Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

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

Miquel Lürling [a,b,*], *Yora Tolman* [a,c]

[a] *Aquatic Ecology & Water Quality Management Group, Department of Environmental Sciences, Wageningen University, P.O. Box 47, 6700 AA Wageningen, The Netherlands*

[b] *Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), P.O. Box 50, 6700 AB Wageningen, The Netherlands*

[c] *Regional Water Authority Delfland, P.O. Box 3061, 2061 DB Delft, The Netherlands*

ARTICLE INFO

Article history:
Received 27 April 2014
Received in revised form
3 August 2014
Accepted 26 August 2014
Available online 6 September 2014

Keywords:
Blooms
Eutrophication control
Microcystis
Cylindrospermopsis
Anabaena
Mitigation

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.

* *Corresponding author*. Aquatic Ecology & Water Quality Management Group, Department of Environmental Sciences, Wageningen University, Droevendaalsesteeg 3a, 6708 PB Wageningen, The Netherlands. Tel.: +31 317 483898; fax: +31 317 419000.
E-mail address: miquel.lurling@wur.nl (M. Lürling).

http://dx.doi.org/10.1016/j.watres.2014.08.043
0043-1354/

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

and Uchida, 2011; Wu et al., 2012): $P = c_{water} \times M_{water} \times \Delta T/\Delta t$, in which c_{water} is the heat capacity of water (4.18 J g^{-1} K^{-1}) and M_{water} is the mass of the water (800 g). The power of the transducers was 0.7 (±0.2, 1 SD) W ($n = 4$).

2.3. Experiments

The experiments were conducted in November and December 2007 in the laboratory of the Aquatic Ecology and Water Quality Management Group of Wageningen University (The Netherlands). Experiments were run in 1 L jars containing 800 mL cyanobacteria or algae suspensions. In every experiment, four jars were exposed continuously to ultrasound by putting a transducer in each, while four other jars remained untreated (controls). The flasks were closed at the top with aluminium foil and placed at 25 °C in 40 μmol quanta m^{-2} s^{-1} provided from the back by fluorescent tubes in a 14:10 h light–dark cycle. Jars were shaken manually once a day.

In the first experiment, *Anabaena* from the stock culture was inoculated in freshly prepared and autoclaved WC-medium at a start concentration of 13 μg L^{-1} chlorophyll-*a* in each of the 800 mL suspensions. The experiment was run for 19 days during which 14 times samples were taken that were analysed on chlorophyll-*a* concentration (μg L^{-1}) and Photosystem II efficiency using a PHYTO-PAM phytoplankton analyser (Heinz Walz GmbH Effeltrich, Germany), biovolume concentration (μm^3 mL^{-1}), particle concentration (# mL^{-1}) and mean particle volume (μm^3) using a cell-counter system (Innovatis Casy® model TT). A subsample taken after eight days was inspected microscopically; filament length and number of cells per filament were determined using a Leica Quantimet 500 MC coupled to a Nikon light microscope. Water quality variables temperature, pH, oxygen concentration and saturation and electric conductivity were measured five times during the experiment, i.e., initially, after four hours, one day, eight days and 17 days.

In the second experiment, *Cylindrospermopsis* from the stock culture was inoculated in freshly prepared and autoclaved WC-medium at a start concentration of 28 μg L^{-1} chlorophyll-*a* in each of the 800 mL suspensions. The experiment was run for 10 days and nine samples were taken for analysis of growth and photosynthesis parameters as describe for the first experiment.

In the third experiment, *Microcystis* was inoculated to reach an initial concentration of 15 μg L^{-1} chlorophyll-*a* in each of the 800 mL suspensions. The experiment was run for seven days during which the cultures were sampled eight times and further processed as describe for the first experiment. Because of wrong system settings, particle counts of samples taken at day 6 were omitted rather than manually recalculated.

In the last experiment, all eight jars were inoculated with the green alga *Scenedesmus* at an initial concentration of 20 μg L^{-1} chlorophyll-*a*. To four jars 15 adult *Daphnia* were added. Transducers were placed in two jars with and two without *Daphnia*. Hence, the experiment consisted of two *Scenedesmus* controls without *Daphnia*, two with *Daphnia* and four ultrasound treatments of which two with and two without *Daphnia*. The experiment was run for five days during which the cultures were sampled five times. Mobility of *Daphnia* was checked and animals on the bottom of the jars were pipetted off and inspected microscopically for movement of thoracic appendages and heart.

Daphnia were reared at 20 °C and transferred to cultures at 25 °C, where ultrasound treatment could further increase the water temperature. To check for a possible effect of temperature on *Daphnia* nine jars containing 800 mL medium and 10 *Daphnia* were divided over three Gallenkamp ORBI-SAFE Netwise Orbital Incubator incubators such that three replicate jars for each temperature were placed at 20 °C, 25 °C and 30 °C. Survival was checked for three days by daily inspection of the jars.

2.4. Data analysis

Each growth and photosynthesis parameter (chlorophyll-*a*, biovolume and particle numbers, mean particle volume and photosystem II efficiency) was analysed by repeated measures ANOVA in the tool pack IBM SPSS Statistics version 19.0.0.1 with treatment (control and ultrasound) as the fixed factor. Growth rates based on the increase in chlorophyll-*a*, biovolume and particle concentrations were derived from iterative fitting of the logistic growth function in the tool pack SigmaPlot 12.3:

$$A_t = \frac{A_0 \cdot K}{A_0 + (K - A_0) \cdot \exp^{(-r \cdot t)}}$$

where A_0 is the initial population size and A_t is the population size at time t, K is the carrying capacity and r is the population growth rate. Growth rates of controls and treatments were compared by *t*-tests in the tool pack SigmaPlot 12.3.

3. Results

3.1. Effects of ultrasound on Anabaena

Biomass indicators chlorophyll-*a* concentration and biovolume concentration were significantly lower in ultrasound treatments compared to controls (Fig. 1A, B; Table A1). Consequently, chlorophyll-based growth rates and biovolume-based growth rates were significantly lower in ultrasound treatments than in controls (Table 1). In contrast, particle concentrations increased more rapidly in ultrasound treatments than in controls (Fig. 1C; Table A1) and particle-based growth rates were significantly higher in ultrasound treatments than in controls (Table 1). The mean particle volume doubled in a few days in controls from around 4000 μm^3 at the start to around 8000 μm^3, while in ultrasound treatments the mean particle volume rapidly decreased to around 370 μm^3 (Fig. 1D). Mean particle volumes were significantly lower in ultrasound than in controls (Table A1), whereas filaments were significantly shorter ($T = 289.0$; $P < 0.001$) in ultrasound (mean ± 1 sd: 28 ± 22 μm; $n = 25$) than in controls (331 ± 333 μm; $n = 10$) having also significantly fewer ($T = 205.0$; $P < 0.001$) cells per filament; on average 7 (±3) cells per filament in ultrasound and 56 (±32) cells per filament in controls. Hence, ultrasound not only reduced the growth rate of *Anabaena*, but also caused filament

