

## Development of a protocol for sampling and analysis of ballast water in Jamaica

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**Abstract:** The transfer of ballast by the international shipping industry has negatively impacted the environment. To design such a protocol for the area, the ballast water tanks of seven bulk cargo vessels entering a Jamaican port were sampled between January 28, 2010 and August 17, 2010. Vessels originated from five ports and used three main routes, some of which conducted ballast water exchange. Twenty-six preserved and 22 live replicate zooplankton samples were obtained. Abundance and richness were higher than at temperate ports. Exchange did not alter the biotic composition but reduced the abundance. Two of the live sample replicates, containing 31.67 and 16.75 viable individuals m<sup>-3</sup>, were non-compliant with the International Convention for the Control and Management of Ships' Ballast Water and Sediments. Approximately 12% of the species identified in the ballast water were present in the waters nearest the port in 1995 and 11% were present in the entire bay in 2005. The protocol designed from this study can be used to aid the establishment of a ballast water management system in the Caribbean or used as a foundation for the development of further protocols. *Rev. Biol. Trop.* 62 (Suppl. 3): 249-257. Epub 2014 September 01.

**Key words:** ballast water, zooplankton, protocol, Jamaica.

The transfer of ballast water allows a vessel to regulate its weight depending on how much cargo is being transported, in order to set the trim, list and overall stability of the vessel. However, such transfer by the international shipping industry has negatively impacted the health of humans, the environment as well as the economy of numerous countries worldwide as global markets encourage the global transfer of aquatic organism across natural barriers (Ruiz, Carlton, Grosholz & Hines, 1997). Monitoring of ballast water to be discharged in any particular country and the subsequent establishment of protocols will therefore be crucial to the future implementation of Ballast Water Management Systems (BWMS) on a large scale. Currently, no protocol has been described for the sampling of ballast water in any Caribbean territory

when most have rich biodiversity of flora and fauna, densely populated coastal towns and cities and economies that depend significantly on the ocean. A suitable protocol could be used to enforce compliance to standards from the International Maritime Organization (IMO) as well as to enable the Caribbean territories to focus their resources on fewer vessels that are more likely to introduce alien, invasive species or 'Harmful Aquatic Organisms and Pathogens' (HAOP). Such a protocol has to be simple and economical so as to be implemented in as many territories as possible.

Successful introduction and establishment of organisms by ballast water discharge in a new habitat is most likely to occur when such organisms are released alive and in high abundances (Hayes, 1998; Olenin, Gollasch,



Jonušas & Rimkutė, 2000) in a singular event. This situation is present amongst bulk cargo vessels which receive raw materials (dry bulk) at a particular port, and have a transfer time less than a month. The transfer time would be the time the vessel would take to leave their off-loading port (uptake ballast water) and reach the up-loading port (discharge ballast water). During this leg of the journey, vessels travel with ballast tanks filled to capacity and with empty cargo holds. Although the conditions within the tanks are harsh, hardy organisms can survive if the transfer time is short (David et al., 2007; Wetseyn & Vink, 2001), allowing the release of live stowaways. Due to the discharge of all the ballast tanks that arrived full, large numbers of live organisms tend to be released. The analysis of ballast water of tanks from these vessels will therefore characterize the greatest threat posed by ballast water transfer so as to prevent another invasion such as the *Perna viridis* in the Kingston Harbour, which was suspected to be introduced from discharged ballast water (Buddo et al., 2003).

The protocol to be outlined can be used to sample ballast water from cargo vessels throughout the Caribbean region. The types of access points, location and management, represent the range to be found on cargo ships that visit the region. Multiple teams can use this protocol to sample other tanks simultaneously.

