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# Sonication of bacteria, phytoplankton and zooplankton: Application to treatment of ballast water

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**Abstract**

We investigated the effect of high power ultrasound, at a frequency of 19 kHz, on the survival of bacteria, phytoplankton and zooplankton, in order to obtain estimates of effective exposure times and energy densities that could be applied to design of ultrasonic treatment systems for ballast water. Efficacy of ultrasonic treatment varied with the size of the test organism. Zooplankton required only 3–9 s of exposure time and 6–19 J/mL of ultrasonic energy to realize a 90% reduction in survival. In contrast, decimal reduction times for bacteria and phytoplankton ranged from 1 to 22 min, and decimal reduction energy densities from 31 to 1240 J/mL. Our results suggest that stand-alone ultrasonic treatment systems for ballast water, operating at 19–20 kHz, may be effective for planktonic organisms >100 μm in size, but smaller planktonic organisms such as phytoplankton and bacteria will require treatment by an additional or alternative system.


*Keywords:* Ultrasound; Ballast water treatment; Bacteria; Phytoplankton; Zooplankton

## 1. Introduction

Nonindigenous vertebrates, invertebrates, plants, algae, bacteria and viruses can all be transported as contents of ships' ballast tanks or cargo holds (for example, Williams et al., 1988; Carlton and Geller, 1993; Smith et al., 1996; Ruiz et al., 2000; Drake et al., 2002). Once introduced, these species can become pests and may do significant harm to marine environments, energy and food supplies, and local economies (see Ruiz et al., 1999; for review). Concern over the impacts of nonindigenous aquatic species has spurred the development of management and treatment methods and regulations, designed to slow or stop the transfer of invaders in ballast water.

Currently, exchange is the most widely applied 'treatment' for ballast water. In ballast water exchange, ballast taken on in coastal areas is replaced with ocean water that is loaded while the vessel is in transit between ports (National Research Council, 1996; Minton et al., 2005; Wonham et al., 2005). The purpose of the exchange is to remove from the ballast tank coastal organisms originating in the departure port, and replace them with oceanic organisms, which may not survive when released in the coastal or fresh waters of the destination port (National Research Council, 1996). Thus, the process of exchange is not necessarily intended to alter the concentration of organisms in a tank, but instead to affect the species structure of the tank community. Results to date suggest that exchange has highly variable effects on the abundance (as opposed to the community structure) of zooplankton, phytoplankton and bacteria found in ballast water (for example, Locke et al., 1991; Smith et al., 1996; Dickman and

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Zhang, 1999; Zhang and Dickman, 1999; Wonham et al., 2001; Drake et al., 2002).

Given that exchange may cause no predictable reduction in the concentration of organisms in ballast water, it would appear to be an unacceptable treatment option for vessels facing the spectre of recently proposed regulations for ballast discharge. The International Maritime Organization's (IMO) new International Convention for the Control and Management of Ships' Ballast Water and Sediments (International Maritime Organization, 2004) sets discharge standards on ballast water based on the abundance of organisms, and not whether they are coastal or oceanic in origin. In the United States, Senate bill S. 363 (the Ballast Water Management Act of 2005) took a similar approach, but set discharge concentrations that were as much as two orders of magnitude lower than the IMO standards. Assuming that these regulations, or others like them, are adopted, the shipping industry will require treatment systems that efficiently remove or inactivate all or nearly all of the organisms resident in ballast water.

A number of approaches or technologies for treatment of ballast water have been considered or evaluated, including thermal techniques (Rigby et al., 1999), deoxygenation (Mountfort et al., 1999; Tamburri et al., 2002), ultraviolet irradiation and filtration/separation (Sutherland et al., 2001; Waite et al., 2003), and advanced oxidation techniques (Cooper et al., 2002). None of these potential solutions are in wide use – for example, treatment systems combining ultraviolet irradiation with filtration have been installed on a small number of ships – and it is not known whether any system now available will consistently and efficiently meet the discharge requirements of developing regulations.

