Safety radius for algae eradication at 200 kHz - 2.5 MHz

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***Abstract*—Algae have been proven to be a severe health hazard to humans, aquatic and semi-aquatic animals. Chemical methods available to control the algae have unwanted side-effects. For this reason, ultrasonic algae control has been under investigation. We measured the eradication effectiveness of ultrasound at three typical centre frequencies. At all three frequencies physical damage to the algae was observed. We conclude that it is possible to eradicate blue-green algae in the clinical diagnostic range. Taking into account the geometry, the low attenuation in water, and the NATO Undersea Research Centre for Human Diver and Marine Mammal Risk Mitigation Rules and Procedures, even at these low voltages, the safe swimming distance is at least several meters away from the sound source.**

I. Introduction

ALGAE are known to cause many health hazards to humans including skin rashes, gastrointestinal, respiratory and allergic reactions, [1] and liver cancer [2]. In addition, blue-green algae may have implications on aquatic and semi-aquatic animals, such as fish [3] and platypus.

There are chemical methods to control certain species of algae, but these have side-effects such as promotion and growth of other species of algae [4] whilst also affecting aquatic life in fresh water ponds and lakes. Therefore, ultrasonic algae control has been under investigation [5]. The frequencies used are as low as 20 kHz and as high as 1.7 MHz. Most commercially available equipment works in the lower ultrasonic range [6]. There have been speculations about the physical mechanism behind the algae eradication, specifically about the role of cavitation.

In this study, we investigate the effectiveness of higher and lower ultrasonic insonification on blue-green algae. From the results, an estimate of the safety radius is made based on current regulations.

Algae strands contain nitrogen-producing cells that make them float. If the membranes of these cells are disrupted by means of ultrasound, the gas may be released analogous to [7], causing the strands to sink. This is a desirable ecological effect. A measure for the safe use of ultrasound is the mechanical index (MI) [8] defined by

$$\mathrm{MI} = \frac{p^-}{\sqrt{f}}, \tag{1}$$

where p^- is the maximum value of peak negative pressure anywhere in the ultrasound field, measured in water but reduced by an attenuation factor equal to that which would be produced by a medium having an attenuation coefficient of 0.3 dBcm^{-1}MHz^{-1}, normalised by 1 MPa, and f is the centre frequency of the ultrasound normalised by 1 MHz [9]. For MI<0.3, the ultrasonic amplitude is considered low. In clinical diagnostics there is a possibility of minor damage to neonatal lung or intestine [9] for 0.3>MI>0.7. These are considered moderate acoustic amplitudes. For MI>0.7, there is a risk of cavitation if gas cavitation nuclei are present, and there is a theoretical risk of cavitation without the presence of cavitation nuclei [9]. The risk increases with MI values above this threshold [9]. These are considered high acoustic amplitudes.

The maximum values to which divers can be exposed to, stated by the NATO Undersea Research Centre for Human Diver and Marine Mammal Risk Mitigation Rules and Procedures [10]. The maximum acoustic pressure to which mammals can be exposed is 708 Pa at frequencies up to 250 kHz. This corresponds to a mechanical index MI$< 0.01 \ll 0.3$.

II. Materials and Methods

To investigate the effect of ultrasonic frequency on algae eradication, three ultrasound transducers were used. A 200-kHz transducer containing a PIC155 single undamped Piezo element (PI Ceramics, Lederhose, Germany), a 1-MHz, PA 188 single undamped element transducer spherically focused at 65 mm (Precision Acoustics Ltd, Dorchester, UK) and a 2.2-MHz transducer containing a Pz37 single undamped element transducer (Ferroperm Piezoceramics A/S, Kvistgård, Denmark).The focal distance of the 2.2-MHz transducer was 73 mm. The design of two transducers is shown in Fig. 1.

The algae used were of the anabaena species. The anabaena were cultured in 2 L of Jaworski's medium at room temperature near a South facing window in an Erlenmeyer flask for 11 days.

