MARITIME AND PORT AUTHORITY OF SINGAPORE
SHIPPING CIRCULAR
NO. 5 OF 2015

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05th March 2015

Applicable to: Shipowners, shipmanagers, operators, and masters of Singapore-registered ships

REPORT OF VOLUNTARILY TRIAL STUDY CONDUCTED BY SINGAPORE ON ONBOARD SAMPLING AND ANALYSIS OF BALLAST WATER

1. This circular shares with the shipping community the data and findings of Singapore's voluntary trial study on onboard sampling and analysis of ballast water for D2 compliance with a view to enabling the smooth implementation of the BWMC when it comes into force.

2. The reports are provided as follows :

a) The executive summary

b) Ship 1 detailed report

c) Ship 2 detailed report

d) Ship 3 detailed report

e) Ship 4 detailed report

f) Ship 5 detailed report

g) Ship 6 detailed report

3. We take this opportunity to thank the shipowners, Master and crew onboard the vessels where the samplings were carried out for their time and support.

4. Singapore will submit a paper to the IMO to share the results of the trial study. We will further inform the shipping community on the submission.

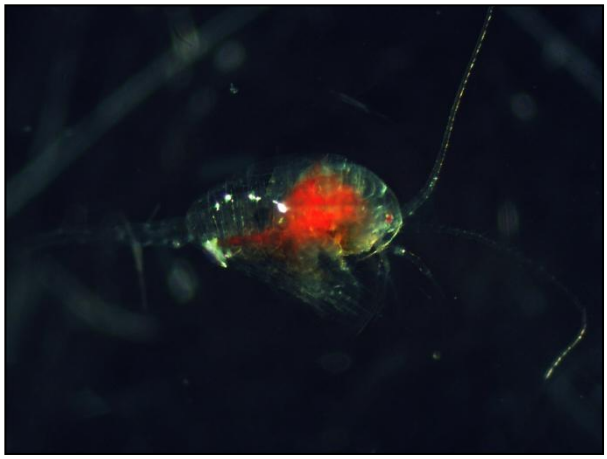
5. Queries relating to this circular should be directed to Mr. Ranabir Chakravarty at Tel: 6375-6210 or email: ranabir_chakravarty@mpa.gov.sg

TAN SUAN JOW
DIRECTOR OF MARINE
MARITIME AND PORT AUTHORITY OF SINGAPORE

BALLAST WATER REPORT

SUMMARY REPORT

SGS



BALLAST WATER REPORT

SUMMARY REPORT

ON BOARD BALLAST WATER COMPLIANCE TESTS

0814-00000 R0

30/09/2014

Prepared by

SGS Testing & Control Services Singapore Pte Ltd

Prepared for

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1. BACKGROUND

In July 2014 the Maritime and Port Authority of Singapore (MPA) charged SGS Testing & Control Services Singapore Pte Ltd (SGS) with the execution of a study of on board sampling and analysis of ballast water for compliance testing within the frame of the Ballast Water Management Convention (BWMC) of the International Maritime Organization (IMO) of the United Nations.

The compliance tests were executed on in total six ships identified by MPA.

It is important to consider that the outcome of this project as reported here was derived from and under specific conditions.

2. OBJECTIVES

The objectives of this study are as follows:

- Assess the feasibility of indicative and detailed (in-depth) analysis of ballast water on board ships under the realistic conditions of time constraints during unloading and loading processes while the ships are berthed in harbors for just one day or even less.
- Allow the Singapore MPA to identify potential practical challenges that vessels may face when MPA will execute its Port State Control role when the convention comes into force.
- Address industry concerns on the performance of IMO type approved Ballast Water Management Systems under normal operating conditions.

3. ON BOARD SAMPLING AND ANALYSIS

The ballast water sampling system allowed for on board sampling with conditions and sample volumes as close to the IMO regulations as possible. The analysis methods developed for use on board were also executed on board all six ships without major problems and without causing delays to the ships. However, there were some minor issues:

- High turbidity of the water from the ballast water tanks can interfere with both indicative and detailed analysis methods.
- There were only single sample points available, which meant the sampling system had to be run in ‘open’ mode, discharging waste water into the bilge. This put a limit on the amount of water that could be sampled, since not too much water was allowed to be discharged into the bilge. There was no provision onboard to collect and retain ballast water. Makeshift drums and portable pumps were used to collect and transfer collected sampled ballast water to the bilges.

(*During the analysis of ship number one a time based approach was initiated. That is to allow ballast water to pass through the sampler for at least one hour. However in this instance the total water generated onboard was approximately 15 m^3 which had to be transferred to the engine room bilges. Due to this enormous volume of water being generated, from ship number two a volume based approach was adopted. That is the total volume of water including flushing was limited to 2 m^3 which was transferred to the engine room bilges. However to ensure enough time to collect as many samples possible during the approximately 1 m^3 of sampled treated ballast water the G2 sampling valve installed onboard had to be throttled to regulate flow.)*

- While all ships were equipped with sampling points, these sampling points were not always designed for ease of access.
- Both pitot tubes and sampling valves were not always optimal. Globe valves were used, which can have an effect on plankton viability. Pitot tubes were of large diameter, making it difficult to sample at a flow speed safe for sampling.
- The PCD of the sampling G2 valve fitted onboard was not always of the same standard for all the vessels. As such prior arrangement of the correct connecting flange for the sampler was needed.
- Crew competence in the understanding of the convention and the expertise in operating and maintaining the equipment is important.
- An average of 6.5 hours was spent onboard for conducting the sampling and analysis.
- There was an additional workload on crew during the time the sample and analysis was being conducted. Almost all vessels sampled, apart from the arrival and cargo operations, ship crew were involved in picking up of stores, stemming bunkers, attending to class surveys etc. As such there may be a possibility of crew not meeting the rest hours requirement by the time the vessel departs. *(Although during this study MPA ensured that no rest hours were compromised)*
- Vessels following procedures like “trim optimization” may find it difficult to follow the holding time requirements of a ballast water treatment system as applicable.
- Arrangements have to be made to ensure that the samples taken to the lab for detailed analysis were maintained at a temperature of between 3-5 degrees centigrade.

4. INDICATIVE AND DETAILED ANALYSIS METHODS

For the interpretation of analysis results, the uncertainty of the methods needs to be considered. For this reason, validation tests of the indicative methods developed by SGS were conducted by third party-laboratories. Using the results of these validation tests the uncertainty of the methods was calculated. These uncertainty calculations were used to determine the uncertainty range of the results of the ATP ≥50 and ATP ≥10 - <50 tests. If the entire uncertainty range of a value is below the D-2 limit value, the value is indicated as 'Compliant'. If the entire uncertainty range of a value is above the D-2 limit value, the value is indicated as 'Non-compliant'. If the uncertainty range of a value overlaps the D-2 limit value, the value is indicated as 'Possibly compliant', see figure 1.

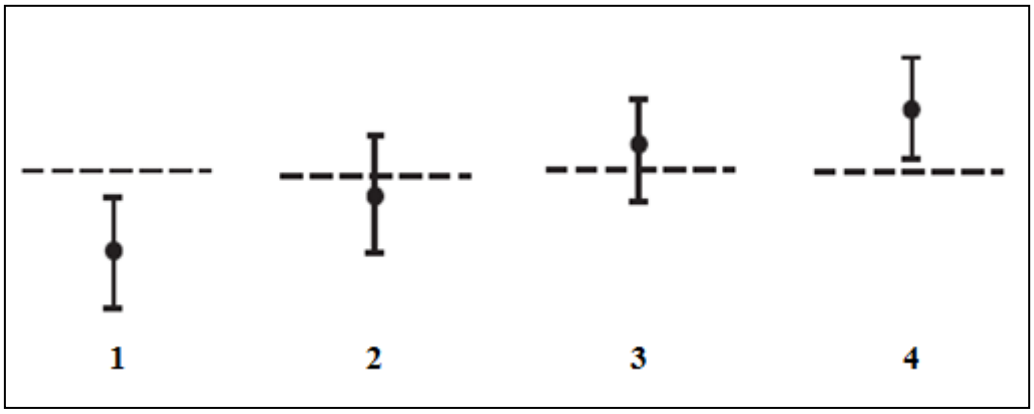

Figure 1: Examples of different compliance situations: Situation 1: Compliance, the value and the uncertainty range are below the limit value. Situation 2: Possible compliance, the value is below the limit value, but the uncertainty range overlaps the limit value. Situation 3: Possible compliance, the value is above the limit value, but the uncertainty range overlaps the limit value. Situation 4: Non-compliance, the value and the uncertainty range are above the limit value.

Since for the detailed analyses results were usually 0, no uncertainty calculations could be performed. The results of indicative and detailed methods did not always provide the same compliance statement, see table 1.

Table 1: Overview of compliance statements according to detailed and indicative methods for all six ships

	Detailed	Indicative
Ship 1	Compliant	Possibly compliant
Ship 2	Non-compliant	Non-compliant
Ship 3	Compliant	Non-compliant
Ship 4	Compliant	Possibly compliant
Ship 5	Compliant	Possibly compliant
Ship 6	Compliant	Possibly compliant

The detailed analysis methods found one of the ships to be non-compliant while the indicative analysis methods found two ships to be non-compliant. The ship which was found to be non-compliant using detailed methods was also found to be non-compliant using the indicative methods. For the other four ships the results of the indicative analyses were all in the uncertainty range of the method and are therefore reported as 'Possibly compliant'. The indicative methods did produce compliant results, but this is not reflected in table 1. Table 2 below provides a more extensive overview of the compliance results, divided not only between detailed and indicative but also between the organism categories ≥50 µm and ≥10 to <50 µm.

Table 2: Overview of compliance statements for the ≥50 µm and ≥10 to <50 µm organism size classes according to detailed and indicative methods for all six ships

	Detailed		Indicative	
	≥50	≥10 to <50	≥50	≥10 to <50
Ship 1	Compliant	Compliant	Compliant	Possibly compliant
Ship 2	Non-compliant	Non-compliant	Non-compliant	Non-compliant
Ship 3	Compliant	Compliant	Non-compliant	Possibly compliant
Ship 4	Compliant	Compliant	Possibly compliant	Possibly compliant
Ship 5	Compliant	Compliant	Possibly compliant	Possibly compliant
Ship 6	Compliant	Compliant	Possibly compliant	Compliant

The indicative methods do not provide false positives for compliance. This matches the principle of 'gross non-compliance' for which indicative methods should be used. However, no limit values for gross non-compliance have so far been determined.

During the testing of ship 5 there was a problem with a contamination in one of the reagents. Improvements to the ATP analysis protocols were made to prevent these problems in the future and these protocols were used during the testing of ship 6, where the ATP analysis provided measurements below the D-2 limit values.

The two different detailed bacteria methods that were tested, the classic bacteria plating according to APHA protocols and the Fluorescent In-Situ Hybridization method, provided very similar results for all ships and samples.

There is no D-2 limit value for total heterotrophic bacteria. The results for this parameter were compared with the limit value from the California standard (< 1,000 cfu/100 mL). Since this is not an IMO limit value exceeding it did not cause non-compliance.

5. COMPLIANCE

One of the six ships tested was found to be non-compliant by both indicative and detailed methods, which means it was non-compliant with the D-2 standard. It is also to be noted that the ship which failed both the indicative and detailed analysis had retained the water inside the ballast tank for only 1 day. Furthermore the ballast water treatment equipment had to be stopped and started a couple of times during the sampling process due to some leakages in the control systems which needed attention. Furthermore, the same ballast water treatment equipment was tested onboard two other vessels for which the detailed analysis results were compliant.

The scale of this study does not allow generalizations to be drawn on the level of compliance of treatment systems.

6. CONCLUSIONS

1. Execution of on board sampling and analysis is possible within the time constraints of vessel loading and unloading in a harbor.
2. Five of the six ballast water treatment systems tested were found to be compliant with the IMO D-2 Ballast Water Performance Standard. The non-compliant system was suffering from technical difficulties during sampling.
3. The indicative methods provided a false positive for non-compliance, but no false negatives.
4. There is no validated protocol for sampling and analysis, for example there are no clearly defined sample volumes and no defined minimum number of samples to be collected.
5. Plankton organisms are sensitive and long transport times can affect viability, because of this reason all analysis were conducted immediately on board ship when possible.
6. Technical issues with the equipments and the reagents used for indicative analysis need to be understood and taken into consideration.

7. ANNEX

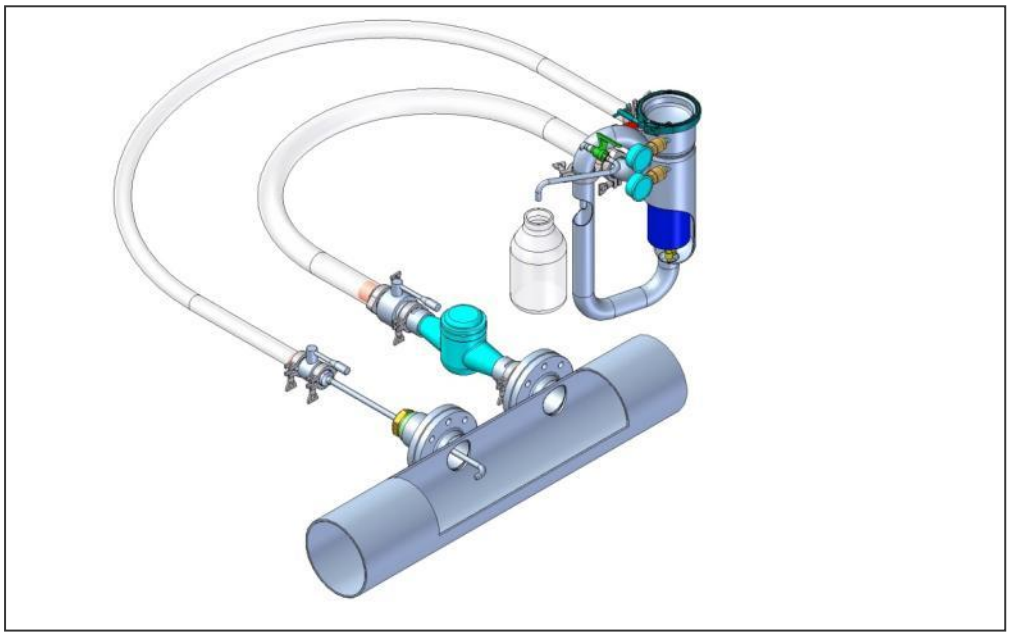

Figure 1: Schematic view of the SGS Ballast Water Sampler v02 (closed system variant)

Figure 2: The SGS Ballast Water Sampler v02

BALLAST WATER REPORT

ON BOARD BALLAST WATER COMPLIANCE TEST

ENVGT01.01

BALLAST WATER REPORT

SHIP 1

ON BOARD BALLAST WATER
COMPLIANCE TEST

0814-00001 R0

02/09/2014

Prepared by

SGS Testing & Control Services Singapore Pte Ltd

Prepared for

Maritime and Port Authority of Singapore

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EXECUTIVE SUMMARY

The ballast water aboard the first ship tested, a Singapore registered 3,600 TEU ship built in 2013, fully complies with the D-2 Ballast Water Performance Standard as defined by the IMO. A short overview of the results is shown in the table below.

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Possibly compliant	Compliant		
Plankton ≥50µm	Compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	>1,000 cfu/100ml		<1,000 cfu/100ml	

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance.

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. There was a problem with the analysis of the first two samples; due to high turbidity of the sample (probably caused by oxidation in some of the pipes) it was not possible to get reliable results for these samples with either indicative or detailed methods. Since these were extra samples outside of normal sampling this does not affect the compliance result.
4. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
5. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC, since the average value of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.

6. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

The paragraphs of this report presents detailed information regarding the execution of the on board compliance test.

1. BACKGROUND

In July 2014 the Maritime and Port Authority of Singapore (MPA) charged SGS Testing & Control Services Singapore Pte Ltd (SGS) with the execution of a study of on board sampling and analysis of ballast water for compliance testing within the frame of the Ballast Water Management Convention (BWMC) of the International Maritime Organization (IMO) of the United Nations.

The compliance tests had to be executed on in total six ships identified by MPA.

This report presents the test results of ship no. 1.

Table 01: Characteristics of the ship

Type	Container Ship
Capacity (TEU)	3,600
Year built	2013

2. REGULATORY FRAMEWORK FOR THE ANALYSIS OF BALLAST WATER

The sampling and analysis of the ballast water should follow, as far as feasible, the relevant regulations published by the International Maritime Organization – IMO within the frame of the Ballast Water Convention 2004:

- G2 : Guidelines for Ballast Water Sampling (G2), October 10th, 2008
  (source: MEPC 58/23, Annex 3, Resolution MEPC.173/58))
- G8 : Guidelines for Approval of Ballast Water Management Systems (G8), October 10th, 2008
  (source: MEPC 58/23, Annex 4, Resolution MEPC.174(58))
- G-PSC : Guidelines for Port State Control under the International Convention for the Control and Management of Ship's Ballast Water and Sediments April 10th, 2014
  (source: III 1/8 IMO Sub-Committee on Implementation of IMO Instruments, 1st Session, Agenda Item 8, Annex 1, document page 7ff)
- BWM.2/Circ.42 : Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)

3. PROJECT OBJECTIVES

The project objectives of this compliance test study are as follows:

- Assess the feasibility of indicative and detailed (in-depth) analysis of ballast water on board ships under the realistic conditions of time constraints during unloading and loading processes while the ships are berthed in harbors for just one day or even less.
- Allow MPA to identify potential practical challenges that vessels may face when MPA will execute its Port State Control role when the convention comes into force.
- Address industry concerns on the performance of IMO type approved Ballast Water Management Systems under normal operating conditions.

4. ANALYTICAL FRAME

4.1 IMO TARGET ORGANISM SIZE CLASSES

In accordance with the IMO regulations and guidelines as listed in paragraph 2 the ballast water onboard the ships was analyzed as to the organism concentrations of the three target organism size classes defined by the IMO :

- Marine plankton organisms with a size of >50µm
- Marine plankton organisms with a size between >10µm and <50µm
- Marine bacteria :
  - *Escherichia coli*
  - Enterococci
  - *Vibrio cholerae*

4.2 PERFORMANCE STANDARDS

The analysis of the ballast water aimed at the verification of compliance with the international performance standards for ballast water to be re-discharged from ships as defined by the IMO within the frame of the International Ballast Water Management Convention 2004:

Table 02: IMO target organism groups and performance standards

IMO Target Size Class	IMO Performance Standard
Plankton ≥50µm	<10 Individuals/m³
Plankton ≥10µm<50µm	<10 Individuals/ml
Escherichia coli	<250 cfu/100ml
Enterococci	<100 cfu/100ml
Vibrio cholerae	<1 cfu/100ml

(cfu : colony forming unit)

4.3 SAMPLING TECHNOLOGY USED

The ballast water samples were taken by the aid of the "*SGS Ballast Water Sampler v02*", which has been connected to the isokinetic sampling port installed in the ballast water pipe system of the ship (see figures 01, 02 and 04 in the annex).

4.4 ANALYTICAL METHODS USED

4.4.1 General Information

Marine organisms are divided in animals (zooplankton), plants (phytoplankton) and bacteria. The D-2 size-classes of ≥50 µm and ≥10 but <50 µm both contain phytoplankton and zooplankton, making it important to use methods that detect both types of organism.

Plankton organisms are sensitive and long transport times can affect viability, because of this reason all analysis were conducted immediately on board ship when possible.

4.4.2 Indicative Methods

Pulse Amplitude Modulation Fluorometry - PAM

The PAM method provides an indication for the presence of only vital phytoplankton in the ballast water sample by measuring the chlorophyll activity in the entire sample. Separate values for phytoplankton ≥50 µm and phytoplankton ≥10 but <50 µm are achieved by filtration of the sample. The PAM method does not allow for a numerical (number of vital marine microalgae/ml) indication. The parameters are concentration of Chlorophyll in the ballast water sample (µg chl/l) and yield (yld), which is expressed without a dimension.

The PAM analysis is executed on board the ship.

