained growth and reproduction of plankton cells in an Australian port; and 4) further spreading via coastal currents or domestic shipping, culminating under suitable environmental conditions in harmful algal blooms impacting on aquaculture operations.

Dinoflagellate cysts act like fine particles (Dale, 1983), so they could accumulate in the sediment of ballast tanks and subsequently get discharge to a port suspended in the ballast water. Aquatic organisms can also settle out of the ballast water and can continue to exist within the sediment, which can survive for long periods after the discharge of the water. They are therefore ideal candidates for being transported from their natural habitat and discharged in another port or area where they may cause damage to the environment, human health, property and resources (G 12, IMO Publication, 2009).

Although dinoflagellate cysts are one of the main concerns that brought ballast water issues to the attention of international community, there have been few discussions on how to monitor dinoflagellate cysts in the ballast water and its sediments. This study would like to emphasize the importance of dinoflagellate cysts in the ballast water management and suggest how to inspect them without any unnecessary detention of ships.

What kinds of organisms are controlled in compliance with BWMC?

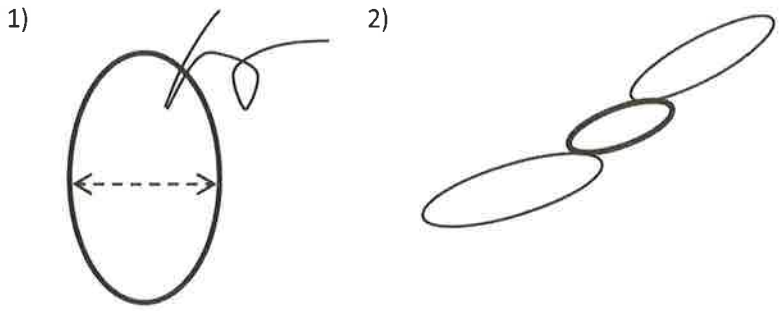
Regulation D-2 of the BWMC (IMO Publication, 2009) stipulates the ballast water performance standard where viable organisms of a certain minimum dimension should not be discharged into the sea in concentrations above a certain limit. Table 1 summarizes the standard, not including indicator microbes as a human health standard.

Table 1. Concentration of size-fractionated viable organisms required for ballast water discharge (| indicates equal to the size, while → is greater than the size).

Size (minimum dimension)	10um →	50um →
Concentration	< 10 viable organisms/ml	< 10 viable organisms/㎥

Guidelines for ballast water sampling (G2) of BWMC define minimum dimension as follows: the minimum dimension of an organism is based upon the dimensions of that organism's body ignoring e.g., the

size of spines, flagella, or antenna. The minimum dimension should therefore be the smallest part of the body, i.e. the smallest dimension between the main body surfaces of an individual when looked at from all perspectives (IMO Publication, 2009). To measure the minimum dimension, G2 gives two examples of spherical shaped organisms and colony forming species, as illustrated in Figure 1.



Certain issues related to monitoring the standards mentioned in Regulation D-2 of BWMC

1. Enumeration

G2 recommends that an indicative analysis of ballast water discharge may be undertaken to establish whether a ship is potentially compliant or non-compliant, prior to testing for compliance with the D-2 standard. Several studies are using plankton nets and/or flow cytometry for size fraction of viable organisms in ballast water.

However certain issues are expected in sampling ballast water with plankton nets. Traditional sampling devices (e.g., plankton nets) are not well suited for use aboard ships, as nets are unwieldy and difficult to manage in the available small spaces, and the water filtered through plankton nets must be managed in the limited spaces of the commercial shipboard environment (First *et al.* 2011). It is also difficult to do any repair or welding job in the ballast tanks due to the small heights of such nets that are approximately in the range of 0.5 to 1.5 meters (Bakalar and Sc. 2011). There are several efforts to develop ballast water analysis device and flow cytometer is one of them; in flow cytometric analysis the cells pass a laser beam one by one at high speed and their individual light scattering and fluorescence properties are recorded to form an optical fingerprint for each cell (Bakalar and Sc. 2011).

However, it is very hard to enumerate the number of viable organisms with those tools because the guidelines require select the smallest part of the body and/or unit to measure the minimum dimension, as shown in Table 1 and Figure 1. For example, a viable organism with minimum dimension less than 50um has high possibility to be size fractionated in greater numbers than if it has spherical shape or spines. Therefore, only microscope must be the ultimate answer for counting the size-fractionated proper cells.

A harmonised sampling approach is also essential to provide consistent compliance tests. Sampling over the entire time of the ballast water discharge would be quite difficult, especially if long sampling times are required over several days or during night (Gollasch and David 2011). They also reported the sequential trials showed different numbers of organisms in the samples taken in the beginning, middle and end of the pumping event, but no consistent trend could be identified.

2. Viability

Not only enumeration of organisms, but also their viability is a crucial point in compliance with D-2 regulation. Viability of an organism can be determined through live/dead judgement by appropriate methods including, but not limited to: morphological change, mobility, staining using vital dyes or molecular techniques (G8). It is very difficult to determine if non-motile cells such as dinoflagellate cysts are live/dead without incubation of the cysts, which might be a time consuming task.
Most dinoflagellate cysts are morphologically very different from the motile stages, therefore, their natural affinities remained unknown until the early 1960s, when palaeontologists discovered living equivalents and showed these to be resting cysts of dinoflagellates (Cho, 2000). Non-motile dinoflagellate cysts have thick walls to be preserved in sediments very long time, whose walls are as thick as used in fossil studies, which makes it very difficult to distinguish them viable/dead at a glance.
During inspection by Port State Control officers (PSCOs), incubation for determining viability of cysts might result in long detention of ships. The BWMC, at the same time, is very specific on undue delay of the ships and stipulates that no undue delay would be caused by the inspection.

Why dinoflagellate cysts are important in ballast water management?

Ecological functions of dinoflagellate cyst formation are considered as follows (Dale 1983): (1) short or long term survival overwinter in lower temperature than the motile cells tolerate, (2) seed bed to ensure a new population of motile stages during favourable intervals, (3) dispersal agents to extend a species range of new territory transported by current, and (4) nuclear replenishment through meiosis, etc.
Previous researches dealt with dinoflagellate cysts as means of transfer of toxic vegetative cells from place to place. Lacasse *et al.* (2013) suggests that some of the cysts of *Alexandrium tamarense* species complex found in the port of Halifax, Nova Scotia, Canada, were introduced through ballast water and sediments, and the highest concentrations of *Alexandrium* cysts were found at a station located near an important ships terminal. Scholin *et al.* (1995) analysed forty-eight cultures from North America, Western Europe, Japan, Australia and Thailand. The ballast water samples showed that viable toxigenic *Alexandrium* cysts were dispersing as a direct result of human activity, and they discovered the occurrence of temperate Asian ribotype *A. catenella* cysts in ballast water from southern Japan, which were identical to those isolated from established populations of this species in both Japan and Australia (Scholin *et al.* 1995). Kotani *et al.* (2006) warned potential events of an invasion by toxic dinoflagellates into Tokyo Bay via ballast water or oceanic water although currently the possibility of paralytic shellfish poisoning (PSP) caused by a bloom of *A. tamarense* and/or *A. catenella* initiated from benthic cysts is considered to be low in Tokyo Bay. Welschmeyer and Maurer (2011) also considered the discharge of ships' ballast water as the primary vector in the spread of aquatic invasive species.
It is said that motile, photosynthetic dinoflagellate cells usually do not survive long voyages in ballast tanks where massive phytoplankton mortalities are incurred one to three days after ballasting (Hallefraeff, 1998). Non-motile cells of dinoflatellates called as dinoflagellate cysts, however, can survive longer in adverse conditions such as in ballast tanks during the voyage and then germinate when they meet good environmental conditions resulting in algal bloom again. Such species, once present in sediments, are available to colonize the water column when the condition becomes suitable for their excystment and growth (Lewis et al. 1999). The report suggested that for many coastal species survival times of at least two years are common for both diatoms and dinoflagellates. For example, there are some limited records that show that marine dinoflagellates can survive between one and five years. It is

also reported, however, after nine years some dinoflagellate cysts could be still cultured.
Genovesi et al (2009) concluded that the short-term exposure to light is an environmental condition that allows for massive synchronized cyst germination, which means the resting cysts have the capability of responding quickly to favourable condition for germination, including release from anoxic sediments. Although cyst-forming dinoflagellates occupy a small proportion of the total number of dinoflagellates, they raise a huge interest because some of the harmful and toxic dinoflagellates produce cysts (Cho, 2000). PSP is a potentially fatal disorder caused by ingesting shellfish that contain high levels of neurotoxins called the saxitoxins, and dinoflagellates of the genus *Alexandrium* are the most numerous and widespread saxitoxin producers and are responsible for PSP blooms (Lilly et al. 2007). Therefore, some ports may consider that it would be important to inspect viable cysts in the discharged ballast water from ships, although no specific requirements are made in the BWMC standards to monitor such cysts.

Dinoflagellate cysts from a monitoring point of view

The International Convention on the Control and Management of Ship's Ballast Water and Sediments was adopted by IMO member States in February 2004, and is commonly known as Ballast Water Management Convention (BWMC) (GEF-UNDP-IMO GloBallast Partnerships Programmes and GESAMP IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2011). The BWMC shall enter into force twelve months after ratification by not less than thirty States, representing not less than thirty-five per cent of the gross tonnage of the world's merchant shipping. The Convention is about to enter into force because the entry into force criteria are almost met with the ratification of thirty eight States representing 30.38% of the world merchant tonnage (IMO, 2013).
The risk of dinoflagellate cysts in the ships' ballast water, being discharged into uncontaminated ports that may cause potential red-tide algal blooms by the successful germination of the cysts is high, however, there have been few discussions on how to detect dinoflagellate cysts in the ballast water and its sediments for compliance with D-2. It is important that the treated ballast water is monitored at regular intervals for viable dinoflagellate cysts in addition to the checking of proper operation of the BWMS and record books by the Port State Control Officers (PSCOs), while we need to strike a balance between the need for uninterrupted operation of ships in ports and to protect the port environment from the invasive species.
The monitoring could enumerate several difficult issues in terms of inspection of the ballast water, such as size-fraction, counting, viability, dinoflagellate cysts, etc. To avoid unnecessary detention of the ships for inspection of the ballast water and sediments, as well as to protect environment in ports from the invasive species by shipping, PSCOs are expected to check appropriate operation of the BWMS and record books, and check BWMS Certification.
One of the issues that will be faced by the PSCOs will be the certainty by which the treatment technology would be able to remove or kill the dinoflagellate cysts, as the technology type approval process mostly rely on seze-based monitoring during the G8 testing. This increases the risk that potentially harmful dinoflagellate cysts might get discharged into the waters if the testing protocol did not specifically consider monitoring for dinoflagellate cysts. It is also very difficult to test treatment technologies for their efficacy in killing or removing these cysts as it is difficult to generate large number of these cysts in experimental conditions. Although some studies have been undertaken which produced the cyst-on-demand protocols (Matheickal et al. 2003), their use in the current testing protocols is almost absent.

Furthermore regular monitoring of remaining sediment in ballast tank is also recommendable. Regular monitoring (e.g. regularly scheduled drydocking) of the remaining sediments for dinoflagellate cysts can give us good information how to manage ballast water and the sediments. The higher concentration of dinoflagellate cysts in the sediments in the ballast tanks may indicate the proportionally higher risk of invasion of harmful dinoflagellates to a port.

Acknowledgements

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A Monitoring System for the Performance of Ballast Water Management Systems

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The system for monitoring a ballast water management system (BWMS) measures filter performance and the biological efficacy of the disinfection unit. It monitors the BWMS efficiency in three sampling locations: before and after pre-treatment, and after disinfection. The system monitors particle density to assess filter functionality and the viability of organisms (microalgae), using it as an indicator of disinfection performance. Both parameters are detected with optical techniques (laser scattering and fluorescence) with a very fast response in real time. Data from tests with the three main disinfection technologies chlorination, ozone and UV treatment are presented. For the latter we also present data on viability and holding time - the die-away relation.

The major advantage of the monitoring system is that the indicative information on the performance of a BWMS is provided directly for a ship's master already on uptake of ballast water in the port, giving her/him continuous BWMS supervision and flexibility of instant reaction, for example:

- to abort a flawed operation, discharge the poorly or untreated ballast water and redo the ballasting with a fully operational (repaired) BWMS
- to continue with the noncompliant ballast water and perform a mid-ocean ballast water exchange (D-1) followed by either:
  - a ballasting with a BWMS repaired en route or
  - seeking a D-2 emergency dispensation from the Port Authority at the destination

Regardless of his choice, the monitoring system can provide with indications that ballast water is or is not in "gross noncompliance" with the Convention. Also, an electronic log will be available on the ballasting operation and the BWMS performance. The electronic BWMS performance log will also allow the crew to supply Port Authorities with the necessary documentation during inspections, in order to prove that there is no basis for a detailed inspection with time consuming sampling and analysis of the ship's ballast water.

The full monitoring system will combine data from many ballasting operations of the individual BWMS in order to improve the performance of the monitoring system. The data collected by the system is stored and analysed in information management facility on-board the vessel. The data may also include additional monitored parameters such as temperature, salinity, vessel specific data, geoposition data and tidal information. The system is initially set to react at pre-set indicator points and provide an instant alarm for the operator of the system, when out of indicator range. The monitoring system can be remote accessed for software updates, parameters upload and adjustment of indicator settings.

Keywords: *ballast water, self-monitoring, indicative, compliance, enforcement*

session B1

Monitoring Tools, Test kit

TRO Monitoring of Active Substances: Challenges and Advancements in Technology

Dan Coker | HF scientific

PEB-BOX: Development of a Test Kit to Analyze the Performance of Treatment Systems on Board of Ships

Louis Peperzak | Royal Netherlands Institute for Sea Research/NIOZ

Molecular Approaches for Monitoring Marine Organisms in Ballast Water

Seungshic Yum | Korea Institute of Ocean Science and Technology

TRO Monitoring of Active Substances: Challenges and Advancements in Technology

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Many ballast water treatment systems use active substances that are oxidants to treat ballast water in order to inactivate potentially harmful invasive species and prevent them from being transported around the globe. There are several types of oxidative active substances currently being used including chlorine, chlorine dioxide, ozone, hydrogen peroxide, or a mixture of these. The ballast water being treated can be a complex matrix with many substances present. Therefore, most of the technologies available to measure active substance concentration report units of Total Residual Oxidant (TRO) as mg/L Cl_2 as there are likely to be a mixture of active substances present following treatment. It is important to utilize a technology that will measure any and all oxidants present to give an accurate concentration value. When these active substances are utilized, their concentrations must be increased to a level that sufficiently inactivates any viable organisms inside the ballast tanks. Some ballast water treatment systems dose up to 10-15 TRO as mg/L Cl_2. Following treatment, the ballast water must be decreased to a near- zero level prior to de-ballasting into a natural body of water to prevent damage to the environment. Current maximum TRO concentration during the de-ballasting process is 0.20 mg/L.

There are three types of continuous monitoring technologies that are commonly used to measure active substance concentration in water. They are oxidation reduction potential (ORP) sensors, amperometric probe sensors, and DPD colorimetric analyzers. These technologies have been available for many years, however were originally designed for municipal water applications. The ballast water treatment application demands that any system intended to accurately measure active substances in complex water matrices at sea must be extremely robust and be able to perform well in harsh marine environments. There are two primary objectives of this paper. The first is to describe the advantages and disadvantages of each technology type including interferences, response times upon start-up, measurement cycle times, typical measurement ranges, sediment, and fouling issues. The second is to provide information and data regarding recent advancements in DPD colorimetric analyzers to specifically address the IMO regulation and the needs of the shipping industry.

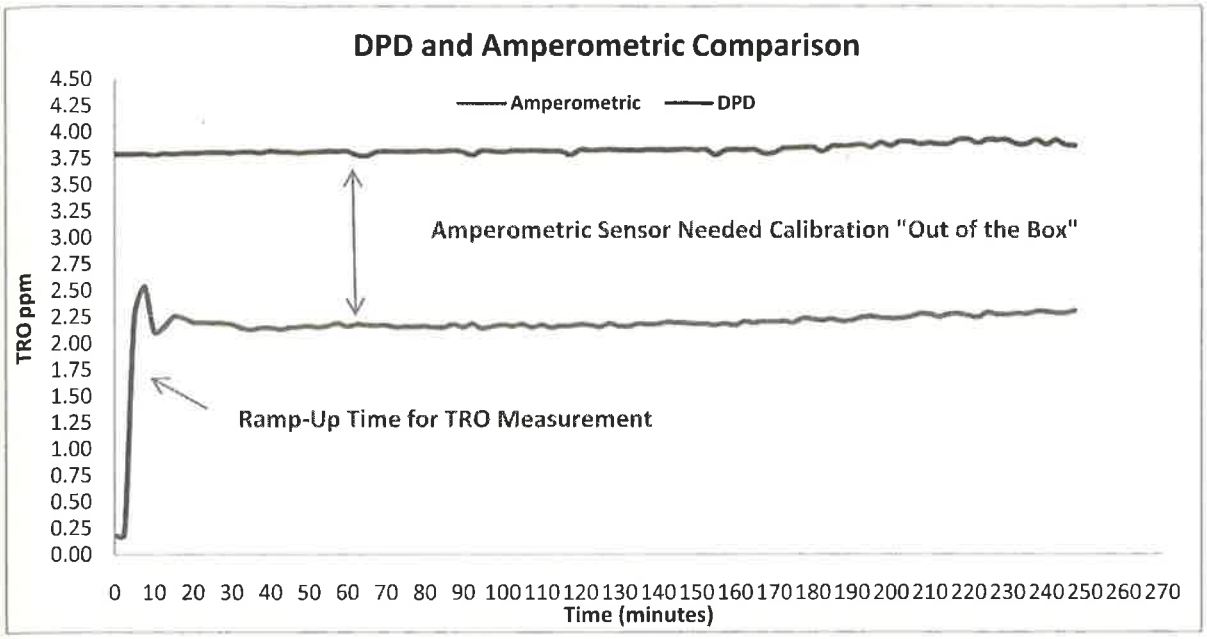
Oxidation reduction potential (ORP) sensors provide a qualitative, indirect method to measure TRO concentration typically measured in millivolt units which must be correlated to TRO mg/L as Cl_2 units. ORP sensors have been used successfully for many years in the swimming pool and spa industry where a consistent concentration of oxidant is desired and the water is of good quality with very low turbidity and color and has a stable pH level. ORP sensor measurements are flow rate, temperature, pH, and salinity dependent and must be re-correlated to TRO if any of these physical parameters change. Physical parameters for ballast water varies greatly depending on a ship's location therefore an ORP sensor likely needs frequent calibration. It is also important to ensure the technology used for TRO measurement has a low detection limit and is accurate at and below 0.20 mg/L which is the maximum concentration limit during de-ballasting. ORP sensors work better when a sufficient amount of oxidant

is present, and may suffer in accuracy at the low level. For these reasons, the next two technologies have been more popular for ballast water TRO monitoring.

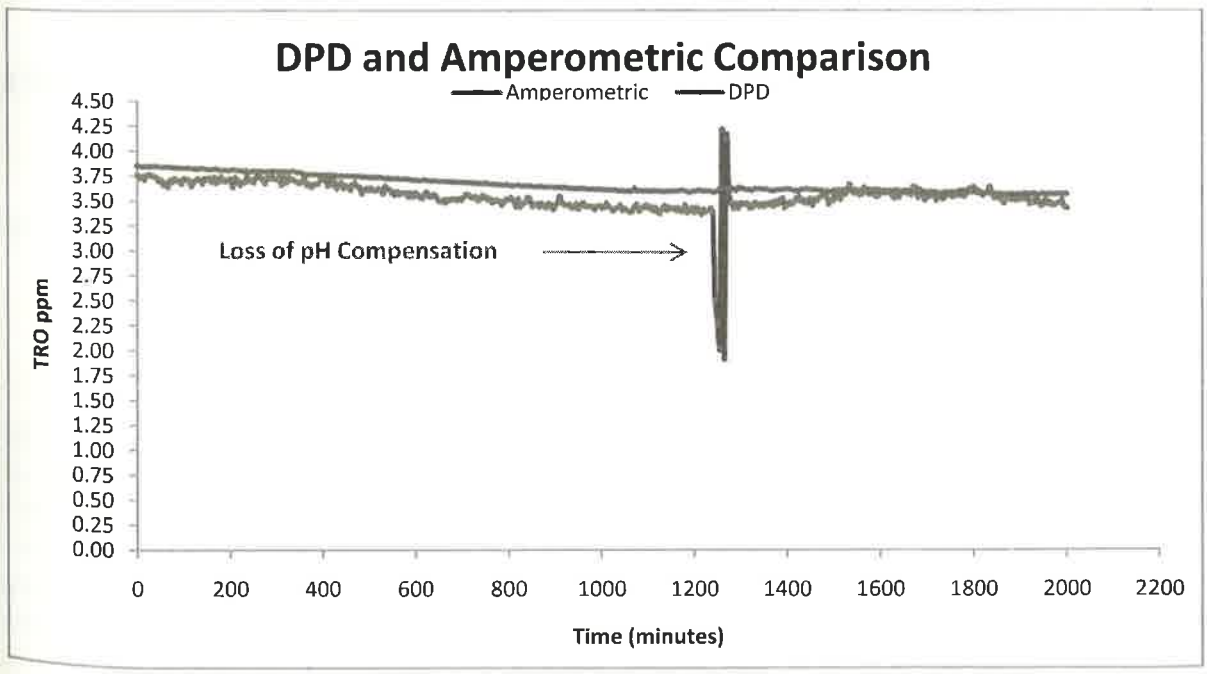
DPD colorimetric analyzers and amperometric sensors provide a direct measurement of TRO. Both technologies have been used for many years in the municipal drinking and wastewater industry for measurement of disinfectant concentration. Below is a table comparing the two technologies:

	DPD Colorimetric	Amperometric
Principle of Operation	Photo Absorbance Proportional to Oxidant Concentration	Electrical Current Proportional to Oxidant Concentration
Advantages	DPD reagent reacts colorimetrically with any and all oxidants Accuracy Independent of Sample pH Accuracy Independent of Temperature Accuracy Independent of Salinity Factory Calibrated Field Re-Calibration Possible, but Not Required Reagent Life 5 Years Prior to Installing Into Analyzer Reagent Life Extended Up To 1 Year When Installed (CLX-EX) Low Detection Limit (0.015 mg/L) Two Measurement Ranges Options: 0-10 mg/L or 0-15 mg/L Direct TRO Measurement (opposed to ORP Indirect) 100% of First Measurement Displayed in 60 seconds Analyzer Equipped with Corrosion Resistant Strainer	No Reagent Required Direct TRO Measurement (opposed to ORP Indirect)
Disadvantages	Standard CLX Requires Reagent (Indicator) Change Monthly	Sensor Must Be Calibrated On a Regular Basis Sensor Can Foul in Poor Quality Water Loss of Accuracy (Drift) Upon Change of pH Loss of Accuracy (Drift) Upon Change of Temperature Loss of Accuracy (Drift) With Improper Flow Difficult to Measure Low/Zero TRO Concentration T_{90} 30 to T_{90} 90 seconds Depending on Manufacturer

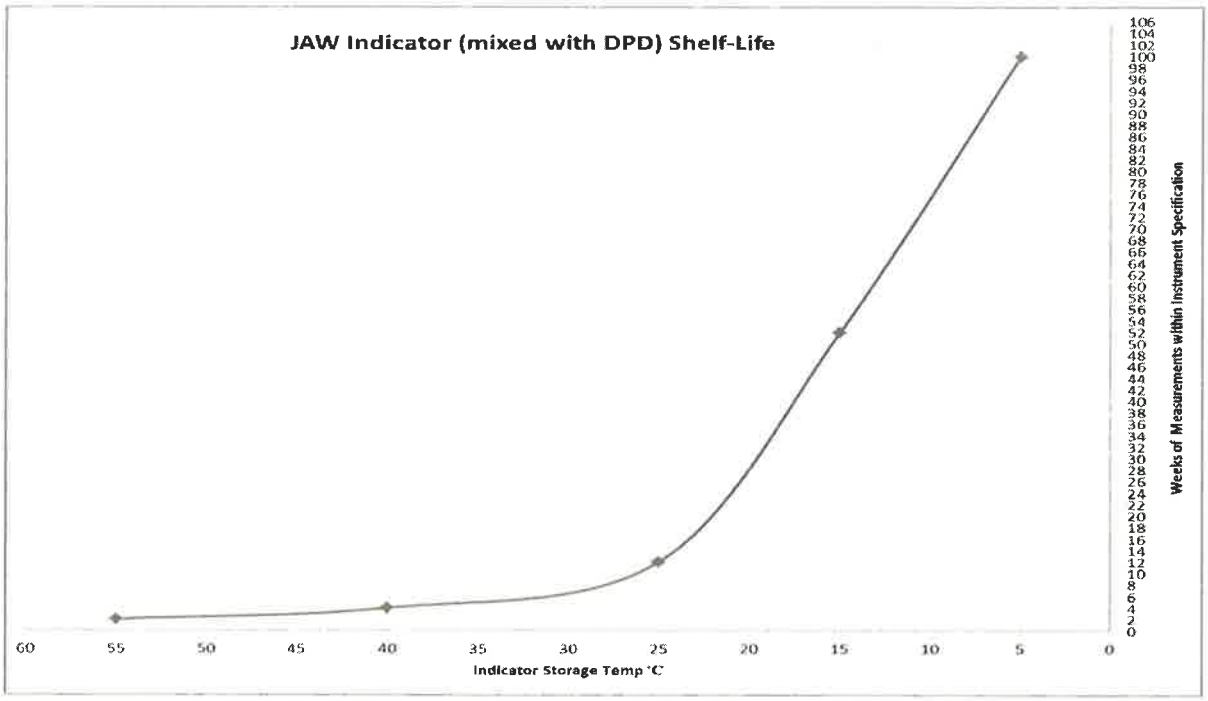
HF scientific manufactures DPD methodology based on-line analyzers and has tested an amperometric based sensor in a side-by-side comparison to the DPD colorimetric analyzer. For test purposes, the same sample water was pumped to each analyzer. The pH of the sample water was 8.1 and contained a mixture of free chlorine and chloramines as TRO.



The DPD analyzer measurement accuracy was confirmed by a laboratory bench method and it was determined that the amperometric sensor needed calibration for accuracy. There was also a 6-8 minute ramp-up time until consistent measurements were obtained using the amperometric sensor. The amperometric sensor was calibrated and the test was resumed using the same sample water. The amperometric sensor provided comparable results until there was a loss of pH compensation. Once restored, the system came back on-line and proved the importance of pH compensation regarding accuracy of TRO measurements. The DPD colorimetric analyzer provided consistent results throughout the test.



There have been several advancements in the DPD colorimetric on-line analyzers to address the marine ballast water application including expansion of TRO measurement range from 0-10 mg/L to 0-15 mg/L, reduction in measurement cycle time from 2.5 minutes to 60 seconds, and software development of "remote standby" that allows the analyzer measurement cycles to start and stop remotely by electronic signal. It also has been determined that by controlling the temperature of the indicator reagent installed in the DPD analyzer, the life of the reagent can be increased from 30 days to 1 year, significantly reducing maintenance. A corrosion resistant strainer is included on some models of the DPD based analyzer to prevent debris such as plankton from entering the system. This feature makes the analyzer more robust in harsh marine environments and poor water quality.



In Conclusion, DPD colorimetric TRO analyzers provide consistent accurate results and with on-going advances to hardware and software provide a robust solution for TRO monitoring.

Keywords: *Active Substances, Amperometric, DPD Analyzers, ORP, TRO*

pEB-BOX: Develpment of a Test Kit to Analyze the Performance of Treatment Systems on Board of Ships

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How can ship-owners test if their ballast water treatment system operates as it should? Building on years of experience in analyzing ballast water quality in type approval land-based and shipboard tests, NIOZ presents a new self-monitoring service called B-box (Figure 1). This Ballastwater-box is a specially designed cool box for the transport of ballast water samples taken by crew members on board of ships.

Figure 1. Sampling ballast water on board the RV Pelagia. The sample bottles are sent in the B-box to the ship by NIOZ. After sampling the bottles are sent back to NIOZ in the cooled B-box in the front. The number and contents (fixatives) of the bottles vary depending on the analyses that have been requested.

The standard B-box is intended for sampling treated discharge water from one ballast water tank. The number of samples taken during discharge normally is three IMO 2004(). To provide an estimate of the efficacy of the treatment system untreated water can also be sampled during ballasting. However, it should be noted that B-box is not a replacement of ship board type approval testing. In other words, B-box can prove that a system was non-compliant with D-2 (organism counts too high) but care should be taken to infer compliance (organism counts lower than D-2). The number of tanks sampled, the number of samples per tank and the analyses that need to be performed are agreed upon before NIOZ sends B-box to a ship.

After sampling by the ship crew the B-box bottles are transported cooled to the NIOZ labs. A continuous logger keeps track of the temperature during transport (Figure 2).

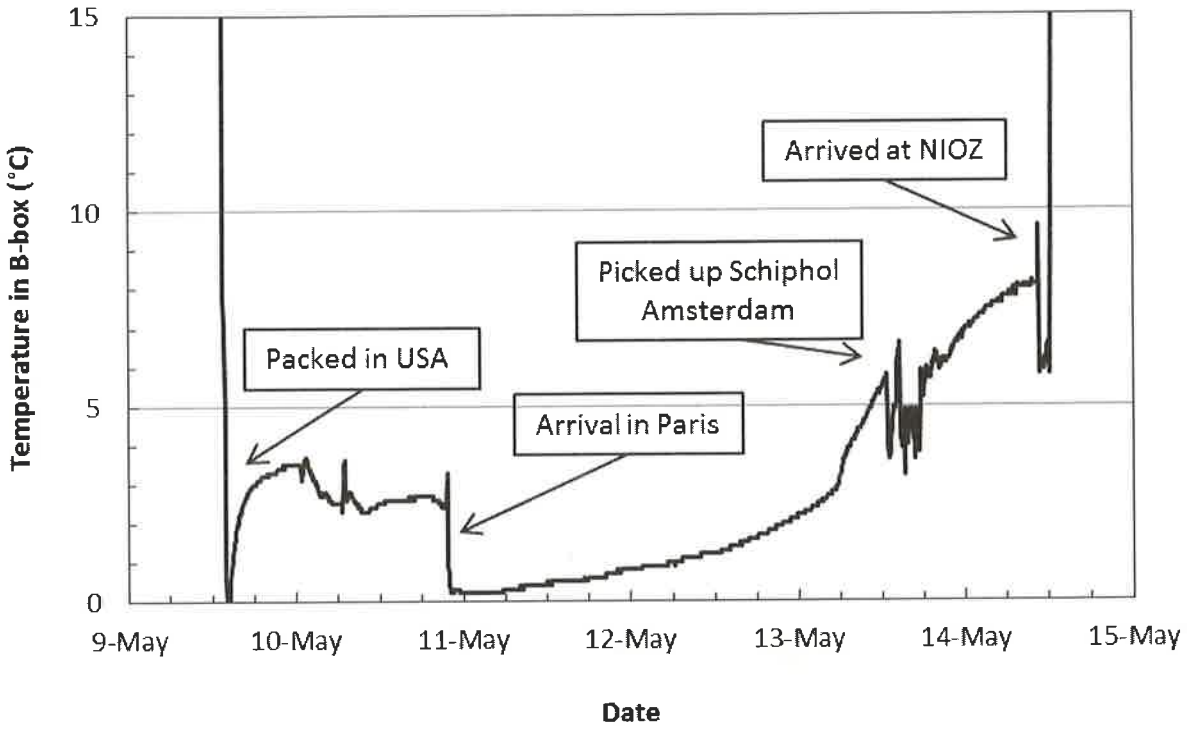

Figure 2. Temperature inside a B-box during transport from the US to Holland. On May 14 2013 the B-box arrived at NIOZ where it was opened and temperature rose to >10°C.

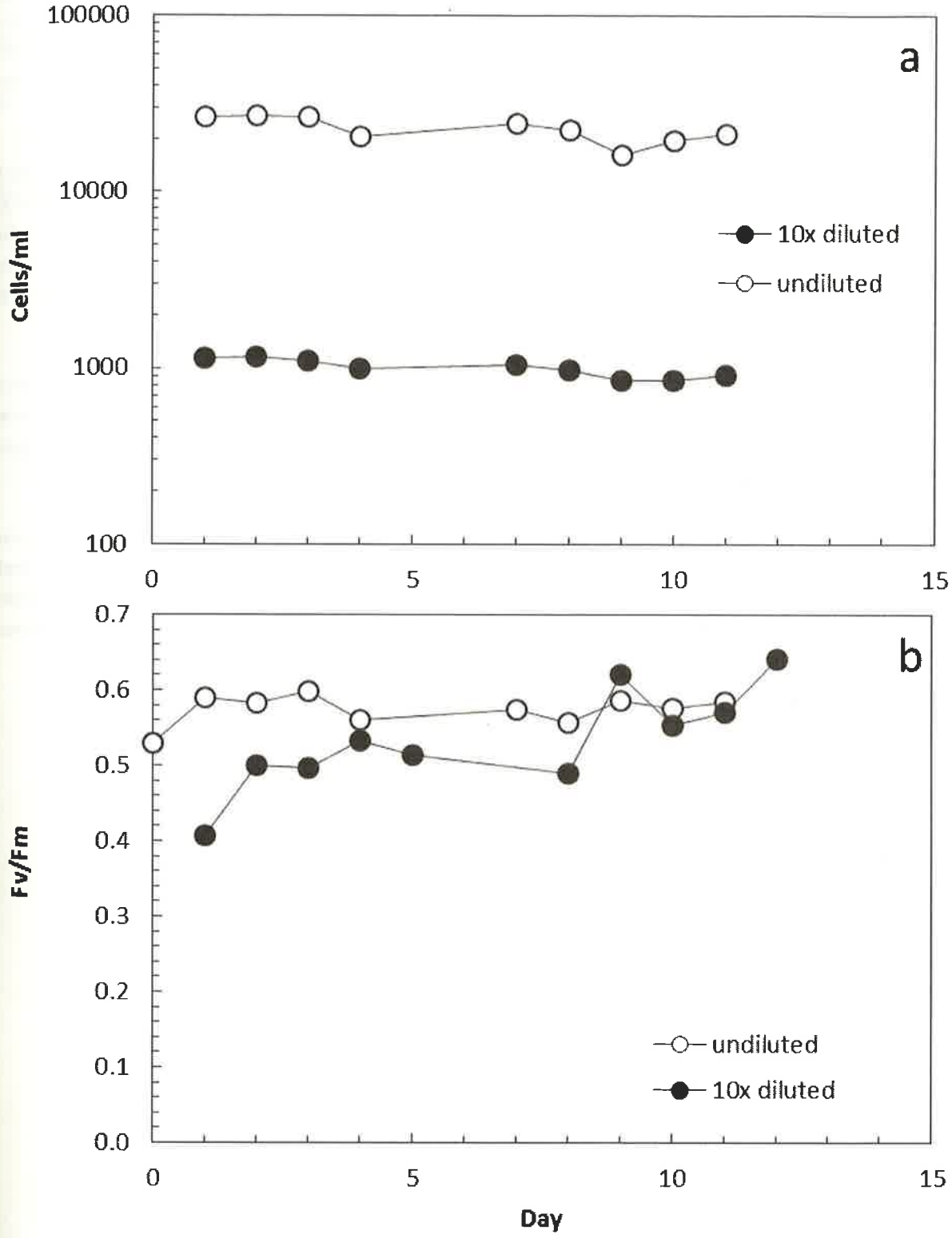

Figure 3. Validation study of the effect of cooled sample storage on phytoplankton cell concentration (a) and on phytoplankton activity (Fv/Fm, b) of undiluted and 10x diluted Wadden Sea samples. Apparently there are no large changes in sample quality for at least 10 days.

The effect of sample storage on various organisms has been examined in validation studies. The effect on phytoplankton appears to be small (Figure 3). The effect of storage and transport can also be measured as part of the B-box project in "Phyto-Check". This check is the comparison of phytoplankton activity on board with a CME technique (for instance Figure 3b) and on arrival at NIOZ using the same type of instrument.

In the NIOZ labs the samples are analyzed according to NIOZ or ETV NSF-International 2010() Standard Operating Procedures. At present the analyses comprise: 10-50 µm organisms, heterotrophic bacteria, variable fluorescence (including "Phyto-Check") and ATP as well as salinity and UV-transmission. An additional analysis is "UV-check" in which the delayed effect of UV-treatment on organism viability is measured. The analysis of >50 µm organisms is possible when large volumes of water can be filtered on board.

B-box can be used by ship-owners, port state control and treatment system manufacturers. The first tests performed in 2013 with a US-based treatment manufacturer indicate that samples can be sent safely and timely with B-box. In addition, tests were carried out with the crew of the RV Pelagia (Figure 1) to test B-box under real world shipping conditions.

Besides testing ballast water, a second goal of the B-Box project is to build confidence in system performance and compliance control between all parties involved in ballast water regulations and treatment. In addition, anonymous statistical analysis of the worldwide collected data will provide more insight in ballast water quality (Figure 4) as well as in the real world efficacy of the various ballast water treatment techniques.

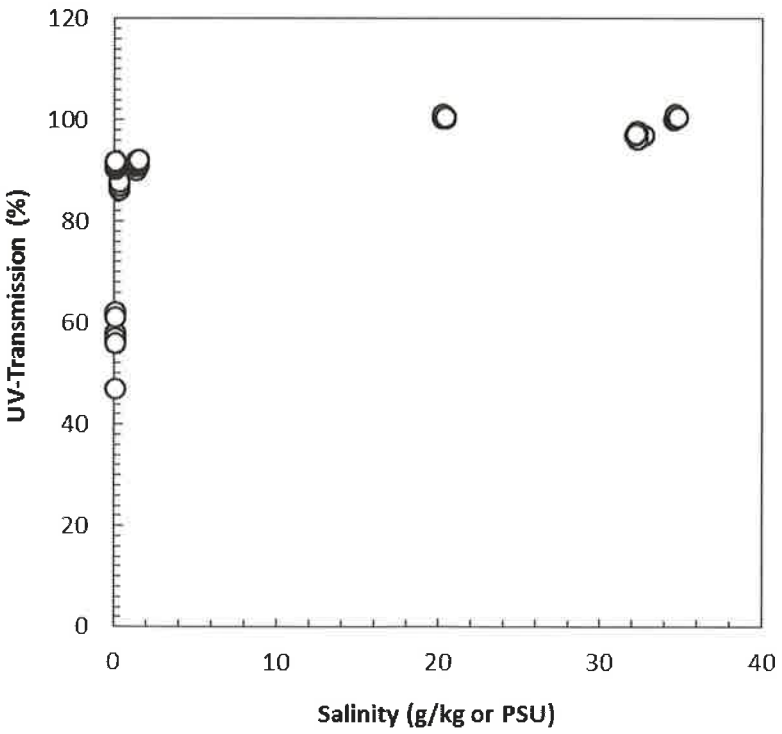

Figure 4. Example of UV-transmission and salinity data collected using B-box samples in 2013.

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Keywords: *ballast water management, BWMS reliability, BWMS efficacy, operational experience, compliance monitoring and enforcement, IMO, ETV, self-monitoring.*

Molecular Approaches for Monitoring Marine Organisms in Ballast Water

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Conventional microscopic method for microorganisms monitoring is tedious, time-consuming and it requires well-trained and disciplined phycologcal investigators. Invention of more quick, accurate and efficient method is necessary to overcome these difficulties. The molecular approaches were carried out to monitor and detect marine organisms in ballast water with ease. The immunochromatography based rapid kit for microalgae detection was developed using monoclonal antibodies raised against a particular protein of *Heterocapsa triquetra,* a candidate of harmful algal bloom in coast of Korea. About 2 ng of purified synthetic protein which was used as antigen can be detected by this rapid kit. The test for field application is now in progress. The introduction of next generation sequencing method for *de novo* mRNA sequencing in two microalgal species, *Alexandrium tamarense* and *Cochlodinium polykrikoides,* both of which are also the harmful algal bloom candidates is now considered to identify and isolate species-specific bio-molecules to develop rapid detection tool.

Introduction

Harmful organism monitoring in ballast has been carried out by identification and conting the cell/individual number using microscopy. This technique requires the experts have learned taxonomic knowledge on the planktonic organisms and is time-cosuming in many ways. Furthermore, the morphological differences between closely related genus and species are often very difficult to be distinguished. Over the last decade, various kinds of method for identifying the marine harmful organisms based on molecular markers, such as nucleic acid sequence of a certain region of DNA, have been developed and adapted for the analysis of plankton communities. Fluorescent in situ hybridization analysis (FISH), quantitative real-time PCR technique, and microarray hybridization have recently proposed as new rapid tools for harmful organisms monitoring. However, even these techniques provide rapid tools with high accuracy and specificity, still they possess comparably complicated preprocessing procedures. In the present study, the possibility of developing the immunochromatography based rapid kit for microalgae detection was carefully tested to overcome the difficulties. For this purpose, *Heterocapsa triquetra* α-tubulin (Htr-tubA) protein gene was selected as a target molecule since the full-length cDNA sequence was available.

