

Literature Review of the Effects of Ultrasonic Waves on Cyanobacteria, Other Aquatic Organisms, and Water Quality

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Executive Summary

Ultrasound technologies have been promoted as an effective means of minimizing cyanobacterial blooms in ponds and lakes, but little is known about the effects of ultrasound on non-target organisms or ecosystem processes when implemented on a large scale in complex natural systems. To better understand possible effects of this technology, we summarize the available scientific literature on the effects of sonication and anti-cyanobacterial, ultrasound devices.

Ultrasound (sound waves at approximately 20 kHz) induces vibrations and ruptures gas vacuoles that control cyanobacterial buoyancy. Cyanobacteria then sink and cannot restore their buoyancy in the lower light levels at the lake bottom. Ultrasound has worked well in short-term, laboratory tests (<30 minutes) to inactivate and sediment cyanobacterial cells. Despite the fact that sonication can destroy cyanobacterial cells and release cyanotoxins to the surrounding water, we could find no large-scale studies that investigated cyanotoxin release.

Many scientific studies have tested ultrasound on organisms, primarily over short periods of time. Sonication effects on non-target organisms could be greater than effects demonstrated in short-term, laboratory studies if anti-algal units are used continuously as recommended by manufacturers. Information on the specific wavelengths and intensities produced by the devices, however, is proprietary and publically inaccessible. Therefore, we reviewed studies which used ultrasound frequencies believed to be similar to those of anti-algal, sonication units on non-cyanobacterial organisms. Effects of sonication on non-target organisms reported include:

- Bacteria – Ultrasound is used to kill bacteria in wastewater treatment and aquaculture facilities. Bacterially mediated nutrient cycles and organic matter processing could be affected by whole-lake sonication.
- Algae – A wide variety of both microalgae and macroalgae are vulnerable to cell injury and death from ultrasound treatments. Because algae provide the foundation of the aquatic food web, ultrasound treatments could have far-reaching effects.

(continued)

- Plants – Treatment of aquatic plants with high frequencies of ultrasound has led to cell membrane disruption and loss of leaves, buoyancy, and vitality.
- Zooplankton – Ultrasound ballast water treatment systems caused high mortality in cladocerans, rotifers, and brine shrimp, reducing them to debris after one to four second exposures.
- Mollusks – Ultrasound is used to kill snails in aquaculture settings and can be used to disable and kill zebra mussels (*Dreissena polymorpha*) at all life history stages.
- Insects – High frequency ultrasound has killed developing fruit flies. Water boatmen (Hemiptera: Corixidae) and caddis fly larvae (Trichoptera) communicate with ultrasound. It is possible that ultrasound devices could interfere with their behavior.
- Amphibians – Amphibian embryonic tissue was destroyed and embryos were killed by exposure to high frequency ultrasound wavelengths.
- Fish – High-frequency ultrasound has been used to deter alewives (*Alosa pseudoharengus*) from power plant intakes. Channel catfish (*Ictalurus punctatus*) fingerlings in aquaculture ponds treated with ultrasound were deterred from feeding and required four hours without treatment in order to feed. Ultrasound makes skin permeable and is used in aquaculture for immersion vaccination. Fish exposed to ultrasound in natural systems could thus be at risk for disease or contaminant uptake because of increased skin permeability.
- Humans – Ultrasound device intensity levels are proprietary information so the effects of the devices on humans are unknown. The owner's manual for one product includes warnings of tissue injury and discourages contact of the transducers with the body. The risk of exposure to lower-level ultrasound is unknown. Exposure to cyanotoxins released from damaged cyanobacterial cells also potentially poses a health risk to humans.

Manufacturers may have additional data on the effects of ultrasonic devices on non-target organisms, but those data are not available to the public. It also is worth noting that if anti-algal, ultrasound devices are not powerful enough to harm non-target organisms, they may also be ineffective against cyanobacteria.

Sonication units are usually coupled with aeration and circulation devices in large-scale systems, which may affect the units' efficacy or impact water quality. Circulation devices may induce the recruitment of inactivated cyanobacterial cells from the sediments into the water column, re-establishing bloom conditions. Coupling sonication with microbubble treatment could potentially lead to cell lysing and toxin release. Ultrasound also can dissociate phosphate from particles, making it available for uptake. Circulation and aeration may increase turbidity, destratify the water column, and facilitate nutrient release from the sediments in some systems.

We reviewed three field studies of sonication in large systems. These studies demonstrated mixed results for chlorophyll and cyanobacterial densities, with the greatest effects when additional flushing and circulation treatments were included. Sediment nutrients increased in one study, while in another sonication may have led to increased nutrients in the water column.

In our review, we found that most sonication studies were laboratory based and short in duration. Although ultrasound has been shown to inactivate cyanobacteria in short-term, small-scale laboratory studies, extrapolating ultrasound's efficacy and safety to longer term, larger scale treatments remains difficult given the lack of field studies and inaccessibility of information on device wavelengths and intensity. Our review found that ultrasound may release cyanotoxins from cyanobacterial cells, pose potential health hazards to humans, adversely affect non-target organisms, have adverse ecological effects on food webs and nutrient processing, and affect recreational fishing opportunities.

All photos in this publication were taken by Gina LaLiberte.



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







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Disclaimer: Mention of trade names and commercial products does not constitute endorsement of their use.

Cyanobacterial bloom-forming taxa common in Wisconsin lakes.



Aphanizomenon.

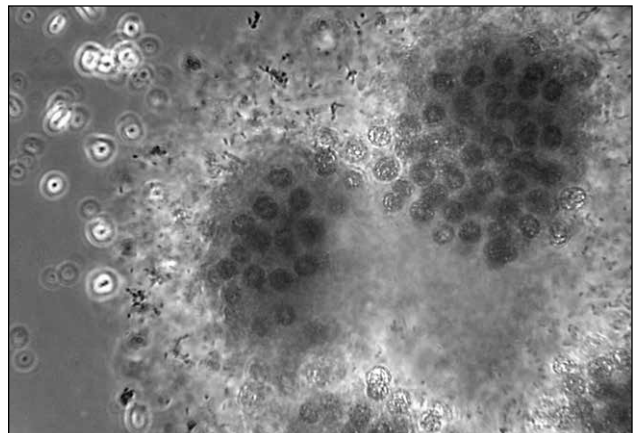
Dolichospermum
(formerly *Anabaena*).

