

Standard Operating Procedures
Collection of Treated Ballast Water Samples using an Inline
Sample Port

Version 1.0

ICES/IOC/IMO Working Group on Ballast and Other Ship Vectors (WGBOSV)

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PURPOSE

These procedures describe how to collect samples of treated ballast water using a ship-supplied sample port and probe (which can vary in size and specifications according to individual installations) and traditional plankton nets. Samples are collected to assess the efficacy of ballast water management systems against Regulation D-2 of the *International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004*. These procedures are recommended to ensure ballast water samples are collected consistently across research groups collecting data during the experience-building phase of the Convention. These methods were developed based on best available science concerning ballast water sampling in recent years (e.g., Cangelosi *et al.* 2011; Gollasch and David 2013, 2017; Wier *et al.* 2015).

SCOPE AND APPLICATION

Ballast water sampling can be conducted by scientists during normal ballast operations to determine if the concentrations of organisms in ballast discharge are above the standards stipulated in Regulation D-2. As per recommendations in the *Guidelines for Ballast Water Sampling (G2)*, “the sampling protocol should result in samples that are representative of the whole discharge of ballast water for any single tank or combination of tanks being discharged” [MEPC 58/23, Annex 3, 6.2.2].

Representative sampling should be conducted “to ensure that a sample contains the same proportions of the various flowing constituents as the flow stream being sampled” using an isokinetic sampling facility that “is designed to separate a subsection of the total flow-stream in a manner that does not encourage or discourage water entry other than that which is otherwise in the cross-section of the sampler opening. In other words, flow streams in the main flow of the pipe should not diverge or converge as they approach the opening of the sampler.” [MEPC 58/23, Annex 3, Part 1, 1.3].

These procedures describe how to collect samples of treated ballast water using a ship-supplied sample port and sample probe, which can vary in size and specifications according to individual installations. These procedures are valid for fully turbulent ballast flows – in most cases, these procedures will not apply to tank stripping flows.

SUMMARY OF PROCEDURES

- Gather essential information from ships' Officers and/or Engineers required to connect scientific sample collection device to ship-supplied sample port
- Determine a target sample flow rate lower than isokinetic flow velocity, with reasonable error bounds, for sampling from a ballast system with set parameters
- Conduct sample collection using a large volume sample collection device and plankton nets for later analysis of organisms in the $\geq 50\text{-}\mu\text{m}$ size range, and a small volume sample collection device for later analysis of organisms in the ≥ 10 to $< 50\text{-}\mu\text{m}$ size range and indicator microbes

- Prepare 10- μ m filtered ballast water for use as sample rinse water
- Split small volume ballast water sample into bottles for later analysis
- Clean/sterilize all scientific sampling equipment for next use

DEFINITIONS

Isokinetic flow rate: the sample flow rate at which the velocity entering the sample probe is equal to the velocity in the main ballast pipe

Sample port: The flanged opening for inserting a sample probe into the ballast discharge pipe; this is ship-installed equipment and should be covered by a closed valve (i.e. full port ball valve or gate valve) or blind flange when not in use

Sample probe: The tube inserted into the ballast pipe through the sample port that provides the ability to connect to external tubing/hose for collection and processing of a ballast water sample

Sample collection device: A device that can concentrate and collect the larger size class of organisms (via a filter or plankton net), collect a whole water sample, or both

HEALTH AND SAFETY

All scientific teams wishing to board commercial ships should be in contact with the local maritime authority which bears responsibility for safety on board ships in regional waters, to ensure that appropriate Safe Work Procedures are in place as part of the scientific program. Safe Work Procedures may need to be established for multiple critical tasks, such as transferring from boat launch to large ship, transiting ship ladders, transiting ship decks during cargo operations as well as for the sampling operations in the engine room, which should consider hazards specific to the ballast water management system being tested - such as exposure to chemicals utilized for treatment or neutralization of the ballast water.

Prior to arrival at ship location, obtain permission from ship owner, ship agent, and ship captain to visit ship for collection of scientific samples, and, obtain permission from port security to visit port property to gain access to ship. Always follow safety protocols and instructions provided by port security, ship agents and/or ship crew. All scientific personnel must sign in to the ships' security list and gain authorization from ships' Officers for all research activities. Obtain approval for usage of all scientific equipment, cameras and cellular devices while on board, mindful of any requirements for intrinsically-safe components.

Ensure that necessary Personal Protective Equipment (PPE) is worn at all times, including safety footwear, hard hats, work gloves and eye protection. Consider need for hearing protection, high-visibility vests and, for boarding/disembarking ship, a personal flotation device. Beware of hazards near the work area(s) such as mooring lines and cargo loading/unloading activities.

PROCEDURES

I. Procedure to connect a sample collection device to a sample port

This procedure describes how to connect a sample collection device to a ship's ballast system to collect a treated ballast water sample. While ISO 11711 suggests the use of a "hot-tap" sample probe supplied by the sampling party, this procedure recognizes that many ships currently have a permanent or semi-permanent sample probe installed at the sample port and that sample probe specifications currently vary across ships. Ideally, the sample port will be fitted with a large ball valve or other fully-ported valve.

1. If possible, send BWMS Sampling Port Survey (Appendix 1) to targeted ship prior to visit to gather essential information required to connect to, and determine appropriate sample flow rate from, the ship-supplied sample port and sample probe; alternatively, complete [or confirm] the survey on arrival to the ship.
2. Be aware that there are likely to be multiple sample ports located on the BWMS intake, on the ballast discharge line(s), and at any additional locations necessary to ascertain the proper functioning of the BWMS. It will typically be desirable to use the sample port located nearest to the point of overboard discharge. Alternatively, justification for sample port selection may be based on location with respect to the undisturbed run-in or run-out distance (undisturbed means without any bend, valve, fittings, etc.).
3. If there is no sample probe in place, determine if there is a suitable sample probe on board (or can be provided by the sample team) that can be installed; if no sample probe is available, the ship cannot currently be sampled. If the presence of a sample probe is unknown or suspect, a small inspection camera can be inserted through a semi-water tight gland to determine the presence/direction/integrity of a sample probe on the wet side of the sample port.
4. Request ship have ready the fittings required to connect a 1-inch (25 mm) hose; Either a 1-inch (25 mm) hose barb or a ¾-inch (20 mm) pipe around which the hose can be fit tightly, is acceptable. If the ship cannot provide this type of connection, the sample team should have a number of flanges and fittings available to adapt to a 1-inch (25 mm) barb fitting. Request ship to advise where sampled ballast water (at least 1 m³) can be disposed of – e.g. into bilge, sent overboard, or returned into ballast water system through a port downstream of the sample port.

