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The efficiency of a new hydrodynamic cavitation pilot system on *Artemia salina* cysts and natural population of copepods and bacteria under controlled mesocosm conditions

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ABSTRACT

A study of the efficiency of hydrodynamic cavitation and separation was carried out to evaluate an innovative, environmentally safe and acceptable system for ballast water treatment for reducing the risk of introducing non-native species worldwide. Mesocosm experiments were performed to assess the morphological changes and viability of zooplankton (copepods), *Artemia salina* cysts, and the growth potential of marine bacteria after the hydrodynamic cavitation treatment with a different number of cycles. Our preliminary results confirmed the significant efficiency of the treatment since more than 98% of the copepods and *A. salina* cysts were damaged, in comparison with the initial population. The efficiency increased with the number of the hydrodynamic cavitation cycles, or in combination with a separation technique for cysts. There was also a significant decrease in bacterial abundance and growth rate, compared to the initial number and growth potential.

1. Introduction

Ballast water is considered, together with hull fouling and aquaculture, as the greatest threat to aquatic environments because of the worldwide transfer of invasive aquatic species between continents and regional seas (David et al., 2013). Recently, more than 986 non-indigenous species (NIS) have been added to the list for the Mediterranean Sea (Zenetos et al., 2012), but only 12 species were said to be directly introduced by ships. However, shipping is assumed to be an important pathway of introduction of another 300 species via ballast water or fouling. Global ballast water discharges in 2013 are estimated to amount to about 3.1 billion tons per year (David, 2014), and a ship's ballast tanks can transfer at least 10,000 different species worldwide (Faimali et al., 2006). Different kinds of non-indigenous organisms can spread (vertebrates, invertebrates, plants, microscopic algae, bacteria, etc.) (Ruiz et al., 2000; Mimura et al., 2005; Khandeparker and Chandrashekar Anil, 2013), and invasions are almost irreversible and could have a negative impact on aquatic ecosystems (Endresen et al., 2004; Flagella and Abdulla, 2005; Flagella et al., 2007; Gollasch, 2007; Kang et al., 2010). They also act as a dispersal mechanism for different human pathogens, such as *Vibrio cholerae* (Seiden and Rivkin, 2014), and could be a significant source of ecological and human risks.

The current international regulations and recommended methods for Ballast Water Management (BWM) were prepared by the International Maritime Organization (IMO) and are known as the BWM Convention 'Convention for the Control and Management of Ships' Ballast Water and Sediments.' The BWM Convention regulates the ways and methods of discharging ballast water from ships, and the methods of ballast water management (IMO, 2004; Bakalar, 2014; IMO, 2014; Lloyd's Register Group Limited, 2014). The Ballast Water Exchange (BWE) Standard (Regulation D-1) includes guidelines and requirements for ships to meet standards with an efficient exchange of ballast water with ocean water (Endresen et al., 2004; ABS, 2010). According to Regulation D-2 of the Ballast Water Management Convention, ships may discharge ballast water when the concentrations of organisms with a minimum dimension of 50 μm or greater are less than 10 viable organisms m^{-3}, less than 10 viable organisms mL^{-1} in the size between 50 and 10 μm, and less than the prescribed concentrations of indicator microbes (*V. cholerae*, *Escherichia coli* and intestinal enterococci). As the IMO's Convention has not yet entered into force, ballast water exchange is still the most widely used method of ballast water management. This method is limited by certain safety issues or geographical conditions, and is not effective to protect ballast water recipient environments from organism introductions. Although ballast water exchange is supposed to reduce some planktonic organisms by 80–95% (Seiden and Rivkin, 2014), it is difficult to reduce the number of bacteria (Seiden et al., 2011; Drake et al., 2007; Hess-Erga et al., 2010; Fykse et al., 2012). Few studies reported the abundances of the

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heterotrophic bacteria and viruses in ballast water tanks (Gollasch et al., 2000a; Ruiz et al., 2000; Burkholder et al., 2007; Ma et al., 2009; Seiden et al., 2010, 2011; Seiden and Rivkin, 2014) and changes in microbial community composition (Drake et al., 2007; Mimura et al., 2005; Seiden et al., 2010; Seiden and Rivkin, 2014; Tomaru et al., 2014). The changes in microbial community composition represent an issue which is necessary to investigate for better understanding of the potential risks associated with the transport of microorganisms in ballast water tanks and complexity of ballast water management. Drake et al. (2007), Seiden et al. (2010), Tomaru et al. (2014), Seiden et al. (2010) and Seiden and coworkers (2011) have monitored bacterial dynamics during transit, and have shown a correlation of bacterial abundance with physical factors such as temperature, dissolved oxygen concentrations, and salinity. The composition of the prokaryotic community in the ballast tank might depend on the global biogeography (Martiny et al., 2006; Ramette and Tiedje, 2007), as well as the weather conditions (Neyland, 2009).

Dominant zooplankton groups determined in ballast water tanks are holo-, mero-, and tychoplankton, and among holoplankton copepods are the most common (Gollasch et al., 2000b; Choi et al., 2005; David et al., 2007; Gruszka et al., 2013). Moreover, Gollasch et al. (2000a) emphasized that harpacticoid copepods are capable of thriving and reproducing in ballast water. Therefore, ballast water tanks can act as incubators for some copepod species during voyages and might have a serious impact on releasing the NIS in coastal waters, bays, or ports (Gollasch et al., 2000a). The cysts in natural marine habitats vary in size and structures, depending on different environmental, physical, chemical, and biological conditions (Paul, 2001; Chen et al., 2011). Transport of long-lived, resistant cysts, especially cysts of some toxic species (e.g. dinoflagellate cysts) (Hamer et al., 2000) by ships became one of the reasons of rising concerns related to global spreading of NIS to new geographical regions. Such species are able to produce a number of compounds which can be accumulated in the food chain and consequently cause different human diseases (Hallegraf, 1995; Hamer et al., 2000).

