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Effects of sonication and advanced chemical oxidants on the unicellular green alga *Dunaliella tertiolecta* and cysts, larvae and adults of the brine shrimp *Artemia salina*: A prospective treatment to eradicate invasive organisms from ballast water

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Abstract

Uptake and release of ship-borne ballast water is a major factor contributing to introductions of aquatic phytoplankton and invasive macroinvertebrates. Some invasive unicellular algae can cause harmful algal blooms and produce toxins that build up in food chains. Moreover, to date, few studies have compared the efficacy of ballast water treatments against different life history phases of aquatic macroinvertebrates. In the present study, the unicellular green alga *Dunaliella tertiolecta*, and three discrete life history phases of the brine shrimp *Artemia salina*, were independently used as model organisms to study the efficacy of sonication as well as the advanced oxidants, hydrogen peroxide and ozone, as potential ballast water treatments. Algal cells and brine shrimp cysts, nauplii, and adults were subjected to individual and combined treatments of sonication and advanced oxidants. Combined rather than individual treatments consistently yielded the highest levels of mortality in algal cells (100% over a 2 min exposure) and in brine shrimp (100% and 95% for larvae and adults, respectively, over a 2 min exposure). In contrast, mortality levels in brine shrimp cysts (66% over 2 min; increased to 92% over a 20 min exposure) were moderately high but consistently lower than that detected for larval or adult shrimp. Our results indicate that a combination of sonication and advanced chemical oxidants may be a promising method to eradicate aquatic unicellular algae and macroinvertebrates in ballast water.

Keywords: Unicellular algae; Brine shrimp; Sonication; Advanced oxidation processes; Ballast water; Invasive species

1. Introduction

Introductions of non-indigenous organisms have transpired for many centuries and the rates of invasion have been increasing exponentially on a global scale (Mooney and Drake, 1986; Drake et al., 1989; Hewitt et al., 1999; Carlton, 2001; Holeck et al., 2004). To date, approximately 50,000 non-indigenous species have been introduced into the United States (Cohen and Carlton, 1998; Ruiz et al., 1999; Hewitt et al., 1999; Pimentel et al., 2000; Lewis et al., 2003). Such introductions often result in unpredictable ecological, economic and social impacts (Drake et al., 1989; Gollasch, 1998; Mooney and Hobbs, 2000; Pimentel, 2002). For example, introduced species may compete with native species for food and spatial resources, induce new behavioral responses in native species (Stachowicz et al., 1999; Hayes and Silwa, 2003), threaten biodiversity and accelerate homogenization and negatively impact marine industries and human health (Bax et al., 2003). Pimentel et al. (2000) estimated that damages from invasive species introductions in the United States alone amount to more than $138 billion per year.

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Ballast water is a significant vector for the transport of non-indigenous plankton (holoplankton and meroplankton) (Gollasch et al., 2000; Ruiz et al., 2000; Kuzirian et al., 2001; Carlton, 1999, 2003). When ballast waters are pumped into ballast tanks they typically contain a wide range of aquatic organisms including bacteria, algae and invertebrates. Since microalgal species are dispersed by the movement of water masses they are easily pumped into and discharged from ballast tanks. Under certain conditions, some unicellular algae may form harmful "blooms" over large areas (van den Bergh et al., 2002). In contrast to unicellular algae, adults of most marine and freshwater invertebrates are not readily taken up in ballast tanks due to their large size and because many have a benthic lifestyle. Nonetheless, many benthic species have planktonic life-history stages that are readily taken up and transported in ballast tanks (Hamer et al., 1998; Tamburri et al., 2002). Moreover, many freshwater and marine invertebrates have life histories that include highly resistant, early resting stages that ensure their long-term survival (e.g., Hairston et al., 1995; Dickman and Zhang, 1999; Hamer et al., 2000; Arnott and Yan, 2002). Such qualities make resting stages particularly effective invasive species as they may readily hatch in new environments that provide favorable environmental conditions (Niimi, 2000).