II. Procedure to Obtain Appropriate Sample Flow Rate through a Sample Collection Device

This procedure describes how to determine the appropriate sample flow rate through a sample collection device to collect a representative sample of ballast water following guidance published by Wier et al. (2015) and Richard et al. 2008. As organisms in ballast water flow do not tend to separate from the flow trajectory like in an oil/water mixture, true isokinetic sampling is not a requirement (Richard et al. 2008). While it would be ideal to collect a ballast water sample using isokinetic and unobstructed sample flows through the sample probe, sampling connections and sampling device, it is impractical to do so under operational conditions due to fluctuations in main ballast flow rate. This procedure establishes a target flow rate, with reasonable error bounds, that are slightly below the isokinetic flow velocity.

Background - IMO-D2 sampling guidance (MEPC 58/23, Annex 3, Part 1):

“4.1 ... Simulations showed that flow transitions from the main stream were best for sample port diameters between 1.5 and 2.0 times the isokinetic diameter (D_{iso}). Ports sized in this range had smooth transitions and pressure profiles that allowed for direct sampling without the need of a pump to induce sample collection.” Wier et al. (2015) revised this guidance to port diameters between 1.0 and 2.0 times the isokinetic diameter.

“4.6 If flow control of the sample flow rate is required, ball, gate and butterfly valve types should be avoided as they may cause significant shear forces which may result in organism mortality. For flow control, it is recommended to use diaphragm valves or similar valve types to minimize sharp velocity transitions. For flow distribution, ball valves may be utilized in such a manner that they are either fully open or fully closed.”

Option A. Look-up table

Use the information in the dynamic look-up table (MS Excel file available at www.ices.dk/community/groups/Pages/WGBOSV.aspx) to determine appropriate sample flow rates given the sample probe diameter, main ballast line diameter, and the range of possible main ballast flow rates. The sample flow rates are determined in reference to the isokinetic flow rate, or the volumetric flow rate at which velocity entering the sample probe is equal to that of the main ballast pipe. Any flow rate for 1-2x D_{iso} will produce a representative sample flow rate (Wier et al. 2015). In the absence of a flow meter installed in the main ballast pipe and immediately accessible to the sampling party at the sampling location, the sample should be taken assuming the main ballast flow rate is $\frac{1}{2}$ of the rated capacity of the pump to begin sampling, this can be increased as the actual flow rate information is relayed to the sampling team.

For parameters outside the scope of the look-up table, or if the table is not available, proceed to *Option B*.

Option B. Sample flow rate formula

The appropriate sample flow rate is determined in reference to the isokinetic flow rate (Q_{iso}), which is dependent on the ballast main pipe diameter (D_m), the sample probe inner diameter (D_{iso}), and the volumetric flow rate through the main ballast pipe (Q_m). It can be calculated using the following equation:

$$\text{Eqn 1} \quad Q_{iso} = Q_m * \left(\frac{D_{iso}}{D_m} \right)^2$$

Wier et al. (2015) recommend sampling at D_{iso} between 1.0-2.0. To determine the lowest sample flow rate ($Q_{iso(min)}$) which will still result in a representative sample at a given main ballast flow rate (Q_m) for a given sample flow rate (Q_{iso}), the maximum flow velocity and rate through a 1-inch sample probe is provided by 1 D_{iso} and the slowest flow velocity which is still representative is garnered by sampling with a 2x D_{iso} or 2-inch sample probe at the exact same flow rate. This flow rate in a 2-inch sample probe is well below the flow rate at which the velocity is isokinetic for a 2-inch sample probe but it still provides a representative sample based on empirical data. Thus, this forms the lower bound of a flow rate range that would be considered representative for a 2-inch sample probe. Extending this logic, to determine the lowest representative sample flow rate for a 1-inch sample probe in a given situation, determine the fastest flow rate of a sample probe that is $\frac{1}{2}$ the diameter of a 1-inch sample probe which can be calculated by modifying equation 1 as follows:

$$\text{Eqn 2} \quad Q_{iso (min)} = Q_m * \left(\frac{D_{iso/2}}{D_m} \right)^2$$

Isokinetic and minimum flow rate

On board ships with permanently installed sample probes, it is not possible to alter the size of the sample probe as described in much of the literature on the subject; one is provided a sample probe of a given size, in a ballast system with set parameters. Sampling at a rate lower than isokinetic flow velocity is paramount for sampling representatively to avoid oversampling due to fluctuations in the main ballast flow. The maximum sample flow rate can be calculated knowing the ballast pipe diameter (D_m), main ballast flow rate (Q_m) and the sample probe diameter (D_{iso}) using equation 1 above. Note that Q_m is the actual main ballast flow in the pipe; in reality this may range over the course of ballast operations. It is best to plan that Q_m may change over the sample collection period and predetermine calculations for representative sample flows for varying Q_m values during the sampling event. One should avoid true isokinetic sampling since fluctuations in Q_m can easily result in sampling flow rates above isokinetic; thus sampling should be conducted at a rate at least 10% below isokinetic to account for this variability, as well as flow rate measurement error. In systems experiencing <10% variation in Q_m , this buffer should increase to account for the variability being experienced. The sampling team needs to monitor Q_m throughout sample collection, using the flow meter installed on the main ballast line or the monitoring system of the BWMS, and be prepared to adjust the rate of sample collection

using the diaphragm valve on the sampling device to ensure that a representative sample flow rate is maintained. The dynamic spreadsheet can be used prior to sampling to create a quick reference sheet of min/max sample flows for a range of Q_m (e.g. 50- m^3/h intervals between 0 and ballast pump capacity) so that adjustments can be made as required while on ship. The dynamic spreadsheet may also be copied to a smartphone or tablet to support calculations in the field.