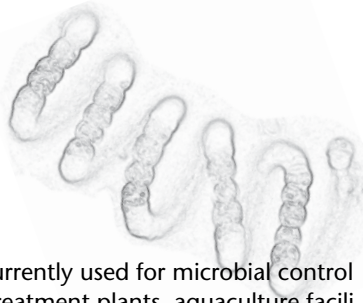


Planktothrix rubescens.



Microcystis.





Introduction

Ultrasound devices are currently used for microbial control and treatment in water treatment plants, aquaculture facilities, reservoirs, and ornamental water bodies such as golf course ponds. Ultrasound's use is promoted for addressing algae and cyanobacteria concerns in ponds and lakes, but little is known about its effects on non-target organisms or ecosystem processes when implemented on a large scale in complex natural systems. Colucci (2010) recently reviewed the existing ultrasound literature to determine feasibility of ultrasound use for algae control in a spring-fed pool in the city of Austin, Texas. Because the federally endangered Barton Springs salamander (*Eurycea sosorum*) lives in the pool, information on ultrasonic impacts to aquatic life was required by the U.S. Fish and Wildlife Service for approval to test the devices. Little specific information was available, and Colucci (2010) concluded that without information about the safety of ultrasonic exposure to aquatic biota and humans, the spring-fed pool was not an appropriate location for testing ultrasonic algae control devices. The lack of readily available information also has made it challenging for the Wisconsin Department of Natural Resources to respond adequately to recent interest in use of ultrasound devices in Wisconsin lakes. To address this situation, we conducted a more comprehensive review of available scientific information on the effects of ultrasound on cyanobacteria (including cyanotoxin releases), non-target aquatic organisms, and water quality.

Ultrasound and Ultrasonic Waves

Ultrasonic waves are waves of sound that are outside the range of human hearing, typically at a frequency of >20,000 Hz (20 kHz). The effects of ultrasonic waves on cells can be divided into two categories, thermal and mechanical, although both types of effects can occur simultaneously. Thermal effects are limited to increased temperature of the cell as a result of absorbing the energy from the ultrasonic waves, while the mechanical effects can vary in manifestation and severity.

The use of ultrasonic waves for algae control capitalizes on the mechanical effects of the sound waves on algae cells. Vibrations caused by sound waves make gas vesicles in the cells resonate. Bubbles form, expand, and contract inside the cells in a process called cavitation. The ultrasound eventually ruptures the bubbles, damaging the cells. The degree of cavitation, and thus the effect on the cell, is regulated by the frequency, intensity, and duration of the sound waves (Rajasekhar et al. 2012b).

Many authors report the intensity used in their studies as the watts (W) supplied to their ultrasound transducer, instead of the power or intensity produced by the waves emitted from the transducer. Joyce et al. (2010) and Rajasekhar et al. (2012b) note that intensities are more correctly given as W/cm^3 or W/mL . Most ultrasound studies, however, do not give intensity using these units, so it is difficult to compare results between studies. Throughout our review, we present ultrasound frequencies and intensities as they are reported by the cited authors.

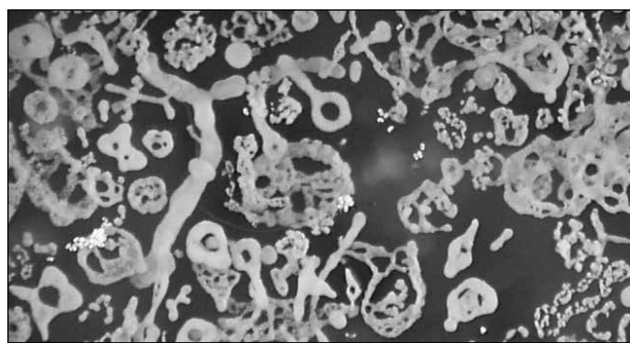
Effects of Ultrasonic Waves on Cyanobacteria

Many planktonic cyanobacteria, including numerous bloom-forming species, regulate their buoyancy using vacuoles, which are filled with a series of gas-filled vesicles. Cavitation leads to vesicle rupture and vacuole collapse (Lee et al. 2001). After the vacuoles collapse, the cyanobacteria can no longer float, so they sink to the lake bottom. In the absence of adequate light at the bottom the cells are unable to restore their vesicles and buoyancy, so they die (Lee et al. 2001). If enough light is available, however, vesicles are able to regenerate and the cyanobacterial cells regain buoyancy control (Walsby 1992).

Other effects of ultrasonic waves on cyanobacteria include disruption of photosynthesis, damage of cell membranes due to lipid peroxidation, and differential susceptibility to ultrasound waves at different stages in the cell division cycle (Ahn et al. 2003, Tang et al. 2004, Zhang et al. 2006).

Ultrasound has been found to effectively inactivate cyanobacteria in simple systems tested in the laboratory. Most laboratory experiments use exposures <10 seconds in duration, and ultrasound frequencies between 20-28 kHz, although some trials used higher frequencies of up to 1.7 MHz. Typically, short exposures led to cavitation followed by sedimentation of treated *Microcystis* cells in the reactor vessels (Lee et al. 2001, Ahn et al. 2003, Joyce et al. 2010, Rajasekhar et al. 2012a, Wu et al. 2012). Some researchers cultured sonicated cells and found reduced re-growth after seven to nine days (Lee et al. 2001, Rajasekhar et al. 2012a).

A few studies examined cyanobacterial taxa other than *Microcystis* and found similar inhibition results. Hao et al. (2004a, b) found inhibition of *Spirulina platensis* at 20 kHz and 1.7 MHz. Rajasekhar et al. (2012a) found greater growth inhibition in *Anabaena circinalis* than *Microcystis aeruginosa* at 20 kHz and 0.085 W/mL , possibly because sonication broke *Anabaena* filaments into small pieces and because *Anabaena* has weaker gas vesicles.



Microcystis.

Effects of Ultrasonic Waves on Cyanotoxin Release in Laboratory Studies

Cyanobacteria are capable of producing a number of toxins which, if ingested in sufficient amounts, can cause illness or even death in humans and animals. These toxins include neurotoxic anatoxins and saxitoxins, cytotoxic cylindrospermopsins, and hepatotoxic microcystins, which are the most commonly occurring cyanotoxins in aquatic systems worldwide (Chorus and Bartram 1999). One of the concerns with any sort of cyanobacterial bloom treatment is the potential release of a large amount of cyanotoxins, particularly in drinking water sources, wildlife habitat, or recreational waters. The potential release of cyanobacterial toxins via ultrasonic treatment of blooms presents a concern for public health, as well as potential impacts to aquatic biota.