Materials and Methods

Generation of recombinant Htr α-tubulin protein

Htr-tubA gene (GenBank/EMBL/DDBJ Accession no. EU153192.1) encoding 454 amino acids α-tubulin protein of *Heterocapsa triquetra* was subcloned by standard reverse transcriptase polymerase chain reaction (RT-PCR) method. The full length of Htr-tubA gene was inserted into EcoRI/NdeI site of pET17b vector (Novagen, Darmstadt, Germany). The resulting clones were transformed into the *Escherichia coli* strain BL21-DE3. The expression of the α-tubulin proteins was induced by 0.1mM IPTG at 25°C

for 6 h. The cells expressing the recombinant proteins were harvested, resuspended in lysis buffer (6M guanidine-HCl, 20mM KH2PO4, 500mM NaCl [pH 7.8]), and then lysed by ultrasonication. After centrifugation of the lysate at 25,000 g, the supernatants were recovered and loaded to nickel affinity chromatography. The column was washed with washing buffer (20mM KH2PO4, 500mM NaCl, 2mM b-mercaptoethanol [pH 7.8]) and eluted with elution buffer (20mM KH2PO4, 500mM NaCl, 300mM imidazole, 2mM b-mercaptoethanol [pH 8.0]). Proteins were quantified with Bradford solution (Bio-Rad, Hercules, CA) and stored at − 70°C until use.

Generation of monoclonal antibodies

To generate mouse monoclonal antibody against Htr-tubA, purified protein (1.5 mg) was used as an immunogen, 1 mg for ELISA assays. Female BALB/c mice (8 weeks old) were immunized intraperitoneally. The primary injection was performed on week 0 and consisted of 100 mg/mouse of immunogen in CFA (Sigma-Aldrich, St. Louis, MO). Booster injections were conducted during week 4. The mouse serum antibody titers were assessed by an indirect ELISA kit (Ab frontier, Seoul, Korea) using a protein antigen. The mouse showing positive immune response activity was subjected to a final boost injection during week 8. The mouse harboring the highest reactivity against protein antigen was sacrificed and splenocytes were isolated from the spleen. The splenocytes were fused to SP2/0 cells and the resulting hybridomas were screened. Hybridomas showing positive reactivity in ELISA were sub-cloned by standard limiting dilution method. The hybridomas producing monoclonal antibody were grown in a 175T flask, and the supernatant was harvested and stored at - 80°C. The isotyping was performed using a Beadlyte-Mouse Immunoglobulin Isotyping Kit (Upstate, Lake Placid, NY).

Results

Production of mouse monoclonal antibodies

Htr-tubA gene encoding 454 amino acids α-tubulin protein of *Heterocapsa triquetra* was cloned into *E. coli* expression vector pET17b to generate mouse monoclonal antibodies. The protein was expressed in *E. coli* under the stimulation of 0.1mM IPTG. The amount of protein obtained using in-chromatography renaturation method was checked on the SDS-PAGE (Fig. 1). Finally we obtained about 9.6 mg of target protein.

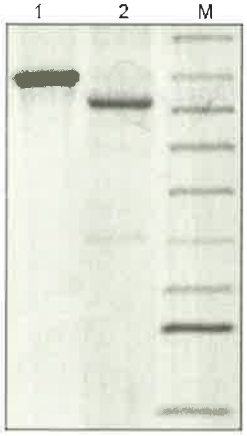

Figure 1. Purification of Htr-tubA protein. The samples were analyzed on Coomassie blue-stained SDS-PAGE. Lane 1, BSA 1 μg; lane 2, purified Htr-tubA protein; M, size marker.

Splenocytes showing the highest serum titer against recombinant VP1 were fused with SP2/0 myeloma cells to form hybridomas. The culture supernatants were screened using indirect ELISA and Western blot analysis, and positive hybridoma cells showing high titer values were sub-cloned by standard limiting dilution. The screening processes were finalized with three stable cell lines—3H8, 1B4, and 6G4 in Htr-tubA. Isotyping using a commercial kit, as described in the Methods section, showed that all of them were identified as IgG1, kappa (data not shown). The properties were further characterized by Western blot and immunofluorescence staining analyses.

Western blot analysis

The obtained mouse monoclonal antibodies were tested to analyze their specificity for immunoblot application. Mouse monoclonal anti-Htr-tubA antibodies readily detected Htr-tubA protein in microalgal species (Fig. 2). The 1B4 clone among clones generated against Htr-tubA showed only specificity to dinoflagellate speices, whereas 6G4 and 3H8 showed reactivity in both of dinoflagellates and diatiom (Fig. 2).

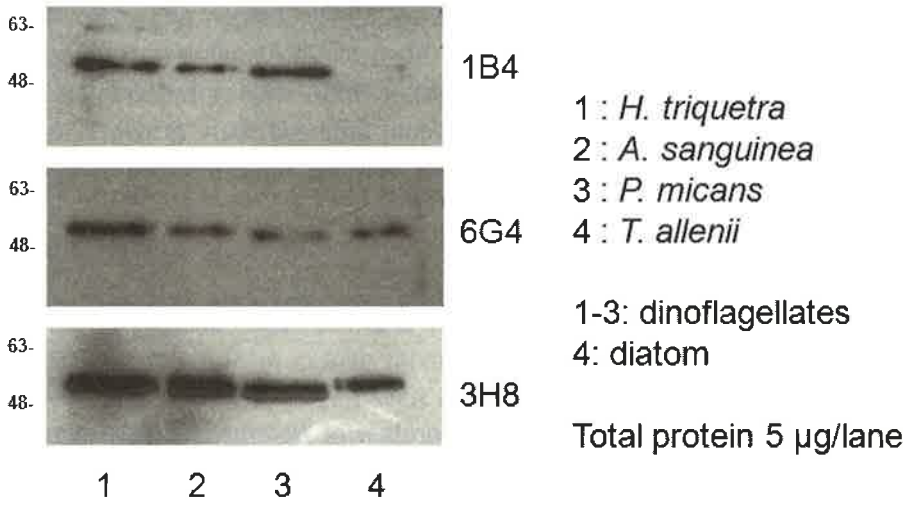

FIG. 2. Western blot analysis using mouse monoclonal antibodies. Total protein (5 µg) extracted from three dinofilagellates (*H. triquetra, Akashiwo sanguine,* and *Pr orocentrum micans*) and one diatom (*Thalassiosira alleni*) were loaded to 12% SDS-PAGE and blotted with the indicated hybridoma culture supernatants.

Keywords: *Microalgae, Immunochromatography, Rapid kit, Next generation sequencing*

session B2

G8 Related Issues and UV

Toward Best Practices for Assessing the Effectiveness of Ultraviolet Radiation for Treatment of Phytoplankton in Ballast Water
John Cullen | Dalhousie University

UV and Ballast Water: The "Live or Dead" Issue
Brian Petri | Trojan Technologies

Regrowth Tests of Planktons after UV-based Ballast Water Treatment
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Experiences in combined testing towards IMO Guidelines G8 and US EPA ETV protocol
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A study on the characteristics of UV disinfection systems during in the process of stripping
Young Chu Ohg | PANASIA Co., Ltd

Toward Best Practices for Assessing the Effectiveness of Ultraviolet Radiation for Treatment of Phytoplankton in Ballast Water

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The purpose of ballast water treatment is to prevent the introduction of potentially invasive species; this can be accomplished by killing them or making them non-viable, i.e., incabable of reproduction. Treatment with ultraviolet radiation in the UVC wavelengths (UVC), a proven technology for drinking water and waste water disinfection, has excellent potential for ballast water applications, but calibration and validation of the method is difficult. The ultimate measure of invasive potential is viability, as organisms unable to reproduce would be unable to colonize in receiving waters, and the direct measure of viability is the ability to grow in culture. Real-time assessments of the effects of UVC on viability can be developed but they must be validated against robust measures of viability based on growth. We describe a framework for estimating the effects of UVC exposure on viability of phytoplankton cells that is based on grow-outs, using the Most Probable Numbers (MPN) technique. Robust quality-control criteria have been developed to minimize the assay time and to minimize both false positive and false negative results. These quality-assurance and quality-control procedures could form the basis of a developing code for best practices in the measurement of UVC effects on the viablity of phytoplankton. Regardless, the generation of robust and repeatable measurements of the effects of UVC on viability make it possible to relate simpler and quicker measurements of UVC effects, i.e., rapid assays, to direct determinations of viability. Toward that end, an assay method has been developed with carefully controlled and replicated experiments on unialgal cultures. It generates repeatable, strain-specific dose-response relationships between UVC exposure and relative reduction in viable cells, along with estimates of the concentration of viable cells after exposure. Application of the method has demonstrated that UV radiation is a highly effective means of rendering phytoplankton incapable of reproduction and thus unable to colonize receiving waters. The resistances of several potentially-invasive species to UVC, including some that form Harmful Algal Blooms (HABs), are comparable to those of bacterial and viral pathogens. As UVC is routinely used to treat these contaminants in drinking and waste waters, the technology is likely to be an effective means of treating invasive phytoplankton in ballast waters.

Keywords: *Assessment, Phytoplankton, Treatment, UV Radiation, Validation*

UV and Ballast Water: The "Live or Dead" Issue

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EXTENDED ABSTRACT

UV is an effective disinfectant, as evidenced by its wide acceptance for treating drinking water, waste water and reuse water around the globe. It's mode of action is very unique, working by damaging DNA in target organisms and preventing their replication. Though the organisms may not be immediately "killed", they are rendered harmless by being made non-viable. Non-viability (or conversely, viability) is a meaningful biological end-point to use for assessing a disinfectant, as it is only through the ability of an organism to replicate that harmful impacts occur (e.g. host infection by viruses, bacteria and protozoans; environmental colonization by invasive species). Said another way, treating organisms to disable their ability to reproduce (to render them non-viable) is the correct damage end point to prevent harmful effects like infections and colonizations.

For phages and bacteria, viability is routinely measured using standard microbiological culturing techniques. This is feasible due to the short generational times (minutes to hours). In essence, organisms are incubated in favourable growth conditions and viable survivors are allowed to propagate over generations, forming plaques or colonies that are each attributable to a single ancestral survivor at the time of sampling. In contrast, viability can be a difficult end point to monitor or measure for more complex organisms with longer generational times. This is in part due to the longer required time frames (and associated burdens on laboratory space and resources), and in part due to unknown growth conditions or complex host systems. These difficulties have led to the use of alternative monitoring techniques; in some cases the fact that different end-points (e.g. death as opposed to non-viability) were being measured was not appreciated, and UV was wrongly reported to be ineffective against certain organisms. The most well known example of this within the UV industry is for *Cryptosporidium* oocysts: required UV doses were reported to be extremely high when vital dyes were used to assess that oocysts were dead (Campbell et al., 1995); when later work was done using infectivity assays (measuring viability end points), UV was shown to be effective at very low doses (Bolton et al., 1998; Clancy et al., 1998; Bukhari et al., 1999; Clancy et al., 2000). A similar situation transpired for the metazoan parasite *Myxobolus cerebralis,* which causes Whirling Disease in salmonid fishes. Early UV dose-response work with vital dyes led to the conclusion that high UV doses were required for inactivation (Hedrick et al., 2000), while later work using a trout infectivity assay showed that UV was effective at very low doses (Hedrick et al., 2007, Hedrick et al., 2012). In the theme of measuring relevant biological end points, Lewis and Whitby (1997, 2000) showed that UV was very effective at typical disinfection doses for preventing *Dreissenid* mussel veligers from attaching to surfaces. Hedrick et al. (2012) did a careful examination of life histories of *M. cerebralis* during host attachment, invasion, and propagation, and showed that UV impacted later stages of the parasite; attempts at early detection of the parasite in the fish hosts through PCR would have led to incorrect conclusions that UV was

ineffective. These examples reinforce the message that to properly evaluate the impact UV for new biological targets, the proper biological end points must be measured.

UV is now being employed as a useful technology in ballast water treatment to prevent the spread of aquatic invasive species. In the United States, ballast water treatment technologies are type-approved by following the ETV Protocol (NSF, 2010), which specifically calls for the use of a combination of FDA and CMFDA stains to evaluate whether organisms in the 10-50 um size class are alive or dead. These stains will penetrate into organisms and be converted into florescent products by functioning esterase systems. Thus, the end point evaluated by these stains is complete organism death where the esterase systems are shut down. Building on the learnings from the use of stains to evaluate UV impacts on *Cryptosporidium* oocysts and *M. cerebralis* actinospores, we compared UV dose-response curves for the phytoplankter *Tetraselmis spp.* (in natural waters, phytoplankton species (autotrophic protists) dominate the community of organisms in the 10-50 um size class) generated using FDA and CMFDA stains, and using a standard culturing technique with marine agar plates. The UV dose response curves differed dramatically when the different end point evaluations were used: *Tetraselmis* appeared to be extremely resistant to UV when evaluated using the stains, while it was relatively susceptible to UV when reproductive viability was assayed (using grow out techniques). The use of stains to evaluate organisms in the 10-50 um size class in the ETV protocol will lead to systems with unnecessarily high UV doses. Grow out assays (MPN culturing) should be used to assess the viability of the mixed community of phytoplankton in the 10-50 um size class samples for evaluating ballast water treatment methods, to show the effectiveness of UV treatment with the relevant biological end point (reproductive ability).

Phytoplankton have generation times in the order of days, so although longer than phage and bacterial culture assays, phytoplankton grow outs can be accomplished in a reasonable time frame. For zooplankton, "culturing" through generations will take unreasonably long and will also be biologically challenging (nurturing zooplankton through various life stages, providing the proper food and reproductive cues is probably an insurmountable challenge). However, UV may cause mortal damage to these animals but not immediate death (sometimes referred to as the "time delay" or "delayed mortality" effect), and assessing the samples after some holding period will allow for this effect to be observed. We also examined the time frames required to observe death for various species of zooplankton following UV treatment.

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Keywords: *ballast water, delayed mortality, most probable number, phytoplankton, UV, viability*

Regrowth Tests of Planktons after UV-based Ballast Water Treatment

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Abstract

International marine transportation has brought up severe ecological and economic issues during last decades because of discharge of ballast water which contains potentially harmful non-indigenous aquatic organisms and/or their cysts. In order to take care of those problems various ballast water treatment systems (BWTS) have been developed in order to disinfect the harmful aquatic organisms and pathogens in ballast water without producing toxic substances during ballasting and de-ballasting. A BWTS, GloEn-Patrol™ which efficiently works by combination of filtration and UV irradiation was developed by Panasia Co., Ltd and approved by International Maritime Organization (IMO). Despite its high disinfection efficiency and environmental safety several buyers and test agencies raised a question on the regrowth possibility of aquatic organisms after disinfection using GloEn-Patrol™. The suspicious question prompted us to reconfirm the disinfection efficiency of GloEn-Patrol™ by regrowth tests of aquatic organisms.

At first, we treated the test organisms (phytoplankton, zooplankton and bacteria) under a collimated beam at different UV intensities to determine the optimal conditions for disinfecting them. A phytoplankton *(Tetraselmis suecica)*, a zooplankton *(Brachionus plicatilis)* and bacteria (*Escherichia coli* and heterotrophic bacteria) showed significantly decreased regrowth rates in proportion to increased UV intensities and no organisms survived at 200 mJ/㎠ in contrast to the controls showing over 90% regrowth rate. Secondary, we tested the test organisms under a pilot-scale treatment system with 5 ㎥/h treatment capacity (one fiftieth of the full-scale treatment system). UV treatment showed no regrowth of planktons tested (*T. suecica* and *B. plicatilis*). Finally, we treated planktons (*T. suecica* and *B. plicatilis*) under a previously approved GloEn-Patrol™ with 250 ㎥/h treatment capacity (full-scale treatment system). Deballasting water sample did not contain any regrown planktons tested.

Introduction

Several types of ballast water management systems (BWMS) have been developed in order to disinfect harmful aquatic organisms and pathogens in ballast water from foreign sea. Among diverse BWMSs, UV treatment system is relatively simple and easy to implement and maintain. The machanism of disinfection by UV light differs considerably from those of chemical disinfection using disinfectants such as chlorine and ozone. The chemical disinfectants inactivate microorganisms by directly destroying or demaging their cellular structures, interfering with metabolism, and hindering biosysthiesis and growth [1]. On the other hand UV light inactivates microorganisms by damaging their nucleic acid, thereby preventing them from replicating. However, partial destroy of their genetic material led buyers to anxiety that organisms once exposed to UV light could resurvive or regrow using the inherent DNA repair system. In fact, several researchers have found the possibility of DNA repair in bacteria which

can be overcome by increasing the damage to the DNA with higher UV doses [2, 3, 4].
In this study, we tested the possibility of regrowth of planktons after UV irradiation using A BWTS, GloEn-Patrol™. GloEn-Patrol™ is developed by Panasia Co., Ltd. and approved by International Maritime Organization (IMO). Approved UV dosage of this system was from 250 to 350 mJ/㎠.

Materials and Methods

Because GloEn-Patrol™ consists of filter unit and UV irradiation unit with middle pressure UV (MPUV) lamps, we established three steps of regrowth test of aquatic microorganisms. First, biodosimetry test was performed again by determining regrowth of planktons at differnet UV dosages. For these tests, a collimated beam apparatus was used to deliver UV light with a near zero degree angle of incidence and relatively hormogenous dose across the surface area. Tested ballast water was prepared by adding a phytoplankton, *Tetraselmis suecica* as a S-type test organism, a zooplankton, *Brachionus plicatilis* as a L-type organism, cultured *Eschericha coli*, autogenous heterotrophic bacteria and sufficient organic substance powder to natural seawater to satisfy the guidelines for approval of BWMS (G8). First, after UV treatment with different dosages, planktons and bacteria were tested for their regrowth. Second, a pilot-scale apparatus that could treat 5 ㎥/h of ballast water was desinged to equip with UV treatment chamber, flow meter and dosimeter excluding filter unit. Considering various holding times at the field, two types of holding times were investigated and UV dosage was set at 300 mJ/㎠. Tested ballast water was prepared with the same method above. Finally, we confirmed the possibility of regrowth of planktons with full-scale BWMS. All steps of the test were regulated by the guidelines for approval of BWMS (G8). UV dosage was set at 300 mJ/㎠.
Regrowth response of bacteria was only examined at the first test (the collimated beam system) because every component of BWMS could not be aseptically maintained at pilot-scale and full-sclae tests. Because of the same reason, we selected the method for confirming regrowth of planktons as the individual concept rather than the population concept; 30 cells of *T. suecica* and 150 individuals of *B. plicatilis* which were certainly confirmed that we could grow or raise in laboratory were picked up by using a capillary pipet. *T. suecica* were incubated in a well plate (1 cell/well) and *B. plicatilis* were put into a well plate (5 individuals/well) and supplied microalgae as food. After two or one week(s) we judged the success of regrowth by counting the number of organisms reproduced. Each regrwoth test was carried out in triplicate.

Results and Discussions

When the ballast water sample was treated with the UV collimated beam apparatus, all aquatic microorganisms tested (phytoplankton, zooplankton and bacteria) did not survived at 200 mJ/㎠ of UV dosage (Fig 1). ED^{50} (50% effective UV dosage) was calculated to 66.4 mJ/㎠ and 57.1 mJ/㎠ for *Tetraselmis suecica* and *Brachionus plicatilis*, respectively.
Becuase GloEn-Patrol™ was approved by IMO with UV dosage range from 250 to 350 mJ/㎠ and treatments at two holding times, we tested the plankton regrowth at zero (immediate second treatement) and five days of holding time at the UV dosage of 300 mJ/㎠ which was the middle of the approved range in the pilot-scale test. At the holding time of zero both phyto- and zooplanktons did not regrow at all, while the control showed over 90% regrowth rate. Meanwhile, at the holding time of five days we stored the ballast water in the tank. Before the storage (Day 0) the regrowth rate of *T. suecica* in the treated sample was 0%, whereas that of *B. plicatilis* was 11.0±9.2%. However, after the second treatment after the five day storage (Day 5), any test microorganisms were not detected

and we could not carry out the regrowth test in treated ballast water (Table 1).
In the full-scale test, every parameter of deballasting water ensured the full compliance with D2 regulation. Both *T. suecica* and *B. plicatilis* did not regrow from the first treatment (Table 2).

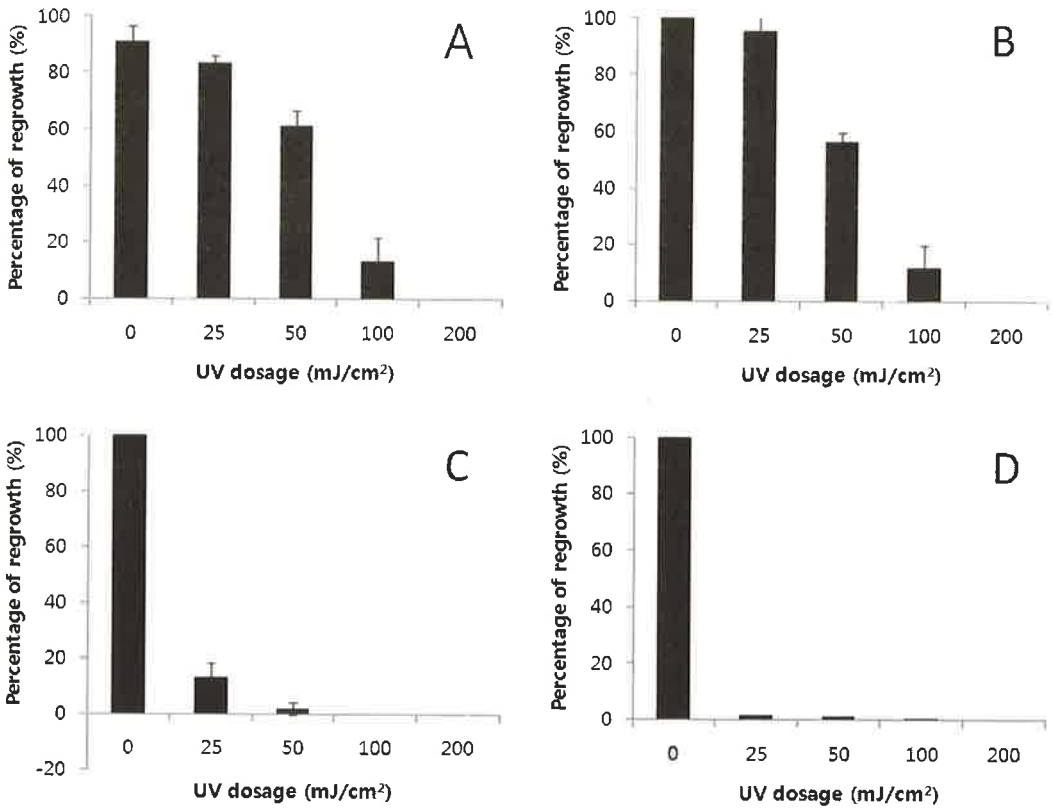

Figure 1. The response of regrowth at various UV dosage with a collimated beam apparatus. A) S-type, a phytoplaknton, *Tetraselmis suecica*, B) L-type, a zooplaknton, *Brachionus plicatilis* C) *Escherichia coli*, and D) heterotrophic bacteria.

Table 1. The response of regrowth after treatement of ballast water in the pilot-scale test at 300 mJ/㎠ of UV dosage

		S-type (*Tetraselmis suecica*)		L-type (*Brahionus plicatilis*)	
		Control	Treated	Control	Treated
Holding time: zero (immediate second treatement)		100.0±0%	0±0%	93.0±3.8%	0±0%
Holding time: five days	Day 0 (the first treatment)	87.0±8.2%	0±0%	100±0%	11.0±9.2%
	Day 5 (the second treatment)	16.7±8.2%	ND1)	100±0%	ND1)

1) Organisms were not detected.

Table 2. The response of regrowth after treatement of ballast water in the full-scale test at 300 mJ/㎠ of UV dosage

		S-type (*Tetraselmis suecica*)		L-type (*Brahionus plicatilis*)	
		Control	Treated	Control	Treated
Holding time: five days	Day 0 (the first treatment)	98.0±1.2%	0±0%	41.0±3.8%	0±0%
	Day 5 (the second treatment)	52.2±4.9%	0±0%	ND1)%	ND1)%

1) Organisms were not detected.

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Keywords: *Ballast water treatment system, Regrowth, Plankton, UV treatment*

Experiences in combined testing towards IMO Guidelines G8 and US EPA ETV protocol

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DHI provides independent performance evaluation of ballast water management systems (BWMS) for the approval process and DHI has no involvement, intellectual or financial, in the mechanics, design or marketing of the products and technologies that are being evaluated. To ensure that DHI's tests are uncompromised by any real or perceived individual or team bias relative to test outcomes, DHI's test activities are subject to rigorous quality assurance (QA), quality control (QC) and documentation. DHI's quality management system is certified according to ISO 9001 by Det Norske Veritas (DNV).

DHI obtained acceptance as sub-laboratory to the accepted Independent Laboratory, DNV Norway, by Letter of Acceptance from the United States Coast Guard (U.S. Coast Guard) dated 11 June 2013.

For an application for final approval, the IMO International Convention for the Control and Management of Ships' Ballast Water and Sediments 0 requires a performance evaluation of BWMS according to the principles laid down in Resolution MEPC.174(58) 0, generally referred to as the IMO G8 guidelines, and, for systems that make use of active substances, also Resolution MEPC.169(57) 0, generally referred to as the IMO G9 guidelines. The purpose of the performance evaluation is to assure that BWMS approved by administrations are capable of meeting the ballast water discharge standard in Regulation D-2 0, also known as the IMO D-2 standard, in land-based and shipboard evaluations and do not cause unacceptable harm to the vessel, crew, environment or public health. The United States Coast Guard Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters 0 (§151.2030) establish a ballast water discharge standard similar to the IMO D-2 standard. According to the United States Coast Guard, the test set-up in land-based test cycles of BWMS must operate as described in the ETV protocol 0.

During 2013 DHI has gained a thorough experience testing according both to the IMO Guideline /2/ as and the US ETV protocol /5/. The main differences between the two guidelines/protocols will be described both in relation to performance of the actual tests, methodologies applied as well as economic aspects.

Keywords: *BWMS testing, IMO G8, US ETV protocol, evaluation methodologies*

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A study on the characteristics of UV disinfection systems during in the process of stripping

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In Ballast Water Management System (BWMS), the process for stripping ballast tanks by the eductor is necessary for removing residual water. Because BWMS's with UV disinfection unit usually include UV re-treatment when de-ballasting, the residual water mixed with driving water have to pass through UV reactor. In this stripping process, the flow-rate in UV reactor occurred by stripping process is significantly lower than occurred by regular process. But the problems, which could be occurred by high dosage from stripping process, have not been fully discussed in previous studies.
Therefore, in this study, performance tests of UV disinfection were conducted by analyzing the characteristics of the UV dosage in various water qualities (transmittance, salinity and temperature) in discharged water made from considered condition for stripping process.

1. Introduction

IMO adopted "International convention for the control and management of ship`s ballast water and sediment" in Feb., 2004 to prevent environmental pollutions from occurring due to ship's ballast water, and to make the convention valid, more than 30 countries which have secured 35% of more of shipping capacity should ratify it[1]. As of now, shipping capacity reached to 33% and 37 countries have ratified to enter into force. In line with the fact, to develop a variety of water treatment techniques to effectively sterilize ship's ballast water, many R&D activities have been briskly conducted in and out of the nation.
Among many treatment ways, the ultraviolet(UV) treatment is relatively simple in its system and easy in maintenance, compare to other ways and further, it doesn't any remnants after treatment in an eco-friendly ways, which is considered to replace or complement chlorine disinfection, and so, it is attracting hot attentions[2]. Moreover, it is considered to be highly efficient not only in saving time of inactivating pathogenic protozoans comparing to chlorine or ozone using ways, but also in its sustaining power[3].
Ultraviolet treatment is to employ a principle that UV on 200~280nm (UV-C) wave length destroys DNA of genes having cell genetic information. This treatment is not a chemical way but a physical one and is not affected much by some factors like temperature or pH, but it is reported that to be highly affected by factors such as turbidity changing UV light transmittance, DOC, and inorganic substances. In case these substances are existent in water, UV light transmittance will be lower to disturb UV light in getting on surface of target organisms and to decrease its effects. For this reason, when conducting ballast water stripping or ballasting operation in muddy water, the disinfection system should be verified to dispel worries of customers about whether it might influence on performances of UV disinfection system.

Therefore, the study analyzed features of UV disinfection system depending on specific circumstances of turbidity, salt level, temperature, and water quality which are worried to cause some effect on performance of UV disinfection system

2. Contents and process of Test

The BWMS, employed in this study, is GloEn-Patrol™ System consisted of filter and UV reactor like in Fig. 1. Pre-treatments are conducted by 50 ㎛ mesh filter and eight MPUV lamps are installed in UV reactor to conduct ballast water disinfection. It is capable of treating 250 ㎥/h and filters and UV reactor are used in ballasting works and only UV reactor in de-ballasting. In the rated flow 250 ㎥/h, rated UV dosage is between 250 ~ 350 mJ/㎠. (The Certificate of Ballast Management System, 2-2010)

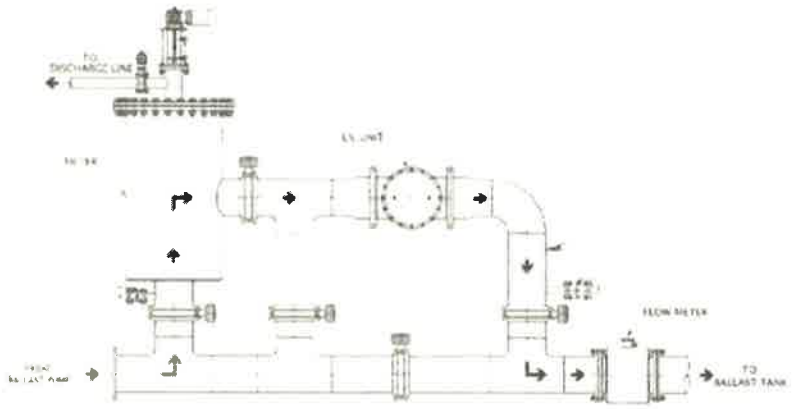

Figure 1. GloEn-Patrol™ System

Stripping operation used a principle to spray high pressured liquid through nozzle, to make a regional vacuum around it to suction liquid, and to discharge it. Fig.2 shows eductor installed inside a test barge. Fig.3 is a diagram showing a stripping operation process using eductor. Residual water in ballast tank is discharged from a vessel through eductor which uses sea water as driving water. Typically, during a stripping operation, it doesn't employ a ballast pump, but GS pump of low capacity and so, its treatment flow rate becomes far more decreased than rated flow, and in this case, as time to stay inside UV reactor gets longer, UV dosage becomes more increased.

Figure 2. Photograph of installed eductor

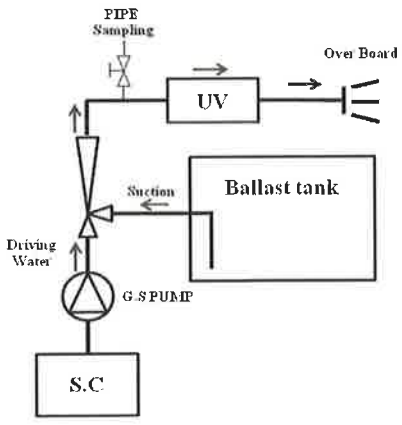

Figure 3. Diagram of stripping process

The test was conducted in a test barge which was manufactured to conduct various performances of BWMS, and it used mudflats to prepare proper turbidity circumstances. Turbidity was arranged by 20, 260, 500 NTU, based on test water flowing into UV reactor, and the test was conducted in a sea water condition and brackish water one respectively, and for the brackish water test, the test water was prepared by mixing fresh water with sea water. The test conducted a stripping operation by 20(85%), 260(30%), and 500(20%) NTU for turbidity and 50 ㎥/h 110 ㎥/h, and 250 ㎥/h for flow rate, and it measured UV dosage inside UV reactor on each condition of turbidity and flow rate. Fig. 4 is test sequence diagram of stripping. The test used a multi sensor meter(Hydrolab MS5, Hach), a turbidity meter(2100P, Hach), and a transmittance meter(Real-UVT, Real Tech) to measure water quality, turbidity and transmittance respectively. UV dosage was calculated from values measured at UV intensity meter(IL-M SUV20.2 Y2 C) installed in UV reactor and from ones measured at flow meter (Endress+Hauser, PROMAG W+PROMAG 10).

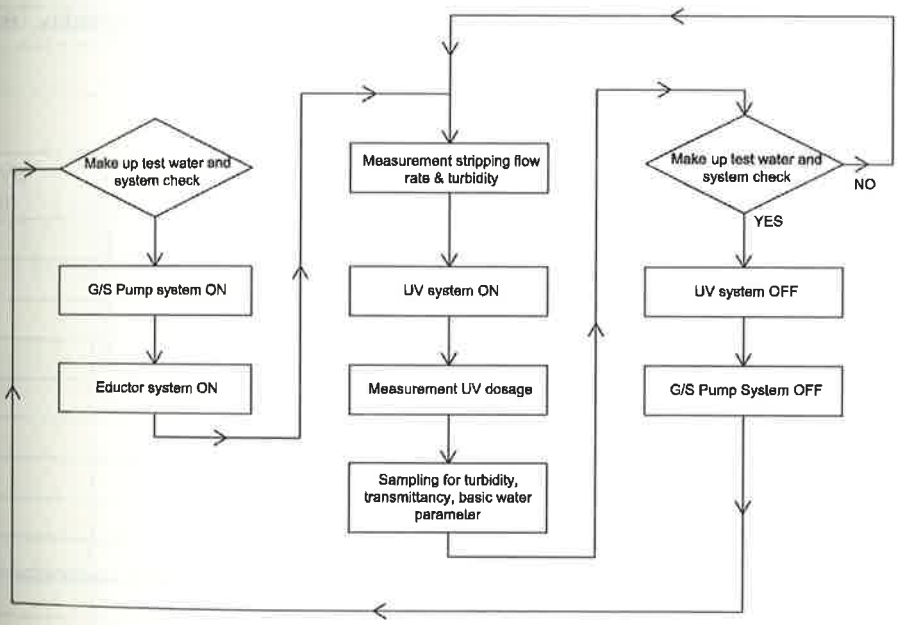

Figure 4. Sequence of a stripping test

Further, the test employed collimated beam, shown in Fig. 5, to observe UV intensity development by temperature changes. The test secured test water with proper salt and turbidity through stripping test and made its penetration depth 10mm in Petri-dish, and it measured UV intensity at 5℃, 25℃ and 40℃ respectively.

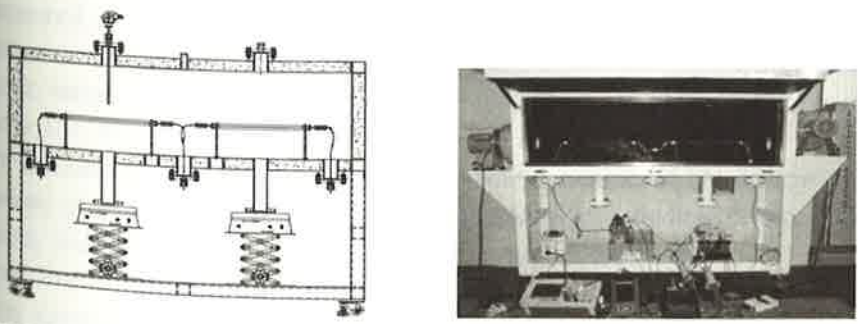

Figure 5. Collimated beam system

3. Results

3.1 In the case of the brackish water

When the conditions were 51.1 ㎥/h of flow rate and 29.1, 298.2 and 421.8 NTU of turbidity, the UV dosage was exceed minimum (250 mJ/㎠). When the conditions were 112.4 ㎥/h of flow rate and 28.6 and 285.3 NTU of turbidity, the UV dosage was exceeded the minimum (250 mJ/㎠). Finally, the conditions were 251.2 ㎥/h of flow rate and the 20.6 NTU of turbidity, the UV dosage was exceeded the minimum (250 mJ/㎠)(Table.1 Brackish water)

3.2 In the case of the sea water

When the conditions were 50.4 ㎥/h of flow rate and 22.0, 285.0 and 418.0 NTU of turbidity, the UV dosage was exceeded the minimum(250 mJ/㎠). When the conditions were 113.2 ㎥/h of flow rate and the 27.7 and 291.2 NTU of turbidity, the UV dosage was exceeded the minimum (250 mJ/㎠). Finally, the conditions were 249.3 ㎥/h of flow rate and the 19.9 NTU of turbidity, the UV dosage was exceed the minimum(250 mJ/㎠).(Table.1 Sea water)

Table 1. UV dosage tests in brackish water and sea water

Test mode	Sampling Tag	Flow rate (㎥/h)	Turbidity (NTU)	UV dosage (mJ/㎠)
Brackish water	BH-50	51.7	421.8	285.2
	BM-50	51.9	298.2	369.8
	BL-50	49.7	29.1	999.0
	BM-110	112.7	285.3	250.0
	BL-110	112.1	28.6	482.8
	BL-250	251.2	20.6	292.8
Sea water	SH-50	50.1	418.0	316.6
	SM-50	53.0	285.2	402.3
	SL-50	48.2	22.0	999.0
	SM-110	113.6	291.2	249.0
	SL-110	112.7	27.7	438.4
	SL-250	249.3	19.9	305.3

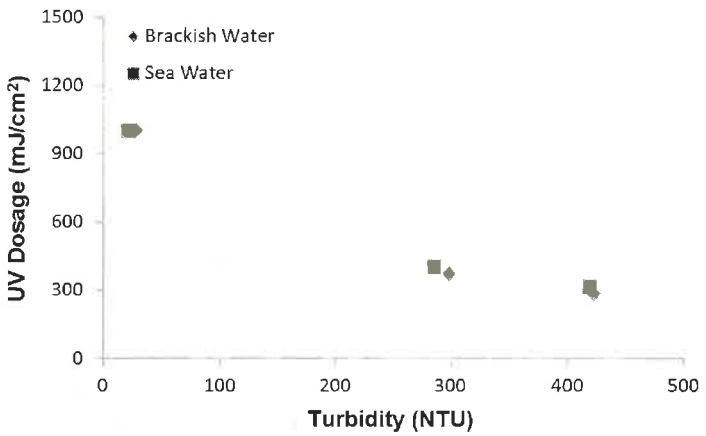

Figure 6. Characteristics of UV dosage by turbidity at 50 ㎥/h of flow rate

3.3 UV intensity test result on the various turbidity and water temperature

Table 2. UV intensity development by temperature

Sample tag	Salinity (PSU)	Turbidity (NTU)	Transmittance (%)	Water temperature (℃)	UV intensity (W/㎡)
GP-BH-5	0.16	424.0	424.0	5.6	28.0
GP-BH-25	0.24	417.3	417.3	25.3	27.0
GP-BH-40	0.26	423.3	423.3	41.5	28.3
GP-BM-5	0.17	288.3	288.3	5.6	43.3
GP-BM-25	0.07	270.7	270.7	25.4	41.3
GP-BM-40	0.17	274.0	274.0	40.4	41.0
GP-BL-5	0.12	20.8	20.8	5.3	89.7
GP-BL-25	0.12	20.3	20.3	25.5	89.7
GP-BL-40	0.13	19.8	19.8	40.4	89.7

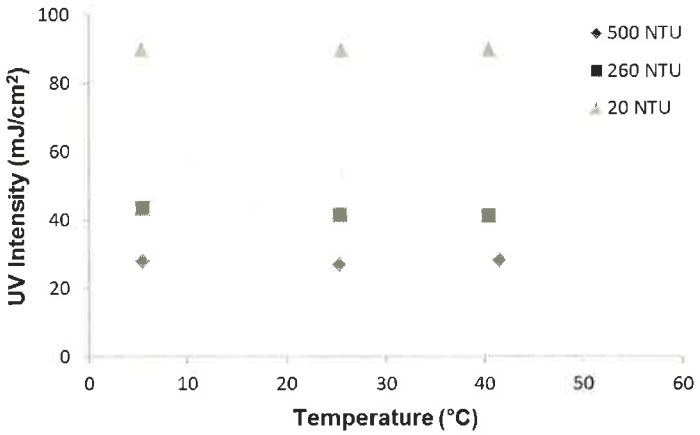

Figure 7. Characteristics of UV intensity by temperature

The test didn't show any significant difference of UV intensity, depending on temperature changes, at Table. 1&2 and Fig. 6&7, and it found that UV intensity was clearly different by changes of turbidity and transmittance.

4. Conclusion

The test arranged turbidity of test water in a same way with the actual stripping operation in the vessel and measured UV dosage, inside UV reactor, depending on flow rate changes, and it finally evaluated whether its performance meets the minimum acceptable condition of UV dosage (250mJ/㎠). Further, the test measured UV intensity values at 5℃, 25℃ and 40℃ of test water temperature to examine how UV intensity depends upon temperature changes.

