GloBal TestNet Member's Methodology Comparison Charts
April 2018

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1. Background

1.1. Introduction

The present set of information has been generated from the information shared over the course of the GloBal TestNet successive annual meetings and was first compiled after the Annual meeting in Istanbul in 2012 (“the Istanbul paper”). Improvement of methodologies applied by members as well as the development of new regulations has promoted an increased transparency in the testing protocols and the present document has been prepared to be updated regularly by the GloBal TestNet members to reflect their testing approaches.

1.2. Documentation regulating testing of ballast water management systems

The testing carried out by the members is done according to the following documentation:

- Guidelines for Approval of Ballast Water Management Systems (G8). Res. MEPC.174(58) & MEPC.279(70)
- Procedure for Approval of Ballast Water Management Systems that Make Use of Active Substances (G9). Res. MEPC. 169(57)
- U.S. Coast Guard. Standards for Living Organisms in Ships’ Ballast Water Discharged in U.S. Waters. 33 CFR Part 151 and 46 CFR Part 162.
- U.S. Environmental Protection Agency, Environmental Technology Verification Program. Generic Protocol for the Verification of Ballast Water Treatment Technology. EPA/600/R-10/146.

Test facilities are listed in all tables in alphabetical order.

2. Water quality and preparation of challenge water during land-based testing

2.1. Water quality background at testing sites

Different facilities face different test water conditions (Table 1). The differences are affected by, e.g., climate, river runoffs, urban influence and impact of resource users. These differences ensure that testing is done under “world-wide conditions” but at the same time this is also a challenge regarding test result comparability.

Table 1: Summary of ambient water parameters in at different land-based test sites.

Test Facility	Temp (°C)	Salinity (PSU)	TSS (mg l^{-1})	POC (mg l^{-1})	DOC (mg l^{-1})	Organisms ≥ 50 µm m^{-3}	Organisms < 50 µm and ≥ 10 µm ml^{-1}	Bacteria ml^{-1}
DHI (DK)	Variable	0 – 33	variable	> 5	> 10	variable	variable	variable
DHI (SG)	28 – 31	<0.3 - 32.2	1.6 – 54	variable	1 - 9	30,000 to 500,000	10 to 300	10^4 - 10^7
GBF (USA)	9-22	0-28	20-100+	0.5-2	2-10	25,000-1,000,000+	100-1,300	> 1,000
GSI (USA)	9 – 22	0 – 1	2 – 21	< 1	6 – 22	100,000 - 3,000,000	25 - 1,200	> 1,000
KIOST (RoK)	2.58 – 30.3	18.5 - 34.0	6.00 – 51.2	0.3 – 9.21	0.3 – 4.49	326 – 663,246	36 – 11,340	0.2 – 12.7 x 10^6
KOMERI[1] (RoK)	4 - 28	0 - 33	Variable	Variable	Variable	1,000 - 500,000	100 - 3,000	40 - 14,634 1,000 - 800,000
MBRIJ (Japan)	9 – 23	31 – 34	1 – 10	<0.1 – 2	1 – 2	10,000 – 300,000	<1 – 200	10,000 – 500,000
MEA-nl (NL)	<1 – 25	<1 – 35	15 – 600	1 - > 6	2 - >6	10,000 – 400,000	500 – 4,000	105 - 107

[1] For the test, water quality used by KOMERI have a wide range. The challenge water is used the natural seawater and fresh waters. The seawater is directly supplied in a nearby sea using the pump, and natural fresh water is indirectly supplied in a nearby river using a tank lorry. For the challenge water, natural viable or living organisms is collected by mechanical concentration method.