Most sonication studies have focused on the genus *Microcystis* and the microcystins produced by these organisms. Lee et al. (2001) analyzed filtrate of ultrasonic-treated *Microcystis* suspensions via high-performance liquid chromatography (HPLC) for microcystins. The suspensions were determined to contain microcystin-LR and microcystin-RR prior to sonication. Suspensions were sonicated for 10 minutes at a frequency of 28 kHz and power of 1,200 W. Microcystin-RR was released after 10 minutes of treatment.

Ma et al. (2005) investigated the dynamics of microcystin release and degradation when *Microcystis* suspensions were treated with a variety of ultrasound frequencies and powers. They found cell destruction and microcystin release after irradiation at 20 kHz and 30-W intensity for five to nine minutes. They found no increase of microcystin release at higher intensities with zero to five minutes of sonication, and a decrease in microcystin levels due to molecule degradation. They found that a low concentration of microcystin was degraded to 35% of original levels after 30 minutes of sonication at 20 kHz and 30 W, but did not investigate this treatment with higher levels of microcystin that would be considered moderate to high risk by the World Health Organization (2003).

Zhang et al. (2006) examined the effects of sonication for five minutes at 25 kHz and 0.32 W/mL on *Microcystis aeruginosa*. They found that sonication degraded extracellular microcystin slightly, and seemed to inhibit microcystin release in the following 14 days when sonicated cells were cultured.

Broekman et al. (2010) found that when low-power ultrasound was applied to bacterial assemblages in tandem with microbubbles from an aeration system, cavitation,

cell inactivation, and lysing occurred at lower power than by ultrasound alone. Rajasekhar et al. (2012b) noted that this method could be effective in treating cyanobacteria in large quantities of water, but that it risks cell lysing and cyanotoxin release.

Rajasekhar et al. (2012a) demonstrated that at all ultrasonic intensities tested (at 20 kHz at 0.043-0.32 W/mL), sonication led to immediate increases in extracellular microcystin in filtrates of cell suspensions. This was true of both longer exposure times (>10 minutes) at low power intensity (0.043 W/mL) and five minutes of sonication at high intensity (0.32 W/mL). Longer exposure times also led to reductions in microcystin concentrations, due to breakdown of microcystin molecules by ultrasound. Rajasekhar et al. (2012a) noted that studies using similar ultrasonic frequencies and intensities may have experienced different results in microcystin releases due to differences in the *Microcystis* strains tested, cellular microcystin content, or resistance of cell walls to sonication. Ultrasound may have very different results in the field than in the laboratory, as natural cyanobacterial assemblages can be genetically diverse (Miller and McMahon 2011).

All of the laboratory-based studies we reviewed limited their ultrasonic treatments of cyanobacteria to relatively short periods. Despite the use of ultrasound devices in recreational water bodies and drinking water reservoirs, we found that no studies that were conducted over long periods in larger systems (see "Field Investigations..." below) had included sampling or analysis for cyanotoxins.

Free Radical and Hydrogen Peroxide Generation

Generation of free radicals and hydrogen peroxide (H_2O_2) from ultrasound treatment of water may play a role in the efficacy of ultrasound treatment. Joyce et al. (2010) noted that in 30-minute laboratory trials of different ultrasound frequencies and powers, at higher frequencies (864 kHz) more free radicals are produced ($\cdot H$ and biocidal $\cdot OH$) which may combine to form hydrogen peroxide and thus enhance the effectiveness of ultrasound treatment in algal inactivation. Although hydrogen peroxide is a naturally occurring byproduct of oxidative metabolism, large amounts of this compound can harm or kill cells.

Commercial Products Using Ultrasonic Technology

Several ultrasonic units marketed for algae removal are available. Small-scale, ultrasonic machines include the LG Sonic[®] line (LG Sonic, <http://www.lgsonic.com/>) and SonicSolutions[®] line (AlgaeControl.US, <http://www.algaecontrol.us/>). The large-scale Jet Streamer[®]/Algae Hunter[®] system marketed by Kapex Manufacturing combines ultrasonic treatment with a water circulation and microbubble oxygenation apparatus to treat cyanobacterial blooms (Yoshinaga and Kasai 2002, Herald 2011).

Large-scale treatments usually require multiple sonication devices and may include additional treatment strategies. Aeration and circulation devices, along with decreasing water residence time through flushing, have been coupled with sonication in large-scale projects

(Nakano et al. 2001, Lee et al. 2002, Ahn et al. 2007). These accessory treatments may potentially exacerbate bloom problems, especially in shallow lakes, or may lead to the lysing of cyanobacterial cells and the release of toxins into the surrounding water.

One working hypothesis for sonication treatment of blooms is that cavitation causes cyanobacterial cells to settle on the lake bottom where, in the absence of light, they are unable to regenerate gas vacuoles and regain buoyancy (Lee et al. 2001). Verspagen et al. (2004), however, found recruitment of *Microcystis* from sediments to the water column was induced by passive processes such as wind-induced mixing in shallow areas and bioturbation of sediments by invertebrates, not by changes in buoyancy. Verspagen et al.'s (2004) study examined naturally sedimented *Microcystis* cells, not cells which had lost buoyancy from sonication-induced vacuole disruption. It is possible, however, that circulation devices could induce cyanobacterial recruitment into the water column from sediments, where exposure to higher light levels would enable rapid vacuole regeneration (Lee et al. 2000) and re-establishment of bloom conditions.

Broekman et al. (2010) investigated sonication control of bacterial populations in industrial water systems. They found that bacterial cells could be lysed at lower ultrasound powers if air microbubbles were added to the treatment. The authors did not specify the makeup of the bacterial assemblages, but since cyanobacteria are true bacteria, they could possibly be subjected to lysing and toxin release if treated with a combination of ultrasound and microbubbles.



Several studies from lakes and ponds provided insight about the feasibility of this approach in large systems.

