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Effect of ultrasonic algae control devices on non-target organisms: a review

Vpliv ultrazvoka za zaviranje rasti alg na netarčne organizme – pregled literature

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Abstract: There is an increasing interest in using ultrasonication in controlling algal (cyanobacterial) blooms and biofouling, a physical method with presumably no adverse effects on non-target organisms, such as fish and zooplankton. At the beginning the use of ultrasound (US) to control algae and biofouling has focused on high-power US causing cavitation; however, due to the potential damage to non-target organisms including marine mammals and human divers, high-power US causing cavitation are not used anymore for algae control in natural environment. Current ultrasonic algae control devices use low-power and thus control algae and biofouling by utilising resonance frequencies and the sound pressure caused by a sound wave propagating through a water column. There are only few studies existing on the effect of US on non-target organisms with incomplete information on wavelengths and intensities of US devices. However, we can conclude that non-cavitation US devices used to control algae and reduce biofouling had no adverse health effects on studied fish species with no feeding and behaviour changes noticed. Caution should be taken when installing US devices in marine locations since they may interfere with communication between sea mammals or may cause adverse effects on fish from subfamily Alosinae, the only known fish able to detect US. The studies dealing with non-cavitation US used to control algae and biofouling on non-target zooplankton have conflicting results from high mortality to no evident effects. Therefore, caution should be taken when using US for counteract algal growth in ponds or lakes, especially in terms of zooplankton and natural balance maintenance.

Key words: algal blooms, algal control, fish, ultrasound, zooplankton

Izvleček: V zadnjih letih narašča zanimanje za uporabo ultrazvoka (UZ) za nadzor prekomerne razrasti alg (cianobakterij) v vodnih telesih in tvorbe biofilma na plovilih, hladilnih napravah in drugih industrijskih objektih. UZ deluje po fizikalnih načelih in po trditvah proizvajalcev nima negativnih vplivov na netarčne organizme, kot so ribe in zooplankton. Prvi poskusi uporabe UZ za zaviranje rasti alg in tvorbe biofilma so potekali z uporabo visoko energijskih UZ, ki povzročajo kavitacijo, vendar se je uporaba le-teh kmalu opustila zaradi morebitnih negativnih vplivov na netarčne organizme, kamor sodijo tudi morski sesalci in potapljači. Danes se za zaviranje rasti alg uporabljajo nizko energijski UZ, ki za kontrolo rasti alg in biofilma uporabljajo resonančne frekvence in zvočni tlak, ki ga povzroča zvočni val s širjenjem skozi vodni stolpec. O vplivu UZ na netarčne organizme obstaja le malo raziskav in še to z nepopolnimi informacijami o karakteristikah testiranih UZ. Kljub temu lahko povzamemo, da nizko energijski UZ, ki se uporabljajo za zatiranje alg in zmanjšanje tvorbe biofilma, nimajo škodljivih vplivov na preučevane vrste rib. Kljub temu priporočamo previdnost pri uporabi UZ v morskem okolju, saj lahko ultrazvočni valovi ovirajo komunikacijo med morskimi sesalci ali škodijo ribam iz poddružine Alosinae - edine znane ribe s sposobnostjo zaznavanja UZ. Raziskave o vplivu nizko energijskih UZ na netarčne zooplanktonske organizme kažejo nasprotujoče si rezultate, od akutnih letalnih učinkov do odsotnosti vpliva. Zato je potrebna previdnost pri uporabi UZ za zmanjševanje rasti alg v ribnikih ali jezerih, zlasti z vidika varovanja zooplanktona in s tem ohranjanja naravnega ravnovesja.

Ključne besede: kontrola alg, prekomerna namnožitev alg, ribe, ultrazvok, zooplankton

Introduction

Current methods to control algal (cyanobacterial) blooms and to reduce their (toxic) by-products include chemical or biological additives, ozonation, activated carbon filters and ultrasonic products (Jarni et al. 2017). A major concern with chemical or biological additives is their environmentally hostile impact and the possible release of toxins when cyanobacterial cells are broken which exacerbate the problem. Therefore, there is an increasing interest in using ultrasonication, a physical method, in controlling algal blooms, which is effective also in degrading the cyanotoxins (Song et al. 2005) when released into water. Although, the effect of the ultrasound (US) on algae is well studied, the knowledge on the safety of the US on non-target organisms, such as fish and zooplankton, is limited. Therefore, this paper focuses on the literature review on the effects of ultrasonication used for counteract algal growth in water bodies on non-target organisms, namely fish and zooplankton.