## MATERIALS AND METHODS

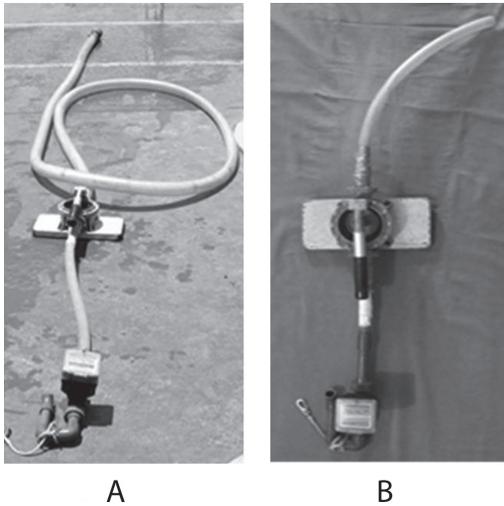
**The Protocol:** The protocol described below was conducted within a study that aimed to characterize the biotic components of ballast tanks that were to be discharged into a Jamaican port (Mitchell, 2012). It serves as a basic methodology that can be used when surveying other bulk cargo vessels at other Jamaican ports and similar ports within the Caribbean.

**Gaining access:** Being able to gain access to the sample is paramount to any study and is one of the steps used to carry out this protocol. Several considerations were included in gaining access to a ballast water sample, which

started with obtaining security clearance to the port of interest. Once obtained, the team requested and acquired permission to enter the port and board the vessel for each sampling occasion. The partnership of a liaison is crucial to the continuation of the study as the project can be explained and permission to sample requested from the Ship Master - Captain, Chief Officer, etc. (Ruiz & Smith, 2005) by someone that is trusted and respected by both sides. Once permission to sample was granted, the Ship Master had to give permission to access either the manhole or the sounding pipe. The Ship Master was also critical in providing information for the ballast water protocol form (Appendix) which was developed specifically for this research.

As advised by AQIS (1998), the designation of a at least one suitable tank (Ruiz & Smith, 2005) as well as the access point was carried out by the Ship Master. Although several starboard tanks were designated, port tanks were requested when the option was available as they were considered to be safer options for sampling as loading and off-loading occurs on the starboard side (Dodgshun & Handley, 1997). The sampling equipment and basic methodology for sampling ballast water from manholes, called Set up 1 (Fig. 1A) were developed from recommendations from MEPC (2005a). Set up 1 was comprised of a 6 m long reinforced hose with an inner diameter of five centimetres and a smooth inner surface. A foot check-valve was clamped to one end of the hose and a diaphragm pump clamped to the other end via a reducer. A second reinforced hose, 32cm long and 3 cm in outer diameter, with a smooth inner surface with 2.5cm in inner diameter, connected the pump to an inline flow meter. The outlet of the flow meter was directed to the opening of the cod-end (cylindrical bottle that receives water from the outlet of the pump and flow meter), which was fitted with a mesh with aperture of 50µm.

**Sampling the ballast water:** Set-up 2 (Fig. 1B) was used to collect samples via sounding pipes. It was designed from the



**Fig. 1.** (A) Set-up 1 and (B) Set-up 2. (A) Sampling equipment used to sample the manhole, consisting of a 20 ft reinforced hose, a diaphragm pump, a smaller reinforced hose and a flow meter. (B) Sampling equipment used to sample the sounding pipe; modified from Set-up 1, consisting of a small reinforced hose, duct tape, diaphragm pump and a flow meter.

**Fig. 1.** (A) Instalación 1 y (B) Instalación 2. (A) El equipo de muestreo utilizado para probar la boca de inspección, que consiste en una manguera de 20 pies, una bomba de diafragma, una manguera reforzada más pequeña y un medidor de flujo reforzados (B) Equipo de muestreo utilizado para el muestreo del tubo de sonda; modificado de instalación 1, que consta de una pequeña manguera reforzada, cinta aislante, bomba de diafragma y un caudalímetro.

equipment used to produce the original set-up (Set-up 1) but was comprised of the smaller hose described above being connected directly to the inline flow meter. The pump and flow meter were kept in the same orientation and as close together as possible. 75% of the 12 samples collected via sounding pipes (vessels 1, 4, 6 and 7) were sampled with Set-up 2. The other 25% were collected using an air-driven pump owned and operated by vessel 4. Vessels with sounding-pipe access should be encouraged to obtain such a pump to facilitate sampling as part of their compliance.

