



**Global TestNet Note:** Revising the Protist Challenge Condition Size Class from  $\geq 10$  and  $< 50$   $\mu\text{m}$  to  $\geq 3$  and  $< 50$   $\mu\text{m}$  in Minimum Dimension for Purposes of Certification Testing

## The Issue

**Global TestNet finds that formulating certification test challenge conditions around the limited ballast water performance standard (BWPS) size classes unproductively diminishes test representativeness, comparability and power. The BWPS does not adequately encompass the range of likely biological challenges that the ballast water management systems (BWMS) could confront (especially in fresh water), and in which it would need to achieve the BWPS in practice.**

## Background

International Maritime Organization (IMO) Type Approval tests of BWMSs in the land-based context are intended to:

- “...provide a uniform interpretation and application of the requirements of regulation D-3 and to:
1. define test and performance requirements for the approval of BWMSs;
  2. assist Administrations in determining appropriate design, construction and operational parameters necessary for the approval of ballast water management systems;
  3. provide guidance to Administrations, equipment manufacturers and ship owners in determining the suitability of equipment to meet the requirements of the Convention and of the environmental acceptability of treated water; and
  4. assure that BWMSs approved by Administrations are capable of achieving the standard of regulation D-2 in land-based and shipboard evaluations and do not cause unacceptable harm to the ship, crew, the environment or public health.” IMO G8 Guidelines (2016).”

United States Coast Guard (USCG) Certification tests of BWMSs in the land-based context are intended to:

“... provide controlled conditions for verifying treatment performance. Land-based BWMS verification testing will be conducted in a manner providing information that is comparable to the maximum practical extent, to ensure that consumers and other stakeholders can make informed choices in selecting appropriate ballast water treatment technology for shipboard installations.” ETV Protocol (2010).”

To serve their purpose, Land-Based BWMS Certification Test challenge conditions must be representative of difficult, if not extreme, natural challenges that could be confronted (other than rarely) by the BWMS in routine use (Hunt et al., 2005). The goal is to prospectively assess BWMS capacity to perform to the BWPS in routine operation globally.

Accordingly, careful selection and application of Land-Based BWMS Certification Test challenge conditions are crucial to assuring relevancy of test outcomes to real-world BWMS performance.

Unfortunately the required biological Intake Challenge Conditions for BWMS Certification Tests are currently defined strictly in terms of the organism categories contained in the regulatory BWPS (Table I—IMO and USCG biological challenge conditions). Specifically, minimum requirements around biological challenge conditions associated with plankton are defined in terms of three discrete size classes of organisms, i.e.,

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Organisms  $\geq 50 \mu\text{m}$ ,  $\geq 10 \mu\text{m}$  to  $< 50 \mu\text{m}$  and  $< 10 \mu\text{m}$ .

In particular, protists  $\geq 3$  and  $< 10 \mu\text{m}$  in minimum dimension are left out of the BWPS, though protist species in this size range include destructive invaders (e.g. microcystis, among others), and can significantly challenge BWMS performance against the BWPS. Though it may be acceptable for these smaller protists to be outside the BWPS and associated compliance monitoring on the assumption that the BWMS will treat them effectively along with regulated size classes, the same logic cannot be applied to Certification challenge conditions, and doing so has not been validated. An absence of information on these sub-sized protists therefore creates a wildcard in BWMS performance assessments via IMO/USCG Type Approval/Certification tests.

Further, limiting the intake challenge condition requirement to the regulatory size class for protist cells also unproductively constrains the ability of test facilities to meet challenge requirements for the protist size class using natural assemblages. The constraint is unproductive because protist cell size by itself is typically not a relevant limiting factor to BWMS process effectiveness. Free-standing cells often pass through filter systems in any case and must be inactivated by secondary treatment in any case. Colonies, irrespective of cells size challenge filtration, irrespective of individual cell size. The requirement of challenge associated with a fraction of the overall protist challenge simply necessitates artificial manipulations of intake water, including reliance on addition of Standard Test Organisms, by TFs. Artificial manipulation of intake challenge biota is known to detract from test representativeness.

### Global TestNet Proposal

Protists  $\geq 3$  and  $< 10 \mu\text{m}$  in minimum dimension are important and readily measured parts of the natural biological load confronting BWMS operation and should be included and accounted for in the required biological challenge conditions for BWMS in Certification/Type Approval Tests. A requirement of a lower number of live cells actually within the regulated size class (e.g., 100/mL, as per the current Control Discharge requirement\*) in the intake stream is sufficient to support a valid context in which to evaluate capacity of the treated discharge to meet the BWPS. As long as this requirement and the Control Discharge requirement relative to the regulated size class are met, the intake challenge can and should be assured through a broader range of protist cell sizes.

Compliance monitoring specifically against the BWPS size classes is an appropriate approach to test BWMS functionality but we feel that certification testing strictly in terms of BWPS size classes is counterproductive. Global TestNet urges the IMO and USCG to reconsider the required challenge conditions for protists. The challenge conditions associated with this organism category should be revised to 1,000 live cells  $\geq 3$  microns and  $< 50$  microns in minimum visible dimension, including  $> 100$  live cells  $\geq 10$  microns in minimum dimension on uptake\*. The requirement for  $> 100$  live cells  $\geq 10$  microns in minimum dimension in Control Discharge should remain in place.

### References:

Hunt CD, Tanis DC, Stevens TG, Frederick RM & Everett RA (2005). Verifying Ballast Water Treatment Performance. Environmental Science & Technology; August 1 2005: 321 – 328. <https://pubs.acs.org/doi/pdf/10.1021/es0533141>; accessed 12 April 2018.