III. Procedure to Collect Ballast Water Samples using a large volume sample collection device and plankton nets, for later analysis of organisms in the $\geq 50\text{-}\mu\text{m}$ size range

The volume required to enumerate organisms present at low concentrations in successfully-treated ballast water with a desired level of precision is dependent on the research objectives as well as the analysis method (for example, the sample concentration factor and number of sub-samples analyzed – see Table 12 in NSF International 2010). This procedure describes how to collect and filter 1 m^3 of ballast water in a continuous manner using a large volume sample collection device and standard plankton nets. The large volume sample collection device can be constructed using a variety of materials – one example is given for illustrative purposes. To determine the amount of time required to collect the sample(s), multiply the sample flow rate ($Q_{iso (min)}$) x sample volume ($1m^3$). The sample collection time should coincide with the ships ballasting operations, and may need readjustment if it does not. It is assumed that no port is available to return sampled water to the ballast pipe after processing is completed -such that waste filtrate must be deposited in the ship's bilge or other suitable location. Alternative procedures using sample collection filter skids and return ports may become available in the future.

Example List of Materials

- 1-inch fitting on sample port (see Section I)
- 1-inch x 20-foot length sample hose on either end to connect sample port to sample device
- hose clamps to secure hose on sample port and sample device
- Large volume sampling device (Appendix 2, Figs. 1,2) consisting of:
 - 1-inch PVC barbed hose fitting
 - 1-inch PVC female NPT adaptor
 - 1-inch Ball valve with 1-inch PVC union connections
 - 4 x 1-inch PVC union connections
 - 1-inch Flow meter with monitor or digital display, battery powered
 - 2 x hose clamps to use as connection for 1-inch hose
 - 1-inch x 4-inch length PVC nipple
 - 1-inch x 2-inch length PVC nipple
 - 10 inches of 1-inch PVC pipe cut into 3-inch and 7-inch sections, glued into place

- Diaphragm valve
- 1-inch, 90° barbed hose fitting
- 1-inch x 14-inch length hose
- Two mounting points for attaching support lines
- Extra O-rings
- 3 x 30-cm diameter Zooplankton net with 50- μm ¹ mesh and cod ends (each numbered 1 through 3)
- 3 x Ropes (having 10 to 15-foot length) to use as support lines for sampling device and nets
- C- clamps to use for attaching support rope if no overhanging structure available
- 6 x carabineers for quick attachment of nets and sampling device to rope supports
- Large sample bin to hold nets during sampling (e.g. 20 gallon (75L) garbage pail) with:
 - 2 x 1-inch bulk head connections for water discharge (see Appendix 2, Fig. 5 for placement)
 - 2 x ball valves
 - two 15-foot lengths of 1-inch hose to direct water into bilge (or else as directed by ship crew)
 - hose clamps for 1-inch hose
- extra batteries for sonde and flow meter
- 1-L hand-pumped pressurized sprayer to rinse nets
- 2 x 500-ml wash bottles to rinse samples for 10- μm filtered ballast water
- 5 L of 10- μm filtered ballast water into sample bottles (see V. Procedure for 10- μm filtered Ballast water)
- 3 x 1-L widemouth Nalgene bottle (line marked at 1-L graduation and pre-labeled for sample (e.g. net 1, net 2, net 3)
- multi-parameter water quality sonde
- tools
 - Nut driver /screw driver to tighten hose clamps
 - 2 x channel locks to tighten sampling device connections (lighter and more compact than pipe wrenches)
 - Adjustable wrench
 - Screw driver to open battery compartment of flow meter, if needed
- field notebook/field sheets and Chain-of-Custody form

¹Note that there is no international standard for plankton mesh sizing – the mesh material and weave type can affect filtration efficacy. Retention efficiency should be verified for plankton nets – a smaller nominal mesh size such as 35- μm can be used to increase capture rate of organisms $\geq 50\text{-}\mu\text{m}$ as long as later analysis methods take into

account possible capture of organisms <50- μm (e.g. microscopy using microbeads for size analysis).

Procedure:

1. Using the information gathered from the BWMS Sampling Port Survey, determine target sample collection flow rate (min/max) using the dynamic look-up table (MS Excel file available at www.ices.dk/community/groups/Pages/WGBOSV.aspx). A range of appropriate flow rates can be calculated ahead of time according to possible combinations of ballast flow and sample flow likely to be encountered. It is recommended to complete sample collection within 60 minutes to avoid negative effects of holding time on organisms in the samples. Depending on the research objectives, it is recommended to collect at least two continuous, sequential samples, totaling at least 1 m³ volume, during ballast water discharge from a single tank or combination of tanks. Again, depending on the research objectives, it may be desirable to conduct ballast water sampling during a certain period of ballast discharge, such as avoiding the initial or final 5 minutes of tank discharge (Gollasch and David 2017), or sampling during discharge of a certain parcel of ballast water (e.g. Bailey and Rajakaruna 2017).
2. Assemble large volume sample collection device (LVSD) as pictured in Appendix 2. This involves tightening NPT thread fittings with channel locks and securing Unions - when tightening unions check that O-rings are in place. Ensure flow meter monitor is on and working (change batteries if necessary).
3. Connect ship's sample port valve to LVSD via 1-inch inner diameter (ID) hose. Secure both ends of the hose using hose clamps.
4. Setup $\geq 50\text{-}\mu\text{m}$ sample filtration system as pictured in Appendix 2:
 - a. Suspend 3 zooplankton nets within sample bin using overhead lines, net hoop should be 4 inches (10 cm) above the lip of the sample bin. A carabiner attached to the top of the net bridal acts as quick attachment/detachment point.
 - b. Suspend LVSD using overhead lines. The out flow point of the LVSD when sampling should sit in the net at least 4 inches (10 cm) below the hoop and within the sample bin. Ideally, the outlet should be below the waterline inside the bin during sample collection.
 - c. Place multi-parameter water quality sonde in sample bin and start recording according to the sonde's standard operating procedures.
 - d. Connect the ball valves and discharge hoses with hose clamps to the sample bin bulkhead connectors. Direct the hoses to the bilge or other discharge location as approved by the Chief Engineer; Close bottom ball valve and fully open top ball valve.
5. Ensure the flow control valves of the LVSD are turned to the closed position (Ball and Diaphragm).
6. Disconnect LVSD at the ball valve union, leaving the closed ball valve attached

to the line connected to the sample port and attach a PVC union with a 1-inch hose barb fitting to the valve.

