

## Protocol to generate representative Samples of Ballast Water from Ballast Water Pipe Systems on board Ships

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The protocol for the sampling of ballast water with the presented sampling system from ballast water pipe systems on board ships is split into five different parts.

### 1 PART 1 : SELECTION OF THE ADEQUATE ISOKINETIC SAMPLING PIPE DIAMETER TO BE USED FOR THE TARGETED BALLAST WATER PIPE SYSTEM

- (1) Get the relevant hydraulic parameters of the targeted ballast water pipe system : (i) diameter of the main ballast water pipe in mm and (ii) volume flow in the main ballast water pipe system as m<sup>3</sup>/hour
- (2) Calculate the resulting flow velocity directly at the filter screen in the filter housing of the sampling system 'Prototype 01' using these equations :

$V_{main}$  (m/s): low velocity in the main ballast water pipe

$d_{main}$  (mm) : diameter of the main ballast water pipe

$Q_{main}$  (m<sup>3</sup>/h): volume flow in the main ballast water pipe

$V_{iso}$  (m/s) : flow velocity in the isokinetic sampling pipe

$d_{iso}$  (mm) : diameter of the isokinetic sampling pipe

$Q_{iso}$  (m<sup>3</sup>/h) : volume flow in the isokinetic sampling pipe

$V_{filter}$  (m/s) : flow velocity directly at the filter screen

$A_{screen}$  (m<sup>2</sup>) : open area of the filter screen 50µm

$$V_{main} = (Q_{main}/3600)/((d_{main}/2)^2 \times 3,14159)/10^6 \quad (1)$$

$$V_{main} = V_{iso} \quad (2)$$

$$d_{iso} = \frac{1}{4} \text{ inch (6,35mm)}, \frac{1}{2} \text{ inch (12,7mm)} \text{ or } 1 \text{ inch (25,4mm)} \quad (3)$$

$$Q_{iso} = ((d_{iso}/2)^2 \times 3,14159/10^6) \times V_{iso} \quad (4)$$

$$A_{filter} = 0,004656 \quad (5)$$

$$V_{filter} = Q_{iso}/A_{screen} \quad (6)$$

- (3) Select the adequate isokinetic pipe, which generates a flow velocity directly at the filter screen of <0,65m/s.

## 2 PART 2 : CONNECTION OF THE SAMPLING SYSTEM 'PROTOTYPE 01' TO THE MAIN BALLAST WATER PIPE SYSTEM

- (1) Equip the universal sampling port with the isokinetic sampling pipe selected in Part 1
- (2) Close the valve of the universal sampling port
- (3) Make sure the main ballast water pipe system is empty
- (4) Open the corresponding flanges in the main ballast water pipe system
- (5) Install the universal sampling port at the corresponding flange in the main ballast water pipe system
- (6) Open all valves of the sampling system 'Prototype 01'
- (7) Connect sampling system 'Prototype 01' and the universal sampling port with the tube
- (8) Install the back flush port at the corresponding flange in the main ballast water pipe system
- (9) Close the valve of the back flush port
- (10) Connect the sampling system 'Prototype 01' and the back flush port with the tube
- (11) Close all valves of the sampling system 'Prototype 01'
- (12) Activate the volume flow in the main ballast water pipe system (= start deballasting procedures)

## 3 PART 3 : SAMPLING OF BALLAST WATER

- (1) Open the valve of the universal sampling port
- (2) Open the valve at the outlet of the sampling system 'Prototype 01'
- (3) Open the valve at the back flush port: ballast water is running through the sampling system 'Prototype 01'
- (4) Open the valve at the lid of the filter housing for de-aeration of the system
- (5) Close the lid valve when water flushes out
- (6) Let the sampling system flush for 10 minutes
- (7) Close the valve at the universal sampling port
- (8) Open the valve at the lid of the filter housing for depressurizing of the system
- (9) Remove the lid of the filter housing
- (10) Insert the filter screen, make sure the bottom valve of the filter screen is closed
- (11) Close the filter housing
- (12) Close the lid valve
- (13) Open the valve at the universal sampling port: ballast water is running through the sampling system 'Prototype 01'
- (14) Immediately record the value in the display of the volume flow meter
- (15) Calculate the volume of ballast water sampled by this equation :