Test Facility	Temp (°C)	Salinity (PSU)	TSS (mg l^{-1})	POC (mg l^{-1})	DOC (mg l^{-1})	Organisms ≥ 50 µm m^{-3}	Organisms < 50 µm and ≥ 10 µm ml^{-1}	Bacteria ml^{-1}
MERC[2] (USA)	4 – 30	0 – 28	3 – 60	1 – 8	1 - 8	80,000 – 1,000,000	500 - 30,000	10,000 - 10,000,000
MRDTC (Japan)	8 – 25	31 – 34	5 – 11	<0.1 – 1.7	1.0 – 1.5	5.8 x 103 – 5.3 x 105	variable	variable
NIOZ (NL)	Variable	20 - 34	5 – 400	5 – 20	1 – 5	10,000 – 1,000,000	100 – 100,000	10,000 – 10,000,000
NIVA (Norway)	4 – 25	0 – 34	1-10	1-3	1-3	50,000 – 300,000	500 – 4,000	>10^3
NRL (USA)	20 – 32	35 – 41	1 – 5 (MM)	2 – 4	2 – 4	50,000 – 180,000	ca. 10 – 200	105 - 107
SWBWTCS (China)	16 – 22	32 – 33	1 – 5	ca. 5	ca. 2	standard met	50 % of standard	standard met
WMR[3] (NL)	0-25	0-20 (34)	1-50 (MM)	2-6	6-15	10^5 - 10^6	600-4000	variable

[2] Three different locations, within the Cheasapeake Bay, with distinct natural biological communities.

[3] Wageningen Marine Research = WMR, formerly known as IMARES.

2.2. Adjustment of water quality parameters during testing

IMO and USCG require certain water conditions to challenge ballast water management systems. Some conditions need to be manipulated to meet these requirements. **Error! Reference source not found.** shows what test facilities do to meet the challenge water conditions.

Table 2: Additives used and methodologies used for challenge water

Test facility	Manipulation of water parameters	Use of Standard Test Organisms (STO)	% of STO	Test tank mixing during hold time	Test soup application
DHI (DK)	+	*Artemia* sp, *Tetraselmis* sp	Up to 90%	+	1000 l and injected
DHI (SG)	Ligno Sulfonate, Sodium Citrate, Corn starch, Kaolin clay	*Artemia* sp, *Tetraselmis suecica*, *Odontella* sp, *Micractinium* sp. and *Chlamydomonas* sp.	0 to 95 %	Bubbling	In tank and injected
GBF	Ligno Sulfonate, Sodium Citrate, Corn starch, Kaolin clay	Ambient phytoplankton grow-out. Ambient zooplankton concentration. Various species.	Up to 90% phytoplankton (ambient organisms). Typically 0% zooplankton.	Air lift in source tank	Organisms mixed into source tank. Water quality 1,000 l injection into uptake water.
GSI	+	+	Up to 90 %	+	Injection separate per organisms and water parameters
Japan	+	*Artemia* sp, *Tetraselmis* sp	Up to 100%	+	500 l, 1 x zoos, 1 x phyto
KIOST	Glucose, Starch, Silica	*Artemia* sp, *Tetraselmis* sp	50 to 90%	+	230 m^3 and/or 430 m^3, i.e. used directly for test (runs tests sequentially)
KOMERI	Carbon	*No. We used only Natural organism*	0	Agitation and/or bubbling	Depends on natural condition (normally 3-30 m^3)
MBRIJ Japan	Carbon, TSS	Brachionus rotundiformis,Tetraselmis sp. Synchaeta sp. Rotaria sp. and Scenedesmus sp.	Up to 90 %	Bubbling	1000 l injected

Test facility	Manipulation of water parameters	Use of Standard Test Organisms (STO)	% of STO	Test tank mixing during hold time	Test soup application
MEA-nl	Carbon lignin/citric acid	Artemia sp (incidentally)	Occasionally up to 20%, otherwise none	+	Injection
MERC	+	-	-	+ optional	1000 l injected
NIOZ	+ (only TSS)	-	-	+	500 l, injection prior treatment system
NIVA	Lignine Sulfonate, Sodium Citrate, Corn starch, Kaolin clay	*Artemia* sp, *Tetraselmis* sp *Chlamydomonas* sp.	Up to 50%	+	500-800 m^3, i.e. used directly for test
WMR	TSS (MM), Salinity above 20 psu	Natural (local) communities	Up to 100 % (usually zero)	+	Feed Tank >700 m^3, only TSS injected

3. Sampling procedures

Table 3: Sampling details, land-based tests for the discharge of treated water, organisms above 50 micron in minimum dimension.