Adenosin-triphosphate method - ATP

Adenosin-triphosphate is a unique energy donor in the physiological processes of all living cells (phytoplankton, zooplankton and bacteria). The ATP method provides the concentration of cellular ATP in the ballast water sample and – based on empirical data - transforms this concentration value (pg cATP/ml) into an indicative numerical concentration value of number of vital organisms. It can be used for organisms ≥50 µm, organisms ≥10 but <50 µm and bacteria. However, it only provides total bacteria numbers and no species identification.

The ATP analysis for all IMO target organism size classes is executed on board the ship.

4.4.3 Detailed (in-depth) Methods

IMO Target Organism Size Class Plankton

The concentration of vital plankton organisms in the ballast water samples was analysed per optical counts under the microscope using a Bogorov chamber for plankton organisms ≥50µm and a Sedgewick Rafter count cell for plankton organisms ≥10µm<50µm.

Three sub-samples of the main sample for plankton organisms ≥50µm were counted and 10 sub-samples of the main sample for plankton organisms ≥10µm<50µm were counted

The microscopic counts of the concentration of viable plankton organisms in the ballast water were executed on board the ship.

IMO Target Organism Size Class Bacteria

Since the detailed (in-depth) analytical methods for the detection of bacteria in ballast water samples, demand a complex laboratory infrastructure the cultivation of human pathogens, these methods can only be executed in land-based laboratories.

Fluorescence-In-Situ-Hybridization Fluorescence Microscopy

This gene probe method analyses the concentration of the IMO target bacteria species *Escherichia coli*, Enterococci and *Vibrio cholerae* in ballast water samples. The time needed from sample to result is approximately 11 hrs.

Classical 24/48 hrs. Incubation Methods

To assess the concentration of total bacteria in the ballast water samples the Heterotrophic Plate Count (HPC) method (APHA 9215) was used.

For the detection of the IMO target bacteria species different methods were used.

Table 03: 24/48 hrs. incubation methods used for the detection of bacteria

Bacteria quantitative :	APHA 9215
Escherichia coli :	APHA 9222G
Enterococci :	APHA 9230
Vibrio cholerae :	APHA 9260

Since there is no limit value for total heterotrophic bacteria, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.

4.5 EXECUTIVE PROTOCOL FOR ON BOARD COMPLIANCE TESTING

4.5.1 Basic Hydraulic Settings

The sampling of live plankton organisms from within the main ballast water pipes on board ships has to ensure, that these plankton organisms are not impacted by the sampling procedures.
Therefore the on board sampling and analysis was proceeded by a meeting with the relevant crew members of the ship to obtain the relevant basic, hydraulic information and to agree on the adequate pump capacity during sampling to exclude impacts on the live plankton organisms.

4.5.2 Establishment of working places

Two working places have been established on board the ship: (i) the *sampling* working place at the sampling port of the main ballast water pipe system and (ii) the analysis working place where the generated samples are further processed, analyzed, labeled and prepared for transport to the land based laboratory for bacterial analysis

4.5.3 Installation of the sampling system

The constructional conditions at the sampling port on the ship required to run the "*SGS Ballast Water Sampler Type 1*" as an open system: the ballast water filtered through the system was not flushed back to the ballast water main pipe system but directed into the bilge of the ship instead. The volume of ballast water that was discharged to the bilge was 15 m^3.

4.5.4 Flushing of the main ballast water pipe system

After the installation of the two working places was finished, the notice to start the de-ballasting procedure was given to the officer in charge.

In order to avoid the contamination of the ballast water the main ballast water pipe system was flushed for 30 minutes.

4.5.5 Flushing of the sampling system

After the flushing of the main ballast water pipe system ended the sampling port valve was opened to direct ballast water through the "*SGS Ballast Water Sampler v02*", without the 50 µm filter installed. The sampling system was flushed with approximately 1.0m³ of ballast water. During this flushing procedure two ballast water samples were taken. These two samples were an additional requirement of MPA and do not form a part of the sampling and analysis for compliance testing.

4.5.6 Sampling of ballast water for compliance test

After the flushing of the sampling system ended, the filter 50 µm filter was installed to sample ballast water for the compliance test. During this sampling procedure 20 ballast water samples of various volumes were taken at the sampling system and brought to the *analysis* workplace in the ship for further processing.

Notice was given to the officer in charge to stop the de-ballasting procedure.

4.5.7 Termination of on board compliance test

The ballast water on board compliance test ended with the de-installation of the *analysis* workplace after finalization of the indicative and detailed analyses of the ballast water samples.

5. RESULTS

5.1 BASIC HYDRAULIC INFORMATION

The basic hydraulic information is displayed in table 04 below.

Table 04: Basic hydraulic information for de-ballasting procedures

Parameter	Value	Dim.
Diameter of ballast water pipe	400	mm
Diameter of isokinetic pipe	25.4	mm
Maximum pump capacity	1,000	m³/h
System pressure in ballast water pipe	3.8	bar
Maximum ballast water volume for de-ballasting	550	m³
Date of ballast water uptake	09.08.2014	date
Holding time of ballast water	3	days
Origin of ballast water	South China Sea	
Minimum possible pump capacity during de-ballasting	150	m³/h
Average pump capacity during de-ballasting	138.6	m³/h
Discharge volume into the bilge	15	m³

5.2 TIME TABLE OF GENERAL ACTIVITIES

The table below presents the time lapse of general activities before, during and after the on board compliance test.

Table 05: Time table of general activities

Time of day	Activity
07:30	Departing from lab to port
08:45	Arrival at port's gate
09:00	Arrival at ship's berth
09:15	Unloading of equipment
09:15	Start interview with ship's crew
09:40	Start setting up working places (sampling and analysis)
11:00	End of setting up working places (sampling and analysis)
11:45	Start of flushing
12:15	End of flushing
13:05	Start of sampling
13:50	End of sampling
14:15	Removal of 50µm stainless steel filter
13:00	Start of analysis
16:15	End of analysis
16:15	Start of de-installation of working places
16:40	End of de-installation of working places
17:40	Alighting from ship
17:40	Loading of material
18:05	Departing from port
18:50	Arrival at Laboratory

5.3 COURSE OF DEBALLASTING PROCEDURE AND SAMPLE RECORDS

The table below presents the general information of the de-ballasting procedure.

Table 06: General information of the de-ballasting procedures

Total duration of de-ballasting (hh:mm)	02:42
Total duration of sampling procedure (hh:mm)	00:34
Generated samples	20
Filtered volume of ballast water (m^3)	4.03

The list below displays the course of the de-ballasting procedures and the sample records.

Time | **Activity**

11:13 Start of de-ballasting, flush of main ballast water pipe system

12:37 Sampling port of main ballast water pipe: sample record

Time	ID	Volume (l)	Purpose
12:37	1, 2	0.5	Turbidity, salinity

12:50 End of flushing of the main ballast water pipe system

12:50 Start of flushing the sampling system (without 50 µm filter installed)

13:09 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
13:09	3, 4	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm[1]

13:12 End of flushing the sampling system

13:14 **Start of sampling for compliance test** (with 50 µm filter installed)

[1] Additional samples included in the sampling scheme by request of MPA

13:14 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
13:14	5	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	6, 7	1.5	Bacterial analysis in land based laboratory
13:21	8	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	9, 10	1.5	Bacterial analysis in land based laboratory
13:25	11	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	12, 13	1.5	Bacterial analysis in land based laboratory
13:38	14	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	15, 16	1.5	Bacterial analysis in land based laboratory
13:43	17	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	18, 19	1.5	Bacterial analysis in land based laboratory

13:48 **End of sampling for compliance test**

13:48 Steel screen filter of sampling system: sample record

Time	ID	Filtered Volume (m^3)	Purpose
13:48	20	4.03 m^3	PAM, ATP plankton ≥50µm, microscopic counts

13:55 End of de-ballasting procedure

5.4 ANALYTICAL RESULTS

5.4.1 Physical parameters

The table below lists the results from the physical analysis of the ballast water

Table 07: Physical parameters of the ballast water

Sample ID	Parameter	Value	Dim.
1, 2	turbidity	> 5	NTU
3-20		< 5	
1	salinity	33.1	PSU
1	temperature	30.6	°C

5.4.2 Indicative analysis: Chlorophyll activity (PAM)

Table 08: Chlorophyll activity in the ballast water

Sample ID	Chl (µg/l)	Yld
1	turbidity too high	
2		
3	0.6	0
4	0.0	0
5	0.4	0
8	0.2	0
11	0.0	0
14	0.6	0
17	0.3	0
20	0.2	0

The ranking of the values is displayed in table 09 below.

Table 09: Ranking of Chlorophyll values

Chlorophyll	Indications
< 0.1 µg/l	Low number of marine microalgae in the sample
>0.1 <1.0 µg/l	Medium number of marine microalgae in the sample
>1.0 µg/l	Large number of marine microalgae in the sample
Yield	**Indications**
< 0.100	The sample contains marine microalgae on a very low vitality level
>0.100 <0.300	The sample contains marine microalgae on a medium vitality level
>0.300	The sample contains marine microalgae on a high vitality level

5.4.3 Indicative analysis: numerical values derived from ATP analysis

Table 10: Indicative concentrations of viable organisms in the ballast water

Sample ID	Bacteria quantitative (cfu/100ml)[2]	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	turbidity too high		
2			
3	925,384	26	not contained[3]
4	647,073	38	
5	388,762	11	
8	3,265,201	13	
11	4,036,508	111	
14	3,310,914	26	
17	456,357	30	
20	not contained[4]		4

[2] Value's are indicated in red when they exceed the California standard, but as explained before exceeding this standard does not cause non-compliance.

[3] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[4] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

Detailed analysis: microscopic counts

Table 11: Concentrations of viable plankton organisms in the ballast water (detailed analysis)

Sample ID	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	turbidity too high	
2		
3	0	not contained[5]
4	0	
5	0	
8	0	
11	0	
14	0	
17	0	
20	not contained[6]	0

5.4.4 Detailed analysis bacteria: classical 24/48 hrs incubation

Table 12: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Bacteria quantitative	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml			
6, 7	3	<1	<1	<1
9, 10				
12, 13	3	<1	<1	<1
15, 16				
18, 19	1	<1	<1	<1

[5] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[6] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

5.4.5 Detailed analysis bacteria: FISH

Table 13: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml		
6, 7	2	1	0
9, 10	10	4	0
12, 13	0	0	0
15, 16			
18, 19	2	0	0

6. PROCESSING OF ANALYTICAL RESULTS

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and uncertainty calculations were applied. These results were compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance, as shown in figure 1.

For the IMO target group "*plankton ≥50µm*" only one large volume sample was taken. Therefore statistical considerations were impossible. The respective statements are derived directly from the analytical results from this single sample.

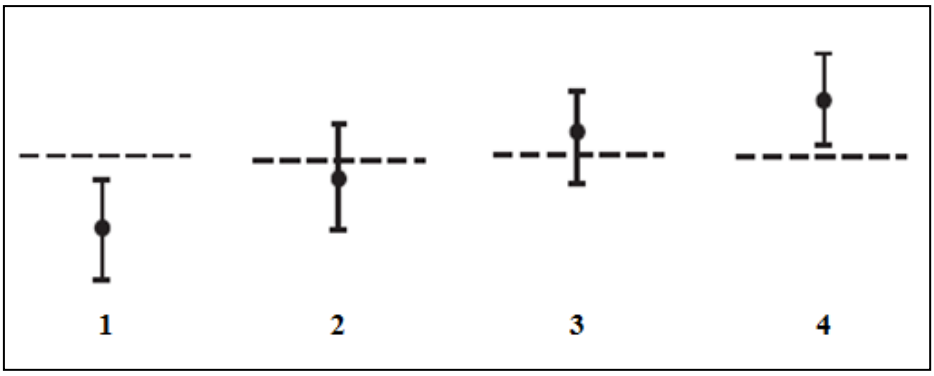


Figure 1: Examples of different compliance situations for indicative analyses. Situation 1: Compliance, the value and the uncertainty range are below the limit value. Situation 2: Possible compliance, the value is below the limit value, but the uncertainty range overlaps the limit value. Situation 3: Possible compliance, the value is above the limit value, but the uncertainty range overlaps the limit value. Situation 4: Non-compliance, the value and the uncertainty range are above the limit value.

Table 14: Final results of IMO target organism groups based on the analytical results

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Possibly compliant	Compliant		
Plankton ≥50µm	Compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/100ml		<1,000 cfu/100ml	

Table 15: Summary of PAM fluorometry, ATP and microscopy results

Sample ID	Chlorophyll (µg/l)	Yield	ATP Bacteria quantitative (cfu/100ml)	ATP Plankton >10µm <50µm (organisms/ml)	Plankton counts >10µm <50µm (organisms/ml)	ATP Plankton ≥50µm (organisms/m³)	Plankton counts ≥50µm (organisms/m³)
1	turbidity too high						
2							
3	0.6	0	925,384	26	0	not contained[7]	
4	0.0	0	647,073	38	0		
5	0.4	0	388,762	11	0		
8	0.2	0	3,265,201	13	0		
11	0.0	0	4,036,508	111	0		
14	0.6	0	3,310,914	26	0		
17	0.3	0	456,357	30	0		
20	0.2	0	not contained[8]			4	0

[7] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[8] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

Table 16: Summary of APHA bacteria incubation analysis and FISH analysis

Sample ID	Bacteria Quantitative (APHA)	Escherichia coli (APHA)	Escherichia coli (FISH)	Enterococci (APHA)	Enterococci (FISH)	Vibrio cholerae (APHA)	Vibrio cholerae (FISH)
	cfu/100ml						
6, 7	3	<1	2	<1	1	<1	0
9, 10			10		4		0
12, 13	3	<1	0	<1	0	<1	0
15, 16							
18, 19	1	<1	2	<1	0	<1	0

7. DISCUSSION

High turbidity interfered with the analysis of the first two samples; the particles in the water were identified as oxidized metals. The problem was resolved by re-routing the water flow through other pipes.

While the indicative and detailed results for the ≥50 micron plankton match, there was a difference in the results for the ≥10 and <50 micron fraction. The ATP method provided a possibly compliant result (average of 36 organisms/mL, 24 organisms/mL when excluding the outlier) while the microscopic counts were compliant (average of 0 organisms/mL). However, it should be kept in mind that ATP is an indicative method, designed to provide an indication of compliance (or an indication of gross non-compliance) which can then be verified by detailed analysis. No limit values for gross non-compliance have been determined as of yet.

The results of the APHA analyses for *Escherichia coli*, Enterococci and *Vibrio cholerae* closely matched the results of the FISH analyses for these same bacteria groups (Table 15). There was, however, a very large difference between the APHA analysis for total heterotropic bacteria and the ATP bacteria analysis. However, it is known that many marine bacteria cannot be cultured using traditional methods (Viable Non-Culturable, VNC). The percentage of culturable bacteria in seawater can be as little as 0.08%[9].

8. CONCLUSIONS

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. There was a problem with the analysis of the first two samples; due to high turbidity of the sample (probably caused by oxidation in some of the pipes) it was not possible to get reliable results for these samples with either indicative or detailed methods. Since these were extra samples outside of normal sampling this does not affect the compliance result.
4. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.

[9] Ferguson et al. 1984 Response of marine bacterioplankton to differential filtration and confinement. Applied and Environmental Microbiology, Volume 47, page 49-55.

5. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC, since the average value of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.
6. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

9. ANNEX

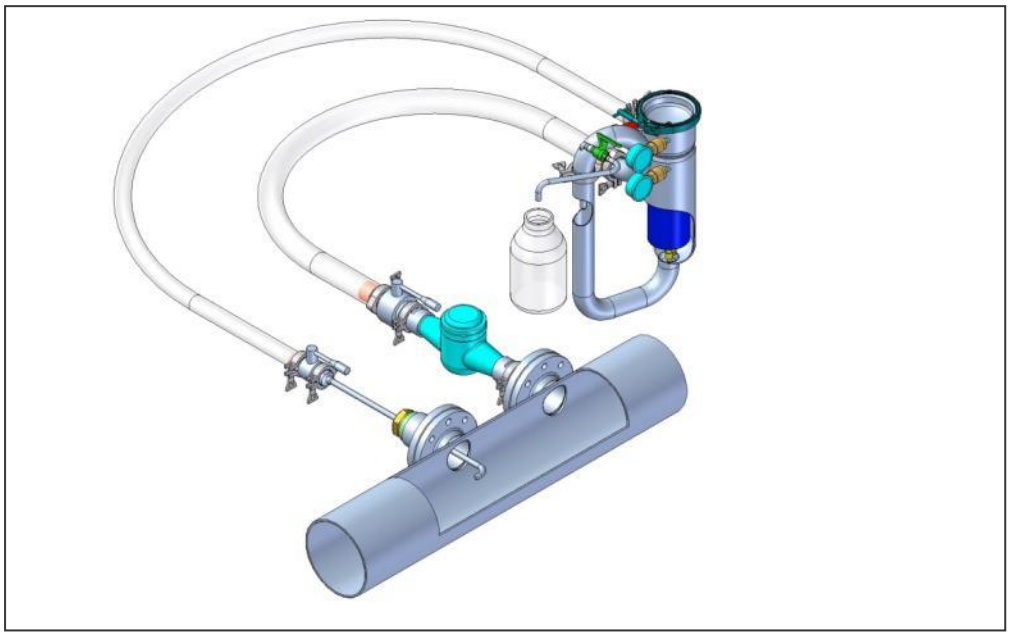


Figure 2: Schematic view of the SGS Ballast Water Sampler v02 (closed system variant)

Figure 3: The SGS Ballast Water Sampler v02

Figure 4: Sampling port on board the ship

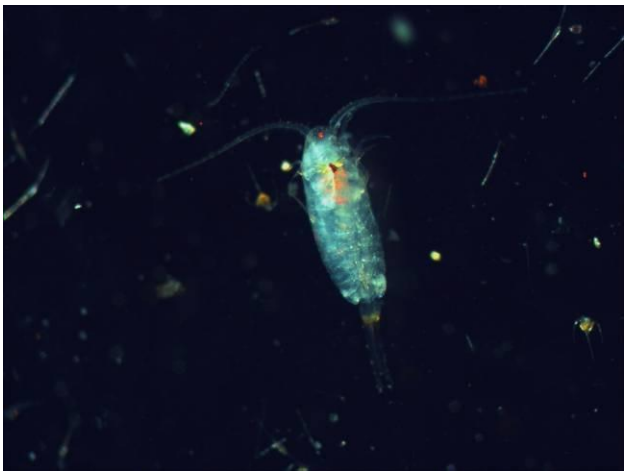
Figure 5: Installed SGS Ballast Water Sampler v02

BALLAST WATER REPORT

ON BOARD BALLAST WATER COMPLIANCE TEST

SGS

ENVGT01.01



BALLAST WATER REPORT

SHIP 2

ON BOARD BALLAST WATER COMPLIANCE TEST

0814-00002 R0

02/09/2014

Prepared by

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EXECUTIVE SUMMARY

The ballast water aboard the second ship tested, a Singapore registered 9,200 TEU ship built in 2013, does not comply with the D-2 Ballast Water Performance Standard as defined by the IMO. A short overview of the results is shown in the table below.

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Non-compliant	Non-compliant		
Plankton ≥50µm	Non-compliant	Non-compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/100 ml		< 1,000 cfu/100 ml	

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance.

1. The ship was found to be non-compliant according to both the indicative and the detailed analysis methods
2. The indicative and detailed methods provided the same compliance statement.
3. The ballast water treatment system was experiencing technical difficulties during the sampling.
4. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
5. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.
6. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

The paragraphs of this report presents detailed information regarding the executed on board compliance test.

1. BACKGROUND

In July 2014 the Maritime and Port Authority of Singapore (MPA) charged SGS Testing & Control Services Singapore Pte Ltd (SGS) with the execution of a study of on board sampling and analysis of ballast water for compliance testing within the frame of the Ballast Water Management Convention (BWMC) of the International Maritime Organization (IMO) of the United Nations.

The compliance tests had to be executed on in total six ships identified by MPA.