$Q_{\text{system}}$  (m<sup>3</sup>/h) : Volume flow in the sampling system 'Prototype 01'

$V_{\text{min}}$  (m<sup>3</sup>) : Volume of ballast water sampled per minute

$$V_{\min} = Q_{\text{system}}/60$$

- (16) Control desired sample volume for the detection of target plankton organisms >50µm
- (17) As soon as desired sample volume is achieved, close the valve at the universal sampling port
- (18) Close the valve at the outlet of the sampling system '*Prototype 01*'
- (19) Close the valve at the back flush port
- (20) Gently open the lid valve of the filter housing
- (21) Open the filter housing
- (22) Remove the filter screen
- (23) Gently rinse the inner surface of the filter screen with artificial seawater top-down
- (24) Hold the bottom valve of the filter screen into a beaker
- (25) Open the bottom valve
- (26) Gently rinse the inner surface of the filter screen into the beaker: the containing organisms represent the total amount of target plankton organisms >50µm in the desired sample volume and the volume rinsed from within the filter screen can enter follow up steps of further analysis by (i) optical counts of viable organisms >50µm or (ii) chemical analysis procedures
- (27) Close the bottom valve of the filter screen
- (28) Replace the filter screen into the filter housing
- (29) Close the filter housing
- (30) Close the lid valve
- (31) Open the valve of the universal sampling port
- (32) Open the valve at the outlet of the sampling system '*Prototype 01*'
- (33) Open the valve at the back flush port: ballast water is running through the sampling system '*Prototype 01*'
- (34) Gently open the lid valve of the filter housing for de-aeration of the system
- (35) Close lid valve when water is flushing out
- (36) Prepare beaker for the desired sample volume for the detection of target plankton organisms >10µm<50µm
- (37) Gently and fully open the valve at the bypass sampling port of the sampling system '*Prototype 01*'
- (38) Let water flush out for a few seconds
- (39) Take the ballast water sample of the desired volume
- (40) Close the bypass valve
- (41) Close the valve at the back flush port
- (42) Close the valve at the outlet of the sampling system '*Prototype 01*'
- (43) Close the valve at the universal sampling port
- (44) Gently open the lid valve of the filter housing for depressurization of the system
- (45) Filter the sample volume taken under step (39) through a nylon mesh of 7µm
- (46) Gently flush the filter residue with artificial seawater into a small beaker: the containing organisms represent the total amount of target plankton organisms >10µm<50µm in the sample volume taken from the bypass of the sampling system '*Prototype 01*'; the volume in the small beaker can enter follow up steps of further analysis by (i) optical examination of

viable organisms >10µm<50µm, (ii) measurements of chlorophyll activity or (iii) chemical analysis procedures

(47) The filtrate generated by step (45) represents a filtered ballast water sample of the volume taken at the bypass valve for further analysis regarding the concentration of bacteria.

(48) Repeat step (1) to (47) for additional ballast water samples

#### **4 PART 4 : DISMOUNTING OF THE SAMPLING SYSTEM**

- (1) Make sure the de-ballast procedures have stopped and the main ballast water pipe is empty
- (2) Open all valves
- (3) Dismantle the universal sampling port
- (4) Disconnect universal sampling port from the sampling system '*Prototype 01*'
- (5) Disconnect the back flush port from the sampling system '*Prototype 01*'
- (6) Remove the lid from the filter housing
- (7) Tilt the sampling system '*Prototype 01*' over and let the system run empty
- (8) Replace the lid of the filter housing
- (9) Dismantle back flush port
- (10) Close both corresponding flanges in the main Ballast water pipe system

#### **5 PART 5 : STORAGE OF THE SAMPLING SYSTEM (IN LANDBASED LABORATORY)**

- (1) Sufficiently rinse all parts of the sampling system with freshwater
- (2) Place ports and connecting tubes into adequate disinfectant solutions for at least one day
- (3) Fill the sampling system '*Prototype 01*' with adequate disinfectant solutions for at least one day
- (4) Place filter screen into adequate disinfectant solutions for at least one day
- (5) Let all parts of the sampling system sufficiently dry out after disinfection