Field Investigations of Ultrasound Applications in Large Systems

Although much of the research on ultrasonic wave effects on cyanobacteria has been conducted in laboratories, there are several studies from lakes, ponds, and reservoirs which provide insight about the feasibility of this approach in large systems. Below we summarize three published field studies (Nakano et al. 2001 and Lee et al. 2002, Ahn et al. 2007, and Purcell et al. 2013) of ultrasonic wave efficacy on cyanobacterial blooms. None of these studies included detailed investigation of cyanobacterial cell sedimentation rates or measurements of algal toxins.

Lake Senba, Japan

A hypereutrophic and high-use recreational waterbody in Japan, Lake Senba (32 ha) has experienced annual cyanobacterial blooms. The shallow depth (average of 1 meter), agricultural watershed, and municipal sewage disposal regime have contributed greatly to the cyanobacterial problem in the lake. A combination of ultrasonic treatment, water jet circulation, and an increased rate of flushing river water through the lake was employed to alleviate the cyanobacterial bloom problem (Nakano et al. 2001, Lee et al. 2002). Ten circulator modules were installed in the lake. Lake water was pumped into a circulator module, irradiated by two 100-W, 200-kHz ultrasound transducers for approximately five seconds, and then ejected from the circulator. The lake was treated and monitored for two years. When lake flushing reached the desired water residence time, chlorophyll-*a*, suspended solids, and transparency were improved. Water quality, however, degraded when the flow rate decreased in the second year of treatment and the lake again experienced high chlorophyll concentrations, increased suspended solids, and decreased transparency. The reduced flow rate in the second year could have allowed sonicated cyanobacteria to settle on the lake bottom, regrow their gas vesicles, and resuspend to reach impaired conditions. Total phosphorus in the water decreased significantly during the treatment period. Total nitrogen was higher than previous years in the first year of treatment but lower in the second year. Chemical oxygen demand (COD) was reported as decreasing in treatment years; peak COD was lower than in the pre-treatment years but during treatment COD in lake water was consistently higher than inflow water. Sediment total nitrogen and total phosphorus generally increased during the treatment period, although levels sampled near the treatment apparatus did not increase. In their conclusions, Nakano et al. (2001) point out that mixing and flushing are important for the prevention of buoyancy renewal and thus for the prevention of the further proliferation of cyanobacteria.

Two Ponds in Gyeryong-si, Chungnam, Korea

Ahn et al. (2007) tested the efficacy of ultrasound in removing cyanobacteria from two eutrophic neighboring ponds (7,000 m³ and 9,000 m³) over a 49-day period from mid-August to the end of September. One pond was untreated and served as a control, while the other pond

was treated with a combination of ultrasonic irradiation (630 W, 22 kHz) and water pumps. Sonication treatments consisted of 85 seconds of irradiation followed by 30 second breaks. Sonication and circulation were halted accidentally from day 7 to day 11 of testing, and then were halted intentionally from day 23 to day 34. Chlorophyll-*a* concentration in the treatment pond was significantly lower than that of the control pond. The treatment pond chlorophyll-*a* concentration, however, quickly rose to the control level when the sonication/pump apparatus was intentionally shut off for 11 days, and the chlorophyll-*a* did not return to lower levels when the apparatus was switched on again. Cyanobacteria immediately became the dominant taxa when the apparatus was shut off in the treatment pond, then diatoms became dominant when treatment resumed. The authors proposed that the persistence of the high chlorophyll concentrations was due to the algal community shifting to a diatom-dominated system that is less susceptible to sonication. Total nitrogen and total phosphorus levels were higher in the treatment pond than in the control pond, though heavy rain caused a landslide in the treatment pond that could be responsible for the higher nutrient levels. The circulation pumps increased turbidity in the treated pond. The proportion of cyanobacteria and the overall algal densities were lower in the treated pond. Treatment may have killed algae other than cyanobacteria, resulting in the lower overall densities, but the authors did not include these data in their paper.

Reservoirs in the United Kingdom

Purcell et al. (2013) tested ultrasound in field trials at reservoirs operated by three United Kingdom water utility companies. Trials in several reservoir sites gave inconsistent results.

Trials at northwest England reservoirs over four years used 40-W, 28-kHz ultrasound transducers. There were no significant differences between cell densities in treated and untreated reservoirs in the northwest reservoirs, possibly because the reservoirs were not sampled on the same dates and because cell densities never exceeded 25,000 cells/mL and thus never reached bloom density. Trials at a southeast England reservoir used 40-W, 40-50-kHz, floating transducers. After five months of treatment with ultrasound, there were no differences in chlorophyll-*a* between treatments and controls, possibly because of methodological artifacts which were not explained in the paper. Green algae and diatoms trended toward lower densities in the sonicated treatment, while cyanobacterial densities were significantly lower in the sonicated treatment. However, when the authors compared the results to the previous three years' algal density data, they concluded that there were no significant differences between the sonication and control treatments.

Trials at East Anglia reservoirs occurred over 27 weeks. The power and frequencies of the ultrasound used were not reported. Chlorophyll-*a* was reduced in the treated reservoir. Although there was no significant difference between cell densities in treated and control reservoirs, there was a trend toward reduction in cyanobacterial and diatom cell densities in the sonicated reservoir, and diatom densities were decreased more than the cyanobacteria.

Biological Effects of Ultrasonic Waves on Non-target Species

Besides inducing cavitation and vacuole collapse in cyanobacterial cells, ultrasonic sound waves can cause harm to other aquatic organisms. We were unable to find publically available studies in which ultrasound devices marketed for cyanobacterial control were tested for potential effects on non-target aquatic organisms. Colucci (2010) found this as well in her literature review, and in correspondence with industry representatives learned that some testing has been conducted, but these studies have not been published nor have the results been made available to the scientific community.

Nevertheless, ultrasound has been tested at varying degrees on aquatic organisms, and studies of its effects on organisms exist from the earliest days of ultrasound use (Harvey and Loomis 1928, cited in Miller 1983a). Ultrasound is used in wastewater treatment (Madge and Jensen 2002), as an anti-biofouling strategy for marine applications (Gómez Olmedilla, 2012), and as a ballast water treatment (Holm et al. 2008). Most trials of sonication effects on organisms are conducted over short periods of time (a few seconds to 20 minutes), but some manufacturers recommend continuous operation of anti-algal sonication devices (LG Sonic, <http://www.lgsonic.com/ultrasonic-algae-control/>, accessed 01 May 2013; Sonic Solutions n.d.). As a result, the exposure of non-target organisms to ultrasound deployed in a lake setting could potentially exceed the exposures tested in laboratory studies. Below we describe some possible effects of ultrasonic waves on non-target aquatic organisms.

