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Sonic cracking of blue-green algae

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Abstract

Algae are aquatic organisms classified separately from plants. They are known to cause many hazards to humans and the environment. Algae strands contain nitrogen-producing cells that help them float (heterocysts). It is hypothesized that if the membranes of these cells are disrupted by means of ultrasound, the gas may be released analogous to sonic cracking, causing the strands to sink. This is a desirable ecological effect, because of the resulting suppressed release of toxins into the water.

We subjected small quantities of blue-green algae of the *Anabaena sphaerica* species to ultrasound of frequencies and pressures in the clinical diagnostic range, and observed the changes in brightness of these solutions over time. Blue green algae were forced to sink at any ultrasonic frequency we studied, supporting our hypothesis that heterocysts release nitrogen under ultrasound insonification in the clinical diagnostic range.

Although the acoustic fields we used to eradicate blue-green algae are perfectly safe in terms of mechanical index, the acoustic pressures surpass the NURC Rules and Procedures by over 35 dB. Therefore, caution should be taken when using these techniques in a surrounding where aquatic or semi-aquatic animals are present.

Key words:

PACS:

1 Introduction

Algae are aquatic, eukaryotic, photosynthetic organisms, ranging in size from single-celled forms to large kelps. Algae are classified separately from plants since they lack true roots, stems, leaves and embryos. Algae are known to cause many health hazards to humans including skin rashes, gastrointestinal,

6 respiratory [1], allergic reactions, [2] and liver cancer [3]. In addition,
7 blue-green algae may have implications on aquatic and semi-aquatic animals
8 [4]. Eutrophication is the increase in chemical nutrients within the ecosystem,
9 causing blooms of algae and plant life and the subsequent decomposition of
10 blue-green algae by bacteria, an oxygen-consuming process [5]. When billions
11 of such bacterial cells die during a bloom, the water becomes oxygen-depleted,
12 killing off oxygen-dependent organisms [6].

13 The main factors that influence algae growth are temperature and light [7,8].
14 At low temperatures and low light conditions, the algae do not photosynthesise
15 and therefore do not bloom. Algae strands contain nitrogen-producing cells
16 that help them float (heterocysts), as demonstrated in Fig. 1. **The heterocysts**
17 **have a diameter between 5 – 7 μm [9].** It is hypothesized that if the membranes
18 of these cells are disrupted by means of ultrasound, the gas may be released
19 analogous to sonic cracking [10], causing the strands to sink. **At the lake bed,**
20 **illumination is lower, thus reducing algae multiplication.** This is a desirable
21 ecological effect, because of resulting suppressed release of toxins into the
22 water.

23 There are chemical methods to control certain species of algae, but these
24 have side-effects such as promotion and growth of other species of algae [11]
25 whilst also affecting aquatic life in fresh water ponds and lakes. Therefore,
26 ultrasonic algae control has been under investigation [12–17]. **In [12–17],**
27 **ultrasonic insonification of different species of algae led to a decrease in algae**
28 **concentrations in the frequency range 20 kHz–1.7 MHz, which is in contrast**
29 **with [18], where ultrasound was observed to strengthen the cell membranes**
30 **of red algae. In these studies, the exact acoustic conditions have not been**
31 **specified other than the frequency and power input. Hence, from the acoustics**

32 point of view, they are not repeatable. More importantly, the mechanism
33 causing algae eradication or membrane disruption had not been investigated.

34 Most commercially available equipment works in the lower ultrasonic range
35 [19]. There have been speculations about the physical mechanism behind
36 the algae eradication, specifically about the role of cavitation. In this study,
37 we investigate the effectiveness of ultrasonic insonification in the clinical
38 diagnostic range on *Anabaena* blue-green algae.

39 A measure for the safe use of clinical diagnostic ultrasound is the mechanical
40 index (MI) defined by

$$41 \quad \text{MI} = \frac{p^-}{\sqrt{f}}, \quad (1)$$

42 where p^- is the maximum value of peak negative pressure anywhere in the
43 ultrasound field, measured in water but reduced by an attenuation factor
44 equal to that which would be produced by a medium having an attenuation
45 coefficient of $0.3 \text{ dBcm}^{-1}\text{MHz}^{-1}$, normalised by 1 MPa, and f is the centre
46 frequency of the ultrasound normalised by 1 MHz [20]. For $\text{MI} < 0.3$, the
47 ultrasonic amplitude is considered low. In clinical diagnostics there is a
48 possibility of minor damage to neonatal lung or intestine [20] for $0.3 < \text{MI} < 0.7$.
49 These are considered moderate acoustic amplitudes. For $\text{MI} > 0.7$, there is a risk
50 of cavitation if gas cavitation nuclei are present, and there is a theoretical risk
51 of cavitation without the presence of cavitation nuclei [20]. The risk increases
52 with MI values above this threshold [20]. These are considered high acoustic
53 amplitudes. According to the NATO Undersea Research Centre (NURC)
54 Human Diver and Marine Mammal Risk Mitigation Rules and Procedures
55 [21], the maximum acoustic pressure to which mammals can be exposed is
56 708 Pa at frequencies up to 250 kHz. This corresponds to a mechanical index

57 $MI < 0.01 \ll 0.3$. In this paper, we have chosen to work in the lower clinical
58 diagnostic range, taking into account both guidelines.