This report presents the test results of ship no. 2.

Table 01: Characteristics of the ship

Type	Container Ship
Capacity (TEU)	9,200
Year built	2013

2. REGULATORY FRAMEWORK FOR THE ANALYSIS OF BALLAST WATER

The sampling and analysis of the ballast water should follow, as far as feasible, the relevant regulations published by the International Maritime Organization – IMO within the frame of the Ballast Water Convention 2004:

- G2 : Guidelines for Ballast Water Sampling (G2), October 10th, 2008
  (source: MEPC 58/23, Annex 3, Resolution MEPC.173/58))
- G8 : Guidelines for Approval of Ballast Water Management Systems (G8), October 10th, 2008
  (source: MEPC 58/23, Annex 4, Resolution MEPC.174(58))
- G-PSC : Guidelines for Port State Control under the International Convention for the Control and Management of Ship's Ballast Water and Sediments April 10th, 2014
  (source: III 1/8 IMO Sub-Committee on Implementation of IMO Instruments, 1st Session, Agenda Item 8, Annex 1, document page 7ff)
- BWM.2/Circ.42 : Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)

3. PROJECT OBJECTIVE

The project objectives of this compliance study are as follows:

- Assess the feasibility of indicative and detailed (in-depth) analysis of ballast water on board ships under the realistic conditions of time constraints during unloading and loading processes while the ships are berthed in harbors for just one day or even less.
- Allow MPA to identify potential practical challenges that vessels may face when MPA will execute its Port State Control role when the convention comes into force.
- Address industry concerns on the performance of IMO type approved Ballast Water Management Systems under normal operating conditions.

4. ANALYTICAL FRAME

4.1 IMO TARGET ORGANISM SIZE CLASSES

In accordance with the IMO regulations and guidelines as listed in para 2 the ballast water onboard the ships was analyzed as to the organism concentrations of the three target organism size classes defined by the IMO:

- Marine plankton organisms with a size of >50µm
- Marine plankton organisms with a size between >10µm and <50µm
- Marine bacteria :
    - *Escherichia coli*
    - Enterococci
    - *Vibrio cholerae*

4.2 PERFORMANCE STANDARDS

The analysis of the ballast water aimed at the verification of compliance with the international performance standards for ballast water to be re-discharged from ships as defined by the IMO within the frame of the International Ballast Water Management Convention 2004:

Table 02: IMO target organism groups and performance standards

IMO Target Size Class	IMO Performance Standard
Plankton >50µm	<10 Individuals/m³
Plankton >10µm<50µm	<10 Individuals/ml
Escherichia coli	<250 cfu/100ml
Enterococci	<100 cfu/100ml
Vibrio cholerae	<1 cfu/100ml

(cfu : colony forming unit)

4.3 SAMPLING TECHNOLOGY USED

The ballast water samples were taken by the aid of the "*SGS Ballast Water Sampler v02*", which has been connected to the isokinetic sampling port installed in the ballast water pipe system of the ship (see figures 01, 02 and 04 in the annex).

4.4 ANALYTICAL METHODS USED

4.4.1 General Information

Marine organisms are divided in animals (zooplankton), plants (phytoplankton) and bacteria. The D-2 size-classes of ≥50 µm and ≥10 but <50 µm both contain phytoplankton and zooplankton, making it important to use methods that detect both types of organism.

Plankton organisms are sensitive and long transport times can affect viability, because of this reason all analysis were conducted immediately on board ship when possible.

4.4.2 Indicative Methods

Pulse Amplitude Modulation Fluorometry - PAM

The PAM method provides an indication for the presence of only vital phytoplankton in the ballast water sample by measuring the chlorophyll activity in the entire sample. Separate values for phytoplankton ≥50 µm and phytoplankton ≥10 but <50 µm are achieved by filtration of the sample. The PAM method does not allow for a numerical (number of vital marine microalgae/ml) indication. The parameters are concentration of Chlorophyll in the ballast water sample (µg chl/l) and yield (yld), which is expressed without a dimension.

The PAM analysis is executed on board the ship.

Adenosin-triphosphate method - ATP

Adenosin-triphosphate is a unique energy donor in the physiological processes of all living cells (phytoplankton, zooplankton and bacteria). The ATP method provides the concentration of cellular ATP in the ballast water sample and – based on empirical data - transforms this concentration value (pg cATP/ml) into an indicative numerical concentration value of number of vital organisms. It can be used for organisms ≥50 µm, organisms ≥10 but <50 µm and bacteria. However, it only provides total bacteria numbers and no species identification.

The ATP analysis for all IMO target organism size classes is executed on board the ship.

4.4.3 Detailed (in-depth) Methods

IMO Target Organism Size Class Plankton

The concentration of vital plankton organisms in the ballast water samples was analysed per optical counts under the microscope using a Bogorov chamber for plankton organisms ≥50µm and a Sedgewick Rafter count cell for plankton organisms >10µm<50µm.

Three sub-samples of the main sample for plankton organisms ≥50µm were counted and 10 sub-samples of the main ample for plankton organisms >10µm<50µm were counted

The microscopic counts of the concentration of viable plankton organisms in the ballast water were executed on board the ship.

IMO Target Organism Size Class Bacteria

Since the detailed (in-depth) analytical methods for the detection of bacteria in ballast water samples, demand a complex laboratory infrastructure the cultivation of human pathogens, these methods can only be executed in land based laboratories.

Fluorescence-In-Situ-Hybridization Fluorescence Microscopy

This gene probe method analyses the concentration of the IMO target bacteria species *Escherichia coli*, Enterococci and *Vibrio cholerae* in ballast water samples. The time needed from sample to result is approximately 11 hrs.

Classical 24/48 hrs. Incubation Methods

To assess the concentration of total bacteria in the ballast water samples the Heterotrophic Plate Count (HPC) method (APHA 9215) was used.

For the detection of the IMO target bacteria species different methods were used.

Table 03: 24/48 hrs. incubation methods used for the detection of bacteria

Bacteria quantitative :	APHA 9215
Escherichia coli :	APHA 9222G
Enterococci :	APHA 9230
Vibrio cholerae :	APHA 9260

Since there is no limit value for total heterotrophic bacteria, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.

4.5 EXECUTIVE PROTOCOL FOR ON BOARD COMPLIANCE TESTING

4.5.1 Basic Hydraulic Settings

The sampling of live plankton organisms from within the main ballast water pipes on board ships has to ensure, that these plankton organisms are not impacted by the sampling procedures.
Therefore the on board sampling and analysis was proceeded by a meeting with the relevant crew members of the ship to obtain the relevant basic, hydraulic information and to agree on the adequate pump capacity during sampling to exclude impacts on the live plankton organisms.

4.5.2 Establishment of working places

Two working places have been established on board the ship: (i) the *sampling* working place at the sampling port of the main ballast water pipe system and (ii) the analysis working place where the generated samples are further processed, analyzed, labeled and prepared for transport to the land based laboratory for bacterial analysis

4.5.3 Installation of the sampling system

The constructional conditions at the sampling port on the ship required to run the "*SGS Ballast Water Sampler v02*" as an open system: the ballast water filtered through the system was not flushed back to the ballast water main pipe system but directed into the bilge of the ship instead. The maximum volume of ballast water that was allowed to enter the bilge was set 2.0m³.

4.5.4 Flushing of the main ballast water pipe system

After the installation of the two working places was finished, the notice to start the deballasting procedure was given to the engineer in charge.

In order to avoid the contamination of the ballast water the main ballast water pipe system was flushed for 30 minutes.

4.5.5 Flushing of the sampling system

After the flushing of the main ballast water pipe system ended the sampling port valve was opened to direct ballast water through the "*SGS Ballast Water Sampler Type 1*". The sampling system was flushed with approximately 1.0m³ of ballast water. During this flushing procedure two ballast water samples were taken. These two samples were an additional requirement of MPA and do not form a part of the sampling and analysis for compliance testing.

4.5.6 Sampling of ballast water for compliance test

After the flushing of the sampling system ended, the system was activated to sample ballast water for compliance test. During this sampling procedure 7 ballast water samples of various volumes were taken at the sampling system and brought to the *analysis* workplace in the ship for further processing. Notice was given to the engineer in charge to stop the de-ballasting procedure.

4.5.7 Termination of on board compliance test

The ballast water on board compliance test ended with the de-installation of the *analysis* workplace after finalization of the indicative and detailed analysis of the ballast water samples.

5. RESULTS

5.1 BASIC HYDRAULIC INFORMATION

The basic hydraulic information is displayed in table 04 below.

Table 04: Basic hydraulic information for de-ballasting procedures

Parameter	Value	Dim.
Diameter of ballast water pipe	450	mm
Diameter of isokinetic pipe	25.4	mm
Maximum pump capacity	1,000	m^3/h
System pressure in ballast water pipe	3.6	bar
Maximum ballast water volume for de-ballasting	2600	m^3
Date of ballast water uptake	15.08.2014	date
Holding time of ballast water	1	days
Origin of ballast water	South China Sea	
Minimum possible pump capacity during de-ballasting	215	m^3/h
Average pump capacity during de-ballasting	183	m^3/h
Maximum allowable discharge volume into the bilge	2.0	m^3

5.2 TIME TABLE OF GENERAL ACTIVITIES

The table below presents the time lapse of general activities before, during and after the on board compliance test.

Table 05: Time table of general activities

Time of day	Activity
11:30	Departing from lab to port
12:30	Arrival at port's gate
12:35	Arrival at ship's berth
13:00	Unloading of equipment
13:00	Start interview with ship's crew
13:15	Start setting up working places (sampling and analysis)
13:45	End of setting up working places (sampling and analysis)
14:13	Start of flushing
14:18	End of flushing
15:10	Start of sampling
15:20	End of sampling
15:50	Removal of 50µm stainless steel filter
15:10	Start of analysis
18:55	End of analysis
19:00	Start of de-installation of working places
19:30	End of de-installation of working places
20:30	Alighting from ship
20:30	Loading of material
21:00	Departing from port
21:45	Arrival at Laboratory

5.3 COURSE OF DEBALLASTING PROCEDURE AND SAMPLE RECORDS

The table below presents the general information of the de-ballasting procedure.

Table 06: General information of the de-ballasting procedures

Total duration of de-ballasting (hh:mm)	01:37
Total duration of sampling procedure (hh:mm)	00:08
Generated samples	21
Filtered volume of ballast water (m^3)	0.985

The list below displays the course of the de-ballasting procedures and the sample records.

Time | **Activity**

13:43 Start of de-ballasting, flush of main ballast water pipe system

De-ballasting was interrupted

Flushing the sampling system

14:13 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
14:13	1, 2	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm[1]

End of flushing the sampling system (without 50 µm filter installed)

15:12 **Start of sampling for compliance test** (with 50 µm filter installed)

15:12 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
15:12	53	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	4, 5	1.5	Bacterial analysis in landbased laboratory
15:13	6	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	7, 8	1.5	Bacterial analysis in landbased laboratory
15:14	9	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	10, 11	1.5	Bacterial analysis in landbased laboratory
15:16	12	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts

[1] Additional samples included in the sampling scheme by request of MPA

	13, 14	1.5	Bacterial analysis in land based laboratory
15:18	15	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	16, 17	1.5	Bacterial analysis in land based laboratory
15:20	18	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	19, 20	1.5	Bacterial analysis in landbased laboratory

15:21 **End of sampling for compliance test**

15:21 Steel screen filter of sampling system: sample record

Time	ID	Filtered Volume (m^3)	Purpose
15:21	21	0.985 m^3	PAM, ATP plankton ≥50µm, microscopic counts

15:21 End of de-ballasting procedure

5.4 ANALYTICAL RESULTS

5.4.1 Physical parameters

The table below lists the results from the physical analysis of the ballast water

Table 07: Physical parameters of the ballast water

Sample ID	Parameter	Value	Dim.
1, 2	turbidity	< 5	NTU
3 - 21		< 5	
1	salinity	31.8	PSU
1	temperature	26.5	°C

5.4.2 Indicative analysis: Chlorophyll activity (PAM)

Table 08: Chlorophyll activity in the ballast water

Sample ID	Chl (µg/l)	YId
1	0.5	0
2	0.8	0.13
3	1	0.10
6	0.8	0
9	0.5	0
12	0.4	0
15	0.1	0
18	0.5	0
21	5.4	0.250

The ranking of the values is displayed in table 09 below.

Table 09: Ranking of Chlorophyll values

Chlorophyll	Indications
< 0.1 µg/l	Low number of marine microalgae in the sample
>0.1 <1.0 µg/l	Medium number of marine microalgae in the sample
<1.0 µg/l	Large number of marine microalgae in the sample
Yield	**Indications**
< 0.100	The sample contains marine microalgae on a very low vitality level
>0.100 <0.300	The sample contains marine microalgae on a medium vitality level
>0.300	The sample contains marine microalgae on a high vitality level

5.4.3 Indicative analysis: numerical values derived from ATP analysis

Table 10: Indicative concentrations of viable organisms in the ballast water

Sample ID	Bacteria quantitative (cfu/100ml)	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	3,586,916		not contained[2]
2	501,508	34	
3	309,201	4	
6	1,055,564	24	
9	3,514,382	117	
12	2,619,131	163	
15	3,739,236	190	
18	3,510,218	228	
21	not contained[3]		522

[2] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[3] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

5.4.4 Detailed analysis: microscopic counts

Table 11: Concentrations of viable plankton organisms in the ballast water (detailed analysis)

Sample ID	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	0	not contained[4]
2	0	
3	6	
6	8	
9	8	
12	35	
15	19	
18	79	
21	not contained[5]	236

5.4.5 Detailed analysis bacteria: classical 24/48 hrs incubation

Table 12: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Bacteria quantitative	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml			
4	2	<1	<1	<1
7	<1	<1	<1	<1
10	1	<1	<1	<1
13	1	<1	<1	<1
16	<1	<1	<1	<1
19	1	<1	<1	<1

[4] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[5] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

5.4.6 Detailed analysis bacteria: FISH

Table 13: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml		
5	2	0	0
8	4	4	0
11	7	2	0
14	1	0	0
17	4	0	0
20	2	0	0

6. RANKING OF ANALYTICAL RESULTS

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and uncertainty calculations were applied. These results were compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance, as shown in figure 1.

For the IMO target group "*plankton ≥50µm*" only one large volume sample was taken. Therefore statistical considerations were impossible. The respective statements are derived directly from the analytical results from this single sample.

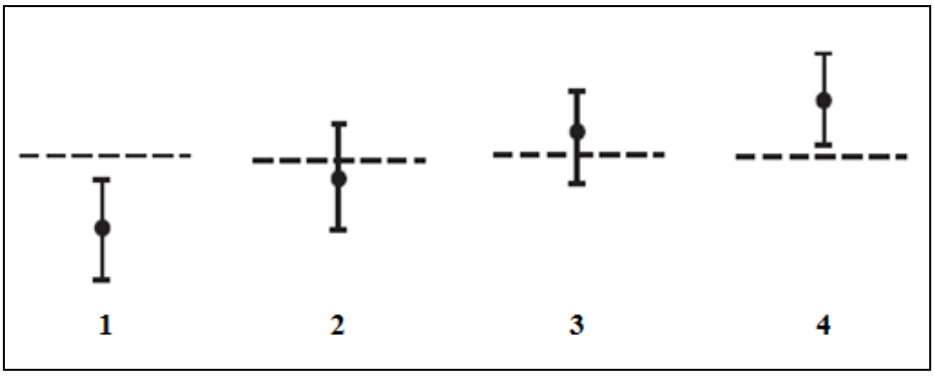

Figure 1: Examples of different compliance situations for indicative analyses: Situation 1: Compliance, the value and the uncertainty range are below the limit value. Situation 2: Possible compliance, the value is below the limit value, but the uncertainty range overlaps the limit value. Situation 3: Possible compliance, the value is above the limit value, but the uncertainty range overlaps the limit value. Situation 4: Non-compliance, the value and the uncertainty range are above the limit value.

Table 14: Final ranking of IMO target organism groups based on the analytical results

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Non-compliant	Non-compliant		
Plankton ≥50µm	Non-compliant	Non-compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/ml		< 1,000 cfu/ml	

Table 15: Summary of PAM fluorometry, ATP and microscopy results

Sample ID	Chlorophyll (µg/l)	Yield	ATP Bacteria quantitative (cfu/100ml)	ATP Plankton >10µm <50µm (organisms/ml)	Plankton counts >10µm <50µm (organisms/ml)	ATP Plankton ≥50µm (organisms/m³)	Plankton counts ≥50µm (organisms/m³)
1	0.5	0	3,586,916		0	not contained[6]	
2	0.8	0.13	501,508	34	0		
3	1	0.1	309,201	4	6		
6	0.6	0	1,055,564	24	8		
9	0.5	0	3,514,382	117	8		
12	0.4	0	2,619,131	163	35		
15	0.1	0	3,739,236	190	19		
18	0.5	0	3,510,218	228	79		
21	5.4	0.25	not contained[7]			522	237

[6] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[7] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

Table 16: Summary of APHA bacteria incubation analysis and FISH analysis

Sample ID	Bacteria Quantitative (APHA)	Escherichia coli (APHA)	Escherichia coli (FISH)	Enterococci (APHA)	Enterococci (FISH)	Vibrio cholerae (APHA)	Vibrio cholerae (FISH)
	cfu/100ml						
4, 5	2	<1	2	<1	0	<1	0
7, 8	<1	<1	4	<1	4	<1	0
10, 11	1	<1	7	<1	2	<1	0
13, 14	1	<1	1	<1	0	<1	0
16, 17	<1	<1	4	<1	0	<1	0
19, 20	1	<1	2	<1	0	<1	0

7. DISCUSSION

The ship was found to be non-compliant according to both the indicative and the detailed analysis methods. Non-compliance was found by both indicative and detailed analysis methods for the ≥50 micron and ≥10 <50 micron fractions.

It is to be noted that the ship had retained the water inside the ballast tank for only 1 day. Furthermore the equipment had to be stopped and started a couple of times during the sampling process due to some leakages in the control systems which needed attention.

The results of the APHA analyses for Escherichia coli, Enterococci and Vibrio cholerae closely matched the results of the FISH analyses for these same bacteria groups (Table 15). There was, however, a very large difference between the APHA analysis for total heterotropic bacteria and the ATP bacteria analysis. However, it is known that many marine bacteria cannot be cultured using traditional methods (Viable Non-Culturable, VNC). The percentage of culturable bacteria in seawater can be as little as 0.08%[8].

8. CONCLUSIONS

1. The ship was found to be non-compliant according to both the indicative and the detailed analysis methods

2. The indicative and detailed methods provided the same compliance statement.

3. The ballast water treatment system was experiencing technical difficulties during the sampling.

4. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.

5. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.

6. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

[8] Ferguson et al. 1984 Response of marine bacterioplankton to differential filtration and confinement. Applied and Environmental Microbiology, Volume 47, page 49-55.