Bacteria

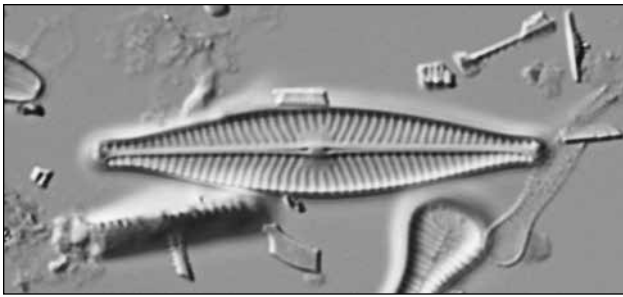
Ultrasound can be used to kill bacteria in water as a disinfection method in wastewater treatment and aquaculture. The physical effects of cavitation inactivate and lyse bacteria (Drakopoulou et al. 2009, Broekman et al. 2010).

Zimba and Grimm (2008) discuss some unpublished research on bacteria in their aquaculture trade magazine article. They tested ultrasound on channel catfish (*Ictalurus punctatus*) fingerlings in tanks and found that sonicated tanks had lower turbidity and lower bacterial counts. They suggest that ultrasound could be used to reduce pathogenic bacteria numbers in aquaculture ponds. If anti-algal, ultrasound devices are capable of killing bacteria in natural systems, this could lead to deleterious effects on bacterial-mediated nutrient cycles and organic matter processing in lakes.

Algae

Algae are the foundation of aquatic food webs, so adverse effects of ultrasonic devices on non-target algal species could have far-reaching effects in aquatic ecosystems. Diatoms in particular are an important, high-quality food source for higher trophic levels.

Appendix A lists 67 algal taxa, mostly identified to genus, which may be killed or otherwise incapacitated by anti-algal, ultrasonic devices. The list includes 13 cyanobacteria, 32 green algae, 16 diatoms, one chrysophyte,



Diatom.

three cryptophytes, and two euglenoid algae. We compiled the appendix from manufacturer and vendor sources, but it is unclear whether this information was taken from scientific literature or from unreleased industry studies.

Ahn et al. (2007) investigated ultrasound devices in ponds containing cyanobacteria, diatoms, and green algae. Chlorophyll-*a* levels and percent cyanobacteria were reduced in the sonicated pond. The authors did not present cell density or biomass data for non-cyanobacterial taxa, so diatoms and green algae may have been killed by the ultrasonic treatment as well, as indicated by the decrease in chlorophyll-*a* in the treated pond.

Holm et al. (2008) investigated sonication of phytoplankton for four minutes at 19 kHz for ballast water treatment. The diatom *Thalassiosira eccentrica* required exposure times of 2.1 to 3.8 minutes at intensities ranging from 14 to 17 W/cm² to kill 90% of cells. The dinoflagellate *Pfiesteria piscicida* required exposure times of 8.1 to 10.4 minutes at intensities ranging from 13 to 19 W/cm² for a 90% reduction in survival.

Rajasekhar et al. (2012a) examined sonication treatment of a small, unicellular coccoid green alga, *Chlorella* sp., at 20 kHz and 0.085 W/mL for zero to 20 minutes. Sonication did not reduce *Chlorella* concentration below the initial concentrations, but the authors note that their results with *Chlorella* may not be representative of all green algae.

A number of other studies have treated green algae with higher ultrasound frequencies (1 MHz to 2 MHz) and found deleterious effects. These include cytoplasmic clumping in *Hydrodictyon*, induction of cellular currents and cavitation in *Nitella*, and emulsification of cell contents and loss of turgor in *Spirogyra* and *Nitella* (Dyer et al. 1976, El'Piner et al. 1965, Goldman and Lepeschkin 1957, Harvey and Loomis 1928, Hopwood 1931; all reviewed in Miller 1983a). These taxa of green algae provide habitat for aquatic invertebrates (J.D. Hall, Department of Botany, Academy of Natural Sciences of Drexel University, Philadelphia, PA, pers. comm.) so their loss from ultrasound treatment could result in reduced invertebrate populations. Additionally, their loss would make more nutrients available for uptake by other primary producers, including cyanobacteria.

🌿 Plants

Numerous studies demonstrate deleterious effects of ultrasound on plants, but many of them use higher frequency ultrasound in the 1 MHz to 2 MHz range so results are more difficult to compare to the 20 kHz frequencies typically thought to be used in anti-algal, sonication devices.

Waterweeds (*Elodea*) were frequently tested, and ultrasonic effects of these higher frequencies include cavitation and cell death (Miller 1983a, 1983b).

Wu and Wu (2007) investigated the effects of a range of frequencies (20 kHz to 2 MHz) on water chestnut (*Trapa natans*). They found that after 10 seconds of ultrasonic waves aimed at a target spot on the plants, the 20 kHz frequency (1.8 MPa acoustic pressure amplitude) caused significant cell membrane disruption leading to loss of leaves, buoyancy, and vitality.

🐞 Zooplankton

Ultrasound has been investigated as a control for zooplankton in ballast water. Holm et al. (2008) tested ultrasound effects on a cladoceran (*Ceriodaphnia dubia*), rotifers (*Brachionus plicatilis*, *B. calyciflorus*, and *Philodina* sp.), and brine shrimp (*Artemia* sp.). Testing was done in a flow-through system and investigated the exposure time and energy density needed to kill 90% of organisms when using an ultrasound frequency of 19 kHz. Holm et al. (2008) found that contact times of one to four seconds and an energy density of 6-19 J/mL resulted in high mortality. Organisms either passed through the system or were "reduced to debris." Microjets within the zooplankton caused by the collapse of cavitation bubbles were the hypothesized cause of zooplankton mortality in the experiments. This 19-kHz treatment was most effective against zooplankton larger than 100 μm, and exposure times below 10 seconds and energy densities less than 20 J/mL resulted in 90% mortality (Holm et al. 2008). Because intensity levels of anti-algal, ultrasonic devices are proprietary information, it is unknown whether the ballast control treatment levels are in a range similar to what is produced by those devices.