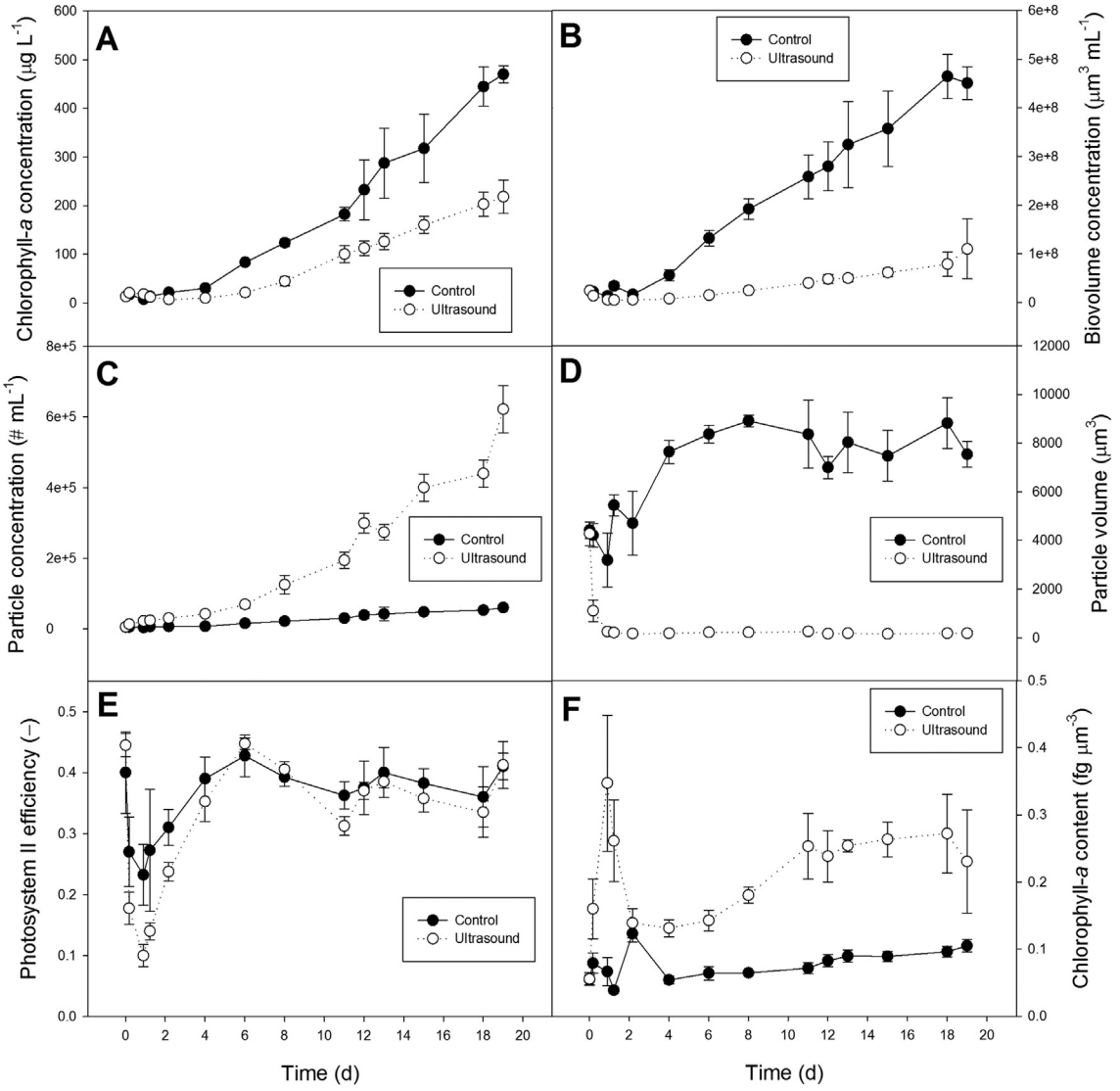

Fig. 1 – Course of the chlorophyll-*a* concentrations (panel A; μg L^{-1}), biovolume concentrations (panel B; μm^3 mL^{-1}), particle concentrations (panel C; # mL^{-1}), mean particle volumes (panel D); μm^3), photosystem II efficiencies (panel E) and chlorophyll-*a* contents (panel F; fg μm^3) of *Anabaena* sp. cultured for 19 days in 800 mL without (Control) or with exposure to low frequency ultrasound (Ultrasound). Error bars indicate 1 SD ($n = 4$).

fragmentation. Nonetheless, biomass still increased over time (Fig. 1A, B) indicating ultrasound was not killing *Anabaena*. This is supported by the Photosystem II efficiency data that, although statistically significantly different between controls and ultrasound treatments, showed a rather comparable pattern in controls and treatments (Fig. 1E). The chlorophyll-*a* content of ultrasound exposed *Anabaena* was double that of non-exposed, i.e., ~0.20 fg μm^{-3} *vs.* ~0.10 fg μm^{-3} (Fig. 1F).

The pH increased from on average 7.7 at the start to 8.4 at the end of the *Anabaena* experiment, but pH was similar in controls and ultrasound treatments (Table 2). Likewise, conductivity (EC) was similar in controls and ultrasound treatments. However, oxygen concentration appeared significantly higher in controls, while temperature was significantly higher in ultrasound treatments (Table 2).

3.2. *Effects of ultrasound on Cylindrospermopsis*

In both controls and treatments the chlorophyll-*a* concentrations increased from ~25 μg L^{-1} at the start to ~250 μg L^{-1} after ten days (Fig. 2A). Because *Cylindrospermopsis* chlorophyll-*a* concentrations were similar in controls and treatments (Fig. 2A; Table A2), also chlorophyll-based growth rates were similar (Table 1). Biovolume concentrations showed a different pattern; biovolume increased in controls, but in ultrasound exposed *Cylindrospermopsis* biovolume declined during the first day of exposure, where after it increased gradually (Fig. 2B). Consequently, biovolume concentrations and biovolume-based growth rates were significantly lower in ultrasound treatments than in controls (Table 1, A2).

Particle concentrations and particle-based growth rates were significantly higher in ultrasound treatments (Fig. 2C;

Table 1 – Growth rates (means ± 1 SD, $n = 4$, in d^{-1}) based on different endpoints, i.e., chlorophyll-*a* concentration, biovolume concentration and the number of particles, for *Anabaena* sp. PCC7122 cultured for 19 days, *Cylindrospermopsis raciborskii* LETC CIRF-01 grown for 10 days and *Microcystis aeruginosa* NIVA-CYA43 for 7 days in the absence (Control) or presence of low frequency ultrasound (Ultrasound). Also included are the results (*t*- and *P* values) of *t*-tests. Significant differences ($P < 0.05$) are indicated in bold.

Endpoint	Control	Ultrasound	*t*- and *P* values
Anabaena growth rates (d^{-1})			
Chlorophyll-*a*	0.28 (±0.02)	0.21 (±0.02)	$t = 4.96$; $P =$ **0.003**
Biovolume	0.28 (±0.03)	0.02 (±0.02)	$t = 6.78$; $P <$ **0.001**
Particles	0.22 (±0.03)	0.36 (±0.01)	$T = 10.0$; $P =$ **0.029**[a]
Cylindrospermopsis growth rates (d^{-1})			
Chlorophyll-*a*	0.31 (±0.06)	0.30 (±0.04)	$t = 0.08$; $P = 0.941$
Biovolume	0.17 (±0.02)	0.13 (±0.02)[b]	$T = 26.0$; $P =$ **0.029**[a]
Particles	0.23 (±0.07)	0.35 (±0.03)	$t = 3.13$; $P =$ **0.020**
Microcystis growth rates (d^{-1})			
Chlorophyll-*a*	0.59 (±0.03)	0.74 (±0.05)	$t = 4.89$; $P =$ **0.003**
Biovolume	0.31 (±0.01)	0.27 (±0.02)	$t = 3.41$; $P =$ **0.014**
Particles	0.30 (±0.02)	0.28 (±0.02)	$t = 2.08$; $P = 0.083$

[a] Mann–Whitney Rank Sum Test because of non-equal variance.
[b] period 0.8 d–10 d.

Table 1, A2). The mean particle volume was significantly lower in ultrasound treatments (Fig. 2D; Table A2).

Photosystem II efficiencies were similar in controls and ultrasound treatments (Fig. 2E; Table A2). The chlorophyll-*a* content of ultrasound exposed *Cylindrospermopsis* was significantly higher than that of non-exposed (Fig. 2F).

3.3. *Effects of ultrasound on Microcystis*

The course of chlorophyll-*a* concentrations in ultrasound exposed *Microcystis* cultures did not differ from that in controls (Fig. 3A; Table A3), but as they were slightly higher in treatments chlorophyll-based growth rates in the ultrasound treatments were significantly higher than in controls (Table 1). Also the increase in biovolume and the number of particles was similar in controls and treatments (Fig. 3B, C; Table A3). Biovolume-based growth rates were only significantly different because of small within group variability, while particle-based growth rates were similar in controls and treatments (Table 1). The mean particle volumes were similar in controls and treatments (Fig. 3D; Table A3). Photosystem II efficiency was slightly higher in treatments compared to controls (Fig. 3E), but statistically significant due to small within group variability (Fig. 3E; Table A3). The chlorophyll-*a* content of non-exposed and ultrasound exposed *Microcystis* was similar (Fig. 3F; Table A3).

Table 2 – Mean values (±1 SD) of water quality variables in the first experiment with *Anabaena* sp. PCC7122 cultured for 19 days in quadruplicates in the absence (Control) or presence of ultrasound (Ultrasound). Also included are the results (*F*- and *P* values) of the between subject effects from repeated measure ANOVAs. Significant differences ($P < 0.05$) are indicated in bold.

