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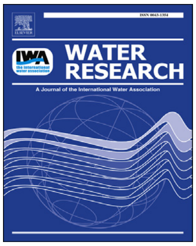

Beating the blues: Is there any music in fighting cyanobacteria with ultrasound?

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ABSTRACT

The hypothesis that cyanobacteria can be controlled by commercially available ultrasound transducers was tested in laboratory experiments with cultures of the cyanobacteria *Anabaena* sp., *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* and the green alga *Scenedesmus obliquus* that were grown in the absence or presence of ultrasound (mix of 20, 28 and 44 kHz). The *Scenedesmus* experiment also included a treatment with the zooplankton grazer *Daphnia magna*. Chlorophyll-*a* and biovolume-based growth of *Anabaena* was significantly lower in ultrasound exposed cultures than in controls. Particle based growth rates were higher in ultrasound treatments. Filaments were significantly shorter in ultrasound exposed cultures reflecting filament breakage. Photosystem II efficiency was not affected by ultrasound. In *Cylindrospermopsis* chlorophyll-*a* based growth rates and photosystem II efficiencies were similar in controls and ultrasound treatments, but biovolume-based growth was significantly lower in ultrasound exposed cultures compared to controls. Despite biovolume growth rates of the filamentous cyanobacteria were reduced in ultrasound treatments compared to controls, growth remained positive implying still a population increase.

In *Microcystis* and *Scenedesmus* growth rates were similar in controls and ultrasound treatments. Hence, no effect of ultrasound on these phytoplankton species was found. Ultrasound should not be viewed “environmental friendly” as it killed all *Daphnia* within 15 min, releasing *Scenedesmus* from grazing control in the cultures. Based on our experiments and critical literature review, we conclude that there is no music in controlling cyanobacteria *in situ* with the commercially available ultrasound transducers we have tested.

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1. Introduction

Nutrient enrichment of surface waters by anthropogenic activity (eutrophication) is a major water quality issue (Roijackers et al., 1998; Smith and Schindler, 2009). Eutrophication of surface waters may lead to several objectionable effects of which cyanobacterial proliferation and formation of surface scum are among the most noticeable ones (Smith et al., 1999; Smith, 2003). Such blooms might be a threat to the health of humans and animals, because cyanobacteria might produce very potent toxins (Codd et al., 2005; Dittmann and Wiegand, 2006).

Over the last decades, eutrophication has increased the frequency and intensity of cyanobacterial blooms (de Figueiredo et al., 2004; Smith and Schindler, 2009; O'Neil et al., 2012). Blooms of cyanobacteria have become a wide spread phenomenon throughout Europe (Chorus, 2001; Mankiewicz et al., 2005; Willame et al., 2005; Mooney et al., 2010) and also represents the summer situation in recreational waters in The Netherlands (Ibelings et al., 2012). In general, cyanobacteria dominate the phytoplankton community in temperate eutrophic lakes, ponds and reservoirs during the warmer periods of the year (Watson et al., 1997), where climate change is expected to further aggravate these symptoms of eutrophication (Paerl and Huisman, 2008; Moss et al., 2011; de Senerpont Domis et al., 2013). Especially summer heat waves might promote cyanobacterial blooms (Jöhnk et al., 2008). This expectation is underpinned by the coincidence of the two hottest summers in Europe – 2003 and 2006, since recording started (Luterbacher et al., 2004; Rebetez et al., 2009), with major cyanobacterial nuisance in The Netherlands. In 2006, more than 100 lakes and ponds in The Netherlands suffered from such heavy blooms that warnings were issued in the media.