The test result is found to meet the minimum acceptable condition of UV dosage (250mJ/㎠) under test circumstances of 48.0~53.5 ㎥/h of flow rate/ 411 NTU of turbidity or less, 111.4~115.7 ㎥/h/ 285 NTU or less, and 246.2~263.0 ㎥/h/ 19.5 NTU or less respectively.

As judging from the study results of brackish water and sea water, it could be confirmed that it didn't show any significant difference in UV dosage upon salt level of test water and in UV intensity

upon temperature changes, either.
The test confirmed that it meets the minimum acceptable condition of UV dosage (250mJ/㎠) on the test conditions of turbidity and transmittance and in result, GloEn-Patrol™ System is highly expected to produce good performances in treating residual water inside ballast tank during the stripping operation.

5. Consideration

A high UV light irradiation over 350 mJ/㎠ was generated under the test conditions of 50 ㎥h of flow rate and 260 NTU of turbidity, 50 ㎥/h and 20 NTU, and 110 ㎥/h and 20 NTU. However, the performance of the UV treatment system was not tested against diverse active substances in this study. Continuous experiments on their toxicity and chemical properties should be conducted in the future.

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Keywords: *BWMS, UV disinfection, Stripping process, Eductor, High UV dosage*

session A3

Port State Control, Compliance Monitoring

Compliance Monitoring and Contingency Measures, Ensuring Successful Ballast Water Management

Kevin J. Reynolds | The Glosten Associates

Sampling and Analysis of Ballast Water on board Ships for Compliance Analysis

Lothar Schillak | SGS S.A. Environmental Services

A Preliminary Optimal Enforcement Strategy for BWMC

Guizhen Zhang | DHI–NTU Centre, Nanyang Environment and Water Research Institute, Interdisciplinary graduate school, Nanyang Technological University

Compliance Monitoring and Contingency Measures, Ensuring Successful Ballast Water Management

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Objectives

The deployment of practical compliance monitoring systems and contingency measures is critical to ensuring successful ballast water management. It is the objective of the program team to develop and deploy such tools for the maritime industry.

Results

The team has developed a shipboard compliance monitoring kit that provides similar confidence intervals as that utilized in land-based testing facilities. This system is capable of capturing three (3) cubic meters in ninety (90) minutes while maintaining iso-kinetic flow rates. Further, the system can be deployed during a ballasting operation. Testing is still undergoing shipboard testing. As such, preliminary results will be presented.

The team has also developed a mobile ballast water treatment system called Ballast Responder. This system allows in-port mobile crews to come aboard the ship and perform treatment in-tank. This relieves the expense and logistics of shore-based or barge-based systems. The results of the shipboard trials will be presented.

Conclusions

The ability to monitor compliance is critical to ensuring proper shipboard integration of ballast water treatment systems. For example, a ship may need to take-on ballast water from a high seachest in order to avoid overwhelming a treatment system with sediment loads at certain drafts. Without a compliance monitoring tool, that feedback may go unnoticed and there would be no change in operational practices or installation configurations.

The ability to execute contingency measures is critical to practical enforcement of any compliance monitoring results. If port state control did not have a reasonable and practical measure available, the only choices would be to stop the ballasting or allow it to continue. A reasonable contingency measure permits a corrective action and then the continuance of the ballasting operation. This results in a protection to the environment and further an incentive for the marine vessel to ensure compliance on the next port call to avoid the delay and expense of the contingency measure.

Ensuring successful ballast water management requires the combination of proven treatment systems, the ability to monitor their performance, and then the safety net of practical contingency measures. Each of these three elements provides feedback, information, and support for the other.

Keywords: *Ballast Water, Compliance, Contingency, Monitoring,... (in Alphabetical order)*

Sampling and Analysis of Ballast Water on board Ships for Compliance Analysis

Report of an International Research and Development Project

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1. Introduction

In 2012 the SGS has been charged with the execution of an international research and development project "Effective new technologies for the Assessment of Compliance with the Ballast Water Management Convention" funded by the Federal German Hydrographic and Maritime Agency.
The project objectives are twofold : (A) the development of an adequate technical system to take representative ballast water samples from within ballast water pipes onboard ships and (B) the development of analytical methods for a rapid, indicative on board ballast water analysis. The project cooperated with partners from Germany, France, Canada, Norway, USA and Australia.

2. Representativeness of ballast water samples

The sampling of ballast water from inside the main pipes on ships has to be orientated along the regulations within the frame of the *'International Convention for the Control and Management of Ships Ballast Water and Sediments 2004'* formulated by the International Maritime Organization of the United Nations – IMO.
To assess the quality through rapid, indicative onboard sampling and analysis the technologies and methods used should ensure that the obtained results are representative and reflect (i) the organism density in the ballast water as well as (ii) the changing quality of the ballast water during the entire deballast procedures.

3. Preconditions for ballast water sampling

The IMO regulations for ballast water sampling demand an isokinetic sampling port installed in the ballast water main pipe of the ships located as close to the discharging sea valve as possible. The isokinetic pipe of the sampling port should be shaped as an "L" with the short sleeve positioned in the centre of the pipe and with the opening facing upstream.
For the sampling system to be developed for onboard use a set of indispensible criteria has been identified :

The adequate onboard sampling system…..

- should adequately separate the organism from ballast water
- should allow for sample volumes in a wide range
- should generate a minimum of waste ballast water
- should be run by easy, rapid procedures
- should be applicable to all ballast water pipes on all ships

This set of major criteria is only fulfilled by a closed sampling system, which pipes the ballast water from the isokinetic sampling port through adequate filtration steps and re-directs the ballast water back to the main ballast water pipe.
The adequate sampling of ballast water as described in the IMO regulations also defines the basic ruling parameter: the flow velocity directly at the filter material, which retains the organisms in the ballast water. Since the IMO standards for maximum admissible density of plankton organisms in ballast water refers to live organisms, it is essential that the live organisms, which enter the sampling system with the ballast water, stay alive and are not killed during the sampling and filtration process.
According to publications and information provided by major, international operating marine biological and oceanographic institutions as well as from engineering departments of major universities in Germany and abroad, the maximum admissible flow velocity directly at the filter material to safely collect plankton organism ranges between 0,45 m/s and 0,65 m/s.

4. Preconditions for rapid, indicative ballast water analysis

Once the international ballast water convention is ratified and comes into force, the state port controls as well as the national coast guard forces are entitled to execute tests of the ballast water on ships, that operate in the territorial waters of a country to assess the compliance with the IMO standards. Ships, which run into harbors to unload and reload their goods, are very restricted in time. As a consequence the execution of ballast water analysis by use of classical methods like optical organism counts and 24/48 hrs. incubation of bacterial samples for the detection of human pathogens are inadequate seen to the fact these methods require much time from sample to result.

Therefore, other, easy applicable analytical methods should be used with less extensive time requirements and rapid steps from sample to result.
These methods could use the concentration of biological substances, which are solely found in living organisms and try to convert and correlate the concentration values into the desired numerical data as the IMO standards for ballast water quality refer to.
In case these analytical methods do not perform a lower sensitivity in the range of the required IMO standards, the obtained data and results may serve as an indication for a malfunction of the ballast water treatment system installed on the ship. In this respect the term *"gross exceedance"*, i.e. an organism density value far above the IMO standard for ballast water quality would give the indication for gross exceedance.
In case the rapid, indicative ballast water analysis for compliance with the IMO ballast water quality standards reveal a gross exceedance, it is very likely, that the analysis of the ballast water has to be repeated, this time by an in-depth-analysis in a landbased laboratory.

5. The new ballast water sampling system

The new ballast water sampling system, which has been developed by SGS within the frame of this international research and development project, can be run as an open or a closed system and consists of three modules, which are connected by tubes when operated. They have been developed in cooperation with naval engineers and were tested under various conditions. The entire system has been validated by an external naval laboratory under real conditions.

(A) A universal sampling port can be mounted to all flanges of ballast water pipe systems down to a minimum size of DN65. Equipped with a cartridge, into which isokinetic pipes with various diameter fit, this universal sampling port can be applied to all ballast water pipe systems on all ships.
(B) The sampling system itself comprises a stainless steel frame, carrying a filter housing for the collection of the plankton organisms >50µm, volume count as well as an inbuilt isokinetic sampling valve, via which the samples for plankton organisms >10µm<50µm and for bacteria are taken. The filter material for the collection of the plankton organisms >50µm is a stainless steel screen with laser perforated round holes of exactly 50µm diameter. Several ball valves installed in the sampling system allow for an easy and safe handling of the system. The system has a small footprint of 40cmx60cm. With a weight of less than 12 kilo the system can easily by transported onboard ships and mounted to the main ballast water pipe system.
(C) The backflush port, too, can be mounted to a large range of different flanges down to a minimum size of DN65. It simply comprises a 2" ball valve screwed into a variable cartridge.

The entire system has also been tested in long time series with a maximum system pressure of 5,5 bar and with volume flows of up to 496m³/h at a maximum operation time of 18 hrs. .
The universal sampling port was tested with three different diameters of the isokinetic pipe : 1", ½" and ¼" under various flow volume regimes. Based on the obtained results the universal sampling port can be used for ballast water pipe system onboard ships up to flow volumes of 8.000m³/h and pipe sizes of up to DN600.

6. The new analytical methods

Within the frame of the international research and development project four different methods were further developed for ballast water analysis :

- Fluorescein-diacetate fluorometry - FDA
- Pulse amplitude modulation fluorometry - PAM
- Adenosin-triphosphate fluorometry - ATP
- Fluorecence-in-situ-hybridisation / fluorescence microscopy- FISH

The focus here will be on the ATP and the FISH method.
Adenosin-triphosphate-ATP is a biological substance, which occurs in all living cells. To assess the concentration of ATP it has to be mobilized from within the cell compartments by adequate techniques. Within the frame of this international research and development project the ATP method has been developed for the analysis of ballast water to detect the three organism size classes plankton >50µm, plankton >10µm<50µm and bacteria. In a preparatory step the organisms are disintegrated followed by the extraction and mobilization of the ATP substance. The results are expressed as pg/volume unit.
With a large number of various test series and with natural plankton and cultured plankton this method has been calibrated for its correlation between the concentration of cellular ATP and the organism densities in ballast water samples. Within the frame of these test series this method was able to detect even a single organism >50µm in the sample.
For plankton organisms >10µm<50µm the method proves a lower sensitivity range of 20 organisms. For the bacteria the ATP method developed for ballast water analysis is able to detect as little as

10 cfu/100ml.
The above presented results have been confirmed by validation tests executed by different external laboratories.
Fluorescence in situ hybridization-FISH uses gene probes to mark the specific bacterial species targetted by the IMO for the ballast water standard. After the application of the gene probes the sample can be qualitatively and quantatively assessed by fluorescence microscopy.
The result of various test series reveal, that the FISH method detects the target bacteria species within in the range of the IMO standards for ballast water quality.

7. Time requirements for rapid onboard sampling and analysis

The time needed for the sampling of ballast water will strongly depend on the desired sample volume as well as on the hydraulic characteristics of the main ballast water pipe and the diameter of the isokinetic pipe subsequently to be used in order to ensure an admissible flow velocity at the filter screen. Thus the time needed for sampling may vary between a few minutes for volumes < 100 l and more than 1 hour for volumes > $1m^3$.
The time needed for the rapid, indicative analytical methods are clearly defined :

- ATP Plankton organisms >50µm : 13-15 min.
- ATP Plankton organisms >10µm<50µm : 13-15 min.
- ATP bacteria (quantitative) : 6 min.
- FISH bacteria (qualitative and quantitative) : 9 hrs.

To asses the time needed for a complete indicative ballast water analysis the sampling system and the analytical methods were tested under real conditions, i.e. with a ballast water treatment system in operation.
Since the ATP method uses the same material, chemicals and technical devices for all of the three target organism size classes, a full, indicative ballast water analysis can be completed within 40 min. and includes these number of samples :

- Bacteria (quantative) : 5 samples
- Plankton >50µm : 2 samples
- Plankton >10µm<50µm : 2 samples

8. Outlook

The sampling system as well as the analytical methods, which have been developed within the frame of this international research and development project will be subject for an informal paper submitted to the IMO for presentation within the frame of the 17th session of the IMO subcommittee Bulk-Liquid and Gases in February 2014. The sampling system as well as the analytical methods developed for ballast water analysis will then be investigated as to their suitability as IMO approved international sampling and analysis methods.

Independent of the further decision by the IMO the sampling system as well as the analytical methods are ready to be applied for performance tests of ballast water treatment systems already installed on ships. At present SGS already executes such performance tests on two large container vessels as well as on several cruise liners and the request for onboard performance testing from various shipping lines, shipowners and producers of ballast water treatment system is increasing.

A Preliminary Optimal Enforcement Strategy for BWMC

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1. Introduction

As the Ballast Water Management Convention 2004 is expected to go into force soon, the compliance, monitoring and enforcement problem becomes increasingly important since simply having BWMC in place is not enough to address the problem of non-indigenous biological species.The BWMC 2004 mainly proposed two methods to mitigate risks of bio-invasion through ballast water: one is ballast water exchange; the other is ballast water treatment. Of course, there are other alternatives, such as the design of ballast free ships or non-ballast ships. However, ballast water exchange is the dominant practice at this moment, which requires ships to exchange ballast water with mid-ocean waters using either the sequential, flow-through, dilution or other exchange methodologies. The logic behind this method is that the coastal biology is less likely to survive in saline and cold water. It is important to recognize that open ocean exchange is not always biologically effective and is not always possible to perform due to ship safety and operational issues involved (Endresen, Lee Behrens et al. 2004). Therefore, according to the timetable of Convention, ballast water exchange will be phased out and eventually (after 2016) all ships are required to install a ballast water treatment system except those granted an exemption, which demands huge capital commitments for ship owners.

Therefore, compliance, monitoring and enforcement (CME) is one of the biggest problems facing the regulation agency (Port State Authority). This means that port state control authorities should take great enforcement efforts to ensure that all ships comply with the convention so that invasion risks are kept to the minimum level. Specificly, in the context of ballast water management convention (phase two), compliance may refers to: (1) initial compliance, in which the objective is to force the regulated source to install the ballast water treatment systems that enable the regulation to be met, (2) compliance with reporting requirements, which aims to force the ships to report truthfully to the authorities, or (3) continuous compliance, which attempts to force the ships to keep discharges within regulatory limits (Harrington 1988). It is quite easy to verify whether there is a treatment system on board the ship. In contrast, more enforcement efforts should be made to ensure that the systems already installed are in operation.

2. The traditional theory of compliance and its limitations

The traditional compliance theory assumes that firms will comply only when the expected cost of violation exceeds that of the benefits. In the case of complying with BWMC, the expected cost of

non-compliance are associated with the potential probability of being caught and penalized when violating. Therefore, the cost of not complying with ballast water convention depends on the level and effectiveness of enforcement. This can be measured by examining an enforcement chain that includes: (1) the probability of a ballast water discharge violation being detected a, (2) the probability of a detected violation resulting in a citation b, (3) the probability of a citation being successfully prosecuted c, and (4) the size of the average assessed penalty d (5) = average "final settlement" amount expressed as the percentage of the average "assessed or schedule-based penalty" the ship owner or ship operator expects to pay e (King and Tamburri 2010). To be specific, the expected costs can be expressed by the following equation:

Expected cost = C = a × b × c × d × e (1)

According to the above equation, for different enforcement schemes, improved detection rate (a) and the increased amount of penalty (d) will definitely increase the level of expected non-compliance cost. In fact, several verification approaches could be used to detect potential violations: self-reporting forms submitted by ship owners, masters or chief engineer plus occasional inspections which incurs little cost for the regulation agency, but the effectiveness of which is not convincing; monitoring by using shipboard sensors, which requires the development of appropriate sensors and the potential cost is medium; direct ballast water sampling which may give the most accurate and reliable result but is both time-consuming and very expensive not to mention the potential delay of the ships (Wright 2012) Wright 2012). In fact, according to Basurko and Mesbahi (2011) to obtain representativeness, the water sample size should at least be:

$$n = \frac{Nz_{a/2}^2 P(1-P)}{(N-1)e^2 + z_{a/2}^2 P(1-P)} \quad (2)$$

where:n = "sample size" , N = "population" size , P = probability of success, $1\text{-}P$) = is the probability of failure (Q) , $z_{a/2}$ = confidence coefficient (for a confidence level of 95%, $z_{a/2}$ =1.96), e = standard sampling error. The volume to be sampled in order to get a reliable result is extremely high. For example, a sample of 905.7 m3 has to be made for a population of 1000 m3 ballast water. Another issue is that ballast water sampling is also time consuming. It may take several days to verify that the species contained in the water are not viable. In such cases, ships will be delayed and the port efficiency will be decreased. The results by King (2010) showed that although ballast water sampling provided the highest level of confidence that violation rate would be detected, the most cost-effective verifying method was the application of indirect monitoring method (use of sensors). However, appropriate monitoring sensors are still not available now.

The benefits of non-compliance, however, mainly include the avoidance of the operational fees. Based on this model, to improve compliance rate, the Port State Control Authority has to inspect frequently enough by to keep the detection rate high enough and to set the penalty arbitrarily high for detected violation. This is almost impossible because, on the one hand, to every inspection incurs an inspection cost for the regulating agency, i.e. port state control. This is especially true for the ballast water regulation, considering the fact that ballast water sampling is not only expensive but also time consuming. No Port State has such large enforcement budget to inspect all ships. Also, there is always an upper limit on the size of fine either stated by the government or to avoid driving the firms into bankruptcy (Harrington 1988). The enforcement budget therefore limits the inspection frequency. As a result, even though the intended administration result is hundred percent compliance, the real compliance rate is far less than that.

3. Enforcement targeting based on the firms' compliance history

One way to solve this problem is to smartly allocate the limited enforcement resource, for example, targeting the inspections according to the compliance history of these ships. To use compliance history of the regulated firms has attracted attention for many scholars in the field of other environmental law enforcement. The pioneer researcher is Harrington(1988), who first introduced an enforcement scheme that treat the regulated firm different based on their compliance history. Harrington's work was further extended by Harford and Harrington (1991), Raymond (1999), Heyes and Rickman (1999), Livernois and McKenna (1999), Lai et al. (2003), Decker (2003), Heyes (1996), Harford (2000), and Friesen (2003). These researchers took a dynamic view of the compliance behavior, which means that enforcement is seen as a repeated game between the firms and the agency. The firms have to decide whether to violate or not, while the agency has a decision to make on whether to initiate an inspection or not. This model distinguish from the traditional model in that it assumes that the agency and the firm can react to each other's action rather than the firm makes the single choice of the violation rate. Thus the reputation of both sides will have some effects on the future compliance action of firm. According to this model, what could be done to improve compliance rate is to set appropriate rules for the game as well as to determine the optimum inspection frequency and amount of penalty. The objective of the agency is to maximize the compliance rate across the industry subjecting to the constrained enforcement budget available.

To be more specific, first all firms are divided into two groups: G_1 (Good Group) and G_2 (Bad Group). The agency has four parameters to use: the size of fine f_1 and f_2; the inspection frequency p_1 and p_2. Of course, f_2 is greater than f_1 and the inspection frequency p_2 is greater than p_1. Firms within both groups could move from one to another based on the action they choose. In fact, a firm may adopt the strategy of complying in both groups f_{cc}, violating in both groups f_{vv}, complying when in G1 while violating in G2 f_{cv} and violating when in G1 while complying in G2 f_{vc}. A rational firm will not only comply because of the expected penalty but also because they want to stay/move to G1 to get the chance to cheat. This provides additional incentive for the ships to comply even when compliance cost exceeds the expected penalty. The payoff matrix for the enforcement game is as follows:

Table 1 Payoff matrix for the enforcement game [5]

	G1		G2	
	Comply	Violate	Comply	Violate
No inspection	c	0	c	0
Inspection	c	f_1,to G2	c,P(toG1)=u	f_2

Meanwhile, the transition probabilities are as follows:

Table 2 Transition probabilities[5]

	COMPLY		VIOLATE	
	G1	G2	G1	G2
G1	1	0	1- p_1	p_1
G2	p_2u	1- p_2u	0	1

In the long term, the transition from G1 to G2 or from G2 to G1 is a Markov process, which means

that the best strategy to adopt is independent of the initial group the firm is in. According to Harrington, The optimal strategy an agency could take is to set the penalties f_1 to be 0 and $f_2$to be K, which is the maximum allowable fine. As to the inspection frequencies, p_2 must be at least Zc/K, where Z is the target compliance rate, c is the compliance cost and K is the maximum allowable fine. In cases where the compliance cost is larger than the maximum allowable fine, then the maximum feasible compliance rate Z is K/c, which also requires p_2 to be 1.

4. Risk assessment and enforcement targeting

Risk assessment is an important decision supporting tool in ballast water management, since not all ballast water discharges pose the same level of bio-invasion risks. The main risk determinants of the vector are: the volume of the released ballast water, the ability of species to survive the voyage, the voyage duration, and the frequency a ship releases ballast water originating from the same donor port (van der Meer 2012) van der Meer 2012). IMO Guidelines for Risk Assessment Under Regulation A-4 of the BWM Convention (G7, 2007) recommend three risk assessment methods: Environmental matching risk assessment, which compares environmental conditions such as salinity, temperature and nutrients between the donor and recipient port; Species' bio-geographical risk assessment, which regards the overlap of species as an indication of environment similarity; Species-specific risk assessment, which evaluates the probability of invasion by historically identified target species.
To avoid unnecessary requirements (burden) for vessels, the selective approach recommends that appropriate BWM measures should vary depending on different levels of risk posed by the intended ballast water discharged. In one instance, such ships may be exempted from BWM requirements provided that the level of risk of such a discharge is acceptable (David and Gollasch 2011) David and Gollasch 2011).
The results of risk assessment could also be integrated into the enforcement targeting model described above. One possible way is to use the dynamic model within each risk groups. That is, to set different compliance rate goal for different risk groups and therefore allocate different amount of enforcement resources accordingly. For example, if the ships are classified into high/medium/low risk groups, the highest compliance rate Z_h will be set for the high risk groups, medium compliance rate Z_m for medium risk groups and lowest compliance rate Z_l for low risk groups. Using the enforcement targeting theory above, it is possible to calculate the optimum inspection rates p as well as the amount of penalty for 'good' groups and 'bad' groups within each risk groups. In this way, inspection costs are further reduced than to treat all ships uniformly.

5. Conclusion

In conclusion, with the ballast water management convention coming into force soon, the monitoring, compliance and enforcement issue becomes more and more important. The traditional way of enforcement doesn't differentiate between firms. For this mode to be successful, the regulator has to employ a lot of inspection resources and to set high level of penalty for violations. However, by using targeting methods which treats the firms that violate more seriously than those comply in the previous period, compliance level could be improved at an enforcement cost as low as reasonable possible.

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session A4

New Scientific and Technological Development in BWM

Ballast Water Treatment Boat (BWTBoat) –Viable Alternative for Ballast Water Management

Sandip Vitthal Patil | Indian Register of Shipping

Rapid Treatment of Ship's Ballast Water with Medium Salinity Using Hydroxyl Radicals Based on IMO Guidelines

Mindong Bai | Xiamen University, Dailan University

Importance of QA & QC in BWMS Approval process

Sathrugnan Karthikeyan | TUV SUD Asia pacific Pte Ltd

Ballast Water Treatment Boat (BWTBoat) -Viable Alternative for Ballast Water Management

Sandip Vitthal Patil[1]

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Introduction

Every year almost more than 10 billion tons of ballast water is exchanged between different ports through International Shipping trade. As design of ballast free ships is not at all possible, the only solution in front of world environment community was to treat the ballast water before discharge. During 1992 UN Conference on Environment (UNCED) and 2002 World Summit on Sustainable Development (WSSD), IMO had been requested to develop rules on ballast water discharge. Thus with united efforts of different countries, the **International Convention for the Control and Management of Ships Ballast Water & Sediments** was adopted by consensus at a Diplomatic Conference at IMO in London on Friday, 13 February 2004.

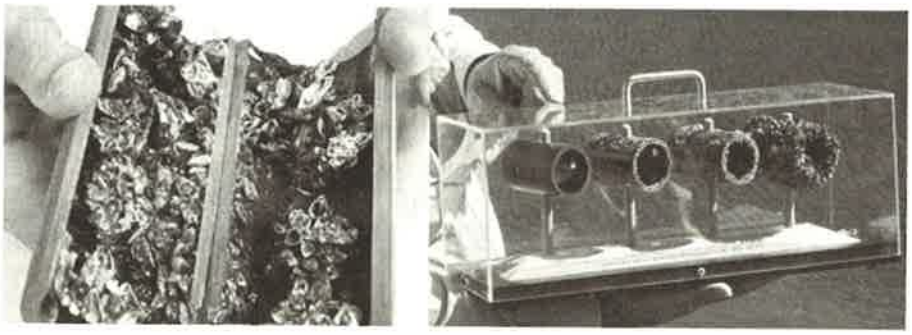

Figure 1. Left photo shows the spread of zebra mussels inside pipe and right one shows the growth of mussels on pipe over a period of time

Current Method of Ballast Water Management

As the only source of ballast water is ships, one unanimous solution emerged as a practical measure, that ships would be fitted with onboard Ballast water treatment system (BWTS). Thus in coming years almost 75000 ships need to be fitted with BWTS to treat water before discharge. The retrofitting cost along with BWTS installation can range from 1 to 3 million dollars per ship.
There are number of problems yet to be resolved with respect to convention's implementation in real world scenario. The main problems are related to real world efficacy of systems, effective implementation of convention and economics. Due to these challenges the ratification is still pending by 4.68% out of 35% tonnage requirement.

Key challenges faced by Shipping Industry & Need of Alternative for Effective Implimentation

- High capital expenditure ($1m to $3m per ship) for BWTS retrofitting of the existing ships, without direct financial return doesn't seem to be sound economic decision

- How one treatment plant can treat all port waters successfully to comply with D-2 standard? Non compliance penalty issue & real world biological efficacy of systems.
- How Class Societies, Port State Control officials will complete survey and certification, sampling and testing of these vast number of vessels every year?
- The % use of BWTS per year against voyage days doesn't look convincing for installation from the huge investment point of view.

Keeping the interests of ship owners, treatment manufacturers, ship yards, port states, Class and IMO in mind, author has developed an Innovative solution of Port based mobile Ballast Water Treatment Boats (BWTBoats) to overcome above challenges. The viability of the BWTBoat in comparison with present approach of convention is described in the following sections of the paper.

Basic theme & overview of BWTBoat Concept

Most of the ships are spending considerable time at sea than the ports and number of ports in turn number of berths there in, are quite lesser than the total number of ships. So it is possible to provide Port-based Mobile Ballast Water Treatment Boats (BWTBoats) to cater service to ships for Ballast Water Management on the shared basis. Thus it eliminates the option of fitment of Ballast water treatment systems onboard Ships. Though IMO came long ahead with the approach of onboard fitment of Ballast Water Treatment Systems, by this reinvented concept of BWTBoats, we can atleast cover Regional and Coastal Trading Ships. This will reduce the burden of 78500 ships for Global Implementation of the BWM convention. Even for some ship types like LNG carriers, we can think of Global use of BWTBoats. Also, to avoid detentions of ships due to non compliance at discharge ports, BWTBoats can be used as contingency measure. From technology point of view, as the Ballast Water treatment systems fitted on the BWTBoats can be customized with respect to ballasting port water quality, there will be lesser chances of non compliance as well as better marine environmental protection at discharge port.

Description for New Approach

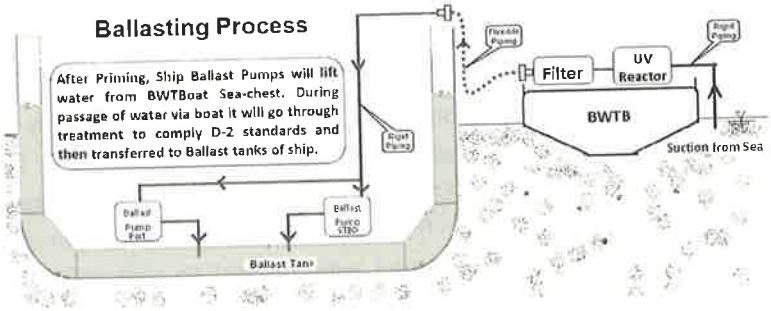

Figure 2. Schematic diagram shows the process of Ballast Water management using BWTBoat

In this concept when ship arrives at port for simultaneous cargo unloading and ballast operation, one boat called Ballast Water Treatment Boat (BWTBoat) will come along side the ship. There will be a modular ballast water treatment system along with control panel, powering generator and necessary piping hose connections on this boat. The crew on the boat will connect the pipe hoses to the suitable ballast water disharge valve on ship deck with flexible pipes. After mooring piping connection and priming, ship ballast pumps will perform ballasting via this boat. So during the passage of ballast

water over the boat the treatment would be carried out to comply with D-2 standard of BWM convention. Here UV based treatment system is taken for example only.

Customisation of Treatment System- Best feature of BWTBoats

In the current approach of BWM convention, treatment systems are getting type approval on the basis of testing with virtually prepared samples covering different range of salinities, temperatures and suspended solids but not with real sea water. So it is one of the biggest hurdle for investment by ship owners that whether these type apporved systems will be able to treat all port waters successfully? Solution to this problem is customisation and BWTBoats can be deployed on this principal.
While deploying a BWTBoat for particular port, the treatment system filters or disinfection units like electrolysis or UV can be chosen or customised with respect to the subject port water. Thus it ensures better protection to environment as well as no fear of non compliance.

BWTBoat Deployment Options

According to berth type based on cargo operation & treatment technology, the options are as follows:

Option 1- {Filtration + Electrolysis/Cl2} Boat at ballasting site + [TRO Neutralizer + UV (optional)] Boat at deballasting site

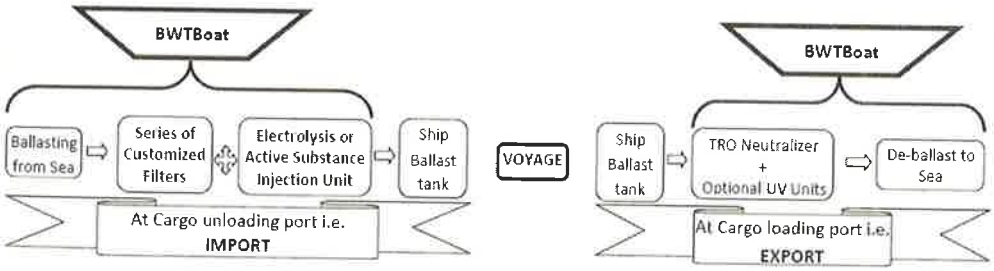

Figure 3. Details of treatment system on BWTBoat with respect to port & overall process

Ships can also fit TRO-neutralizer unit onboard to avoid dependency over deballasting port facility. This option can be utilised for Oil Tankers (11000-world fleet) and Bulk carriers (9800-world fleet).

Option 2- {Filtration + UV} Boat @ballasting site + {Filtration + UV} Boat @deballasting site

Ships such as container ships (5500), general cargo vessel (16000) and car carriers having lesser voyage time and frequent loading & unloading, need a treatment system which can provide immediate disinfection. UV radiation treatment is the best option for this purpose. Various customized options can also be arranged based on the ship-segment and port characteristics by adopting this approach.

Number of BWTBoats for Implementation

Global Study

Number of berths in a particular port, are very important to calculate the number of BWTBoats. There are approximately 2500 ports in the world connected with International Trade. Study of the World's

top 150 busiest ports wrt handling of deadweights, was carried out. Total number of berths in all global ports can be as follows: = 10650 (for 150 busiest ports) + 23500 (for remaining 2350 ports) => 34150 berths. Based on study, an average of 63% berth occupancy has been observed. Hence number of BWTBoats required globally (approx.) = 10650 * 0.63 + 23500 * 0.63 => 6603 + 14570 => 21173. This number is much lesser than the onboard fitment of the BWTS over the 75000 vessels. Hence by adopting this approach total investment will be much lesser.

Regional Study

World shipping trade can be divided into as many 16 regions as follows

1	Australasia	9	South East Asia
2	Caribbean	10	UK - Continent - Baltic
3	East and South Africa	11	US East Coast
4	East Coast South America	12	US Gulf
5	Far East	13	US West Coast
6	Great Lakes - St. Lawrence	14	West Africa
7	Gulf - Red Sea - India	15	West Coast South America
8	Mediterranean- black sea	16	Norwegian Sea

Some ships ply all over the world where as some ply among only one or more regions. In current study, analysis of ship movements and port calls has been done for **Gulf-red sea-India-Far east-South east Asia-Australasia** region using **IHS Fairplay Database**. In Asian region there are approximately 31000 ships and 1063 ports in 27 countries. So project outcome gives the number of ships which are only plying in the above Asian region. Also project outcome gives the number of BWTBoats required to be deployed in all the regional ports for catering the Regional Ships. Thus regional ships can get exemption from onboard fitment of Ballast Water Treatment Systems by deploying sufficient numbers of BWTBoats for Ballast water management on shared basis.

IMO SUBMISSIONS

MEPC 65 Submissions and Outcome

A brief concept paper was submitted by INDIA to IMO on 22nd March 2013. The paper ref. no is MEPC65/2/20. Ballast Water review group invited INDIA to submit detailed report with operational details, suitability of the concept and survey & certification of BWTBoat

MEPC 66 Submission

A detailed report containing technical as well as operational explanation about implementing BWTBoat cocept will be submitted by INDIA to IMO-MEPC-66. Report will also include **Guidelines for Floating Mobile Ballast Water Treatment Facilities** and Survey-Certification of BWTBoats.

Conclusion & Implmentation Process

Step 1- With data analysis of ship movements and port international consortium or regional Port MoU can decide number of ports where BWTBoat can be deployed.

Step 2- With respect to ship traffic over years, Risk assessment of a particular port will be done

to identify number of BWTBoats and dangerous species need to be disinfected at uptake.

Step 3- Port will invite BWT manufacturers to give demonstration for proving the compatibility of their equipment to comply with D-2 standard. Port, Ship owners associations and Class societies can decide customized designs to suit the port water for better disinfection

Step 4- Port authority may invite the tender for 'x' number of BWTBoats with above customized configuration.

Step 5- The investment will be done by either local ship owners or international owners or port itself.

Step 6- The BWTBoats will be deployed according to the special guidelines issued by MEPC BWM convention for BWTBoats.

Step 7- BWTBoat start giving service to ships and charging fee in proportion to power spent for running ballast water treatment plant.

Thus **BWTBoat** approach is an **UNITED SHARED GREEN** approach for achieving **Environmental Protection** as well as **Sustainable development Goal** of **IMO**.

Rapid Treatment of Ship's Ballast Water with Medium Salinity Using Hydroxyl Radicals Based on IMO Guidelines

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Invasive aquatic species are considered to be one of the greatest threats to global marine bio-diversity and ecosystems, as well as a significant threat to coastal economic and public health. Accordingly, it is now widely recognized that the discharge of ship's ballast water is the main cause for the worldwide transfer of harmful marine organisms. There are 3000–4000 types of aquatic invasive species in ballast water transferred per day worldwide, 0.3 billion live *dinoflagellate* resting spores in only one ballast tank, and 2.25×10^4 cells/mL phytoplankton spores in the sediments of ballast tank beds. The International Convention for the Control and Management of Ship's Ballast Water and Sediments was adopted by the International Maritime Organization (IMO) in 2004, and discharge standards (Regulation D-2) limiting maximum concentrations of living organisms that could be released with ballast water have been proposed. To achieve the above discharge standards, technology developers and manufactures around the world are advancing onboard ballast water treatment systems that use methods such as filtration + UV radiation, ozonation and chlorination. However, certain problems are associated with these methods. For UV radiation, two grades of mechanical filtration are usually used for the treatment of ballast water, which results in very high energy consumption. Additionally, the UV lamps used for this process need to be cleaned periodically and are easily broken. Ozonation is considered to be effective for ballast water treatment, but produces bromate and other undesirable by-products that may cause adverse environmental impacts. For electrolysis of seawater, the efficiency is affected by water conditions (e.g., salinity, pH, temperature), and the treatment effect on medium-salinity seawater is especially poor due to insufficient sodium hypochlorite.

In the present study, the rapid treatment of ship's ballast water with medium salinity was achieved with hydroxyl radicals (•OH) generated from a strong ionization discharge (SID) combined with numbers of micro-streamer and micro-glow discharges. As shown in Fig. 1, in the micro-streamer channels, parameters such as the electric field, electron energy, and electron density increase sharply in several tens of nanoseconds. As a result, the electron density reaches levels up to 10^{14} cm^{-3}. In the micro-glow discharge channel, there is very strong electrical field intensity in the cathode fall zone, which exists continuously throughout the discharge cycle. The secondary electron emission similar to γ processing is triggered in the cathode surface, and the electron density of non-equilibrium plasma discharge is increased. When alternating between the micro-streamer and micro-glow discharge modes, a strong ionization discharge is obtained at atmospheric pressure. In the electric field of strong ionization discharge, electrons could obtain sufficient energy to break down the chemical bonds of gas molecules.

When the SID discharge method is used, the electrons have an average energy of 10 eV, which results in enough electrons to produce sufficient energy to dissociate O_2 (dissociation potential, 8.4 eV) or ionize O_2 and H_2O in the gas state (ionization potential, 12.5 and 12.6 eV, respectively). High

concentrations of reactive oxygen species such as O_2^+, $O(^1D)$, O, O_2^-, $O_2(a^1\triangle_g)$, and O_3 are produced in the SID plasma reactor. The oxygen species, O_2^+, O_3, O_2^-, H_2O^+, and H_2O_2, were transferred into the gas/liquid dissolver with a portion of the ballast water to form dissolved •OH. The •OH in seawater undergo very complicated chain reactions that result in their rapid (ns) conversion into other active pieces such as HO_2^-, $O_2•^-$, $O_3•^-$, $HO_3•$, and $O_2^+H_2O$, which are collectively referred to as total reactive oxidant (TRO). High-concentration TRO was then injected into the liquid/liquid mixer in the main pipe to kill harmful organisms and pathogens while conveying medium salinity ballast water.

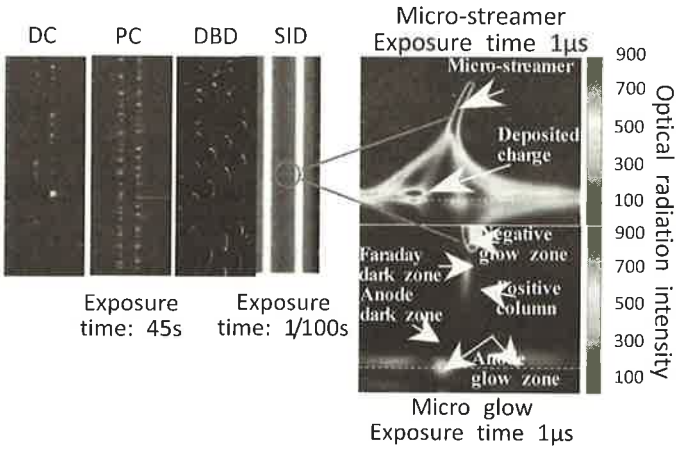

Figure 1. Comparison photos of several gas discharge intensity

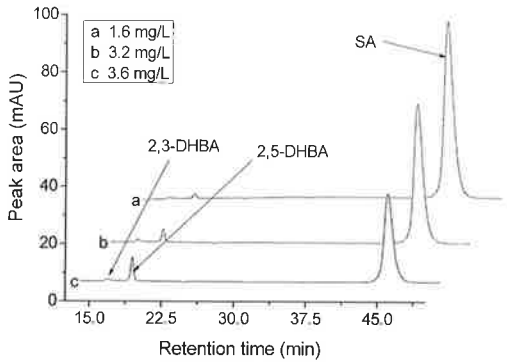

Figure 2. Liquid chromatogram of SA hydroxyl compound

Table 1. Data for Killing the Introduced Organisms in Ship's Ballast Water

Introduced Organisms	0h		48h		120h	
	Control (×10 cells/mL)	·OH Treated (cells/mL)	Control (×10 cells/mL)	·OH Treated (cells/mL)	Control (×10cells/mL)	·OH Treated (cells/mL)
TRO (mg/L)	----	1.7±0.01	----	0.36±0.01	----	ND*
K.mikimotoi	200±10	0±0	150±8	0±0	70±4	0±0
P. micans	200±10	1±1	160±7	0±0	80±6	0±0
S.costatum	210±7	3±1	170±10	0±0	120±6	0±0
T.rotula	200±12	0±0	170±9	0±0	120±9	0±0
H.akashiwo	210±12	1±1	160±9	0±0	120±8	0±0
Total	1020±21	5±1	810±18	0±0	510±11	0±0
Bacteria	Control ($\times10^2$cfu/mL)	·OH Treated (cfu/100mL)	Control ($\times10^2$ cfu/mL)	·OH Treated (cfu/100mL)	Control ($\times10^2$ cfu/mL)	·OH Treated (cfu/100mL)
E. coli	52±9	25±2	49±7	10±1	46±9	10±1
I.enterococci	31±4	12±3	30±7	7±2	27±3	5±1
Heterotrophic bacteria	158±14	150±16	156±10	51±6	153±7	42±4

* ND = not detected.