7. Once the treatment system has been started and treated ballast water is flowing through the ballast discharge pipe sampling can begin. The actual flow rate through the ship's main ballast line should be verified using the flow meter installed on the main ballast line or the monitoring system of the BWMS. Wait at least one minute before starting sample collection to allow the main ballast line to flush; note that some BWMS maintenance procedures keep potable water or cleaning solution in the BWMS lines when not in use.
8. Fully open the ship's sample port isolation valve.
9. Direct the LVSD ball valve either to the bilge or sample bin and then slowly open the LVSD ball valve to fully open and flush the sample collection line for approximately 30 seconds.
10. Close the LVSD ball valve and reconnect it to the LVSD.
11. Inform all sampling team members that sampling is about to commence.
12. Re-open the ball valve on the LVSD after it is connected.
13. Slowly open the flow control valve (diaphragm). Water should start to flow through the LVSD (direct water into the bilge or sample bin outside of the net).
14. Read the flow rate on the flow meter on the LVSD and adjust the flow rate to the target sample flow rate range as per step 1 by adjusting the flow control valve.
15. To start sample collection, place the outlet of the LVSD into the first plankton net (net 1) and record the start time and initial volume from the flow meter.
16. Continually monitor the main ballast flow and the sample device flow meter, and adjust the flow control valve to maintain a sample flow rate within the target flow rate range (as calculated in the look-up table) throughout sample collection.
17. Continually monitor the water depth in the sample bin, adjusting the lower ball valve to maintain a constant water level in the sample bin with some water always draining out of the top bulkhead connector. This top ball valve should be fully open at all times. Watch for air-locks forming in the discharge hose which will prevent water from draining into the bilge.
18. Once the target sample volume is achieved for the first net, switch LVSD to net 2 (and subsequently net 3, if required) and continue sampling. Record time and volume of water collected for each net sample.
19. After last sample is completed, close the ball valve on the LVSD and record the stop time and final volume sampled.
20. Close ball valve on lower bulkhead connector to retain some water in the sample bin (to keep the net cod-ends submerged; this water will also be used to rinse nets and produce filtered rinse water).
21. Close ship's sample port isolation valve.
22. With sampling complete, condense $\geq 50\text{-}\mu\text{m}$ samples by rinsing net 1 with a hand

- pumped pressurized sprayer filled with water from the sample bin (spraying only the outside of the net)
23. Once the sample has been condensed within the cod end, remove the cod end and rinse the sample into a labeled 1-L sample container using a wash bottle filled with 10- μ m filtered ballast water (see V. Procedure for 10- μ m filtered ballast water) and top up to the 1 L mark with 10- μ m filtered ballast water
 24. Repeat steps 22 and 23 for remaining nets. At minimum, the sample bottles must be labeled with the date and sample number. If more than one ship is being sampled, add additional information (e.g. ship name, time).
 25. Send ≥ 50 - μ m samples to lab with completed Chain of Custody forms.
 26. Clean up and remove sampling station:
 - a. With the sample port isolation valve closed, disconnect the 1-inch hose from the sample port and drain any residual water in the sample bin (including any extra water produced as part of the sample processing) into the bilge or other location approved by the Chief Engineer.
 - b. Break down all sampling equipment and pack up for exiting ship
 27. Rinse the sample collection device and hose with tap water off ship: fully open the flow control valve and allow at least 20 L of water to flush through the device and 20 L through the hose; gently shake out the device and hose to remove excess water. Store until needed again.
 28. Sample bottles can be cleaned according to section VII. Procedure for cleaning bottles, carboys and other equipment, and dried for re-use.

IV. Procedure to Collect Whole Water Ballast Samples using a small volume sample collection device, for later analysis of organisms in the ≥ 10 to < 50 - μm size range and indicator microbes

This procedure describes how to collect whole water samples of ballast water in an isokinetic, continuous manner using components initially selected by the U.S. Navy Research Lab (Moser et al. in preparation). This small volume sample collection device (SVSD) is used in conjunction with the large volume sample device (LVSD) described in section III. The small volume sample collection device can be constructed using a variety of materials – one example is given for illustrative purposes.

A. Using the SVSD in conjunction with the LVSD

Example List of Materials (in addition to those listed above in Section III)

- Small volume sampling device (Appendix 2, Figs. 1-2) consisting of:
 - 1-inch tee fitting with FTP fittings
 - 1-inch x 17-inch length PVC pipe
 - 3-mm ID sample probe, compression fitting, and adaptors to 1-inch
 - 2 to 3 foot length of $\frac{1}{4}$ -inch ID tubing for 3-mm sample probe to flow meter
 - 5 to 10 foot length of $\frac{1}{4}$ -inch ID tubing to go to collection carboy
 - $\frac{1}{4}$ -inch flow meter
 - $\frac{1}{4}$ -inch x 2-inch length male NPT to male NPT nipple
 - $\frac{1}{4}$ -inch ball valve with female NPT connections (or pinch valve)
 - $\frac{1}{4}$ -inch barbed hose fitting
 - hose clamps for $\frac{1}{4}$ -inch tubing
 - mount to hold flow meter on to sample device
- 1-2 carboys (15L volume)

Procedure:

1. Determine minimum and maximum flow rate for the small volume sample via the MS Excel file available at www.ices.dk/community/groups/Pages/WGBOSV.aspx. Select a target flow rate within this range where 10 L will be collected in the same time that 1 m³ will be collected using the target flow rates of the LVSD (See section III).
2. Assemble SVSD as pictured in Appendix 2, Figs. 1-3 as an integrated unit of the LVSD. Note that the tee connection for the 3-mm sample probe must be installed downstream of at least 17 inches of straight LVSD pipe to allow for flow development. Also, the 3-mm sample probe should sit in the middle facing upstream inside the 1-inch straight pipe (Appendix 2, Fig. 3). The compression fittings allow for adjusting and tightening the probe in place.
3. Connect $\frac{1}{4}$ -inch inner diameter tubing from the mini sample probe in the extended LVSD to the inlet of the SVSD. Secure both ends of the tubing to a barbed fitting using hose clamps. Set up carboy(s) for ≥ 10 to < 50 - μm samples

