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Assessing the viability of microorganisms in the ballast water of vessels transiting the North Atlantic Ocean

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ABSTRACT

Testing phytoplankton viability within ballast tanks and receiving waters of ballast water discharge remain understudied. Potentially harmful dinoflagellates and diatoms are transported via ballast water to Galveston Bay, Texas (USA), home to three major ports: Houston, Texas City and Galveston. Ballast water from vessels transiting the North Atlantic Ocean was inoculated into treatments representing low and high salinity conditions similar to the Ports of Houston and Galveston respectively. Phytoplankton in ballast water growout experiments were deemed viable and showed growth in low and mid salinities with nutrient enrichment. Molecular methods identified several genera: *Dinophysis, Gymnodinium, Gyrodinium, Heterocapsa, Peridinium, Scrippsiella, Chaetoceros* and *Nitzschia*. These phytoplankton genera were previously identified in Galveston Bay except *Scrippsiella*. Phytoplankton, including those capable of forming harmful algal blooms leading to fish and shellfish kills, are transported to Galveston Bay via ballast water, and are viable when introduced to similar salinity conditions found in Galveston Bay ports.

1. Introduction

The introduction of invasive species to new regions via ballast water (BW) has caused detrimental impacts to coastal communities and ecosystems (Ruiz et al., 1997, 2015; Gollasch et al., 2000, 2015; Carlton and Ruiz, 2003; Muirhead et al., 2014). Organisms such as bacteria, phytoplankton, and ciliates, are frequently transported across natural barriers via BW and, if conditions are favorable may become invasive species (Zaiko et al., 2015; Burkholder et al., 2007; Drake et al., 2002; Smayda, 2002). Most notably invaders transferred via BW include: the European zebra mussel (*Dreissena polymorpha*) in the Great Lakes, USA (Hebert et al., 1989), the Atlantic comb jelly (*Mnemiopsis leidyi*) in the Black Sea (Pereladov, 1983) and the toxic dinoflagellate (*Gymnodinium catenatum*) in the Pacific Ocean near Tasmania, Australia (Hallegraeff and Bolch, 1992).

Many studies have examined the biological community within BW while the vessels are underway (Lavoie et al., 1999; Gollasch et al., 2000; Burkholder et al., 2007). More recently research has started to focus on species viability (ex. phytoplankton) when introduced to the receiving waters (Zaiko et al., 2015; Baek et al., 2011; Kang et al., 2010; Pertola et al., 2006). Survival and subsequent success of organisms post BW discharge, increase when donor and receiving regions are environmentally similar as seen in coastal ports located along similar latitudes (Carlton, 1996; Vermeij, 1991).

BW transfer facilitates the dispersal of nonindigenous across environmental filters or barriers (ex. water circulation inter-specific competition) that would naturally prevent their distribution (Colautti and MacIsaac, 2004). Phytoplankton species taken onboard a vessel are bypassing the first natural filter of dispersal. The transport of viable cells to a new region via BW represents the bypassing of the second stage. Once organisms are discharged into new waters, surviving, adapting (to biotic and abiotic factors) and reproducing and must take place to result in a successful establishment (Ono et al., 2000). By assessing the viability of introduced species in conditions post-BW discharge, we strive to further understand the survivability of organisms which may pose invasion threat.

The population in Texas (USA) is expected to double by 2050, with coastal communities experiencing the bulk of this growth (TWDB, 2007). With this development, comes an increase in impervious surfaces, more septic and/or waste water treatment plant discharge, elevated groundwater nutrients, increased atmospheric deposition from transportation sources, etc., all leading to increased runoff and subsequent elevated nutrient loading into the bays and estuaries (Quigg et al., 2009; Greene et al., 2014; Dorado et al., 2015). With this increase in urban development and changes in land use comes the challenge of managing eutrophication in Galveston Bay, the largest and most commercially and recreationally important estuary in Texas

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(Lester and Gonzalez, 2011). Changes in nutrient availability may enhance the opportunity for non-indigenous phytoplankton species to successfully establish in Galveston. In addition to the increased growth in the region, there is a predicted 78% increase in the median total BW discharge after the completion of the Panama Canal expansion in early 2016 (Muirhead et al., 2014). With this increased BW discharge there is an expected rise in the likelihood of introductions of nonindigenous species along the Gulf coast (Muirhead et al., 2014). Introduced species of phytoplankton have the potential to become invasive causing harmful algal blooms, impacting ecosystem services, including the productive oyster and fishing industries (Lester and Gonzalez, 2011; Quigg, 2011). Steichen et al. (2015) recently provided the first reporting of two dinoflagellates (*Takayama* and *Wolozynskia*) in the Bay. While it is not certain these genera were introduced to Galveston Bay via BW, similar concerns have been reported worldwide, especially in those estuaries which are home to major ports (Bax et al., 2003; Ruiz et al., 2015).

Galveston Bay is home to 3 deep-water ports including: the Port of Houston which is the 10th largest port in the world and 2nd largest in the US in terms of overall waterborne tonnage in 2012, the Port of Texas City (8th largest in the US), and the Port of Galveston (see Steichen et al., 2012 for more details). Another challenge facing Galveston Bay is the expansion of the Panama Canal, set for completion in 2016; significantly larger vessels will enter the bay with greater frequency and after shorter transit times. Due to the increased carrying capacity of these Post-Panamax vessels (i.e. supertankers and larger container vessels), larger volumes of BW will be discharged per event with a corresponding increased number of propagules, increasing the potential success rate of introduced species (Casas-Monroy et al., 2015; Muirhead et al., 2014; Ruiz et al., 2015).