As a consequence of the media attention in 2006 around cyanobacteria issues, in subsequent years Dutch water authorities were confronted with a number of (commercial) parties that claimed to have fix-it-all solutions for the cyanobacteria-related problems. A heavily promoted product in the Netherlands following the 2006 heat waves was the use of ‘Effective Microorganisms (EM)’, which were embedded in the so-called ‘mudballs’ or ‘Bokashi-balls’. However, controlled experiments revealed they were far from efficient in controlling cyanobacteria (Lürling et al., 2009, 2010). Concurrently, The National and Regional Water Authorities were approached by suppliers of ultrasound devices to control cyanobacteria in Dutch surface waters. The potential of ultrasound in controlling cyanobacteria is based on laboratory studies showing clear effects of ultrasound on cyanobacterial growth, the collapse of gas vesicles, cell wall disruption and disturbance of the photosynthetic activity (Wu et al., 2011; Rajasekhar et al., 2012b). However, these studies have used relatively high ultrasound intensities, which are difficult to apply in lakes and ponds (Rajasekhar et al., 2012b).

Because of the uncertainties on the efficacy of commercially available ultrasound devices, we have performed controlled experiments in the laboratory testing the hypothesis that commercially available ultrasound transducers strongly reduce cyanobacteria biomass. The manufacturer of the ultrasound transducers we've used stated that “phytoplankton would be killed within one week” (http://flexidal.be/nl/produktenvanflexidal_algen.asp?rubriek=algen&fotoid=8; last accessed August 2nd 2014). Moreover, it is stated that no detrimental effects of ultrasound on humans, animals and plants have been found (http://flexidal.be/nl/uitlegoverdeproduktenvanflexidal_algen.asp?paginaid=5&rubriek=algen, last accessed August 2nd 2014). Inasmuch as ultrasound is claimed “environmental friendly” (Rajasekhar et al., 2012b), we also tested the hypothesis that the emitted ultrasound is safe to non-target organisms as by expecting no deleterious effect of ultrasound on the zooplankton grazer *Daphnia*.

2. Materials and methods

2.1. *Organisms*

The cyanobacteria *Anabaena* sp. Lemmermann 1896 strain PCC7122, *Cylindrospermopsis raciborskii* (Woloszýnska) Seenayya et Subba Raju 1972 strain LETC CIRF-01 and *Microcystis aeruginosa* (Kützing) Kützing 1846 strain NIVA-CYA43 and the green alga *Scenedesmus obliquus* (Turpin) Kützing 1833 strain SAG276/3a were maintained in 250 mL Erlenmeyer flasks containing 100 mL modified WC (Woods Hole modified CHU10)-medium (Lürling and Beekman, 2006) closed with a cellulose stopper. The flasks were placed at 25 °C in 40 μmol quanta m^{-2} s^{-1} provided in a 14:10 h light–dark cycle. Stock cultures were transferred to fresh medium every two to three weeks.

The zooplankton grazer *Daphnia magna* Straus 1820 has been cultured in the laboratory in 1 L jars containing 800 mL artificial RT-medium (Tollrian, 1993). Three times a week *Daphnia* cultures received about 4 mg C L^{-1} of the green alga *S. obliquus* from a continuous culture (grown at 20 °C in continuous light of about 100 μmol photons m^{-2} s^{-1} and with a dilution rate of 1.0 d^{-1}).

2.2. *Ultrasound*

Four ultrasound devices (Flexidal AL-10) were purchased commercially. According to the manufacturer these transducers are applied commonly in ponds (http://flexidal.be/nl/produktenvanflexidal_algen.asp?rubriek=algen&fotoid=8; last assessed August 2nd 2014). The inscription on the devices indicates the transducers might produce ultrasound in the range 300 Hz to 200 kHz. The device contains a Sunpower SPS-025–024 power supply with a maximum power of 26.4 W (Sunpower Technology Corp, Taiwan). All transducers were analysed in the laboratory on the produced electronic frequencies using an Agilent 54622D Mixed Signal Oscilloscope. Detected waves were not sinusoid, but block or square waves at frequencies of ~20 kHz, ~28 kHz and ~44 kHz. One transducer also produced sound at ~12 kHz. The transducers have a diameter of 5 cm.

The acoustic power (*P*) of the transducers was determined following standard calorimetric procedure by measuring the increase in water temperature (ΔT) of 800 mL demineralized water over exposure time (Δt) using the equation (e.g., Kikuchi