- and secure sample tube from the SVSD into the bilge.
4. Ensure the flow control valves of the SVSD are turned to the closed position.
 5. Start water flow as described in Section III steps 7-12.
 6. Slowly open the SVSD flow control valve. Water should start to flow through the SVSD to the bilge or location approved by the chief engineer.
 7. Read the flow rate on the flow meter on the SVSD and adjust the flow rate to the target sample flow rate range as per step 1 by adjusting the flow control valves. To minimize the possibility of sample contamination, run at least 2 L of water through the SVSD and associated tubing before beginning sample collection.
 8. To start sample collection, follow Section III steps 13+ and secure the open end of the discharge tubing from the outlet of the SVSD into the sample carboy and record the start time.
 9. Continually monitor the flow meter and adjust the flow control valve to maintain a sample flow rate at the target rate throughout collection for the SVSD. At the same time, monitor the water level in the carboy(s) so as not to overfill it. Switch the outlet tubing to another carboy, as required.
 10. Once the target sample volume is achieved for the LVSD, completely close the isolation valve on the LVSD and record the stop time and final volume sampled. Remove the SVSD discharge tubing from the collection carboy and secure the lid.
 11. Split ≥ 10 to < 50 - μm samples for later analysis as directed in VI. Procedure to split ballast sample for analysis (if required).
 12. Send ≥ 10 to < 50 - μm samples to lab with completed Chain of Custody forms.
 13. With the ship's sample port isolation valve closed, disconnect the $\frac{1}{4}$ -inch tubing from the ship's sample port and drain any residual water in the SVSD and associated tubing into the bilge or other location approved by the Chief Engineer.
 14. Take apart all sampling equipment and pack up for exiting ship.
 15. Once off the ship rinse the SVSD with tap water: fully open the flow control valve and allow at least 20 L of water to flush through the device; gently shake to remove excess water. Store the sample collection device until it is needed again.
 16. Wash carboys following section VII. Procedure for cleaning bottles, carboys and other equipment. Allow to dry, and re-use.

V. Procedure for 10- μ m filtered Ballast water

This procedure describes how to filter ballast water through 10- μ m mesh for use during rinsing of ≥ 50 - μ m samples into sample bottles.

List of Materials:

- Sieve with 10- μ m nitex mesh (minimum 4-inch diameter recommended)
- One 5 to 10-L bucket or carboy to hold filtered water
- 1-L measuring cup (or equivalent container) to scoop water

Procedure:

1. Using water remaining in the sample bin, use measuring cup to scoop water into sieve.
2. Scoop required amount (e.g. 5 L) of sample bin water through the 10- μ m sieve and collect into a separate labeled bucket (or carboy).
3. The 10- μ m filtered ballast water can then be used to rinse the ≥ 50 - μ m samples into sample bottles and to top up the samples to 1 L.

VI. Procedure to split ballast sample for analysis

This procedure describes how to mix and split the whole water ballast sample for analysis by multiple analytical approaches.

List of Materials:

- Nitrile Gloves
- 1 – 2 carboys (at least 10 L) of sample water
- Sample bottles

Preparation:

1. Put on gloves

Procedure:

1. Determine how the sample bottles will be filled based on the number of sample carboys collected and the number of sample bottles to be filled. The sample water needs to be distributed evenly across all bottles. For example, if two full carboys are collected, half-fill each bottle from one carboy and then top up the other half with the second carboy. If only one carboy is collected half-fill all the bottles first, then fill the second half.
2. Prior to adding water to each sample bottle gently invert the carboy five times (with cap closed tightly); cap the sample bottles between fills to avoid spillage. If two people are available one can gently invert the carboy while the other person uncaps and recaps the bottles.
3. Top up each sample bottle to the fill line, cap tightly and place in cooler for later analysis.

VII. Procedure for cleaning bottles, carboys and other equipment

This procedure describes how to clean bottles and other equipment after sampling so that the equipment can be re-used for the next sampling trip

List of Materials:

- Large plastic container with lid or large sink
- Bleach (and Safety Data Sheet (SDS), as required)
- Drying rack (optional)
- Tap (potable) water
- Chemical resistant gloves
- Safety glasses
- Lab coat

Sample bottles, pails, sieves, carboys, squeeze bottles etc.

Preparation:

1. Put on lab coat, gloves and safety glasses.
2. Prepare dilute bleach bath having concentration of 100-200 ppm – (e.g. add 2-4 ml of 5.25% household bleach to 1 L of water).

Cleaning Equipment:

1. Rinse delicate equipment (fabrics, rubber – collapsible carboys) quickly with bleach water.
2. Soak hardy equipment (glass, metal, plastic) in bleach bath for 60 minutes.
3. After bleaching, rinse all equipment with tap/potable water 3 times and let air-dry.
4. Prior to re-use, bottles should be rinsed with sample water 3 times to remove any residues.

Nets and cod ends

Preparation:

1. Put on lab coat, gloves and safety glasses.
2. Prepare dilute bleach bath having concentration of 5 % bleach (by volume) – e.g. add 50 ml of 5.25% household bleach to 1L of water. Use enough water to cover nets completely.

Cleaning Equipment:

1. Submerge the nets into the bleach bath for 15 minutes. Ensure that all the fabric is submerged into the bath water.
2. Cod ends should be submerged for only 2-3 minutes, then removed for rinsing.
3. After bleaching, rinse all equipment with tap/potable water 3 times and let air-dry. Rinse down the fabric of the nets by starting at the ring and working towards the cod end attachment. Alternatively, the nets can be soaked in clean water for a few minutes.

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Appendix 1: BWMS Sampling Port Survey

All ships with a ballast water management system (BWMS) should also have a sampling port for collection of samples to assess compliance with the D-2 standard in the *International Convention for the Control and Management of Ships' Ballast Water and Sediments, 2004*. As sampling port configuration is currently variable across ships, please complete the following survey in order to plan what equipment will be needed to collect a sample on your ship.