While the set-up of the equipment varied according to the type of access point available, (either a manhole or a sounding pipe),

both arrangements had the diaphragm pump ultimately leading to the flow meter. The equipment that was used to sample from a manhole access point varied according to the position and number of hoses used, with the reinforced hose #1 forming the inlet, fitted with a foot check valve at the end. The reinforced hose #2 was positioned between the pump and the flow meter. Reinforced hose #1 was graduated at 0.25m intervals so that the depth to which the hose was deployed could be known.

The manhole was the preferred access point, but it was not always available for sampling as the opening of manholes of Ballast tanks which are filled to capacity and are therefore pressurized, would not be safe (Sutton, Murphy, Martin & Hewitt, 1998). The sounding pipes were only sampled if the manhole was not available and the pressure within the tank allowed water to either overflow or remain at the top near the lid. There were four tanks which were accessed from pressurized manholes. Sampling of such tanks was done after the pressure was released, either by discharging a small volume of water from the tank outlet or by slowly releasing the cover of the manhole until the pressure was low enough to safely remove the manhole lid, the second of which was more time consuming. In one instance, the location of the released water was deemed to create an unsafe environment during the loading process, as explained by AQIS (1998). Therefore, the 5th top-side tank from the bow on the port-side (TST P 5) was used for sampling on subsequent voyages of that particular vessel. When sampling using the manhole, the depth at which the sample was retrieved was kept constant at relatively half of the maximum depth of the ballast tank (Ruiz & Smith, 2005). The entire equipment was flushed with ballast water from each tank for 2 - 5 mins (approximately 40 l) at the beginning of the retrieval of each sample (Dodgshun & Handley, 1997; Murphy, Ruiz & Sytsma, 2005) so as to remove any material from previous samples.

A 20L bucket was placed beneath the cod-end to collect the filtered water. Sampling was done in replicates of two. The filtered water for

each replicate was then transferred to a squeeze bottle (Dodgshun & Handley, 1997), which was used to wash the residue from the mesh into a 250mL plankton sample bottle, which did not contain any preservative. Dodgshun and Handley (1997). The salinity of the ballast water of each sample was tested using a refractometer by collecting a 2L bottle of filtrate and carrying it back to lab along with the plankton samples. This water could also be used for other chemical tests. The sample-set was therefore comprised of two replicates of live zooplankton and two replicates of preserved zooplankton. Each replicate was obtained by filtering a known volume of 15m<sup>3</sup> or 16m<sup>3</sup> of ballast water. Miller et al. (2011) supports the statistical accuracy of using several trials of 7m<sup>3</sup> each, when testing the level of compliance of ballast water to IMO viability standards once subjected to ballast water treatment systems. Separate cod-ends and meshes were used to collect the live and to-be-preserved replicates. The bottles were colour-coded with a blue tape for the live sample and with brown tape for the preserved sample. Labels were not completed on site so as to minimise use of ships time for sampling. Each sample set was collected in approximately forty minutes. Dodgshun and Handley (1997) expressed the importance of keeping the sample collection time to a minimum so as not to delay normal shipping operations. The impervious cone that was included in the Ballast Water Sampling Kit (MEPC, 2005a; Mitchell, 2012) was not used as the flow rates used in this study (9-20L min<sup>-1</sup>) did not require the precaution of reducing the velocity at which the sample left the flow meter. Additionally, the height of the cone prevented it from being fully extended at the sampling site.