Facility	Sampling point location	OET or sequences	Sample port	Volume	Duration sample collection	Method	Concentrated sample volume	Second concentrated sample volume	% of sample volume analysed	Max time concentrated sample storage	Time end of collection to end of analysis
DHI (DK)	In-line										
DHI (SG)	In-line	3 replicates, continuous	G2-isokinetic, Pitot-tube, 3 sampling port	> 1000 L (3 samples in parallel)	30 - 60 min	Nets 35µm	1000 ml		100 % (discharge)	<2h	< 6 hours
GBF	In-line	OET	G2-isokinetic, Pitot-tube, 1 sampling point, use flow splitter for 3 parallel samples	> 1000 L (3 samples)	Ca 2 hours		400 ml	60 ml	100	< 1.5 hours	< 1.5 hours
GSI	In-line	OET	G2-isokinetic, Pitot-tube, 2/3 sampling point	> 3000 L (3 samples)	Up to 1 hour		1000 ml	Organism density dependent (or dilution)	100 % or counts at least xx orgs	< 2 hours	< 2 hours
Japan	In-line	3 sequences	G2-isokinetic, Pitot-tube, 3 sampling point	> 1000 L (for each sequence)	> 1 hour		> 500 ml	-	100	5 mins	< 6 hours
KIOST	In-line	OET divided in 3, or continuous sequences	G2-isokinetic, Pitot-tube, 1 sampling point	> 1000 L (3 samples) or max. 7ton	Ca. 1 hour	35 ㎛ (diagonal size), 1L container	1000 ml	100 ml	100	< 2 hours	< 2 hours

Facility	Sampling point location	OET or sequences	Sample port	Volume	Duration sample collection	Method	Concentrated sample volume	Second concentrated sample volume	% of sample volume analysed	Max time concentrated sample storage	Time end of collection to end of analysis
KOMERI	In-line	Continuous	G2-isokinetic, Pitot-tube, one sampling point	10,000 to 15,000 L [1,000 L, 3 samples are also available]	Ca. 1 hour	30 µm (diagonal)	≥ 3,000 ml [1,000 ml, 3 samples are also available]	1,000 ml (final) [20-100 ml is also avalable]	According to EPA ETV Protocol[4] [100]	< 2 hours	< 2 hours
MBRIJ Japan	In-line	OET divided in 3 continuous sequences	G2-isokinetic, Pitot-tube, 3 sampling point	> 1000 L (for each sequence)	> 1 hour		> 500 ml	10-50 ml	100	< 30 mins	< 6 hours
MEA-nl	In-line	3 continuous sequences	G2-isokinetic, Pitot-tube, 1 sampling point	> 3000 L as >3 * >1000 L time-integrated samples	Whole treatment time period		< 500 ml	Organism density dependent (or dilution)	100 % for treated	< 6 hours	< 6 hours
MERC	In-line	OET consistent with ETV	G2-isokinetic, Pitot-tube, 1 sampling point	> 7000 L for treated discharge and 3000 L for others	1 to 2 hours depending on flow rate				100	< 2 hours	
NIOZ	In-line	OET divided in 3 continuous sequences	G2-isokinetic, Pitot-tube, 1 sampling point	> 1000 L (for each sequence)	Ca. 1 hour		250-750 ml	-	100	2-4 hours	< 6 hours
NIVA	In-line	>3 continuous sequences	G2-isokinetic, Pitot-tube, 2 sampling	> 1000 L (3 consecutiv	>3x 6-15 mins per sample	Nets 35µm in 1m3	100 ml	-	100	5 mins	< 2-6 hours

[4] US EPA ETV Protocol, Generic protocol for the verification of ballast water treatment technology (EPA/600/R-10/146, September 2010)