Definitions:

Sample port: The flanged opening for inserting a sample probe into the ballast discharge pipe; this is ship-installed equipment and should be covered by a closed valve (i.e. full port ball valve or gate valve) or blind flange when not in use

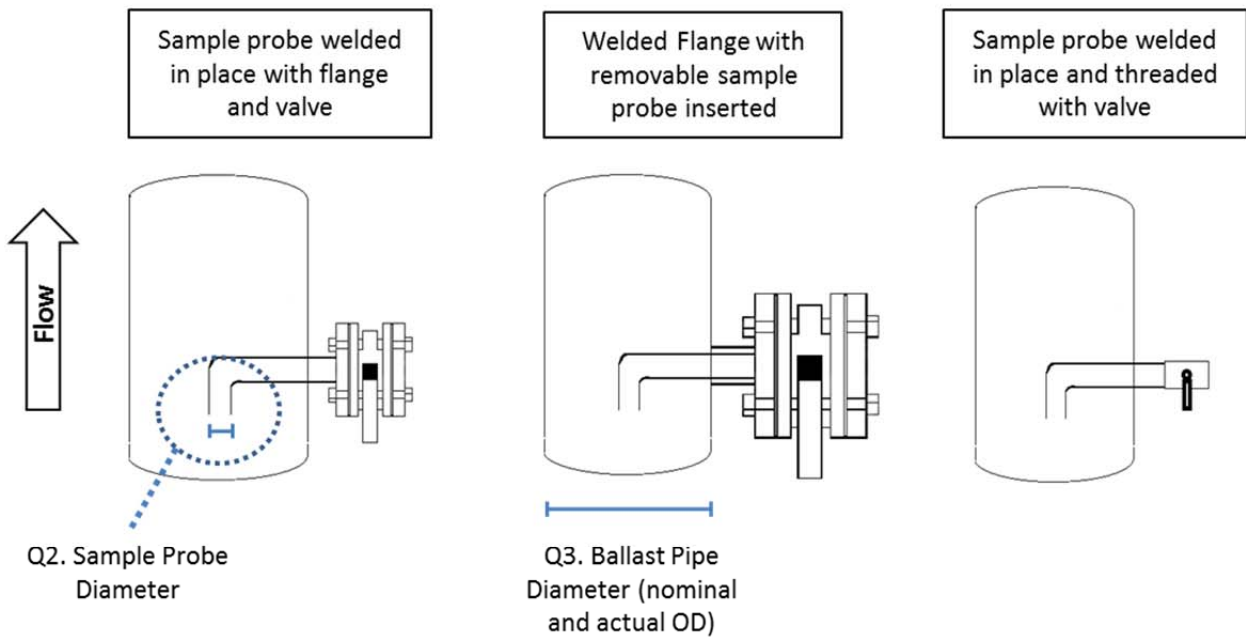
Sample probe: The tube inserted into the ballast pipe through the sample port that provides the ability to connect to external tubing/hose for collection and processing of a ballast water sample

Note: there are likely to be multiple sample ports located on the BWMS intake, on the ballast discharge line(s), and at any additional locations necessary to ascertain the proper functioning of the BWMS. It will typically be desirable to use the sample port located nearest to the overboard discharge. *Please refer to attached figure of sample ports to complete this survey.*

Ship Name:	IMO number:		
BWMS Manufacturer and Model:			
Describe the location of the Sample Port nearest to the overboard discharge: (e.g. Downstream of BWMS, high side of ballast pump, close to overboard discharge)			
<i>If available, attach ballast system diagram identifying sample port locations</i>			
1. Describe the Sample Port Configuration:			
Valve installed:	<input type="checkbox"/> YES	<input type="checkbox"/> NO	
Valve DN:	<input type="checkbox"/> MM <input type="checkbox"/> INCHES		
Type of Valve:	<input type="checkbox"/> Ball	<input type="checkbox"/> Butterfly	<input type="checkbox"/> Gate
	<input type="checkbox"/> Other (Specify:)		
Valve connection type:	<input type="checkbox"/> flanged	<input type="checkbox"/> threaded	
Blind flange installed:	<input type="checkbox"/> YES	<input type="checkbox"/> NO	
If flanged, provide description: e.g. JIS 10K50A (PCD 120mm)			
If threaded connection type available:	<input type="checkbox"/> male	<input type="checkbox"/> female	
Valve connection nominal size:	<input type="checkbox"/> MM <input type="checkbox"/> INCHES		

Valve connection pipe OD (actual):	<input type="checkbox"/> MM <input type="checkbox"/> INCHES		
Valve connection standard:	<input type="checkbox"/> NPT	<input type="checkbox"/> BSP	<input type="checkbox"/> Other (Specify:)
If other connection type, please describe: e.g. Nakijima 50A			
<i>If available, attach photos of the sample port (close-up) and general location</i>			
2. Describe the Sample Probe Configuration:			
Sample probe installed:	<input type="checkbox"/> YES	<input type="checkbox"/> NO	<input type="checkbox"/> UNKNOWN
If yes, sample probe diameter:	<input type="checkbox"/> MM <input type="checkbox"/> INCHES		
<i>If available, attach dimensioned drawing of the flange and valve set up</i>			
3. Describe the Ship's Main Ballast Line Configuration at the sample port:			
Ballast pipe nominal diameter:	<input type="checkbox"/> MM <input type="checkbox"/> INCHES		
Ballast pipe OD (actual):	<input type="checkbox"/> MM <input type="checkbox"/> INCHES		
Number of Ballast Pumps:			
Ballast Pump capacity (single pump):	<input type="checkbox"/> m ³ /hr <input type="checkbox"/> GPM		
4. Can ship engineers provide a 25mm (1-inch) hose barb fitting at the sample port for us to connect to?	<input type="checkbox"/> YES	<input type="checkbox"/> NO	
If no, what fittings are required?			
5. Can the ship accept at least 1 m³ of water into the bilge?	<input type="checkbox"/> YES	<input type="checkbox"/> NO	
If no, can an alternative be provided, such as a non-oily/ non-greasy submersible pump of 1/3 to 1/2 HP; Connections and hose to connect to a water return port or drain downstream of the planned sample port; and power and ability to easily start and stop the pump without leaving the immediate area of the sample port.	<input type="checkbox"/> YES	<input type="checkbox"/> NO	
6. Describe sample port accessibility (check box below):			
<input type="checkbox"/> Ideal	Easy to access with clear area at least 1 x 1 x 2.5 m (W x L x H)		
<input type="checkbox"/> Challenging:	<input type="checkbox"/> Below Deck Plating <input type="checkbox"/> Accessible only by ladder <input type="checkbox"/> Minimal Clearance		
Thank you for completing this survey. Forward survey, diagrams and photos to [Insert Contact Info for Project Leader Here]			