🐌 Mollusks

Ultrasound has been found to be effective in killing snails which serve as parasite hosts in aquaculture settings. Goodwiller and Chambers (2012) sonicated ramshorn snails (*Planorbella trivolvis*) in a tank at a frequency of 20 kHz and power up to 89 W (the specific power levels they used were unreported). Snails were placed five to 13 cm from the sonicator and in tests of five to 120 seconds of sonication of groups of 10 snails, 0 to 100% of snails died, with 40% dead after 30 seconds and 70% after 60 seconds. Death was hypothesized to be from internal injuries, as the sonication produced clouds of cavitation bubbles. Additional experiments that were run over 90-second intervals appeared to kill 35% of snails outright and mortally wounded an additional 33% of snails, which died within four days of the conclusion of the experiment.

Ultrasound has been investigated as a method for zebra mussel (*Dreissena polymorpha*) control, although frequencies below ultrasound are most often used (Kowalewski et al. 1993, Donskoy and Ludyanskiy 1995). Donskoy and Ludyanskiy (1995) cite research in which ultrasound ranging from 20 kHz to 380 kHz was used to induce cavitation and mortality in veliger, juvenile, and adult zebra mussels. No information on the effects of ultrasound on native mussel glochidia (juvenile life stages) could be found.

Insects

Miller (2007) cites a study by Child and Carstensen (1982) in which pulsed ultrasound (peak intensity 10-20 W/cm², 2 MHz), destroyed cell membranes and killed cells of fruit flies (*Drosophila*) as eggs hatched and larvae developed gas-filled respiratory channels. Child and Carstensen (1982) hypothesized that the ultrasound affected the gas bodies within the respiratory channels. These are higher frequencies than those typically believed to be used by anti-algal, sonication devices, but these studies indicate that sonication could have deleterious effects on insects.

Some aquatic insects are known to communicate with ultrasonic sound. Water boatmen (Hemiptera: Corixidae: *Micronecta*) produce courtship songs which are partially in ultrasonic range (approximately 5-22 kHz) (Sueur et al. 2011). Caddisfly larvae (Trichoptera: Hydropsychidae: *Cheumatopsyche*, *Diplonecta*, and *Hydropsyche*) produce ultrasonic sounds which serve as territorial displays (Silver 1980). Ultrasound generated by anti-algal, ultrasonic devices potentially could interfere with aquatic insect communication and behavior.

Amphibians

Amphibian embryonic tissue was destroyed and amphibian embryos suffered mortality after being exposed to ultrasonic waves (Sarvazyan et al. 1982, Pashovkin et al. 2006). Sarvazyan et al. (1982) irradiated common frog (*Rana temporaria*) and African clawed frog (*Xenopus laevis*) eggs and tissue at 0.88 MHz and at average intensities of 0.025-0.1 W/cm². Pashovkin et al. (2006) employed a variety of frequencies and durations and induced almost complete mortality of *Rana temporaria* embryos after five to 15 minutes at 0.88 MHz and 0.2-0.7 W/cm². While these studies used ultrasound frequencies which exceed the frequencies usually employed in cyanobacterial studies, they demonstrate a potentially deleterious effect of sonication on amphibians.

Fish

Despite the use of anti-algal, ultrasonic units in aquaculture ponds, we found no publically available information that addressed the effects of non-medical, ultrasound uses on fish and only a small number of papers that dealt with behavioral responses to ultrasound.

Some marine fish (Clupeidae: cod [*Gadus morhua*], herring and shad [*Alosa aestivalis*, *A. sapidissima*, *Clupea harengus*], and Gulf menhaden [*Brevoortia patronus*]) can detect ultrasound (up to 180 kHz), which elicits anti-predator behavior (Astrup 1999, Popper et al. 2004), but research on ultrasound detection in freshwater fish is scarce. Ultrasound (122-128 kHz, 190 dB) has been used as a method to deter alewives (*Alosa pseudoharengus*) from a Lake Ontario power plant intake (Ross et al. 1993, 1996). Alewives are members of the same family that includes the marine fish species known to detect ultrasound. In addition to using the inner ear for ultrasound detection (Popper et al. 2004), the lateral line, swim bladder, or receptors in the epidermis may also play roles in ultrasound detection in fish (Astrup 1999).

Zimba and Grimm (2008) noted in a trade magazine article that in tank trials with channel catfish fingerlings, continuous operation of ultrasound devices deterred fish from feeding. Their trials were modified to allow a four-hour period without ultrasound treatment around the feeding time. Continuous operation of ultrasound devices could interfere with fish feeding or other behavior in a natural setting.

Ultrasound enhances uptake of particles into cells by inducing cavitation and by widening intercellular spaces, thus increasing permeability of the skin (e.g., sonication at 3 MHz and 2.2 W/cm²; Frenkel et al. 2000a). This effect of ultrasound has been used for a variety of applications in aquaculture, including transport of silver chloride nanoparticles (Frenkel et al. 2000b) and vaccination. Fernandez-Alonso et al. (2001) used ultrasound (24 seconds at 40 kHz and 40 W in a small bath sonicator) to transfer viral hemorrhagic septicemia plasmids into trout fingerlings as a form of immersion vaccination.

Zohar et al. (1991) note in their U.S. patent that for fish, crustaceans, and mollusks, compounds which can be administered with their ultrasound-enhanced method include proteins, nucleic acid sequences, antibiotics, anti-fungals, steroids, vitamins, nutrients, minerals, hormones, and vaccines. They state that frequencies and intensities used to implement molecule transfer range from 20 kHz to 10 MHz and 0 to 3 W/cm². Exposures of a few minutes are sufficient, and they consider excessive exposure as being greater than one hour (Zohar et al. 1991). It is possible that fish in natural systems could be at risk for disease or possibly environmental contaminant uptake if their ultrasound exposure is great enough to induce epidermal permeability.