The culture was split equally into four 250-mL Perspex beakers: one control beaker and one for each transducer. The transducers were inserted separately in each beaker with the acoustic focus within the sample. Each transducer was turned on for 1 hour. The transducers were subjected to 16-Vpp square pulses at a 11.8-kHz pulse repetition rate transmitted by a V1.0 pulser-receiver (Sonemat, Coventry, UK). A digital picture of the solution was taken every five minutes using an EOS 350D digital photo camera (Canon Inc, Tokyo, Japan). The lighting and exposure settings were controlled and maintained throughout the insonification. Full manual settings were used: ISO 100, Exposure Time 1/50 s, F Number 3.50, Focal Length 18mm, No flash, Centre weighted metering mode, Custom white balance B4, 0 Shift. The digital

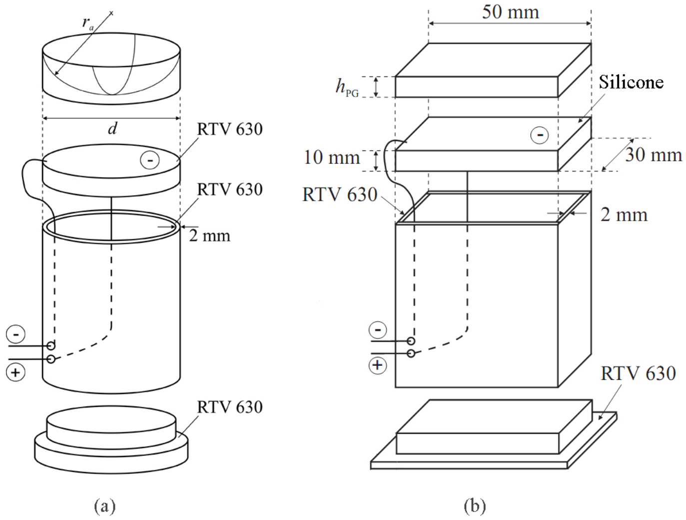

Fig. 1. (a) Undamped 2.2-MHz ultrasound transducer with $d = 1$" diameter and $r_{\mathrm{a}} = 35$ mm acoustic lens. (b) Undamped 200-kHz ultrasound transducer with h_{PG} = 10 mm.

images were converted to 8-bit grey scale. On both control and insonified solution, a square area of 160 x 160 pixles was taken and the average was grey scale depth was calculated using MATLAB (The Mathworks, Natick, MA). The change in shade from the first image taken just before insonification was calculated and graphed for both control sample and insonified samples.

Every 10 minutes a 20-μL sample was taken from the insonified solution. These were looked at under a CHA microscope (Olympus Corporation, Tokyo, Japan) and photographed. From these photographs, cell deterioration and chlorophyll damage was determined. To investigate the effect of ultrasound on chlorophyll, fluorescent light was used. When fluorescent light is projected onto chlorophyll it is absorbed and re-emitted as a red glow. The red glow denotes that the chlorophyll is still active and can photosynthesize, thus the algae strands are still alive.

After one hour insonification, twenty four 1-mL samples were removed from each insonified solution and put into a culture dish to see the effect of ultrasound on growth. Twenty-four control samples were taken. The culture dish was left in sunlight for 30 days.

III. Results and Discussion

Fig. 4 shows the microscopic effect off ultrasound on the floating bodies in the algae solution. From 0 minutes to 60 minutes of insonification, no change was seen in the physical structure of the algae for the whole frequency range. Illuminating the algae with fluorescent light showed that the ultrasound had no effect on the chlorophyll activity for the whole frequency range. The active chlorophyll shows that the algae strands are still alive and able to photosynthesize.

Examples of increased clarity after insonification and increase in amount of sunken bodies can be seen in Fig. 2 and Fig. 3 respectively.

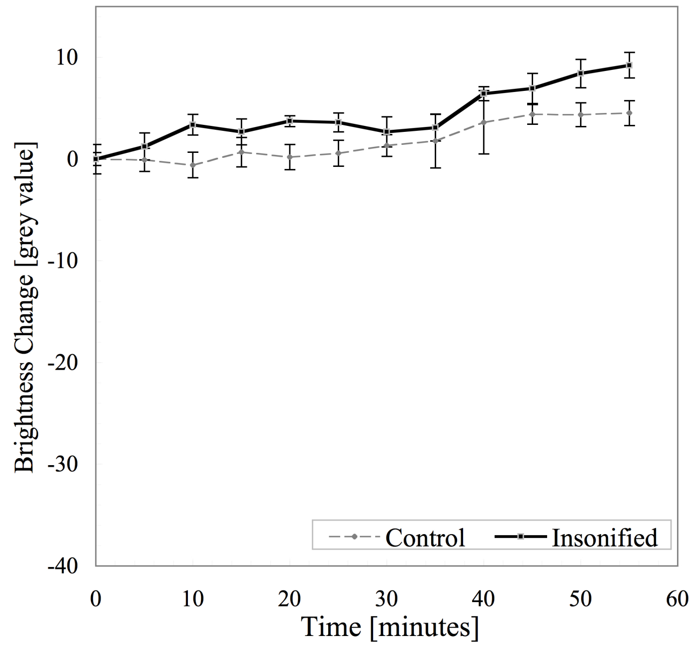

Fig. 2. Water clarity as a function of ultrasonic exposure time for a 1.0 MHz frequency. Higher values show greater clarity.

