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All aboard! A biological survey of ballast water onboard vessels spanning the North Atlantic Ocean

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ABSTRACT

Global movement of nonindigenous species, within ballast water tanks across natural barriers, threatens coastal and estuarine ecosystem biodiversity. In 2012, the Port of Houston ranked 10th largest in the world and 2nd in the US (waterborne tonnage). Ballast water was collected from 13 vessels to genetically examine the eukaryotic microorganism diversity being discharged into the Port of Houston, Texas (USA). Vessels took ballast water onboard in North Atlantic Ocean between the Port of Malabo, Africa and Port of New Orleans, Louisiana, (USA). Twenty genera of Protists, Fungi and Animalia were identified from at least 10 phyla. Dinoflagellates were the most diverse and dominant identified (*Alexandrium, Exuviaella, Gyrodinium, Heterocapsa, Karlodinium, Pfiesteria and Scrippsiella*). We are reporting the first detection of Picobiliphytes, Apusozoa (*Amastigomonas*) and Sarcinomyces within ballast water. This study supports that global commerce by shipping contributes to long-distance transportation of eukaryotic microorganisms, increasing propagule pressure and invasion supply on ecosystems.

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1. Introduction

Over the last century, propagule pressure of non-indigenous (NIS) species has increased, especially in estuaries and coastal waters. Ballast water (BW) is thought to be the primary vector in dispersal and introduction of NIS species of aquatic organisms to ports around the world (Minton et al., 2005; Verling et al., 2005) and new invasion pathways are developing as a result of increasing trade and expanding shipping transport routes (Wonham, 2006). Shipping not only has the ability to increase the frequency but also the volume of these introductions (Lodge, 1993). BW transport from port to port has been attributed to the movement of organisms from their native habitat across natural barriers to new environments (Carlton and Geller, 1993; Drake et al., 2002; Hallegraeff, 1993; Smayda, 2002). After multiple introductions, a species is more likely to become an established NIS or even an invasive species (Wonham, 2006; Carlton, 1985). Zebra and quagga mussels and Chinese mitten crab have already become successful invaders as a result of BW discharge (Benson et al., 2012; Richerson, 2013; Cohen and Carlton, 1997). Although biodiversity may not increase or decrease in the area of interest the species composition may change from native to non-native biota. Maintaining the native biodiversity within natural systems is important to the resilience and the stability of the communities (Holling, 1973).

BW is taken onboard vessels to maintain vessel stability while in transit and then must be managed (i.e. exchanged) before being discharged into other coastal locations. BW exchange is conducted in overseas waters (>200 nm from any shoreline) to replace the biologically diverse coastal water in the BW tanks that may have been taken onboard in the port of origin. For the empty and refill method of BW exchange to be effective, 100% of the BW must be emptied in the open ocean before the tank can be refilled (USCG, 2012). For an efficient exchange utilizing the flow-through method, open ocean water equaling three times the volume of the ballast tank capacity must be pumped through the BW tank. When these management methods are conducted properly, 99% of the initial coastal water should be replaced and over 90% of the coastal zooplankton can be removed from the ballast tanks (Minton et al., 2005; Ruiz et al., 2000). However, there is an exception to the rule for management of BW. Vessels sailing within 200 nm from shore do not have to conduct BW exchange and can discharge their BW 'coastwise' directly into port.

With increased global distribution of phytoplankton via BW, the communities within port ecosystems have the potential to become altered and biotically homogenized (Drake and Lodge, 2004; Rahel, 2002). Invasive species of phytoplankton must undergo a three-step process before they can successfully invade a habitat

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including: a regional translocation (i.e. BW transport), colonization (i.e. algal bloom) and achievement of competitive dominance (i.e. native biota displaced by invasive species) (Smayda, 2002). For example, the movement and subsequent invasion of the dinoflagellate *Prorocentrum minimum* to new regions within the Baltic Sea is has been linked to BW dispersal (Olenina et al., 2010). This invasion of *P. minimum* was shown to displace the native phytoplankton community within the Baltic Sea (Olenina et al., 2010). *P. minimum*, as many other types of phytoplankton, is capable of tolerating a wide range of salinities as well as producing viable cysts after spending time (>3 months) in unfavorable conditions such as those in a ballast tank (Grzebyk et al., 1997). Dinoflagellates can enter a resting or dormant cyst stage in unfavorable conditions, and/or can switch feeding modes (autotrophic ↔ mixotrophic ↔ heterotrophic) increasing their ability to survive long journeys within ballast tanks (Hallegraeff, 1998; Hallegraeff and Bolch, 1992). Cysts allow dinoflagellates to withstand environmental changes, remaining viable for up to 10 years or more until growth conditions are favorable again (Ribeiro et al., 2011). Not all diatoms and dinoflagellates in the vegetative life stage are capable of tolerating the same variations in environmental conditions that they can while in the cyst stage. The generation time of phytoplankton typically will last from hours to a few days. If the phytoplankton enters into the cyst life stage, they can remain viable for upwards of 100 years (Ribeiro et al., 2011).