9. ANNEX

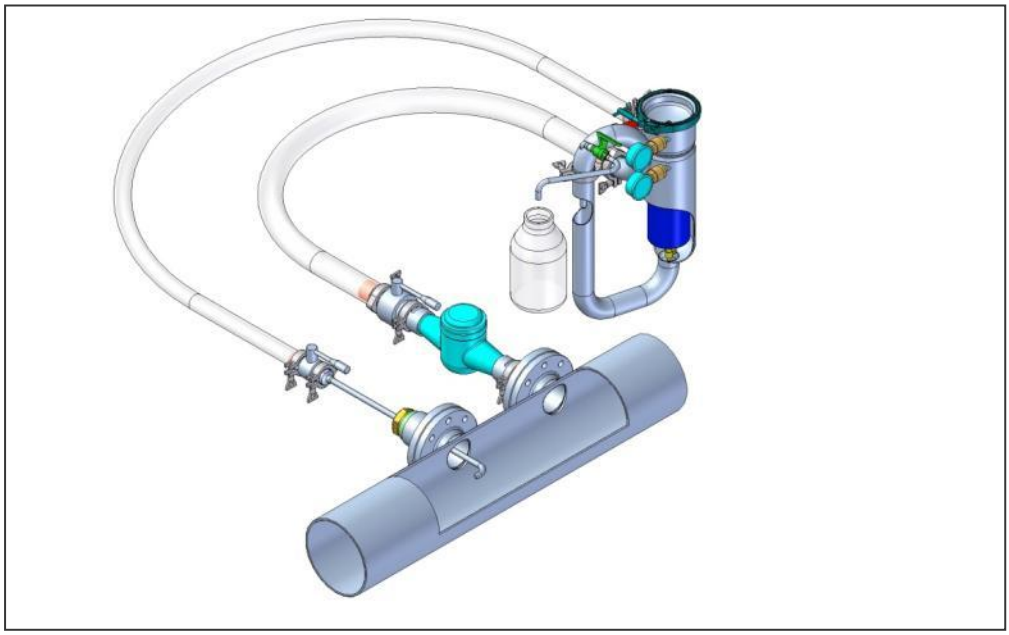

Figure 2: Schematic view of the SGS Ballast Water Sampler v02 (closed system variant)

Figure 3: The SGS Ballast Water Sampler v02

Figure 4: Sampling port on board the ship

Figure 5: Installed SGS Ballast Water Sampler v02

BALLAST WATER REPORT

ON BOARD BALLAST WATER COMPLIANCE TEST

ENVGT01.01

BALLAST WATER REPORT

SHIP 3

ON BOARD BALLAST WATER COMPLIANCE TEST

0814-00003 R0

02/09/2014

Prepared by

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EXECUTIVE SUMMARY

The ballast water aboard the third ship tested, a Singapore registered 3,566 TEU ship built in 2013, fully complies with the D-2 Ballast Water Performance Standard as defined by the IMO. A short overview of the results is shown in the table below.

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Possibly compliant	Compliant		
Plankton ≥50µm	Non-compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/100 ml		< 1,000 cfu/100 ml	

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance.

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
4. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.
5. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

The paragraphs of this report present detailed information regarding the executed on board compliance test.

1. BACKGROUND

In July 2014 the Maritime and Port Authority of Singapore (MPA) charged SGS Testing & Control Services Singapore Pte Ltd (SGS) with the execution of a study of on board sampling and analysis of ballast water for compliance testing within the frame of the Ballast Water Management Convention (BWMC) of the International Maritime Organization (IMO) of the United Nations.

The compliance tests had to be executed on in total six ships indentified by MPA.

This report presents the test results of ship no. 3.

Table 01: Characteristics of the ship

Type	Container Ship
Capacity (TEU)	3,566
Year built	2013

2. REGULATORY FRAMEWORK FOR THE ANALYSIS OF BALLAST WATER

The sampling and analysis of the ballast water should follow, as far as feasible, the relevant regulations published by the International Maritime Organization – IMO within the frame of the Ballast Water Convention 2004:

- G2 : Guidelines for Ballast Water Sampling (G2), October 10th, 2008
  (source: MEPC 58/23, Annex 3, Resolution MEPC.173/58))
- G8 : Guidelines for Approval of Ballast Water Management Systems (G8), October 10th, 2008
  (source: MEPC 58/23, Annex 4, Resolution MEPC.174(58))
- G-PSC : Guidelines for Port State Control under the International Convention for the Control and Management of Ship's Ballast Water and Sediments April 10th, 2014
  (source: III 1/8 IMO Sub-Committee on Implementation of IMO Instruments, 1st Session, Agenda Item 8, Annex 1, document page 7ff)
- BWM.2/Circ.42 : Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)

3. PROJECT OBJECTIVE

The project objectives of this compliance study are as follows:

- Assess the feasibility of indicative and detailed (in-depth) analysis of ballast water on board ships under the realistic conditions of time constraints during unloading and loading processes while the ships are berthed in harbors for just one day or even less.
- Allow the MPA to identify potential practical challenges that vessels may face when MPA will execute its Port State Control role when the convention comes into force.
- Address industry concerns on the performance of IMO type approved Ballast Water Management Systems under normal operating conditions.

4. ANALYTICAL FRAME

4.1 IMO TARGET ORGANISM SIZE CLASSES

In accordance with the IMO regulations and guidelines as listed in para 2 the ballast water onboard the ships was analyzed as to the organism concentrations of the three target organism size classes defined by the IMO:

- Marine plankton organisms with a size of >50µm
- Marine plankton organisms with a size between >10µm and <50µm
- Marine bacteria :
    - *Escherichia coli*
    - Enterococci
    - *Vibrio cholerae*

4.2 PERFORMANCE STANDARDS

The analysis of the ballast water aimed at the verification of compliance with the international performance standards for ballast water to be re-discharged from ships as defined by the IMO within the frame of the International Ballast Water Management Convention 2004:

Table 02: IMO target organism groups and performance standards

IMO Target Size Class	IMO Performance Standard
Plankton >50µm	<10 Individuals/m³
Plankton >10µm<50µm	<10 Individuals/ml
Escherichia coli	<250 cfu/100ml
Enterococci	<100 cfu/100ml
Vibrio cholerae	<1 cfu/100ml

(cfu : colony forming unit)

4.3 SAMPLING TECHNOLOGY USED

The ballast water samples were taken by the aid of the "*SGS Ballast Water Sampler v02*", which has been connected to the isokinetic sampling port installed in the ballast water pipe system of the ship (see figures 01, 02 and 04 in the annex).

4.4 ANALYTICAL METHODS USED

4.4.1 General Information

Marine organisms are divided in animals (zooplankton), plants (phytoplankton) and bacteria. The D-2 size-classes of ≥50 µm and ≥10 but <50 µm both contain phytoplankton and zooplankton, making it important to use methods that detect both types of organism.

Plankton organisms are sensitive and long transport times can affect viability, because of this reason all analysis were conducted immediately on board ship when possible.

4.4.2 Indicative Methods

Pulse Amplitude Modulation Fluorometry - PAM

The PAM method provides an indication for the presence of only vital phytoplankton in the ballast water sample by measuring the chlorophyll activity in the entire sample. Separate values for phytoplankton ≥50 µm and phytoplankton ≥10 but <50 µm are achieved by filtration of the sample. The PAM method does not allow for a numerical (number of vital marine microalgae/ml) indication. The parameters are concentration of Chlorophyll in the ballast water sample (µg chl/l) and yield (yld), which is expressed without a dimension.

The PAM analysis is executed on board the ship.

Adenosin-triphosphate Fluorometry - ATP

Adenosin-triphosphate is a unique energy donor in the physiological processes of all living cells (phytoplankton, zooplankton and bacteria). The ATP method provides the concentration of cellular ATP in the ballast water sample and – based on empirical data - transforms this concentration value (pg cATP/ml) into an indicative numerical concentration value of number of vital organisms. It can be used for organisms ≥50 µm, organisms ≥10 but <50 µm and bacteria. However, it only provides total bacteria numbers and no species identification.

The ATP analysis for all IMO target organism size classes is executed on board the ship.

4.4.3 Detailed (in-depth) Methods

IMO Target Organism Size Class Plankton

The concentration of vital plankton organisms in the ballast water samples was analysed per optical counts under the microscope using a Bogorov chamber for plankton organisms ≥50µm and a Sedgewick Rafter count cell for plankton organisms >10µm<50µm.

Three sub-samples of the main sample for plankton organisms ≥50µm were counted and 10 sub-samples of the main ample for plankton organisms >10µm<50µm were counted

The microscopic counts of the concentration of viable plankton organisms in the ballast water were executed on board the ship.

IMO Target Organism Size Class Bacteria

Since the detailed (in-depth) analytical methods for the detection of bacteria in ballast water samples, demand a complex laboratory infrastructure the cultivation of human pathogens, these methods can only be executed in land based laboratories.

Fluorescence-In-Situ-Hybridization Fluorescence Microscopy

This gene probe method analyses the concentration of the IMO target bacteria species *Escherichia coli*, Enterococci and *Vibrio cholerae* in ballast water samples. The time needed from sample to result is approximately 11 hrs.

Classical 24/48 hrs. Incubation Methods

To assess the concentration of total bacteria in the ballast water samples the Heterotrophic Plate Count (HPC) method (APHA 9215) was used.

For the detection of the IMO target bacteria species different methods were used.

Table 03: 24/48 hrs. incubation methods used for the detection of bacteria

Bacteria quantitative :	APHA 9215
Escherichia coli :	APHA 9222G
Enterococci :	APHA 9230
Vibrio cholerae :	APHA 9260

Since there is no limit value for total heterotrophic bacteria, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.

4.5 EXECUTIVE PROTOCOL FOR ON BOARD COMPLIANCE TESTING

4.5.1 Basic Hydraulic Settings

The sampling of live plankton organisms from within the main ballast water pipes on board ships has to ensure, that these plankton organisms are not impacted by the sampling procedures.
Therefore the on board sampling and analysis was proceeded by a meeting with the relevant crew members of the ship to obtain the relevant basic, hydraulic information and to agree on the adequate pump capacity during sampling to exclude impacts on the live plankton organisms.

4.5.2 Establishment of working places

Two working places have been established on board the ship : (i) the *sampling* working place at the sampling port of the main ballast water pipe system and (ii) the analysis working place where the generated samples are further processed, analyzed, labeled and prepared for transport to the landbased laboratory for bacterial analysis

4.5.3 Installation of the sampling system

The constructional conditions at the sampling port on the ship required to run the "*SGS Ballast Water Sampler v02*" as an open system: the ballast water filtered through the system was not flushed back to the ballast water main pipe system but directed into the bilge of the ship instead. The maximum volume of ballast water that was allowed to enter the bilge was set 2.0m³.

4.5.4 Flushing of the main ballast water pipe system

After the installation of the two working places was finished, the notice to start the de ballasting procedure was given to the engineer in charge.

In order to avoid the contamination of the ballast water the main ballast water pipe system was flushed for 30 minutes.

4.5.5 Flushing of the sampling system

After the flushing of the main ballast water pipe system ended the sampling port valve was opened to direct ballast water through the "*SGS Ballast Water Sampler v02*". The sampling system was flushed with approximately 1,0m³ of ballast water. During this flushing procedure two ballast water samples were taken. These two samples were an additional requirement of MPA and do not form a part of the sampling and analysis for compliance testing.

4.5.6 Sampling of ballast water for compliance test

After the flushing of the sampling system ended, the system was activated to sample ballast water for compliance test. During this sampling procedure 7 ballast water samples of various volumes were taken at the sampling system and brought to the *analysis* workplace in the ship for further processing. Notice was given to the engineer in charge to stop the de-ballasting procedure.

4.5.7 Termination of on board compliance test

The ballast water on board compliance test ended with the de-installation of the *analysis* workplace after finalization of the indicative and detailed analysis of the ballast water samples.

5. RESULTS

5.1 BASIC HYDRAULIC INFORMATION

The basic hydraulic information is displayed in table 04 below.

Table 04: Basic hydraulic information for de-ballasting procedures

Parameter	Value	Dim.
Diameter of ballast water pipe	360	mm
Diameter of isokinetic pipe	25.4	mm
Maximum pump capacity	750	m³/h
System pressure in ballast water pipe	2.6	bar
Maximum ballast water volume for de-ballasting	3,049	m³
Date of ballast water uptake	18.08.2014	date
Holding time of ballast water	8	days
Origin of ballast water	South East Asian Port	
Minimum possible pump capacity during de-ballasting	150	m³/h
Average pump capacity during de-ballasting	152	m³/h
Maximum allowable discharge volume into the bilge	2.0	m³

5.2 TIME TABLE OF GENERAL ACTIVITIES

The table below presents the time lapse of general activities before, during and after the on board compliance test.

Table 05: Time table of general activities

Time of day	Activity
15:40	Departing from lab to port
16:30	Arrival at port's gate *(ship is delayed)*
18:00	Arrival at ship's berth
18:50	Unloading of equipment
19:00	Start interview with ship's crew
19:10	Start setting up working places (sampling and analysis)
19:40	End of setting up working places (sampling and analysis)
20:10	Start of flushing
20:28	End of flushing
20:45	Start of sampling
21:25	End of sampling
21:55	Removal of 50µm stainless steel filter
20:30	Start of analysis
23:00	End of analysis
23:00	Start of de-installation of working places
00:00	End of de-installation of working places
00:30	Alighting from ship
00:30	Loading of material
01:00	Departing from port *(break for dinner)*
02:30	Arrival at Laboratory

5.3 COURSE OF DEBALLASTING PROCEDURE AND SAMPLE RECORDS

The table below presents the general information of the de-ballasting procedure.

Table 06: General information of the de-ballasting procedures

Total duration of de-ballasting (hh:mm)	02:02
Total duration of sampling procedure (hh:mm)	00:20
Generated samples	15
Filtered volume of ballast water (m^3)	0.9987

The list below displays the course of the de-ballasting procedures and the sample records.

Time | **Activity**

19:00 Start of deballasting, flush of main ballast water pipe system

20:10 Start of flushing the sampling system (without 50 µm filter installed)

20:14 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
20:14	1	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm[1]
20:16	2		

20:28 End of flushing the sampling system

20:42 **Start of sampling for compliance test** (with 50 µm filter installed)

20:47 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
20:47	3	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	4, 5	1.5	Bacterial analysis in land based laboratory
20:51	6	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	7, 8	1.5	Bacterial analysis in land based laboratory
20:55	9	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	10, 11	1.5	Bacterial analysis in land based laboratory
20:59	12	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts

[1] Additional samples included in the sampling scheme by request of MPA

	13, 14	1.5	Bacterial analysis in land based laboratory

21:02 **End of sampling for compliance test**

21:03 End of de-ballasting

21:25 Steel screen filter of sampling system: sample record

Time	ID	Filtered Volume (m^3)	Purpose
21:25	15	0.9987 m^3	PAM, ATP plankton ≥50µm, microscopic counts

5.4 ANALYTICAL RESULTS

5.4.1 Physical parameters

The table below lists the results from the physical analysis of the ballast water

Table 07: Physical parameters of the ballast water

Sample ID	Parameter	Value	Dim.
1	turbidity	< 5	NTU
2		< 5	
1	salinity	32.7	PSU
1	temperature	25.9	°C

5.4.2 Indicative analysis: Chlorophyll activity (PAM)

Table 08: Chlorophyll activity in the ballast water

Sample ID	Chl (µg/l)	Yld
1	0	0
2	0.5	0
3	0.4	0
6	0.5	0
9	0.1	0
12	0.5	0
15	0.8	0

The ranking of the values is displayed in table 09 below.

Table 09: Ranking of Chlorophyll values

Chlorophyll	Indications
< 0.1 µg/l	Low number of marine microalgae in the sample
>0.1 <1.0 µg/l	Medium number of marine microalgae in the sample
<1.0 µg/l	Large number of marine microalgae in the sample
Yield	**Indications**
< 0.100	The sample contains marine microalgae on a very low vitality level
>0.100 <0.300	The sample contains marine microalgae on a medium vitality level
>0.300	The sample contains marine microalgae on a high vitality level

5.4.3 Indicative analysis: numerical values derived from ATP analysis

Table 10: Indicative concentrations of viable organisms in the ballast water

Sample ID	Bacteria quantitative (cfu/100ml)	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	2,942,787	170	not contained[2]
2	2,247,338	53	
3	1,876,218	12	
6	1,490,891	55	
9	1,830,532	22	
12	1,931,984	17	
15	not contained[3]		117

[2] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[3] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

5.4.4 Detailed analysis: microscopic counts

Table 11: Concentrations of viable plankton organisms in the ballast water (detailed analysis)

Sample ID	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	0	not contained[4]
2	0	
3	0	
6	0	
9	0	
12	0	
15	not contained[5]	9

5.4.5 Detailed analysis bacteria: classical 24/48 hrs incubation

Table 12: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Bacteria quantitative	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml			
4	2	<1	<1	<1
7	18	<1	<1	<1
10	10	<1	<1	<1
13	8	<1	<1	<1

[4] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[5] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

5.4.6 Detailed analysis bacteria: FISH

Table 13: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml		
5	2	0	0
8	2	0	0
11	1	0	0
14	1	0	0

6. RANKING OF ANALYTICAL RESULTS

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and uncertainty calculations were applied. These results were compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance, as shown in figure 1.

For the IMO target group "*plankton ≥50µm*" only one large volume sample was taken. Therefore statistical considerations were impossible. The respective statements are derived directly from the analytical results from this single sample.

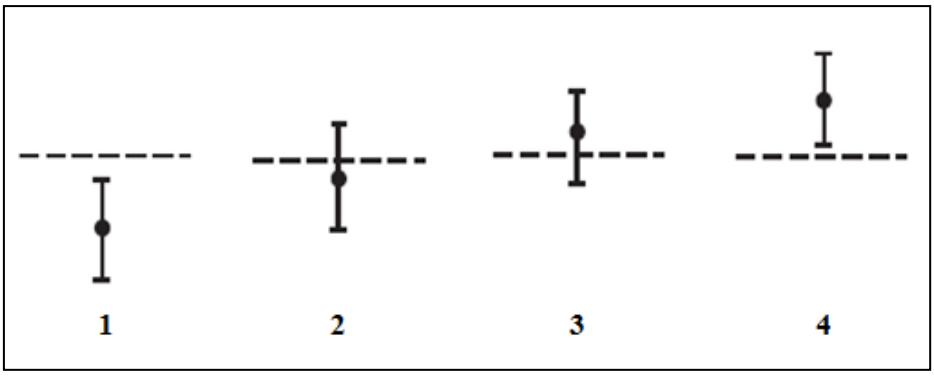


Figure 1: Examples of different compliance situations for indicative analyses: Situation 1: Compliance, the value and the uncertainty range are below the limit value. Situation 2: Possible compliance, the value is below the limit value, but the uncertainty range overlaps the limit value. Situation 3: Possible compliance, the value is above the limit value, but the uncertainty range overlaps the limit value. Situation 4: Non-compliance, the value and the uncertainty range are above the limit value.

Table 14: Final ranking of IMO target organism groups based on the analytical results

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Possibly compliant	Compliant		
Plankton ≥50µm	Non-compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/ 100 ml		< 1,000 cfu/100 ml	

Table 15: Summary of PAM fluorometry, ATP and microscopy results

Sample ID	Chlorophyll (µg/l)	Yield	ATP Bacteria quantitative (cfu/100ml)	ATP Plankton >10µm <50µm (organisms/ml)	Plankton counts >10µm <50µm (organisms/ml)	ATP Plankton ≥50µm (organisms/m³)	Plankton counts ≥50µm (organisms/m³)
1	0	0	2,942,787	170	0	not contained[6]	
2	0.5	0	2,247,338	53	0		
3	0.4	0	1,876,218	12	0		
6	0.5	0	1,490,891	55	0		
9	0.1	0	1,830,532	22	0		
12	0.5	0	1,931,984	17	0		
15	0.8	0	not contained[7]			117	9

[6] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[7] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

Table 16: Summary of APHA bacteria incubation analysis and FISH analysis

Sample ID	Bacteria Quantitative (APHA)	Escherichia coli (APHA)	Escherichia coli (FISH)	Enterococci (APHA)	Enterococci (FISH)	Vibrio cholerae (APHA)	Vibrio cholerae (FISH)
	cfu/100ml						
4, 5	2	<1	2	<1	0	<1	0
7, 8	18	<1	2	<1	0	<1	0
10, 11	10	<1	1	<1	0	<1	0
13, 14	8	<1	1	<1	0	<1	0

7. DISCUSSION

The indicative and detailed results for the ≥50 micron and the ≥10 <50 micron plankton fractions did not match. The indicative ATP method provided a non-compliant result for the ≥50 micron fraction and a possibly compliant result for the ≥10 <50 micron fraction while the microscopic counts were compliant.

It should be kept in mind that ATP is an indicative method, designed to provide an indication of compliance (or an indication of gross non-compliance) which can then be verified by detailed analysis. No limit values for gross non-compliance have been determined as of yet.