Facility	Sampling point location	OET or sequences	Sample port	Volume	Duration sample collection	Method	Concentrated sample volume	Second concentrated sample volume	% of sample volume analysed	Max time concentrated sample storage	Time end of collection to end of analysis
			ports	e samples)		sampling tanks					
NRL	In-line	OET	G2-isokinetic, Pitot-tube, 1 sampling point	> 1000 L (5 to 10 m³)	Ca. 1 hour		1000 ml	500 ml	100	< 6 hours	< 5 hours
WMR	In-line	OET divided in 3 continuous sequences	G2-isokinetic, Pitot-tube, 1 sampling point	3x1000 L	Ca. 50 mins	Collect 1000 L sample, sieved (50µm) and concentrated	3x200 ml (sample storage)	-	100	< 6 hours	< 6 hours

Table 4: Sampling details, ship-board tests for the discharge of treated water, organisms above 50 micron in minimum dimension.

Facility	Sampling point location	OET or sequences	Volume	Duration sample collection	Concentrated sample volume	Second concentrated sample volume	% of sample volume analysed	Max time concentrated sample storage	Time end of collection to end of analysis	Flowmeter	Method details
David Consult (Slovenia)	In-line	(OET or) 3 sequences	> 1000 L (3 OET samples in parallel or 1 sequence in each beginning, middle and end)	Dependent on vessel specifics , typically 30 mins to 1 hour	250 ml	100 ml	20-100	15-60 mins	< 6 hours	Flowmeter capacity 20-200 L/min	50 µm mesh (diagonal dimension) in a sampling bin of ca. 200 L capacity
DHI (DK)	In-line	OET	> 1000 L (3 samples)	Dependent on vessel specifics	1000 ml		100	2 hours	< 6 hours		
DHI (SG)	In-line	3 replicates, continuous	> 1000 L (3 samples)	Dependent on vessel specifics	1000 ml		100	<2 hours	< 6 hours	yes	Plankton nets 35 µm
GBF	In-line	OET	> 1000 L (3 samples)	Ca 2 hours	400 ml	60 ml	100	< 1.5 hours	< 1.5 hours		
GoConsult (Germany)	In-line	(OET or) 3 sequences	> 1000 L (3 OET samples in parallel or 1 sequence in each beginning, middle and end)	Dependent on vessel specifics , typically 30 mins to 1 hour	250 ml	100 ml	20-100	15-60 mins	< 6 hours	Flowmeter capacity 20-200 L/min	50 µm mesh (diagonal dimension) in a sampling bin of ca. 200 L capacity
GSI	In-line	Pending	> 1000 L (3 samples)	Dependent on vessel specifics	1000 ml	Organism density dependent	100 % or counts at least xx	< 2 hours	< 2 hours		

Facility	Sampling point location	OET or sequences	Volume	Duration sample collection	Concentrated sample volume	Second concentrated sample volume	% of sample volume analysed	Max time concentrated sample storage	Time end of collection to end of analysis	Flowmeter	Method details
						(or dilution)	orgs				
Japan	In-line	3 sequences	> 1000 L (9 samples)	Dependent on vessel specifics , typically 20 min	> 500 ml	-	100	Ca. 15 mins	< 6 hours		
KE,Japan	In-line	Beginning, middle, end	>1000L (9 samples)	Dependent on vessel specifics, typically 10 to 30 min	<1000ml	100ml	100	<30mins	< 6 hours		
KIOST	In-line	Beginning, middle, end, uncontinuous	> 1000 L (9 samples)	Dependent on vessel specifics, typically ca. 1 hour	1000 ml	100 ml	100	< 1 hour	< 6 hour		
KOMERI	In-line	Beginning, middle, end, semi-continouos	3,000 L (3 samples) [1,000 L x 9 samples]	Dependent on vessel specifics, typically ca. 1 hour	1000 ml	20 - 100 ml	100	< 1 hour	< 5 hours		
MBRIJ	In-line	3 sequences	> 1000 L (9 samples)	Dependent on vessel specifics , typically 4 to 10 min	> 500 ml	50 ml	100	< 30 mins	< 6 hours	Flowmeter capacity 20-250 L/min	Used of 50 µm mesh (diagonal dimension) net in a sampling plastic buckets (70 L capacity).
MEA-nl	In-line	3 sequences (OET)	> 3000 L (3 samples)	Depends on ship. Min 1 hour	< 500 mL		100 % for treated	< 6 hours	< 6 hours		50 µm mesh (diagonal dimension) in a