Common Sample Port Configurations:



Q1. Common Connection Types:

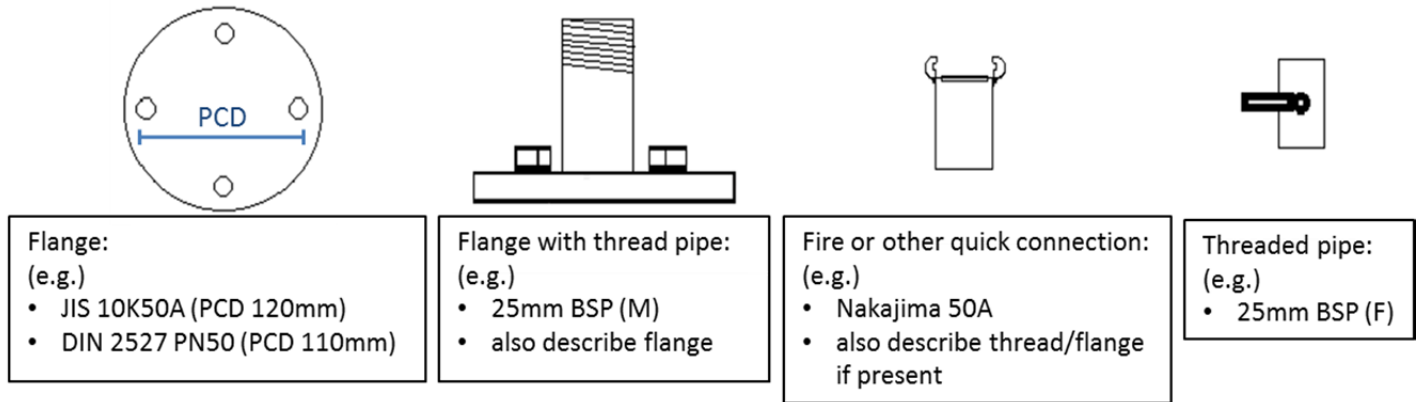


Figure 1. Examples of sample ports showing information required for survey.