International Maritime Organization (2016). Guidelines for Approval of Ballast Water Management Systems (G8), Resolution MEPC.279(70). Adopted on 28 October 2016.

USEPA (2010). Generic Protocol for the Verification of Ballast Water Treatment Technology. Version 5.1. EPA/600/R-10/146. Produced for the USEPA, Environmental Technology Verification Program in Conjunction with U.S. Coast Guard, Environmental Standards Division and U.S. Naval Research Laboratory, Center for Corrosion Science and Engineering. NSF International, Ann Arbor, Michigan.

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**Table 1:** Comparison of Key Land-Based Test Parameters; IMO vs ETV

Parameter	Subcategory	IMO G8	ETV Generic Protocol
Organisms to be Evaluated	Organisms $\geq 50 \mu\text{m}$	Naturally occurring, or cultured species that may be added to the test water.	Ambient assemblage supplemented by the addition of organisms.
	Organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Naturally occurring, or cultured species that may be added to the test water.	Ambient assemblage supplemented by the addition of organisms.
	Organisms $< 10 \mu\text{m}$	Naturally occurring, or cultured species that may be added to the test water.	Ambient assemblage supplemented by the addition of organisms.
Intake Organism Diversity & Density	Organisms $\geq 50 \mu\text{m}$	Total density of preferably $10^6$ individuals but not less than $10^5$ individuals per $\text{m}^3$ ; at least 5 species from at least 3 different phyla/divisions.	Minimum of $10^5$ organisms/ $\text{m}^3$ with at least 5 species present across 3 phyla.
	Organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Total density of preferably $10^4$ individuals but not less than $10^3$ individuals per mL; at least 5 species from at least 3 different phyla/divisions.	Minimum of $10^3$ organisms/mL with at least 5 species present across 3 phyla.
	Organisms $< 10 \mu\text{m}$	Heterotrophic bacteria should be present in a density of at least $10^4$ living bacteria per mL.	Minimum concentration of $10^3$ /mL as culturable aerobic heterotrophic bacteria.
Water Quality of Intake/Source Water	Salinity	$< 3$ PSU 3 - 32 PSU $> 32$ PSU	Fresh: $< 1$ PSU Brackish: 10-20 PSU Salt: 28-36 PSU
	Temperature	N/A	4 – 35 °C.
	Dissolved Organic Matter	$< 3$ PSU: $> 5$ mg/L as DOC 3 - 32 PSU: $> 5$ mg/L as DOC $> 32$ PSU: $> 1$ mg/L as DOC	$\geq 6$ mg/L as DOC
	Particulate Organic Matter	$< 3$ PSU: $> 5$ mg/L as POC 3 - 32 PSU: $> 5$ mg/L as POC $> 32$ PSU: $> 1$ mg/L as POC	$\geq 4$ mg/L as POC
	Mineral Matter	N/A	$\geq 20$ mg/L
	Total Suspended Solids	$< 3$ PSU: $> 50$ mg/L 3 - 32 PSU: $> 50$ mg/L $> 32$ PSU: $> 1$ mg/L	$\geq 24$ mg/L
Discharge Organism Density	Organisms $\geq 50 \mu\text{m}$	Less than 10 viable organisms per $\text{m}^3$ greater than or equal to $50 \mu\text{m}$ in minimum dimension for treated water; more than 100 viable organisms per $\text{m}^3$ greater than or equal to $50 \mu\text{m}$ in minimum dimension for control water.	Treatment efficacy will be determined by the measurement of living ambient organism concentrations in the treatment discharge. Minimum concentration in control tank discharge is 100 live organisms/ $\text{m}^3$ .
	Organisms $\geq 10 \mu\text{m}$ and $< 50 \mu\text{m}$	Less than 10 viable organisms per mL less than $50 \mu\text{m}$ in minimum dimension and greater than or equal to $10 \mu\text{m}$ in minimum dimension for treated water; more than 100 viable organisms per mL less than $50 \mu\text{m}$ in minimum dimension and greater than or equal to $10 \mu\text{m}$ in minimum dimension for control water.	Treatment efficacy will be determined by the measurement of living ambient organism concentrations in the treatment discharge. Minimum concentration in control tank discharge is 100 live organisms/mL.
	Organisms $< 10 \mu\text{m}$	Less than 1 colony forming unit (cfu) per 100 mL or less than 1 cfu per 1 g (wet weight) zooplankton of Toxicogenic <i>Vibrio cholerae</i> (O1 and O139), less than 250 cfu per 100 mL of <i>E. coli</i> , and less than 100 cfu per 100 mL of intestinal <i>Enterococci</i> for treated water; more than 10 cfu per 100 mL or more than 10 cfu per 1 g (wet weight) zooplankton of Toxicogenic <i>Vibrio cholerae</i> (O1 and O139), more than 2500 cfu per 100 mL of <i>E. coli</i> , and more than 1000 cfu per 100 mL of intestinal <i>Enterococci</i> for control water.	Treatment efficacy will be determined by the measurement of living ambient organism concentrations in the treatment discharge. Minimum concentration in control tank discharge is $5 \times 10^2$ /mL.

**\*NOTE:** For most test facilities, there is known protist die-off in the retention tank. Therefore, as a practical matter, it will be necessary for those facilities to boost the intake numbers of live cells  $\geq 10$  microns in minimum dimension well above 100/mL to create a cushion sufficient to assure 100 live cells/mL in control discharge in the regulatory size class.