To measure the •OH radicals in ballast water, SA was applied in the gas/liquid dissolver. The rapid reaction between SA and •OH radicals resulted in the formation of 2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-dihydroxybenzoic acid (2,5-DHBA). The •OH concentration was then determined by measuring the production of these two compounds through high-performance liquid chromatography. As shown

in Fig. 2, the peaks of 2,3-DHBA and 2,5-DHBA could be easily identified upon liquid chromatography, confirming that •OH radicals existed in ballast water. In this experiment, the concentrations of injected TRO were 1.6, 3.2, and 3.6 mg/L, respectively. As more TRO were injected, higher peak areas of 2,3-DHBA and 2,5-DHBA appeared upon liquid chromatography. Overall, these findings indicate that the concentration of TRO is very important to the production of •OH radicals.

A series of •OH treatment experiments for ship's ballast water with medium salinity were conducted in a 10 t/h of treatment system based on IMO Guidelines. Five species of algae from three different phyla and three types of bacteria were used in these experiments. As shown in Table 1, the initial total algae content was 1.02×10^4 cells/mL. After •OH treatment, the content was reduced to 5 cells/mL, which is below the D-2 discharge standard of the IMO (<10 cells/mL). After 2 or 5 days of storage, no living algae were detected in the treated tank, while there were 0.81×10^4 and 0.51×10^4 cells/mL of algae, respectively, in the control tank. The concentrations of *E. coli*, I. enterococci, and heterotrophic bacteria after •OH treatment were greatly decreased to 25, 12, and 150 cfu/100 mL, respectively. And the concentrations of *E. coli* and I. enterococci were below the D-2 ballast water standard of the IMO for all days investigated. However, the levels of bacteria in the control tank decreased slightly during the 5-day storage period. These results indicate that the organisms in the medium-salinity ballast water could be effectively treated using •OH in compliance with the D-2 ballast water standard of the IMO.

The effect of TRO concentration on the killing efficiency of algae is shown in Fig. 3. When the TRO was 1.5 mg/L, the killing efficiency was almost 100% for the total algae content of 0.5×10^4 cells/mL, while at a TRO of 1.7 mg/L, the killing efficiency was almost 100% for the algae content of 1.0×10^4 cells/mL and at a TRO of 2.0 mg/L, the killing efficiency was almost 100% for the algae content of 1.5×10^4 cells/mL. These results differ greatly from those obtained by Oemcke et al., who reported that an ozone dosage of 5–11 mg/L was required for 10^4 inactivation of the dinoflagellate alga Amphidinium sp. For electrolysis BWTS, a free chlorine dosage of approximately 7–10 mg/L is required to meet the ballast water discharge standard according to the application dossier approved by the MEPC. When compared with chlorine and ozone treatment of ship's ballast water, the •OH radicals could kill the oceanic algae with higher killing efficiency at a lower dosage of 2.0 mg/L.

Fig. 4 shows the effects of •OH radicals on different algae after various lengths of time. When the •OH killing time was 6 s at sampling point F, the remaining four species of algae were reduced to <10 cells/mL (D-2 ballast water discharge standard) with almost 100% killing efficiency. Different findings were reported by Juretic et al., who showed that 100% mortality never occurred within 24 h of ozonation for inactivation of non-indigenous species in seawater. However, we found that effective killing of the oceanic organisms in the course of conveying ballast water on board by •OH treatment required 6 s. This occurred because biochemical reaction of •OH radicals belongs to the dissociative radical reaction, and the reaction rate constant of •OH radicals is 10^9 L/(mol·s), which is 10^7 times higher than that of O_3 and 10^{10} times higher than that of chlorine.

The qualities changes of medium-salinity ballast water after •OH treatment were investigated in this study. The salinity, temperature, and pH were almost completely unchanged after •OH treatment and 5 days of storage. However, as shown in Fig. 5, the TSS of medium-salinity ballast water was greatly reduced from 63.3 to 11.6 mg/L (81.7%) after •OH treatment, after which it was continuously slightly reduced to 11.4 and 10.5 mg/L within 2 and 5 days of storage. The TOC was greatly decreased by

63.1% from 12.93 to 4.77 mg/L after •OH treatment, then continuously decreased slightly to 4.49 and 4.31 mg/L after 2 and 5 days of storage. The DOC and POC were also sharply reduced after •OH treatment. These findings indicate that the quality of ballast water with medium salinity and heavy pollution was greatly improved. The entire treatment process was in accordance with the principle of advanced oxidation technology (AOT).

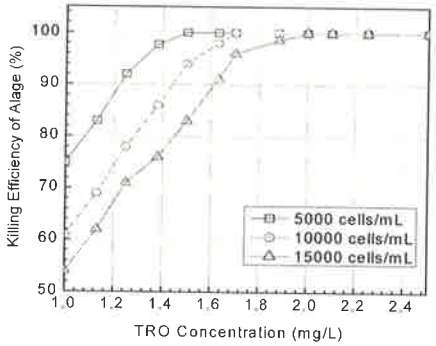

Figure 3. Effect of TRO concentration on killing efficacy

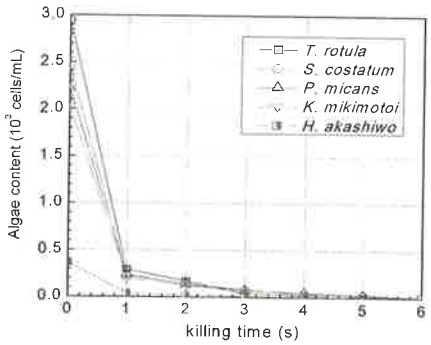

Figure 4. Effect of •OH killing time on different algae

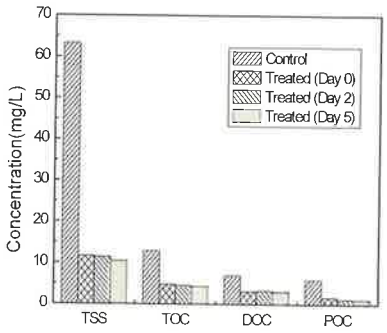

Figure 5. Quality Changes of Medium Salinity Ballast Water in control and treated ballast water on days 0, 2 and 5

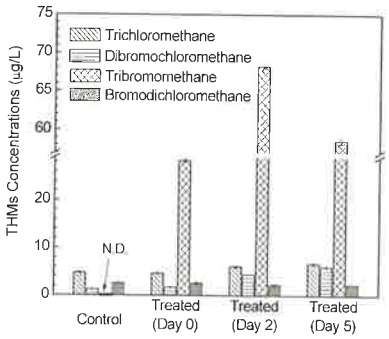

Figure 6. Concentrations of trihalomethanes in control and treated ballast water on days 0, 2 and 5

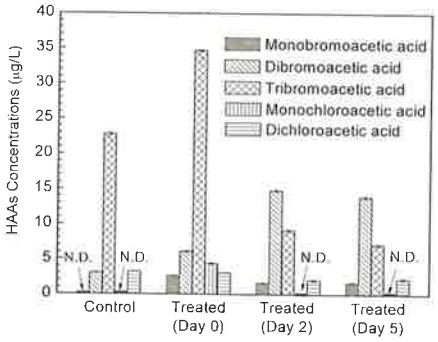

Figure 7. Concentrations of haloacetic acids in control and treated ballast water on days 0, 2 and 5

The possible relevant chemicals (RCs) produced from the reactions between •OH and natural organic matter were also analyzed. The levels of trichloromethane (TCM), dibromochloromethane (DBCM), dichlorobromomethane (DCBM) and tribromomethane (TBM) are shown in Fig. 6. The concentrations of TCM, DBCM and DCBM were not changed significantly after •OH treatment (day 0), or during the 5-day storage period. However, the concentration of TBM was immediately increased to 28.3μg/L after treatment, and then increased to the maximum of 68.2 μg/L after 2 days of storage, and decreased to 58.5 μg/L after 5 days of storage. Overall, the measured levels of the four THMs were within the World Health Organization (WHO) drinking water standard in all samples (TBM =100 μg/L, DBCM = 100 μg/L, DCBM = 602 μg/L, TCM=300 μg/L).

The levels of the five detected haloacetic acids (HAAs) are shown in Fig. 7. After •OH treatment, all HAAs except for dibromoacetic acid (DBAA) decreased with increasing storage time. The concentration of DBAA was 6.08 μg/L immediately after treatment, increased to the maximum of 14.8 μg/L after 2 days of storage, and then decreased to 13.9 μg/L after 5 days of storage. The total concentration of HAAs was 50.8 μg/L immediately after treatment, which is within the WHO drinking water standard (sum of MCAA, DCAA and TCAA = 270 μg/L).

In summary, five species of algae from three different phyla and three types of bacteria were killed by •OH radicals in compliance with the D-2 ballast water discharge standard. Ship's ballast water could be rapidly treated onboard during ballast water discharge with only 6 s required. Meanwhile, the quality of ballast water with heavy pollution was greatly improved. Furthermore, the concentrations of the measured RCs were within the World Health Organization drinking water standard, indicating that ballast water after •OH treatment is safe to oceanic environments. Compared with the current methods, the method of •OH treatment of ship's ballast water developed herein is an effective technology that is practical for application in oceanic ships in the future.

Keywords: *strong ionization discharges, hydroxyl radical, ship's ballast water, medium salinity, D-2 ballast water standard, killing time, water quality, relevant chemicals*

Importance of QA & QC in BWMS Approval process

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Background:

Shipping plays a major role in world trade, carrying approximately 90% of all internationally traded goods. Ballast water is critical for the stability and structural integrity of a cargo ship when it is partly or completely empty. But as ships fill up their ballast tanks, they take in water surrounding the ship – and with it come the organisms living in that water. These unwanted stowaways – marine and freshwater fish larvae and small fish, crustaceans, algae, invertebrates, and even viruses and bacteria – are then let out into a new environment when the ship reaches the next port and discharges its ballast to load cargo. The introduction of new species can often expand unhindered and have large and detrimental consequences on the new host ecosystem, affecting the productivity of fisheries and aquaculture, as well as the economy and livelihoods of communities dependent upon the invaded area's biodiversity. In 2004, the International Maritime Organization's (IMO) has introduced a new *Convention on the Control and Management of Ships' Ballast Water and Sediments* (Ballast Water Convention) which is widely accepted by governments and the global shipping industry as the only international instrument that can prevent trading ships from continuing to spread harmful invasive species via transfers of unmanaged ballast water.

Eversince ballast water management convention adopted (2004), there is a constant increase in number of ballast water treatment systems and their approvals. A company offering a treatment process must have the process approved by Flag administration before they can be installed in any ships. The approval is done through a lengthy testing scheme (both land based and shipboard) by an independent third party organization (facility). The testing of ballast water treatment system have numerous requirements (intake water quality, system flow rate, run time, holding volume, holding time, chemical and biological analysis etc) to fullfil in order to accept their test result. A small error in any one of the step may affect the integrity of the final result. Therefore, it is critical to adopt stringent Quality Assurance-Quality Control protocols in every testing organization to ensure that the findings coming out of the test facility is quality assured, reliable and fit for the intended purpose. Final verification by Flag adminstration or classical society is allowing another layer of verification before the final acceptance of the test results. This paper will present the important QA-QC elements that are normally adopted in a testing facility and discuss their relevance. The paper will discuss about Quality objectives and data indicators. In particular, It will be discussed how the quality objectives and data indicators are set for a testing facility. Further the interpretation of these QA-QC data will be discussed with some classical examples.

Methodologies:

The QA process involves establishment of proper Quality management system, its implimentatin and

monitoring to evaluate its effectiveness. Quality control is mostly considered as part of monitoring process to ensure its conformance against the requirements or set objectives. For QMS, ISO 9001 is widely used by testing organization. Further, the organization might consider additional standards such as ISO 17025/GLP for laboratory related activities. The factors verified during testing include biological treatment performance, operational and maintenance, predictability and acceptability and environmental acceptability/safety. Monitoring and evaluation are normally performed through internal and external audits.

Quality objectives and quality data indicators should be established as part of QA process in every test facility. As part of monitoring and evaluation, the actual set of performance data should be verified against Quality Data Indictors. The entire work flow process should be verified thoroughly to ensure that the final results are quality assured and fit for the intended purpose.

Discussion

BWMS approval consist of both land based testing to test the system's biological efficacy as per D2 discharge standard and shipboard testing to confirm that the system is workable in real environmental condition. The testing and approval process will take 6 – 12 months depending upon the stability of the system performance. The typical approval process is shown in Fig 1.

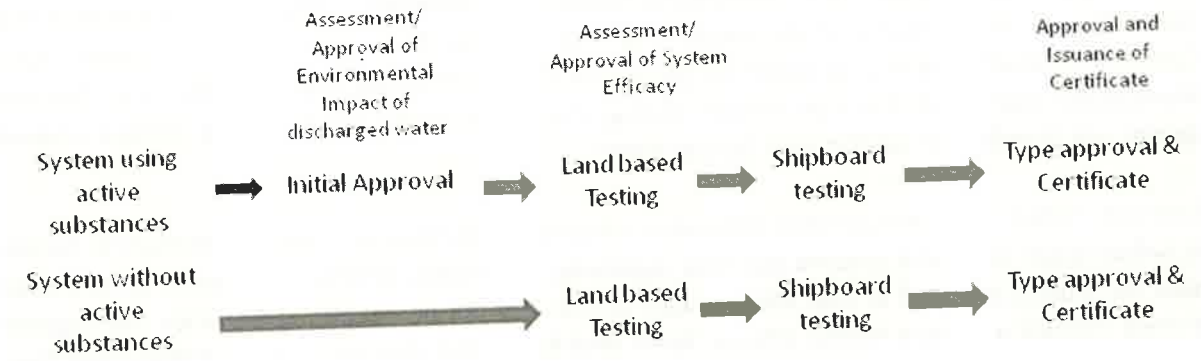

Figure 1. Summary of Approval pathway of BWMS

QA-QC involves data verification, data validation and data integrity. Data verification needs to be performed for all activities related BWMS testing. This includes SOPs, training record, sample collection, analysis data, etc. The internal verification as part of routine QA-QC and external verification as part of external audit are required. Data validation is typically performed by person(s) independent of the activity which is being validated. The appropriate degree of independence is an issue that can be determined on a program specific basis. At a minimum, it is preferable that the validator does not belong to the same organizational unit with immediate responsibility for producing the data set. Data integrity is to make sure that the data were generated through actual performance. Data validators should watch for signs that may indicate improper field and laboratory practices. The following sections provide examples of abuse and warning signs that a data validator should recognize.

Data verification tools can be anything that will allow the verifier to ensure conformity against the requirement and completeness. The tools can be as many as possible such as sample log sheet, sample raw data sheet, final results calculation etc. These checks should allow us to confirm consistency and

acceptable QC results. Data suitability should be checked against quality objectives and quality data indicators. If the results are deviated, the corrective measures taken to assure quality of the final results should be verified. In summary, QA-QC elements are critical in a BWMS approval process. If proper QA-QC objectives and indicators are not established, the interpretation of data and acceptance will be difficult and therefore the quality of final may be questionable. Therefore it is important to establish these objectives and indicators. Similarly, QA-QC protocols should be strictly followed and verified internally and externally to ensure the results coming out of a testing facility are quality assured.

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session B3

Latest Information on Biological Invasion and Their Impacts

Establishment of National Ballast Water Information System and Analysis of Ballast Water Discharge in Republic of Korea

Eun-Chan KIM | Korea Institute of Ocean Science & Technology

Mnemiopsis leidyi Invasion into the Caspian Sea, Vulnerabilities and Mitigation Measures

Reza Shahifar | Iran Fisheries Organization

High search efforts to discern foreign risky species through port baseline surveys at international Korean seaports

Jung-Hoon Kang | Korea Institute of Ocean Science & Technology

Establishment of National Ballast Water Information System and Analysis of Ballast Water Discharge in Republic of Korea

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In order for the port states to manage ship's ballast water discharge in accordance with the IMO Ballast Water Management Convention, it is necessary to understand the amount, frequency and details of the ballast water discharge. According to the Article 5 (the Entry Report) of the Ballast Water Management Act of Republic of Korea [1], all of incoming ships need to report their ballast water discharge. The article stipulates that "Any ship entering into jurisdictional waters after taking up ballast water on board the ship at any waters other than jurisdictional waters shall file an entry report with the Minister of Land, Transport and Maritime Affairs, as prescribed by Ordinance of the Ministry of Land, Transport and Maritime Affairs". This Act shall enter into force on the date on which the International Convention for the Control Management of Ships' Ballast Water and Sediments takes effect in Republic of Korea.

Prior to the implementation of the Ballast Water Management Act of Republic of Korea, Korea Institute of Ocean Science & Technology (KIOST, former KORDI) has been developing various technologies for the national strategy such as establishment of ballast water information system, survey on status of ballast water discharge and monitoring on various species in ports, as part of the Government projects [2]. The national ballast water information system is linked with the geographic information system and, the database consists of shipping records, ballast water discharge records, port environmental data, risky species taxonomic and distribution data and geographic data.

There are three major databases in ballast water information system: Ballast Water DB, Environment DB and Species DB. The ballast water database is comprised of the vessel, shipping and cargo information and ballast water loading/unloading information. All of these information are uploaded on-line in realtime and can be processed by any web browser. The information and the web-format ballast water reporting forms can be accessed from anywhere by internet. And it enhances collaboration process through such an easily accessible information sharing system. The shipping and cargo information could be connected to the Port-MIS of the SP-IDC (Shipping and Port Integrated Data Center) which is a port total information management system. The database is being operated by the Ministry of Oceans and Fisheries of Republic of Korea. They manage records such as ship's first arrival reports, port facility use, controlling conditions, entering and exiting of cargoes, customs, and departure reports.
The environment database consists of various environmental information on ports and coasts, such as the temperatures, salinity, pH, Chl-a, DO, COD, TSS, Silicase, Nitrite, Ammonium et al. These environmental information are updated off-line, by using the data of Korea Hydrographic and Oceanographic Administration, Korea Meteorological Administration for instance or National Fisheries Research and Development Institute.
The species database includes information on various species of both inside and outside of the main ports. The information on these species are updated off-line with the data of annual seasonal monitoring

results and/or reference survey results.
This ballast water information system was applied to the environmental comparison and risk assessment of the ports. KIOST conducted port risk assessments through the GloBallast Program method which had slightly been modified by KIOST, and also species-specific method which was orginally constructed by CSIRO, by using this ballast water information system [3]. Figure 1 shows a sample result of a port risk assessment which was conducted by using the GloBallast Program method.

The reporting of ballast water discharge is not yet mandatory by the law in Republic of Korea as the IMO Convention has not come into effect. Hence, at present, the amount of ballast water discharge and intake cannot directly be calculated but it is only possible to estimate it by multiplying the empirical ratio by the amount of net cargo loading or unloading. KIOST investigated the relationships between the discharge/intake of ballast water and loading/unloading of cargo of various incoming ships at the selected ports throughout last several years.

Based on the survey results, the ratio between the amount of ballast water discharge and the amount of net loading, which is the amount of loading subtracted from the amount of unloading, has been predicted. The amount of ballast water discharge was calculated by multiplying the ratio by the amount of net loading which was provided by the shipping data of SP-IDC of Korea [4].
Figure 2 and 3 show the amount of ballast water which had been discharged at all ports of Korea in 2012 by the ships recently arrived from other Continents or foreign Countries. About 42 million tons of ballast water were discharged at the Korean Ports by the ships arrived from other countries. Among them, 94% were from Asia, 3% from Oseania, 2% from Europe and 1% from North America. Ships from Europe were mostly from far east of Russia.
Figure 4 shows the amount of ballast water discharged by various types of ships arrived from foreign countries at all ports of Korea in 2012 . And figure 5 shows the amount of ballast water intaken by the ships departing to other continents, at all ports of Korea in 2012 . About 179 million tons of ballast water were intaken in Korean Ports by the ships departing to other countries. Among them, 70% were from Asia, 17% from Oseania, 7% from Europe and 3% from North America. It was discovered

that the amount of intaken ballast water were four times larger than the discharged ballast water at the ports in Republic of Korea.

Port States of Republic of Korea will be able to collect accurate statistics of ballast water discharge once the Ballast Water Management Act enters into force and all of the incoming ships obligatorily submit the ballast water reporting form to the Port States.

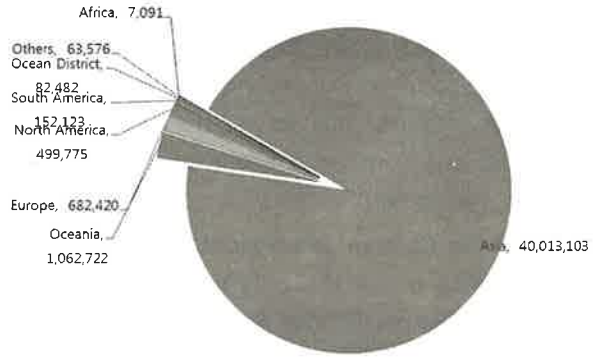

Figure 2. The Amount of Ballast Water Discharge Arrived from Other Continents

Figure 3. The Amount of Ballast Water Discharge Arrived from Foreign Countries

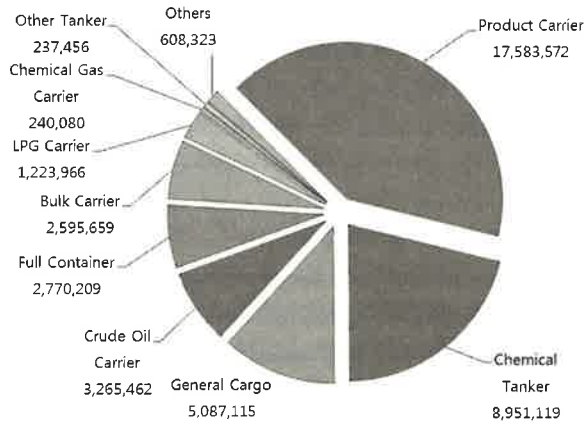

Figure 4. The Amount of Ballast Water Discharge by Various types of Ships from Foreign Countries

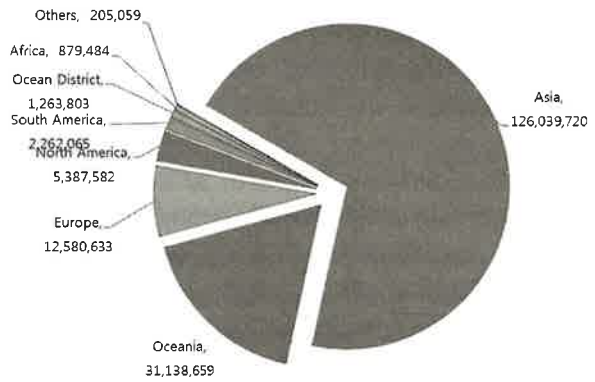

Figure 5. The Amount of Ballast Water Intake by the Ships Departing to Other Continents

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Keywords: *Ballast Water, Information System, Discharge, IMO, Port State*

Mnemiopsis leidyi Invasion into the Caspian Sea, Vulnerabilities and Mitigation Measures

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Abstract:

In 1998 fishermen reported unknown gelatinous species in the Caspian Sea which it was identified as *Mnemiopsis leidyi (M.leidyi)* in November 1999. The *M.leidyi* is not the only invasive species in the Caspian Sea, but this is the species which most negatively impacted the state of the sea since 2001. Base on *M.leidyi* biology, this species originally belong to the west part of North and South America, which was transferred into the Black Sea in 1980 and then Caspian Sea in 1998, by ballast water. The long term effects of *M.leidyi* in the sea are yet difficult to predict but it has significantly changed the physical, chemical and biological parameters of the Sea.

By the summer 2000 *M.leidyi* had already well adapted itself in the Caspian Sea and started actively reproducing and spreading widely in the Southern and Middle of the Sea. In October 2000, it was first discovered in the Northern Caspian as well. In 2001, a sharp increase in the abundance of *M.leidyi* was observed in most areas of the Sea. In 2002 the abundance of *M.leidyi* increased even more but in 2003, sharp decrease of *M.leidyi* abundance in the entire Caspian followed due to the cold winter and spring, when water temperature was lower by 1- 2º C compared to previous years. In fact after 2002 when *M.leidyi* spread entirely the sea, its abundance and biomass has decreased and found a stable trend since 2003. This happened most probably because of limited availability of zooplanktons as a food for *M.leidyi*. The mass development of *M.leidyi* especially in the south of the Caspian Sea has been associated with decrease in mesozooplankton density and increase in number amplitude of phytoplankton blooms. According to Iranian scientist's reports minimum 24 different species of zooplanktons disappeared from the Sea while these planktons are the main food of some Clupeidae family. The impact of *M.leidyi* on the Caspian Sea ecosystem has been even worse than in the Black Sea due to the greater sensitivity of this enclosed basin. Adverse impacts from *M.leidyi* could be listed as the following:

1. Again the fish collapse was the most apparent problem in the ecosystem. Striking decreases were observed in the pelagic (mainly sprat Clupeonella spp.) fishery of all countries bordering the Caspian Sea: almost a 50% decrease in the kilka catches of both Iranian, Azerbaijan and Russian fisheries had occurred during 1999 and 2001. During spring and summer of 2001, mass (estimated as 250,000 tons, or 40% of the population) mortalities of sprat were reported at the sea surface (Davis et al., 2003). The fish catch value was halved again in 2002, resulting in great economic losses (Kideys et al., 2004, 2005). Fishermen even stopped fishing during most part of 2003, due to lack of fish (Fazli and Roohi 2003).

2. Sharp decrease in fish catch became a big problem for thousands people earning livelihood from

sprat fishery. The economical loss from sprat fishery alone is hundreds million Euros per year. Most of the fishermen in Iran, who once took loans from banks for starting to a business with promising outlook, cannot now pay their debts and may even end up in prison. Their problem was even at headlines on BBC World TV in 23rd July 2001.

3. Not only pelagic fishes, but also some large predators feeding on these fish such as white sturgeon Huso huso and the endemic Caspian seal Phoca caspica are also suffering from significant population decrease. As reported by the media, the mass deaths of Caspian seals (Phoca caspica) occurred in the northern Caspian Sea during the spring of 2000. There is strong evidence that the epizootic disease observed in seals during the spring of 2000 was caused by under nourishment (Davis et al., 2003). Significantly decreased pregnancy and fat content in seal population were lso reported. The white sturgeon, that is famous for the quality of its caviar, mainly depends on sprat as food Hashemian and Roohi 2004).

4. Biodiversity of the Caspian is important as most of species occur only in this sea all over the world (i.e. endemic). Not only is the quantity of zooplankton reported to decrease sharply, but also the number of species. For example, number of zooplankton (copepod and cladocerans) species during 2001-2002 was only 3 compared to 22 species in 1995 or 1996! The consequences of such reduction could be very significant for the ecosystem (Roohi et al., 2010).

5. Due to decreased levels of zooplankton, eutrophication (too much plant production) started to be a significant problem for this ecosystem. Global chlorophyll distribution obtained via remote sensing display the Caspian Sea as one of the most eutrophic regions in the world in recent years, in contrast to years before *M.leidyi* invasion (Roohi et al., 2008a, b).

In Order to solving the problems during the Caspian Environment Program (CEP) Iranian Fisheries Research Organization emergently accept the responsibility of developing and implementation of series research to find the solution to control of *M.leidyi*. After three years study and research and by use of international and regional experiences, Iran recommended use of biological method for control of *M.leidyi*. Iran recommendation contained a biological control by intentionally introduction of Beroe ovate into the Sea. The cost of works on biological control over distribution of *M.leidyi* in the Azov Sea and the Black Sea basin was evaluated at some millions of dollars or at 1-1.5% of the annual damage sustained merely as a result of the loss of catch. This decision has never consensus among Caspian Sea littoral states and without any usage has remained up to now. The following properties make Beroe ovata the most preferential predator:

- Low salinity tolerance (the lower tolerance threshold of Beroe ovata is between 7.2-4.5% and that of *M.leidyi* – approx. 3%). Since *M.leidyi* penetrates into the Azov Sea every year, causing outburst and dying during autumn-winter period, the major area of fighting it is water area of the Black Sea, which, if judged by salinity tolerance, could be totally acclimatized both by *M.leidyi* and Beroe ovata.
- Reproduction of Beroe ovata begins when its body is 2.5-3.0 sm at the age of one month, fertility of pubertal individuals makes up several thousands of ovum daily (like *M.leidyi*)
- Beroe ovata is an obligate eater of comb-jellies (*M.leidyi* and Pleurobrachia);
- The events of 1997-2000 proved that Beroe succeeds in surviving in conditions of the Black Sea round the year just like *M.leidyi*, i.e. there always exists a possibility of preservation of the population (female culture).

Mnemiopsis leidyi photo by: ROOHI, A. Beroe ovate photo by: S.Vostokov

Information on development cycles of Beroe ovata and *M.leidyi* in a natural habitat as well as observations on biology of invasive species in the Azov and the Black Sea basin enable to formulate the following statements predetermining effective use of *Beroe ovata* as a regulator of *M.leidyi* populations in water basins-recipients:

- prompt and positive effect – sharp reduction of the populations of *M.leidyi* takes place when *Beroe ovata* is numerous (irrespective of the fact whether the majority is presented by large specimens or whitebait);
- Similar dynamics of the populations of comb-jellies under studies is preserved in natural habitats and water basins of introduction: first *M.leidyi* sharply increases its populations, reaching maximum figures, and then close to that maximum level there appear outbursts of *Beroe ovata* which eats away *M.leidyi* within 3-4 weeks.

However after accidental introduction of *M.leidyi* into the Caspian Sea, the catch of these species decreased more than six times during three years. Two species of them completely disappeared from the region and many of fishing vessels and related industries completely bankrupted and closed. Only in I.R.Iran around 150 fishing vessels and 30 factories were closed and 2000 people lost their jobs. Although the amount of prejudice in the Caspian Sea ecosystem and the social economical damages have not calculated but only I.R.Iran government has spent more than 30 Million US$, for buy back of fishing vessels, paying their loans to Banks and compensate the vessels damages and related industries to close their business and stars another jobs. This is notable that Kilkas are one of the main foods of Sturgeons and the only Mammal of the Sea (Caspian Phoca or Caspian Seal) which are located in endangered levels now. Although the amount of annually catch of kilkas and their composition were changed and in comparison with 1998 common kilka was dominated in catch and replaced with Anchovy Kilka but in total the amount of Kilkas catch decreased from 98000 tons in 1998 to around 20000 tons in average and has found a stable amount of catch since 2005.

Nudularia sp. Blooms in August 2005 which distributed in 20000 Km^2

By an ecosystem base management approach the present state of the Caspian Sea is not in a safe condition and is calls for an immediate control of the *M.leidyi* population, which biological control have recommended by Iranian scientists since 2005. The stocks of many fish species is completely have damaged and many zooplanktons disappeared from the sea while happening of alga blooms by distribution of Nudolaria. However, by all means prevention is better than cure. It is much less costly to prevent an introduction than to cope with the consequences and to eradicate an already introduced species. For this reason, besides focused on technology to prevent introductions, developing and implementing some management measures are urgently necessary. Yet, technology will never be able to prevent the introduction of all species. Therefore, it is essential to conduct navigation control and monitoring in a timely and systematic manner in order to combating against any invasion species introductions. For the Caspian Sea Volga Don channel is the only way to enter into the Caspian Sea. For this in this meeting we seriously recommend all the vessels should be controlled before any enter into the Caspian Sea in Volga Don River. Also the prepared plan by I.R.Iran for biological control of *M.leidyi* should be urgently implemented in the Sea by supports of Caspian Sea littoral states and international and regional organization such as UNEP, UNDP, IMO, World Bank, GEF, Tehran Convention and *etc.*

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Key words: Ballast water, *Bereo ovata,* Caspian Sea, Iran Fisheries, *Mnemiopsis leidyi (M.leidyi)*

High search efforts to discern foreign risky species through port baseline surveys at international Korean seaports

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As concern about biological invasions via ballast water grows, our institute has taken several approaches to mitigate intentional and accidental transfer of harmful aquatic organisms and pathogens via ship ballast water. This study was designed to search foreign and harmful species and their environmental characteristics from port environment which is vulnerable to ballast water discharged from commercial ships. Communities of phyto- and zooplankton have been investigated seasonally at seaports of Incheon, Gwangyang, Pusan and Ulsan in Korea from 2007 to 2009. Eleven seaports were investigated seasonally in 2010. Sixty-one species of potentially risky phytoplankton consisted of eight toxic species and fifty-three red-tide species. Distributional features of the red-tide phytoplankton were correlated positively with the physical factors such as temperature, salinity, dissolved oxygen and pH ($p<0.05$). However, distribution of the toxic species was affected positively by chemical factors like nitrate and chemical oxygen demand ($p<0.01$). From the viewpoint of risky species, multiple regression analysis indicated that temperature, salinity, dissolved oxygen and total suspended solid were better predictors of the variation in *Acartia* copepods at the seaports during the study than chlorophyll-*a* concentration when food was not limiting condition. Abundances of *Noctiluca scintillans* observed at the three surveyed ports did not significantly ($p>0.01$) affect the concentration of dissolved oxygen(DO) in the surface layer, indicating that the species abundances were not enough to cause reduction of dissolved oxygen during the study period. Gwangyang seaport is recognized as the most suitable environment for a wide range of *N. scintillans* blooming compared Incheon and Ulsan seaports. And additional scientific endeavor for understanding the potential risk of the bloom-forming species will be presented during the Ballast 2013.

Introduction

A wide variety of coastal ecosystems have been influenced by natural and anthropogenic disturbances, such as diverse climatic features, changes in hydrological characteristics, eutrophication, overfishing, and the introduction of nonnative species. Of these, the introduction of foreign species transported in ship ballast is recognized as a great threat, one that can alter the biodiversity and structure of the receiving aquatic ecosystem. A port environment is vulnerable to ballast water discharged from commercial ships, which contains intake water from foreign waters. Once a foreign population is successfully established in a port, the system may become a source for subsequent introductions through passive range expansion or secondary spread through further human transfer. The problem of invasions in major harbors is a growing concern, but little is known about the occurrence pattern or dynamics of phytoplankton and zooplankton to discriminate foreign species from the community native to the port. The distributional characteristics of normally occurring plankton species and the related

environmental features are indispensable information for distinguishing unwanted species from the plankton community inhabiting in the major ports of Korea. Therefore, as part of the Port Environmental Risk Assessment Technology (PERAT) project, this study investigated the species composition and distribution of plankton, and the related environmental characteristics in seasonal baseline surveys conducted at major seaports from 2007 to 2010. Here, the occurrence lists of potential risky plankton are described on a seasonal basis, and the relationship between environmental variability and distribution of the plankton was examined during the study period.

Methods and materials

Plankton samples were taken on seasonal basis at four seaports during the port baseline surveys of 2007-2009 and eleven seaports during 2010. Baseline surveys were conducted the R.V. "Jangmok" at Ulsan and were carried out using the ships of opportunity at other seaports. Stations for analysis of the spatial distribution in plankton population and its related environmental factors are selected considering characteristics of the inner and outer ports. The number and position of stations sampled at each port were identical. For zooplankton, samples were collected by using same gear (60 cm in diameter and 200㎛ mesh size) of standard type net. The net was hauled vertically through whole depth at a speed of 60 cm s^{-1} at inner stations with shallow depth of approximately 10m, while the net was vertically hauled through the surface mixed layer at relatively deep outer stations. Surveys were made in the daytime and always at the same tidal period, starting at high tide from the innermost station. Filtered volume was calculated from the flowmeter reading with Hydrobios digital meters (Hydro-Bios Model 438-115). After sampling, samples were preserved immediately with borax-neutralized formaldehyde (Final conc. 5%) and identified under a stereomicroscope (ZEISS Model Stemi-2000C). For phytoplankton, seawater was sampled at surface waters and preserved with Lugol's solution at final concentration of 3%. To identify and enumerate diatoms and dinoflagellates, seawater samples were concentrated and analysed under light microscopy using a Sedgwick Rafter counting chamber. Vertical profiles of temperature and salinity were obtained using a SBE 911plus CTD and the data were binned into 1 m depth. Chlorophyll-a (chl-*a*) concentrations in the surface waters were determined using a fluorometer (Turner Designs 10-AU) following Parsons et al.(1984). Seawaters for analysis of chemical oxygen demand were sampled and determined base on Parsons et al.(1984). The total suspended solid matter was measured after filtration through 0.7 μm pore filter, according to APHA (1985). Dissolved oxygen and pH were determined with DO meter (YSI-58) and pH meter (Orion 3-star). For statistical analysis, temperature and salinity were depth-averaged considering the depths towed with zooplankton net. Water samples collected from surface waters were considered for analysis of concentration of chl-*a*, DO and TSS. All statistical analysis was conducted using SPSS 10.0 statistical software packages.

Results and discussion

Total potential risky phytoplankton were summarized into sixty-one species consisting of fifty-three red-tide species and eight toxic species. Thirty-eight diatoms, twenty-one dinoflagellates, one euglenoids and blue-green algae comprised of total risky species. Relatively high number of risky species (35-45) occurred at seaports located in the Yellow Sea, and the next number of species (29-32) did at seaports in the South Sea, and the low number of species (~28) were observed at seaports in the East Sea. At saposrts Incheon, Gwangyang, Pusan and Ulsan from 2007 to 2009, *Skeletonema* spp., as eurythermal and euryhaline species, occurred ubiquitously dominantly at all seaports. Distribution of the species was positively corrrelated with pH and DO cocnetration (p<0.05). And the next dominant species such

as *Chaetoceros debilis, Ch. socialis, Detonula pumila, Leptocylindrus danicus,* and *Thalassiosira nordenskioeldii* were significantly correlated with temperature, salinity, pH, DO, and nutrients ($p<0.05$). *Pseudo-nitzschia pungens* producing domoic acid occurred with high abundances in summer and autumn, and was positively correlated with concentration of nitrate and silicate during the study period. *Alexandrium* sp. producing paralytic shellfish poison occurred at seaports of Incheon and Pusan in summer, with high correlation to COD concentration. *Dinophysis acuminata* producing diarrhetic shellfish poison showed up at seaport Ulsan in summer and autumn, with positive correlation with phosphate and COD ($p<0.01$). Toxin producing phytoplannkton is likely correlated with chemical parameters such as nutrients and COD compared to red-tide species affected by physical parameters like temperature and salinity.