Variable	Control	Ultrasound	*F*- and *P* values
pH	8.0 (0.4)	7.8 (0.3)	$F_{1,6} = 2.09$; $P = 0.198$
Conductivity (µS cm^{-1})	260 (12)	256 (7)	$F_{1,6} = 1.10$; $P = 0.336$
Oxygen (mg L^{-1})	10.1 (2.1)	9.4 (1.2)	$F_{1,6} = 11.4$; $P =$ **0.015**
Temperature (°C)	24.9 (1.8)	27.1 (1.6)	$F_{1,6} = 25.8$; $P =$ **0.002**

3.4. *Effects of ultrasound on* Scenedesmus *and* Daphnia

In the controls, pure ultrasound treatments and in the combined *Daphnia*-ultrasound treatments the course of the chlorophyll-*a*, biovolume and particle concentrations were similar (Fig. 4A–C; Table A4). However in the presence of only *Daphnia* chlorophyll-*a*, biovolume and particle concentrations were significantly lower than in the other treatments (Fig. 4A–C; Table A4). Tukey's post hoc comparison tests revealed that these growth parameters were significantly lower in the pure *Daphnia* treatments ($P < 0.05$). The different effect of the pure *Daphnia* treatment and the combined *Daphnia*-ultrasound treatment on the *Scenedesmus* biomass indicators was caused by the detrimental effect of ultrasound on *Daphnia*. When exposed to ultrasound all *Daphnia* died within 15 min, whereas all animals remained alive in the controls.

The mean particle volumes were similar among cultures (Fig. 4D). Photosystem II efficiency was lower in the *Daphnia* treatments at the end of the experiment (Fig. 4E). The chlorophyll-*a* content of *Scenedesmus* in the pure *Daphnia* treatments was lower than that of the controls, while in the just ultrasound exposed cultures it was significantly higher (Tukey's test) than in the controls (Fig. 4F; Table A4).

After 24 h, *Daphnia* survival was 100% at all three temperatures. After two days on average 90% of the animals were alive at 20 °C, 93% at 25 °C and 57% at 30 °C, while after three days 87% was alive at 20 °C, 74% at 25 °C and 17% at 30 °C (Fig. 5).

4. Discussion

The results of this study are not in favour of the hypothesis that cyanobacteria can be controlled by using the commercially available ultrasound devices we have tested.

The transducers we have used are sold on the market and claimed of being effective over 10–12 m and clearing ponds of phytoplankton within one week (http://flexidal.be/nl/produktenvanflexidal_algen.asp?rubriek=algen&fotoid=8; last assessed, August 2nd 2014). However, in all four our laboratory experiments the devices were not capable of clearing the 800 mL exposed to ultrasound. Inasmuch as in larger volumes significantly less power is transmitted, the impact on cyanobacteria will be far less (Rajasekhar et al., 2012b). Hence, it is unlikely that such devices will reduce cyanobacteria in ponds.

The results of our experiments seem in conflict with the numerous positive reports on highly effective control of cyanobacteria by ultrasound as reviewed in Wu et al. (2011) and Rajasekhar et al. (2012b). The latter authors pointed out that most laboratory-based studies applied relatively high ultrasound intensities that cannot be used in lakes or ponds,

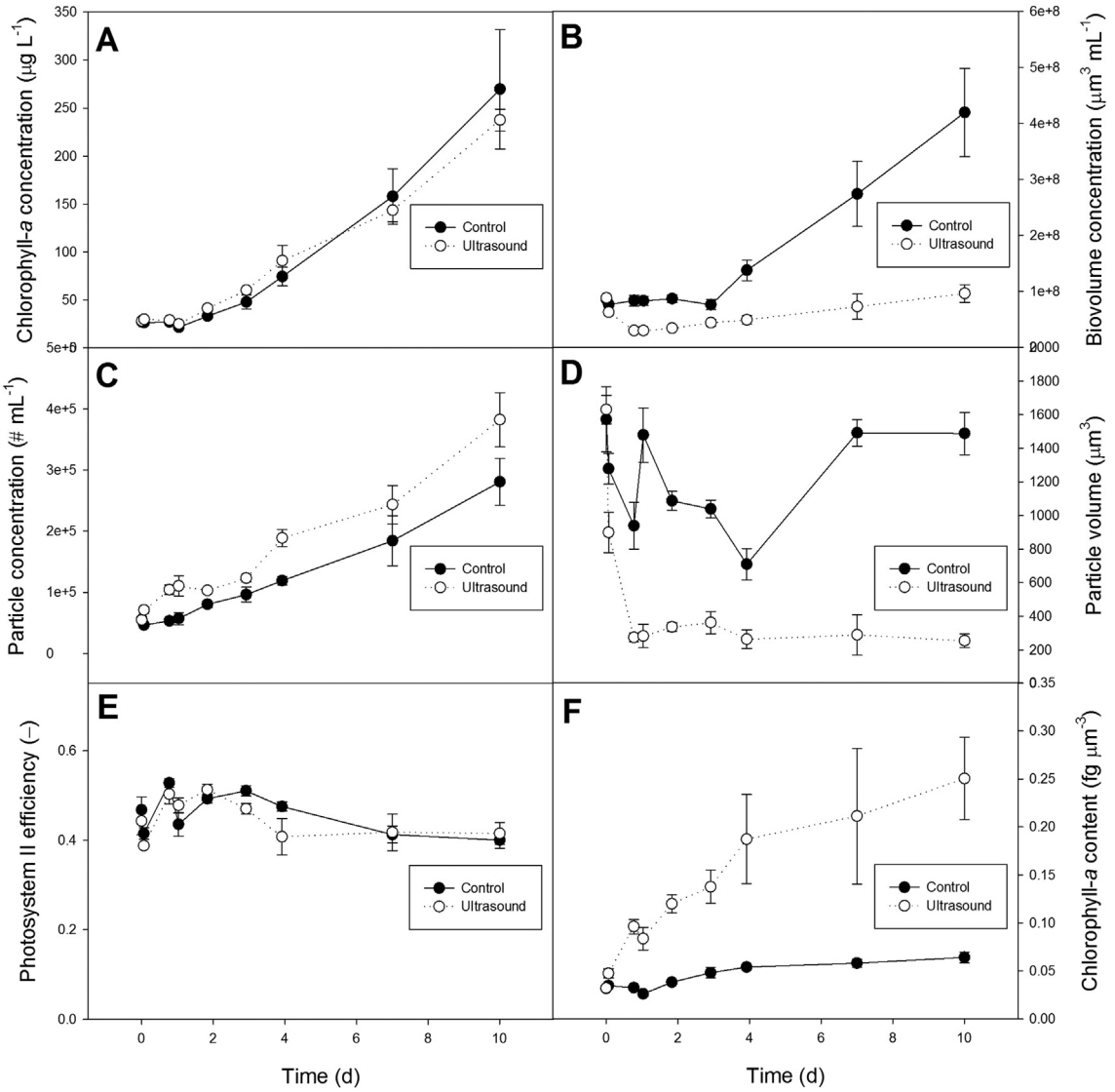

Fig. 2 – Course of the chlorophyll-*a* concentrations (panel A; µg L^{-1}), biovolume concentrations (panel B; μm^3 mL^{-1}), particle concentrations (panel C; # mL^{-1}), mean particle volumes (panel D); μm^3), photosystem II efficiencies (panel E) and chlorophyll-*a* contents (panel F; fg μm^3) of *Cylindrospermopsis raciborskii* cultured for 10 days in 800 mL without (Control) or with exposure to low frequency ultrasound (Ultrasound). Error bars indicate 1 SD ($n = 4$).

because of less power transmission in larger volumes and consequently less impact on cyanobacteria (Rajasekhar et al., 2012b). In our experiment, we used transducers that produced an acoustic power of 0.7 W yielding and intensity of 8.5×10^{-4} W mL^{-1}. Comparing our experiments (in which cultures were exposed continuously to ultrasound) with laboratory-based studies on ultrasound at comparable frequencies revealed that all other studies applied relatively short exposure duration, at intensities dozens to hundreds times higher (Table 3). That higher intensities are capable of killing cyanobacteria is evident from additional trials we have performed with a Branson Digital Sonifier 450 – a device that is used for disrupting cells, bacteria, spores or tissues (*see* Supplementary information). All *M. aeruginosa* could be killed at 4 W mL^{-1} in 4 repeated exposures of 30 s each, at 8 W mL^{-1} three cycles were needed, while at 10 W mL^{-1} two repeated exposures sufficed. Hence, there is a clear correlation between power and time needed to kill *Microcystis* cells. The mode of action is through cavitation (Joyce et al., 2003; Rajasekhar et al., 2012b). The observed increased mortality of *M. aeruginosa* with higher intensities (*see* Supplementary information) is supported by studies run at 580 kHz, where 0.0018 W mL^{-1} caused 13.2% reduction, 0.0210 W mL^{-1} led to 36.8% reduction and 0.0490 W mL^{-1} resulted in 47.4% reduction (Joyce et al., 2010). Some commercial suppliers of ultrasound to treat lakes, ponds and aquaria acknowledge that cavitation is the mode of action in most devices that are used to clean or sterilise samples using high power (e.g., http://www.lgsonic.com/lg-sonic-vs-cavitation/, last accessed August 2nd 2014): "*Most ultrasonic algae control devices based on cavitation use relatively low ultrasonic frequencies but a very high power …. For most devices that use cavitation, the power is known to be at least 0.015 W/cm*^{3}." They also pointed out that for their commercial available transducers "*the occurrence of cavitation can be*