59 2 Materials and Methods

60 To investigate the effect of diagnostic ultrasound on algae eradication, three
61 ultrasound transducers were used. A 200-kHz undamped single element
62 transducer containing a PIC155 Piezo crystal (PI Ceramics, Lederhose,
63 Germany), a PA 188 (Precision Acoustics Ltd, Dorchester, UK) 1-MHz
64 undamped single element transducer, and a 2.2-MHz undamped single element
65 transducer containing a Pz37 Piezo crystal (Ferroperm Piezoceramics A/S,
66 Kvistgård, Denmark). The focal distance of the 2.2-MHz transducer was
67 73 mm. The design of two transducers is shown in Fig.2. The transducers
68 were subjected to 16-Vpp square pulses at a 11.8-kHz pulse repetition rate
69 transmitted by a V1.0 pulser-receiver (Sonemat, Coventry, UK). Low acoustic
70 amplitudes were used in order to comply with $MI < 0.3$ [20]. The acoustic
71 amplitudes were measured in a separate water tank in the acoustic foci of
72 the transducers with a 0.2-mm needle hydrophone (Precision Acoustics Ltd,
73 Dorchester, UK) connected to a TDS 420A four channel digital oscilloscope
74 (Tektronix Inc, Beaverton, USA). The peak-negative acoustic pressures were
75 40 kPa for the 1-MHz transducer and 68 kPa for the 2.2-MHz transducer,
76 respectively, *i.e.*, $MI \ll 0.1$.

77 The algae used were of the *Anabaena* species. The *Anabaena* were **obtained**
78 **from an outside lake and** cultured in 2 L of Jaworski's medium [22] at room
79 temperature near a South-facing window in an Erlenmeyer flask for 11 days.
80 The solution was put in a Swirtlock2000 autoclave (Astell Scientific, Kent,
81 UK) at 15-lb pressure for 15 minutes.

82 *Brightness measurements*

83 To measure the time-dependent change in brightness of water containing
84 blue-green algae, the culture was split equally into four 250-mL Perspex
85 beakers: one beaker for each transducer and one control beaker. The
86 transducers were inserted separately in each beaker with the acoustic
87 focus within the sample. Each transducer was turned on for 1 hour. The
88 experimental setup is shown in Fig. 3. A digital photograph of the solution
89 was taken every five minutes using an EOS 350D digital photo camera (Canon
90 Inc, Tokyo, Japan). The lighting and exposure settings were controlled and
91 maintained throughout the insonification. Full manual settings were used:
92 ISO 100, exposure time 1/50 s, F number 3.50, focal length 18 mm, no flash,
93 centre weighted metering mode, custom white balance, B4,0 shift. The digital
94 photographs were converted to 8-bit grey scale. On the photographs of the
95 insonified solution and control solution, a square area of 160×160 pixels was
96 selected, whose average grey scale depth was calculated using MATLAB[®]
97 (The Mathworks[™], Natick, MA). The change in shade between the first image
98 taken just before insonification and each sequential image after insonification
99 was calculated and graphed for all 18 insonified and control samples. A white
100 sheet was placed behind the beakers to maintain a constant background. The
101 grey scale of the sheet was also measured and used to calibrate the results. In
102 total, 575 measurements were analysed from 122 photographs.

103 *Viability measurements*

104 Every 10 minutes, a 20- μ L sample was taken from the insonified solution.
105 Samples were put on a test slide and observed through a CHA microscope

106 (Olympus Corporation, Tokyo, Japan) with a 10× objective lens (Olympus
107 Corporation, Tokyo, Japan). Digital photographs were captured from the
108 microscope's eye piece using an FE-230 digital camera (Olympus Corporation,
109 Tokyo, Japan). Automatic settings were used with Super Macro mode and a
110 -1.0 exposure adjustment. From these digital images, cell deterioration and
111 chlorophyll damage were determined. To investigate the effect of ultrasound
112 on the viability of the cells, fluorescent light was used. When fluorescent
113 light is projected onto chlorophyll, it is absorbed and re-emitted as a red
114 glow. The red glow denotes that the chlorophyll is still active and can
115 photosynthesize, thus the algae strands are still alive. **Fluorescent light was**
116 **used as the method to check the viability of the chlorophyll, since it is a**
117 **standardized and accepted method in detecting chlorophyll activity in plants**
118 **[23,24]. Fluorescent light was projected onto these samples for no more than**
119 **10 seconds in order to capture the digital image. We may assume that there**
120 **are no disadvantageous effects of the fluorescent light on the algae [25,26]. The**
121 **samples were discarded after being exposed to fluorescent light.** Three trials
122 of each frequency were performed. One hundred and thirty-one photographs
123 were taken of the microscopic cell structure.