Galveston Bay is the largest estuary in Texas (Gulf of Mexico, USA), and is highly productive in terms of oyster and seafood production (brown and white shrimp, blue crabs and oysters) second only to Chesapeake Bay in the US (Martin et al., 1996; Lester and Gonzalez, 2011). Studies in this ecosystem are important given the frequency and magnitude of ship traffic and BW discharge into its three ports (Galveston, Houston and Texas City). Steichen et al. (2012) reported that more than 45,000 vessels traveled across Galveston Bay between 2005 and 2010, discharging a total of 1.2×10^8 metric tons of BW into the Bay itself. BW discharge was found to be an important propagule source of dinoflagellates based on the origin of vessels arriving to Galveston Bay from both domestic and foreign ports of origin (Steichen et al., 2012). Galveston Bay receives more BW discharge than both Chesapeake and San Francisco Bays combined (Steichen et al., 2012; Steichen, 2013). This is important considering the Chesapeake and San Francisco bays are two highly invaded estuarine systems (Cohen and Carlton, 1995, 1998).

The goal of this study was to identify the eukaryotic diversity (18S rDNA community primer) including diatoms and dinoflagellates (18s rDNA specific primers), within BW tanks of vessels entering Galveston Bay. To accomplish this, we examined BW from vessels crossing the northern Atlantic Ocean from the Port of Malabo on the West African coast to the Port of New Orleans, Louisiana, (USA). A highly diverse aquatic eukaryotic community, from diatoms and dinoflagellates, to fungi and copepods, was revealed from within the BWs tanks of vessels entering Galveston Bay.

2. Methods

2.1. Sample collection

A shipping agent working at a terminal within the POH collected BW samples from vessels. The shipping agent communicated with the vessel captains and BW samples were given on a voluntary basis. Vessels were sampled at various times between May 2007 and March 2010 (Table 1). Per request, the shipping agent and the identity of the vessels remain anonymous. The captains of provided a BW report regarding the time and location (latitude, longitude) of where the BW was taken onboard. All ships sampled were general cargo vessels. Ships were labeled S1 through S13 corresponding to the location where BW exchanges occurred prior to entering the POH is shown in Fig. 1. The vessels that were sampled in the POH had traveled westward across the North Atlantic Ocean (Fig. 1; Table 1). Samples were collected in a dark acid-washed container and placed on ice for transport to the laboratory. The BW samples were filtered onto a 0.22 μm Sterivex GP (Millepore) cartridge filter using a Masterflex peristaltic pump and tubing (Cole Parmer Instrument Company, Vernon Hills, IL). The filter was stored at −80 °C until DNA extraction was performed. Salinity of the BW sample was measured using a refractometer; all salinity results will be presented on the unit-less practical salinity scale (Table 1).

2.2. Classification of coastwise or overseas BW

BW is managed or exchanged in various locations within the coastal and open ocean environments. The National Ballast Information Clearinghouse (NBIC) has developed two categories to better describe the origin of BW. When BW management is conducted or BW is taken onboard a vessel within the Exclusive Economic Zone (EEZ; <200 nm of any shoreline) the BW is termed "coastwise". Vessels that take on or manage BW beyond the EEZ (>200 nm from a shoreline) the BW is termed "overseas". These two categories are based on the definitions used in BW reports submitted and cataloged by the National Ballast Information Clearinghouse of coastwise and overseas. This criterion was applied to the BW samples in this study (Table 1).

2.3. Genetic analysis

2.3.1. Extraction of nucleic acids

Genomic DNA was extracted from filters with a cetyltrimethylammonium-bromide (CTAB; 3%)-chloroform isoamyl-alcohol method modified from Doyle and Doyle (1987). Quality and quantity of DNA was determined spectrophotometrically (Nanodrop-1000 spectrophotometer).

2.3.2. PCR amplification of 18S rDNA

PCR reactions were performed in 50 μL volumes containing approximately 150 ng of template DNA, 10× PCR reaction buffer with 15 mM $MgCl_2$ (Roche Applied Science, Manheim, Germany), 50 μM of each deoxynucleotide, 0.1% bovine serum albumin 1 U Roche *Taq* DNA polymerase (Roche Applied Science, Manheim, Germany), 10 μM of each primer, and 0.5 μL dimethyl sulfoxide. PCR cycling was conducted using an Eppendorf Mastercycler gradient thermal cycler. Primer sets were selected based on the proven success in amplifying target DNA from environmental water samples and they can be used to identify a wide range of organisms (Giovannoni et al., 1988; Godhe et al., 2008; Oldach et al., 2000; van Hannen et al., 1998; Wang et al., 2005). The primer sets used were designed to identify organisms to the phyla level and in some cases the quality of the DNA allowed resolution to species level (van Hannen et al., 1998). In addition to targeting the more general aquatic eukaryotic community we wanted to target dinoflagellates and diatoms more specifically.

To identify the aquatic eukaryotic community, we used the primer set developed by van Hannen et al. (1998): 1427F (5′-TCTGTGATGCCCTTAGATGTTCTGGG-3′) with a 40-bp GC-rich clamp and 1616R (5′-GCGGTGTGTACAAAGGGCAGGG-3′). The PCR temperature cycling conditions for the 1427F/1616R primer set were: 1 denaturing step at 94 °C for 5 min followed by 25 cycles of 94 °C for 0.5 min, 52 °C for 1 min and 68 °C for 1.5 min and a final extension step of 68 °C for 10 min (van Hannen et al., 1998).

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