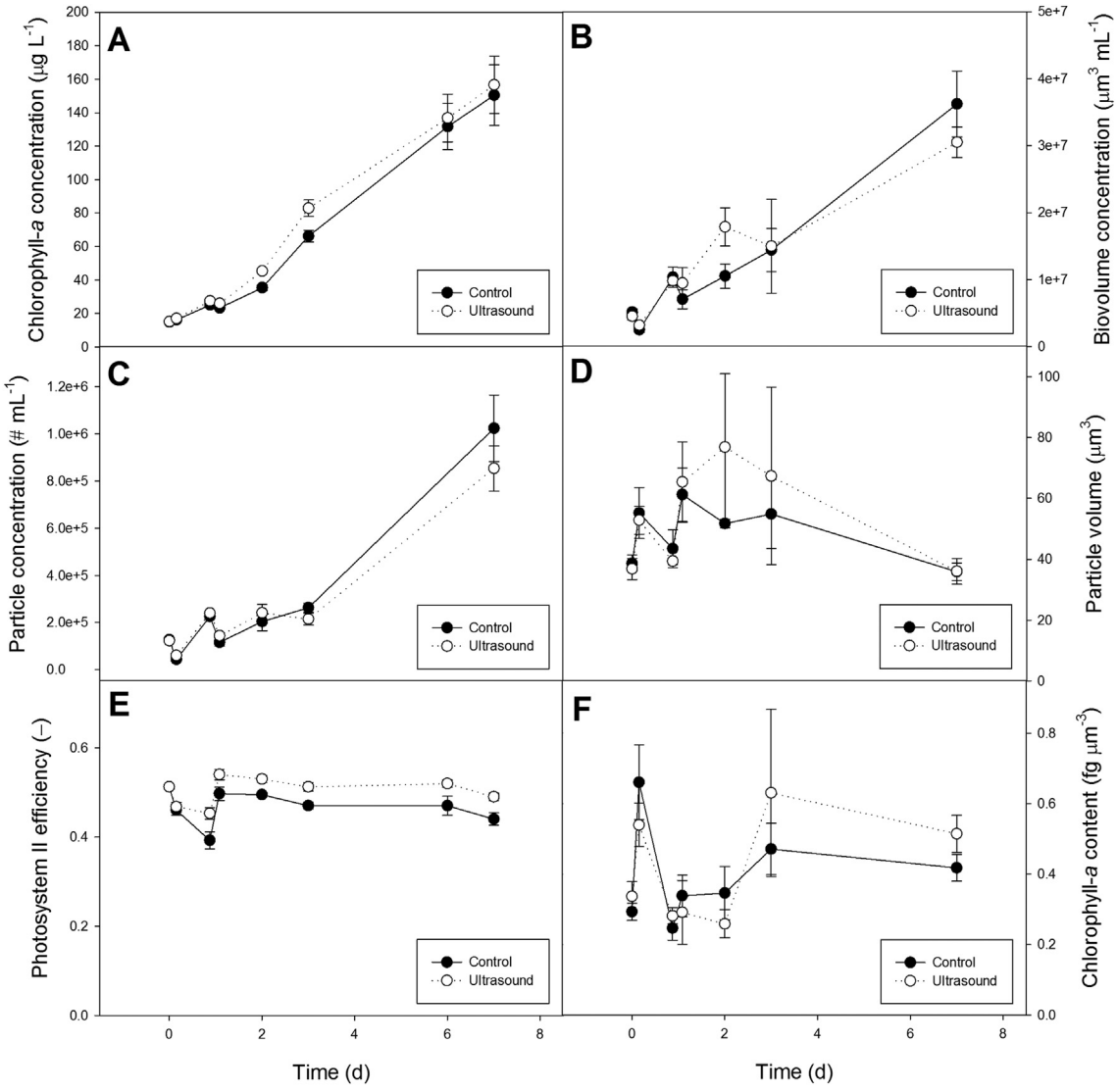

Fig. 3 – Course of the chlorophyll-*a* concentrations (panel A; µg L^{-1}), biovolume concentrations (panel B; μm^3 mL^{-1}), particle concentrations (panel C; # mL^{-1}), mean particle volumes (panel D); μm^3), photosystem II efficiencies (panel E) and chlorophyll-*a* contents (panel F; fg μm^3) of *Microcystis aeruginosa* cultured for 7 days in 800 mL without (Control) or with exposure to low frequency ultrasound (Ultrasound). Error bars indicate 1 SD ($n = 4$).

disregarded" and that the mode of action "*is purely based on killing algae by bringing them in resonance*" (http://www.lgsonic.com/lg-sonic-vs-cavitation/, last accessed August 2nd 2014). In our study, no proof was obtained for such killing by resonance, but we found clear evidence that high power – and presumably cavitation – is efficient in killing cyanobacteria (*see* Supplementary information). However, with high power and cavitation as mode of action there is no reason to expect solely effects on phytoplankton. High power ultrasound is also used for disinfection of ballast water or raw water for drinking water preparation, where it may inactivate motile plankton (Hoyer and Clasen, 2002) or kill zooplankton, especially larger cladocerans (Holm et al., 2008). Cavitation could also damage fish skin (Frenkel et al., 1999) and effects on macrophytes have been reported (Wu and Wu, 2006).

Inasmuch as we did not find any effects of the commercially available ultrasound devices in relatively small volumes, there is no reason to expect effects on larger scale, *in situ*. In fact, this finds support in field trials with comparable devices that have been conducted in The Netherlands in 2007. No evidence of an effect of ultrasound on cyanobacteria or phytoplankton could be found (Govaert et al., 2007; Kardinaal et al., 2008). The study of Govaert et al. (2007) was conducted in two identical ponds of which one was treated with ultrasound produced by a Flexidal AL-50 transducer, while the other one served as control. During the four months of operation chlorophyll-*a* concentrations in the control were around 64 (±13) µg L^{-1} and in the ultrasound treatment around 69 (±26) µg L^{-1} (data digitally extracted from Fig. 2 in Govaert et al., 2007). Moreover, no difference in phytoplankton composition was found (Govaert et al., 2007). Kardinaal et al. (2008) described two other field trials in The Netherlands; one in the Southwest of the Netherlands in a harbour area near Tholen and the other one in a bay of recreational area De Gouden Ham near