124 *Post-insonification growth measurements*

125 To measure the effect of ultrasound on post-insonification blue-green algae
126 growth, twenty-four 1-mL samples were removed from **three solutions that had**
127 **been subjected to 1 hour of insonification with 200-kHz, 1.0-MHz and 2.2-MHz**
128 **ultrasound, respectively,** and put into a culture tray with 96 compartments.
129 Twenty-four control samples were taken. The culture tray was left in sunlight
130 for 30 days. The grey scale depth was measured for each compartment.

131 3 Results and Discussion

132 Figure 4 shows the microscopic effect of ultrasound on floating bodies in the
133 algae solution. From 0 minutes to 60 minutes of insonification, no change was
134 seen in the physical structure of the algae for the whole frequency range.
135 Illuminating the algae with fluorescent light showed that the ultrasound
136 had no effect on the chlorophyll activity for the whole frequency range.
137 The active chlorophyll shows that the algae strands are still alive and
138 able to photosynthesise after 60 minutes insonification. This indicates that
139 the ultrasound transmitted does not affect the chlorophyll-containing cells
140 themselves, which is desirable since no toxins are released this way.

141 However, Fig. 5 shows that at all frequencies, for the floating bodies, the
142 insonified samples showed greater brightness than the control samples. For
143 the sunken bodies, all insonified samples showed reduced clarity as compared
144 to the control samples. Thus, the ultrasound has caused the algae to sink. For
145 example, after 60 minutes, the beakers subjected to 200-kHz insonification
146 were $92 \pm 12\%$ brighter than the control samples, contrasted by the beaker
147 bottoms, which were $53 \pm 27\%$ darker than the control samples.

148 Clearly, the algae that were floating in the beaker dropped to the bottom at a
149 faster rate than the control sample. This has been attributed to the disruption
150 of the floating bodies by the ultrasound. This is supported by Fig. 6, which
151 shows that the sunken bodies still have active chlorophyll but contain very
152 few heterocysts.

153 Figure 7 shows the viability of the culture 30 days after insonification in
154 terms of sample brightness. At 200 kHz, 1.0 MHz, and 2.2 MHz, the samples

155 were $39 \pm 14\%$, $45 \pm 17\%$, and $46 \pm 17\%$ brighter than the control samples,
 156 respectively. All samples were significantly brighter than the control samples,
 157 even at the lower boundaries of the standard deviation. Thus, these results
 158 support the hypothesis that the algae that have sunk are less capable of
 159 multiplying. Hence, insonification may prevent algae bloom.

160 Our results can be interpreted as follows. When a heterocyst is subjected to
 161 an ultrasound pulse, the bubble encapsulated by it expands, in our frequency
 162 range [27] during the rarefaction phase of the ultrasound. If the acoustic
 163 amplitude is sufficiently high, the encapsulating membrane cannot withhold
 164 the bubble from further expanding, resulting in its rupture. This phenomenon
 165 is similar to the sonic cracking of micrometer-sized membrane-encapsulated
 166 bubbles observed in [10]. Sonic cracking exclusively occurs during the
 167 expansion phase of a bubble [28].

168 The resonance frequency f_0 of a an encapsultaed microbubble is given by [29]:

$$169 \quad f_0 = \frac{1}{2\pi} \sqrt{\left(\frac{3\Gamma}{R_0^2 \rho}\right) \left(p_0 + \frac{2\sigma}{R_0} + \frac{2\chi}{R_0}\right) - \left(\frac{2\sigma + 6\chi}{R_0^3 \rho}\right)}, \quad (2)$$

170 where p_0 is the ambient pressure, R_0 is the bubble radius, Γ is the polytropic
 171 exponent of the gas, ρ is the liquid density, σ is the surface tension, and χ
 172 is the elasticity of the encapsulation [29]. Using $p_0=1.013 \times 10^5$ Pa, $R_0=3\mu\text{m}$,
 173 $\Gamma=1.4$, ρ is 998 kgm^{-3} , $\sigma=0.072 \text{ Nm}^{-1}$, and assuming that the membrane
 174 elasticity is similar to that of a lipid encpsulation, $\chi=0.044 \text{ Nm}^{-1}$ [30], we
 175 can estimate $f_0 \approx 1 \text{ MHz}$ for Anabaena heterocysts used in our experiments.
 176 Since the greatest change in clarity was seen at this particular frequency, we
 177 can safely state that ultrasound insonification close to heterocyst resonance
 178 frequency leads to a more effective eradication.