Fig. 2 shows a faster and constant increase in brightness over time compared to the control experiment. The insonified sample maintained a greater brightness throughout the whole insonification period. At 55 minutes the insonified sample is 54% clearer than the control sample. Similar results were obtained for the whole frequency range.

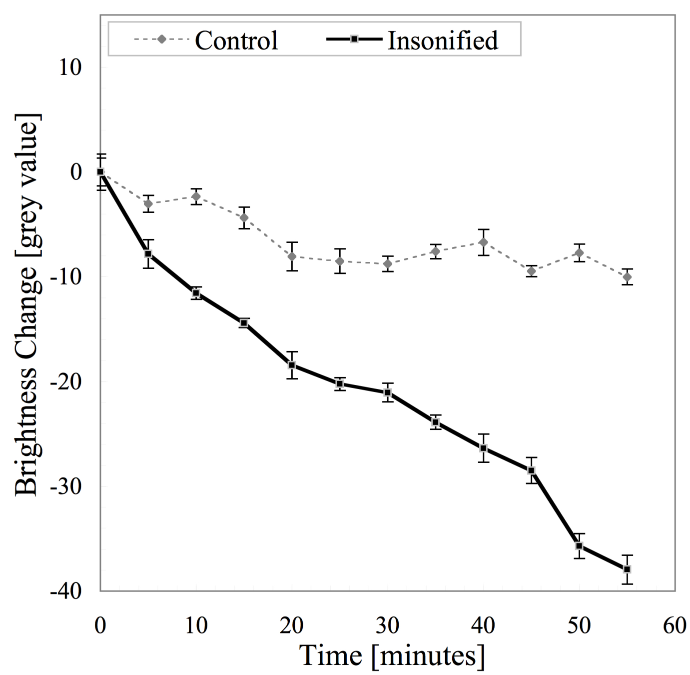

Fig. 3. Water clarity at the base of the beaker as a function of ultrasonic exposure time for a 1.0 MHz frequency. Lower values show more sunken algae.

Fig. 3 shows shows a decrease in brightness over time compared to the control experiment. The insonified sample has gathered algae at the bottom of the beaker at a greater rate than that of the control experiment. More algae had sunk to the bottom of the beaker as compared to the control experiment throughout the whole duration of the insonification. At 55 minutes the insonified sample was 280% darker than the control sample showing greater amount of algae had sunk than that in the control sample. Similar results were seen throughout the whole frequency range. Clearly, the algae that were floating in the beaker dropped to the bottom at a faster rate than the control sample. This is attributed to the disruption of the floating bodies by the ultrasound.

The grey scale measurements for the culture dish can be summarised as follows. At 200 kHz, 1.0 MHz and 2.2 MHz, the samples were 15%, 41% and 34% brighter respectively, as compared to the control sample. These results support the hypothesis that the algae that have sunk are less capable of multiplying. Hence, insonification may prevent algae bloom.

In the acoustic focus, the highest sound pressure was calculated to be 219 kPa at 2.2 MHz, *i.e.*, mechanical index of MI<0.15. Clearly, these values are much lower than the cavitation threshold. Comparing the acoustic outputs of the transducers to the NATO Undersea Research Center Human Diver and Marine Mammal Risk mitigation Rules and Procedures, *i.e.*, 708 Pa between 31.5 kHz and 250 kHz, at very close distances the threshold for safe diving is surpassed. From *in-situ* measurements of commercial equipment, it is estimated that the safe radius for swimmers is 15 m [6]. Although the worst-case mechanical index close to the transducers is MI<0.3, some acoustic pressures determined are higher than those allowable by the NATO Undersea Research Center Human Diver and Marine Mammal Risk mitigation Rules and Procedures.