The results of the APHA analyses for *Escherichia coli*, Enterococci and *Vibrio cholerae* closely matched the results of the FISH analyses for these same bacteria groups (Table 15). There was, however, a very large difference between the APHA analysis for total heterotropic bacteria and the ATP bacteria analysis. However, it is known that many marine bacteria cannot be cultured using traditional methods (Viable Non-Culturable, VNC). The percentage of culturable bacteria in seawater can be as little as 0.08%[8].

8. CONCLUSIONS

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
4. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.
5. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

[8] Ferguson et al. 1984 Response of marine bacterioplankton to differential filtration and confinement. Applied and Environmental Microbiology, Volume 47, page 49-55.

9. ANNEX

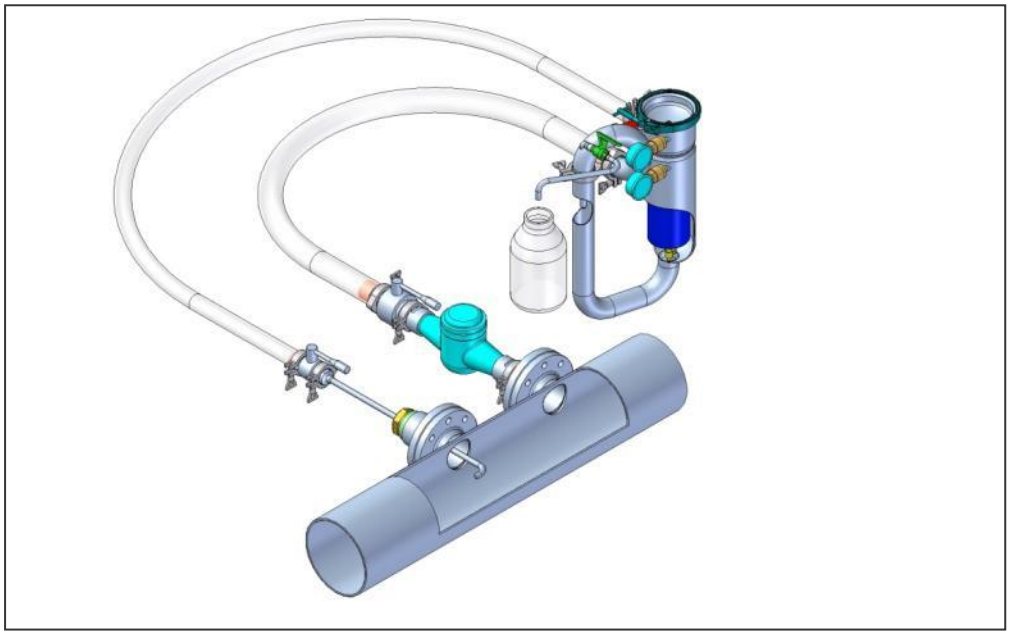


Figure 2: Schematic view of the SGS Ballast Water Sampler v02 (closed system variant)

Figure 3: The SGS Ballast Water Sampler v02

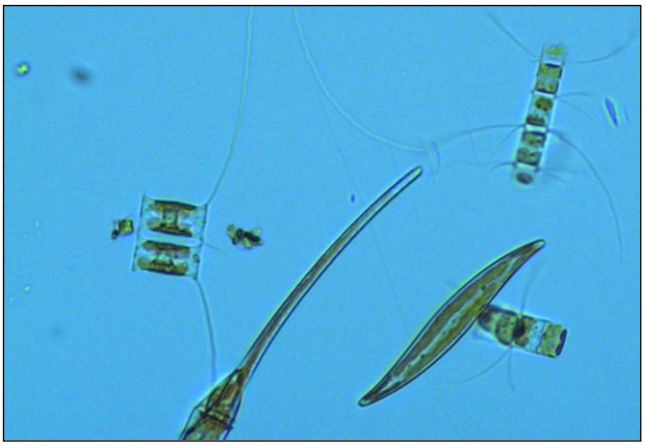
Figure 4: Sampling port on board the ship

Figure 5: Installation of SGS Ballast Water Sampler v02

BALLAST WATER REPORT

ON BOARD BALLAST WATER COMPLIANCE TEST

SGS



BALLAST WATER REPORT

SHIP 4

ON BOARD BALLAST WATER COMPLIANCE TEST

0814-00004 R0

08/09/2014

Prepared by

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EXECUTIVE SUMMARY

The ballast water aboard the fourth ship tested, a Singapore registered 14,000 TEU ship built in 2014, fully complies with the D-2 Ballast Water Performance Standard as defined by the IMO. A short overview of the results is shown in the table below.

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Possibly compliant	Compliant		
Plankton ≥50µm	Possibly compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/100 ml		< 1,000 cfu/100 ml	

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance.

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
4. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.
5. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

The paragraphs of this report present detailed information regarding the executed on board compliance test.

1. BACKGROUND

In July 2014 the Maritime Port Authority of Singapore (MPA) charged SGS Testing & Control Services Singapore Pte Ltd (SGS) with the execution of a study of on board sampling and analysis of ballast water for compliance testing within the frame of the Ballast Water Management Convention (BWMC) of the International Maritime Organization (IMO) of the United Nations.

The compliance tests had to be executed on in total six ships indentified by MPA.

This report presents the test results of ship no. 4.

Table 01: Characteristics of the ship

Type	Container Ship
Capacity (TEU)	14,000
Year built	2014

2. REGULATORY FRAMEWORK FOR THE ANALYSIS OF BALLAST WATER

The sampling and analysis of the ballast water should follow, as far as feasible, the relevant regulations published by the International Maritime Organization – IMO within the frame of the Ballast Water Convention 2004:

- G2 : Guidelines for Ballast Water Sampling (G2), October 10th, 2008
  (source: MEPC 58/23, Annex 3, Resolution MEPC.173/58))
- G8 : Guidelines for Approval of Ballast Water Management Systems (G8), October 10th, 2008
  (source: MEPC 58/23, Annex 4, Resolution MEPC.174(58))
- G-PSC : Guidelines for Port State Control under the International Convention for the Control and Management of Ship's Ballast Water and Sediments April 10th, 2014
  (source: III 1/8 IMO Sub-Committee on Implementation of IMO Instruments, 1st Session, Agenda Item 8, Annex 1, document page 7ff)
- BWM.2/Circ.42 : Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)

3. PROJECT OBJECTIVE

The project objectives of this compliance study are as follows:

- Assess the feasibility of indicative and detailed (in-depth) analysis of ballast water on board ships under the realistic conditions of time constraints during unloading and loading processes while the ships are berthed in harbors for just one day or even less.
- Allow the MPA to identify potential practical challenges that vessels may face when MPA will execute its Port State Control role when the convention comes into force.
- Address industry concerns on the performance of IMO type approved Ballast Water Management Systems under normal operating conditions.

4. ANALYTICAL FRAME

4.1 IMO TARGET ORGANISM SIZE CLASSES

In accordance with the IMO regulations and guidelines as listed in para 2 the ballast water onboard the ships was analyzed as to the organism concentrations of the three target organism size classes defined by the IMO:

- Marine plankton organisms with a size of >50µm
- Marine plankton organisms with a size between >10µm and <50µm
- Marine bacteria :
    - *Escherichia coli*
    - Enterococci
    - *Vibrio cholerae*

4.2 PERFORMANCE STANDARDS

The analysis of the ballast water aimed at the verification of compliance with the international performance standards for ballast water to be re-discharged from ships as defined by the IMO within the frame of the International Ballast Water Management Convention 2004:

Table 02: IMO target organism groups and performance standards

IMO Target Size Class	IMO Performance Standard
Plankton >50µm	<10 Individuals/m³
Plankton >10µm<50µm	<10 Individuals/ml
Escherichia coli	<250 cfu/100ml
Enterococci	<100 cfu/100ml
Vibrio cholerae	<1 cfu/100ml

(cfu : colony forming unit)

4.3 SAMPLING TECHNOLOGY USED

The ballast water samples were taken by the aid of the "*SGS Ballast Water Sampler v02*", which has been connected to the isokinetic sampling port installed in the ballast water pipe system of the ship (see figures 01, 02 and 04 in the annex).

4.4 ANALYTICAL METHODS USED

4.4.1 General Information

Marine organisms are divided in animals (zooplankton), plants (phytoplankton) and bacteria. The D-2 size-classes of ≥50 µm and ≥10 but <50 µm both contain phytoplankton and zooplankton, making it important to use methods that detect both types of organism.

Plankton organisms are sensitive and long transport times can affect viability, because of this reason all analysis were conducted immediately on board ship when possible.

4.4.2 Indicative Methods

Pulse Amplitude Modulation Fluorometry - PAM

The PAM method provides an indication for the presence of only vital phytoplankton in the ballast water sample by measuring the chlorophyll activity in the entire sample. Separate values for phytoplankton ≥50 µm and phytoplankton ≥10 but <50 µm are achieved by filtration of the sample. The PAM method does not allow for a numerical (number of vital marine microalgae/ml) indication. The parameters are concentration of Chlorophyll in the ballast water sample (µg chl/l) and yield (yld), which is expressed without a dimension.

The PAM analysis is executed on board the ship.

Adenosin-triphosphate Fluorometry - ATP

Adenosin-triphosphate is a unique energy donor in the physiological processes of all living cells (phytoplankton, zooplankton and bacteria). The ATP method provides the concentration of cellular ATP in the ballast water sample and – based on empirical data - transforms this concentration value (pg cATP/ml) into an indicative numerical concentration value of number of vital organisms. It can be used for organisms ≥50 µm, organisms ≥10 but <50 µm and bacteria. However, it only provides total bacteria numbers and no species identification.

The ATP analysis for all IMO target organism size classes is executed on board the ship.

4.4.3 Detailed (in-depth) Methods

IMO Target Organism Size Class Plankton

The concentration of vital plankton organisms in the ballast water samples was analysed per optical counts under the microscope using a Bogorov chamber for plankton organisms ≥50µm and a Sedgewick Rafter count cell for plankton organisms >10µm<50µm.

Three sub-samples of the main sample for plankton organisms ≥50µm were counted and 10 sub-samples of the main ample for plankton organisms >10µm<50µm were counted

The microscopic counts of the concentration of viable plankton organisms in the ballast water were executed on board the ship.

IMO Target Organism Size Class Bacteria

Since the detailed (in-depth) analytical methods for the detection of bacteria in ballast water samples, demand a complex laboratory infrastructure the cultivation of human pathogens, these methods can only be executed in landbased laboratories.

Fluorescence-In-Situ-Hybridization Fluorescence Microscopy

This gene probe method analyses the concentration of the IMO target bacteria species *Escherichia coli*, Enterococci and *Vibrio cholerae* in ballast water samples. The time needed from sample to result is approximately 11 hrs.

Classical 24/48 hrs. Incubation Methods

To assess the concentration of total bacteria in the ballast water samples the Heterotrophic Plate Count (HPC) method (APHA 9215) was used.

For the detection of the IMO target bacteria species different methods were used.

Table 03: 24/48 hrs. incubation methods used for the detection of bacteria

Bacteria quantitative :	APHA 9215
Escherichia coli :	APHA 9222G
Enterococci :	APHA 9230
Vibrio cholerae :	APHA 9260

Since there is no limit value for total heterotrophic bacteria, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.

4.5 EXECUTIVE PROTOCOL FOR ON BOARD COMPLIANCE TESTING

4.5.1 Basic Hydraulic Settings

The sampling of live plankton organisms from within the main ballast water pipes on board ships has to ensure, that these plankton organisms are not impacted by the sampling procedures.
Therefore the on board sampling and analysis was proceeded by a meeting with the relevant crew members of the ship to obtain the relevant basic, hydraulic information and to agree on the adequate pump capacity during sampling to exclude impacts on the live plankton organisms.

4.5.2 Establishment of working places

Two working places have been established on board the ship : (i) the *sampling* working place at the sampling port of the main ballast water pipe system and (ii) the analysis working place where the generated samples are further processed, analyzed, labeled and prepared for transport to the landbased laboratory for bacterial analysis

4.5.3 Installation of the sampling system

The constructional conditions at the sampling port on the ship required to run the "*SGS Ballast Water Sampler Type 1*" as an open system: the ballast water filtered through the system was not flushed back to the ballast water main pipe system but directed into the bilge of the ship instead. The maximum volume of ballast water that was allowed to enter the bilge was set 2.0m³.

4.5.4 Flushing of the main ballast water pipe system

After the installation of the two working places was finished, the notice to start the de-ballasting procedure was given to the engineer in charge.

In order to avoid the contamination of the ballast water the main ballast water pipe system was flushed for 30 minutes.

4.5.5 Flushing of the sampling system

After the flushing of the main ballast water pipe system ended the sampling port valve was opened to direct ballast water through the "*SGS Ballast Water Sampler v02*". The sampling system was flushed with approximately 1.0m³ of ballast water. During this flushing procedure two ballast water samples were taken. These two samples were an additional requirement of MPA and do not form a part of the sampling and analysis for compliance testing.

4.5.6 Sampling of ballast water for compliance test

After the flushing of the sampling system ended, the system was activated to sample ballast water for compliance test. During this sampling procedure 7 ballast water samples of various volumes were taken at the sampling system and brought to the *analysis* workplace in the ship for further processing. Notice was given to the engineer in charge to stop the de-ballasting procedure.

4.5.7 Termination of on board compliance test

The ballast water on board compliance test ended with the de-installation of the *analysis* workplace after finalization of the indicative and detailed analysis' of the ballast water samples.

5. RESULTS

5.1 BASIC HYDRAULIC INFORMATION

The basic hydraulic information is displayed in table 04 below.

Table 04 : Basic hydraulic information for de-ballasting procedures

Parameter	Value	Dim.
Diameter of ballast water pipe	400	mm
Diameter of isokinetic pipe	25.4	mm
Maximum pump capacity	1,000	m³/h
System pressure in ballast water pipe	4.8	bar
Maximum ballast water volume for de-ballasting	1156	m³
Date of ballast water uptake	06.09.2014	date
Holding time of ballast water	1	days
Origin of ballast water	Strait of Malacca	
Minimum possible pump capacity during de-ballasting	200	m³/h
Average pump capacity during de-ballasting	209	m³/h
Maximum allowable discharge volume into the bilge	2.0	m³

5.2 TIME TABLE OF GENERAL ACTIVITIES

The table below presents the time lapse of general activities before, during and after the on board compliance test.

Table 05: Time table of general activities

Time of day	Activity
08:30	Departing from lab to port
09:15	Arrival at port's gate *(ship is delayed)*
09:45	Arrival at ship's berth
09:50	Unloading of equipment
09:55-10:15	Start interview with ship's crew
10:30	Start setting up working places (sampling and analysis)
11:00	End of setting up working places (sampling and analysis)
11:43	Start of flushing
12:05	End of flushing
12:20	Start of sampling
13:09	End of sampling
14:00	Removal of 50µm stainless steel filter
12:00	Start of analysis
15:30	End of analysis
15:30	Start of de-installation of working places
15:55	End of de-installation of working places
16:00	Alighting from ship
16:20	Loading of material
16:35	Departing from port *(break for dinner)*
17:00	Arrival at Laboratory

5.3 COURSE OF DEBALLASTING PROCEDURE AND SAMPLE RECORDS

The table below presents the general information of the de-ballasting procedure.

Table 06: General information of the de-ballasting procedures

Total duration of de-ballasting (hh:mm)	02:30
Total duration of sampling procedure (hh:mm)	00:49
Generated samples	28
Filtered volume of ballast water (m^3)	0.962

The list below displays the course of the de-ballasting procedures and the sample records.

Time | **Activity**

10:40 Start of de-ballasting, flush of main ballast water pipe system

11:43 Start of flushing the sampling system (without 50 µm filter installed)

11;50 Bypass port of sampling system : sample record

Time	ID	Volume (l)	Purpose
11.50	1	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm[1]
11:57	2		

12:05 End of flushing the sampling system

12:20 **Start of sampling for compliance test** (with 50 µm filter installed)

12:25 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
12:25	3	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	4 - 7	2.0	Bacterial analysis in land based laboratory
12:35	8	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	9 - 12	2.0	Bacterial analysis in land based laboratory
12:43	13	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	14 - 17	2.0	Bacterial analysis in land based laboratory
12:58	18	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts

[1] Additional samples included in the sampling scheme by request of MPA

	19 - 22	2.0	Bacterial analysis in land based laboratory
13:06	23	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	24 - 27	2.0	Bacterial analysis in land based laboratory

13:09 **End of sampling for compliance test**

13:10 End of de-ballasting

14:00 Steel screen filter of sampling system: sample record

Time	ID	Filtered Volume (m^3)	Purpose
14:00	28	0.962 m^3	PAM, ATP plankton ≥50µm, microscopic counts

5.4 ANALYTICAL RESULTS

5.4.1 Physical parameters

The table below lists the results from the physical analysis of the ballast water

Table 07: Physical parameters of the ballast water

Sample ID	Parameter	Value	Dim.
1	turbidity	< 5	NTU
2		< 5	
1	salinity	32.4	PSU
1	temperature	31.0	°C

5.4.2 Indicative analysis: Chlorophyll activity (PAM)

Table 08: Chlorophyll activity in the ballast water

Sample ID	Chl (µg/l)	YId
1	0.3	0
2	0	0
3	0.1	0
8	0.4	0
13	0.1	0
18	0.1	0
23	0.2	0
28	0.2	0

The ranking of the values is displayed in table 09 below.

Table 09: Ranking of Chlorophyll values

Chlorophyll	Indications
< 0.1 µg/l	Low number of marine microalgae in the sample
>0.1 <1.0 µg/l	Medium number of marine microalgae in the sample
<1.0 µg/l	Large number of marine microalgae in the sample
Yield	**Indications**
< 0.100	The sample contains marine microalgae on a very low vitality level
>0.100 <0.300	The sample contains marine microalgae on a medium vitality level
>0.300	The sample contains marine microalgae on a high vitality level

5.4.3 Indicative analysis: numerical values derived from ATP analysis

Table 10: Indicative concentrations of viable organisms in the ballast water

Sample ID	Bacteria quantitative (cfu/100ml)	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	5,083,680	84	not contained[2]
2	4,426,285	37	
3	2,108,076	10	
8	1,015,439	21	
13	914,489	13	
18	2,244,656	15	
23	2,484,165	21	
28	not contained[3]		26

[2] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[3] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

5.4.4 Detailed analysis: microscopic counts

Table 11: Concentrations of viable plankton organisms in the ballast water (detailed analysis)

Sample ID	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	0	not contained[4]
2	0	
3	0	
8	0	
13	0	
18	0	
23	0	
28	not contained[5]	0

5.4.5 Detailed analysis bacteria: classical 24/48 hrs incubation

Table 12: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Bacteria quantitative	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml			
4	3	<1	<1	<1
5	2	<1	<1	<1
6	1	<1	<1	<1
7	21	<1	<1	<1
9	2	<1	<1	<1
10	1	<1	<1	<1
11	3	<1	<1	<1

[4] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[5] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

12	<1	<1	<1	<1
14	1	<1	<1	<1
15	68	<1	<1	<1
16	3	<1	<1	<1
17	2	<1	<1	<1
19	8	<1	<1	<1
20	88	<1	<1	<1
21	4	<1	<1	<1
22	1	<1	<1	<1
24	1	<1	<1	<1
25	3	<1	<1	<1
26	<1	<1	<1	<1
27	1	<1	<1	<1

5.4.6 Detailed analysis bacteria: FISH

Table 13: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml		
4	3	1	0
9	0	0	0
14	2	1	0
19	1	1	0
24	3	2	0

6. RANKING OF ANALYTICAL RESULTS

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and uncertainty calculations were applied. These results were compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance, as shown in figure 1.

For the IMO target group "*plankton ≥50µm*" only one large volume sample was taken. Therefore statistical considerations were impossible. The respective statements are derived directly from the analytical results from this single sample.