**Sample analysis:** The samples were carried to the lab where processing was initiated within 1 hour after collection. The labelling of each bottle was then completed with sample number, type (live/preserved), replicate number, date of collection and the volume filtered. The live samples (Part I) were placed in the refrigerator at a temperature of 5°C which had

the effect of slowing down the metabolism of the specimens. This allows the live animals to survive until they can be processed as slowing them down also retarded predation. Part II of the samples was preserved in 8% formalin also within an hour of arriving at the lab. The ballast water collected after removal of organisms, was filtered further through a 0.7µm Glass Fibre filter paper and used for salinity determination and The analysis of the live samples was completed within 6 hours of its collection. Viable counts were conducted where viability was determined by motility. MEPC (2005b) indicated that viability could be determined by morphological change, mobility, staining using vital dyes or molecular techniques.

Counts were done in two stages starting with the larger and faster plankton, which were counted at x 60 magnification. At a higher magnification, such plankton would swim out of the field of view and may easily be recounted. The second stage was done at x 500 magnification, where much smaller plankton were counted easily within the field of view. Regulation D-2 of the compliance standards (IMO, 2011) only requires a viability count that indicates whether there are more or less than 10 viable organisms of 10µm and 50µm minimum dimensions per cubic meter. The collection and study of organisms with a minimum dimension of 10µm was outside the scope of this study. This count provides an estimate of the conditions within the tank, whether or not they are suitable to sustain a standard abundance of life. A total abundance count was not done for the live samples.

The preserved samples were analysed after the live sample analysis was completed. Total counts were done for each taxonomic group of zooplankton observed. Counts were not taken for empty exoskeletons and damaged organisms, thus excluding plankton that were unlikely to be alive within the ballast tank prior to sampling or preservation. Whole sample counts were done for 93% of the samples. The remaining 7% were two replicates from separate samples that were too dense and so sub samples were enumerated (1/8 and 1/4 of the samples, respectively). It was deemed

important to do as many complete counts as possible as the relative abundance of organisms in most of the samples was low. Each new organism was recorded by the use of codes, which were based on the first sample in which it was found, whether the sample was live-(L) or preserved-(P), the particular replicate and its order of appearance. The organisms observed were identified to the species level, where possible. This was difficult as many of the organisms were larvae of marine organisms which are difficult to identify to species (Sutton et al., 1998).

A reference collection of isolated specimens was created with each new specimen in small glass jars with glycerol and 10% formalin. The bottles were labelled with the specimen-code created, a short description, date, source details and collector's initials. Each specimen was either documented by notes, drawing, and photographs or by a combination. The following printed and web-source identification keys were used: Rammner (1939), Klie (1944a), Klie (1944b), Farran (1951), Davis (1955), Lovegrove (1956), Forneris (1957), Naylor (1957a), Naylor (1957b), Berzins (1960a), Berzins (1960b), Berzins (1960c), Berzins (1960d), Hadfield (1964), Ryland (1965), Be

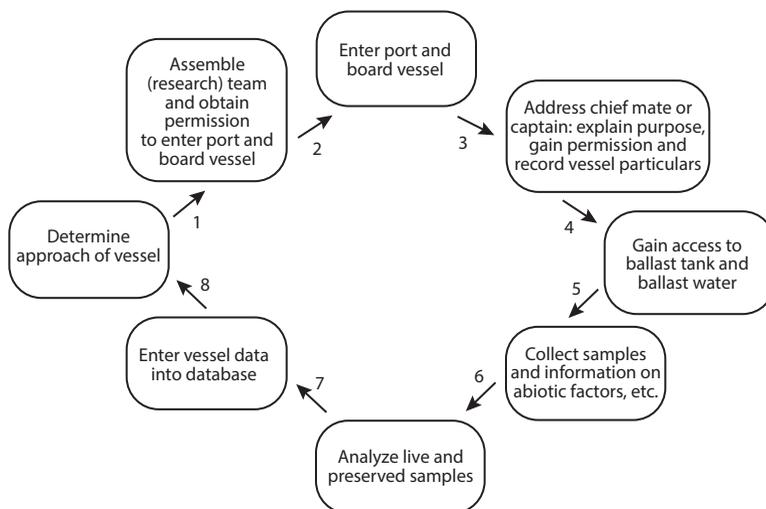
(1967), Bottazzi and Nencini (1969), Marshall (1969a), Marshall (1969b), Harding and Smith (1974), Newell and Newell (1977), Gerber (2000), Smith (2001), Wallace and Snell (2001), Razouls et al. (2005-2011).