Facility	Sampling point location	OET or sequences	Volume	Duration sample collection	Concentrated sample volume	Second concentrated sample volume	% of sample volume analysed	Max time concentrated sample storage	Time end of collection to end of analysis	Flowmeter	Method details
											sampling bin
MERC	In-line	OET	1000 to 3000 ml	Dependent on vessel specifics			100	< 2 hours	< 6 hours		
NIOZ	No ship-board tests										
NIVA	In-line	> 3 successive continuous sequences	> 1000 L (>3-9 samples)	Dependent on vessel specifics typically 10 mins per sequence	100 ml	-	100	<2 hours	< 6 hours	flow measurement with or without flowmeter	Plankton nets 35 µm
NRL	No ship-board tests										
PML Applications	In-Line	OET	>1000L broken into 10 minute sub-samples	Dependent on vessel	100ml	Organism density dependent (or dilution)	100	<2 hours	<2 hours	Flowmeter capacity 20-200 L/min	50 µm mesh (diagonal dimension) in a sampling bin of ca. 100 L capacity
WMR	No ship-board tests										

4. Plankton analyses for land-based and ship-board testing

4.1. Methods for counting and assessing viability

Table **5: Methods for counting organisms and viability assessment.**

Test facility	Greater than 50 µm	10 – 50 µm Concentration (C) No Concentration (NC)	Counts	Resting stages
David Consult	Organism movement, organism integrity, poking	(C and preferably NC) FDA/CMFDA stain	Epi-fluorescence microscopy	Rarely encountered, if found numbers noted
DHI (DK)	Movement, poking	(NC) FDA/CMFDA, MPN and Lugol's samples	Microscope Fluorescence for MPN	Counted, viability assessment not always possible
DHI (SG)	Movement, poking	(NC) FDA/CMFDA, MPN and Lugol's samples	Microscope Fluorescence for MPN	Rarely encountered Not counted
GBF	Movement, poking	(NC) FDA/CMFDA, MPN and Lugol's samples, other corroborative assays (e.g. flow cytometry, PAM, BWI)	Microscope Fluorescence for MPN	Rarely encountered Not counted
GoConsult	Organism movement, organism integrity, poking	(C and preferably NC) FDA/CMFDA stain	Epi-fluorescence microscopy	Rarely encountered, if found numbers noted
GSI	Response to stimulus (poking, light), Lugol's preserved samples	(C) FDA stain, Lugol's preserved samples	Microscope	No viability assessment, counted
Japan	Organism movement, organism integrity, poking	(C) Organism movement, organism intergrity	Microscope	Incubation (not used yet)
KE,Japan	Movement with poking	(C and NC) Cell integrity, Organism movement	Microscope	Incubation with light for a few days
KIOST	Organism integrity, stain (Neutral Red), poking	(C and NC) Growth experiments, FDA stain, FDA + CMFDA-stain, organism movement	Microscope	Not looked at
KOMERI	Organism integrity, stain (Neutral Red), poking. Direct count and judge as	(C and NC) Growth experiments, FDA/CMFDA-stain, organism movement, MPN.	Microscope, Fluorescent microscope	Not looked at, All egg stages are counted as living cells