US, a sound with a frequency of 20 kHz or above, is beyond the limits of human hearing. According to frequency, US is divided into three categories including low frequency US with a frequency of 20–100 kHz, high frequency US with a frequency of 0.1–1 MHz and diagnostic US with a frequency of 1–500 MHz. Low frequency US is commonly used in chemically important systems in which chemical and physical changes are desired, as it has the ability to cause acoustic cavitation, a phenomenon, where US causes the formation of bubbles that implode upon themselves, causing intense heat (4,500–7,500 °C) and pressure (approximately 10,000 Bar) (Askokkumar et al. 2007). In acoustic cavitation microscopic gas bubbles that are generally present in a liquid will be forced to oscillate due to an applied acoustic field. If the oscillation amplitude is large enough cavitation bubbles may appear within the liquid bulk (Dular et al. 2016). 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. 2012). Whenever low frequency and high intensity US is applied to liquids, for example in applications as sonar, industrial processing, and bio-medical research, cavitation may occur (Neppiras 1984). Lower sound pressure limit for appearance of acoustic cavitation is at sound pressure amplitude of about 2 kPa or 186 dB re 1 μPa (Margulis 1995).

US technologies are effective means of minimizing algal (cyanobacterial) blooms in freshwater bodies, minimizing biofilm formation in industrial applications, and preventing biofouling on ships (Lee et al. 2001, Zhang et al. 2006, Krivograd Klemenčič and Griessler Bulc 2010). At first the use of US to control algae has focused on high-power US causing cavitation (Lee et al. 2001, Zhang et al. 2006, Joyce et al. 2010). However, the potential damage to non-target organisms, including marine mammals and human divers, has been one of the reasons why high-power US causing cavitation is not an ideal solution for algae control in natural environment. This is why current ultrasonic algae control devices use low-power and thus do not control algae through the high pressures and temperatures associated with cavitation; but instead by utilising resonance frequencies and the sound pressure caused by a sound wave propagating through a water column (Lowe 2011). It was discovered that certain ultrasonic sound vibrations in the water produce critical resonance frequencies of algae gas vesicles, vacuoles, and plasmalemma cell lining. Exposure to these sounds cause the cell membranes to break or tear (Rajasekhar et al. 2012). Although low-power US is believed to be safer to non-target organisms the knowledge about the effects of low-power US on non-target organisms when implemented on a large scale in complex natural systems is limited.

The US algae control devices currently available on the market are operating in the range from 20–200 kHz with low-power and in contrast to US used in industry and medicine applications do not induce cavitation effect.

Effect of ultrasound on fish

Sensory systems in fish

Fishes, like other vertebrates, have a variety of different sensory systems that enable them to glean information from the world around them. Vision is often most useful when a fish is close to the source of the signal, in daylight, and when the water is clear. However, vision does not work well at night or in deep waters. Chemical signals can be highly specific (e.g., a particular pheromone used to indicate danger). However, chemical signals travel slowly in still water and diffusion of the chemicals depends upon currents. Therefore, chemical signals are not directional and, in many cases, they may diffuse quickly to a non-detectable level. As a consequence, chemical signals may not be effective over long distances (Popper 2008). In contrast, acoustic signals in water travel very rapidly, travel great distances without substantially attenuating (declining in level) in open water, and they are highly directional. Thus, acoustic signals provide the potential for two animals to communicate quickly (Zelick et al. 1999, Popper et al. 2003). Since sound is potentially a good source of information, fishes have evolved two sensory systems to detect acoustic signals, and therefore many species use sound for communication (e.g., mating, territorial behaviour) (Zelick et al. 1999). The two systems are the ear, for detection of a sound from 20 Hz to 1 kHz or more, and the lateral line for detection of hydrodynamic signals (water motion) from less than 1 Hz to 100 or 200 Hz (Zelick et al. 1999).

If a fish can hear a biologically irrelevant human-generated sound (e.g., sonar, ship noise, US for algae control) it might interfere with the ability of fish to detect other biologically relevant signals. In effect, anthropogenic sounds and explosions (e.g., detonations) may affect behaviour, and result in short and long-term tissue damage, but only at significantly high levels. Fish hearing can be affected by the presence of a background noise that is in the same general frequency band as the biologically relevant signal (Fay and Megela-Simmons 1999, Popper et al. 2003). In the case the background noise is increased due to human-generated sources it may be harder for a fish to detect the biologically relevant sounds needed for its survival (Popper 2008). According to Popper (2008) the effects of military mid- and high-frequency active sonars on fishes has not been studied yet.