Salinity readings were taken to determine if ballast water exchange (BWE) was conducted, as outlined in Regulation D-1 of the compliance standard of the convention (IMO, 2011). The salinity was determined by the use of an optical refractometer ( $\pm 0.01$  ppt). All data were added to spread-sheets for graphing and analysis. All information obtained on the particulars and procedures of the vessel was also added to the database.

## RESULTS

The protocol is represented as a cyclic flow chart (Fig. 2) which facilitates easy communication. The first step consists of making initial contact with the vessel as it prepares to dock to the recording of the data obtained, which is the final stage to be considered for that particular vessel.

Abundance and richness of the samples were higher than observed in ballast water from previous studies conducted at temperate



**Fig. 2.** Steps for the Ballast Water Sampling and Analysis Protocol.  
**Fig. 2.** Pasos para el Muestreo de Agua de Lastre y el Protocolo de Análisis.

TABLE 1  
Summary of results gleaned by using the Ballast Water Sampling and Analysis Protocol

CUADRO 1  
Resumen de los resultados del Muestreo de Agua de Lastre y el Protocolo de Análisis

	Protocol Data		Literature
Abundance	13 445 individuals (ind.)	1 349 individuals (ind.)	Drake and Lodge 2007
	448.167 ind. m <sup>-3</sup>	154.467 ind. m <sup>-3</sup>	David <i>et al.</i> 2007
	Copepoda, Mollusca, Cnidaria	Annelida, Mollusca, Crustacea (Copepoda, etc.)	
Richness	16 phyla	12 phyla, 11 invertebrate phyla	Chu <i>et al.</i> 1998 Locke <i>et al.</i> 1993
Impact of Source	Port-composition remained	Port-composition remained	Taylor and Bruce 1999
	Abundance was reduced	Abundance was reduced	Levings <i>et al.</i> 2004

ports. The total organisms counted in this study was 13 445 individuals and the density obtained was 448.167 individuals m<sup>-3</sup>. Therefore, the observed abundance is greater than the expected abundance. Ballast water exchange did not alter the biotic composition of the tank but it did reduce the abundance of organisms in the tank (Table 1). Fifteen phyla were identified consisting of nineteen taxonomic groups. The three most species-rich adult groups were Copepoda, then Rotifera, then Cladocera. Tintinnida was the only group that was more abundant than the group Copepoda when both adults and juveniles counts were combined. Two of the live sample replicates, containing 31.67 viable individuals m<sup>-3</sup> and 16.75 viable individuals m<sup>-3</sup>, were non-compliant with the Standard Regulation D-2 of the International Convention for the Control and Management of Ships' Ballast Water and Sediments. Approximately 12% of the species identified in the ballast water were present at the station nearest the port in 1995 and 11% were present in the entire bay in 2005.

Salinity readings fell within the expected range of the general salinity regime of the source point of the water. For instance, ballast water from tanks that were filled from within a river (not near the mouth) had a salinity that was also between 0-5 ppt. However, two samples did not have salinity readings that coincided with their source point. One sample had a reading of 9ppt with a source point

salinity regime of 36-40 ppt and a source port salinity regime of 18-35ppt. The other sample had a reading of 36ppt with a source point and port salinity regime of 0-5ppt.

## DISCUSSION

The discharge of ballast water containing viable organisms is considered the ultimate step in the transfer of species from ballast water operations. All mitigation strategies are therefore aimed at managing such an outcome, from reducing the likelihood of taking up threat species, the removal of such species either by exchange or by applying treatments aimed at reducing viability within the tank as well as reducing the likelihood of viable species. Therefore, any ballast water study should not be conducted without accessing the viability levels of the tanks sampled.