179 The quick decrease in live algae is similar to that in previous studies [12–17].
180 We assume that the correlation between (high)frequency and algae eradication
181 in these studies is related to the ultrasound proximity to heterocyst resonance
182 as well.

183 According to the NURC Rules and Procedures [21], the maximum acoustic
184 pressure to which mammals can be exposed is 708 Pa at frequencies up to
185 250 kHz. The transducers used had acoustic pressures of 40 kPa and 68 kPa at
186 driving frequencies 1.0 MHz and 2.2 MHz, respectively. These pressures surpass
187 the NURC Rules and Procedures by over 35 dB.

188 4 Conclusion

189 At any ultrasonic frequency we studied, blue-green algae were forced to
190 sink. This supports our hypothesis that heterocysts release nitrogen under
191 ultrasound insonification in the clinical diagnostic range. As supported by
192 previous studies, under identical pulse length and pulse repetition, eradication
193 is most effective close to heterocyst resonance, at a driving frequency of
194 roughly 1 MHz.

195 Although the acoustic fields we used to eradicate blue-green algae are safe in
196 terms of mechanical index, the acoustic pressures surpass the NURC Rules
197 and Procedures by over 35 dB. Therefore, caution should be taken when using
198 these techniques in a surrounding where aquatic or semi-aquatic animals are
199 present.

200 5 Acknowledgments

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202 Research Pump Priming Fund. We would like to thank John Adams, The
203 University of Hull, for support with the experimental setup.

204 **References**

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280 **List of Figures**

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283 there is no chlorophyll in the body. Each frame corresponds to
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287 200-kHz ultrasound transducer with modification layer $h_{\text{PG}} =$
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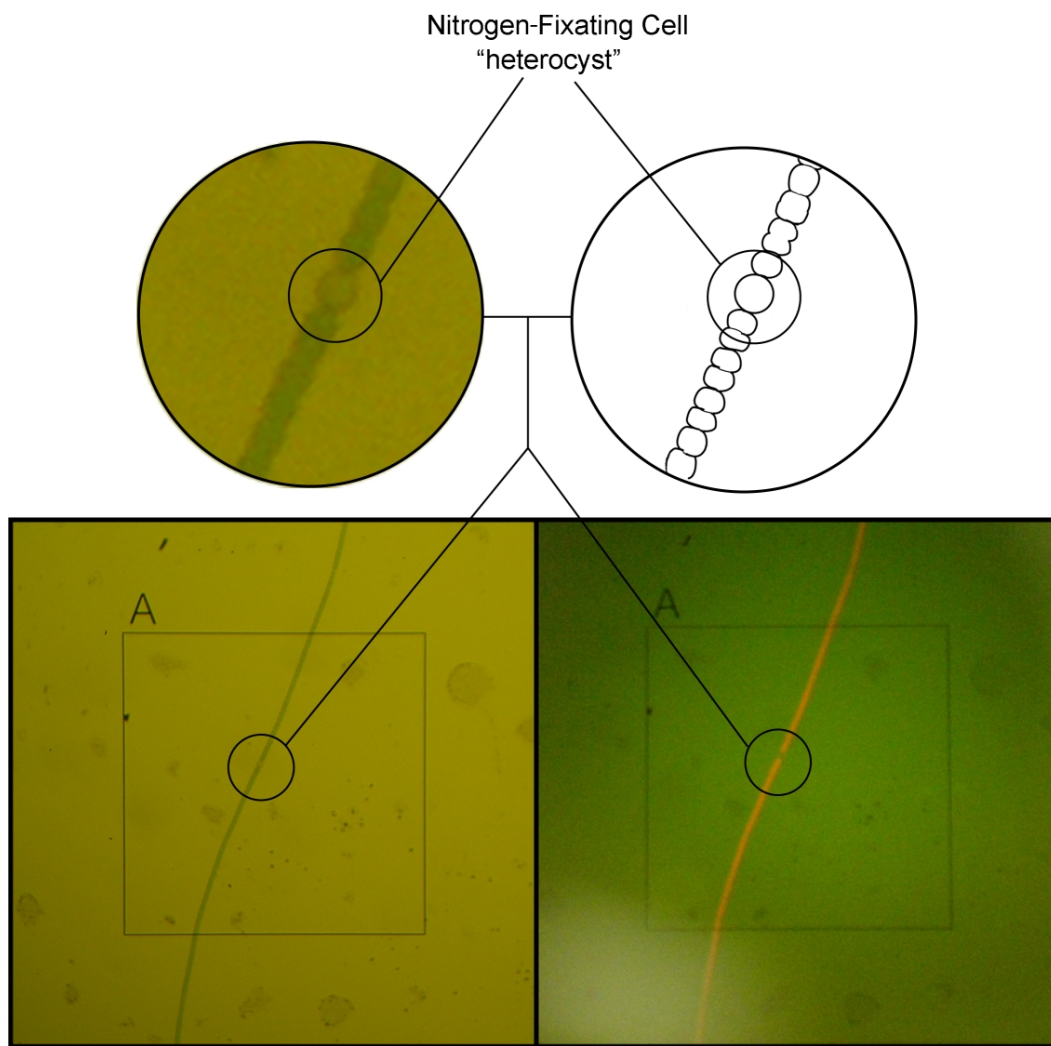


Fig. 1. Nitrogen-fixating body in *Anabaena sphaerica* algae. Under fluorescent light the body does not illuminate red, proving there is no chlorophyll in the body. Each frame corresponds to $565 \times 565 \mu\text{m}^2$.