Detection of ultrasound by fish

It is known that fish species are not producing communication sounds within the ultrasonic frequency range (Bass and Ladich 2008). Fish, with few exceptions from family Clupeidae described below, cannot hear sounds above 3–4 kHz, and the majority of fish species are only able to detect sounds to 1 kHz (Hawkins 1981, Fay 1988, Popper 2008). In contrast, a healthy young human can detect sounds to about 20 kHz, while dolphins and bats can detect sounds to well over 100 kHz (Popper 2008).

In 1982 it was discovered that ultrasonic sonar (about 160 kHz) caused behavioural responses in migrating *Alosa sapidissima* (Kynard and O'Leary 1990). Ten years later Nestler et al. (1992) and

Dunning et al. (1992) reported that high frequency sounds at 110–140 kHz and with high intensities (180 dB re 1 µPa) were effective in deterring two fish species belonging to the family Clupeide (subfamily of Alosine (shads)) from power plant intakes: blueback herring (*Alosa aestivalis*) and alewife (*Alosa pseudoharengus*). Mann et al. (1997) measured the audiogram of *Alosa sapidissima* which confirmed that the species could detect sound in the ultrasonic frequency range up to 180 kHz. Later behavioural and physiological studies showed that additional species belonging to the subfamily Alosine can detect and respond to US. These include gulf menhaden (*Brevoortia patronus*) (Mann et al. 2001) and two species of European shad, *Alosa fallax fallax* (Gregory et al. 2007) and *Alosa alosa* (Wilson et al. 2008). Wilson et al. (2011) found out that the response of Alosine to US is an antipredatory response against echolocating toothed whales and this is why the fish always turn away from the sound source. According to Plachta et al. (2004) the ultrasonic pathway in Alosine appears to be a feature-rich US detector that is likely to be adapted (e.g., frequency, intensity) to odontocete echolocation signals.

According to known data the ability to detect US is limited to the subfamily Alosinae and has not been found in other fish species from the family Clupeidae, for e.g., from the subfamily Clupeinae (Mann et al. 2001, 2005) or the subfamily Dorosomatinae (Narins et al. 2013). It also does not appear that fishes from other families are able to detect US, although very few hearing studies have tested this ability (Narins et al. 2013). One study conditioned *Gadus morhua* to ultrasonic pulses at 38 kHz with a threshold for detection of 204 dB re 1 µPa (Astrup and Møhl 1993). A follow-up study by Schack et al. (2008) found that unconditioned *Gadus morhua* did not show any behavioural or physiological response when exposed to the same type of stimulus generated with the same equipment as used in the study performed by Astrup and Møhl (1993).

Effects of ultrasound used for counteract algal growth and biofouling on fish

Summary of the effects caused by the US devices used to control algae and reduce biofouling on fish is shown in Table 1. De Lange (2007) conducted a research on the effects of US used for cyanobacteria control in surface waters on different fish: bream, silver bream, bass, common roach (*Rutilus rutilus*), ruffle (*Gymnocephalus cernua*), common rudd (*Scardinius erythrophthalmus*), tenches (*Tinca tinca*), and pikes (*Esox lucius*). Bream, bass, and silver bream are names for several species or higher taxonomic groups of fish. However, in De Lange (2007) Latin names or more exact names for fish species are not reported; therefore, we cannot know which species of fish from the groups bream, bass, and silver bream were used for the research. Results showed that the fish population was evenly distributed across two basins, with and without the US, even after 4 months of US operation revealing that US had no effect on fish migration between the two basins. Also, the length of fish in both basins did not differ significantly. Given that the fish in the basin with US did not massively flee, it can be concluded that the US with the applied load (no data is provided about the type, frequencies or intensities of the US used) is not noticeable or considered unsafe for tested fish species. Furthermore, no excessive mortality of fish has been observed in the basin where the US was used.

Oyib (2009) reported on reduction of cage net fouling at a salmon-farming facility by US (LG Sonic, 12 W, dual core multi frequency technology, 20–200 kHZ) installed in a salmon cage with nets covered with marine fouling organisms. As reported by Oyib (2009) there were no detectable changes in salmon behaviour during the US exposure of 28 days.