Table 1. Port specific distribution of potentially risky phytoplankton from eleven Korean seaports (IC; Incheon, PT; Pyeongtaek, DS; Daesan, GS; Gunsan, MP; Mokpo, GY; Gwangyang, MS; Masan, BS; Busan, US; Ulsan, PH; Pohang, DH; Donghae, DA; domoic acid, PSP; paralytic shellfish poisoning, DSP; diarrhetic shellfish poisoning, ○; indicates occurrence)

Potentially risk species												
Bacillariophyceae	Character	Distribution										
		IC	PT	DS	GS	MP	GY	MS	BS	US	PH	DH
Actinoptychus senarius	Red tide	○	○	○	○	○	○	○	○	○	○	
Asterionellopsis glacialis	Red tide	○	○	○	○	○	○	○	○	○	○	
Asterionellopsis kariana	Red tide	○	○	○	○	○						○
Bacillaria paxillifer	Red tide	○	○	○	○	○			○	○	○	
Cerataulina pelagica	Red tide				○		○	○	○			○
Chaetoceros danicus	Red tide	○		○	○	○		○				
Chaetoceros debilis	Red tide	○	○	○	○	○	○	○	○	○	○	○
Chaetoceros didymus	Red tide	○	○	○	○	○	○	○	○	○	○	○
Chaetoceros pseudocurvisetus	Red tide		○	○		○				○	○	
Chaetoceros socialis	Red tide	○		○	○	○		○		○	○	○
Coscinodiscus jonesianus	Red tide	○	○	○	○	○						
Coscinodiscus perforatus	Red tide									○	○	
Coscinodiscus wailesii	Red tide			○	○				○			
Cylindrotheca closterium	Red tide	○	○	○	○	○	○	○	○	○	○	○
Detonula pumila	Red tide					○	○		○	○	○	
Ditylum brightwellii	Red tide	○	○	○	○	○		○	○	○	○	
Eucampia zodiacus	Red tide	○	○	○	○	○			○	○	○	
Guinardia flaccida	Red tide	○		○	○	○		○		○	○	
Leptocylindrus danicus	Red tide	○	○	○	○	○	○	○	○	○	○	○
Melosira nummuloides	Red tide	○				○						
Pseudo-nitzschia americana	Red tide		○									
Pseudo-nitzschia delicatissima	Red tide and Toxic (DA)			○	○	○						
Pseudo-nitzschia delicatissima complex	Red tide and Toxic (DA)	○		○	○	○	○	○	○	○	○	○
Pseudo-nitzschia pungens	Red tide and Toxic (DA)	○	○	○	○	○	○	○	○	○	○	○
Pseudo-nitzschia seriata complex	Red tide	○	○	○	○			○		○	○	
Rhizosolenia imbricate	Red tide			○	○				○			○
Rhizosolenia setigera	Red tide		○		○							○
Skeletonema spp.	Red tide	○	○	○	○	○	○	○	○	○	○	○
Thalassionema nitzschioides	Red tide	○	○	○	○	○	○	○	○	○	○	○
Thalassiosira anguste-lineata	Red tide	○		○		○		○				
Thalassiosira curviseriata	Red tide	○	○		○	○	○	○				
Thalassiosira delicatula	Red tide	○	○	○		○	○		○	○	○	○
Thalassiosira eccentrica	Red tide	○	○	○	○	○	○	○	○	○	○	○
Thalassiosira gravida	Red tide	○						○		○		
Thalassiosira nordenskioeldii	Red tide	○	○	○	○	○	○	○	○	○	○	○
Thalassiosira pacifica	Red tide			○		○	○	○		○	○	○
Thalassiosira punctigera	Red tide	○		○	○	○	○	○	○			
Thalassiosira rotula	Red tide	○	○	○	○	○	○	○	○	○	○	○

Dinophyceae												
Akashiwo sanguinea	Red tide	○		○	○	○			○	○	○	○
Alexandrium sp.	Red tide and Toxic (PSP)	○	○					○	○			
Neoceratium furca	Red tide	○			○							○
Neoceratium fusus	Red tide	○		○						○	○	
Chattonella sp.	Red tide	○	○	○	○	○	○			○	○	
Cochlodinium polykrikoides	Red tide	○			○	○						
Dinophysis acuminata	Red tide and Toxic (DSP)			○				○		○		
Gonyaulax scrippsae	Red tide		○						○			
Gonyaulax spinifera	Red tide					○	○					
Heterosigma akashiwo	Red tide	○	○	○	○	○	○	○	○	○	○	○
Karenia brevis	Red tide and Toxic (neurotoxins)						○					
Karenia mikimotoi	Red tide and Toxic (unknown toxin)	○	○	○	○	○		○		○	○	○
Karlodinium veneficum	Red tide and Toxic (unknown toxin)	○	○	○	○	○	○	○	○			○
Katodinium glaucum	Red tide	○			○		○			○	○	
Prorocentrum balticum	Red tide					○						
Prorocentrum minimum	Red tide	○	○	○	○	○		○	○	○	○	○
Prorocentrum triestinum	Red tide	○	○	○	○	○	○	○	○	○	○	
Protoperidinium bipes	Red tide		○		○	○	○		○			○
Protoperidinium leonis	Red tide		○		○	○	○	○		○	○	○
Protoperidinium pallidum	Red tide					○	○					○
Protoperidinium pyriforme	Red tide			○		○						○
Cyanophyceae												
Anabeana sp.	Red tide	○	○		○							
Chrysophyceae												
Dictyocha fibula	Red tide	○	○	○	○	○	○			○	○	

Table 2. Port specific distribution of potentially risky zooplankton from eleven Korean seaports (IC; Incheon, PT; Pyeongtaek, DS; Daesan, GS; Gunsan, MP; Mokpo, GY; Gwangyang, MS; Masan, BS; Busan, US; Ulsan, PH; Pohang, DH; Donghae, ○; indicates occurrence)

Potentially risky species												
Zooplankton	Character	Distribution										
		IC	PT	DS	GS	MP	GY	MS	BS	US	PH	DH
Ctenophore larvae	Strong invasive, Omnivorous						○		○	○		
Thaliacea larvae	Strong invasive, Omnivorous								○	○		
Hydromedusae	Strong predator	○	○	○	○	○	○	○	○	○	○	○
Noctiluca scintillans	Red tide species	○	○	○	○		○	○	○	○	○	○
Acartia omorii	Eurythermal and Euryhaline, Potentially strong transfer ability by dormant eggs	○					○	○	○	○	○	○
Acartia hongi	Stenohaline, Potentially international or intra-coastal transfer	○	○	○	○	○	○		○			
Pseudodiaptomus inopinus	Brackish water species, Salinity is the most important factor to its moment					○						

Among the abovementioned potentially risky zooplankton, distribution of ctenophore and thaliacean larvae, which possess strong invasive and omnivorous characters, are only limited to three seaports. And hydromedusae known as strong predator showed occurrence throughout the all seaports but showed very low abundances that could not mention significant distributional pattern. *Pseudodiaptomus inopinus* with strong invasibility as brackish water species showed very limited occurrence at seaport Mokpo. Thus, *Noctiluca scintillans* and *Acartia* copepods were selected for analysis of correlation with environmental parameters due to their consistent occurrence and high abundances. Here, *Acartia omorii* is distributed widely in coastal and offshore waters of the Yellow, South, and East seas of Korea, Japanese waters, and even in the Southern Bight of the North Sea. The disjunct distribution likely stems not only from the eurythermal and euryhaline characters of *A. omorii*, but also due to transport in ballast water. To avoid unfavorable conditions such as low oxygen, the copepod produces diapause

eggs, which can hatch if conditions become favorable. Diapause eggs produced in the harsh environment of a ballast tank may have played a role in the introduction of *A. omorii* into distant waters via transport in ship ballast water. *A. omorii* is common and widespread in coastal and offshore waters, while *A. hongi* is confined to brackish or coastal waters in the Yellow Sea, indicating that it is endemic to the Yellow Sea. *A. hongi* consistently occurred at Incheon, which is its original habitat. Unexpectedly, *A. hongi* was also abundant at Gwangyang in May and August 2007 and some were found at Ulsan in May 2008. The seaports, which have different regional characteristics based on large marine ecosystem regions (http://www.lme.noaa.gov/), are affected by local river discharges. This disjunct occurrence in Korean waters might have resulted from intraregional transport in ballast water because the copepod was confined to the coastal waters of the Yellow Sea. However, it did not occur consistently in Gwangyang and Ulsan, indicating that the species failed to become established at the two receiving ports. Seasonal distributional patterns of marine dinoflagellate *Noctiluca scintillans* internationally recognized as harmful species and the related environmental factors were surveyed at Incheon, Gwangyang and Ulsan seaports from 2007 to 2009. Abundances of *N. scintillans* observed at the surveyed seaports did not significantly ($p>0.01$) affect the concentration of dissolved oxygen in the surface mixed layer, indicating that the species abundances were not enough to cause reduction of dissolved oxygen during the study period. However, consistent occurrence of high abundances (10,000 to 40,000 inds·m^{-3}) indicated that Gwangyang seaport may provide the most suitable environment for *N. scintillans* blooming compared to other seaports.

Keywords: *Port environment, Phytoplankton, Risky species, Seaport, Zooplankton*

session B4

Installation, Verification, Other Emerging Issues

Lesson learned from Operation Experiences

Mike JW Lee | Techcross

Scaling-up Issues of Ballast Water Treatment Systems and the Need for Verification of CFD Models

Goran Goranović | DHI Water & Environment (S) Pte. Ltd.

Lesson learned from Operation Experiences

Mike JW Lee

Director / Marketing & Sales Div., Techcross

The total number of contracting States to the BWM Convention has now reached 37, representing 30.32% of the world tonnage after ratification by Germany in June and ratification of the IMO Ballast Water Convention is round the corner, and ship owners and operators concerns are everywhere. Not only there are a range of systems to choose from, their operation and performance is still in question, which could cause uncertainty in achieving operability and compliance.

Techcross is one of the frontiers in BWMS market with early Type Approved system based on full-flow electrolysis. However, even though we have delivered the vessels more than 150 installed with ECS, not many owners operate the system regularly. Fortunately, we have been receiving operation track records from a few number of ships operated by owners and from continuous operation of ECS since 2011, we could have ensured the system durability and safety by applying upgraded features considering even mis-operation of ship crew.

The system operation and reliability depend on proper installation of the system by vessel either at E/R , P/R or on-deck space, ballast piping arrangement, and others as system should be carefully installed in consideration of all of various factors. Training and operation by ship crew will be also important factor to ensure safety and compliance.

Techcross would like to share lessons learned from experience on installation and operation of BWMS on following points :

Case study: What has been learned from initial BWMS designs, installation and operation?

Lessons learned from early installation and operational experiences for the Electro-Cleen System by Techcross.

- BWMS is the system not only working as a system itself but also working with many of existing equipments including Ballast Control Panel Ballast pump, valves, level gauge at ballast tanks, Alarm Monitoring System(AMS) and Vessel Management System (VMS) / ICMS (Integrated Control and Monitoring System)

- Before installation of BWMS on board, Thorough review with shipyard design from hull, machinery, electrical division

- Even though we have delivered the vessels more than 150 installed and commissioned with ECS, not many owners operate the system regularly.

- Fortunately, we have been receiving operation track records from a few number of ships operated by owners and from continuous operation of ECS since 2011, we have ensured the system

durability and safety by applying upgraded features considering even mis-operation of ship crew.

- There have been issues on proper sampling of the treated water from main ballast pipeline for TRO sensor units.

- Insulation of electric equipment at E/R. communication line failure, filter clogging, inflow rate changes and others affecting BWMS operation as well as the performance of BWMS to comply with D-2 standards at various water condition

The system operation and reliability depend on proper installation of the system by vessel either at E/R , P/R or on-deck space, ballast piping arrangement, and others as system should be carefully installed in consideration of all of various factors. Training and operation by ship crew will be also important factor to ensure safety and compliance.

- The biggest concern we had was system performance efficacy to comply with the D-2 standards.

- We have taken safety features into the system, however, experience from operation of the system by ship crew demonstrated something could go wrong if they do not operate the system as intended

- ECS is controlled by Window based HMI software from Chief Officer(C/O) at CCR(Cargo Control Room) when the system located and operated at Engine Room(E/R). Bulk carriers often control BWMS from Engine Control Room (ECR)

- Many of vessels operate Ballasting and Discharging by C/O only as unman attended operation is normal procedure these days.

- When the system is stand-by all the time including voyage after stopping the ballasting and system from CCR via communication line, there could be a chance to cause system operation by misconduct or failure of the part or component.

- Two of the vessels installed our system had the failure of system by misconduct and we went back to secure shut-down function even if there is misconduct by ship crew. We take this as a very important factor to be reviewed and required procedure to secure safety of ship and ship crew.

- Safety Assessment review with the Classifications and upgraded safety modifications of Electro-Cleen System.
  - HAZOP with DNV and ABS
  - FMEA and Software verification with KR and ABS
  - Installation of Independent hardware checking device on Ballast Pump and inlet valve and bypass valve signals.
  - HMI (Human Machine Interface) Software verification by 3rd party
  - Additional installation of water level sensor and temperature sensor inside of electrolysis chamber to secure no power supply to the chamber when isolated valves are closed and not filled with water.

- Pressure switch to cut power when internal pressure increase
- Pressure relief valve as a final safety measure

Agenda to be shared with all stakeholders going forwards.

- Safety, Safety, Safety
- Training on new system to ship crew is not easy. On-board field training on a regular basis is required.
- BWMS convention requirement could change present normal practice on board.
- Gravity Ballasting and De-ballasting vs. Data log requirement.
- Communication and co-work between CCR and E/R during ballasting and de-ballasting operation.
- Many number of valve control before and after of ballasting and de-ballasting operation
- Regulation require for additional equipment to BWMS : Uninterrupted Power Supply for data logging
- There still are issues on operation of BWMS to be reviewed and cleared to shipowners as well as BWMS makers.

Scaling-up Issues of Ballast Water Treatment Systems and the Need for Verification of CFD Models

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Keywords: *CFD models, scaling-up, test approval, UV systems, verification*

Introduction

Type approval of Ballast Water Management Systems allows for certain degrees of scaling: both land-based and shipboard systems are tested for a single Total Rated Capacity (TRC), and the test then serves as basis for approving larger or smaller TRC's. According to paragraph 2.3.14 of Annex 2 of Guidelines G8, such scaling can be done by the manufacturer by using mathematical modelling and/or calculations to document that the scaling will not change the functioning and effectiveness of the system [1].

While significant efforts are put in place in Guidelines G8 to describe how different tests are carried out, no further guidelines, specifications or standard protocols exist on the choice and prescribed accuracy of the models used for scaling the systems. Typically, computational fluid dynamics (CFD) modelling of full Navier-Stokes equation is used to model various levels of detail of complex flows, but often using different approximations especially for turbulence [2]. In addition, realistic BWT flows are characterized by a number of physical and chemical variables, such as temperature, sediments, salinity, UV fields [3] etc., which are generally flow and geometry dependent, and all of which impact survival rate of unwanted microorganisms. Hence, there can be large uncertainties in modelling the survival rates of organisms depending on the level of flow approximations and/or the level of details involved. A sole reliance on results that are not carefully verified could lead to completely wrong assessments of designs.

UV systems are commonly used for BWT and are particularly dependent on models for scale-up since no in-situ measurements of UV field alone can reveal the killing rate of organisms (an integral dose is needed, which differs in different types of flows, [4]).

Scaling-up

Consider the schematic of a cylindrical UV reactor of length L and radius R, containing N lamps, Fig. 1. Critical places which contribute to low doses are those which contribute short resident times and/or small UV irradiation (shaded areas). In the process of scaling-up, one needs to ensure that these volumes diminish. We make an estimate to observe the salient features in the scaling-up of the system.

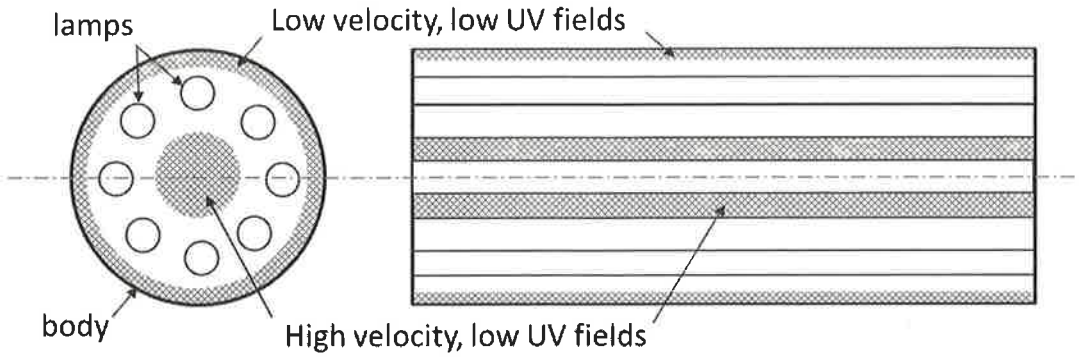

Figure 1. Schematic of a UV reactor with shaded parts indicating areas of low dosage.

Incident radiation around a single lamp is given by

$$G \sim \frac{\varepsilon^{-\lambda r}}{r}, \qquad \text{Eq. (1)}$$

where r is distance away from the surface of the lamp and λ the UV absorption coefficient. Eq. 1 shows that intensity drops off steeply away from the lamp, determined mainly by the exponential term. If lamps are more or less evenly distributed in the reactor, typical distance between them is

$$\chi \cong \frac{R}{\sqrt{N}} - a, \qquad \text{Eq. (2)}$$

where a is a single lamp's radius and R and N defined above. Further, typical time spent by a particle in the system is

$$T = \frac{L}{\overline{U}}, \qquad \text{Eq. (3)}$$

where $\overline{U}$ is mean turbulent velocity and L the reactor's length. We can now write for the accumulated dose per particle somewhere in the center of the reactor

$$D = \int_O^T G dt \cong GT \sim \frac{\varepsilon^{-\lambda\left(\frac{R}{\sqrt{N}} - a\right)}}{\frac{R}{\sqrt{N}} - a} N \frac{L}{\overline{U}} \cdot \qquad \text{Eq. (4)}$$

Eq. 4 shows how the dose on a single particle is affected by the system's variables. It depends inversely on velocity (flow rate) and exponentially on radius (size). Hence, any scaling-up causes a reduction in the dose. This can be counteracted by adding more lamps which increases the number of sources in the system and decreases the average spacing between the lamps (the dominant effect). By summing up the individual contributions of Eq. 4 one arrives at a distribution of doses among a population of particles. This is highly dependent on geometry and flow and hence requires a more detailed analysis.

CFD simulations

A more precise treatment requires numerical simulations to account for the facts that turbulent flow randomizes velocities, particularly around obstacles, and that the overall UV fields are made-up by

superposition from all sources and depend on precise geometry. We briefly outline major steps in performing a CFD simulation and emphasize particular issues in simulating UV reactors.

Geometry and meshing

Simplification and subsequent meshing (discretization of computational domain) of complex geometries are crucial steps before simulations can run. For example, in L-shapeed UV reactors recirculations develop in the outgoing (bent) channel. If the channel is made long enough, the flow will settle and enable simple boundary conditions to be assigned at the outlet. For typical ballast-water reactors number of necessary mesh elements, including the refinements around UV lamps, range up to several millions.

Flow models

Beside choosing and calibrating turbulence models, boundary conditions on solid surfaces (so-called wall functions) must ensure correct logarithmic velocity profiles. This is critical in UV reactors as the residence time depends inversely on the velocity, Eq. 3. To demonstrate complexity of the models we write the governing equation for most widely used $k-\varepsilon$ turbulence model [2]. They include averaged continuity and momentum equations, Eqs. (5) and (6), an equation for the kinetic energy k of turbulent fluctuations, Eq. (7), and an equation for turbulent dissipation ε at smallest scales, Eq. (8). Calculations of turbulent properties provide estimates of the effective turbulent viscosity, Eq. (9), which then determines turbulent shear forces.

$$\nabla \cdot (\rho \bar{u}) = 0, \qquad \text{Eq. (5)}$$

$$\rho\left((\bar{u} \cdot \nabla)\bar{u} + \overline{(u' \cdot \nabla)u'}\right) = -\nabla \bar{p} + \mu \nabla^2 \bar{u}, \qquad \text{Eq. (6)}$$

$$\rho \bar{u} \cdot \nabla k = P_k - \rho\varepsilon + \nabla\left(\left(\mu + \frac{\mu_T}{\sigma_k}\right)\nabla k\right); P_k = -\mu_T\left(\frac{\partial \bar{u}_l}{\partial x_j} + \frac{\partial \bar{u}_j}{\partial x_i}\right)\frac{\partial \bar{u}_l}{\partial x_j}, \qquad \text{Eq. (7)}$$

$$\rho \bar{u} \cdot \nabla \varepsilon = C_{s1} P_k \frac{\varepsilon}{k} - \rho C_{s2} \frac{\varepsilon^2}{k} + \nabla\left(\frac{\mu_T}{\sigma_\varepsilon}\nabla\varepsilon\right), \qquad \text{Eq. (8)}$$

$$\mu_T = \rho C_\mu \frac{k^2}{\varepsilon}, \qquad \text{Eq. (9)}$$

$$C_\mu = 0.009;\ C_{s1} = 1.44;\ C_{s2} = 1.92;\ \sigma_k = 1.0;\ \sigma_\varepsilon = 1.3 \qquad \text{Eq. (10)}$$

UV fields

The largest deviations in accumulated dose happen from use of different models for UV fields, which use different ways to approximate the exponential factor, Eq. 1. Unfortunately, calculations of UV fields in complex geometries are extremely tedious because of the integro-differential nature of the governing equations in the most general case (including production, absorption and scaterring of radiation) [3]. Accuracy of the radiation field models, such as Discrete Ordinates Method (DOM) or P1 (based on spherical harmonics) depends on particular type of geometry, scales and absorption media. Differences can be rather significant, Fig. 2. Hence, one needs to understand the limitations and applicability of such approximations.

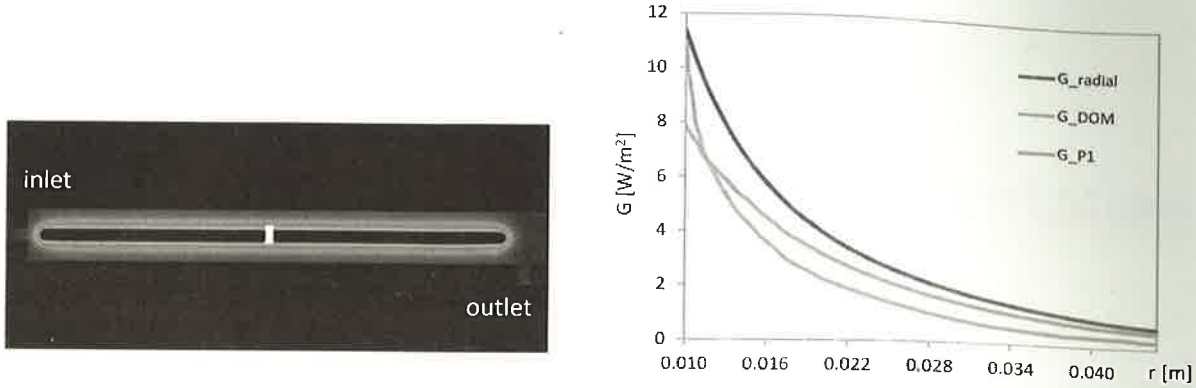

Figure 2 a) Contours of UV field intensity in a BWT reactor with one UV lamp (cross-section shown) b) Different models for UV intensities produce significant variations in radial UV distribution (along the white line in a). Which one should be used? In a scaled-up reactor (more lamps), variations in the overall UV dose would also scale-up.

Microbe decay

Decay of microbes can be calculated in two ways. In the Lagrangian approach, the accumualted UV dose is calculated for each particle as it passes outlet, Eq. 4. In Eulerian approach, a concentration field is set-up to decay under UV field as it is transported through the reactor, Fig.3. The number of surviving microbes is usually approximated by the exponential law

$$N_m = N_{m0} e^{-kG\bar{t}} \qquad \text{Eq. (11)}$$

where the decay rate constant k depends on microbes present in the ballast water. This is usually available form literature or from experimental (in-house) water analyses. Eq. 11 is applicable to both Lagrangian and Eulerian approach.

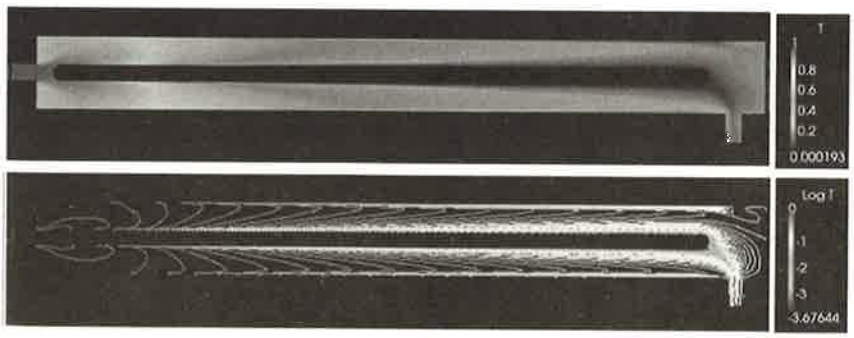

Figure 3.
Decaying concentration of microbes in the single lamp UV reactor (upper panel) and its logarithm (lower panel) for k=0.1 ㎠/mJ (single stranded RNA virus).

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[1] Ballast water management convention and the guidelines for its implementation, Internal Maritime Organization, London, 2009 edition

[2] D. C. Wilcox, Turbulence modeling for CFD, DCW Industries Inc., La Cañada, USA, 2nd printing 1994

[3] M. Modest, Radiative Heat Transfer, Elsevier Inc., Amsterdam, 3rd ed. 2013

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Poster Presentation

Consideration of Acceptable Discharge Concentrations of Relevant Chemicals in BWMS Using Electrolysis

Soo-Yeon Im | Korea Marine Equipment Research Institute

Birth of a New Test Facility off Long-standing Expertise in BWT; Concept, Challenges and Successes

Etienne J. Brutel de la Riviere | MEA-nl BV

Re-growth of Heterotrophic Bacteria Following Treatment with Ballast Water Management System

Keun-Hyung Choi | Ballast Research Center, Korea Institute of Ocean Science and Technology

A Modeling Study of Survivability of Phytoplankton Species in Ballast Water

Jeong-Hwan OH | Korea Institute of Ocean Science and Technology

Re-growth Ability and Species Composition of Phytoplankton in International Commercial Ship's Ballast Water

Moonho Son | Korea Institute of Ocean Science and Technology

Effect of •OH Radicals on Viability of Marine Microplankton and Bacteria in Ships' Ballast Water

Xiangying Meng | Dalian Maritime University

Risk Assessment based Exemptions from Ballast Water Management – The Intra-Baltic Study

Stephan Gollasch | GoConsult

Superiority of the System Treated by Medium Pressure Ultra-Vilot and PLASMA

Kwang-Moon, Lee | SAMKUN CENTURY CO., LTD

Effective New Equipment for Indicative Analysis Using Pulse Counting of FDA Fluorescence

Akiko Nakata | SATAKE Corporation

Pre-Loadng Onshore Ballast Water Treatment System (PreOBWTS): A Viable Proposition for Developing Economies

Lawrence A. Kuroshi | Nigerian Ports Authority, Pollution Control Department, Dockyard, Port Harcourt

The Appropriate Test Vessels Type and Medium Volume To meet the Validation (OECD 201) of Algal Growth Inhibition Test with Marine Algae Skeletonema costatum

Myung-Baek Shon | Marine Eco-technology Institute Co., Ltd.

Chemical Speciation and Fate in Electrolysed Ballast Water under G8 Test

Ji-Hyun Lee | Korea Testing & Research Institute

Optimization of Analysis Method for Total Residual Oxidant in Ballast Water Management System

Ji-Hyun Lee | Korea Testing & Research Institute

In-Line Water Sampling Methods for Representative Sampling of Ballast Water

Jeong-Kyeong Park | Korea Marine Equipment Research Institute

Is There Any Assurance?

Isabel van der Star | MEA-nl

Effect of Disinfection for High TRO Concentration without the Filter System

Sangho Park | Samsung Heavy Industries

Consideration of Acceptable Discharge Concentrations of Relevant Chemicals in BWMS Using Electrolysis

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Introduction

In order to prevent the introduction on the marine environment of unwanted aquatic organisms and pathogens from ships' ballast water and sediment discharges, the International Convention for the Control and Management of Ships' Ballast Water and Sediments was adopted by the International Maritime Organization (IMO) in 2004. Accordingly, Ballast Water Management Systems (BWMS) have been developed using a variety of treatment technologies, such as filtration, ultraviolet irradiation, ozonization, electrolysis and chemical injection. Regulation D-3.2 of the Convention states that BWMS that use Active Substances (AS) or Preparations shall be approved by the IMO, based on a procedure for the approval of BWMS that make use of Active Substances (G9).

Through G9, developed BWMS must determine the acceptability of AS in their BWMS applications with respect to ship safety, human health, and the health of the aquatic environment. The GESAMP-BWWG noted that many Relevant Chemicals (RCs) are commonly found in treated ballast water, irrespective of the technology used in the BWMS. Based on the occurrences, frequencies, and concentrations of RCs, the GESAMP-BWWG has selected 18 substances of RCs and has created the first phase of the database. The substances currently in the database include mostly halogenated acetic acids (HAAs) and trihalomethanes (THMs). To date, most BWMS approved by the IMO have been developed using electrolysis. In accordance with the GESAMP-BWWG database, discharge concentrations of RCs in approved BWMS using electrolysis have been reviewed and presented in non-confidential document.

Material and method

In order to consider acceptable discharge concentration of RCs, 8 of BWMS using electrolysis and approved IMO Final Approval was selected from 54th to 65th of MEPC and discharge concentration was selected the highest concentration on discharge treated water from each BWMS. Using selected discharge concentration, Predicted environmental concentration (PEC) was calculated by MAMPEC BW 3.0. And dermal, inhalation and oral exposure level of worker and general population were calculated in accordance with Methodology for information gathering and conduct of work of the GESAMP-BWWG (IMO Circular BWM.2/Circ.13/Rev.1).

Also predicted no effect concentration (PNEC) and Derived No Effect Levels (DNEL) were calculated using Information on the GESAMP-BWWG Database of chemicals most commonly associated with treated ballast water (IMO MEPC 65/INF.14).

From these results, PEC/PNEC ratio and risk characterization ratios (RCRs) of dermal, inhalation and

oral exposure of worker and general population were calculated, RCRs were calculated as below;

RCR=Exposure/Derived No Effect Levels (DNEL)

Results

Table 1 shows that detected HAAs frequencies of dibromoacetic acid, monochloroacetic acid, and tribromoacetic acid are higher than those of other HAAs, and detected THMs consist mostly of bromoform. Among HAAs, the highest concentrations are given for bromochloroacetic acid (13 μg/L), dibromoacetic acid (63 μg/L), dichloroacetic acid (2.59 μg/L), monobromoacetic acid (191 μg/L), monochloroacetic acid (495 μg/L), tribromoacetic acid (970 μg/L) and trichloroacetic acid (5.67 μg/L); among THMs, the highest concentrations are given for bromoform (670 μg/L), chloroform (3.87 μg/L), dibromochloromethane (21 μg/L) and dichlorobromomethane (8.54 μg/L); among other substances, the highest concentrations are given for dibromoacetonitrile (3.42 μg/L) and trichloropropane (2.3 μg/L). The substances of the highest concentration are tribromoacetic acid (970 μg/L), bromoform (670 μg/L) and monochloroacetic acid (495 μg/L). The concentrations of chloroform (3.87 μg/L) and dichlorobromomethane (8.54 μg/L) are lower in principle.

Table 1. Concentrations of RCs

Substances	Concentration (μg/L)							
	BWMS 1	BWMS 2	BWMS 3	BWMS 4	BWMS 5	BWMS 6	BWMS 7	BWMS 8
Haloacetic acids (HAAs)								
Bromochloroacetic acid	13	1.8	< 1.0	9.83	3.9	9.39	NA[a]	0.31
Dibromoacetic acid	50	63	7.9	26.7	20.7	2.97	NA	26
Dichloroacetic acid	1.15	< 1.0	< 1.0	1.22	1.08	2.59	NA	NA
Monobromoacetic acid	14.5	1	< 1.0	3.21	2.79	191	NA	NA
Monochloroacetic acid	1.08	< 1.0	< 1.0	43.5	151	495	NA	NA
Tribromoacetic acid	13.66	970	66	6.67	15.1	13.1	NA	NA
Trichloroacetic acid	ND[b]	< 1.0	< 1.0	5.67	1.38	0.53	NA	NA
Trihalomethanes (THMs)								
Bromoform	500	310	450	126	486	124	230	670
Chloroform	ND	< 0.5	< 4.0	0.54	3.87	ND	NA	< 0.1
Dibromochloromethane	21	8.7	14.8	6.17	15.6	9.3	15	18
Dichlorobromomethane	0.98	7.9	0.49	0.35	8.54	2.57	1	0.8
Others								
Dibromacetonitrile	NA	NA	< 10	< 0.008	3.42	0.28	NA	0.8
Trichloropropane	NA	NA	< 4	< 0.01	NA	NA	2.3	NA

[a]NA, Not applicable
[b]ND, Not detected

As the results of PEC/ PNEC ratios calculation using the result of Table 1, all PEC/PNEC ratios of RCs were below 1 except for monochloroacetic acid (2.02E+00, ≥ 43.5 μg/L), dibromoacetonitrile(1.67E+00, ≥ 3.42 μg/L) and trichloropropane (1.19E+01, ≥ 2.3 μg/L) (Table 2).

Table 2. PEC/PNEC ratio of RCs

Substances	BWMS 1	BWMS 2	BWMS 3	BWMS 4	BWMS 5	BWMS 6	BWMS 7	BWMS 8
Haloacetic acids (HAAs)								
Bromochloroacetic acid	2.19E-02	3.03E-03	< 1.68E-03	1.66E-02	6.56E-03	1.58E-02	NA[a]	5.22E-04
Dibromoacetic acid	1.96E-01	2.46E-01	3.09E-02	1.04E-01	8.09E-02	1.16E-02	NA	1.01E-01
Dichloroacetic acid	1.35E-02	< 1.17E-02	< 1.17E-02	1.43E-02	1.27E-02	3.03E-02	NA	NA
Monobromoacetic acid	2.44E-02	1.68E-03	< 1.68E-03	5.40E-03	4.69E-03	3.21E-01	NA	NA
Monochloroacetic acid	5.02E-02	< 4.64E-02	< 4.64E-02	2.02E+00	7.02E+00	2.29E+01	NA	NA
Tribromoacetic acid	6.13E-03	4.35E-01	2.97E-02	3.00E-03	6.78E-03	5.88E-03	NA	NA
Trichloroacetic acid	NA	< 4.48E-04	< 4.48E-04	2.55E-03	6.20E-04	2.38E-04	NA	NA
Trihalomethanes (THMs)								
Bromoform	5.61E-02	3.48E-02	5.05E-02	1.42E-02	5.46E-02	1.40E-02	2.58E-02	7.53E-02
Chloroform	NA	< 2.30E-05	< 1.85E-04	2.49E-05	1.78E-04	NA	NA	< 4.61E-06
Dibromochloromethane	3.06E-02	1.27E-02	2.16E-02	8.98E-03	2.27E-02	1.35E-02	2.19E-02	2.62E-02
Dichlorobromomethane	9.82E-05	7.91E-04	4.91E-05	3.51E-05	8.55E-04	2.58E-04	1.00E-04	8.01E-05
Others								
Dibromacetonitrile	NA	NA	< 4.87E+00	< 3.91E-03	1.67E+00	1.37E-01	NA	3.91E-01
Trichloropropane	NA	NA	< 2.07E+01	< 5.15E-02	NA	NA	1.19E+01	NA

[a]NA, Not applicable

RCRs of dermal, inhalation and oral exposure for worker and general population were below 1 in all detected concentrations (Table 3 ~ Table 7).

Table 3. RCR of dermal exposure for worker

Substances	BWMS 1	BWMS 2	BWMS 3	BWMS 4	BWMS 5	BWMS 6	BWMS 7	BWMS 8
Haloacetic acids (HAAs)								
Bromochloroacetic acid	9.45E-09	1.31E-09	< 7.26E-10	7.15E-09	2.83E-09	6.83E-09	NA[a]	2.25E-10
Dibromoacetic acid	6.29E-07	7.92E-07	9.93E-08	3.35E-07	2.60E-07	3.73E-08	NA	3.26E-07
Dichloroacetic acid	8.78E-09	< 7.62E-09	< 7.62E-09	9.32E-09	8.25E-09	1.98E-08	NA	NA
Monobromoacetic acid	1.88E-07	1.30E-08	< 1.30E-08	4.16E-08	3.62E-08	2.48E-06	NA	NA
Monochloroacetic acid	1.40E-08	< 1.30E-08	< 1.30E-08	5.64E-07	1.96E-06	6.41E-06	NA	NA
Tribromoacetic acid	1.45E-08	1.03E-06	7.01E-08	7.08E-09	1.60E-08	1.39E-08	NA	NA
Trichloroacetic acid	NA	< 1.06E-09	< 1.06E-09	6.02E-09	1.46E-09	5.63E-10	NA	NA
Trihalomethanes (THMs)								
Bromoform	1.02E-06	6.29E-07	9.14E-07	2.56E-07	9.88E-07	2.53E-07	4.67E-07	1.36E-06
Chloroform	NA	< 2.23E-09	< 1.79E-08	2.41E-09	1.73E-08	NA	NA	< 4.47E-10
Dibromochloromethane	3.04E-08	1.26E-08	2.14E-08	8.92E-09	2.25E-08	1.34E-08	2.18E-08	2.60E-08
Dichlorobromomethane	6.46E-09	5.20E-08	3.23E-09	2.31E-09	5.63E-08	1.70E-08	6.59E-09	5.27E-09
Others								
Dibromacetonitrile	NA	NA	< 5.51E-08	< 4.42E-11	1.89E-08	1.54E-09	NA	4.42E-09
Trichloropropane	NA	NA	< 2.45E-08	< 6.08E-11	NA	NA	1.41E-08	NA

[a]NA, Not applicable

Table 4. RCR of inhalation exposure for worker

Substances	BWMS 1	BWMS 2	BWMS 3	BWMS 4	BWMS 5	BWMS 6	BWMS 7	BWMS 8
Haloacetic acids (HAAs)								
Bromochloroacetic acid	3.58E-08	4.96E-09	< 2.75E-09	2.71E-08	1.07E-08	2.59E-08	NA[a]	8.53E-10
Dibromoacetic acid	4.49E-07	5.66E-07	7.10E-08	2.40E-07	1.86E-07	2.67E-08	NA	2.34E-07
Dichloroacetic acid	5.82E-06	< 5.06E-06	< 5.06E-06	6.17E-06	5.46E-06	1.31E-05	NA	NA
Monobromoacetic acid	1.10E-05	7.55E-07	< 7.55E-07	2.42E-06	2.11E-06	1.44E-04	NA	NA
Monochloroacetic acid	1.78E-08	< 1.65E-08	< 1.65E-08	7.16E-07	2.49E-06	8.15E-06	NA	NA
Tribromoacetic acid	1.85E-09	1.31E-07	8.94E-09	9.03E-10	2.04E-09	1.77E-09	NA	NA
Trichloroacetic acid	NA	< 4.09E-09	< 4.09E-09	2.32E-08	5.64E-09	2.17E-09	NA	NA
Trihalomethanes (THMs)								
Bromoform	3.07E-01	1.90E-01	2.76E-01	7.73E-02	2.98E-01	7.60E-02	1.41E-01	4.11E-01
Chloroform	NA	< 7.55E-03	< 6.04E-02	8.16E-03	5.85E-02	NA	NA	< 1.51E-03
Dibromochloromethane	1.60E-01	6.65E-02	1.13E-01	4.71E-02	1.19E-01	7.10E-02	1.15E-01	1.37E-01
Dichlorobromomethane	1.24E-02	1.00E-01	6.22E-03	4.44E-03	1.08E-01	3.26E-02	1.27E-02	1.02E-02
Others								
Dibromacetonitrile	NA	NA	< 1.06E-07	< 8.49E-11	3.63E-08	2.97E-09	NA	8.49E-09
Trichloropropane	NA	NA	< 4.72E-03	< 1.18E-05	NA	NA	2.71E-03	NA

[a]NA, Not applicable

Table 5. RCR of dermal exposure for general population

Substances	BWMS 1	BWMS 2	BWMS 3	BWMS 4	BWMS 5	BWMS 6	BWMS 7	BWMS 8
Haloacetic acids (HAAs)								
Bromochloroacetic acid	9.05E-09	1.25E-09	< 6.96E-10	6.85E-09	2.72E-09	6.54E-09	NA[a]	2.16E-10
Dibromoacetic acid	6.03E-07	7.59E-07	9.51E-08	3.21E-07	2.49E-07	3.57E-08	NA	3.13E-07
Dichloroacetic acid	8.42E-09	< 7.31E-09	< 7.31E-09	8.94E-09	7.90E-09	1.89E-08	NA	NA
Monobromoacetic acid	1.80E-07	1.24E-08	< 1.24E-08	3.99E-08	3.47E-08	2.37E-06	NA	NA
Monochloroacetic acid	1.34E-08	< 1.24E-08	< 1.24E-08	5.40E-07	1.88E-06	6.14E-06	NA	NA
Tribromoacetic acid	1.39E-08	9.85E-07	6.71E-08	6.79E-09	1.54E-08	1.33E-08	NA	NA
Trichloroacetic acid	NA	< 1.01E-09	< 1.01E-09	5.77E-09	1.40E-09	5.39E-10	NA	NA
Trihalomethanes (THMs)								
Bromoform	9.74E-07	6.03E-07	8.76E-07	2.46E-07	9.47E-07	2.42E-07	4.48E-07	1.31E-06
Chloroform	NA	< 2.14E-09	< 1.71E-08	2.31E-09	1.66E-08	NA	NA	< 4.28E-10
Dibromochloromethane	2.92E-08	1.21E-08	2.05E-08	8.55E-09	2.16E-08	1.29E-08	2.09E-08	2.49E-08
Dichlorobromomethane	6.19E-09	4.99E-08	3.10E-09	2.21E-09	5.39E-08	1.62E-08	6.31E-09	5.05E-09
Others								
Dibromacetonitrile	NA	NA	< 5.28E-08	< 4.24E-11	1.81E-08	1.48E-09	NA	4.24E-09
Trichloropropane	NA	NA	< 2.34E-08	< 5.83E-11	NA	NA	1.35E-08	NA

[a]NA, Not applicable

Table 6. RCR of inhalation exposure for general population

Substances	BWMS 1	BWMS 2	BWMS 3	BWMS 4	BWMS 5	BWMS 6	BWMS 7	BWMS 8
Haloacetic acids (HAAs)								
Bromochloroacetic acid	8.25E-09	1.14E-09	< 6.34E-10	6.24E-09	2.47E-09	5.96E-09	NA[a]	1.97E-10
Dibromoacetic acid	1.04E-07	1.30E-07	1.64E-08	5.53E-08	4.29E-08	6.15E-09	NA	5.38E-08
Dichloroacetic acid	1.34E-06	< 1.17E-06	< 1.17E-06	1.42E-06	1.26E-06	3.02E-06	NA	NA
Monobromoacetic acid	2.52E-06	1.74E-07	< 1.74E-07	5.59E-07	4.86E-07	3.32E-05	NA	NA
Monochloroacetic acid	4.10E-09	< 3.79E-09	< 3.79E-09	1.65E-07	5.73E-07	1.88E-06	NA	NA
Tribromoacetic acid	4.26E-10	3.03E-08	2.06E-09	2.08E-10	4.71E-10	4.09E-10	NA	NA
Trichloroacetic acid	NA	< 9.42E-10	< 9.42E-10	5.34E-09	1.30E-09	4.99E-10	NA	NA
Trihalomethanes (THMs)								
Bromoform	7.06E-02	4.38E-02	6.36E-02	1.78E-02	6.87E-02	1.75E-02	3.25E-02	9.47E-02
Chloroform	NA	< 1.74E-03	< 1.39E-02	1.88E-03	1.35E-02	NA	NA	< 3.48E-04
Dibromochloromethane	3.70E-02	1.53E-02	2.60E-02	1.09E-02	2.75E-02	1.64E-02	2.64E-02	3.17E-02
Dichlorobromomethane	2.87E-03	2.31E-02	1.43E-03	1.02E-03	2.50E-02	7.52E-03	2.93E-03	2.34E-03
Others								
Dibromacetonitrile	NA	NA	< 2.44E-08	< 1.96E-11	8.36E-09	6.84E-10	NA	1.96E-09
Trichloropropane	NA	NA	< 1.09E-03	< 2.72E-06	NA	NA	6.25E-04	NA

[a]NA, Not applicable

Table 7. RCR of oral exposure for general population

Substances	BWMS 1	BWMS 2	BWMS 3	BWMS 4	BWMS 5	BWMS 6	BWMS 7	BWMS 8
Haloacetic acids (HAAs)								
Bromochloroacetic acid	6.20E-06	8.59E-07	< 4.76E-07	4.69E-06	1.86E-06	4.48E-06	NA[a]	1.48E-07
Dibromoacetic acid	6.22E-05	7.84E-05	9.82E-06	3.31E-05	2.57E-05	3.69E-06	NA	3.23E-05
Dichloroacetic acid	1.20E-06	< 1.04E-06	< 1.04E-06	1.27E-06	1.12E-06	2.69E-06	NA	NA
Monobromoacetic acid	1.51E-05	1.04E-06	< 1.04E-06	3.34E-06	2.91E-06	1.99E-04	NA	NA
Monochloroacetic acid	1.13E-06	< 1.04E-06	< 1.04E-06	4.53E-05	1.58E-04	5.15E-04	NA	NA
Tribromoacetic acid	7.62E-06	5.40E-04	3.69E-05	3.73E-06	8.43E-06	7.31E-06	NA	NA
Trichloroacetic acid	NA	< 2.62E-07	< 2.62E-07	1.49E-06	3.62E-07	1.39E-07	NA	NA
Trihalomethanes (THMs)								
Bromoform	2.35E-03	1.45E-03	2.11E-03	5.92E-04	2.28E-03	5.83E-04	1.08E-03	3.15E-03
Chloroform	NA	< 2.00E-06	< 1.60E-05	2.16E-06	1.55E-05	NA	NA	< 4.01E-07
Dibromochloromethane	4.09E-05	1.69E-05	2.88E-05	1.20E-05	3.03E-05	1.81E-05	2.92E-05	3.49E-05
Dichlorobromomethane	6.16E-06	4.96E-05	3.08E-06	2.20E-06	5.36E-05	1.62E-05	6.28E-06	5.03E-06
Others								
Dibromacetonitrile	NA	NA	< 4.43E-06	< 3.55E-09	1.52E-06	1.24E-07	NA	3.55E-07
Trichloropropane	NA	NA	< 4.19E-05	< 1.04E-07	NA	NA	2.41E-05	NA

[a]NA, Not applicable

Discussion

Acceptable discharge concentrations of the substances were identified using the PEC/PNEC and RCR calculation. Among HAAs, acceptable discharge concentration limits are given for bromochloroacetic

acid (≤ 13 μg/L), dibromoacetic acid (≤ 63 μg/L), dichloroacetic acid (≤ 2.59 μg/L), monobromoacetic acid (≤ 191 μg/L), monochloroacetic acid (< 43.5 μg/L), tribromoacetic acid (≤ 970 μg/L), and trichloroacetic acid (≤ 5.67 μg/L); among THMs, acceptable discharge concentration limits are given for bromoform (≤ 670 μg/L), chloroform (≤ 3.87 μg/L), dibromochloromethane (≤ 21 μg/L), and dichlorobromomethane (≤ 8.54 μg/L); among other substances, acceptable discharge concentration limits are given for dibromoacetonitrile (< 3.42 μg/L) and trichloropropane (< 2.3 μg/L). Itsuggests that the RCs produced by BWMS, which is using electrolysis treatment, have no adverse effects on human health and on the aquatic environment at the concentrations reported the IMO.