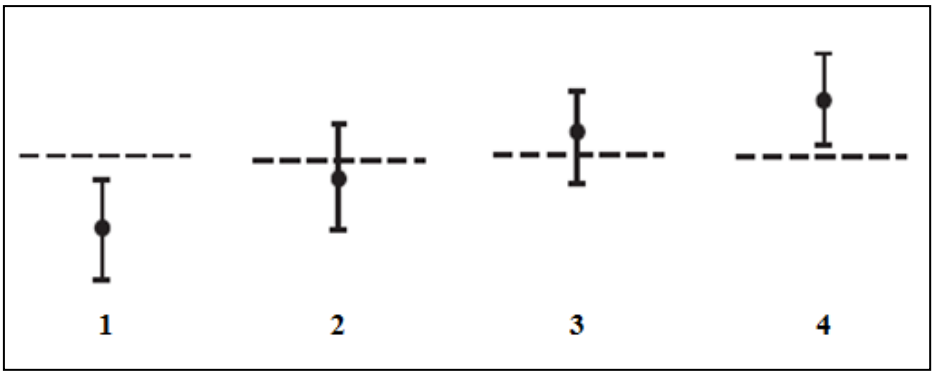


Figure 1: Examples of different compliance situations for indicative analyses: Situation 1: Compliance, the value and the uncertainty range are below the limit value. Situation 2: Possible compliance, the value is below the limit value, but the uncertainty range overlaps the limit value. Situation 3: Possible compliance, the value is above the limit value, but the uncertainty range overlaps the limit value. Situation 4: Non-compliance, the value and the uncertainty range are above the limit value.

Table 14: Final ranking of IMO target organism groups based on the analytical results

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Possibly compliant	Compliant		
Plankton ≥50µm	Possibly compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/100 ml		< 1,000 cfu/100 ml	

Table 15: Summary of PAM fluorometry, ATP and microscopy results

Sample ID	Chlorophyll (µg/l)	Yield	ATP Bacteria quantitative (cfu/100ml)	ATP Plankton >10µm <50µm (organisms/ml)	Plankton counts >10µm <50µm (organisms/ml)	ATP Plankton ≥50µm (organisms/m³)	Plankton counts ≥50µm (organisms/m³)
1	0.3	0	5,083,680	84	0	not contained[6]	
2	0	0	4,426,285	37	0		
3	0.1	0	2,108,076	10	0		
8	0.4	0	1,015,439	21	0		
13	0.1	0	914,489	13	0		
18	0.1	0	2,244,656	15	0		
23	0.2	0	2,484,165	21	0		
28	0.7	0	not contained[7]			26	0

[6] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[7] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

Table 16: Summary of APHA bacteria incubation analysis and FISH analysis

Sample ID	Bacteria Quantitative (APHA)	Escherichia coli (APHA)	Escherichia coli (FISH)	Enterococci (APHA)	Enterococci (FISH)	Vibrio cholerae (APHA)	Vibrio cholerae (FISH)
	cfu/100ml						
4	3	<1	3	<1	1	<1	0
5	2	<1		<1		<1	
6	1	<1		<1		<1	
7	21	<1		<1		<1	
9	2	<1	0	<1	0	<1	0
10	1	<1		<1		<1	
11	3	<1		<1		<1	
12	<1	<1		<1		<1	
14	1	<1	2	<1	1	<1	0
15	68	<1		<1		<1	
16	3	<1		<1		<1	
17	2	<1		<1		<1	
19	8	<1	1	<1	1	<1	0

20	88	<1		<1		<1	
21	4	<1		<1		<1	
22	1	<1		<1		<1	
24	1	<1	3	<1	2	<1	0
25	3	<1		<1		<1	
26	<1	<1		<1		<1	
27	1	<1		<1		<1	

7. DISCUSSION

The indicative and detailed results for the ≥50 micron and ≥10 <50 micron plankton fractions did not match. The indicative ATP method provided a possibly compliant result for these fractions while the microscopic counts were compliant.

It should be kept in mind that ATP is an indicative method, designed to provide an indication of compliance (or an indication of gross non-compliance) which can then be verified by detailed analysis. No limit values for gross non-compliance have been determined as of yet.

The results of the APHA analyses for *Escherichia coli*, Enterococci and *Vibrio cholerae* closely matched the results of the FISH analyses for these same bacteria groups (Table 15). There was, however, a very large difference between the APHA analysis for total heterotropic bacteria and the ATP bacteria analysis. However, it is known that many marine bacteria cannot be cultured using traditional methods (Viable Non-Culturable, VNC). The percentage of culturable bacteria in seawater can be as little as 0.08%[8].

8. CONCLUSIONS

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
4. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.
5. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

[8] Ferguson et al. 1984 Response of marine bacterioplankton to differential filtration and confinement. Applied and Environmental Microbiology, Volume 47, page 49-55.

9. ANNEX

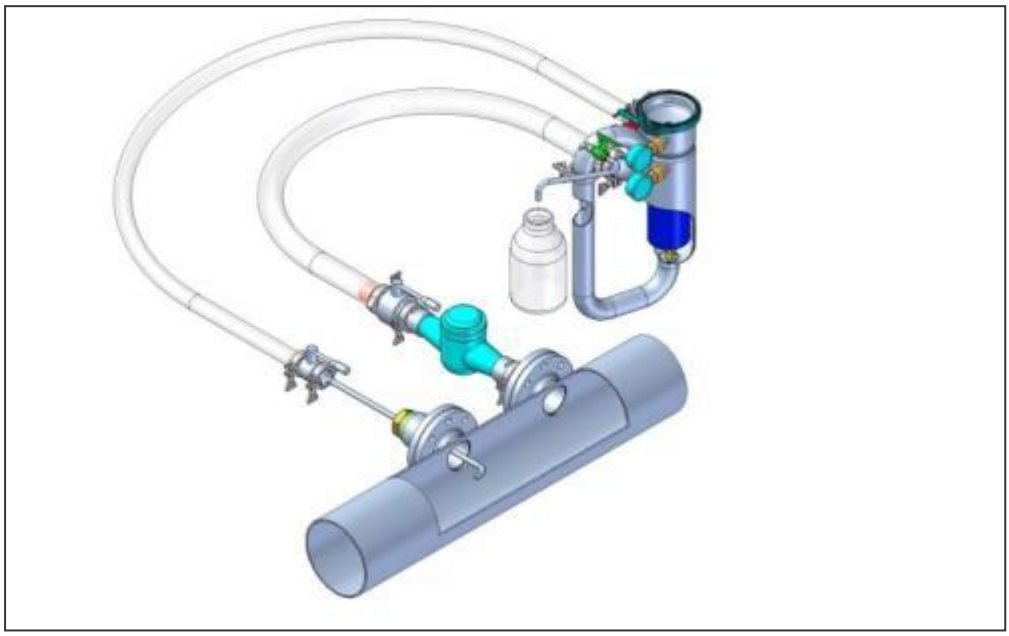


Figure 2: Schematic view of the SGS Ballast Water Sampler v02 (closed system variant)

Figure 3: The SGS Ballast Water Sampler v02

Figure 4: Sampling port on board the ship

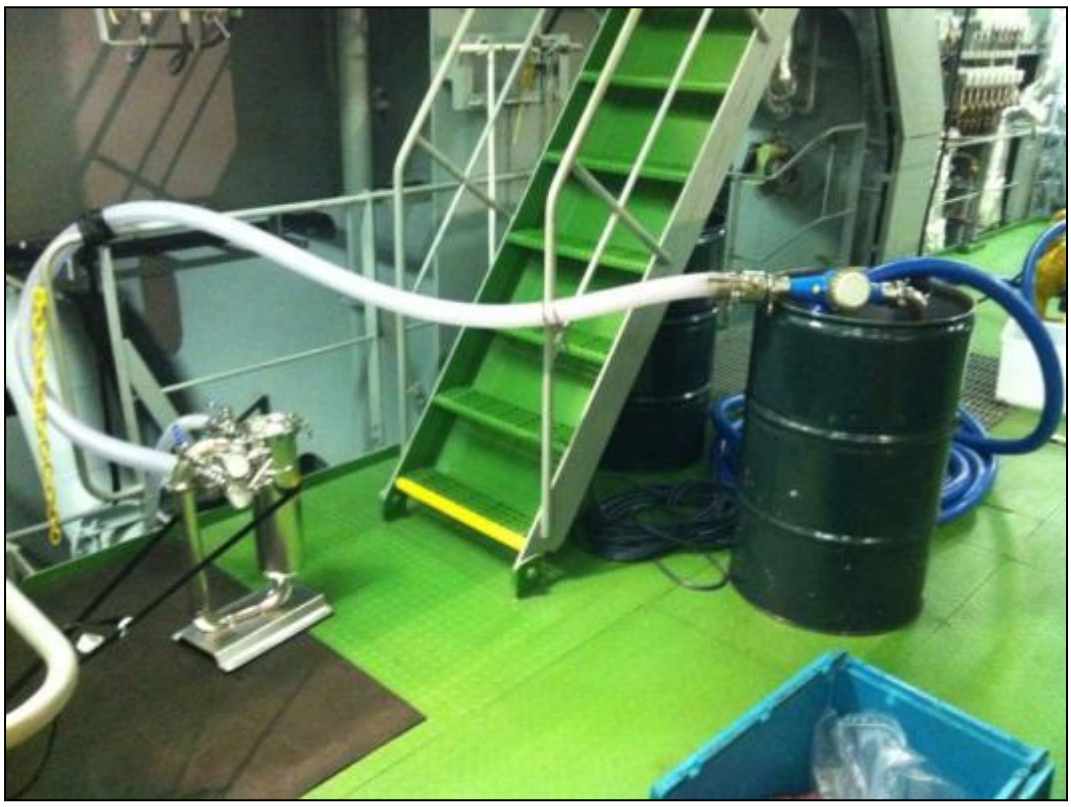


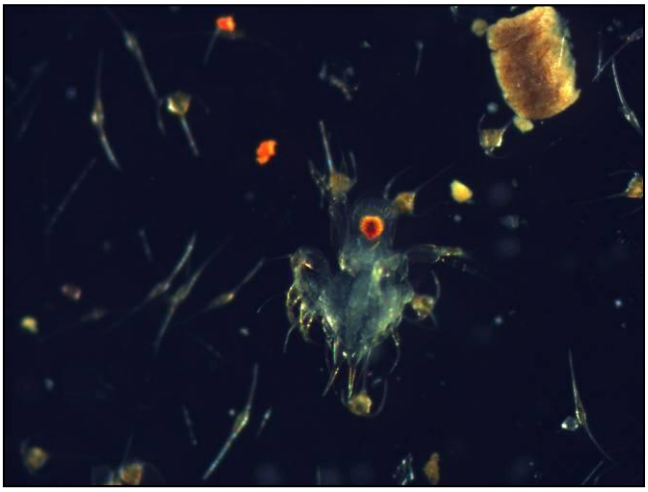
Figure 5: Installation of SGS Ballast Water Sampler v02

BALLAST WATER REPORT

ON BOARD BALLAST WATER COMPLIANCE TEST

SGS

ENVGT01.01



BALLAST WATER REPORT

SHIP 5

ON BOARD BALLAST WATER COMPLIANCE TEST

0814-00005 R0

10/09/2014

Prepared by

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EXECUTIVE SUMMARY

The ballast water aboard the fifth ship tested, a Singapore registered 9,200 TEU ship built in 2013, fully complies with the D-2 Ballast Water Performance Standard as defined by the IMO. A short overview of the results is shown in the table below.

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Possibly compliant	Compliant		
Plankton ≥50µm	Possibly compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/100 ml		< 1,000 cfu/100 ml	

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance.

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
4. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.
5. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

The paragraphs of this report present detailed information regarding the executed on board compliance test.

1. BACKGROUND

In July 2014 the Maritime and Port Authority of Singapore (MPA) charged SGS Testing & Control Services Singapore Pte Ltd (SGS) with the execution of a study of on board sampling and analysis of ballast water for compliance testing within the frame of the Ballast Water Management Convention (BWMC) of the International Maritime Organization (IMO) of the United Nations.

The compliance tests had to be executed on in total six ships indentified by MPA.

This report presents the test results of ship no. 5.

Table 01: Characteristics of the ship

Type	Container Ship
Capacity (TEU)	9,200
Year built	2013

2. REGULATORY FRAMEWORK FOR THE ANALYSIS OF BALLAST WATER

The sampling and analysis of the ballast water should follow, as far as feasible, the relevant regulations published by the International Maritime Organization – IMO within the frame of the Ballast Water Convention 2004:

- G2 : Guidelines for Ballast Water Sampling (G2), October 10th, 2008
  (source: MEPC 58/23, Annex 3, Resolution MEPC.173/58))
- G8 : Guidelines for Approval of Ballast Water Management Systems (G8), October 10th, 2008
  (source: MEPC 58/23, Annex 4, Resolution MEPC.174(58))
- G-PSC : Guidelines for Port State Control under the International Convention for the Control and Management of Ship's Ballast Water and Sediments April 10th, 2014
  (source: III 1/8 IMO Sub-Committee on Implementation of IMO Instruments, 1st Session, Agenda Item 8, Annex 1, document page 7ff)
- BWM.2/Circ.42 : Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)

3. PROJECT OBJECTIVE

The project objectives of this compliance study are as follows:

- Assess the feasibility of indicative and detailed (in-depth) analysis of ballast water on board ships under the realistic conditions of time constraints during unloading and loading processes while the ships are berthed in harbors for just one day or even less.
- Allow the MPA to identify potential practical challenges that vessels may face when MPA will execute its Port State Control role when the convention comes into force.
- Address industry concerns on the performance of IMO type approved Ballast Water Management Systems under normal operating conditions.

4. ANALYTICAL FRAME

4.1 IMO TARGET ORGANISM SIZE CLASSES

In accordance with the IMO regulations and guidelines as listed in para 2 the ballast water onboard the ships was analyzed as to the organism concentrations of the three target organism size classes defined by the IMO:

- Marine plankton organisms with a size of >50µm
- Marine plankton organisms with a size between >10µm and <50µm
- Marine bacteria :
    - *Escherichia coli*
    - Enterococci
    - *Vibrio cholerae*

4.2 PERFORMANCE STANDARDS

The analysis of the ballast water aimed at the verification of compliance with the international performance standards for ballast water to be re-discharged from ships as defined by the IMO within the frame of the International Ballast Water Management Convention 2004:

Table 02: IMO target organism groups and performance standards

IMO Target Size Class	IMO Performance Standard
Plankton >50µm	<10 Individuals/m³
Plankton >10µm<50µm	<10 Individuals/ml
Escherichia coli	<250 cfu/100ml
Enterococci	<100 cfu/100ml
Vibrio cholerae	<1 cfu/100ml

(cfu : colony forming unit)

4.3 SAMPLING TECHNOLOGY USED

The ballast water samples were taken by the aid of the "*SGS Ballast Water Sampler v02*", which has been connected to the isokinetic sampling port installed in the ballast water pipe system of the ship (see figures 01, 02 and 04 in the annex).

4.4 ANALYTICAL METHODS USED

4.4.1 General Information

Marine organisms are divided in animals (zooplankton), plants (phytoplankton) and bacteria. The D-2 size-classes of ≥50 µm and ≥10 but <50 µm both contain phytoplankton and zooplankton, making it important to use methods that detect both types of organism.

Plankton organisms are sensitive and long transport times can affect viability, because of this reason all analysis were conducted immediately on board ship when possible.

4.4.2 Indicative Methods

Pulse Amplitude Modulation Fluorometry - PAM

The PAM method provides an indication for the presence of only vital phytoplankton in the ballast water sample by measuring the chlorophyll activity in the entire sample. Separate values for phytoplankton ≥50 µm and phytoplankton ≥10 but <50 µm are achieved by filtration of the sample. The PAM method does not allow for a numerical (number of vital marine microalgae/ml) indication. The parameters are concentration of Chlorophyll in the ballast water sample (µg chl/l) and yield (yld), which is expressed without a dimension.

The PAM analysis is executed on board the ship.

Adenosin-triphosphate Fluorometry - ATP

Adenosin-triphosphate is a unique energy donor in the physiological processes of all living cells (phytoplankton, zooplankton and bacteria). The ATP method provides the concentration of cellular ATP in the ballast water sample and – based on empirical data - transforms this concentration value (pg cATP/ml) into an indicative numerical concentration value of number of vital organisms. It can be used for organisms ≥50 µm, organisms ≥10 but <50 µm and bacteria. However, it only provides total bacteria numbers and no species identification.

The ATP analysis for all IMO target organism size classes is executed on board the ship.

4.4.3 Detailed (in-depth) Methods

IMO Target Organism Size Class Plankton

The concentration of vital plankton organisms in the ballast water samples was analysed per optical counts under the microscope using a Bogorov chamber for plankton organisms ≥50µm and a Sedgewick Rafter count cell for plankton organisms >10µm<50µm.

Three sub-samples of the main sample for plankton organisms ≥50µm were counted and 10 sub-samples of the main ample for plankton organisms >10µm<50µm were counted

The microscopic counts of the concentration of viable plankton organisms in the ballast water were executed on board the ship.

IMO Target Organism Size Class Bacteria

Since the detailed (in-depth) analytical methods for the detection of bacteria in ballast water samples, demand a complex laboratory infrastructure the cultivation of human pathogens, these methods can only be executed in landbased laboratories.

Fluorescence-In-Situ-Hybridization Fluorescence Microscopy

This gene probe method analyses the concentration of the IMO target bacteria species *Escherichia coli*, Enterococci and *Vibrio cholerae* in ballast water samples. The time needed from sample to result is approximately 11 hrs.

Classical 24/48 hrs. Incubation Methods

To assess the concentration of total bacteria in the ballast water samples the Heterotrophic Plate Count (HPC) method (APHA 9215) was used.

For the detection of the IMO target bacteria species different methods were used.

Table 03: 24/48 hrs. incubation methods used for the detection of bacteria

Bacteria quantitative :	APHA 9215
Escherichia coli :	APHA 9222G
Enterococci :	APHA 9230
Vibrio cholerae :	APHA 9260

Since there is no limit value for total heterotrophic bacteria, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.

4.5 EXECUTIVE PROTOCOL FOR ON BOARD COMPLIANCE TESTING

4.5.1 Basic Hydraulic Settings

The sampling of live plankton organisms from within the main ballast water pipes on board ships has to ensure, that these plankton organisms are not impacted by the sampling procedures.
Therefore the on board sampling and analysis was proceeded by a meeting with the relevant crew members of the ship to obtain the relevant basic, hydraulic information and to agree on the adequate pump capacity during sampling to exclude impacts on the live plankton organisms.

4.5.2 Establishment of working places

Two working places have been established on board the ship : (i) the *sampling* working place at the sampling port of the main ballast water pipe system and (ii) the analysis working place where the generated samples are further processed, analyzed, labeled and prepared for transport to the land based laboratory for bacterial analysis

4.5.3 Installation of the sampling system

The constructional conditions at the sampling port on the ship required to run the "*SGS Ballast Water Sampler v02*" as an open system: the ballast water filtered through the system was not flushed back to the ballast water main pipe system but directed into the bilge of the ship instead. The maximum volume of ballast water that was allowed to enter the bilge was set 2.0m³.

4.5.4 Flushing of the main ballast water pipe system

After the installation of the two working places was finished, the notice to start the de-ballasting procedure was given to the engineer in charge.

In order to avoid the contamination of the ballast water the main ballast water pipe system was flushed for 30 minutes.

4.5.5 Flushing of the sampling system

After the flushing of the main ballast water pipe system ended the sampling port valve was opened to direct ballast water through the "*SGS Ballast Water Sampler v02*". The sampling system was flushed with approximately 1.0m³ of ballast water. During this flushing procedure two ballast water samples were taken. These two samples were an additional requirement of MPA and do not form a part of the sampling and analysis for compliance testing.

4.5.6 Sampling of ballast water for compliance test

After the flushing of the sampling system ended, the system was activated to sample ballast water for compliance test. During this sampling procedure 7 ballast water samples of various volumes were taken at the sampling system and brought to the *analysis* workplace in the ship for further processing. Notice was given to the engineer in charge to stop the de-ballasting procedure.

4.5.7 Termination of on board compliance test

The ballast water on board compliance test ended with the de-installation of the *analysis* workplace after finalization of the indicative and detailed analysis' of the ballast water samples.