Test facility	Greater than 50 µm	10 – 50 µm Concentration (C) No Concentration (NC)	Counts	Resting stages
	living cell for all egg stage			
MBRIJ	Movement, stain (Neutral Red)	(C and NC) stain (Neutral Red or FDA/CMFDA), Movement, Growth experiments (MPN)	Nomal-microscope and Fluorescence-microscope	Incubation(Zooplankton eggs and Phytoplankton cyst)
MEA-nl	Organism movement, organism integrity, poking	(C and NC) Stain (C, live/dead stains, i.e. (CM)FDA), photosynthetic efficiency/ biomass (phytoplankton NC), direct counts phytoplankton (flow cytometry) Regrowth and MPN (NC)) 4 * concentrated; for PAM automated unconcentrated	Epi-fluorescence microscopy, flow cytometry Active fluorometry (phytoplankton), Standard Microscopy	rarely encountered, will be visible during regrowth
MERC	Movement with poking (ETV)	(C and NC) FDA+CMFDA stain (ETV), fixed (Lugol's) samples for archiving samples, QA/QC	Microscope	Optional - extended observations for recovery and movement, egg hatching, and FDA+CMFDA staining for large diatoms
NIOZ	Neutral red, chloroplast, integrity of cell, poking	(C and NC) Stain (live/dead stains, i.e. Sytox Green), photosynthetic activity, minimum theoretical number (20 day experiment)	Automatic counting equipment	Incubation
NIVA	Movement, poking	(NC) FDA/CMFDA, MPN	Microscope	(Not seen any)
PML Applications	Organism movement, organism integrity, poking	FDA/CMFDA stain, fixed (Lugol's) samples for archiving samples (NC)	Std Microscopy & Epi-fluorescence microscopy	Rarely encountered, if found numbers noted
WMR	Movement, cell integrity, poking (recovery)	(NC) cell integrity, stain, MPN	Microscope	Incubation with light for a few days

4.2. Sizing of organisms

There are two fundamentally different principles to identify the minimum dimension. One way to do this is to measure the maximum width of the smallest visible axis of the organism excluding cilia, spikes and appendages. In the other approach the smallest dimension of the smallest visible axis is measured.

For organisms forming chains and colonies, single cells are measured and counted.

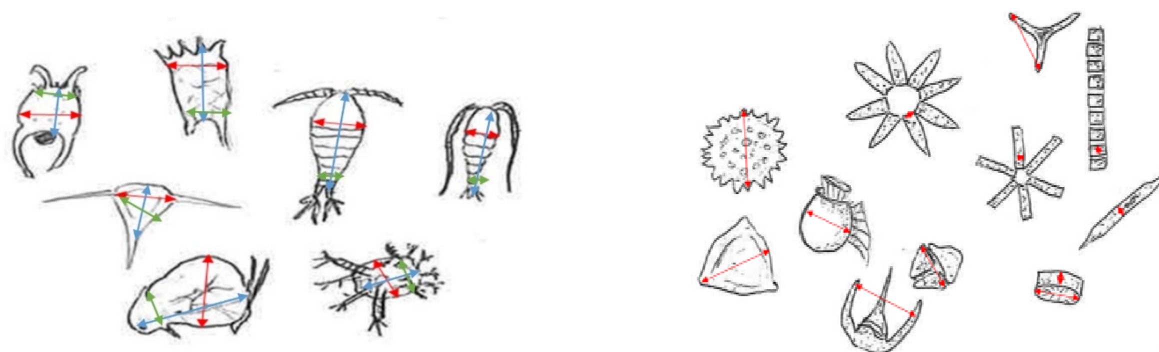

Figure 1: Examples how to measure the organism size. Red line maximum "body" dimension on smallest axis, green line minimum "body" dimension on the smallest axis and blue line maximum dimension in length of the organism.

Table 6: Method used to measure minimum dimension.

Test facility	Minimum dimension measurement		Test facility	Minimum dimension measurement
David Consult	Minimum size on the smallest visible axis		KOMERI	Minimum dimension of main body on the latitudinal axis. Transapical (main body) axis width
DHI (DK)			MBRIJ	Minimum size on the smallest visible axis
DHI (SG)	Maximum size on the smallest visible axis		MEA-nl	Measuring size and size fractionation, flow cytometry
GBF	Maximum size on the smallest visible axis		MERC	Maximum size on the smallest visible axis
GoConsult	Minimum size on the smallest visible axis		NIOZ	
GSI	Maximum size on the smallest visible axis		NIVA	Minimum size on the smallest visible axis
Japan			PML Applications	Minimum size on the smallest visible axis
KIOST	Minimum size on the smallest visible axis		WMR	Maximum size on the smallest visible axis