Table 1: The list of effects caused by the ultrasound (US) devices used to control algae (cyanobacteria) and to reduce biofouling on non-target fish and zooplankton.
Tabela 1: Seznam učinkov, ki jih povzročajo ultrazvočne (US) naprave za zaviranje rasti alg in biofilma na netarčne ribe in zooplankton.

US effect	Test organisms	US characteristics	Literature
No long-term effect on fish migration or mortality	Bream, silver bream, bass, *Rutilus rutilus, Gymnocephalus cernua, Scardinius erythrophthalmus, Tinca tinca, Esox lucius*	No data on the type, power and frequency of the US used	De Lange 2007
No changes in salmon behaviour during the US exposure of 28 days	Salmon	LG Sonic, 12 W, 20–200 kHZ	Oyib 2009
No long-term effect noticed on the body weight increase, fish productivity, feeding, and behaviour during the US exposure of 1 year	*Cyprinus carpio*	LG Sonic Tank, range 50 m, 12 W, 20–200 kHZ	Griessler Bulc et al. 2011
Continuous US exposure deterred fish from feeding	*Ictalurus punctatus*	No data on the type, power and frequency of the US used	Zimba and Grimm 2008
No effect noticed on the body weight increase, fish productivity, feeding, and behaviour	Juvenile *Cyprinus carpio*	LG Sonic SSS, range 10 m, 11 W, 20–200 kHZ	Krivograd Klemenčič and Griessler Bulc 2013
No effect noticed on the fish productivity, feeding, and behaviour	*Cyprinus carpio*	LG Sonic Tank, range 70 m, 13 W, 20–200 kHz	Krivograd Klemenčič and Griessler Bulc 2015
No long-term effect noticed on fish physiology during the US exposure of 30 days	*Cyprinus carpio*	SOFCHEM TWIN-f system, 15 W, 23–46 kHZ	Techer et al. 2017
Acute lethal effect	*Daphnia magna*	Flexidal AL-10, acoustic power 0.7 W, $8.5x10^{-4}$ W mL^{-1}, 12–200 kHz	Lürling and Tolman 2014a
Acute lethal effect	*Daphnia magna*	Flexidal AL-05, acoustic power 0.63 W, 20–44 kHz	Lürling and Tolman 2014b
Acute lethal effect	*Daphnia magna*	Flexidal AL-50, US characteristics not reported	Govaert et al. 2007
No acute effect noticed	*Daphnia* ssp.	Pool Tec 10", Huges Sonic Systems, power not reported, 110-240 V, 45–60 kHz	Hedge 2013
No acute nor long-term effect noticed	*Daphnia* ssp.	producer and power not reported, ~580 kHz	Hedge 2013
No acute effect noticed	Juvenile and adult *Daphnia magna*	LG Sonic e-line, 25 W, 20–100 kHz	Klemenčič and Krivograd Klemenčič 2021

Griessler Bulc et al. (2011) studied a water treatment system for common carp (*Cyprinus carpio*) which included among other treatment devices also US. The research was conducted in two fish ponds, namely an experimental pond with treatment, including a roughing filter, a glass fibre filter, a UV-C unit, and a US device and a control pond without any treatment. The US device was a low-power commercially available US transducer (LG Sonic® Tank, range 50 m, 12 W, with dual core multi frequency technology, 20–200 kHZ) floatingly installed in the corner of the experimental pond. The research was performed continuously for more than one year and during the whole experiment the US device was switched on. The results showed that in the experimental pond with US rearing conditions for common carps were better with higher body weight increase and higher fish productivity than in the pond without treatment. It can be concluded that the US device showed no negative effect on fish regarding body weight increase and fish productivity. Moreover, fish mortality was the same in both ponds correlated with transportation stress at the beginning of the experiment. The authors of the research did not report any feeding problems or behaviour changes in fish in the pond with US. On the contrary, in the research performed by Zimba and Grimm (2008) in tank trials with channel catfish fingerlings (*Ictalurus punctatus*), continuous operation of the US devices deterred fish from feeding. Their trials were therefore modified to allow a four hour period without US treatment around the feeding time. Turning off the US signal during the feeding resulted in the fish feeding and no further adverse effects. However, in the research performed by Zimba and Grimm (2008) no information is available on the main characteristics of the US used for trials, namely intensity, power or frequencies. Therefore, it is very hard to compare both studies.