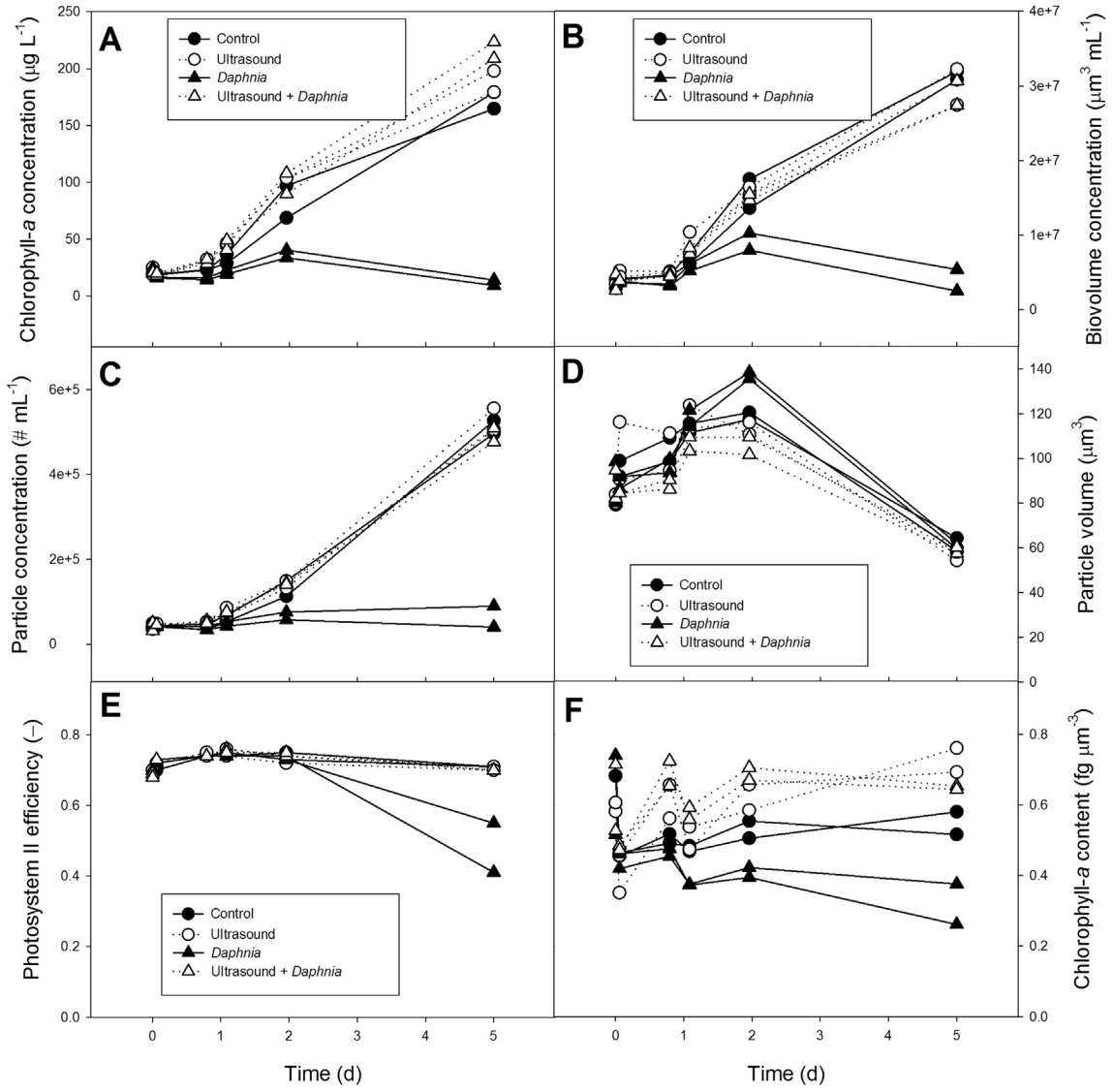

Fig. 4 – Course of the chlorophyll-*a* concentrations (panel A; µg L^{-1}), biovolume concentrations (panel B; μm^3 mL^{-1}), particle concentrations (panel C; # mL^{-1}), mean particle volumes (panel D); μm^3), photosystem II efficiencies (panel E) and chlorophyll-*a* contents (panel F; fg μm^3) of *Scenedesmus obliquus* cultured in duplicates for 5 days in 800 mL without (Control) or with exposure to low frequency ultrasound (Ultrasound) and also in presence of the cladoceran grazer *Daphnia magna* (*Daphnia*; Ultrasound + *Daphnia*).

the river Maas. Surface scums and high *Microcystis* densities were observed on both sites despite the ultrasound treatment and the authors concluded that ultrasound was not effective in reducing cyanobacteria (Kardinaal et al., 2008). There are no data available to determine ultrasound intensities in these field trials, but a few field studies using even higher power units - 10 units of 2 times 100 W in a 365.000 m^3 reservoir (Lee et al., 2002) and one 630 W unit in 9000 m^3 pond (Ahn et al., 2007) gave no support for strong cyanobacteria control by ultrasound. Where the control pond in Ahn et al. (2007) was also dominated by diatoms and the treated pond already at start had significantly lower chlorophyll-a concentration than the control, the results should be met critically. Likewise, Lee et al. (2002) described and reported that chlorophyll-a concentrations were lower in the two years of ultrasound treatment, which, however, finds no support in the data as chlorophyll-a concentrations (digitally extracted from Fig. 4 in Lee et al., 2002), yielded 81 (±56) µg L^{-1} before and 74 (±42) µg L^{-1} during ultrasound.

Some of the reviewed studies (Table 3) showed good growth of the ultrasound treated cyanobacteria in subsequent days (e.g., Ahn et al., 2003; Hao et al., 2004a; Rajasekhar et al., 2012a). In our experiment with *Anabaena*, biovolume-concentration decreased during the first day of the experiment (Fig. 1B), but a parallel line analysis revealed that this was not different in controls and ultrasound treatments ($F_{1,2} = 0.48$; $P = 0.560$). However, in the *Cylindrospermopsis* experiment only in the treatments a decrease in biovolume concentration during the first day was observed (Fig. 2B), which was significantly different from the controls ($F_{1,4} = 11.8$; $P = 0.027$). However, here after *Cylindrospermopsis* grew with a biovolume-based growth rate of 0.13 d^{-1} (Table 1). Restricting

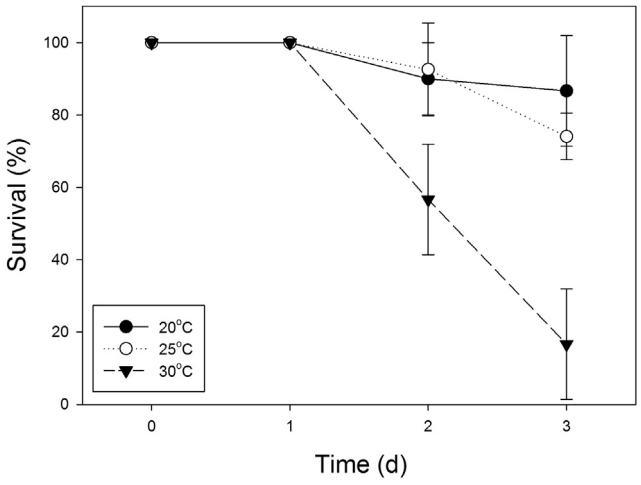

Fig. 5 – Survival (%) of *Daphnia magna* over three days in 800 mL medium placed at three different temperatures (20, 25 and 30 °C). Error bars indicate 1 SD ($n = 3$).

our experiment to only one day would have yielded a reduction based on biovolume in this cyanobacterium, whereas growth, albeit lower than in controls, was evident from the longer incubation. Nevertheless, it is very doubtful that the same effect will be achieved when the transducers are placed in larger volume such as ponds or lakes, because of less power transmittance and consequently far less impact on cyanobacteria (Rajasekhar et al., 2012b).

Although biovolume-based growth inhibition was observed in the filamentous cyanobacteria subjected to ultrasound, growth rates remained positive, whereas no effect on *Microcystis* and the green alga *Scenedesmus* was found. These results are in agreement with those reported by Purcell (2009) who classified *M. aeruginosa* and *Scenedesmus subspicatus* as non-susceptible to ultrasound, while the filamentous cyanobacteria *Aphanizomenon flos-aquae, Anabaena flos-aquae* and the filamentous diatom *Melosira* sp. were highly susceptible to ultrasound. Rajasekhar et al. (2012a) reported *Anabaena circinalis* being more susceptible than *M. aeruginosa* which in turn was more susceptible to ultrasound than *Chlorella*. The latter authors explained the differences from presence of gas vesicles in the cyanobacteria and weaker gas vesicles in *Anabaena* (Rajasekhar et al., 2012a), where the underlying assumption is that ultrasound caused resonance and subsequent rupture or collapse of gas vesicles (Rajasekhar et al., 2012b). The resonance frequency (f_0) of gas bubbles can be estimated with the equation (Kotopoulis et al., 2009):

$$f_0 = \frac{1}{2\pi}\sqrt{\left(\frac{3\gamma}{R_0^2\rho}\left(p_0 + \frac{2\sigma}{R_0} + \frac{2\chi}{R_0}\right) - \left(\frac{2\sigma + 6\chi}{R_0^3\rho}\right)\right)}$$

In which γ is the polytropic exponent of the gas (1.39 for air), R_0 is the radius of the bubble (μm), ρ is the density of the surrounding liquid (1000 kg m^{-3}), p_0 is the ambient pressure (10^5 Pa), σ is the surface tension of the surrounding medium (N m^{-1}) and χ is the membrane elasticity (N m^{-1}). Inasmuch as the contribution of surface tension and membrane elasticity

Table 3 – Overview of laboratory-based ultrasound studies using frequencies in the same range as our study, including test-species, ultrasound intensity (in W mL^{-1};# = W), duration and main effect.