5. RESULTS

5.1 BASIC HYDRAULIC INFORMATION

The basic hydraulic information is displayed in table 04 below.

Table 04 : Basic hydraulic information for de-ballasting procedures

Parameter	Value	Dim.
Diameter of ballast water pipe	450	mm
Diameter of isokinetic pipe	25.4	mm
Maximum pump capacity	1,000	m^3/h
System pressure in ballast water pipe	4.8	bar
Maximum ballast water volume for deballasting	1600	m^3
Date of ballast water uptake	07.09.2014	date
Holding time of ballast water	2	days
Origin of ballast water	South China Sea	
Minimum possible pump capacity during deballasting	300	m^3/h
Average pump capacity during deballasting	333	m^3/h
Maximum allowable discharge volume into the bilge	2.0	m^3

5.2 TIME TABLE OF GENERAL ACTIVITIES

The table below presents the time lapse of general activities before, during and after the on board compliance test.

Table 05: Time table of general activities

Time of day	Activity
08:30	Departing from lab to port
09:20	Arrival at port's gate *(ship is delayed)*
09:25	Arrival at ship's berth
09:25	Unloading of equipment
09:40-10:00	Start interview with ship's crew
10:10	Start setting up working places (sampling and analysis)
10:25	End of setting up working places (sampling and analysis)
11:00	Start of flushing
12:05	End of flushing
11:25	Start of sampling
11:55	End of sampling
13:15	Removal of 50µm stainless steel filter
11:05	Start of analysis
14:05	End of analysis
13:20	Start of de-installation of working places
13:40	End of de-installation of working places
14:15	Alighting from ship
14:30	Loading of material
15:10	Departing from port
16:00	Arrival at Laboratory

5.3 COURSE OF DEBALLASTING PROCEDURE AND SAMPLE RECORDS

The table below presents the general information of the de-ballasting procedure.

Table 06: General information of the de-ballasting procedures

Total duration of de-ballasting (hh:mm)	01:40
Total duration of sampling procedure (hh:mm)	00:30
Generated samples	28
Filtered volume of ballast water (m^3)	0.999

The list below displays the course of the de-ballasting procedures and the sample records.

Time | **Activity**

10:25 Start of de-ballasting, flush of main ballast water pipe system

10:50 Start of flushing the sampling system (without 50 µm filter installed)

11:00 Bypass port of sampling system : sample record

Time	ID	Volume (l)	Purpose
11.00	1	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm[1]
11:00	2		

11:15 End of flushing the sampling system

11:25 **Start of sampling for compliance test** (with 50 µm filter installed)

11:31 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
12:31	3	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	4 - 7	2.0	Bacterial analysis in land based laboratory
11:37	8	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	9 - 12	2.0	Bacterial analysis in land based laboratory
11:43	13	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	14 - 17	2.0	Bacterial analysis in land based laboratory
11:49	18	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts

[1] Additional samples included in the sampling scheme by request of MPA

	19 - 22	2.0	Bacterial analysis in land based laboratory
11:54	23	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	24 - 27	2.0	Bacterial analysis in land based laboratory

11:55 **End of sampling for compliance test**

11:58 End of de-ballasting

13:15 Steel screen filter of sampling system: sample record

Time	ID	Filtered Volume (m^3)	Purpose
13:15	28	0.999 m^3	PAM, ATP plankton ≥50µm, microscopic counts

5.4 ANALYTICAL RESULTS

5.4.1 Physical parameters

The table below lists the results from the physical analysis of the ballast water

Table 07: Physical parameters of the ballast water

Sample ID	Parameter	Value	Dim.
1	turbidity	< 5	NTU
2		< 5	
1	salinity	33.4	PSU
1	temperature	30.0	°C

5.4.2 Indicative analysis: Chlorophyll activity (PAM)

Table 08: Chlorophyll activity in the ballast water

Sample ID	Chl (µg/l)	YId
1	0.2	0
2	0.4	0
3	0.4	0
8	0.1	0
13	0.4	0
18	0.3	0
23	0.1	0
28	0.2	0

The ranking of the values is displayed in table 09 below.

Table 09: Ranking of Chlorophyll values

Chlorophyll	Indications
< 0.1 µg/l	Low number of marine microalgae in the sample
>0.1 <1.0 µg/l	Medium number of marine microalgae in the sample
<1.0 µg/l	Medium number of marine microalgae in the sample
Yield	**Indications**
< 0.100	The sample contains marine microalgae on a very low vitality level
>0.100 <0.300	The sample contains marine microalgae on a medium vitality level
>0.300	The sample contains marine microalgae on a high vitality level

5.4.3 Indicative analysis: numerical values derived from ATP analysis

Table 10: Indicative concentrations of viable organisms in the ballast water (The high values for samples 2, 3, 8, 13 and 18 were tracked to a contaminated batch of reagents. These values were not used in the compliance calculations)

Sample ID	Bacteria quantitative (cfu/100ml)	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	5,017,620	57	not contained[2]
2	4,254,475	257	
3	8,077,201	298	
8	3,052,887	160	
13	5,274,288	90	
18	7,021,434	200	
23	1,041,465	9	
28	not contained[3]		16

[2] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[3] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

5.4.4 Detailed analysis: microscopic counts

Table 11: Concentrations of viable plankton organisms in the ballast water (detailed analysis)

Sample ID	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	0	not contained[4]
2	0	
3	0	
8	0	
13	0	
18	0	
23	0	
28	not contained[5]	0

5.4.5 Detailed analysis bacteria: classical 24/48 hrs incubation

Table 12: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Bacteria quantitative	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml			
4	1	<1	<1	<1
5	48	<1	<1	<1
6	5	<1	<1	<1
7	3	<1	<1	<1
9	2	<1	<1	<1
10	24	<1	<1	<1
11	1	<1	<1	<1

[4] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[5] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

12	36	<1	<1	<1
14	16	<1	<1	<1
15	13	<1	<1	<1
16	36	<1	<1	<1
17	44	<1	<1	<1
19	68	<1	<1	<1
20	3	<1	<1	<1
21	44	<1	<1	<1
22	32	<1	<1	<1
24	<1	<1	<1	<1
25	1	<1	<1	<1
26	<1	<1	<1	<1
27	<1	<1	<1	<1

5.4.6 Detailed analysis bacteria: FISH

Table 13: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml		
4	0	0	0
9	1	0	0
14	2	1	0
19	1	0	0
24	1	1	0

6. RANKING OF ANALYTICAL RESULTS

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and uncertainty calculations were applied. These results were compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance, as shown in figure 1.

For the IMO target group "*plankton ≥50µm*" only one large volume sample was taken. Therefore statistical considerations were impossible. The respective statements are derived directly from the analytical results from this single sample.

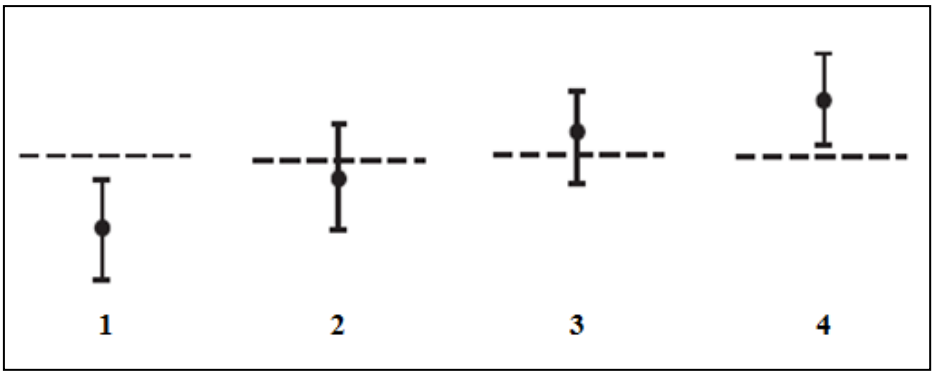

Figure 1: Examples of different compliance situations for indicative analyses: Situation 1: Compliance, the value and the uncertainty range are below the limit value. Situation 2: Possible compliance, the value is below the limit value, but the uncertainty range overlaps the limit value. Situation 3: Possible compliance, the value is above the limit value, but the uncertainty range overlaps the limit value. Situation 4: Non-compliance, the value and the uncertainty range are above the limit value.

Table 14: Final ranking of IMO target organism groups based on the analytical results

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Possibly compliant	Compliant		
Plankton ≥50µm	Possibly compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/100 ml		< 1,000 cfu/100 ml	

Table 15: Summary of PAM fluorometry, ATP and microscopy results

Sample ID	Chlorophyll (µg/l)	Yield	ATP Bacteria quantitative (cfu/100ml)	ATP Plankton >10µm <50µm (organisms/ml)	Plankton counts >10µm <50µm (organisms/ml)	ATP Plankton ≥50µm (organisms/m³)	Plankton counts ≥50µm (organisms/m³)
1	0.2	0	5,017,620	57	0	not contained[6]	
2	0.4	0	4,254,475	257	0		
3	0.4	0	8,077,201	298	0		
8	0.1	0	3,052,887	160	0		
13	0.4	0	5,274,288	90	0		
18	0.3	0	7,021,434	200	0		
23	0.1	0	1,041,465	9	0		
28	0.7	0	not contained[7]			16	0

[6] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[7] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

Table 16: Summary of APHA bacteria incubation analysis and FISH analysis

Sample ID	Bacteria Quantitative (APHA)	Escherichia coli (APHA)	Escherichia coli (FISH)	Enterococci (APHA)	Enterococci (FISH)	Vibrio cholerae (APHA)	Vibrio cholerae (FISH)
	cfu/100ml						
4	1	<1	0	<1	0	<1	0
5	48	<1		<1		<1	
6	5	<1		<1		<1	
7	3	<1		<1		<1	
9	2	<1	1	<1	0	<1	0
10	24	<1		<1		<1	
11	1	<1		<1		<1	
12	36	<1		<1		<1	
14	16	<1	2	<1	1	<1	0
15	13	<1		<1		<1	
16	36	<1		<1		<1	
17	44	<1		<1		<1	
19	68	<1	1	<1	0	<1	0

20	3	<1		<1		<1	
21	44	<1		<1		<1	
22	32	<1		<1		<1	
24	<1	<1	1	<1	1	<1	0
25	1	<1		<1		<1	
26	<1	<1		<1		<1	
27	<1	<1		<1		<1	

7. DISCUSSION

The indicative and detailed results for the ≥50 micron and the ≥10 but <50 micron plankton fractions did not match. The indicative ATP method provided a possibly compliant result for these fractions while the microscopic counts were compliant.

It should be kept in mind that ATP is an indicative method, designed to provide an indication of compliance (or an indication of gross non-compliance) which can then be verified by detailed analysis. No limit values for gross non-compliance have been determined as of yet.

There was a contamination in one of the reagents for the ATP analysis. Because of this the results for samples 2, 3, 8, 13, and 18 for the indicative ≥10 <50 micron analysis were not taken into the compliance calculation. Since this is only apparent when the calculations are made it went undetected on board ship. The analysis protocols have now been improved to prevent this occurring in the future.

The results of the APHA analyses for *Escherichia coli*, Enterococci and *Vibrio cholerae* closely matched the results of the FISH analyses for these same bacteria groups (Table 15). There was, however, a very large difference between the APHA analysis for total heterotropic bacteria and the ATP bacteria analysis. However, it is known that many marine bacteria cannot be cultured using traditional methods (Viable Non-Culturable, VNC). The percentage of culturable bacteria in seawater can be as little as 0.08%[8].

8. CONCLUSIONS

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
4. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.

[8] Ferguson et al. 1984 Response of marine bacterioplankton to differential filtration and confinement. Applied and Environmental Microbiology, Volume 47, page 49-55.

5. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

9. ANNEX

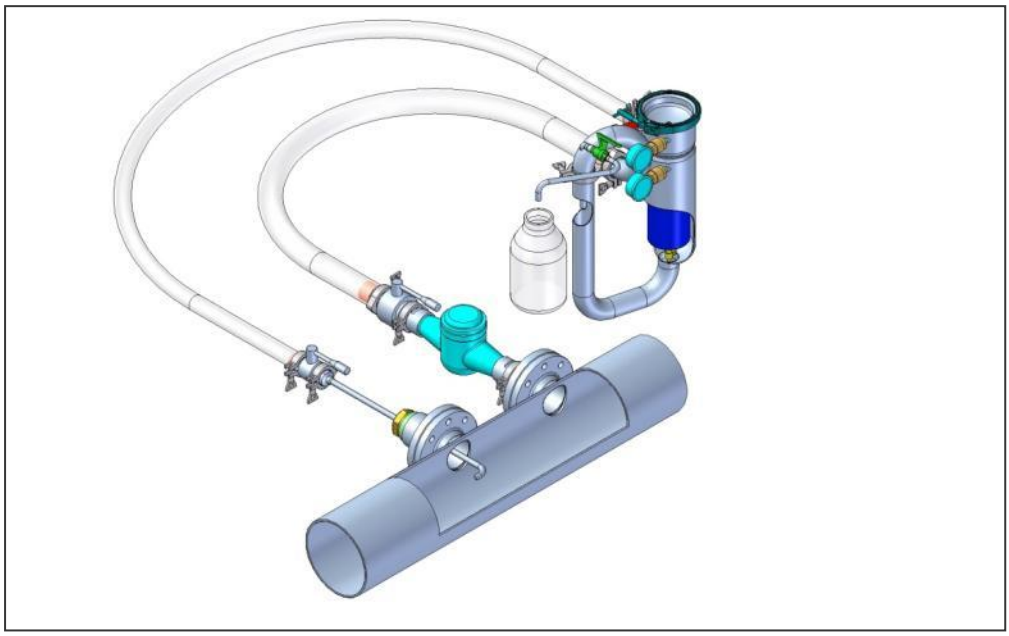

Figure 2: Schematic view of the SGS Ballast Water Sampler v02 (closed system variant)

Figure 3: The SGS Ballast Water Sampler v02

Figure 4: Sampling port on board the ship

Figure 5: Installation of SGS Ballast Water Sampler v02

BALLAST WATER REPORT

ON BOARD BALLAST WATER COMPLIANCE TEST

SGS

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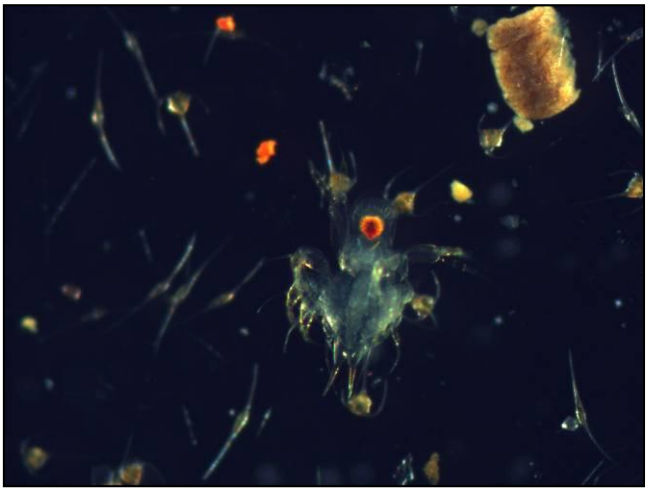

BALLAST WATER REPORT

SHIP 6

ON BOARD BALLAST WATER COMPLIANCE TEST

0814-00005 R0

10/09/2014

Prepared by

SGS Testing & Control Services Singapore Pte Ltd

Prepared for

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EXECUTIVE SUMMARY

The ballast water aboard the sixth ship tested, a Singapore registered 9,200 TEU ship built in 2013, fully complies with the D-2 Ballast Water Performance Standard as defined by the IMO. A short overview of the results is shown in the table below.

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Compliant	Compliant		
Plankton ≥50µm	Possibly compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/100 ml		< 1,000 cfu/100 ml	

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance.

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
4. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.
5. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

The paragraphs of this report present detailed information regarding the executed on board compliance test.

1. BACKGROUND

In July 2014 the Maritime and Port Authority of Singapore (MPA) charged SGS Testing & Control Services Singapore Pte Ltd (SGS) with the execution of a study of on board sampling and analysis of ballast water for compliance testing within the frame of the Ballast Water Management Convention (BWMC) of the International Maritime Organization (IMO) of the United Nations.

The compliance tests had to be executed on in total six ships indentified by MPA.

This report presents the test results of ship no. 6.

Table 01: Characteristics of the ship

Type	Container Ship
Capacity (TEU)	9,200
Year built	2013

2. REGULATORY FRAMEWORK FOR THE ANALYSIS OF BALLAST WATER

The sampling and analysis of the ballast water should follow, as far as feasible, the relevant regulations published by the International Maritime Organization – IMO within the frame of the Ballast Water Convention 2004:

- G2 : Guidelines for Ballast Water Sampling (G2), October 10th, 2008
  (source: MEPC 58/23, Annex 3, Resolution MEPC.173/58))
- G8 : Guidelines for Approval of Ballast Water Management Systems (G8), October 10th, 2008
  (source: MEPC 58/23, Annex 4, Resolution MEPC.174(58))
- G-PSC : Guidelines for Port State Control under the International Convention for the Control and Management of Ship's Ballast Water and Sediments April 10th, 2014 (source: III 1/8 IMO Sub-Committee on Implementation of IMO Instruments, 1st Session, Agenda Item 8, Annex 1, document page 7ff)
- BWM.2/Circ.42 : Guidance on ballast water sampling and analysis for trial use in accordance with the BWM Convention and Guidelines (G2)

3. PROJECT OBJECTIVE

The project objectives of this compliance test study are as follows:

- Assess the feasibility of indicative and detailed (in-depth) analysis of ballast water on board ships under the realistic conditions of time constraints during unloading and loading processes while the ships are berthed in harbors for just one day or even less.
- Allow the MPA to identify potential practical challenges that vessels may face when MPA will execute its Port State Control role when the convention comes into force.
- Address industry concerns on the performance of IMO type approved Ballast Water Management Systems under normal operating conditions.

4. ANALYTICAL FRAME

4.1 IMO TARGET ORGANISM SIZE CLASSES

In accordance with the IMO regulations and guidelines as listed in para 2 the ballast water onboard the ships was analyzed as to the organism concentrations of the three target organism size classes defined by the IMO:

- Marine plankton organisms with a size of >50µm
- Marine plankton organisms with a size between >10µm and <50µm
- Marine bacteria :
    - *Escherichia coli*
    - Enterococci
    - *Vibrio cholerae*

4.2 PERFORMANCE STANDARDS

The analysis of the ballast water aimed at the verification of compliance with the international performance standards for ballast water to be re-discharged from ships as defined by the IMO within the frame of the International Ballast Water Management Convention 2004:

Table 02: IMO target organism groups and performance standards

IMO Target Size Class	IMO Performance Standard
Plankton >50µm	<10 Individuals/m³
Plankton >10µm<50µm	<10 Individuals/ml
Escherichia coli	<250 cfu/100ml
Enterococci	<100 cfu/100ml
Vibrio cholerae	<1 cfu/100ml

(cfu : colony forming unit)

4.3 SAMPLING TECHNOLOGY USED

The ballast water samples were taken by the aid of the "*SGS Ballast Water Sampler v02*", which has been connected to the isokinetic sampling port installed in the ballast water pipe system of the ship (see figures 01, 02 and 04 in the annex).

4.4 ANALYTICAL METHODS USED

4.4.1 General Information

Marine organisms are divided in animals (zooplankton), plants (phytoplankton) and bacteria. The D-2 size-classes of ≥50 µm and ≥10 but <50 µm both contain phytoplankton and zooplankton, making it important to use methods that detect both types of organism.

Plankton organisms are sensitive and long transport times can affect viability, because of this reason all analysis were conducted immediately on board ship when possible.