In this study, we report that phytoplankton transported to Galveston Bay via BW were viable in a number of treatments where BW was combined with water of lower salinity. Molecular approaches were utilized to target the dinoflagellates and diatoms from the growout treatments and BW samples. Phytoplankton growth increased when introduced to waters of lower salinity and higher nutrient concentrations relative to the waters within the ballast tanks. When larger volumes of BW were introduced to our treatments (simulating increased propagule number), there was a parallel increase in the overall phytoplankton biomass.

2. Methods

2.1. Sample collection

The initial BW sample was collected by a shipping agent who collaborated with the vessel captains that volunteered to provide a BW sample. One (1) 20 L BW sample was collected from each vessel for each respective experiment. BW for all treatments within each of the three experiments was an aliquot from the initial 20 L BW sample. The sampled vessels had conducted a BW exchange in the North Atlantic Ocean while en route to the Port of Houston from the Port of Malabo, (Malabo, Equatorial Guinea, Western Africa): GE1 (14°04′N, 068°51′W), GE2 (01°37′N, 032°39′W) and GE3 (05°55′N, 021°55′W) (Fig. 1). BW age at the time of sampling ranged from GE1: 49 days, GE2: 16 days and GE3: 20 days. BW samples were collected in dark, acid-washed containers and retrieved within 24 h of collection and transported to the laboratory on ice. BW salinity was measured with a refractometer and reported using the unit-less practical salinity scale. Salinities of the BW samples ranged from 30 to 38 on a unitless scale (Table 1). Mean salinities of Port of Houston and Port of Galveston 'during the study period (2007–2009) were 10 ($\pm$5.22; $n = 20$) and 28 ($\pm$4.60; $n = 18$) and therefore used for the low salinity (LS) and high salinity (HS) treatments respectively (see Steichen et al., 2014).

2.2. Phytoplankton growout experiments (GEs)

GEs were designed to test the growth of phytoplankton in ballast tanks when exposed to changing salinity and nutrient regimes. These are analogous to nutrient addition bioassay or resource limitation bioassays performed to assess nutrient limitation (Fisher et al., 1999; Quigg, 2011). Low and high salinity treatments represent average salinities observed in the Port of Houston (avg. salinity 10) and Port of Galveston (avg. salinity 28) respectively (Steichen et al., 2014). The San Jacinto River flows directly into the Port of Houston producing higher average nutrient concentration and lower salinities compared to Port of Galveston (~25 miles south in Galveston Bay) which is more influenced by the Gulf of Mexico (Fig. 1). Gulf seawater was pumped to our facility (average salinity = 33) and filtered through a 0.22 μm Sterivex cartridge filter and autoclaved (121 °C; 40 min). Sterile distilled water was used to dilute the higher salinity gulf water for the lower and high salinity

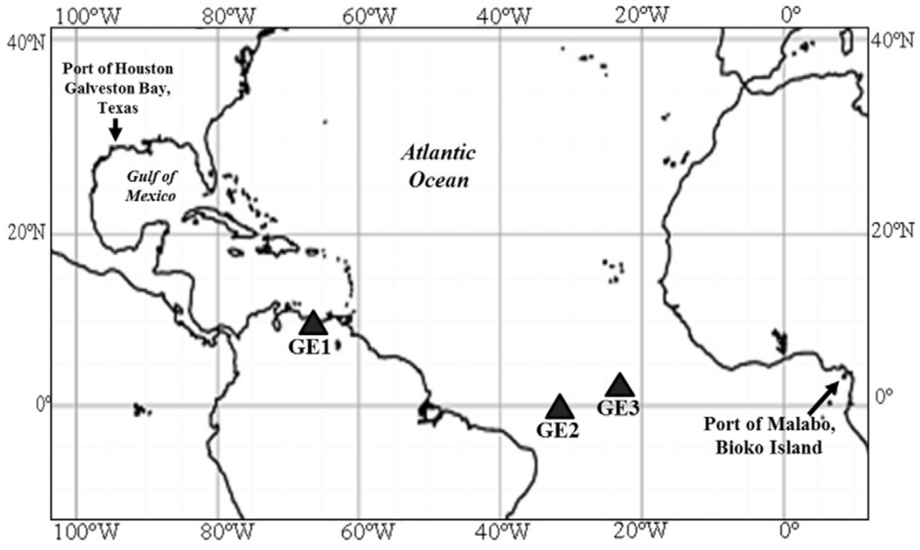

Fig. 1. Map showing the location of ballast water exchange before BW water sample were collected from each vessel. The location where BW was exchanged include: GE1 (14°04′N, 068°51′W), GE2 (01°37′N, 032°39′W) and GE3 (05°55′N, 021°55′W). Apex of the black triangle indicates reported location of ballast exchange. The Port of Houston (29°36′39.96″N; 95°1′18.12″W) and Port of Malabo (3°46′35.4″N/8°45′19.8″E) are shown by black arrows.