Nowadays, researchers are striving to develop different ballast water treatment technologies with the aim of finding an appropriate solution to reduce the risk of unwanted invasions to the greatest extent. Today, very different technologies are used, alone or in combination (Perrins et al., 2006; McCollin et al., 2007; Holm et al., 2008; Gregg et al., 2009; Liebich et al., 2012; IMO, 2014; Lloyd's Register Group Limited, 2014). All technologies have advantages and disadvantages: common environmentally dangerous effects, high installation costs, high energy consumption, complexity, problems in performance and installation on ship, or even the inability to damage certain organisms. (Gregg et al., 2009; Werschkun et al., 2014).

One of the relatively new methods used in ballast water treatment is hydrodynamic cavitation (Sawant et al., 2008; Cvetković et al., 2015). Despite the fact that hydrodynamic cavitation breaks the cells of unicellular or multicellular organisms, and therefore represents a well-known and widely used method in science, engineering, and different industrial processes (Gogate, 2002; Sawant et al., 2008; Brujan, 2011; Ozonek, 2012), it is still insufficiently explored and applied for ballast water treatment. Only a few authors (Kato, 2003; Sawant et al., 2008; Ranade et al., 2009) have carried out experiments with different pilot devices in different working conditions to determine the effectiveness of hydrodynamic cavitation on different kinds of aquatic organisms. Four types of the ballast water treatment systems using hydrodynamic cavitation were approved by the Administration for fitting systems on board to date (IMO, 2014; Lloyd's Register Group Limited, 2014; Cvetković et al., 2015). These systems used cavitation mainly as a step in the treatment, and are usually combined with different chemicals to increase the efficiency of the treatment. Although they meet the regulations prescribed by IMO, mainly because of the addition of chemicals, such technologies have a negative impact on the environment (influence on aquatic organisms), economy (high costs and its maintenance, high energy consumption), and safety (special education is needed for the crew to handle the devices and the chemicals) (Joo-won, 2010; Lloyd's Register Group Limited, 2014).

The objective of this research was to test the effectiveness of a developed and innovative ballast water treatment pilot system that is based on a combination of hydrodynamic cavitation technology and a separation phase (as pre-treatment phase). Our goal was to develop an economical, ecological, safe and acceptable treatment that would also be able to meet the limited working conditions required on ships (especially low working pressures which rely on the properties of the ship's pumps). We tested the effectiveness of the pilot system on assessing the viability of different key marine organisms after the treatment. The natural zooplankton samples (copepods) were chosen as test organisms — representatives for multicellular organisms, the cultured *Artemia salina* cysts as representatives for the resting stages of organisms, and the natural bacteria as the representatives of unicellular organisms.

2. Materials and methods

2.1. The pilot system for ballast water treatment

The pilot system for ballast water treatment consists of the following parts: a container for the storage of sea water (mesocosm), a centrifugal pump, pipes for water circulation, and a chamber for hydrodynamic cavitation (Fig. 1). The seawater from the mesocosm was pumped through the pipes to the chamber for the hydrodynamic cavitation treatment (Fig. 1) and was discharged back to the container after each cycle. The total volume of seawater treated with the pilot system was 150 L (100 L in the mesocosm and 50 L in the chamber and pipes). The pressure on the entrance of the chamber for the hydrodynamic cavitation varied between 1.8 and 2.8 bar (0.8 and 1.8 bar in the relative value). The flow rate of the seawater through the system was 15 $m^3\ h^{-1}$, and each cycle lasted 0.6 min. The entire amount of seawater (150 L) re-circulates through the system during one experiment. For each experiment 100 cycles were performed per hour. The system may operate with or without the separation phase (separator), which has an outlet at the bottom of the chamber and whose openness is adjusted by the valves ('open valve' means the open process of separation). The water enters tangentially into the chamber, and due to the formation of the centrifugal and gravitational forces within the chamber, all particles with a density greater than water are suppressed by the vortex inside the chamber towards the wall of the chamber. They continue to glide down the wall, and when the separation phase is open they are ejected through the outlet at the bottom of the system. The rest of the particles, which had not been previously ejected through the outlet, pass through the mechanism for generation of the hydrodynamic cavitation. After the hydrodynamic treatment, water returns through the central tube to the mesocosm.

2.2. Experimental set-up

The efficiency of the pilot system was tested on the natural population of planktonic marine organisms in seawater collected in the Gulf of Trieste (northern Adriatic Sea). Experiments for each size fraction of plankton organism were performed separately, under identical technical parameters of the pilot system during the hydrodynamic cavitation process in all experiments (see Section 2.1.). Altogether, five experiments with zooplankton samples, six experiments with *A. salina* (Crustacea, Anostraca) cysts, and six experiments for bacteria were carried out. The first experiments were performed in October 2013 and repeated in May 2014.

Before each experiment, the 150 L mesocosm was filled with seawater (it took about 15 min to fill the mesocosm), was then well mixed,