Humans

The safety of ultrasound use for medical applications should not be extrapolated to other situations. The acoustic pressure generated in one study to disrupt and sink cyanobacterial cells exceeded the maximum acoustic pressure allowed by the NATO Undersea Research Centre (NURC) by over 35 decibels, despite being within the safe mechanical index range used for diagnostic ultrasound (NURC 2006, Kotopoulis et al. 2009). Kotopoulis et al. (2009) tested higher frequencies (200 kHz to 2.5 MHz) than those believed to typically be produced by anti-algal, ultrasound devices. The maximum acoustic pressure exposure to human divers and marine mammals allowed by NURC is 708 Pa at frequencies up to 250 kHz (Kotopoulis et al. 2009). Exceeding these levels, as Kotopoulis et al. (2009) did to burst cyanobacterial vacuoles, could cause serious damage to divers and aquatic mammals, and the authors urge caution in using their cyanobacteria removal techniques when aquatic animals are present. It is not clear if these devices also pose a risk of tissue damage to humans swimming near the devices, or if they may cause cumulative effects from repeatedly swimming in the lower intensity ultrasonic treatment area. Kotopoulis et al. (2008), in a conference abstract, report that if ultrasound in the clinical diagnostic range from 200 kHz to 2.5 MHz is used for algal eradication, the safe swimming distance

would be several meters away from the ultrasound source. These frequencies are higher than most used in cyanobacterial treatment investigations. Ultrasound device intensity levels are proprietary information, so we are unable to determine whether the intensity levels required for scaling up lower frequency treatments for larger systems would have an effect on humans.

Tissue damage from cavitation is a potential risk with ultrasound exposure at certain frequencies, intensities, and lengths of exposure. The owner's manual for Sonic Solutions Algae Control Systems (Sonic Solutions, n.d.) includes the following warning in the safety information:

“7. WARNING – Risk of injury. May cause tissue damage. DO NOT place the transducer against your head or chest while the device is operating.”

The release of cyanobacterial toxins from burst or damaged cyanobacterial cells poses a potential risk to human health from operation of these devices in lakes. Current guidance to the public advises them to visually assess water bodies and to avoid ingestion of water if cyanobacterial scums or turbid, “pea soup” conditions are present, as those conditions represent high to very high risk of adverse health effects (World Health Organization 2003; Wisconsin Department of Health Services, www.dhs.wisconsin.gov/eh/bluegreenalgae/understandingalgae.htm). Hypothetically, if an ultrasonic device were powerful enough to remove an algal scum in a short period of time, recreational users could be presented with a situation in which toxins were present but the absence of the scum did not indicate risky conditions for exposure.

Impacts on Water Quality

Sonication and the supplementary treatments with which it may be coupled may adversely affect water quality. Ahn et al. (2003) noted increases in total dissolved phosphorus and orthophosphate in sonicated pond enclosures, and attributed this to ultrasound's ability to dissociate phosphate from particles. Long-term treatment could thus fuel additional algal growth. Ahn et al. (2007) found that in a pond treated with a combination of sonication and circulation, the water pumps increased turbidity. Circulation and aeration devices may de-stratify the water column, which in some systems may enhance nutrient release from lake sediments, further impacting water quality and promoting algal blooms (Hupfer and Lewandowski 2008, James 2012).

Conclusions

Most studies of ultrasound on cyanobacteria are short, laboratory-based studies. It is difficult to draw conclusions on the effects of the continuous operation of anti-algal, ultrasound devices in large aquatic systems from the few field studies that are available in the peer-reviewed, publicly-available scientific literature. Additionally, there is a lack of information on wavelengths and intensities used by the devices because that remains proprietary information. We reviewed studies using ultrasound frequencies believed

to be in a range similar to those generated by anti-algal, ultrasound devices. Sonication does appear to inactivate cyanobacteria in very short-term, small laboratory experiments. Ultrasound intensity and duration, however, will likely be different when these devices are used in natural systems. Intensity may effectively be lower with larger volumes of water. The effects of continuously operated units as recommended by manufacturers (LG Sonic, <http://www.lgsonic.com/ultrasonic-algae-control/> accessed 01 May 2013; Sonic Solutions n.d.) may differ from those of five to 10-minute laboratory trials.

Concerns for the use of sonication technology include the potential release of cyanotoxins from lysed cyanobacterial cells. This would pose a hazard not only to the organisms living in or foraging in the lake, but to humans and their pets recreating on the water as well. Ultrasound is used in recreational waters and drinking water reservoirs (Purcell et al. 2013), but data on algal toxins in large systems treated with ultrasound are absent from the scientific literature.

The devices themselves may pose potential health hazards. Depending on the duration, intensity, and proximity to swimmers, ultrasonic algae control technology could cause harm to humans. One study found that the acoustic pressure generated to burst cyanobacterial vacuoles greatly exceeded the criteria proposed by NATO for divers and aquatic mammals (NURC 2006, Kotopoulis et al. 2009). The owner's manual for one manufacturer's devices warns that tissue damage could result if the transducer is placed against the head or chest while operating and states that the device should always be unplugged before cleaning or handling (Sonic Solutions n.d.). If the sonication device assemblage in a lake is prominent or noticeable, such as the swan-shaped Lake Senba units (Nakano et al. 2001, Lee et al. 2002), people could be drawn to them out of curiosity and receive high dosages of ultrasound irradiation while swimming near them.

We have reviewed numerous scientific studies which detail the negative effects of ultrasound on aquatic organisms. The ecological effects should also be considered, particularly changes to aquatic food webs if high-quality food sources such as diatoms or zooplankton are killed. Effects on recreational opportunities should also be considered if fish will not feed when exposed to ultrasonic waves.

If ultrasonic devices truly are effective in large systems, our review of ultrasound effects on non-target organisms indicates that they potentially could affect adversely a great number of non-target species in lakes, as well as potentially pose some risk to humans using lakes for recreation. On the other hand, if the devices are not powerful enough to cause harm to aquatic organisms, they may not be effective against cyanobacteria either. Mason (2007) advocates ultrasound use in environmental remediation and protection as a link between “green” chemistry, “green” engineering, and physics. Ultrasound does offer potential for treating the conditions caused by eutrophication, but the biology and ecology of aquatic organisms and their habitats must be considered as well.