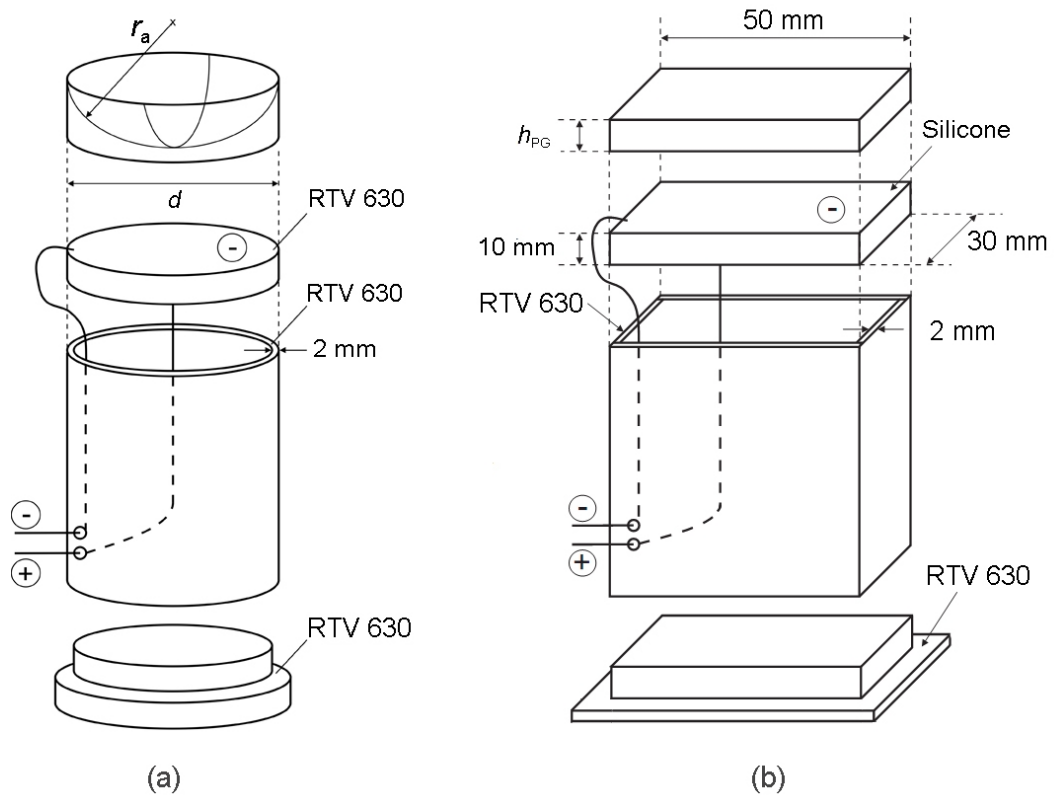


Fig. 2. (a) Undamped 2.2-MHz ultrasound transducer with $d = 1''$ diameter and $r_a = 35$ mm acoustic lens. (b) Undamped 200-kHz ultrasound transducer with modification layer $h_{PG} = 10$ mm.