4.4.2 Indicative Methods

Pulse Amplitude Modulation Fluorometry - PAM

The PAM method provides an indication for the presence of only vital phytoplankton in the ballast water sample by measuring the chlorophyll activity in the entire sample. Separate values for phytoplankton ≥50 µm and phytoplankton ≥10 but <50 µm are achieved by filtration of the sample. The PAM method does not allow for a numerical (number of vital marine microalgae/ml) indication. The parameters are concentration of Chlorophyll in the ballast water sample (µg chl/l) and yield (yld), which is expressed without a dimension.

The PAM analysis is executed on board the ship.

Adenosin-triphosphate Fluorometry - ATP

Adenosin-triphosphate is an unique energy donor in the physiological processes of all living cells (phytoplankton, zooplankton and bacteria). The ATP method provides the concentration of cellular ATP in the ballast water sample and – based on empirical data - transforms this concentration value (pg cATP/ml) into an indicative numerical concentration value of number of vital organisms. It can be used for organisms ≥50 µm, organisms ≥10 but <50 µm and bacteria. However, it only provides total bacteria numbers and no species identification.

The ATP analysis for all IMO target organism size classes is executed on board the ship.

4.4.3 Detailed (in-depth) Methods

IMO Target Organism Size Class Plankton

The concentration of vital plankton organisms in the ballast water samples was analysed per optical counts under the microscope using a Bogorov chamber for plankton organisms ≥50µm and a Sedgewick Rafter count cell for plankton organisms >10µm<50µm.

Three sub-samples of the main sample for plankton organisms ≥50µm were counted and 10 sub-samples of the main ample for plankton organisms >10µm<50µm were counted

The microscopic counts of the concentration of viable plankton organisms in the ballast water were executed on board the ship.

IMO Target Organism Size Class Bacteria

Since the detailed (in-depth) analytical methods for the detection of bacteria in ballast water samples, demand a complex laboratory infrastructure the cultivation of human pathogens, these methods can only be executed in land based laboratories.

Fluorescence-In-Situ-Hybridization Fluorescence Microscopy

This gene probe method analyses the concentration of the IMO target bacteria species *Escherichia coli*, Enterococci and *Vibrio cholera* in ballast water samples. The time needed from sample to result is approximately 11 hrs.

Classical 24/48 hrs. Incubation Methods

To assess the concentration of total bacteria in the ballast water samples the Heterotrophic Plate Count (HPC) method (APHA 9215) was used.

For the detection of the IMO target bacteria species different methods were used.

Table 03: 24/48 hrs. incubation methods used for the detection of bacteria

Bacteria quantitative :	APHA 9215
Escherichia coli :	APHA 9222G
Enterococci :	APHA 9230
Vibrio cholerae :	APHA 9260

Since there is no limit value for total heterotrophic bacteria, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.

4.5 EXECUTIVE PROTOCOL FOR ON BOARD COMPLIANCE TESTING

4.5.1 Basic Hydraulic Settings

The sampling of live plankton organisms from within the main ballast water pipes on board ships has to ensure, that these plankton organisms are not impacted by the sampling procedures.
Therefore the on board sampling and analysis was proceeded by a meeting with the relevant crew members of the ship to obtain the relevant basic, hydraulic information and to agree on the adequate pump capacity during sampling to exclude impacts on the live plankton organisms.

4.5.2 Establishment of working places

Two working places have been established on board the ship: (i) the *sampling* working place at the sampling port of the main ballast water pipe system and (ii) the analysis working place where the generated samples are further processed, analyzed, labeled and prepared for transport to the land based laboratory for bacterial analysis

4.5.3 Installation of the sampling system

The constructional conditions at the sampling port on the ship required to run the "*SGS Ballast Water Sampler v02*" as an open system: the ballast water filtered through the system was not flushed back to the ballast water main pipe system but directed into the bilge of the ship instead. The maximum volume of ballast water that was allowed to enter the bilge was set 2.0m³.

4.5.4 Flushing of the main ballast water pipe system

After the installation of the two working places was finished, the notice to start the de-ballasting procedure was given to the engineer in charge.

In order to avoid the contamination of the ballast water the main ballast water pipe system was flushed for 30 minutes.

4.5.5 Flushing of the sampling system

After the flushing of the main ballast water pipe system ended the sampling port valve was opened to direct ballast water through the "*SGS Ballast Water Sampler v02*". The sampling system was flushed with approximately 1.0m³ of ballast water. During this flushing procedure two ballast water samples were taken. These two samples were an additional requirement of MPA and do not form a part of the sampling and analysis for compliance testing.

4.5.6 Sampling of ballast water for compliance test

After the flushing of the sampling system ended, the system was activated to sample ballast water for compliance test. During this sampling procedure 7 ballast water samples of various volumes were taken at the sampling system and brought to the *analysis* workplace in the ship for further processing. Notice was given to the engineer in charge to stop the de-ballasting procedure.

4.5.7 Termination of on board compliance test

The ballast water on board compliance test ended with the de-installation of the *analysis* workplace after finalization of the indicative and detailed analysis of the ballast water samples.

5. RESULTS

5.1 BASIC HYDRAULIC INFORMATION

The basic hydraulic information is displayed in table 04 below.

Table 04: Basic hydraulic information for de-ballasting procedures

Parameter	Value	Dim.
Diameter of ballast water pipe	450	mm
Diameter of isokinetic pipe	25.4	mm
Maximum pump capacity	1,000	m^3/h
System pressure in ballast water pipe	5.13	bar
Maximum ballast water volume for de-ballasting	1600	m^3
Date of ballast water uptake	11.09.2014	date
Holding time of ballast water	2	days
Origin of ballast water	Indian Ocean	
Minimum possible pump capacity during de-ballasting	154	m^3/h
Average pump capacity during de-ballasting	190	m^3/h
Maximum allowable discharge volume into the bilge	2.0	m^3

5.2 TIME TABLE OF GENERAL ACTIVITIES

The table below presents the time lapse of general activities before, during and after the on board compliance test.

Table 05: Time table of general activities

Time of day	Activity
18:00	Departing from lab to port
18:45	Arrival at port's gate *(ship is delayed)*
19:40	Arrival at ship's berth
19:45	Unloading of equipment
19:50	Start interview with ship's crew
19:50	Start setting up working places (sampling and analysis)
20:15	End of setting up working places (sampling and analysis)
20:30	Start of flushing
21:18	End of flushing
21:43	Start of sampling
22:06	End of sampling
22:10	Removal of 50µm stainless steel filter
21:00	Start of analysis
00:15	End of analysis
00:15	Start of de-installation of working places
00:40	End of de-installation of working places
00:45	Alighting from ship
00:55	Loading of material
01:00	Departing from port
02:00	Arrival at Laboratory

5.3 COURSE OF DEBALLASTING PROCEDURE AND SAMPLE RECORDS

The table below presents the general information of the de-ballasting procedure.

Table 06 : General information of the de-ballasting procedures

Total duration of de-ballasting (hh:mm)	01:16
Total duration of sampling procedure (hh:mm)	00:23
Generated samples	28
Filtered volume of ballast water (m^3)	0.999

The list below displays the course of the de-ballasting procedures and the sample records.

Time | **Activity**

20:25 Start of de-ballasting, flush of main ballast water pipe system

20:30 Start of flushing the sampling system (without 50 µm filter installed)

21:00 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
21:05	1	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm[1]
21:10	2		

21:18 End of flushing the sampling system

21:43 **Start of sampling for compliance test** (with 50 µm filter installed)

21:49 Bypass port of sampling system: sample record

Time	ID	Volume (l)	Purpose
21:49	3	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	4 - 7	2.0	Bacterial analysis in land-based laboratory
21:52	8	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	9 - 12	2.0	Bacterial analysis in land-based laboratory
21:56	13	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	14 - 17	2.0	Bacterial analysis in land-based laboratory
21:59	18	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts

[1] Additional samples included in the sampling scheme by request of MPA

	19 - 22	2.0	Bacterial analysis in land-based laboratory
22:05	23	0.5	PAM, ATP bacteria, ATP plankton >10µm<50µm. microscopic counts
	24 - 27	2.0	Bacterial analysis in land-based laboratory

22:06 **End of sampling for compliance test**

22:10 End of de-ballasting

23:15 Steel screen filter of sampling system: sample record

Time	ID	Filtered Volume (m^3)	Purpose
23:15	28	0.999 m^3	PAM, ATP plankton ≥50µm, microscopic counts

5.4 ANALYTICAL RESULTS

5.4.1 Physical parameters

The table below lists the results from the physical analysis of the ballast water

Table 07: Physical parameters of the ballast water

Sample ID	Parameter	Value	Dim.
1	turbidity	< 5	NTU
2		< 5	
1	salinity	34.5	PSU
1	temperature	30.3	°C

5.4.2 Indicative analysis: Chlorophyll activity (PAM)

Table 08: Chlorophyll activity in the ballast water

Sample ID	Chl (µg/l)	Yld
1	0.8	0.09
2	0.2	0
3	0.5	0
8	0.4	0
13	0.5	0
18	0.2	0
23	0.4	0
28	1.2	0.08

The ranking of the values is displayed in table 09 below.

Table 09: Ranking of Chlorophyll values

Chlorophyll	Indications
< 0.1 µg/l	Low number of marine microalgae in the sample
>0.1 <1.0 µg/l	Medium number of marine microalgae in the sample
<1.0 µg/l	High number of marine microalgae in the sample
Yield	**Indications**
< 0.100	The sample contains marine microalgae on a very low vitality level
>0.100 <0.300	The sample contains marine microalgae on a medium vitality level
>0.300	The sample contains marine microalgae on a high vitality level

5.4.3 Indicative analysis: numerical values derived from ATP analysis

Table 10: Indicative concentrations of viable organisms in the ballast water

Sample ID	Bacteria quantitative (cfu/100ml)	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	361,801	3	not contained[2]
2	267,996	2	
3	401,513	5	
8	370,628	4	
13	362,906	9	
18	934,849	2	
23	331,521	1	
28	not contained[3]		9

[2] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[3] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

5.4.4 Detailed analysis: microscopic counts

Table 11: Concentrations of viable plankton organisms in the ballast water (detailed analysis)

Sample ID	Plankton >10µm <50µm (organisms/ml)	Plankton ≥50µm (organisms/m³)
1	0	not contained[4]
2	0	
3	0	
8	0	
13	0	
18	0	
23	0	
28	not contained[5]	0

5.4.5 Detailed analysis bacteria: classical 24/48 hrs incubation

Table 12: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Bacteria quantitative	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml			
4	<1	<1	<1	<1
5	<1	<1	<1	<1
6	<1	<1	<1	<1
7	<1	<1	<1	<1
9	<1	<1	<1	<1
10	<1	<1	<1	<1
11	<1	<1	<1	<1

[4] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[5] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

12	<1	<1	<1	<1
14	<1	<1	<1	<1
15	1	<1	<1	<1
16	<1	<1	<1	<1
17	<1	<1	<1	<1
19	<1	<1	<1	<1
20	<1	<1	<1	<1
21	<1	<1	<1	<1
22	<1	<1	<1	<1
24	<1	<1	<1	<1
25	<1	<1	<1	<1
26	<1	<1	<1	<1
27	<1	<1	<1	<1

5.4.6 Detailed analysis bacteria: FISH

Table 13: Concentrations of bacteria in the ballast water (detailed analysis)

Sample ID	Escherichia coli	Enterococci	Vibrio cholerae
	cfu/100ml		
4	3	0	0
9	0	2	0
14	4	1	0
19	3	0	0
24	2	0	0

6. RANKING OF ANALYTICAL RESULTS

For the IMO target groups "*bacteria*" and "*plankton >10µm<50µm*" several samples were taken. The average of these samples was calculated and uncertainty calculations were applied. These results were compared to the D-2 Ballast Water Performance Standard limit values to determine compliance or non-compliance, as shown in figure 1.

For the IMO target group "*plankton ≥50µm*" only one large volume sample was taken. Therefore statistical considerations were impossible. The respective statements are derived directly from the analytical results from this single sample.

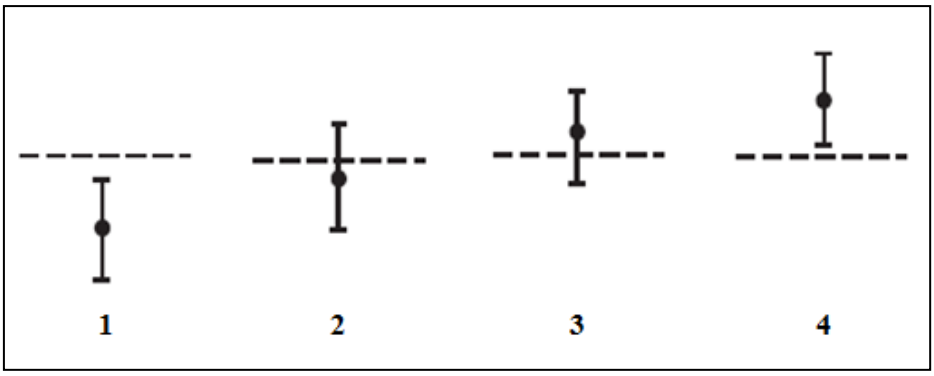

Figure 1: Examples of different compliance situations for indicative analyses: Situation 1: Compliance, the value and the uncertainty range are below the limit value. Situation 2: Possible compliance, the value is below the limit value, but the uncertainty range overlaps the limit value. Situation 3: Possible compliance, the value is above the limit value, but the uncertainty range overlaps the limit value. Situation 4: Non-compliance, the value and the uncertainty range are above the limit value.

Table 14: Final ranking of IMO target organism groups based on the analytical results

IMO Target Group	Indicative	Detailed		
	ATP Method	Microscopic Counts	APHA Methods	FISH Method
Plankton >10µm<50µm	Compliant	Compliant		
Plankton ≥50µm	Possibly compliant	Compliant		
Escherichia coli			Compliant	Compliant
Enterococci			Compliant	Compliant
Vibrio cholerae			Compliant	Compliant
Non IMO target group				
Bacteria quantitative	> 1,000 cfu/100 ml		< 1,000 cfu/100 ml	

Table 15: Summary of PAM fluorometry, ATP and microscopy results

Sample ID	Chlorophyll (µg/l)	Yield	ATP Bacteria quantitative (cfu/100ml)	ATP Plankton >10µm <50µm (organisms/ml)	Plankton counts >10µm <50µm (organisms/ml)	ATP Plankton ≥50µm (organisms/m³)	Plankton counts ≥50µm (organisms/m³)
1	0.8	0.09	361,801	3	0	not contained[6]	
2	0.2	0	267,996	2	0		
3	0.5	0	401,513	5	0		
8	0.4	0	370,628	4	0		
13	0.5	0	362,906	9	0		
18	0.2	0	934,849	2	0		
23	0.4	0	331,521	1	0		
28	1.2	0.08	not contained[7]			9	0

[6] These samples were taken behind the 50 µm filter and therefore do not contain organisms ≥50 µm

[7] These samples were taken from the 50 µm filter and therefore do not contain organisms <50 µm

Table 16: Summary of APHA bacteria incubation analysis and FISH analysis

Sample ID	Bacteria Quantitative (APHA)	Escherichia coli (APHA)	Escherichia coli (FISH)	Enterococci (APHA)	Enterococci (FISH)	Vibrio cholerae (APHA)	Vibrio cholerae (FISH)
	cfu/100ml						
4	<1	<1	3	<1	0	<1	0
5	<1	<1		<1		<1	
6	<1	<1		<1		<1	
7	<1	<1		<1		<1	
9	<1	<1	0	<1	2	<1	0
10	<1	<1		<1		<1	
11	<1	<1		<1		<1	
12	<1	<1		<1		<1	
14	<1	<1	4	<1	1	<1	0
15	1	<1		<1		<1	
16	<1	<1		<1		<1	
17	<1	<1		<1		<1	
19	<1	<1	3	<1	0	<1	0

20	<1	<1		<1		<1	
21	<1	<1		<1		<1	
22	<1	<1		<1		<1	
24	<1	<1	2	<1	0	<1	0
25	<1	<1		<1		<1	
26	<1	<1		<1		<1	
27	<1	<1		<1		<1	

7. DISCUSSION

The indicative and detailed results for the ≥50 micron plankton fraction did not match. The indicative ATP method provided a possibly compliant result for this fraction while the microscopic counts were compliant.

It should be kept in mind that ATP is an indicative method, designed to provide an indication of compliance (or an indication of gross non-compliance) which can then be verified by detailed analysis. No limit values for gross non-compliance have been determined as of yet.

The results of the APHA analyses for *Escherichia coli*, Enterococci and *Vibrio cholerae* closely matched the results of the FISH analyses for these same bacteria groups (Table 15). There was, however, a very large difference between the APHA analysis for total heterotropic bacteria and the ATP bacteria analysis. However, it is known that many marine bacteria cannot be cultured using traditional methods (Viable Non-Culturable, VNC). The percentage of culturable bacteria in seawater can be as little as 0.08%[8].

8. CONCLUSIONS

1. The ship was found to be compliant according to the detailed analysis methods.
2. There was a difference in compliance statement between the indicative and detailed methods. It is recommended to determine a threshold value above which detailed analysis will be initiated.
3. Since there is no limit value for total heterotrophic bacteria in the D-2 Ballast Water Performance Standard, the bacteria limit value from the California Standard of <1,000 cfu/100mL is used. Since this value is not part of the D-2 standard it can be exceeded without causing non-compliance.
4. It is recommended that the volume of sample to be sampled and the number of samples need to be defined for standardized application during PSC. Since average of all samples determines compliance or non-compliance. If only one sample is used then a vessel may be unduly penalised.
5. It is a challenge to dispose waste water generated after sampling. It is recommended to install an additional valve next to the G2 sampling valve for a closed loop sampling.

[8] Ferguson et al. 1984 Response of marine bacterioplankton to differential filtration and confinement. Applied and Environmental Microbiology, Volume 47, page 49-55.

9. ANNEX

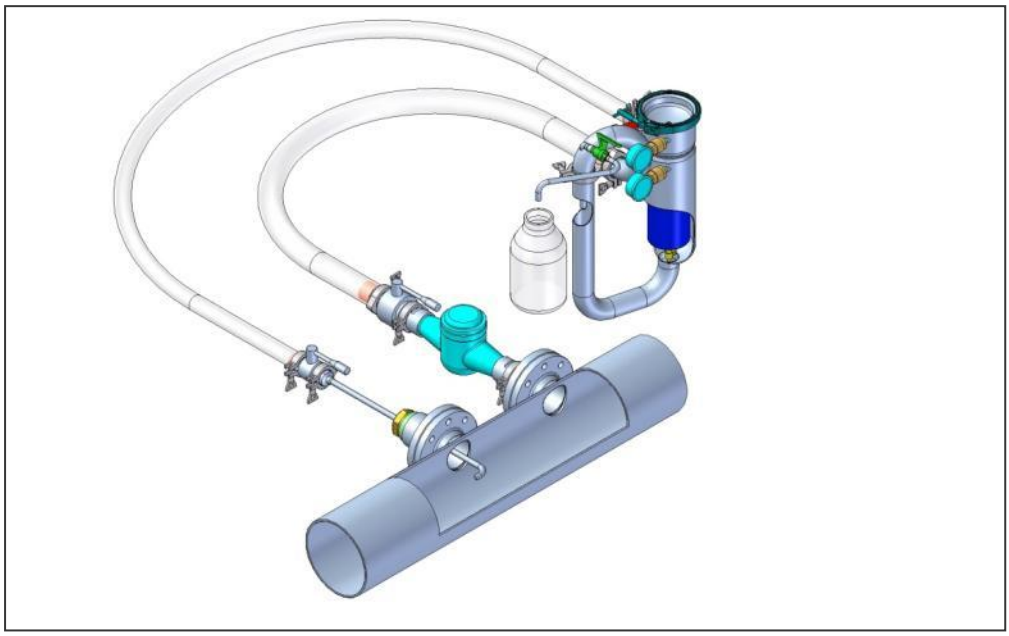

Figure 2: Schematic view of the SGS Ballast Water Sampler v02 (closed system variant)

Figure 3: The SGS Ballast Water Sampler v02

Figure 4: Installed SGS Ballast Water Sampler v02

Figure 5: Sampling using the SGS Ballast Water Sampler v02