Obtaining permission from the shipping agencies involved was the most critical step as providing access to ballast water was voluntary due to the current absence of legislation about ballast water management and regulation in Jamaica. However, with or without legislation, once access to the port is obtained permission must still be sought from the Ship Master to sample any ballast tank and its contents (Dodgshun & Handley, 1997). Barring this, sampling of any ballast tank could not be done despite any arrangement carried out before.

The urgent need for protocols is indicated by the findings of this study, in that total number of organisms counted from Ballast tanks was 10 times higher than that of Drake & Lodge (2007b) who counted a total of 1 349 individuals. Densities were twice those previously reported by David et al. (2007) who found 154.467 individuals m<sup>-3</sup>. This may be due to the higher abundance and diversity of organisms within the tropics. Salinity variations in the ballast tanks also pointed to varied practices carried out by the vessel. Salinity is critical as it can confirm the carrying out of exchanges, especially where source ports are of very different salinities. All these findings point to the critical need for ballast water assessments to be conducted in the Caribbean and other tropical areas.

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#### RESUMEN

**Desarrollo de un protocolo de muestreo y análisis de agua de lastre en Jamaica.** La transferencia de lastre por el transporte marítimo internacional ha impactado negativamente el ambiente. Con el fin de diseñar un protocolo, los tanques de agua de lastre de siete barcos carga en el puerto jamaicano fueron muestreados entre el 28 de enero del 2010 y el 17 de agosto del 2010. Contenedores provenían de cinco puertos, utilizan tres rutas principales, algunos de los cuales conllevan un intercambio de agua de lastre. Se obtuvieron 26 muestras de zooplankton preservado y 22 vivo durante este periodo. La abundancia y riqueza de las muestras fue superior que en clima templado. El intercambio de agua de lastre no alteró la composición biótica pero disminuyó la abundancia. Dos de las muestras vivan, contenían 31.67 y 16.75 individuos viables m<sup>-3</sup>, no compatibles con el Reglamento D-2 estándar de la Convención Internacional para el control y manejo del agua de lastre y sedimentos de barcos. Aproximadamente el 12% de las especies identificadas en el agua de lastre estaban presentes en las aguas del puerto más cercano en 1995 y

11% estaban presentes en toda la bahía en el año 2005. El protocolo diseñado a partir de este estudio puede ser utilizado para ayudar al establecimiento de un sistema de gestión del agua de lastre en el Caribe o usado como una base para el desarrollo de futuros protocolos.

**Palabras clave:** agua de lastre, zooplankton, protocolo, Jamaica.

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TABLE 2  
Form used to record information from the vessel being sampled

CUADRO 2  
Formulario utilizado para registrar la información de la embarcación muestreada

BALLAST DATA FORM

Name of Vessel: \_\_\_\_\_

IMO number: \_\_\_\_\_

IMO certificate (if applicable): \_\_\_\_\_

Tank Type: \_\_\_\_\_

Tank Capacity: \_\_\_\_\_

Tank Volume: \_\_\_\_\_

Tank Depth: \_\_\_\_\_

Salinity: \_\_\_\_\_

Ballast water management plan: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Ballast Water Exchange Protocol: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Source- [Discrete: Mixed: Unknown]: \_\_\_\_\_

Time and date of uptake [Bay/Ocean]: \_\_\_\_\_  
\_\_\_\_\_

Previous ports:

1. \_\_\_\_\_

2. \_\_\_\_\_

3. \_\_\_\_\_

4. \_\_\_\_\_

Sampling method: \_\_\_\_\_

Volume of water sampled: \_\_\_\_\_

Stage of Discharged: \_\_\_\_\_

Voyage duration: \_\_\_\_\_ [DAYS]