IV. Conclusion

It is possible to eradicate blue-green algae in the clinical diagnostic range. Taking into account the geometry,the low attenuation in water, and the NATO Undersea Research Centre for Human Diver and Marine Mammal Risk Mitigation Rules and Procedures, even at these low voltages, the safe swimming distance is at least several meters away from the sound source.

V. Acknowledgments

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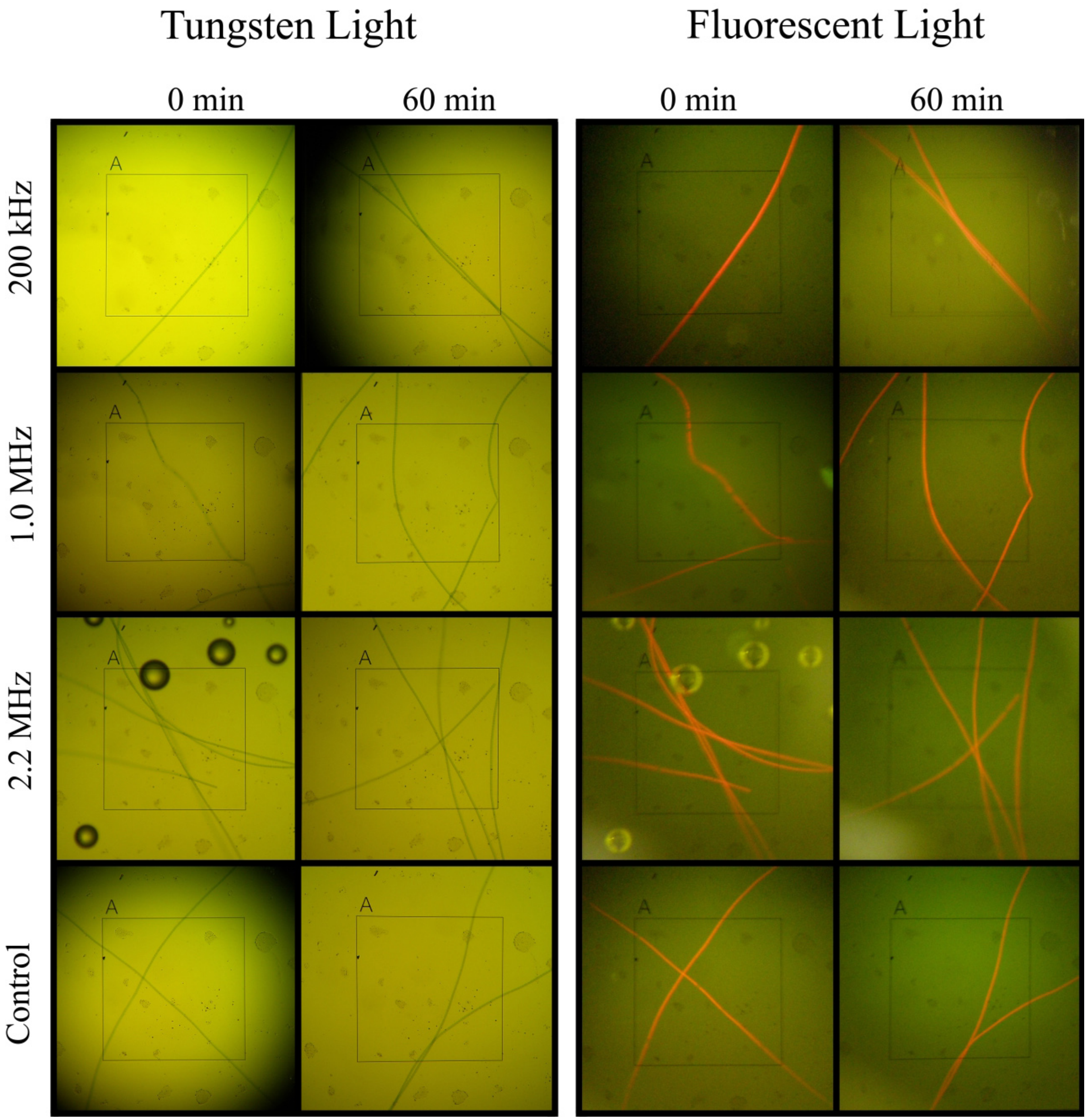

Fig. 4. Microscopic image sequence showing the effect of 200 kHz – 2.5 MHz ultrasound on algae. Each frame corresponds to 565 × 565 μm^2. Under fluorescent light the red shows active chlorophyll.