Appendix 2: Photos of example sampling devices

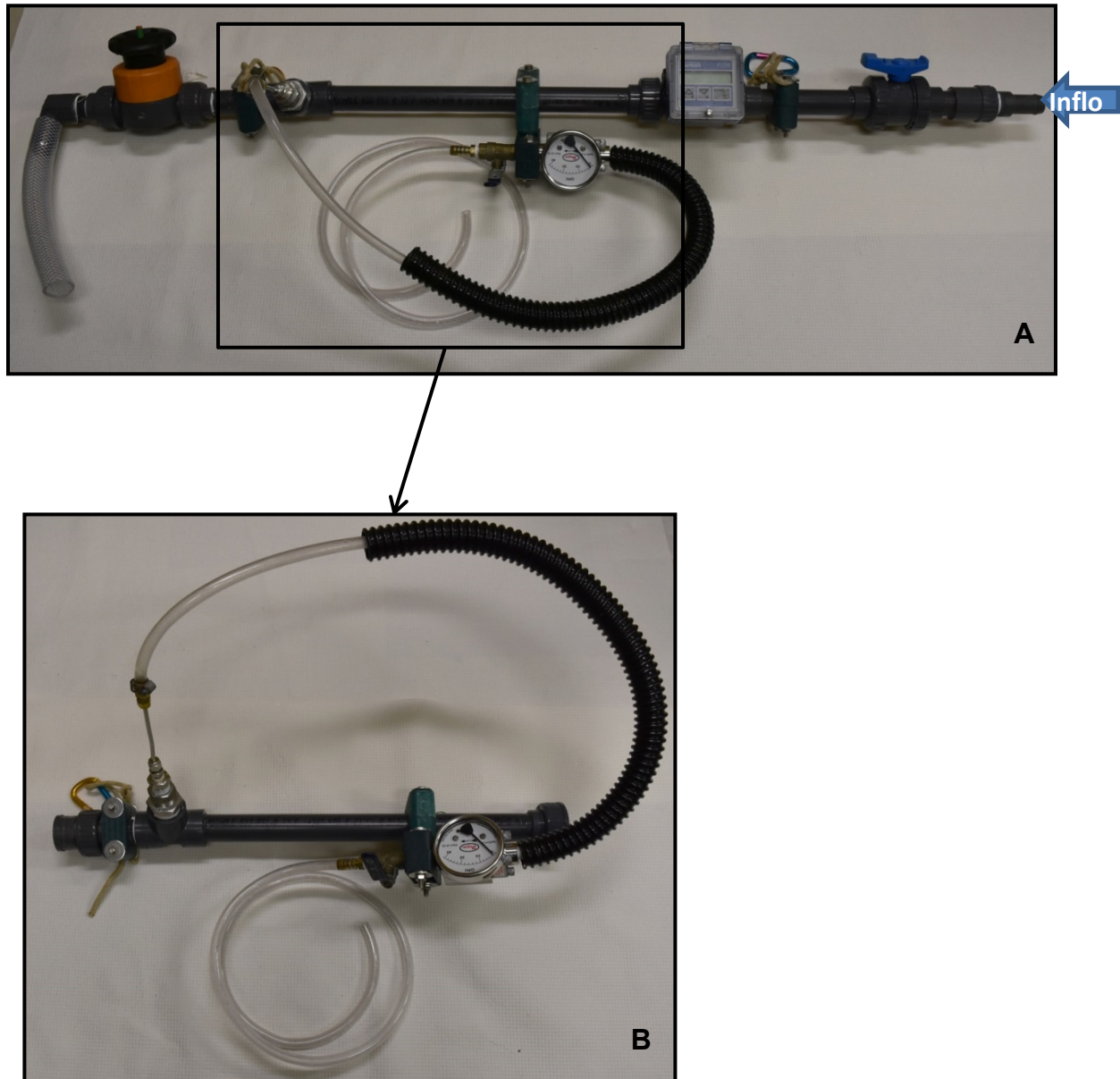
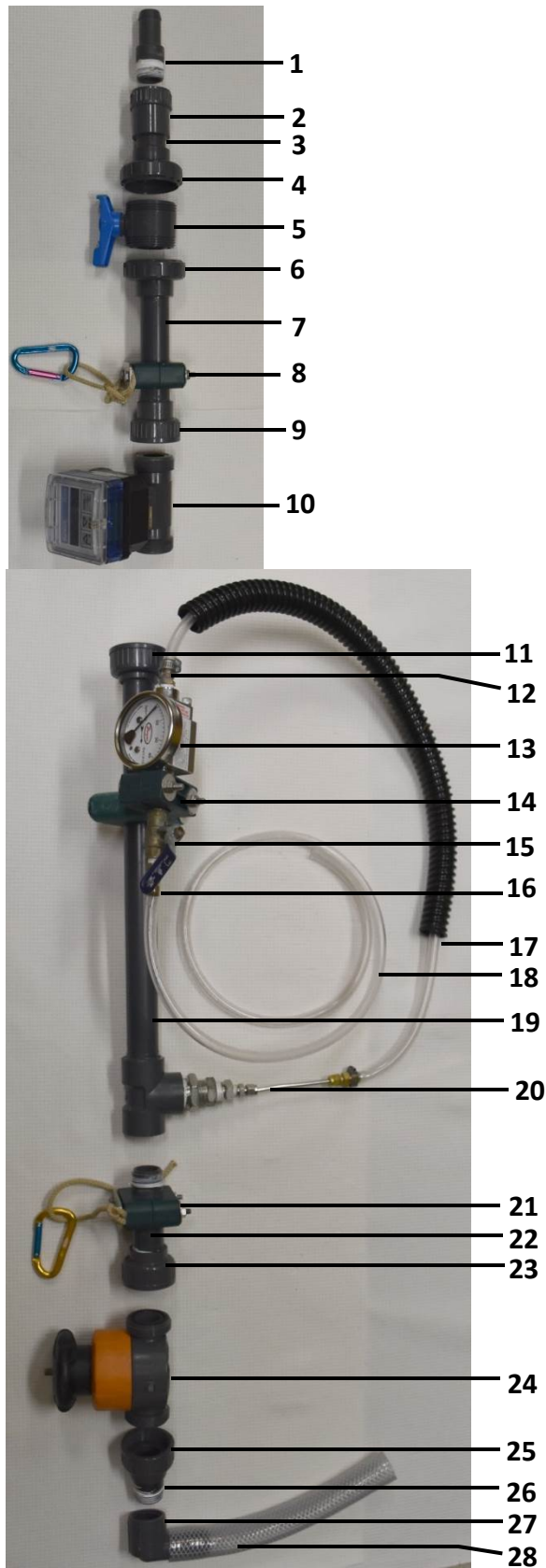
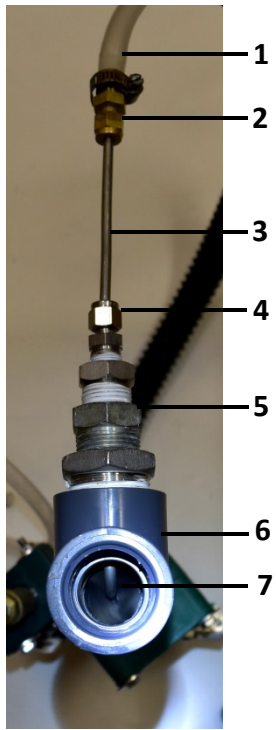


Figure 1. (A) The large volume sample collection device combined with small volume sample collection device (enlarged in inset photo B).



- 1 - 1-inch barbed hose fitting
- 2 - 1-inch female NPT adaptor
- 3 - 3 inches of 1-inch PVC pipe
- 4 - PVC union connection for ball valve
- 5 - 1-inch Ball valve
- 6 - PVC union connection for ball valve
- 7 - 7 inches of 1-inch PVC pipe
- 8 - mount to support sampling device while sampling
- 9 - 1-inch PVC union connection
- 10 - 1-inch Flow meter with monitor
- 11 - 1-inch PVC union connection
- 12 - ¼-inch barbed hose fitting
- 13 - ¼-inch flow meter
- 14 - mount for flow meter with ¼-inch x 2-inch length male NPT to male NPT nipple underneath
- 15 - ¼-inch ball valve with female NPT connections
- 16 - ¼-inch barbed hose fitting
- 17 - ¼-inch x 3 foot length ID tubing from 3-mm sample probe to flow meter fitted with 1-inch corrugated tubing for support
- 18 - ¼-inch x 5 foot length ID tubing to collection vessel
- 19 - 1-inch x 17-inch length PVC pipe
- 20 - 3-mm sample probe with compression fitting and adaptors to 1-inch fitted into a 1-inch 3-way Tee connection (see Fig 3. below)
- 21 - mount to support sampling device while sampling
- 22 - 1-inch x 4-inch PVC nipple
- 23 - 1-inch PVC union connection
- 24 - Diaphragm valve
- 25 - 1-inch PVC union connection
- 26 - 1-inch x 2-inch PVC nipple
- 27 - 1-inch, 90° barbed hose fitting
- 28 - 1-inch by 14-inch length hose

Figure 2. Large and small sample collection devices, disassembled, with parts labelled.



- 1 - ¼-inch ID tubing
- 2 - 1/8-inch compression fitting
- 3 - 3 mm sample probe
- 4 - 1/8-inch compression fitting
- 5 - adaptors from compression fitting to 1-inch
- 6 - 1-inch PVC Tee fitting

Figure 3. Small volume sample collection device showing 3 mm sample probe.



Figure 4. Photographs of sample filtration system using plankton nets.



Figure 5. Sample bin with bulk head connectors for out flow of sample ballast water into the bilge.