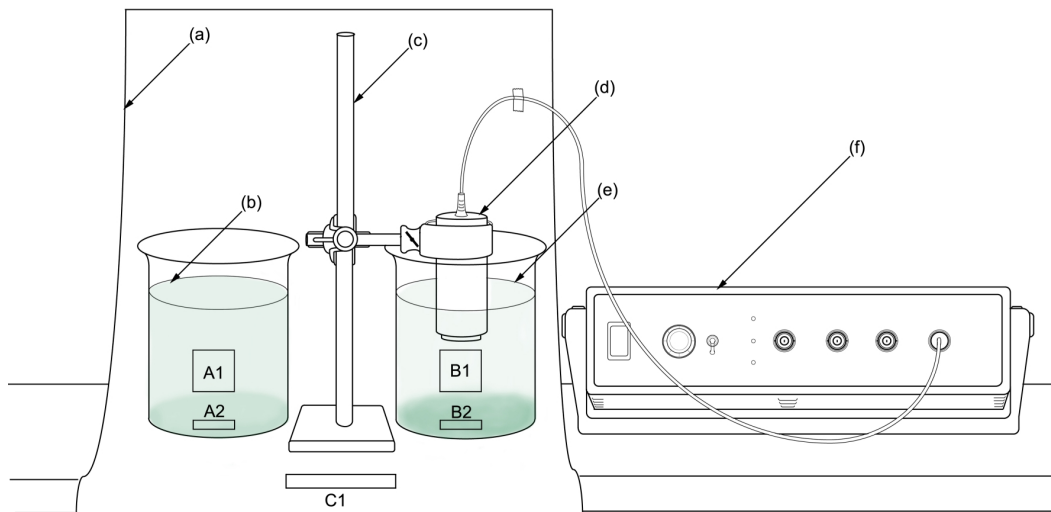


Fig. 3. Experimental setup: (a) white paper sheet; (b) control sample; (c) clamp stand; (d) transducer; (e) insonified sample; (f) pulser-receiver. Areas A1 – B2 represent brightness measurement areas, C1 represents the calibration area.

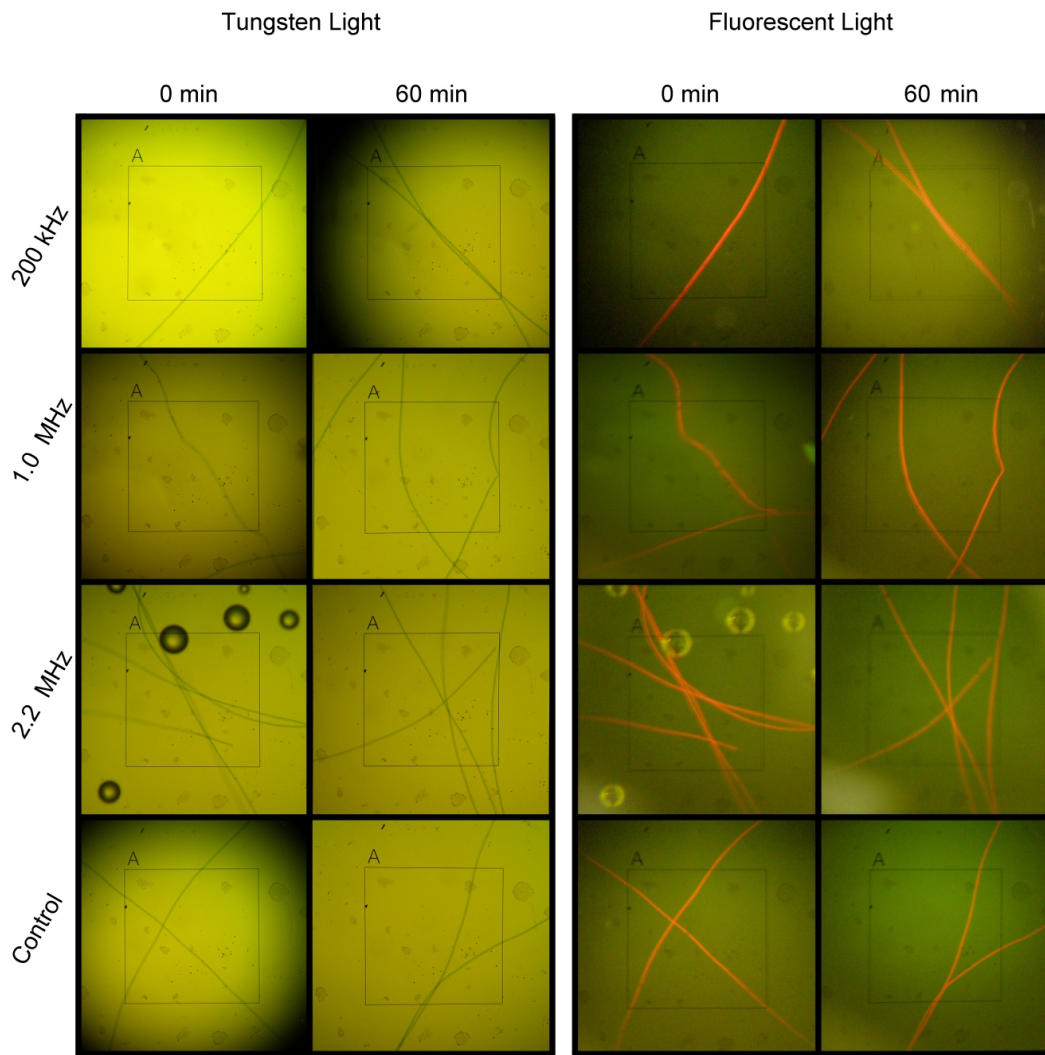


Fig. 4. Microscopic image sequence showing the effect of 200 kHz – 2.5 MHz ultrasound on the floating bodies of algae. Each frame corresponds to $565 \times 565 \mu\text{m}^2$.