Species	Frequency	Intensity (W mL^{-1})	Duration	Effect	Reference
Arthrospira platensis	20 kHz	0.0875	Max 5 min	Max 44% lower OD_{560}	Hao et al., 2004a
Arthrospira platensis	20 kHz	0.025/0.05	5 min/6 min	Max 43%/45% lower OD_{560}	Hao et al., 2004b
	20 kHz	0.075/0.1	7 min/8 min	Max 48% lower OD_{560}	Hao et al., 2004b
Microcystis aeruginosa	20 kHz	600#	2 × 2 min day^{-1}	No reduction, no growth	Ahn et al., 2003
Anabaena flos-aquae	20 kHz	0.1	Not specified	Growth stimulation	Thomas et al., 1989
Selenastrum capricornutum	20 kHz	0.2	Not specified	No effect 1x US, inhibition daily US	Thomas et al., 1989
Spirulina maxima	20 kHz	0.0727	5 s day 1 and 7	Growth stimulation	Al-Hamdani et al., 1998
	20 kHz	0.0727	5 s every other day	Growth inhibition	Al-Hamdani et al., 1998
	20 kHz	0.1455	5 s every other day	Growth inhibition	Al-Hamdani et al., 1998
	20 kHz	0.1636	5 s every other day	Growth inhibition	Al-Hamdani et al., 1998
Anabaena flos-aquae	20 kHz	0.2	5 min day^{-1}	46% more cells after 12 days	Francko et al., 1990
Selenastrum capricornutum	20 kHz	0.2	5 min day^{-1}	No effect	Francko et al., 1990
Anabaena flos-aquae	20 kHz	0.1190	5 min day^{-1}	Growth stimulation during 7 days	Francko et al., 1994
Microcystis aeruginosa	25 kHz	0.32	5 min	Growth inhibition; 86% cell reduction	Zhang et al., 2006
Microcystis aeruginosa	20 kHz	0.0403	30 min	39% cell reduction	Wu et al., 2012
Microcystis aeruginosa	20 kHz	0.043	5, 10, 15, 20 min	25% cell reduction	Rajesekhar et al., 2012
	20 kHz	0.085	5, 10, 15, 20 min	38% cell reduction	Rajesekhar et al., 2012
	20 kHz	0.139	5, 10, 15, 20 min	58% cell reduction	Rajesekhar et al., 2012
	20 kHz	0.186	5, 10, 15, 20 min	63% cell reduction	Rajesekhar et al., 2012
	20 kHz	0.32	5, 10, 15, 20 min	67% cell reduction	Rajesekhar et al., 2012
Anabaena circinalis	20 kHz	0.085	5, 10, 15, 20 min	Cell density reduction	Rajesekhar et al., 2012
Microcystis aeruginosa	20 kHz	0.085	5, 10, 15, 20 min	Cell density reduction	Rajesekhar et al., 2012
Chlorella sp.	20 kHz	0.085	5, 10, 15, 20 min	Cell density reduction	Rajesekhar et al., 2012
Microcystis aeruginosa	20 kHz	0.0178	5, 10, 20, 30 min	5% reduction in OD_{680}	Joyce et al., 2010
	40 kHz	0.0213	5, 10, 20, 30 min	4% increase in OD_{680} (30 min)	Joyce et al., 2010
Microcystis aeruginosa	20 kHz	2#	1 min	5% reduction in absorption	Qui et al., 2012
	40 kHz	2#	1 min	7% reduction in absorption	Qui et al., 2012

can be considered negligible, they can be ignored (Zhang et al., 2006; Rajasekhar et al., 2012a), yielding:

$$f_0 = \frac{1}{2\pi R_0}\sqrt{\left(\frac{3\gamma}{\rho}(p_0)\right)}$$

With this equation the resonance frequency can be calculated, but as pointed out by (Rajasekhar et al., 2012b) in several studies the accuracy of calculated resonance frequencies is doubtful. The shape of the gas vesicles is assumed to be spherical, while in reality they have the form of a hollow cylindrical tube (Walsby and Hayes, 1989). In *M. aeruginosa* they have a diameter of 60–70 nm and maximum length of around 600 nm (Walsby, 1994; Dunton and Walsby, 2005). This implies that assuming gas vesicles in *M. aeruginosa* up to 1 μm (Hao et al., 2004a,b; Zhang et al., 2006) or gas vacuoles of 3–5 μm (Tang et al., 2004) greatly underestimated frequencies needed to evoke resonance. For example, a sphere with a diameter of 5 μm would require 1.3 MHz, a 1 μm sphere 6.5 MHz, a 0.6 μm sphere 11 MHz, a 0.1 μm sphere 65 MHz and a 60 nm sphere 109 MHz. Therefore, it seems highly unlikely that low frequency ultrasound, as has been used in our study, will provoke resonance of gas vesicles and subsequent collapse of gas vesicles in the cyanobacteria.

Gas vesicles have a low density (60–210 kg m^{-3}) and provide cyanobacteria cells with buoyancy (Walsby, 1994). Loss of gas vesicles would increase cyanobacteria settling rates, however, in our study no accumulations at the bottom of ultrasound treated jars were observed. Sonic cracking of heterocysts in *Anabaena* at frequencies of 200 kHz, 1.0 MHz and 2.2 MHz has been reported as an alternative explanation for loss of buoyancy (Kotopoulis et al., 2009). In contrast, in *Anabaena* cultures that had been irradiated at a comparable frequency of 862 kHz only heterocysts remained (Purcell, 2009). Sonic cracking of heterocysts would result in shorter filaments as in *Anabaena* single heterocysts usually are separated by vegetative cells (Golden and Yoon, 1998). In our controls single heterocysts were separated by on average 23 vegetative cells ($n = 30$), while in ultrasound treatments filaments were much shorter, but more importantly heterocysts were still present. Therefore, filament shortening was not the result of heterocyst destruction. Likewise, Purcell (2009) found that at low energy inputs ultrasound caused filament shortening by breakage with only a limited amount of cell lysis, while at higher energy inputs more severe breakage occurred with increasing amounts of cell lysis.

The magnitude of cell lysis in the filamentous cyanobacteria used in our study is unknown. The significantly lower biovolume-based growth rates in ultrasound exposed filamentous cyanobacteria could be a result of filament breakage. In addition, ultrasound could also cause interruption of photosynthetic activity and cell division (Rajasekhar et al., 2012b) resulting in lower growth. Lee et al. (2001) reported reduced photosynthetic activity in ultrasound exposed cyanobacterial material that was collected on a filter and on which they determined chlorophyll fluorescence using a Mini-PAM. They stated that the effect of ultrasound on photosynthetic activity was species dependent (Lee et al., 2001). However, assuming their depicted chlorophyll-*a* fluorescence reflected efficiency of whole-chain photosynthetic electron transport (Kromkamp and Forester, 2003), there is stronger difference between two identical assays, i.e., ~55% reduction and a ~25% reduction both for *M. aeruginosa* irradiated for 2 min with 28 kHz at 1200 W, than between *M. viridis* and *M. aeruginosa* (Lee et al., 2001), suggesting more factors were involved. In our study, no effect of ultrasound on photosystem II efficiency was found, but this is no guarantee that there was not an effect on the physiology (Parkhill et al., 2001) or no cell death (Franklin et al., 2009). If ultrasound would have had an "immediate effect on photosynthetic activity" (Lee et al., 2001), it would also be expected in *M. aeruginosa* and *S. obliquus* and translated in lower growth, but this was not the case in our experiments. Based on an enclosure study Ahn et al. (2003) concluded that a decreased pH and dissolved oxygen concentration indicated that ultrasonication inhibited photosynthesis by the algae. However, in that study in the control the chlorophyll-*a* concentration doubled and cell density more than doubled without causing a change in pH (Ahn et al., 2003). Moreover, in both their control and treatment oversaturation remained, whereas after 6 days oxygen concentrations were again similar in the control and ultrasound treatment (Ahn et al., 2003), which is not in favour of an ultrasound inhibited photosynthesis. In our *Anabaena* experiment, dissolved oxygen concentrations indicated oversaturation in both controls (122%) and ultrasound exposed treatments (118%) also indicating absence of an effect on photosynthesis.

Ultrasound had no effect on pH and conductivity. Ahn et al. (2003) reported that conductivity was 50 μS cm^{-1} higher in their ultrasound enclosure compared to the control, but looking at their results revealed that this was caused by a decrease in the control and not the result of an ultrasound induced increase in the treatment enclosure.

Ultrasound caused a significant warming of about 2.2 °C, which is comparable to the 3 °C warming reported from an enclosure study (Ahn et al., 2003). In a previous study, the chlorophyll-based growth rate of the *Anabaena* strain we've used was about 25% lower at 27.5 °C than at 25 °C (Lürling et al., 2013). In that view the 20% lower growth observed here in the ultrasound treatments could also be a result of higher temperature (on average 27.1 °C) compared to controls (on average 24.9 °C). *C. raciborskii* expressed higher growth rates at higher temperatures (Lürling et al., 2013), but in ultrasound treatments (and thus probably at elevated temperature) chlorophyll-based growth was more or less similar to that in controls. Nonetheless, the effect on biovolume-based growth of the two filamentous species was far more pronounced than that on chlorophyll-based growth (*see* Table 1). The difference between these two endpoints is most probably a result of ultrasound causing filament shortening by breakage and cell lysis at the break points (Purcell, 2009), thereby releasing pigments in the medium. This might explain the observed higher chlorophyll-*a* concentrations per unit biovolume in these filamentous species, which is further corroborated by the observed identical chlorophyll-*a* content of non-exposed and ultrasound exposed unicellular *Microcystis* that is not susceptible to 'filament' breakage. The *Microcystis* strain we used was dominated by uni- and bicells. Although its typical appearance as large colonies in the field could be affected in our experimental system leading to a

declumping (*cf.* Joyce et al., 2003), we do not expect a large effect on viability simply because our experiment already revealed that *Microcystis* uni/bicells are not killed with the commercially available transducers. The field trials performed in The Netherlands in 2007 unequivocally demonstrated that the large colonies of *Microcystis* and surface accumulations could not be prevented by comparable ultrasound devices as the ones we have tested (Kardinaal et al., 2008). Again providing evidence that the supposed mechanism of gas vesicle rupture was not occurring.