Table 8. Acceptable discharge concentration limits

Substances	Concentration (μg/L)
Haloacetic acids (HAAs)	
Bromochloroacetic acid	≤ 13
Dibromoacetic acid	≤ 63
Dichloroacetic acid	≤ 2.59
Monobromoacetic acid	≤ 191
Monochloroacetic acid	< 43.5
Tribromoacetic acid	≤ 970
Trichloroacetic acid	≤ 5.67
Trihalomethanes (THMs)	
Bromoform	≤ 670
Chloroform	≤ 3.87
Dibromochloromethane	≤ 21
Dichlorobromomethane	≤ 8.54
Others	
Dibromacetonitrile	< 3.42
Trichloropropane	< 2.3

It suggests that the RCs produced by BWMS, which is using electrolysis treatment, have no adverse effects on human health and on the aquatic environment at the concentrations reported the IMO. However, chemical behaviors of specific substances in aquatic environment haven't been verified ideally therefore, continuous research and studies will have to follow.

To identify accurate acceptable discharge concentrations of RCs generated from electrolysis treatment, further studies will consider the factors affecting the concentrations of RCs, such as the quality of test (challenge) waters, so as to evaluate the acceptability of BWMS, the characteristics of electrodes, electrical power consumption, and so on.

Birth of a New Test Facility off Long-standing Expertise in BWT; Concept, Challenges and Successes

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Early 2012, with a small group of experts that since 2002 had built up their expertise in the field of ballast water treatment and detection technologies. , we decided to take up the challenge to start our own, privately owned, test facility for ballast-water management systems. We were convinced that we could meet the challenges and improve ballast water testing on its merits, due to the natural resources and conditions in combination with a very experienced team. A major threat was not to sacrifice our independency and objectivity when commercializing this type of venture.
Based on the confidence of potential principals and with a very enthusiast team, we made plans for the facility, identified required skills and competences for the short- and the long term goals, made the necessary financial arrangements and developed strategies for the future. But plans only are not enough and work forces were empowered. The enterprise mainly required courage and common sense. Finally, or as a start, we could welcome our first principals August 2012.
Although there is not a fixed recipe for such adventure, we can rely on a strong and enthusiastic team, capable of beating challenges and of assist principals from the BWT market to make significant steps ahead.
Nevertheless, the main aim of the enterprise is to remain objective and to give advice to all parties involved, either on demand or under our own steam.

In our paper we will exemplify which choices had to be made and why as well as the main highlights of the trajectory.

Re-growth of Heterotrophic Bacteria Following Treatment with Ballast Water Management System

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Testing biological efficacy of ballast water management system (BWMS) requires not only sampling right after treatment but also time-delayed sampling (normally five days after treatment) to test any significant re-growth of test organisms in the treated water. Heterotrophic bacteria are generally the most abundant living organisms found in ballast water. Although they are not listed as an organism for testing the effectiveness of BWMS, they are often difficult to remove and thus could be a useful surrogate of the efficacy of any BWMS. In this study, we examined re-growth of heterotrophic bacteria in seawater treated with BWMS in a land-based test facility. The test water was augmented following the International Maritime Organization's Guideline 8 ("IMO soup"). Heterotrophic bacteria samples were taken at day 0 and 5 in both control and treatment lines. The results show that all BWMS treatments significantly reduced heterotrophic bacteria following treatments at day 0 with nearly no viable heterotrophic bacteria detected. However, heterotrophic bacteria numbers were significantly greater at samples from treated water taken at day 5 than those at day 0. We also present bacterial taxonomy with DNA analysis to show which heterotrophic bacteria regrew and are potentially resistant to ballast water treatment. These results could have broad implications on management of ships ballast water in a global effort for stemming marine bioinvasions through ballast water.

Keywords: *ballast water management system, heterotrophic bacteria, molecular fingerprints, regrowth*

A Modeling Study of Survivability of Phytoplankton Species in Ballast Water

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Most phytoplankton species introduced by ship's ballast water discharge tend to be extinct, but some species have ability to survive in sites for ballast water discharge and potential to disturb the native ecosystem. For a successful ballast water management, therefore, it is necessary to know how to survive in the Ballast Tank and if the invasive species introduced by ballast water can survive and regrow under the environmental conditions of surrounding water column. In this study, Hydrodynamic Eutrophication Model-3D (HEM-3D) simulated the behavior of the phytoplankton and compared the model result with results of laboratory experiments under the limited condition. Also, we simulated survivability of phytoplankton species introduced by ballast water discharge as a function of environmental conditions (i.e., light, water temperature and etc.). The model results showed that some species were able to survive and regrow under specific ranges of limited environmental conditions.

Keywords: *Ballast Water, Invasive Species, EFDC Model*

Re-growth Ability and Species Composition of Phytoplankton in International Commercial Ship's Ballast Water

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The aim of this study is to assess the importance of ballast water discharge as a vector for the introduction of exotic species into Korean coastal and port water. Also, we examined to understand the impacts of environmental factors on the survival success of introduced species by ship's ballast water in laboratory experiments. The duration time of ballast water in each ship was ranged from 1 to 365 days. The numbers of species and phytoplankton standing crops in uploaded ballast water were related to the duration time of ballast water; the phytoplankton population densities in ship's ballast water of short duration time was significant. Of these, the most diverse taxonomic groups were diatoms. In the laboratory study, the *in vivo* fluorescence of phytoplankton viability in *Spring Lyra* gradually increased with increasing nitrate and phosphate. Phytoplankton in new (9 days), medium (31 days) and old (365 days) ballast water successfully survived under the nutrient typical of shipside water and F/2 medium at 15°C and 20 °C, whereas phytoplankton in ballast water treatment did not survive even in optimal temperature. Colonization process was dominated by diatoms, *Skeletonema coastatum* for *Spring Lyra, Thalassiosira pseudonana* and *Thalassiosira* for *Han Yang, Thalassiosira pacifica* and *Odentella aurita* for *Modern Express*, and *Chaetoceros pseudocurvisetus* and *Pseudo-nitzschia seriata* for *Asian Legend*. The successful establishment of non-native species was also related to nutrient richness. In particular, *T. pseudonana* mainly dominated at all salinity conditions (0 to 30 psu). In combinations experiments of temperature and salinity on *T. pseudonana* monoculture, the organism did not grow between 5 and 10°C in the culture media of freshwater, whereas they have grow ability even in low temperature(5°C) in the salinity of brackish and marine. Also, *T. pseudonana* cells are significantly grown with increasing of temperature (5-30°C). Our laboratory design can be applied as a practical tool to assess the survival possibility of invasive autotrophic phytoplankton introduced into Korean coastal and port water.

Keywords: *ballast water, nutrients, phytoplankton, laboratory study*

Introduction

The ballast water of ships has been implicated in the introduction of planktonic organisms into novel areas. Given that ballast water can harbor organisms from diverse taxonomic groups that originate from various bio-geographic regions, the introduction of harmful species from ballast water can cause serious problems in ecosystems.

During the past two decades, many studies into ballast water have tended to focus on analyzing species collected before and after ballast water is exchanged at the end of a voyage to assess the importance of ballast water as a vector. Although such studies can provide information on introduced species in ballast water, these studies are not sufficient to indicate successful establishment or the population growth of the species after introduction to a novel ecosystem. In particular, resting stages of plankton, such as spores, that are transported or produced within ballast water have an important ecological role as the seed populations, which suggests that planktonic cells might impose significant 'propagule pressure' on aquatic systems.

Some organisms that survive the harsh environment of ballast tanks might face environmentally unfamiliar recipient waters after being discharged. The likelihood of invasion is significantly increased when invasive species are introduced into the favorable environmental conditions of ports, where the water is often rich in nutrients. Modeling population growth under various environmental conditions in laboratory experiments can provide important information on the survival potential of species after their introduction into recipient waters.

In order to understand the effects of environmental factors on the survival success of introduced species, we monitored biotic and abiotic factors in ballast and shipside harbor waters and assessed the success of phytoplankton from ballast water with respect to colonization under different conditions.

Material and methods

Ballast water uploaded from coastal waters of other countries or ports located in different bioregions was targeted. Ship crews, with assistance of port authorities (Port State Control), were interviewed to gather information on the ballasting site and record logs. The ship's specific details and log notes related to ballasting and de-ballasting were acquired. Information on ballasting dates, sites and names of vessels, which was dominated by chemical tankers, is shown in Table 1. For targeted ships, biological and environmental factors were examined in the ballast water tanks through a manhole on board and we also investigated only environmental factors in shipside harbor water.

Table 1. Ballast tank information of ships surveyed from 25 to 27 May at Pyeongtaek Port.

Destination (port)	Ship name	Tonnage (GRT)	Kind of ship	Ballasting date	Duration time (day)	Ballasting site and source port
Pyeongtaek	*Queen Gingdao*	16,485	Passemger	2010.05.24	1	Weihai, China
Pyeongtaek	*Hamilton Strait*	9,040	Container	2010.05.22	3	Lianyunsang, China
Pyeongtaek	*Han Yang*	4,237	Container	2009.05.26	365	Shanghai, China
Pyeongtaek	*Modern Express*	33,831	Car carrier	2010.04.06	41	Pacific ocean exchange
Pyeongtaek	*Asian Legend*	55,680	Car carrier	2010.04.26	31	Iquique, Chile

Temperature, salinity and oxygen conditions in ballast water and shipside harbor water were measured by a YSI 6600. Phytoplankton samples were collected by gently towing a 20-mm mesh plankton net (diameter: 30 cm, length 70 cm) obliquely from tank bottom layer to the surface. Two net samples were collected from each tank. One sub-sample was used to identify species composition. The others were used to examine the phytoplankton survival potential. Water samples were collected with a bucket from the surface layer in the ballast water tank and shipside harbor water. The ballast tank and shipside harbor samples were kept in dark conditions in autoclaved 2L bottles to determine inorganic nutrients (silicate, nitrate + nitrite, and phosphate) and pH. A sub-samples was used to measure pH levels with a pH meter. Sub-samples for the estimation of dissolved inorganic nutrients were filtered through a GF/F filter. Nutrient concentration was analyzed using a nutrient auto-analyzer (Bran Luebbe,

AACE-6). In addition, sub-samples netted to determine phytoplankton species composition in the ballast water were immediately fixed with 5% Lugol solution and stored in the dark prior to cell identification. These cells were counted and identified using a Sedgwick-Rafter chamber.

The study experiments were designed to investigate survival potential of introduced phytoplankton from ballast water after discharge into the coastal waters of Korea. The effects of phytoplankton survival were investigated for introduced ballast water, which was directly introduced in Pyeongtaek port without exchange in oceanic water, from three vessels. For three vessels *(M/V Hanyang, Modern Express,* and *Asian Leged*), an experiment was designed to mimic the resource- limited waters of the ballast tank as well as the nutrient-rich eutrophic water of harbor and coastal waters in which nutrients are plentiful. The media for incubation comprised three different resources ballast water; shipside harbor seawater; and nutrient rich-water of F/2 medium, which is representative of coastal eutrophication. The shipside and ballast water for four vessels was filtered using a 0.2 μm membrane filter. Two liters of filtrate was placed into 2L autoclaved polycarbonate (PC) bottles. The F/2 medium was prepared using filtered shipside harbor seawater in Pyeongtaek ports. Before the inoculation of phytoplankton stocks for survival experiments, large zooplankton were excluded by gentle filtering through a 100 μm Nitex mesh. The concentrated stock was added to each flask and the culture bottles were placed into an incubator at 10, 15 and 20°C under illumination conditions of 60 μmol m-2 s-1 provided by a 12 L: 12 D cycle. Also, the initial density of phytoplankton was equally adjusted using a fluorometer (Turner-Designs 10-AU).

Results and discussion

Environmental parameters in ballast and shipside harbor waters are important to understand the survival potential of introduced species. Environmental parameters in ballast and shipside harbor waters are shown in Table 2. Differences in water temperature between ballast and shipside harbor water were relatively small, with ballast water being slightly higher than shipside water, except for the *M/V Modern Express* and *Asian Legend.*

Table 2. Results of temperature, salinity, dissolved oxygen (DO), and nutrients in shipside port water and ballast water of ships berthed at Pyeongtaek Port.

Ship name	Waters	Temp. (°C)	Sal. (psu)	DO (mg L^{-1})	pH	Nutrients (mM)		
						DSi	DIN	DIP
Queen Gingdao	SPW	14.9	26.11	7.51	8.12	8.13	76.49	0.97
	BTW	**12.3**	**29.11**	**8.25**	**8.28**	**7.33**	**26.96**	**0.19**
Hamilton Strait	SPW	15.7	26.02	8.05	8.30	7.31	40.96	0.51
	BTW	**13.9**	**22.92**	**9.03**	**8.21**	**40.66**	**64.15**	**1.16**
Han Yang	SPW	15.8	26.18	7.92	8.13	19.29	51.85	0.29
	BTW	**14.3**	**10.55**	**0.66**	**8.13**	**11.59**	**93.53**	**N.D**
Modern Express	SPW	15.3	26.69	7.90	8.16	12.43	47.34	0.58
	BTW	**15.5**	**8.54**	**9.47**	**8.38**	**27.64**	**30.68**	**0.04**
Asian Legend	SPW	15.3	26.69	7.90	8.16	12.43	47.34	0.58
	BTW	**16.2**	**33.81**	**7.69**	**8.49**	**16.30**	**29.97**	**1.77**

SPW: shipside port water, BTW: ballast tank water, which is indicated in bold
DSi: silicate; DIN (dissolved inorganic nitrogen): nitrate+ nitrite, Ammonium; DIP: phosphate

The salinity of the ballast water was relatively low compared with that of shipside harbor water, except for the *M/V Queen Gindao* and *Asian Legend.* In particular, the salinity of the ballast water in the *M/V Modern Express* was dramatically low (approximately 8 PSU) compared with that in the other ships. Differences in dissolved oxygen (DO) between ballast and shipside harbor water were

relatively large, and ranged from 0.66 to 9.03 mg L^{-1}. The pH of ballast and shipside harbor water ranged from 8.12 to 8.49with higher values in ballast than in harbor water. The levels of nutrients in ballast water differed significantly between the ships.

Differences in phytoplankton growth between temperature and nutrient conditions (ballast water, shipside harbor water, and F/2 medium) were significant (Table 3). On the *M/V Han Yang*, phytoplankton did not survive in ballast water at any temperature conditions. At 10°C, the *in vivo* fluorescence of phytoplankton did not detect at any treatments, At 15 and 20°C, the phytoplankton cultured in F/2 medium and shipside harbor water gradually increased after 8 days. This was mainly achieved by the centric diatoms *Thalassisira* spp., *S. costatum*-like spp. and the unidentified pinnate diatoms under nutrient-rich conditions. Several diatom species, such as *Detonula pumila* and *Odentella aurita* successfully survived on the *M/V Mordern Express*. On the *M/V Asian Legend*, although phytoplankton well grewn at 10°C 15°C and 20°C under F/2 meidum abd shipside water treatments, they did not survive in ballast water at any temperature conditions. Under all treatments, *Chaetoceros* spp. mainly dominated, followed by *Thalassiosira* spp..

Table 3. Observed species compositions at last phase of inoculated phytoplankton in ballast water, shipside port water, and F/2 medium.

Ship name	F/2 meidum	Shipside water	Ballast water
Han Yang	*Fragilaria sp.* (c) *Thalassiosira curviseriata* (b*,c*) *Thalassiosira pseudonana* (c*) Unidentified pinnate diatom (< 10 μm) (b)	*Skeletonema costatum* (c) *Thalassiosira curviseriata* (b*,c*) *Thalassiosira pseudonana* (c*)	No observation
Mordern Express	*Detonula pumila* (b) *Odontella aurita* (a,b,c*) *Thalassiosira pacifica* (a*,b*)	*Odoentella aurita* (c*) *Thalassiosira pacifica* (a*,b*)	No observation
Asian Legend	*Chaetoceros pseudocurvisetus* (a*,b*) *Chaetoceros* sp.(a) *Leptocylindrus minimum* (a) *Thalassiosira* spp. (a,b,c*) *Navicula* sp. (b) Unidentified pennate diatoms (< 10 μm) (c*)	*Chaetoceros* sp.(a) *Leptocylindrus minimum* (b) *Skeletonema costatum* (b) *Thalassiosira* spp. (c*) *Diploneis* sp. (c*) Unidentified pennate diatoms (< 10 μm) (b,c*)	No observation

a:10°C, b:15°C, c: 20°C, *: dominant species

In summary, the four main findings of laboratory culture experiments conducted in this study were: (1) a small number of introduced phytoplankton cells might play an important role in establishing in local aquatic ecosystems; (2) introduced diatom species might have suitable characteristics for colonization under nutrient-rich water; (3) autotrophic phytoplankton species can survive for up to two weeks in aphotic ballast tanks; and (4), as in the centric diatom *T. pseudonana*, tolerance for salinity is an important trait for the successful establishment of introduced phytoplankton species. The interpretation of the ecological implications of the study will provide important information on risk management in terms of the introduction of non-indigenous species from the ballast water of ships.

Effect of ·OH Radicals on Viability of Marine Microplankton and Bacteria in Ships' Ballast Water

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A ·OH ballast water-treatment system was designed and the efficacy of ·OH radicals in killing harmful marine plankton (*Karenia mikimotoi, Alexandrium tamarense, Skelrtonema costatum, Thalassiosira rotula* and *Heterosigma akashiwo*) and bacteria (*Escherichia coli,* Intestinal *Enterococci* and Heterotrophic bacteria) have been determined in our laboratory. The strong discharge plasma was filled in two micro-gaps. Two α-Al_2O_3 dielectric layers were covered on the discharge electrode of Ag thin plate. The self-made power supply was applied on the discharge electrodes. The O_2 and H_2O at gas state were introduced into the ·OH plasma generator. ·OH radicals were produced by a series of plasma reactions and then were dissolved into ballast water to kill the microplankton. During ·OH treatment, total reactive antioxidant (TRO) was defined as all the oxidants which could kill harmful aquatic organisms and pathogens. After ·OH treatment with TRO at 2.0 mg/L, the total content of 5 species of algae was reduced from 1.0×10^4 cells/mL to 5 cells/mL which was less than the D-2 discharge standard of International Maritime Organization (IMO) (<10 cells/mL). When the concentration of TRO was about 1.1 mg/L, the maximum concentration of killed algae was about 2000 cells/mL. When the concentration of TRO was about 1.4 mg/L, the maximum concentration of killed algae was 5000 cells/mL. The content of heterotrophic bacteria was 0.65×10^4 cfu/mL (Colony Forming Units per milliliter) considering as the total content of bacteria. After ·OH treatment, the colony numbers of *E. coli,* Intestinal *Enterococci* and heterotrophic bacteria were decreased to 57 cfu/100mL, 13 cfu/100mL and 5 cfu/100mL, respectively. It also meeted the requirement of D-2 discharge standard of IMO. The content of chlorophyl-a was decreased by 90%, which was primary reason leading the death of microalgae. The DNA strands of the 5 species of algae were broken after ·OH treatment. The length of DNA strands was mainly between 200 bp to 1000 bp. Those DNA strands could not express any proteins and the algae cells could not repair themselves. The quality of the ballast water was considerably improved after treated and no secondary pollution to aquatic organisms and environment. During a storage time of 5 days, no re-growth of plankton and bacteria was observed. The content of chlorophyl-a was decreased by 100%. TRO levels had declined below 0.2 mg/L. When the concentration of TRO was 1.9 mg/L, the maximum concentration of killed algae was about 10 000 cells/mL. The results showed that the D-2 ballast water discharge standard of the IMO was satisfied. The treatment of ship's ballast water using ·OH

radicals is an advanced oxidation method, which realizes the goal of zero emission and zero pollution in the process of producing OH· radicals and killing organisms in ship's ballast water.

Keyword: *Bacteria, Ballast water treatment system, Microplankton,·OH Radicals, water*

Risk Assessment based Exemptions from Ballast Water Management - The Intra-Baltic Study

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Global requirements to prevent the transfer of harmful aquatic organisms and pathogens with ballast water were set by the International Convention for the Control and Management of Ship's Ballast Water and Sediments (BWM Convention) . The BWM Convention includes that vessels on certain routes can be exempted from BWM requirements based on risk assessment (RA). RA needs to be conducted according to the International Maritime Organisation Guidelines for Risk Assessment under Regulation A-4 of the BWM Convention (G7 Guidelines). With the BWM Convention nearing its entry into force vessels will need to comply with requirements and the interest to conduct RA for exemptions becomes important. This paper presents a RA model for exemptions that might be granted in intra-Baltic shipping based on BWM Convention, G7 Guidelines as an elementary framework and also noting the regionally agreed Helsinki Commission RA guidance for exemptions for Baltic countries. We discuss the RA methods and elements that were selected for the application of ballast water management exemptions in intra-Baltic shipping. This is worldwide the first RA model for ballast water management exemptions under the provisions of the BWM Convention. The current lack of reliable information regarding alien and cryptogenic species, as well as human pathogens present in port areas of the ballast water donor and recipient points were found as most limiting factor to conduct RA. However, this study may be of particular interest for regional seas with similar RA relevant features, such as intensive shipping and different salinities throughout the sea.

Keywords: *ballast water management, Baltic Sea, exemptions, risk assessment*

Superiority of the System Treated by Medium Pressure Ultra-Vilot and PLASMA

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The IMO was adopted "International Convention For The Control And Management Of Ships' Ballast Water And Sediments", in 2004. During the past 10 years, many manufacturers of the world have developed various ballast water treatment technology and these systems have engaged the fierce competition in the global market. And IMO was expected that the convention will enter into force shortly, the competition is expected to be even more heightened. Hence, SAMKUN CENTURY company has applied complex treatment method and more efficient and reliable of the system will be introduced. "ARA PLASMA" ballast water treatment system consisted of PLASMA+MPUV has excellent performance for removal or disinfection to marine plants, animals and microbes in varied environmental conditions and in the ocean which has infinite resources. Our system doesn't generate secondary pollutant and by product, so it efficiently protect indigenous organism. In particular, PLASMA complements the disadvantage of MPUV under high turbidity condition, in addition there has a dual effect by reducing the burden that the filter should remove the small particles. Until recently, the system has carried turbidity test and has acquired varied data under different turbidity condition.

Keywords: *Ballast water, ARA PLASMA BWTS,Turbidity*

Effective New Equipment for Indicative Analysis Using Pulse Counting of FDA Fluorescence

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Backgrounds

In the Guidance for potential compliance with the D-2 standard*, several methods for indicative analysis have been reported. MEPC has recognized both advantages and disadvantages of these methods. SATAKE Corporation in Japan developed equipment with FDA's staining and a photomultiplier tube as a detector. The equipment counts pulse of the fluorescence from stained viable organisms and estimates the number of viable organisms using the pulse counts with an appropriate threshold. It is developed under the concept to seek high-accuracy, high-sensitivity and to avoid interferences of background fluorescence, which could be mainly caused by leakage from viable organisms.
In this paper, technical information and results of performance tests of the equipment are provided.

Viable Organisms Analyzing Equipment

Figure 1 shows exterior of the equipment. We consolidated all devises (pump, detection unit, touch panel display and CPU) in a single portable casing, all system can be easily handled onboard. The equipment can analyze organisms of $\geq$10μm and <50μm in minimum dimension and organisms of $\geq$50μm in minimum dimension, However, manual preparation process with filtering will be needed for both categories. After setting vessel containing the prepared 100ml sample to the holder, adequate FDA solution is added manually and set to closure in detection unit. Hereafter, all process will be automatically operated. After definite period of time for FDA staining, sample is stirred by a magnetic stirrer. When the viable organisms pass through the detection part, organism emits green fluorescence, which is excited by blue light LED.
Figure 2 shows a schematic view of the detection unit. Figure 3 shows a schematic counting process using pulse signal from photomultiplier tube. The fluorescence is continuously detected with photomultiplier tube as a voltage. In Figure3, only pulses, whose detected intensity is greater than the appropriate thresholds will be counted as a viable organism. Whole analysis is automatically operated. Then the concentration of organism is displayed on the touch panel display.
FDA solution is prepared by mixing with reagent grade dimethylsulfoxide(DMSO) at a concentration of 1mM. The solution was kept cold and in the dark until used. Then 1ml of FDA solution is added

to 100ml sample (approximately 100-fold dilution). For Analysis of ≥50μm organism total operation will be completed in 13 minutes (10 minutes for FDA staining and 3 minutes for detection), and for analysis of ≥10μm and <50μm organism, it will be completed in 31 minutes (30 minutes for FDA staining and 1 minutes for detection), respectively.

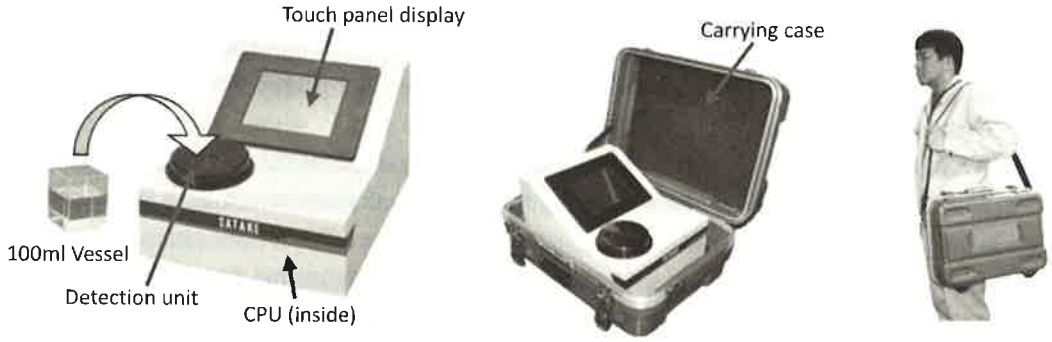

Figure 1. Viable organisms analyzing equipment

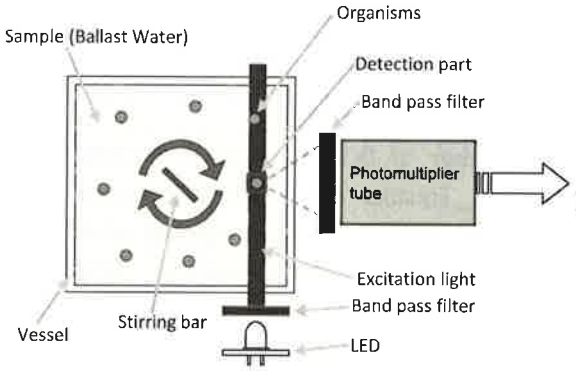

Figure 2. Detection unit (view from above)

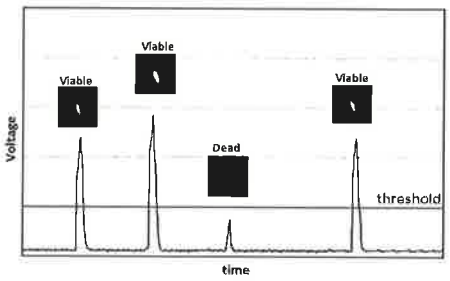

Figure 3. Pulse obtained from photomultiplier tube

The specific features of the equipment are;

1. High-accuracy: there is high linear correlation between pulse counting and manual counting,
2. High-sensitivity: one viable organism per 100ml sample can be detected,
3. High-reliability: detection without interference caused by background fluorescence,
4. Rapidity: result can be provided in 30 minutes,
5. Portability: sample can be analyzed onboard, even at the sampling facility, and
6. Simplicity: easy operation after putting a reagent into a sample.

Materials and Methods

1. Correlation between pulse counting and manual counting

Organisms of L size range (larger than 50 μm) were collected at Imari Bay in Japan in December 2012 by filtering seawater slowly not to give damage to the organisms using a plankton-net cloth of 35 μm opening. Then, the filtrate was again filtered through a net with an opening of 7 μm to collect organisms of S size range (larger than or equal to 10 μm and smaller than 50 μm). Collected

organisms were stained by FDA and their pulses were counted using methods described above. For a manual counting, G8 guideline was applied. Lastly, the correlation between two counting was observed.

2. High-sensitivity as detection of one viable organism per 100ml sample

Two mimic BW samples of 100ml purely clean seawater was provided for the test; one is content one viable organism, the other is content without organisms. Both samples were stained by FDA and their pulses were counted using methods described above. Results of two counting were compared.

Interference caused by background fluorescence

Two mimic BW samples with one viable organism *(Brachionus plicatilis)* per 100ml were provided for the test; one is purely content clean seawater, the other is contaminated with disrupted particles of organism by ultrasonic. The concentration of disrupted organism (*Chattonella antiqua,* phytoplankton) was 100 indi. per ml. Also, two control samples without viable organisms per 100ml were provided; one is purely content clean seawater, the other is contaminated with disrupted particles of organism by ultrasonic. The concentration of disrupted organism (*Chattonella antiqua,* phytoplankton) was 100 indi. per ml. Results of the counting were compared.

Results and Discussion

1. Correlation between pulse counting and manual counting

In the test using organisms of L size range, the number of 0-1122 pulses was obtained against concentration of 0-180 indi. per 100ml by manual counting. Equation of Y = 6.194X (Correlation coefficient of 0.996) was calculated. In the test using organisms of S size range, the number of 0-1113 pulses was obtained against concentration of 0-924 indi. per 100ml by manual counting. Equation of Y = 1.196X (Correlation coefficient of 0.999) was calculated (see Figure 4). From this result, it is said that a high linear correlation was observed between pulse counting and manual counting in both L and S size range. Therefore, this equipment can be applied to BW sample at the range of concentrations mentioned above.

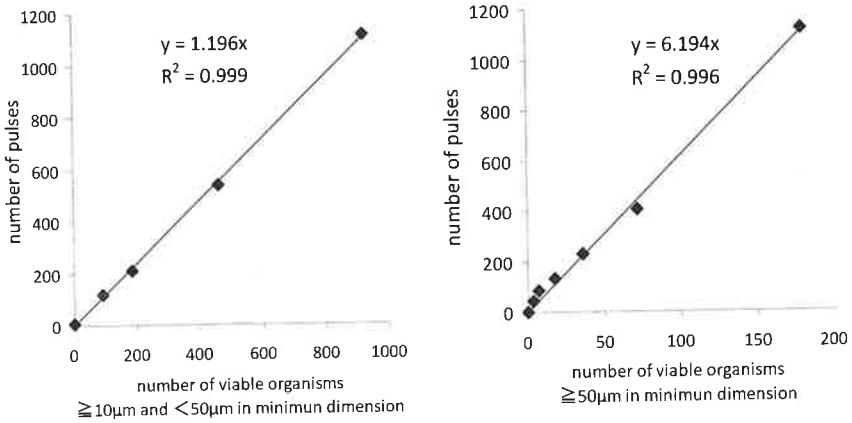

Figure 4. Correlation between pulse counting and manual counting

2. Detection ability of one viable organism per 100ml sample

The number of pulses obtained from sample of one viable organism was 13.20(Average of 10 tests).

Standard deviation was 1.93. On the other hand, the number of pulses obtained from sample without viable organism was 0(Average of 10 tests) (see Table 1). From this result, it can be said that the equipment could detect one single viable organism per 100ml sample.

3. Interference caused by background fluorescence

The number of pulses obtained from sample of one viable organism with purely content clean seawater was 13.20(Average of 10 replicates). Standard deviation was 1.75. On the other hand, the number of pulses obtained from sample of one viable organism with disrupted particles of organism by ultrasonic was 13.00(Average of 10 replicates). Standard deviation was 2.91. Also, the number of pulses obtained from both control samples without viable organisms were 0(Average of 10 tests) (see Table 2). There was no difference between the two pulses counting with one viable organism. Therefore, interference caused by background fluorescence can be cancelled with an appropriate threshold.

From these three studies, applicability to treated ballast water analysis has been validated at the laboratory level. Since the equipment is using pulse counting method with appropriate threshold, background fluorescence does not interfere with the counting. Therefore, the method can avoid leading to false positive/negative.

Table 1. Detection ability of one viable organism per 100ml sample

Test No.	0 Viable	1 Viable
1	0	12
2	0	12
3	0	11
4	0	14
5	0	12
6	0	12
7	0	16
8	0	17
9	0	13
10	0	13
Average	0	13.20
S.D.	0	1.93

Table 2. Interference caused by background fluorescence caused from disrupted organisms

	Control		Test	
Test No.	0 Viable		1 Viable	
	without disrupted organisms	disrupted organisms	without disrupted organisms	disrupted organisms
1	0	0	12	11
2	0	0	14	11
3	0	0	13	10
4	0	0	16	11
5	0	0	14	13
6	0	0	15	17
7	0	0	10	10
8	0	0	14	14
9	0	0	12	18
10	0	0	12	15
Average	0	0	13.20	13.00
S.D.	0	0	1.75	2.91

Conclusion

In the test of correlation between pulse counting and manual counting, since high linier correlation was observed in both L and S size range, it was verified that the equipment meets "High-accuracy". Secondly, in the test of detection ability of one viable organism per 100ml sample, since there was no difference between the number of pulses obtained from sample of one viable organism and sample without viable organism, it was verified that the equipment meets "High-sensitivity". Thirdly, in the test of interference caused by background fluorescence, since there was no difference between the number of pulses obtained from sample of one viable organism with purely content clean seawater and sample of one viable organism with disrupted particles of organism by ultrasonic, it was verified that the equipment meets "High-reliability".

From results of these three tests, it is said that this equipment allows anyone to evaluate BW sample

with high-sensitivity, high-accuracy and high-reliability. In addition, because the equipment was developed under the concept with rapidity, portability and simplicity, it can be one of the effective indicative analysis methods.

Keywords: *background fluorescence, bulk FDA, FDA, fluorescence pulse counting, indicative analysis.*

* Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines(G2) (BWM.2/Circ42)

Pre-Loadng Onshore Ballast Water Treatment System (PreOBWTS): A Viable Proposition for Developing Economies

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OBJECTIVES

The discharge of Harmful Aquatic Organisms and Pathogens (HAOP) found in ships ballast water from one port environment to another can have severe ecological, environmental and economic consequences, especially when they transform into marine pests. This informs the necessity to investigate the viability of a novel treatment concept known as Pre-loading Onshore Ballast Water Treatment System (PreOBWTS) which has the potential to curtail the transfer of these organisms from a source harbour.

CONCEPTUAL FRAMEWORK

Description of Pre-Loading Onshore Ballast Water Treatment System (Preobwts)

PreOBWTS is a preventative and Last Port of Call (LPOC) solution and it allows for the treatment of the harbour water of the port before it is uploaded as ballast water into a ship (Figure 1). The proposed system is preferred by this study because the conditions of the host port (referred to in this study as last port of call or LPOC) is relatively stable and the biological, chemical and physical characteristics of the port are well known to the port authority. The system, therefore, is aimed at removing planktons that are characteristically native or resident in that port aquatic environment before the water is loaded as ballast into the ballast water tank of the ship.

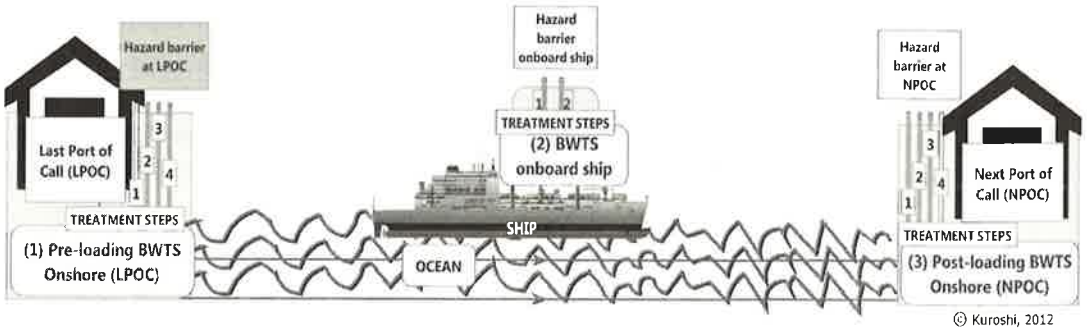

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Figure 1. Ballast Water Treatment Options- the onshore treatment options (pre-loading {1} and post-loading {3}) both having more treatment steps or hazard barriers than the shipboard treatment {2} model.