Despite the fact that ultrasound is known to kill bacteria and zooplankton, its applicability to treatment of ballast water is unclear. The National Research Council (1996) deemed that ultrasonic treatment was not feasible for ballast water. Mesbahi (2004) exposed a mix of zooplankton and phytoplankton, as would be found in a typical ballast tank community, to ultrasound and obtained less than a 40% reduction in zooplankton and 71% reduction in chlorophyll *a*. Moreover, the cost of this ultrasonic treatment per volume of ballast water was unacceptably high (see Table 3 in Mesbahi, 2004).

The objective of this research was to quantify treatment parameters that could be employed to design more effective, larger-scale ultrasonic systems capable of operating at flow rates approximating those associated with the uptake or discharge of ballast water. We determined contact times and levels of ultrasonic intensity or energy density necessary to kill a range of organisms, from bacteria to large zooplankton, representative of those occurring in ballast tanks. By individually testing cultures of species spanning a range of sizes or morphological characteristics, we were also able to identify types of organisms that might be more *versus* less affected by ultrasonic treatment.

## 2. Materials and methods

### 2.1. Ultrasonic treatment apparatus

Ultrasonic treatment systems typically employ piezoceramic transducers. New magnetostrictive materials such as TERFENOL-D may offer potentially important advantages over piezoceramics for transduction of ultrasound (Bright, 2000). These new materials could provide a basis for more cost-effective treatment systems, utilizing ultrasound either alone or in combination with other technologies. The ultrasonic treatment apparatus in our experiments employed a transducer (model AU-12, Etrema Products, Inc., Ames, IA) of TERFENOL-D driving a 13.34 cm long titanium horn with a 1.26 $cm^2$ circular terminal face. Operating frequency was approximately 19 kHz. During operation the transducer was cooled with forced air. Power was supplied to the transducer by a Hewlett-Packard HP3325B function generator through a linear amplifier (LVC2016; AE Techron Inc., Elkhart, IN). Characteristics of the power supplied to the transducer–AC voltage, current, and phase angle – were monitored using a Fluke 123 Industrial Scopemeter (Fluke Corporation; Everett, WA), and logged to a personal computer. Output of the function generator was monitored and adjusted manually in order to maintain constant power and high efficiency. Efficiency of energy transmission to the test medium was determined calorimetrically, by observing temperature increase over time for artificial seawater and freshwater sonicated in an insulated beaker. Ultrasonic intensity ($I$) of the particular treatment regime was then calculated as

$$I = (W \cdot CE)/1.26$$

where $I$ is the ultrasonic intensity (in W/$cm^2$), $W$ is the power output of the function generator in watts, $CE$ is the calorimetric efficiency (as a proportion), and 1.26 is the area of the terminal face of the horn.

### 2.2. Experiments with bacteria

Bacteria tested included *Vibrio cholerae* ATCC 15748, *Escherichia coli* ATCC 11775, *Enterococcus avium* ATCC 14025, and *Cobetia marina* ATCC 35142. Spores of *Bacillus globigii* (syn. *Bacillus subtilis* var. *niger*) were also treated. *C. marina* was grown in marine broth (Difco Laboratories, Detroit, MI). All others were grown in trypticase soy broth (Sigma–Aldrich Co., St. Louis, MO). Cultures were grown overnight, inoculated into fresh media, harvested in mid-log-phase ($A_{600} \sim 0.5$–$0.8$) by centrifugation, and washed and resuspended in synthetic seawater (salinity approximately 29‰, pH 8.2). Cultures were inoculated to approximately 2–5 $\times 10^6$ colony forming units per mL (CFU/mL) in synthetic seawater for sonication testing. All sonication trials were carried out under static conditions within a jacketed glass reaction vessel (#9850, Ace Glass, Inc., Vineland, NJ). Samples (0.1 mL) were drawn at intervals during the