A major feature of many cyanobacterial taxa is the capability to produce potent toxins, which makes cyanobacterial blooms and surface scums a threat to environmental health and public safety (Codd et al., 2005; Funari and Testai, 2008). The toxins are largely contained within the cyanobacterial cells, until lysis or damage of the cells liberates them (Steffensen et al., 1999). Hence, ultrasound induced filament shortening by breakage and cell lysis at the break points (Purcell, 2009), could lead to release of toxins in the water. In this study, no toxins have been measured, but further studies could include analysis of cyanotoxins as these compounds, when dissolved, could potentially affect aquatic organisms that would not readily ingest cyanobacteria (e.g., Pavagadhi et al., 2012).

Ultrasound is considered "environmental friendly" (Rajasekhar et al., 2012b) and also the manufacturer of the transducers we've used stated that no deleterious effect of ultrasound on humans, animals and plants have been found. However, surprisingly few studies have been undertaken to examine the effect of ultrasound on non-target organisms such as *Daphnia*. Our experiment, clearly showed that ultrasound from the commercially available transducers was acute lethal to *Daphnia*. All exposed animals died within 15 min (while all controls survived) and this rapid death could not be explained from differences in temperature between control and treated jars. Also Wells (1968) found that short exposure of *D. magna* to 3 MHz was lethal to the animals, while a Russian study reported on immediate death of *Daphnia* in 50, 500 and 1000 kHz (Kamenskii, 1970). Hence, claims that ultrasound is "environmental friendly" (Rajasekhar et al., 2012b) and can be considered a "green solution" (Wu et al., 2011) find no support in the literature and are not supported at all by our study, as we found a clear and fast lethal impact of ultrasound produced by commercially available transducers. Based on evaluation of the published literature and the proposed underlying effects of ultrasound on cyanobacteria, together with the outcomes of our experiments, we conclude that there is no music in fighting cyanobacteria nuisance with commercially available ultrasound.

5. Conclusions

Based on the results of this study it can be concluded that:

- Ultrasound from the commercial transducers caused growth reduction in filamentous cyanobacteria *Anabaena* and *Cylindrospermopsis*.
- Ultrasound caused filament breakage in *Anabaena*.
- Ultrasound had no effect on *Microcystis*
- Ultrasound did not clear the water from phytoplankton.
- Ultrasound was acutely harmful to the zooplankton grazer *D. magna*.
- There is no music in fighting cyanobacteria nuisance with commercially available ultrasound transducers.

Acknowledgements

We thank Wendy Beekman for assistance during the experiments. This study was supported by the Ministry of Transport, Public Works and Water Management, Directorate – General for Public Works and Water Management.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2014.08.043.

REFERENCES

Ahn, C.-Y., Park, M.-H., Joung, S.-H., Kim, H.-S., Jang, K.-Y., Oh, H.-M., 2003. Growth inhibition of cyanobacteria by ultrasonic radiation: laboratory and enclosure studies. Environ. Sci. Technol. 37 (13), 3031–3037.

Ahn, C.-Y., Joung, S.-H., Choi, A., Kim, H.-S., Jang, K.-Y., Oh, H.-M., 2007. Selective control of cyanobacteria in eutrophic pond by a combined device of ultrasonication and water pumps. Environ. Technol. 28, 371–379.

Al-Hamdani, S., Burnett, C., Durrant, G., 1998. Effect of low-dose ultrasonic treatment on *Spirulina maxima*. Aquac. Eng. 19, 17–28.

Chorus, I., 2001. Cyanotoxins: Occurrence, Causes, Consequences. Springer Verlag, Berlin Heidelberg New York, p. 357.

Codd, G.A., Morrison, L.F., Metcalf, J.S., 2005. Cyanobacterial toxins : risk management for health protection. Toxicol. Appl. Pharmacol. 203 (3), 264–272.

de Senerpont Domis, L.N., Elser, J.J., Gsell, A.S., Huszar, V.L.M., Ibelings, B.W., Jeppesen, E., Kosten, S., Mooij, W.M., Roland, F., Sommer, U., Van Donk, E., Winder, M., Lürling, M., 2013. Plankton dynamics under different climatic conditions in space and time. Freshw. Biol. 58 (3), 463–482.

Dittmann, E., Wiegand, C., 2006. Cyanobacterial toxins – occurrence, biosynthesis and impact on human affairs. Mol. Nutr. Food Res. 50 (1), 7–17.

Dunton, P.G., Walsby, A.E., 2005. The diameter and critical collapse pressure of gas vesicles in *Microcystis* are correlated with GvpCs of different length. FEMS Microbiol. Lett. 247, 37–43.

Figueiredo de, D.R., Azeiteiro, U.M., Esteves, S.M., Gonçalves, F.J.M., Pereira, M.J., 2004. Microcystin-producing blooms-a serious global public health issue. Ecotoxicol. Environ. Saf. 59, 151–163.

Francko, D.A., Taylor, S.R., Thomas, B.J., Mclntosh, D., 1990. Effect of low-dose ultrasonic treatment on physiological variables in *Anabaena flos-aquae* and *Selenastrum capricornutum*. Biotechnol. Lett. 12 (3), 219–224.

Francko, D.A., Al-Hamdani, S., Joo, G.-J., 1994. Enhancement of nitrogen fixation in *Anabaena flos-aquae* (Cyanobacteria) via low-dose ultrasonic treatment. J. Appl. Phycol. 6, 455–458.

Franklin, D.J., Choi, C.J., Hughes, C., Malin, G., Berges, J.A., 2009. Effect of dead phytoplankton cells on the apparent efficiency of photosystem II. Mar. Ecol. Prog. Ser. 382, 35–40.