METHODS

The study covered sampling of Port Harcourt Harbour water in Nigeria. The field samples were subjected to laboratory analysis. Inferential statistics was employed to determine the relationships between the physicochemical properties of sampling stations (Table 1) and organisms' density (see Figures 2 & 3). Literature on ballast water treatment research were reviewed, and the most viable treatment options for Port Harcourt Harbour (Nigeria) based on the field results obtained were discovered to be treatment combinations that could remove most of the species found in the study area, especially; *Alexandrium minutum, Acartia clausi, Pseudocalanus elongatus, Tortanus sp.,* and *Oncaea sp.,* which are non-indigenous to North America; one of the Harbour's leading trading regions in the world.

RESULTS

Table 1. Physicochemical Properties of Study Area

S/N	STATION CODE	PARAMETERS (mean)						
		PH	COND (μscm^{1})	TURB (NTU)	TEMPT (°C)	SALINITY(0/00)	DO (mg/l)	TDS (mg/l)
1	NP1	7.51	33600	3.0	29.1	21.2	6.7	23520
2	NP2	7.73	33700	3.0	29.2	21.3	6.6	23590
3	OK1	7.63	34600	1.0	29.1	21.9	7.7	24220
4	OK2	7.64	34900	1.0	29.0	22.1	7.7	24430

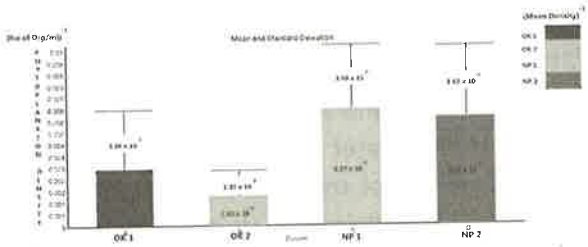

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Figure 2. Summary of Mean and SD of plankton Density in General Cargo Terminal (NP 1 & NP 2) and Oil Terminal (OK 1 & OK 2) of Port Harcourt Harbour.

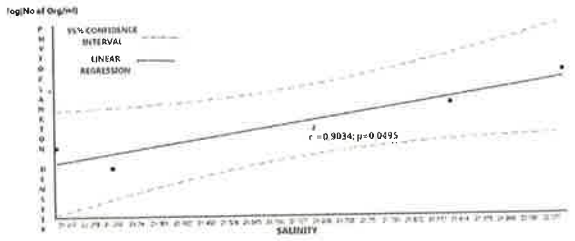

Figure 3. Total Phytoplankton Density log (mg/l) as a function of Salinity.

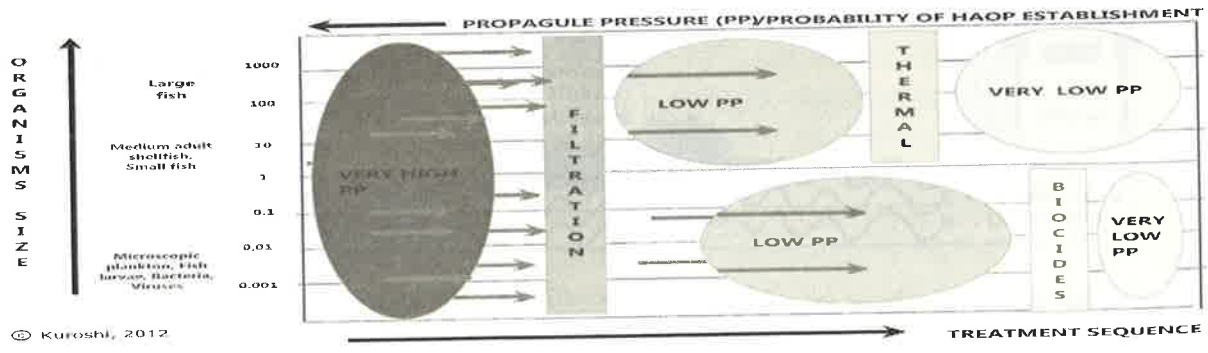

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Figure 4. Relationship between proposed treatment sequence for the Study Area and Propagule Pressure /Probability of HAOP Invasion.

DISCUSSION

Physicochemical Properties of Harbour Water and Plankton Survivability

A very significant difference exists between the plankton densities of the stations sampled (ANOVA, F_{calc}=6.650; df=3, 52; p=0.0007 see Figure 2). A strong positive correlation exists between plankton density and salinity (Regression analysis, r^2=0.9034, df=3, p=0.0495; see Figure3). This shows that altering some physicochemical conditions of the water will significantly affect HAOP survivability (Figure 3). Therefore, by deploying a three stage treatment process proposed in this study (Figure 4), the probability of transfer of HAOP from the host port will be sufficiently minimized so as to meet the minimum requirements of the BWM Convention, 2004.

Potential Financial Deliverables of PreOBWTS to Ports Authority

The 6 leading seaports in Nigeria from ship traffic records of year 2010 for example, handled approximately 5,000 ocean-going vessels. By transposing a conceptual design and estimates for onshore Ballast Water treatment developed by Australian Quarantine and Inspection Services (AQIS, 1993) and California Association of Ports Authority (CAPA, 2000), onshore treatment systems with approximated storage capacity of greater than 120,000 MT shall be required per port to serve the about 5,000 ocean going vessels that visit these ports annually. Pumping rate for each treatment system should not be less than 4,000 MT/h.

Studies by AQIS (1993) have shown that the average annual capital cost of shipboard treatment of ballast water per ship with discharge capacity of 500,000MT per year is about $2,040,844. Whereas annual capital cost of onshore treatment per ship with similar annual discharge capacity is four times less at about $556,594.

The PreOBTWS has taken care of some of the downsides of the existing onshore treatment system (i.e. Post-loading treatment system) in areas such as, *ship lightering*- where ships discharge ballast water to lower draft in order to enter a shallow channel or cross over a shallow bar. The system assures safe discharge of ballast water at any location as the ballast water has already been treated before it was loaded prior to voyage, which is not the case with post loading (Kuroshi,2012). Also, issues regarding *ship delays* at ports in order to offload ballast water into treatment systems does not arise in the case of the PreOBWTS. It is safe to discharge the ballast water into surrounding water because it has already been treated (seeFigure5). *Space limitation* in the port for the project need not be a serious concern as the system can be remotely sited and by means of piping system can serve the visiting ships in the harbour.

The Port Authorities through private or joint venture initiative could provide ballast water treatment services to the visiting ocean going ships, by charging a set environmental levy. If the Port Authority in Nigeria for example, could charge an environmental levy of about $40 per 1000MT of treated ballast water supplied to visiting ocean-going ships as against the approximately $82 per 1000MT in cost for shipboard treatment using figures adjusted to June 2010 US dollars (see AQIS, 1993): because it will require an onshore treatment system one ninth ($1/9^{th}$) of that cost to treat 1,000MT (i.e. $9 per 1,000MT). The Port Authority will be making about $30 dollars (about300%) in profit per 1000MT and saving the visiting ships (i.e. shipping companies) about $40 (about50%) in cost of shipboard treatment per 1000MT, aside the other advantages of the visiting ship having a reduced port turnaround time due to less port state control BWM convention compliance functions. This is certainly a win-win proposition for both the Port Authority and the visiting ship.

TABLE 2: Summary Of Treatment Cost Estimates And Profit Using 2010 Ship Traffic Record From Six Major Sea Ports In Nigeria.

PORT	OCEAN GOING SHIP TRAFFIC IN 2010	APPROX. TOTAL BALLAST WATER TO BE TREATED PER ANNUM (MT)	COST FOR TREATMENT PER ANUM ($)	REVENUE GENERATION FROM TREATMENT ($)	PROFIT ($)
Apapa	1,563	781,500,000	7,033,500	31,260,000	23,445,000
Tin Can Island	1,607	803,500,000	7,231,500	32,140,000	24,105,000
Rivers Port	471	235,500,000	2,119,500	9,420,000	7,065,000
Onne	785	392,500,000	3,532,500	15,700,000	11,775,000
Delta	337	168,500,000	1,516,500	6,740,000	5,055,000
Calabar	199	99,500,000	895,500	3,980,000	2,985,000
GRAND TOTAL	4,962	2,038,200,000	22,329,000	95,658,000	71,743,500

The Port Authority could make approximately about $ 71,743,500 in profit from all the six major sea ports in Nigeria, if the environmental levy charged is $40 per 1000MT of treated ballast water (see Table 1).

Port Authority's Responsibility for PreOBWTS

The port authority from Figure 5 decides the type of treatment system A to be installed in the port that is based on the specific baseline information on the port. This unique baseline information should guide the port authority in deciding whether to go for a single treatment system C or a combination of systems B and what kind of combination is appropriate for the harbour.

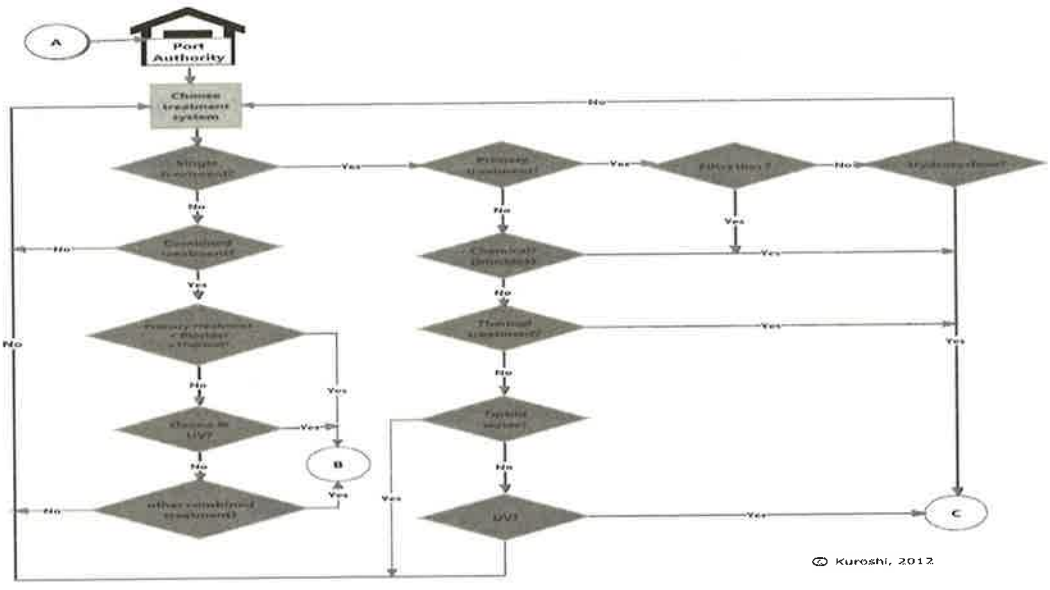

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Figure 5. Port Authority's (Port of call) Onshore Ballast Water Management Decision Flowchart Model.

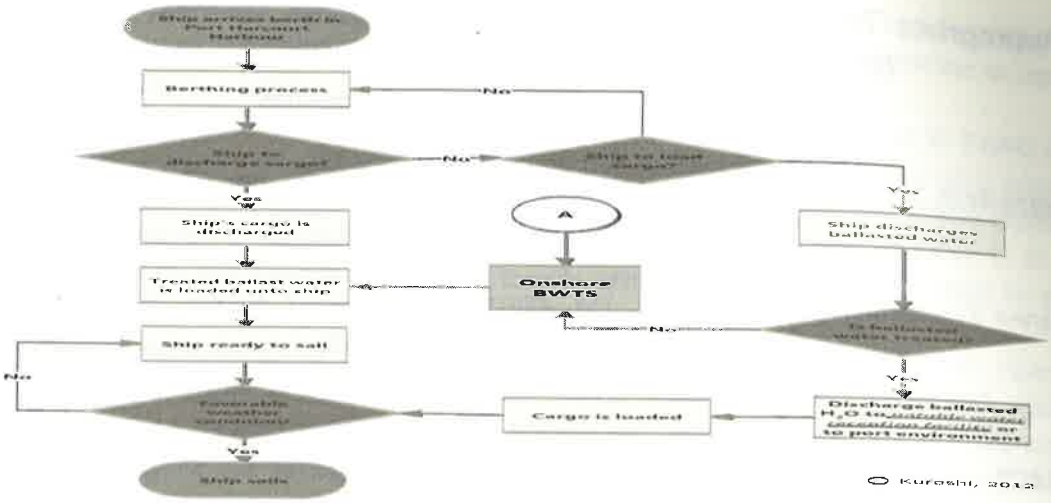

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Figure 6. Ship's Onshore Preloading Ballast Water Management Decision Flowchart Model.

CONCLUSION

A three stage shore treatment combination process was therefore, proposed by the study for employment in Port Harcourt Harbour with respect to the harbours unique biological and physicochemical characteristics. The first stage should involve filtration of the harbour's sea water to remove the larger organisms, mainly zooplankton. It should be followed by a stage of heating of the harbour's water (>38°C) to remove larger zooplanktons that have escaped the filtration process (Figure4). The third stage should involve the use of biocides which has a strong lethal effect on a lot of phytoplankton and bacteria. And finally, the treated sea water is pumped into the visiting ship as treated ballast water.

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Kuroshi, L.A. (2012). Onshore ballast water treatment stations: A harbour specific vector management proposition. *Thesis Submitted to the World Maritime University, Malmo, Sweden for the Award of a Master of Science Degree.*

Keywords: *Ballast Water Management, Harmful Aquatic Organisms and Pathogens (HAOP), Planktons, Pre-loading Onshore Ballast Water Treatment System (PreOBWTS), Shipboard Ballast Water Treatment .*

The Appropriate Test Vessels Type and Medium Volume To meet the Validation (OECD 201) of Algal Growth Inhibition Test with Marine Algae *Skeletonema costatum*

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ABSTRACT

To find the appropriate test vessel type and medium volume for algal growth inhibition test with marine algae *Skeletonema costatum,* 30, 40, 50 and 65% of capacity of 250 mL glass flask (GF-250), 250 mL & 50 mL cell culture flask (CCF-250 and CCF-50) and 50 mL centrifuge tube (CT-50) were filled with f/2 medium and 15 mL test tube (TT-15) was filled with 10 mL of it. *S. costatum* was inoculated in the each experiment vessel which initial cells density was 5,288±731 (mean±S.D.) cells/mL. The experiment vessels incubated at 20℃ under the continuous light (day light fluorescent lamp, 61.5 μmol/m^2·s) in shaking incubator (60 rpm) for 72 hour, and cell density in each experiment vessel measured every 24±2 hour. Average specific growth rate (ASGR), mean coefficient of variation for section-by-section specific growth rates (mCV-SBSSGR), coefficient of variation of average specific growth rates (CVASGR) of *S. costatum* and pH variation of medium were compared with test validation of OECD 201.

ASGR, mCV-SBSSGR, CVASGR of *S. costatum* and pH variation in GF-250 containing 80 mL (GF-250-80), 100 mL (GF-250-100), 125 mL (GF-250-125) and 160 mL (GF-250-160) of f/2 medium were 1.45±0.19~1.48±0.08 day^{-1} (mean±S.D.), 12.43~16.81%, 4.26~6.29% and 0.24±0.12~0.37±0.15 (mean±S.D.), respectively. It was indicated that all experiments were met with test validation of OECD 201.ASGR, mCV-SBSSGR, CVASGR and pH variation in CCF-250-80, 100, 125 and 160 were 1.44±0.14~1.57±0.17 day^{-1}, 25.00~58.17%, 2.93~7.89% and 0.75±0.30~0.99±0.24, respectively. ASGR, mCV-SBSSGR, CVASGR and pH variation in CCF-50-16, 20, 25 and 33 were 1.46±0.07~1.54±0.10 day^{-1}, 25.27~35.96%, 4.89~7.92% and 0.71±0.28~0.86±0.33, respectively. ASGR, mCV-SBSSGR, CVASGR and pH variation in CT-50-16, 20, 25 were 1.40±0.06~1.45±0.07 day^{-1}, 21.41~26.04%, 3.04~4.89% and 0.73±0.23~0.86±0.28, respectively. ASGR, mCV-SBSSGR, CVASGR and pH variation in TT-15-10 was 1.42±0.05 day^{-1}, 30.50%, 3.70% and 0.69±0.21, respectively.

The CVASGR and mCV-SBSSGR in some part of experimental medium volume of CCF-250 and CCF-50 were not met with test validation of OECD 201. The experiment results indicated that GF-250 and TT-15 are appropriate test vessels for algae growth inhibition test with *S. costatum.* The proper range of medium volume of GF-250 is 30~65% and 10 mL of medium for 15 mL test tube is suitable.

1. INTRODUCTION

For developing the ballast water management system (BWMS), BWMS has to meet the requirements of regulation D-2 of the Ballast Water Management Convention and simultaneously, chemical analysis, aquatic toxicity test and environmental risk assessment for discharge water treated by BWMS that make use of active substance and preparations have to conduct and assess to protect receiving

environment or human health. The data set of aquatic toxicity test should be included with multiple test species (a fish, an invertebrate and plant) and carried by relevant OECD guideline or equivalent ((G9, Resolution MEPC 126(53)).

Until October 2012, 42 BWMS was received Basic or Final approval from IMO and 35 BWMS of them were use of active substance, and electrolysis disinfection technologies had been mainly used in the BWMS (IMO, 2013). For discharge water treated by BWMS using electrolysis disinfection technologies, most sensitive organism of aquatic toxicity test was algae, *Skeletonema costatum* (Shon et al., 2013). Therefore the results and reliability of aquatic toxicity test with algae is very important to evaluate the discharge water treated by BWMS making active substances.

In newly revised methodology for G9, test validation criteria of aquatic toxicity test with algae should be taken into requirements of OECD guideline 201 (OECD, 2006) and/or ISO 10253 (ISO, 2006). The criteria of it in newly revised methodology for G9 are that specific growth rate is more than 0.92 day^{-1} within the 72-hour test period, and the mean coefficient of variation for section-by-section specific growth rate must not exceed 35 %, and the coefficient of variation of average specific growth rate in the replicates during the whole test period must not exceed 7% (IMO, 2012).

The selection of the test vessel is one thing of many important components to meet the test validation in the aquatic toxicity test. It is related to meet the test validation that good & stable growth rate in closed system could be obtained through the good gas exchange to prevent increasing pH by CO_2 uptake. Good turbulence and high surface/volume ratio in the test vessel vary depending on the surface area and movement of test medium that is affected by the shape of test vessels and volume of the test medium. Therefore, the aim in this study was to find the appropriate test vessel type and medium volume for algal growth inhibition test with marine algae *Skeletonema costatum.*

2. MATERIALS AND METHODS

2.1 Test Vessels & Medium Volume

30, 40, 50 and 65% of capacity of 250 mL glass flask (GF-250), 250 mL & 50 mL cell culture flask (CCF-250 and CCF-50) and 50 mL centrifuge tube (CT-50) were filled with f/2 medium and 15 mL test tube (TT-15) was filled with 10 mL of it (Table 1). All medium volume of each test vessel was 12 replicates.

Table 1. Test vessel of capacity and medium volume

Test vessel	Medium volume (mL) (proportion of capacity (%))
250 mL Glass flask	80 (30), 100 (40), 125 (50), 160 (65)
250 mL Cell culture flask	80 (30), 100 (40), 125 (50), 160 (65)
50 mL Cell culture flask	16 (30), 20 (40), 25 (50), 33 (65)
50 mL Centrifuge tube	16 (30), 20 (40), 25 (50), 33 (65)
15 mL Test tube	10

2.2 Test Organism & Experiment Condition

Experiments carried out with the diatom *Skeletonema costatum* (Strain No.: LB 308) obtained from UTEX (the University of Texas at Austin the culture collection of Algae 205 W), and maintained in batch culture for several months at our laboratory. Batch culture vessels were incubated at 20±1 ℃ under the continuous light (day light fluorescent lamp, 61.5 $\mu mol/m^2 \cdot s$). The experiment condition during 72 hour was same with batch culture condition in shaking incubator (60 rpm).

2.3 Measurement & Calculation

Cell density

S. costatum was inoculated in the each experiment vessel which initial cells density was 5,288±731 cells/mL (mean±S.D.). The cell density measured by manual counting the 1 mL aliquot from experiment vessel on Sedgwick-Rafter chamber at every 24±2 h.

pH

pH was measured at initial and final of the experiment using pH meter (ORION, Thermo Fisher Scientific Inc., USA).

Growth rate

Growth rate was calculated from measurement of cell density as fallowed equation; $r = (\ln X_j - \ln X_i)/(t_j - t_i)$, where, r = growth rate of *S. costatum*, X_j = cell density at time j, X_i = cell density at time i.
Section-By-Section specific growth rate (SBSSGR) calculated as the specific growth rates for each day during the course of the test, average specific growth rate (ASGR) calculated as the specific growth rates for 72 hour.
The coefficient of variation (CV) for SBSSGR and ASGR calculated as fallowed equation; CV=standard deviation/mean x 100.

3. RESULTS

3.1 Growth Rate

Growth rate of *Skeletonema costatum* in 250 mL glass flask (GF-250), 50 mL cell culture flask (CCF-50), 50 mL centrifuge tube (CT-50) and 15 mL test tube (TT-15) containing all experiment medium volume was continually-stably increased (Figure 1a, c, d) but it of medium volume 80, 100 and 120 mL in 250 mL cell culture flask (CCF-250) showed rapid growth during 24~48 hrs and then growth rate during 48~72 hrs was less than 24~48 hrs (Figure 1b).
The ASGR of *S. costatum* in test vessels during 72 hour was 1.40±0.06 ~ 1.55±0.14 day^{-1} (mean±S.D.) and ASGR was met with OECD validation which was over 0.92 day^{-1} within the 72 hour experiment period (Table 2). The coefficient of variation of ASGR (CVASGR) was ranged from 2.93% to 9.15%. CVASGR in CCF-250 containing 80 (CCF-250-80) and 120 mL (CCF-250-120), and in CCF-50 containing 20 (CCF-50-20) and 25 mL (CCF-50-25) was not met with OECD validation which must not exceed 7% (Table 2).
Average SBSSGR of *S. costatum* in test vessels was 1.40±0.34 ~ 1.55±0.64 day^{-1} which was similar the ASGR within the 72 hour and mean CV of SBSGR (mCV-SBSSGR) was ranged from 12.43 to 58.17 % (Table 2). mCV-SBSSGR of in CCF-250-80, 100, 120 and CCF-50-20 was not met with OECD validation which must not exceed 35% (Table 2).

3.2 pH

pH variation is not descirbed in the test validation of OECD 201 but incubation codition in the OECD 201 require that the pH of the control medium should not increase by more than 1.5 units during the test. pH variation of all experimantal medium volume in the test vessels was ranged from 0.06 to 1.32(Figure 2) and that was met with reqirement of OCED 201.

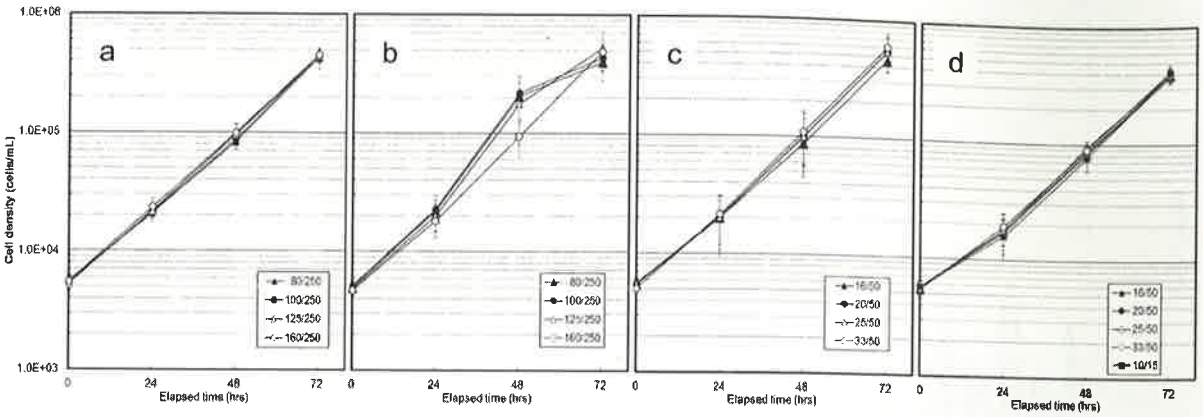

Figure 1. Growth curves of medium volume with test vessel type (the legend indicated medium volume/test vessel volume; a: 250 mL glass flask; b: 250 mL cell culture flask; c: 50 mL cell culture flask; d: 50 mL centrifuge tube and 15 mL test tube).

Table 2. Section-by-section growth rate (SBSGR) and specific growth rate (SGR) during whole experiment period (72 hours)

Test vessel	Medium volume(mL)	SBSGR		SGR during 72hours	
		Mean±S.D.	Mean C.V.	Mean±S.D.	C.V.
250 mL Glass flask	80	1.45 ±0.24	16.81	1.45±0.09	6.29
	100	1.47±0.22	14.97	1.47±0.08	5.22
	125	1.48±0.19	12.68	1.48±0.08	5.50
	160	1.47±0.18	12.43	1.47±0.06	4.26
250 mL Cell culture flask	80	1.45±0.84	58.17	1.45±0.11	7.89
	100	1.47±0.75	50.68	1.47±0.08	5.11
	125	1.55±0.64	41.54	1.55±0.14	9.15
	160	1.55±0.39	25.00	1.55±0.05	2.93
50 mL Cell culture flask	16	1.46±0.44	29.91	1.46±0.07	4.89
	20	1.46±0.53	35.96	1.46±0.11	7.80
	25	1.47±0.50	34.08	1.47±0.12	7.92
	33	1.54±0.39	25.27	1.54±0.10	6.72
50 mL Centrifuge tube	16	1.43±0.37	26.04	1.43±0.07	4.89
	20	1.40±0.34	24.37	1.40±0.06	4.44
	25	1.42±0.34	23.81	1.42±0.04	3.04
	33	1.45±0.31	21.41	1.45±0.07	4.63
15 mL Test tube	10	1.42±0.43	30.50	1.42±0.05	3.70

2.3 Measurement & Calculation

Cell density

S. costatum was inoculated in the each experiment vessel which initial cells density was 5,288±731 cells/mL (mean±S.D.). The cell density measured by manual counting the 1 mL aliquot from experiment vessel on Sedgwick-Rafter chamber at every 24±2 h.

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3. RESULTS

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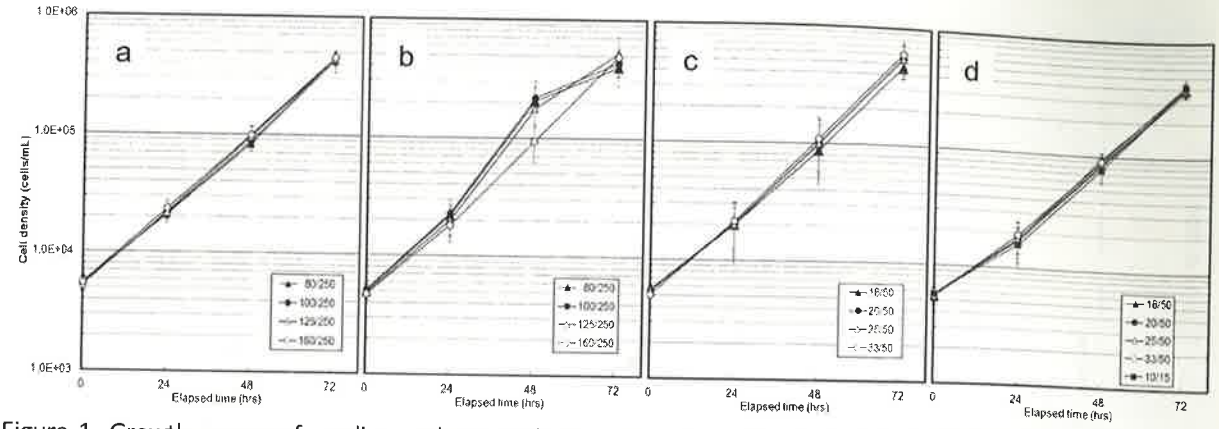
3.2 pH

pH variation is not descirbed in the test validation of OECD 201 but incubation codition in the OECD 201 require that the pH of the control medium should not increase by more than 1.5 units during the test. pH variation of all experimantal medium volume in the test vessels was ranged from 0.06 to 1.32(Figure 2) and that was met with reqirement of OCED 201.



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	125	1.55±0.64	41.54	1.55±0.14	9.15
	160	1.55±0.39	25.00	1.55±0.05	2.93
50 mL Cell culture flask	16	1.46±0.44	29.91	1.46±0.07	4.89
	20	1.46±0.53	35.96	1.46±0.11	7.80
	25	1.47±0.50	34.08	1.47±0.12	7.92
	33	1.54±0.39	25.27	1.54±0.10	6.72
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	20	1.40±0.34	24.37	1.40±0.06	4.44
	25	1.42±0.34	23.81	1.42±0.04	3.04
	33	1.45±0.31	21.41	1.45±0.07	4.63
15 mL Test tube	10	1.42±0.43	30.50	1.42±0.05	3.70

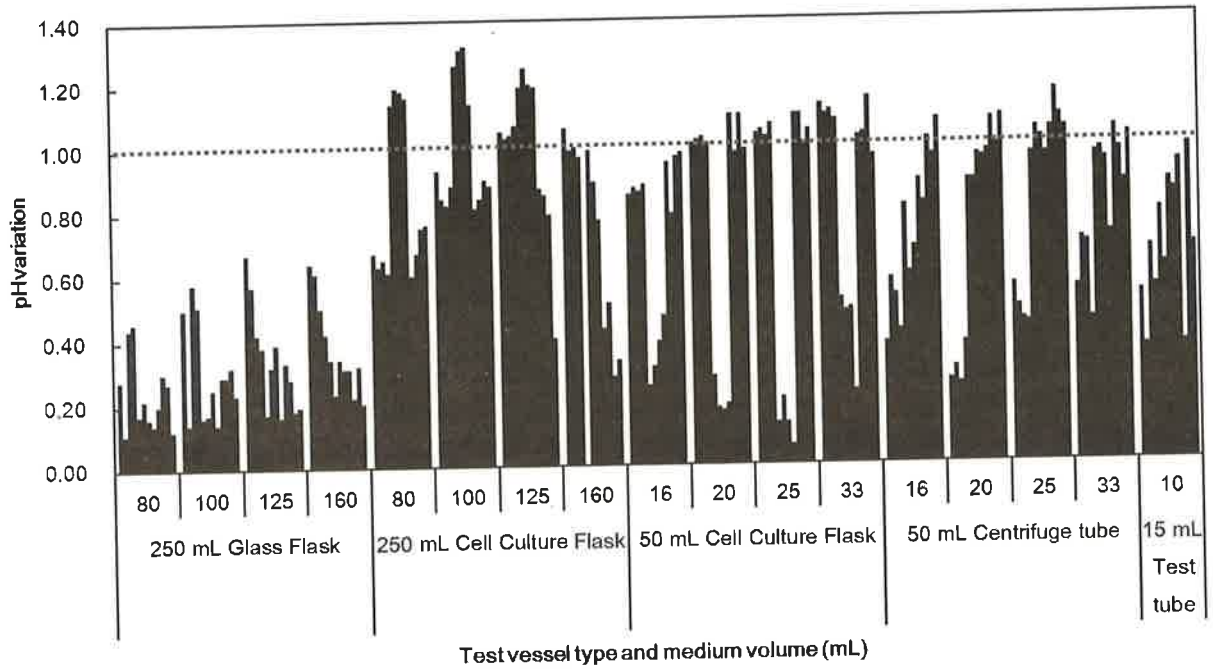

Figure 2. pH variation in the growth experiment, depending on medium volume and test vesssel type (Red dotted line: limitation suggested by ISO 10253).

4. DISCUSSION

ASGR of the test validity of OECD 201 means minimum test organism's rate of the growth, and mCV-SBSSGR and CVASGR of it mean that test organism has to grow stably and continually at a steady rate. ASGR in all experiments that were more than 0.92 day^{-1} at the end of the experiment were met with test validity of OECD 201, but the CVASGR and mCV-SBSSGR in some part of experimental medium volume of CCF-250 and CCF-50 were not met with test validation of OECD 201. These results indicate that test vessels of GF, CCF, CT and TT type are an appropriate culture vessel for *S. costatum* but not appropriate test vessel for CCF and CT type.

To prevent increasing pH, the use of shaking for good gas exchange is recommended in OECD 201 and ISO 10253. And in this study, sili-stopper on glass flask was used and it played role the ventilation between in and out of glass flask without making bacteria or virus's way into a glass flask. Other test vessels for gas exchange had disadvantage because they were closed with the cap. pH of the test medium is related to CO_2 availability and photosynthesis of algae, and these are related to algae growth. pH increases as a result of CO_2 uptake when the carbonate system is displaced from its equilibrium with air, and this happens if the CO_2 mass transfer across the air/water interphase into the culture is smaller than the CO_2 utilization rate (Arensberg et. al., 1995). At high pH levels the availability of CO_2 may become limiting to marine phytoplankton growth and photosynthesis (Chen and Durbin, 1994). pH variation in the algae growth inhibition test of OECD 201 and ISO 10253 is limited at 1 (ISO 10253) or 1.5 (OECD 201) unit. If the conservative criterion of pH variation of ISO 10253 was applied, test vessel of CCT and CT type is high possibility not to meet the test validation (Figure 2).

The cross sections depending on shape of the test vessels were varied and the movement of medium depending on the cross section of each test vessel was changed. While medium movement in GF was eddy as well mixing, CCF, CF and TT were left and right shake and making the splash in the

test vessel because they were placed in landscape in shaking incubator for securing high surface/volume ratio to get good gas exchange. The movement of medium might affect to stable growth rate of test organism and pH variation in the test vessel. The experiment results indicated that GF-250 and TT-15 are appropriate test vessels for algae growth inhibition test with *S. costatum*. The proper range of medium volume of GF-250 is 30~65% for and 10 mL of medium for 15 mL test tube is suitable.

5. REFERENCE

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Keywords: *algae growth inhibition, test validation, test vessels*

Chemical Speciation and Fate in Electrolysed Ballast Water under G8 Test

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1. Introduction

As the International Convention for the Control and Management of Ships' Ballast Water and Sediments was proposed by the International Maritime Organization (IMO) in 2004, various ballast water treatment technologies such as electrolysis, ozonation and ultraviolet oxidation have been developed to protect marine environment from invasion of non-indigenous species. However, ballast water management systems (BWMS) using active substance(s) have to be obtained approval by the Marine Environment Protection Committee (MEPC) for the safe use of active substance and it's by-products. According to the Procedure for Approval of Ballast Water Management Systems that Make Use of Active Substances (G9), principal regulation by-products are trihalomethanes (THMs), haloacetonitriles (HANs), haloacetic acids (HAAs), bromate and so on.

In this study, various disinfection by-products (DBPs) including regulation species and emerging compounds were analyzed in ballast water to evaluate their formation and fate in BWMS using electrolysis system. For this, the analysis results were based on data reported in non-confidential documents from IMO. Also, several organic matter parameters and system conditions were surveyed in the BWMS to evaluate formation and fate of DBPs.

2. Materials and methods

All samples were collected from two land base test facility for more than 200 ton and five pilot scale test facility having electrolysis system in Korea. These systems were one electrochlorination and other electrolysis types. The sampling points were test water, control water and treated water tanks by IMO Regulation D-2 (Ballastwater performance standard), and the sample volume was 20 L. At the same time, total residual oxidants (TRO) and free residual oxidants (FRO) were measured on sites (day 0). To investigat more accurate chemical speciation, fate and formation, control water and treated water were surveyed at day 1 and 5. Especially, at day 5, neutralized treated water was collected to confirm chemical pattern by neutralization. During the sampling period, the water samples were stroed in control and treated tanks. The samples were kept at 4℃ after adding preservation reagents, and analyzed within 14 days. Target compounds for this study were divided by cause substances and DBPs. The cause substances are DOC, UV-254, specific UV absorbance (SUVA), molecular weight size distributions and NOM (fulvic acid, humic acid, protein). DBPs are including THMs, HANs, HAAs, chloral hydrate, chloropicrin, bromate, halopehnols (HPhs) and halogenated volatile organic compounds (HVOCs). Whole procedure from sampling to reporting was performed based on US EPA methods or ISO standards. Besiedes the cause substances and DBPs, basic water parameters such as pH, water temperature and

ORP were determined.

3. Results and discussion

3.1. Occurrence of DBPs in electrolysis BWMS

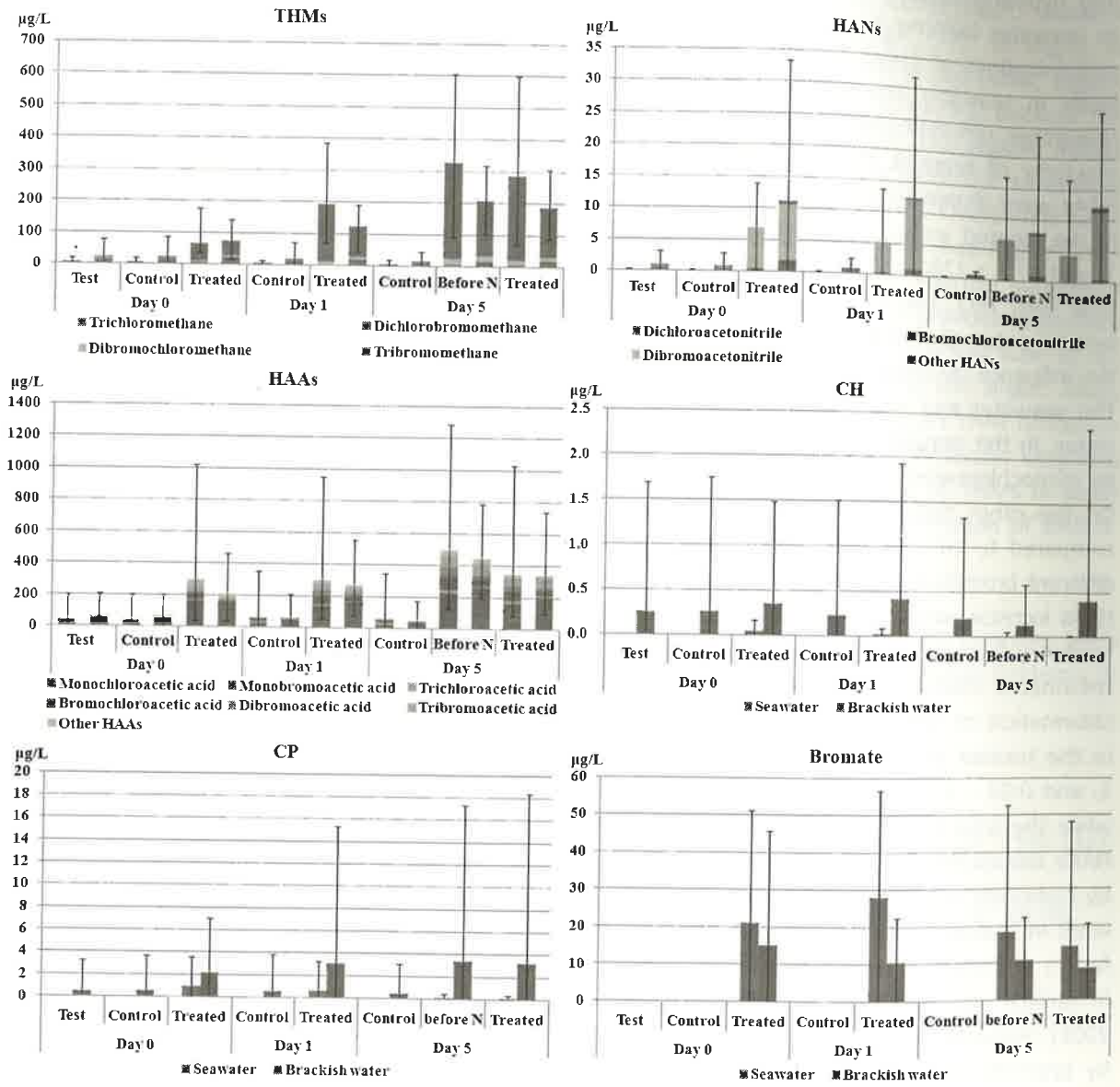

Figure 1. The concentrations of DBPs in electrolysis BWMS (S: seawater, B: brackish water, Before N: before neutralization).

Figure 1 shows the concentrations of DBPs in seawater and brackish water from the electrolysis BWMS. THMs, HAAs, HANs, CH, CP and bromate had the increase tendencies during the electrolysis, while HPhs and other HVOCs showed insignificant patterns. In the test water samples, the concentrations of DBPs varied with sampling time and sites. THMs were found in all treated water samples. Overall, THMs in the treated water showed the increase pattern as time passed (day 0: 15.2 – 177 µg/L, day 1: 0, 42.3 – 383 µg/L, day 5: 72.3 – 606 µg/L). Previous studies reported that the reaction time is a positive factor of THM formation (Pourmoghaddas and Stevens, 1995; Nikolaou et al., 2004).