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Chrysophytes (Kingdom Chromista, Phylum Ochrophyta)									
<i>Tribonema</i> sp.									♦
Cryptophytes (Kingdom Chromista, Phylum Cryptophyta)									
<i>Cryptomonas erosa</i> Ehrenberg								♦	♦
<i>Cryptomonas</i> sp.	♦							♦	
<i>Rhodomonas lacustris</i> var. <i>nannoplantctica</i> (Skuja) Javornický (reported as <i>Rhodomonas minuta</i>) ¹	♦							♦	♦
Euglenoid Algae (Kingdom Protozoa, Phylum Euglenophyta)									
<i>Euglena</i> sp.							♦		♦
<i>Phacus</i> sp.	♦							♦	
Green Algae (Kingdom Plantae, Phylum Charophyta & Phylum Chlorophyta)									
<i>Acutodesmus acuminatus</i> (Lagerheim) Tsarenko (reported as <i>Scenedesmus acuminatus</i> [sic])	♦							♦	♦
<i>Acutodesmus obliquus</i> (Turpin) Hegewald & Hanagata (reported as <i>Scenedesmus obliquus</i>)	♦						♦	♦	
<i>Ankistrodesmus falcatus</i> (Corda) Ralfs									♦
<i>Aphanochaete</i> sp.									♦
<i>Botryococcus braunii</i> Kützing								♦	♦
<i>Chara</i> sp.									♦
<i>Chlamydomonas</i> sp.	♦								♦
<i>Chlorella</i> sp.	♦								♦
<i>Chloromonas botrys</i> Pascher									♦
<i>Cladophora</i> sp.	♦							♦	
<i>Closterium</i> sp.	♦								♦
<i>Coelastrum</i> sp.	♦								♦
<i>Cosmarium</i> sp.	♦								♦
<i>Crucigenia</i> sp.									♦
<i>Desmodesmus abundans</i> (Kirchner) Hegewald (reported as <i>Scenedesmus abundans</i>)									♦
<i>Desmodesmus quadricaudatus</i> (Turpin) Hegewald (reported as <i>Scenedesmus quadricauda</i>)	♦								♦
<i>Dictyosphaerium</i> sp.	♦								♦
<i>Gloeocystis</i> sp.	♦								♦
<i>Lagerheimia</i> sp.	♦								♦

(continued on next page)

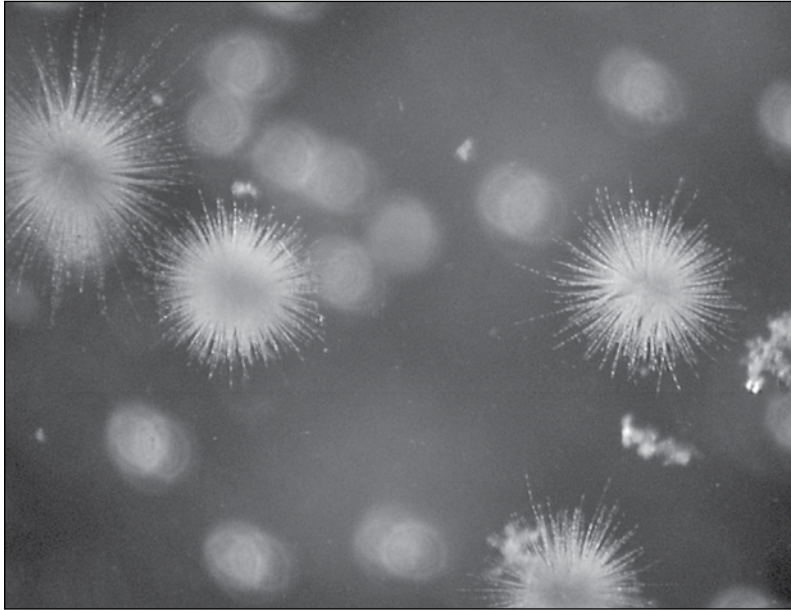
¹This is a marine species. *Plagioselmis nannoplantctica* (Skuja) Novarino, Lucas & Morrall may instead be the correct species (it was transferred from *Rhodomonas minuta* var. *nannoplantctica* Skuja).

(continued from page 11)

Source:*	1		2			3			4		
	Killed	75-95% effectiveness	Not killed	Affected	Partial or 75% effectiveness	Resistant [sic]	100% effectiveness	75-95% effectiveness	50% effectiveness	Controlled	Not controlled
Effects of sonication vs cyanobacteria and algae species:											
Green Algae (Kingdom Plantae, Phylum Charophyta & Phylum Chlorophyta) continued											
<i>Micractinium</i> sp.	♦			♦			♦		♦	♦	
<i>Nitella</i> sp.			♦			♦			♦		♦
<i>Oedogonium</i> sp.	♦										
<i>Oocystis pusilla</i> Hansgirg	♦			♦			♦			♦	
<i>Oocystis</i> sp.	♦										
<i>Pediastrum</i> sp.						♦			♦		
<i>Pithophora</i> sp.											♦
<i>Pseudopediastrum boryanum</i> (Turpin) E. Hegewald (reported as <i>Pediastrum boryanum</i>)											
<i>Sphaerocystis Schroeteri</i> Chodat											
<i>Spirogyra</i> sp.	♦						♦			♦	
<i>Staurastrum</i> sp.											
<i>Stigeoclonium</i> sp.	♦									♦	
<i>Ulothrix</i> sp.	♦									♦	

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Gloeotrichia.



Planktonic algae.

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Abbreviations

cm = centimeter
dB = decibel
J = joule
kHz = kilohertz
MHz = megahertz
mL = milliliter
MPa = megapascal
Pa = pascal
µm = micrometer
W = watt

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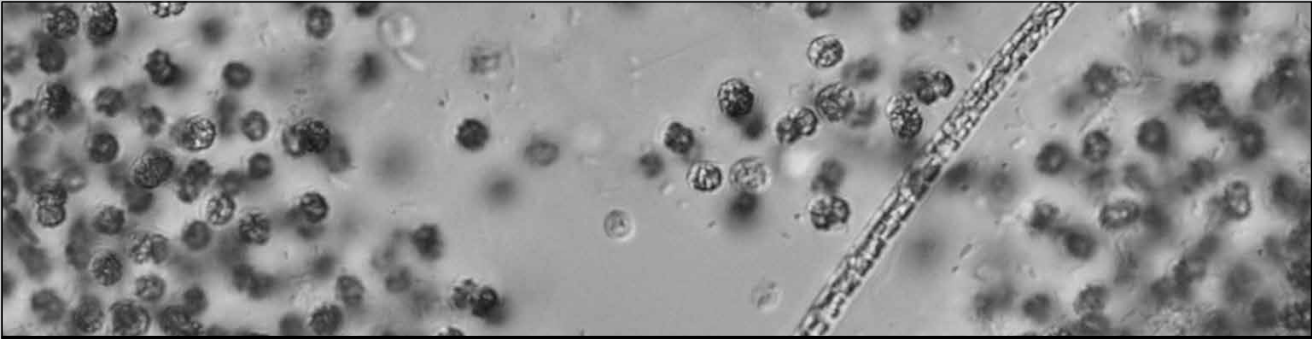
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