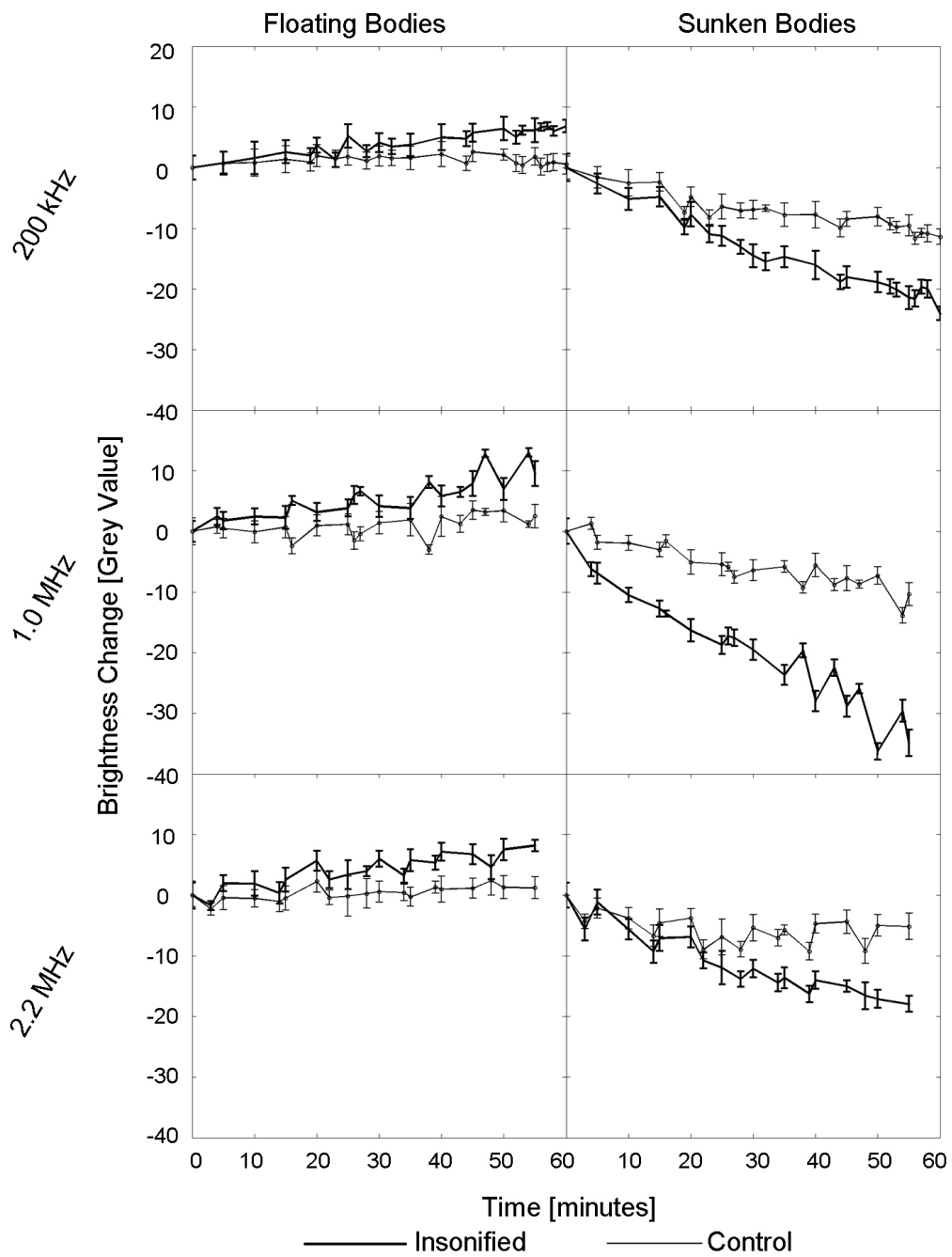


Fig. 5. Water brightness as a function of ultrasonic exposure time for samples containing floating algae or sunken algae.