Especially, in the treated water, tribromomethane had much higher levels compared to other three THMs. This distribution of THMs was similar to other researches on chlorination of seawater (Allonier et al, 1999; Delacroix et al., 2013; Sun et al., 2009; Werschkun et al., 2012). Brominated THMs are more dominantly formed because hypochlorite (OCl-) and hypochlorous acid (HOCl) change rapidly into hypobromite (OBr-) and hypobromous acid (HOBr) in water containing bromide ion (Br-) such as seawater (Allonier et al, 1999; Taylor, 2006). In the treated water, the seawater had higher total concentrations of THMs than the brackish water. This seems to be related to different bromide ion levels in seawater and brackish water (Pourmoghaddas and Stevens, 1995; US EPA, 1981). These researches reported that total THMs increase with increasing bromide level owing to more effective reactivity of bromine than chlorine.

HAAs were dominant compound group generated from the electrolysis facilities together with THMs. In the treated water, the levels of HAAs were 35.9 – 1020 μg/L at day 0, 55.5 – 945 μg/L at day 1, and 87.1 – 1284 μg/L at day 5. In principal, the concentrations of HAAs were proportional to contact time with TRO. The reaction time is concerned as a factor of HAA formation (WRRI, 2008). In the treated water, the seawater had higher total concentrations of HAAs than the brackish water, indicating the influence by bromide ion similar to the result of THMs (Sun et al., 2009; Uyak and Toroz, 2007). The seawater and brackish water showed somewhat different distribution tendencies in the treated water. In the seawater at day 5, bromochloroacetic acid was the most dominant compound, followed by monochloroacetic acid, dibromoacetic acid, monobromoacetic acid, tribromoacetic acid and others. On the other hand, in the brackish water at day 5, monochloroacetic acid has a high proportion compared to tribromoacetic acid, bromochloroacetic acid and other HAAs. This may be related to different bromide levels between seawater and brackish water. According to several studies, brominated HAAs increase with increasing bromide level in broad outlines, while chlorinated HAAs have the decrease tendency (Sun et al., 2009; Uyak and Toroz, 2007). Also, Cowman and Singer (1996) reported that chlorinated HAAs are gradually transformed into bromochlorinated or brominated species during chlorination of water containing bromide ion.

In the treated water, the levels of HANs were 0.03 – 33.3 μg/L at day 0, 0.03 – 31.8 μg/L at day 1, and 0.02 – 27.5 μg/L at day 5. At day 0, HANs were generated through the electrolysis facilities, while the total levels decreased during the residence time in general. Kim et al. (2002) reported that HANs decreased with contact time after 48 h, and the reason may be due to decomposition of HANs by hydrolysis and additional reactions with residual chlorine (Nikolaou et al., 2004b). HANs had lower levels in seawater than brackish water unlike THMs and HAAs, showing the influence by specific formation factors of HAAs (i.e., protein precursor) unconnected with THMs or HAAs (Reckhow et al., 2001). Generally, in surface water, dichloroacetonitrile is regarded as a major HAN (Kim et al., 2002; Reckhow et al., 2001). However, in this study, dibromoacetonitrile was the most dominant because of the influence by bromide ion in seawater and brackish water.

Chloral hydrate, chloropicrin and bromate were generated from the chlorination system. Their concentration ranges showed maximum 2.36 μg/L of chloral hydrate, 18.3 μg/L of chloropicrin, and 56.5 μg/L of bromate in the treated samples including neutralization water. As chloral hydrate ($C_2H_3Cl_3O_2$) and chloropicrin (Cl_3CNO_2) are chlorinated species, they had lower levels in seawater than brackish water. On the other hand, the concentrations of bromate were higher in the seawater than the brackish water because bromide ion is an essential factor of bromate formation pathway (Xin et al., 2008). It is known that chloral hydrate and chloropicrin are closely connected with algae during their formation (Fang et al., 2010; Lui et al., 2012). Especially, chloropicrin may be occurred from chlorination of algal organic material such as amino acids like HANs (Lui et al., 2012). Therefore, chloral hydrate and chloropicrin need to be consistently monitored in the electrolysis BWMS.

3.2. Formation characteristics of DBPs according to water parameters

Various by-products are generated from the reaction of organic matter and TRO (Hua and Reckhow, 2007). Especially, for BWMS test in brackish water, starch as an organic matter is added into test ballast water to maintain certain level by G8 (organism: min. 3 phyla/5 species, phytoplankton: min. 1,000 inds./mL, zooplankton: min. 100,000 inds./ton, heterotrophic bacteria: min. 10,000 cells/L; seawater standard - DOC, POC, TSS: 1mg/L; brackish water standard - DOC, POC: 5mg/L, TSS: 50 mg/L). In this study, SUVA values were mostly lower 1 or 2, indicating strong predominance of hydrophilic organic matter. The control water presented slight increase tendency of SUVA as time passed. Because the control water contains glucose, starch and algae, various metabolic substances and organic matter may be generated from algal growth cycle (Huang et al., 2009). Especially, low molecular substances (hydrophilic) may have higher occurrence compared to high molecular (hydrophobic). Chlorinated water generally shows smaller SUVA than raw water (Reckhow et al.), but treated water had higher SUVA than control water in some cases of this study. This seems to be related to high molecular substances such as polysaccharide released from extinct algae (Huang et al., 2009). According to previous researches, hydrophilic organic matter induces higher reactivity of HAAs than hydrophobic portion (Lee et al., 2003). In contrast, formation of THMs increases in hydrophobic organic matter (Liang and Singer, 2003). In many cases of this study, HAA formation potential (mean 64%) was greater than THM formation potential (mean 40%) like the preceding. This result was also identified by normal molecular weighting distribution that molecular weighting distribution of HAAs was higher than those of THMs in some treated water. It seems that this study is useful for efficient development and operation of BWMS.

3.3. Emerging DBPs from electrolysis system

In this study, emerging DBPs such as perchlorate, formaldehyde, acetaldehyde and nitrosamines were additionally analyzed. As the result, perchlorate (maximum 5.86 μg/L) had the increase tendency in some cases, while the trend of formaldehyde and acetaldehyde was insignificant.

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OPTIMIZATION OF ANALYSIS METHOD FOR TOTAL RESIDUAL OXIDANT IN BALLAST WATER MANAGEMENT SYSTEM

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1. Introduction

As the International Convention for the Control and Management of Ships' Ballast Water and Sediments was proposed by the International Maritime Organization (IMO) in 2004, various ballast water treatment technologies such as electrolysis, ozonation and ultraviolet oxidation have been developed to protect marine environment from invasion of non-indigenous species. However, Ballast Water Management Systems (BWMS) using active substance(s) have to be obtained approval by the Marine Environment Protection Committee (MEPC) for the safe use of active substance and its by-products. The active substances are quantified with the total residual oxidant (TRO) as Cl2. Therefore, TRO measurement is very important factor for operating BWMS.

Generally, TRO are measured as residual chlorine using DPD (N,N-diethyl-p-phenylenediamine) colorimetric method, DPD titrimetric method, iodometric method, amperometric titration method, and o-tolidine colorimetric method. Among the methods, DPD colorimetric method is widely used because of its accuracy and expediency. According to the expert assessment of analysis methods by the American Water Works Association (AWWA), DPD colorimetric method got higher point than DPD titrimetric method, iodometric method, amperometric titration method, and syringaldazine (FACTS) method (Shin et al., 2007). But, these analysis methods are mostly applied for drinking water and low saline water. The American Public Health Association (APHA) recommend analysis of TRO in seawater instead of residual chlorine, because several seawater elements such as bromide, iodine and ammonium ion are transformed into Cl2, HClO, ClO-,HBrO, BrO- after reaction with TRO (Wang et al., 2008). Actually, it is hard to obtain accurate TRO result in seawater using DPD colorimetric method due to relatively low color reaction by salt. Like this, seawater has interferences on TRO analysis compared with surface water. Therefore, in this study, TRO analysis method in seawater was optimized by searching interference factors and comparing other analysis method under various water conditions.

2. Experimental

2.1. DPD methods

DPD analyzers were using CLX Online TRO & Chlorine Monitor (HF scientific, USA) and Pocket ColorimeterTM (HACH, Germany). For offline method, 1 mL aliquot of standard solution of chlorine was diluted to 50 mL. DPD total chlorine reagent pillow (HACH, Germany) was added. The mixture was thoroughly shaken at room temperature for 2 min. The solution was measured at 515 nm.

2.2. Al-Okab and Syed's method

1 mL aliquot of standard solution of chlorine was diluted to 40 mL. 2 mL of 0.025% (w/v) PNZ (phenoxazine) solution, 1 mL of 0.05% (w/v) NTA (4-nitro-2-(trifluoromethyl)aniline) solution, 2 mL of 5 M HCl and 2 mL of 0.5% (w/v) hexadecylpyridinium chloride monohydrate were added to a series of 50 mL volumetric flask. The mixture was thoroughly shaken at a room temperature until the red color was formed almost instantaneously. The solution was measured at 520 nm against the corresponding reagent blank prepared under identical condition.

3. Results and discussion

3.1 Effect of sodium chloride (NaCl)

In order to check the influence of NaCl, 1, 5 and 10 mg/L of TRO were injected in ultrapure water and 3.4% (w/v) NaCl solution. For TRO standard solution, 1000 mg/L of solution was made by melting calcium hypochlorite in ultrapure water, then diluted to 10 mg/L again. CLX Online TRO & Chlorine Monitor (HF scientific, USA) and Pocket Colorimeter™ (HACH, Germany) were used for measurement of TRO solutions. As the result of comparison between Pocket Colorimeter™ and CLX Online TRO & Chlorine Monitor (5 and 10 mg/L), the difference of measured values was within 10%. As shown in Table 1, ultrapure water showed average 103% of recovery rate and 3.4% (w/v) NaCl solution showed average 91.3% of recovery rate in CLX Online TRO & Chlorine Monitor. Even though the difference was slight, ultrapure water had more accurate recovery rate than 3.4% (w/v) NaCl solution.

Table 1. Measured concentrations of TRO in ultra pure water and 3.4% NaCl solution (CLX Online TRO & Chlorine Monitor)

1mg/ L TRO		5 mg/ L TRO		10 mg/ L TRO	
Ultra pure water	3.4 % NaCl	Ultra pure water	3.4 % NaCl	Ultra pure water	3.4 % NaCl
1.07	0.85	5.28	4.64	9.87	9.06
1.07	0.85	5.30	4.65	9.90	9.17
1.06	0.90	5.29	4.81	9.94	9.46
1.06	0.91	5.26	4.75	10.11	9.06
1.05	0.89	5.31	4.78	9.91	9.30
1.04	0.88	5.28	4.76	10.03	8.52
1.03	0.86	5.22	4.80	9.96	9.18
Avg : 1.05	Avg : 0.88	Avg :5.27	Avg : 4.76	Avg : 9.96	Avg : 8.15

3.2. Effect of salinity

After melting Daigo's Artificial Seawater SP (Nihon seiyaku, Japan) in ultrapure water, the artificial seawater was diluted to 7, 20 and 34 psu, respectively. 10 mg/L of TRO was spiked into dilluted artificial seawater, and recovery rate was measured (Table 2). As the result of DPD method, in higher salinity of artificial seawater, the recovery rate of TRO was lower. Moberg and Karlberg (2000) reported that iron ion and copper ions are the interference substances of DPD method. Al-Okab and Syed (2008) also reported that bromide, chloride, nitrate, sulfate, potassium, lead and iron ions are the substances that interfere to measure TRO. It is considered that chloride (17,000 mg/L) and bromide (60 mg/L) which are relatively abundant in artificial seawater worked as disturbance factors (Asano et al., 2012). Whereas, it showed Al-Okab and Syed's method is almost not influenced by salt concentration compared to DPD method.

Table 2. Concentrations and recoveries of TRO by different salinities of artificial seawater

Salinity (psu)	DPD method (offline)	Al-Okab and Syed's method
	Conc., mg/L (recovery, %)	Conc., mg/L (recovery, %)
7	8.26 (82.6)	11.13 (111.3)
20	7.79 (77.9)	11.20 (112.0)
34	7.49 (74.9)	11.02 (110.2)

3.3. Effect of chloride and bromide

Among many interfering substances, TRO recovery rate by chloride and bromide ions was examined. Also, the effect of bromide and chloride mixture was estimated. Concentration range of chloride and bromide was set based on levels of chloride (approximately 20,000 mg/L) and bromide (approximately 70 mg/L) measured in actual Korean sea water (Shim et al., 2009). Chloride and bromide were diluted with ultrapure water respectively to 3000 ~ 21000 mg/L, and 10 ~ 70 mg/L. TRO was measured by DPD method and Al-Okab and Syed's method. Firstly, as the result of DPD method, TRO recovery rate was 64.6% when concentration of chloride was 3000 mg/L, and 51.2% in 21000 mg/L (Table 3). When concentration of bromide was 10 mg/L, TRO recovery rate was 74.2%, and 53.7% in 70 mg/L. When concentration of chloride/bromide mixture was 3000/10 mg/L, recovery rate was 65.5%, and 52.8% in 18000/60 mg/L. Thus, it could be confirmed that recovery rate of TRO decreased with increasing chloride and bromide levels in DPD method.

Table 3. Absorbance and TRO values by difference chloride and bromide concentrations (DPD method)

Chloride			Bromide			Chloride and bromide			
mg/L	Abs (515 nm)	Recovery (%)	mg/L	Abs (515 nm)	Recovery (%)	Cl- (mg/L)	Br- (mg/L)	Abs (515 nm)	Recovery (%)
3000	0.0359	64.6	10	0.0413	74.2	3000	10	0.0364	65.5
9000	0.0342	61.6	20	0.0395	71.0	6000	20	0.0334	60.2
12000	0.0324	58.4	30	0.0366	65.9	9000	30	0.0326	58.7
18000	0.0312	56.2	40	0.0349	62.8	12000	40	0.0313	56.4
21000	0.0284	51.2	50	0.0350	63.0	15000	50	0.0307	55.3
			60	0.0349	62.8	18000	60	0.0293	52.8
			70	0.0298	53.7				

Table 4 shows the result of measurement using Al-Okab and Syed's method. When concentration of chloride is 3000 mg/L, recovery rate was 92.1%, and 79.0% in 21000 mg/L. When concentration rate of bromide was 10 mg/L, recovery rate was 98.1%, and 82.0% in 70 mg/L. When concentration of chloride/bromide mixture was 3000/10 mg/L, recovery rate was 89.7%, and 73.9% in 21000/70 mg/L. Al-Okab and Syed's method had smaller decrease range from influence of interfering ions compared to DPD method. However, decrease tendency of TRO recovery rate by interfering ion was the same.

Table 4. Absorbance and TRO values by difference chloride and bromide concentrations (Al-Okab and Syed's method)

Chloride			Bromide			Chloride, Bromide mixture			
mg/L	Abs (515 nm)	Recovery (%)	mg/L	Abs (515 nm)	Recovery (%)	Cl- (mg/L)	Br- (mg/L)	Abs (515 nm)	Recovery (%)
3000	0.0342	92.1	10	0.0362	98.1	3000	10	0.0344	89.7
6000	0.0315	84.1	20	0.0342	92.1	6000	20	0.0298	79.0
9000	0.0341	91.8	30	0.0323	86.4	9000	30	0.0285	75.1
12000	0.0334	89.7	40	0.0299	79.3	12000	40	0.0297	73.3
15000	0.0295	78.1	50	0.0263	68.5	15000	50	0.0268	70.0
18000	0.0321	85.9	60	0.0308	82.0	18000	60	0.0266	69.4
21000	0.0298	79.0	70	0.0308	82.0	21000	70	0.0281	73.9

4. Conclusions

In this study, TRO was measured in samples containing different chloride and bromide levels using DPD colorimetric method and Al-Okab and Syed method respectively. As the result, from low to high salinity water (about 5 – 34 psu), TRO showed decrease tendency. Also, DPD's color reaction had interference by salt such as copper and ferrous. Although Al-Okab and Syed method had about 20% higher TRO recoveries than DPD method, it is hard to be applied it because hexadecylpyridinium chloride monohydrate used in Al-Okab and Syed method is an acute toxicity matter. Thus, it is needed to reduce interruption factors for more accurate TRO analysis in seawater, for example calibration considering interference ions, pre-filter installation for removing turbidity, and so on.

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IN-LINE WATER SAMPLING METHODS FOR REPRESENTATIVE SAMPLING OF BALLAST WATER

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1. Introduction

In 2004, the International Convention for the Control and Management of Ships' Ballast Water and Sediments (BWMC) was adopted by the International Maritime Organization (IMO). The BWMC will enter into force 12 months after the ratification by 30 States, representing 35 per cent of the world merchant shipping tonnage (IMO, 2004). As of 10th of September 2013, 37 States had ratified the BWMC, representing 30.32% of the world merchant shipping tonnage (IMO, Status of Conventions), which means that the entery into force of the Convention is so close. The Port State Control (PSC) of each flag state will commence the inspection of BWMS performance after the entry into force of the BWMC, but the ratification of the BWMC has been delayed due to uncertain evaluation and monitoring standard methods to comply with Regulation D-2. Thus, it is necessary to prepare the representative sampling methods for PSC inspection. When determining the compliance with Regulation D-2, the G2 guidelines require sampling regimes to provide samples that are representative of the whole discharge of ballast water (Carney et al., 2013). Ballast water passes through various types of pipelines such as L-type, U-type and S-type from uptake to discharge. The in-line sampling should be collected at fully developed water flow region to assure representativeness because irregular velocity and turbulence is normally induced when ballast water passes through a curved pipeline. In this study, to determine the representative in-line sampling methods, entrance distance of fully developed flow depending on the various curved pipeline types were analyzed using computational fluid dynamic (CFD) simulation.

2. Materials and methods

2.1 Curved-type pipelines on ships

We have investigated the types of pipeline that were actually installed on ships, which are container carrier, product & chemical tanker and bulk carrier using drawings from Hyundai Heavy Industries (HHI) (Table 1). Four types of curved pipelines have been installed on the ships such as `L'- curved shape (L-type), `U'- curved shape (U-type), `S'- curved shape (S-type) and `twice curved `S' shape (S2-type) at the main discharge pipelines (Fig. 1).

Table 1. Details of ship's information

Number of Ship	Kind of Ship	DWT	Ballast Pump Capacity
2233	Container carrier	13,100 TEU	1,000 m^3/h
HMD 4027	Container carrier	1850 TEU	300 m^3/h
-	Product & Chemical tanker	52 K	750 m^3/h
HMD 6143	Bulk carrier	56 K	900 m^3/h

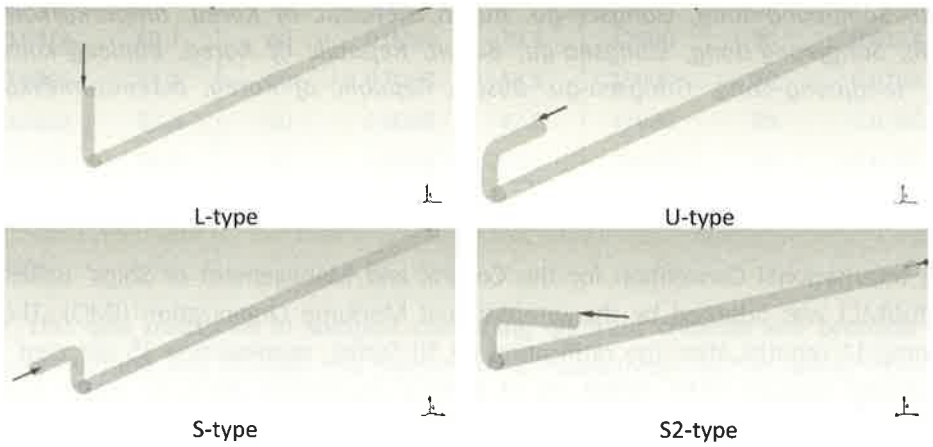


Figure 1. Four types of curved-shaped pipeline insatlled on actual ship.

2.2 Analysis of Computational Fluid Dynamics (CFD)

2.2.1 Conditions of CFD simulation

To study the fluid flow of four curved shaped pipeline for ballast water sampling, the commercial application SST (Shear Stress Transport) was used. Boundary conditions of the flow, wall and inlet were at a steady state, no slip condition and normal velocity (1, 2, 3 and 4 m/s). And the length of the entrance distance was 10 m and the main pipe inner diameter was 200 mm. To identify the entrance distance of fully developed flow, velocity field, streamline and velocity vector was analyzed.

2.2.2 Installation of Grid Structure

To reduce turbulence length that occurs when ballast water passes through a curved-shape pipeline, a grid structure was installed near the inlet of each pipeline and analysis was conducted (Fig. 2.). The maximum velocity in a pipeline was set at 4 m/s and the length of the entrance distance was 10 m and main pipe inner diameter was 200 mm.

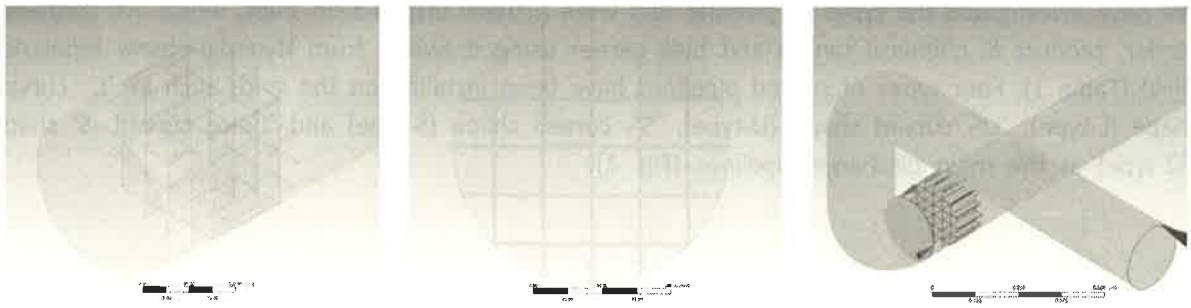


Fig. 2. Gird structure and installtion in the pipeline `S2- type'.

3. Results

3.1 Fully developed flow region in pipelines of various shapes

CFD simulations were performed to determine the fully developed flow, as dependent on the four types of curved pipelines (L-type, U-type, S-type and S2-type) and in-line velocities (1, 2, 3 and 4 m/s). Straight entrance length was required to induce a fully developed flow as follows; according to the velocity of 1, 2, 3 and 4 m/s, L-type required 6.6 m, 7.4 m, 8.0 m, and 8.4 m; U-type required 6.6, 7.5, 8.0, and 8.4 m; S-type required 6.7, 7.5, 8.0, and 8.4 m, and; S2-type required 6.7, 7.5, 8.0, and 8.4 m, respectively (Table 2).

Table 2. Required straight entrance length for fully developed flow depending on curved shapes of pipeline and in-line velocity

Type of pipeline	In-line velocity [m/s]	Required straight entrance length [m]
L	1	33.20 D[1] [6.6 m]
	2	37.23 D [7.5 m]
	3	39.80 D [8.0 m]
	4	41.77 D [8.4 m]
U	1	33.19 D [6.6 m]
	2	37.28 D [7.5 m]
	3	39.89 D [8.0 m]
	4	41.85 D [8.4 m]
S	1	33.27 D [6.7 m]
	2	37.31 D [7.5 m]
	3	39.81 D [8.0 m]
	4	41.86 D [8.4 m]
S2	1	33.28 D [6.7 m]
	2	37.35 D [7.4 m]
	3	39.95 D [8.0 m]
	1	33.20 D [8.4 m]

[1]Main pipeline inner diameter: 200 mm

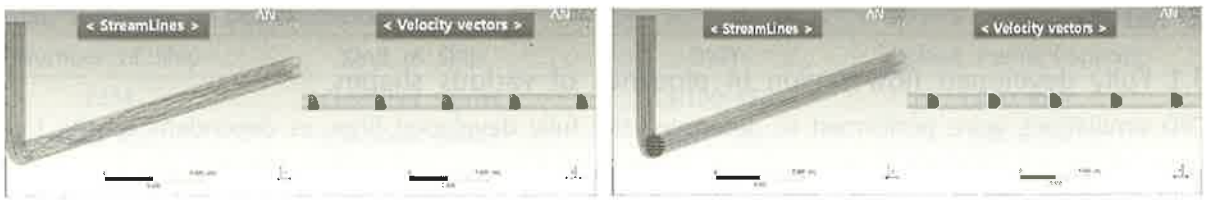
3.2 Installation of grid structure

Fluid flow was analyzed to reduce the straight length of fully developed flow in the pipeline after the grid structure was installed. The result showed that turbulent on all four types of curved pipelines were reduced. The straight entrance length was required to reduce turbalance was minimum 2.6 m-long and maximum 3.8 m-long (Table 3).

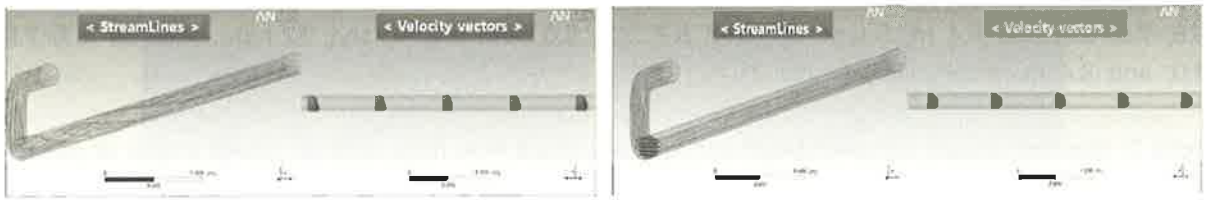
Table 3. Required straight entrance length for fully developed after installation of grid structure

Type of pipeline	In-line velocity [m/s]	Required straight entrance length [m]
L	4	13 D[1] [2.6 m]
U	4	18 D [3.6 m]
S	4	17 D [3.4 m]
S2	4	19 D [3.8 m]

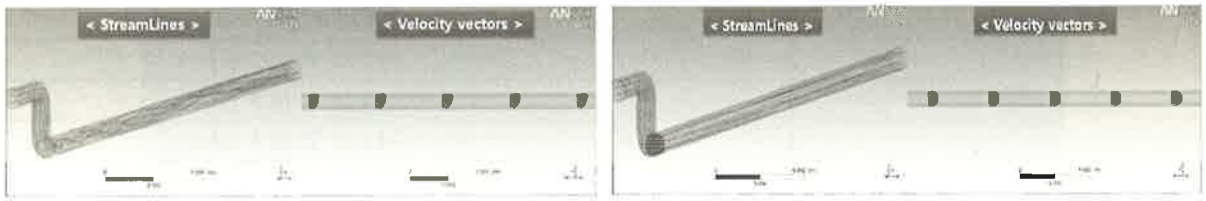
[1]Main pipeline inner diameter: 200 mm



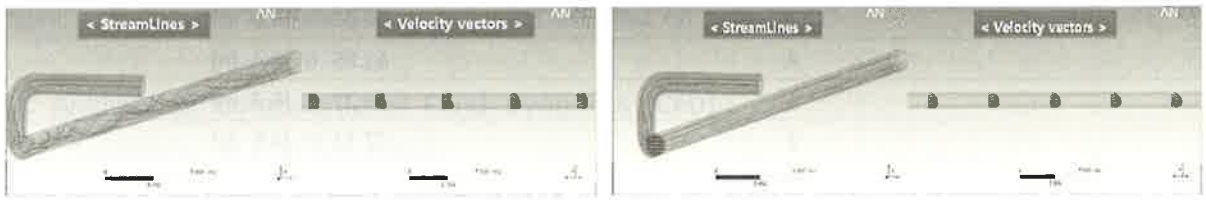
L-type pipeline (right) and L-type installed grid structure (left), in-line velocity 4 m/s



U-type pipeline (right) and U-type installed grid structure (left), in-line velocity 4 m/s



S-type pipeline (right) and S-type installed grid structure (left), in-line velocity 4 m/s



S2-type pipeline (right) and S2-type installed grid structure (left), in-line velocity 4 m/s

Fig. 3. Shapes of pipelines and characteristics of fluid flow (velocity field, streamlines, velocity vectors).

4. Discussion

The straight length required for fully developed flow in all the types of curved pipelines increased as velocity increased. But there was no significant difference in entrance lengths for the fully developed flow between the four types of curved pipelines. For a representative sampling of any type of curved pipeline, a straight pipeline is required at least 8.4 m-long. However, in practice, the installation of 8.4 m-long straight pipes seems unrealistic for a ship on account of space constraints.

To shorten the length, gird structures were installed at the pipeline entrances, which reduced the circulation of turbulence. The results of the fully developed region show that the grid structure installation reduced the length maximum 4.6 m from the location where fluid passed the structures. Previous studies on sampling port design determined that sampling ports should be located as close to the overboard outlet as possible. For turbulent flow in pipe, the true isokinetic sampling is actually impossible to implementation of the convention, due to the high variability and constant fluctuations in instantaneous flow, and highly turbulent flows have significantly long entrance lengths that require long straight sections to fully develop (Richard et al., 2008). However, the results of this study show that required straight pipeline length for fully developed flow reduced from 8.4 m to 3.8 m-long with the gird structure in the pipeline (Table 3, Fig. 3). It suggest that if the sampling port installed in 3.8 m-long at the straight pipeline from curved pipeline with the grid structure, it will be able to perform the

representative sampling.

It is important that not only are effective treatment systems developed, but also that representative sampling methods for ensuring D-2 compliance are determined. Development of representative sampling for ballast water is necessary to avoid unduly detention of ships and dispute with flag states caused by uncertain sampling methods after entry into force of the convention. Also, this development needs for shipowners who are unquestionably chose their BWMS without worrying about sampling methods.

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Is There Any Assurance?

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Ballast Water Management Systems (BWMS) need to be tested to become certified. In the near future, for systems once installed on-board a ship, also testing of BWMS for compliance control will be required. Therefore it is crucial to specify the sampling and analytical techniques used to test such BWMS. In this perspective Quality Control and Quality Assurance are important to assure uniform, adequate and comparable results all over the world. Our presentation will in more detail explain the challenges of testing BWMS when there is no standard method available, while never having exactly similar ballast water conditions.

Keywords: *Ballast Water, Ballast Water management Systems, Quality Assurance, Sampling*

Effect of Disinfection for High TRO Concentration without the Filter System

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Waste-tire-derived crumb rubber was utilized as filter media to develop an efficient filter for ballast water treatment. In this study, the effects of disinfection without filtration on the performance of the filtration were investigated. The removal efficiencies of turbidity, phytoplankton and zooplankton, and head loss development were monitored during the filtration process The marine organisms and microorganisms tested could be killed effectively by sodium hypochlorite, with inactivation efficacies exceeding 77.8%~100% without filter system. The result showed that the biological efficacy dissatisfaction met the IMO regulation D-2 ballast water performance standard for discharged ballast water. However, these filtration techniques alone did not meet the criteria for removing indigenous organisms from ballast water.

1. Introduction

Ballast water is defined as the water carried by ships to ensure stability, trim and structural integrity. If ship is empty cargo, it will fill with ballast water whereas if it loads cargo, the ballast water will be discharged. The International Maritime Organization(IMO) estimates that at least 70,000 different species are being carried are microorganisms, phytoplankton, zooplankton, etc. And most of them do not survive the journey or the new environmental conditions where they are discharged. (Engracia et al., 2013)

The introduction of unwanted alien species causes significant damage to ecological, economic and human health welfare around the globe (National Research Council, 1996; Bax et al.,2003). Mills et al. (1993) reported that over 140 alien species have been introduced to the Great Lakes of North American. Ballast water is believed to be the primary vector of unintentional transfers into the Great Lakes and other US and Canadian waters (Parsons,2003). Ribera and Boudouresque (1995) estimated that zebra mussel alone caused damages of $5,000 million from 1986 to 1995, by fouling fishing nets, boat hulls, and buoys, and blocking water intakes of power plants, water treatment plants, and other industries. The invasion of the American Atlantic coast comb jelly fish, Asian clam, and European crab, caused the collapse of fisheries in various regions (National Research Council, 1996; Bax et al., 2003). To alleviate the impacts of alien species invasion, ballast water management and treatment are essential. The newly adopted International Ballast Water Convention sets maximum concentrations of 10 viable organisms per m3 for organisms larger than 50 lm and 10 viable organisms per mL for organisms larger than 10 lm and less than 50 lm in the minimum dimension (International Maritime Organization, 2004). The convention also sets maximum concentrations for indicator microbes: one colony forming unit (cfu) per 100 mL for toxicogenic Vibrio cholerae (O1 and O139), 250 cfu per 100 mL for Escherichia coli and 100 cfu per 100 mL for intestinal Enterococci. The National Research Council (1996) evaluated a variety of approaches for removing indigenous organisms from ballast water. Among these approaches, filtration was recommended as the most promising technology. However, filtration alone may be inefficient for the removal of small size organisms (e.g., bacteria and viruses) in ballast water.

In a previous study by the authors, an innovative filtration technology, using waste-tire-made crumb

rubber as filter media, was developed to remove indigenous organisms from ballast water (Tang et al., 2006a,b). Compared with conventional granular media filters crumb rubber filters weigh less, which is an important consideration for shipboard application.

However, Auto back-flushing filtration alone did not achieve the target removal of invasive species proposed by the International Maritime Organization (2004) in these studies. It is well-known that filtration coupled with particle destabilization (e.g., coagulation) are effective processes for reducing turbidity, particle counts, viruses, bacteria and parasites (Nasser et al., 2002). It is also known that dual-media filtration generally has better filtration performance and reduced head loss development. This study aimed to evaluate the disinfection efficacies of several marine aquatic organisms in ballast water.

2. Test methods

2.1 Experimental procedure

The disinfection tested in this study was composed of non-filtration configured in parallel followed by a sodium hypochlorite disinfection process for the destruction of the microorganisms present in ballast water (Figure 1). In batch experiments, samples were taken at 0, 4 and 24 hour and analyzed with regard to the population of *Artemia* sp., Oyster Larvae and Mussel edulis. The influence of total residual chlorine was also studied adding the appropriate amounts of 300ppm sodium hypochlorite stock solution to the effluent vessel in order to achieve levels of 10, 15 and 20ppm (mg/L free residual chlorine).

2.2 Disinfection efficacy test

For the disinfection efficacy test, Over 50㎛ sized organisms (*Artemia* sp., mainly zooplankton), Oyster Larvae and mussel edulis were elected as probe species.

Each sample for the disinfection efficacy test was collected in triplicate at 3 times intervals when 0hour, 4hours, 24hours, according to the guideline of IMO (G8). The sample was classified into 'control water' and 'treated water', indicating the samples taken before and after the BWTS, respectively.

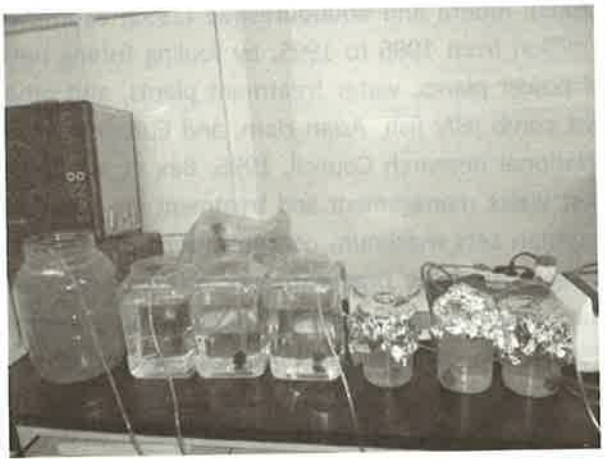

Figure 1. Experimental set-up

Figure 2. Test organism

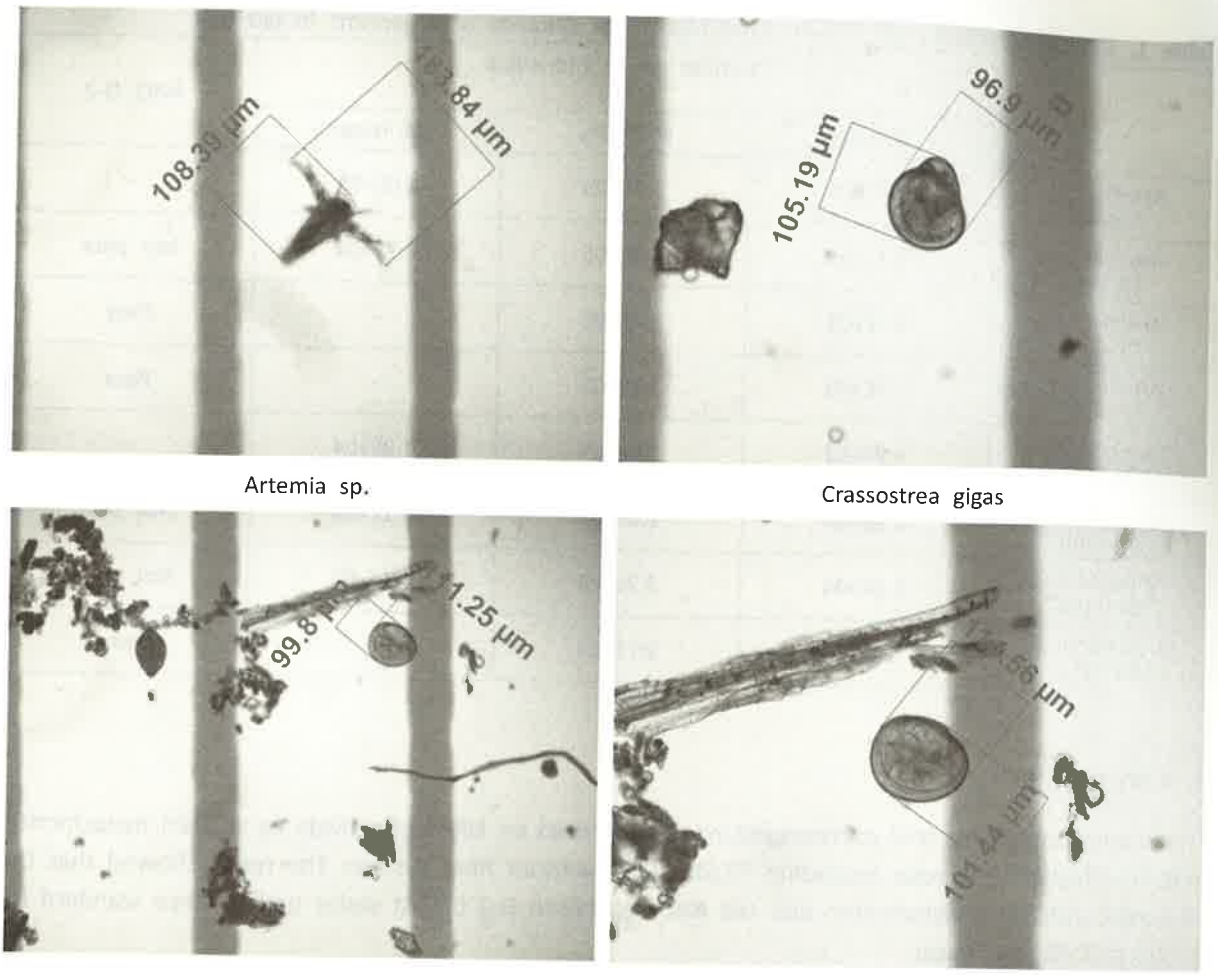

Figure 3. Microphotographs of the target organisms.

3. Results and discussion

3.1 Disinfection efficacy

The disinfection efficacy tests were performed at lab test. Table 1 shows the disinfection efficacy of the lab test with seawater. As shown in the results, *Artemia sp.* were removed by up to 23.5%, 25.5% and 27.5% respectively, after 4 hours. Oyster Larvae and mussel edulis were removed by up to 12.5%, 14.5% and 16.5% respectively, after 4 hours.

Artemia sp. were removed by up to 84.1%, 100% and 100% respectively, after 24 hours. Oyster Larvae and mussel edulis were removed by up to 77.8%, 96.6% and 100% respectively, after 24 hours. After 24 hours, 41.2~46.9% of the plankton and microorganisms tested in the control water (untreated) were inactivated.

Table 1. Biological efficacy of sodium hypochlorite for removal of organisms in lab test

	Number of an individual ($/m^3$)			IMO D-2
	0 hour	4 hours	24 hours	
Artemia sp. Control	1.7E+05	1.4E+05	1.0E+05	-
Artemia sp. 10 ppm	1.7E+05	1.3E+05	2.7E+04	Not pass
Artemia sp. 15 ppm	1.7E+05	1.2E+05	-	Pass
Artemia sp. 20 ppm	1.7E+05	1.1E+05	-	Pass
Oyster/Mussel Control	3.2E+04	3.0E+04	1.9E+04	-
Oyster/Mussel 10 ppm	3.2E+04	2.8E+04	7.1E+03	Not pass
Oyster/Mussel 15 ppm	3.2E+04	2.7E+04	1.1E+03	Not pass
Oyster/Mussel 20 ppm	3.2E+04	2.7E+04	-	Pass

4. Conclusion

The marine organisms and microorganisms tested could be killed effectively by sodium hypochlorite, with inactivation efficacies exceeding 77.8%~100% without filter system. The result showed that the biological efficacy dissatisfaction met the IMO regulation D-2 ballast water performance standard for discharged ballast water.

Consequently, the BWMS employing the combination of filter and disinfection treatment system is a clean and effective technology, which can safely treat ballast water and will not affect marine aquatic environments.

Keywords: *ballast water, filter, filtration, disinfection*

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