A variety of options are being considered to reduce, if not completely eradicate, high impact invasive species that are transported via ballast water (e.g., Thresher and Kuris, 2004; Faimali et al., 2006). The International Maritime Organization (IMO) has mandated a ballast water exchange at sea policy to prevent the transport and introduction of non-indigenous species (IMO, 2004). However, mid-ocean exchanges of ballast water may endanger ships when conducted in rough seas (Carlton, 1992; Kuzirian et al., 2001). As a result, there is considerable interest in developing alternative ballast water treatments (Kuzirian et al., 2001; Waite et al., 2003). Technologies that are currently being investigated as primary treatments include the application of heat, pressure, filtration, ultrasonics, cyclone or hydrotech-drum settling, and cyclonics (Tsou et al., 1991; Rigby et al., 1999; Parsons and Harkins, 2000; Kuzirian et al., 2001; Parsons and Harkins, 2002; Tamburri et al., 2002). Lately, a variety of secondary treatments coupled with primary ballast water treatments have also been investigated. These include the use of biocides, advanced oxidants, electric pulse or pulse plasma and ultraviolet (UV) light (Kuzirian et al., 2001; Waite et al., 2003; Faimali et al., 2006).

One important aspect of a prospective ballast water treatment is that it should be effective against a wide range of organisms. In a previous study, we have demonstrated that sonication, ozone and hydrogen peroxide can be used successfully to inactivate a model bacterium (*Vibrio alginolyticus*) (Ananthanarayanan, 2005). In an effort to optimize a practical, environmentally acceptable and cost-effective method of treating ballast water, the present study tests the efficacy of individual and combined treatments of sonication, hydrogen peroxide and ozone on the inactivation of the green alga *Dunaliella tertiolecta*, as well as the cyst, larval, and adult life history phases of the brine shrimp *A. salina*.

2. Materials and methods

2.1. Algal culture and maintenance

The alga *D. tertiolecta* (Chlorophyceae), commonly found in saline waters (Hall and Golding, 1998), was selected as a model organism because of its wide distribution, lack of clumping behavior and ease of culture, and because vital staining techniques can be used readily to evaluate mortality. *D. tertiolecta* (Strain # CCMP1320), was cultured to stationary phase in gently aerated artificial seawater (ASW) enriched with Guillard's *f*/2 medium in 2000 ml glass flasks (Andersen, 2005). Concentrations of algal cells in culture were determined using a hemocytometer.

2.2. Brine shrimp culture and maintenance

Cysts of the brine shrimp *A. salina* were purchased from Argent Laboratories (Argentemia Grade I Gold Label). The cysts used in the experimental and control treatments were tested at a concentration of 0.4 g dry cysts in 2.0 l of artificial seawater (ASW) (28 ppt). For larval cultures, cysts (at the above concentration) were maintained in 2.5 l glass beakers equipped with air stones and an overhead lamp to provide a constant temperature of ~28 °C. Cysts subsequently hatched into naupliar larvae within a period of 24 h and were then maintained for an additional 24 h prior to use in experiments. Hatched naupliar larvae were placed on an *ad libitum* diet of the dried cyanobacterium *Spirulina* spp. (Superpetz, premium). In order to prepare cultures of adults, a lower concentration of 0.2 g wt dry cysts in 2 l of ASW was used to allow for lower adult densities per ml ASW. Cysts were cultured as above until hatching and subsequently fed an *ad libitum* diet of the cyanobacterium *Spirulina* spp. Developing shrimp were transferred to clean glass beakers every 4 days to prevent fouling due to the accumulation of food and feces. All adult brine shrimp experiments were conducted using 30-day-old adult shrimp.

2.3. Treatment techniques

Algal cells, adjusted to the standardized concentration, were transferred to 2-l polypropylene experimental bottles. Individual treatments consisted of exposing algal cells and brine shrimp to sonication, hydrogen peroxide or ozone over 5, 10, 15 and 20 min exposure time periods. Combined treatments of sonication and hydrogen peroxide, sonication and ozone, hydrogen peroxide and ozone and, sonication with hydrogen peroxide and ozone were similarly tested over 5, 10, 15 and 20 min exposure time periods.