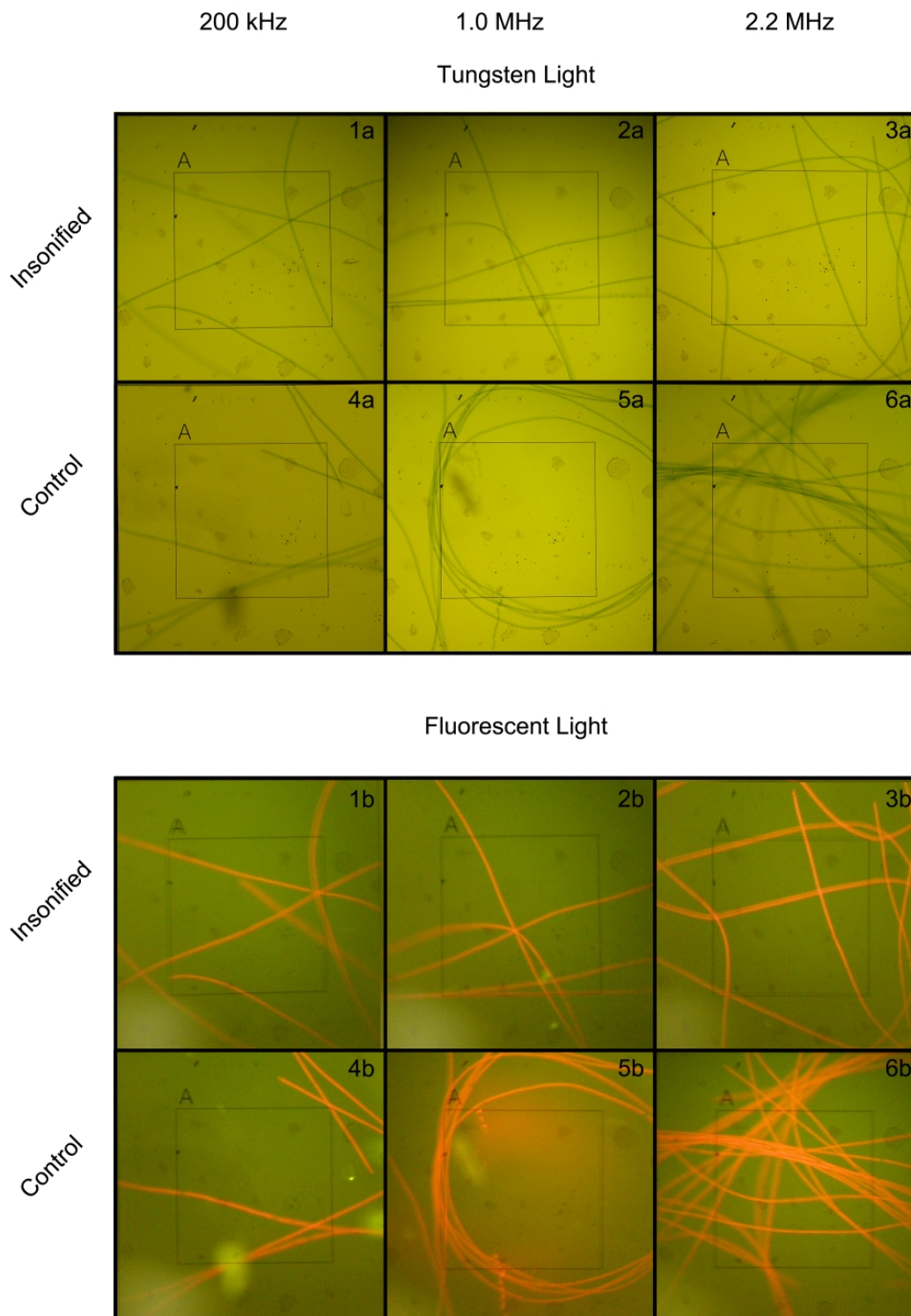


Fig. 6. Microscopic image sequence of sunken algae Each frame corresponds to $565 \times 565 \mu\text{m}^2$.

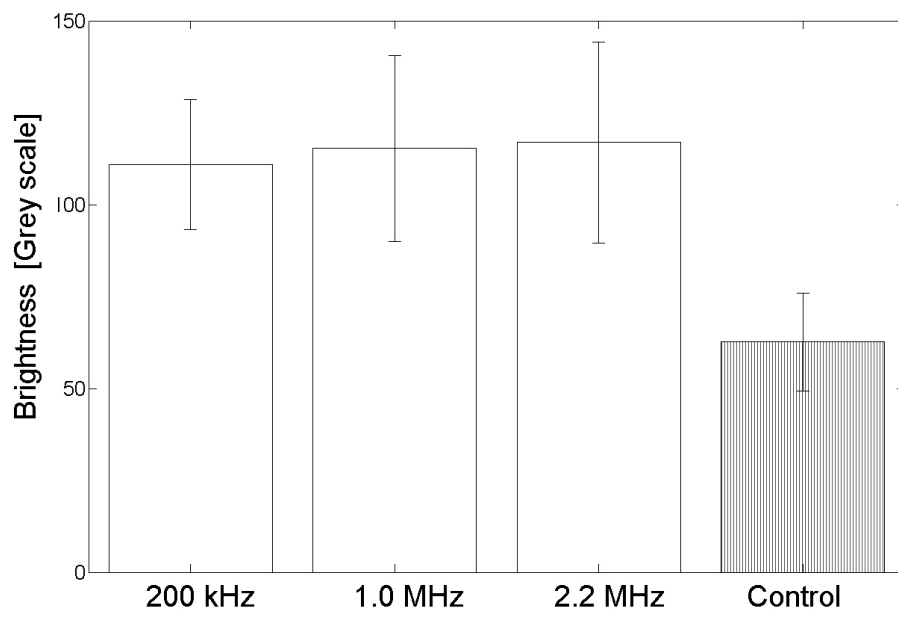


Fig. 7. Water clarity 30 days after insonification.