Frenkel, V., Kimmel, E., Iger, Y., 1999. Ultrasound-induced cavitation damage to external epithelia of fish skin. Ultrasound Med. Biol. 25 (8), 1295–1303.
Funari, E., Testai, E., 2008. Human health risk assessment related to cyanotoxins exposure. Crit. Rev. Toxicol. 38, 97–125.
Golden, J.W., Yoon, H.-S., 1998. Heterocyst formation in *Anabaena*. Curr. Opin. Microbiol. 1, 623–629.
Govaert, E., Vanderstukken, M., Muylaert, K., 2007. Evaluatie Van Effecten Van Ultrasone Straling Op Het Ecosysteem. KULeuven Kortrijk, p. 20 (In Dutch).
Hao, H., Wu, M., Chen, Y., Tang, J., Wu, Q., 2004a. Cavitation mechanism in cyanobacterial growth inhibition by ultrasonic irradiation. Colloids Surf. B Biointer. 33, 151–156.
Hao, H., Wu, M., Chen, Y., Tang, J., Wu, Q., 2004b. Cyanobacterial bloom control by ultrasonic irradiation at 20 kHz and 1.7 MHz. J. Environ. Sci. Health A 39, 1435–1446.
Holm, E.R., Stamper, D.M., Brizzolara, R.A., Barnes, L., Deamer, N., Burkholder, J.M., 2008. Sonication of bacteria, phytoplankton and zooplankton: application to treatment of ballast water. Mar. Pollut. Bull. 56, 1201–1208.
Hoyer, O., Clasen, J., 2002. The application of new technologies in the water treatment process of a modern waterworks. Water Sci. Technol. Water Supply 2, 63–69.
Ibelings, B.W., Stroom, J.M., Lürling, M.F.L.L.W., Kardinaal, W.E.A., 2012. Netherlands: risks of toxic cyanobacterial blooms in recreational waters and guidelines. In: Chorus, I. (Ed.), Current Approaches to Cyanotoxin Risk Management, Risk Management and Regulations in Different Countries. Federal Environment Agency, Dessau Roβlau, Germany, pp. 82–96.
Jöhnk, K.D., Huisman, J., Sharples, J.P., Sommeijer, B., Visser, P.M., Stroom, J.M., 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Glob. Change Biol. 14, 495–512.
Joyce, E., Phull, S.S., Lorimer, J.P., Mason, T.J., 2003. The development and evaluation of ultrasound for the treatment of bacterial suspensions. A study of frequency, power and sonication time on cultured *Bacillus* species. Ultrason. Sonochem. 10, 315–318.
Joyce, E.M., Wu, X., Mason, T.J., 2010. Effect of ultrasonic frequency and power on algae suspensions. J. Environ. Sci. Health, Part A Toxic Haz. Subst. Environ. Eng. 45 (7), 863–866.
Kamenskii, I.V., 1970. Influence of ultrasound on eggs and larvae of some fish trematodes. Byulleten' Vsesoyuznogo Instituta Gel'mintologii Im. 4, 47–50 (In Russian).
Kardinaal, E., De Haan, M., Ruiter, H., 2008. Maatregelen ter voorkoming blauwalgen werken onvoldoende. H_2O 7, 4–7 (In Dutch).
Kikuchi, T., Uchida, T., 2011. Calorimetric method for measuring high ultrasonic power using water as a heating material. J. Phys. Conf. Ser. 279, 012012.
Kotopoulis, S., Schommartz, A., Postema, M., 2009. Sonic cracking of blue-green algae. Appl. Acoust. 70, 1306–1312.
Kromkamp, J.C., Forester, R.M., 2003. The use of variable fluorescence measurements in aquatic ecosystems: differences between multiple and single turnover measuring protocols and suggested terminology. Eur. J. Phycol. 38, 103–112.
Lee, T.J., Nakano, K., Matsumara, M., 2001. Ultrasonic irradiation for blue-green algae bloom control. Environ. Technol. 22, 383–390.
Lee, T.J., Nakano, K., Matsumara, M., 2002. A novel strategy for cyanobacterial bloom control by ultrasonic irradiation. Water Sci. Technol. 46, 207–215.
Lürling, M., Beekman, W., 2006. Palmelloids formation in *Chlamydomonas reinhardtii*: defence against rotifer predators? Ann. Limnol. 42, 65–72.
Lürling, M., Tolman, Y., Euwe, M., 2009. Mitigating cyanobacterial blooms: how effective are 'effective microorganisms'? Lakes Reserv. Res. Manag. 14, 353–363.
Lürling, M., Tolman, Y., van Oosterhout, F., 2010. Cyanobacteria blooms cannot be controlled by Effective Microorganisms (EM®) from mud- or Bokashi-balls. Hydrobiologia 646, 133–143.
Lürling, M., Eshetu, F., Faassen, E., Kosten, S., Huszar, V.L.M., 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. Freshw. Biol. 58 (3), 455–462.
Luterbacher, J., Dietrich, D., Xoplaki, E., Grosjean, M., Wanner, H., 2004. European seasonal and annual temperature variability, trends, and extremes since 1500. Science 303, 1499–1503.
Mankiewicz, J., Komárková, J., Izydorczyk, K., Jurczak, T., Tarczynska, M., Zalewski, M., 2005. Hepatotoxic cyanobacterial blooms in the Lakes of Northern Poland. Environ. Toxicol. 20, 499–506.
Mooney, K.M., Hamilton, J.T.G., Floyd, S.D., Foy, R.H., Elliot, C.T., 2010. Initial studies on the occurrence of cyanobacteria and microcystins in Irish lakes. Environ. Toxicol. 26, 566–570.
Moss, B., Kosten, S., Meerhoff, M., Battarbee, R.W., Jeppesen, E., Mazzeo, N., Havens, K., Lacerot, G., Liu, Z., De Meester, L., Paerl, H., Scheffer, M., 2011. Allied attack: climate change and eutrophication. Inland Waters 1, 101–105.
O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmful Algae 14, 313–334.
Paerl, H.W., Huisman, J., 2008. Blooms like it hot. Science 320, 57–58.
Parkhill, J.-P., Maillet, G., Cullen, J.J., 2001. Fluorescence-based maximal quantum yield for PSII as a diagnostic of nutrient stress. J. Phycol. 37, 517–529.
Pavagadhi, S., Gong, Z., Hande, M.P., Dionysiou, D.D., de la Cruz, A.A., Balasubramanian, R., 2012. Biochemical response of diverse organs in adult *Danio rerio* (zebrafish) exposed to sub-lethal concentrations of microcystin-LR and microcystin-RR: a balneation study. Aquat. Toxicol. 109, 1–10.
Purcell, D., 2009. Control of Algal Growth in Reservoirs with Ultrasound. PhD-thesis. Cranfield University.
Qui, Y., Rong, F., Yang, F., Li, J., Long, H., Wu, W., 2012. Ultrasound frequency impacts on the removal of indigenous blue-green algae taken from Lake Taihu. Adv. Mater. Res. 383-390, 3758–3762.
Rajasekhar, P., Fan, L., Nguyen, T., Roddick, F.A., 2012a. Impact of sonication at 20 kHz on *Microcystis aeruginosa, Anabaena circinalis* and *Chlorella* sp. Water Res. 46, 1473–1481.
Rajasekhar, P., Fan, L., Nguyen, T., Roddick, F.A., 2012b. A review of the use of sonication to control cyanobacterial blooms. Water Res. 46, 4319–4329.
Rebetez, M., Dupont, O., Giroud, M., 2009. An analysis of the July 2006 heatwave extent in Europe compared to the record year of 2003. Theor. Appl. Climatol. 95, 1–7.
Roijackers, R., Aalderink, R.H., Blom, G., 1998. Eutrophication research. State of the art: inputs, processes, effects, modelling, management. Water Sci. Technol. 37, 1–250.
Smith, V.H., 2003. Eutrophication of freshwater and coastal marine ecosystems. A global problem. Environ. Sci. Pollut. Res. 10, 126–139.
Smith, V.H., Schindler, D.W., 2009. Eutrophication science: where do we go from here? Trends Ecol. Evol. 24, 201–207.
Smith, V.H., Tilman, G.D., Nekola, J.C., 1999. Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. Environ. Pollut. 100, 179–196.
Steffensen, D., Burch, M., Nicholson, B., Drikas, B., 1999. Management of toxic blue-green algae (cyanobacteria) in Australia. Environ. Toxicol. 14, 183–195.
Tang, J.W., Wu, Q.Y., Hao, H.W., Chen, Y., Wu, M., 2004. Effect of 1.7 MHz ultrasound on a gas-vacuolate cyanobacterium and a gas-vacuole negative cyanobacterium. Colloids Surf. B: Biointer. 36, 115–121.

Thomas, B.J., McIntosh, D., Taylor, S.R., Francko, D.A., Ownby, J., 1989. Effect of low-dose ultrasonic treatment on growth rates and biomass yield of *Anabaena flos-aquae* and *Selenastrum capricornutum*. Biotechnol. Tech. 3 (6), 389–392.

Tollrian, R., 1993. Neckteeth formation in *Daphnia pulex* as example of continuous phenotypic plasticity: morphological effects of *Chaoborus* kairomone concentration and their quantification. J. Plankton Res. 15, 1309–1318.

Walsby, A.E., 1994. Gas vesicles. Microbiol. Rev. 58, 94–144.

Walsby, A.E., Hayes, P.K., 1989. Gas vesicle proteins. Biochem. J. 264, 313–322.

Watson, S.B., McCauley, E., Downing, J.A., 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. Limnol. Oceanogr. 42, 487–495.

Wells, P.N.T., 1968. The effect of ultrasonic irradiation on the survival of *Daphnia magna*. Exp. Biol. 49, 61–70.

Willame, R., Jurczak, T., Iffly, J.F., Kull, T., Meriluoto, J., Hoffmann, L., 2005. Distribution of hepatotoxic cyanobacterial blooms in Belgium and Luxembourg. Hydrobiologia 551, 99–117.

Wu, J., Wu, M., 2006. Feasibility study of effect of ultrasound on water chestnuts. Ultrasound Med. Biol. 32 (4), 595–601.

Wu, X., Joyce, E.M., Mason, T.J., 2011. The effects of ultrasound on cyanobacteria. Harmful Algae 10, 738–743.

Wu, X., Joyce, E.M., Mason, T.J., 2012. Evaluation of the mechanisms of the effect of ultrasound on *Microcystis aeruginosa* at different ultrasonic frequencies. Water Res. 46, 2851–2858.

Zhang, G., Zhang, P., Wang, B., Liu, H., 2006. Ultrasonic frequency effects on the removal of *Microcystis aeruginosa*. Ultrason. Sonochem. 13, 446–450.