Krivograd Klemenčič and Griessler Bulc (2013) compared at a lab-scale two treatment systems for fish farms (a) a system with a constructed wetland and a US device, and (b) a system with a constructed wetland in order to find out the effect of US on fish productivity and behaviour. Model fish was juvenile common carp. The US device used was a commercially available US transducer (LG Sonic® SSS, range 10 m, 11 W, with dual core multi-frequency technology). The research showed no negative effect of the US device on fish, resulting in higher body weight increase and higher fish productivity in the system with the US unit compared to the control system without the US. There was no fish mortality observed during the experiment nor authors stated any difference in feeding habits or behaviour of fish in the tank with the US. Another research was performed in 2015 by the same authors (Krivograd Klemenčič and Griessler Bulc 2015) with the similar type of low-power non-cavitation US device (LG Sonic® Tank, range 70 m, 13 W, 20–200 kHz) and the same test organism common carp. Again, in the pond with treatment system, including the US device, no negative effects of the US on fish were noticed, resulting in higher fish productivity compared to the fish pond without treatment. In addition, no adverse effects were noticed on fish.

Techer et al. (2017) studied the effect on the fish physiology of a long-term exposure to anti-cyanobacterial US on the common carp (*Cyprinus carpio*). Two-years-old carps were chronically (for 30 days) exposed to a low-power US. The used US system consisted of a dual-frequency US device emitting continuous signals and powered by a solar floating platform with sound pressure level (SPL) up to 187 dB re 1μPa. There were two transducers operating independently. They were immersed at a depth of around 0.5 m with one emitting at an average frequency of 23 kHz and the other at 46 kHz. The two frequencies are simultaneously transmitted using the two transducers mounted adjacent one another, with a distance of 10 cm between them. The supplied power to the transducers was initially fixed at 15 W. According to the manufacturer Sofchem (France) the TWIN-f® ultrasonic system, which was used for the experiments, has been especially developed for algae growth inhibition. After seven and 30 days of exposure to ultrasonication, fish were sacrificed, condition factor indices were determined, and a panel of biochemical markers linked to fish physiological homeostasis was assessed, encompassing (i) hepatic antioxidant enzyme biomarkers, i.e., total superoxide dismutase (total SOD), catalase (CAT), total glutathione peroxidase (total GPx), and glutathione S-transferase (GST) activities, (ii) lactate dehydrogenase activity related to cellular energetic metabolism, and (iii) circulating cortisol levels subsequent to stress. Results showed that carps were not affected by US exposure when exposed in floating cages in fish ponds over a 30-day period. Cortisol levels slightly increased over the duration of the experiment, but its variation did not show US exposure related stress. Moreover, an overall diminution of the expression levels of different biomarkers was reported during the experiment including cellular antioxidant enzyme activities such as superoxide dismutase, glutathione peroxidase, catalase and glutathione S-transferase, and lactate dehydrogenase. Subtle changes in these biomarkers were dependent on the type of enzyme activity and especially of the origin of fish (i.e., sampled pond) regardless of the presence of US equipment, reflecting thereby fish adaptation to local environmental conditions in each pond. In conclusion, this study does not provide indication that ultrasonication in the aforementioned conditions affects the welfare and physiological homeostasis of carps.

Effects of ultrasound used for other applications on fish

Summary of the effects caused by the US devices used to control algae and reduce biofouling on zooplankton is shown in Table 1. Duchene (2016) reported that US deployed underwater directly into fish pens has a lethal effect on juvenile stages of the Chilean sea lice (*Caligus rogercresseyi*). The application as reported by Duchene (2016) is not harmful to the fish (salmon) or marine mammals due to the low-power (20 W) and low frequencies (20 KHz) used per transmitter. However, no tests were performed to confirm this statement.

According to Frenkel et al. (2000a) US at therapeutic intensity levels enhances uptake of particles into fish cells by widening intercellular spaces, thus increasing permeability of the skin (e.g., sonication at 3 MHz, intensity 2.2 W cm^{-2}, power 11 W). The first signs of biological effects in the sonicated tissues were observed at 1.7 W cm^{-2} or 8.5 W and at sonication time of 90 s with the ultrasonic beam perpendicular to the skin surface and the distance of 15 cm between the fish and the transducer. This effect of US 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 US (24 s 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) noted that for fish, crustaceans, and molluscs, compounds which can be administered by their US-enhanced method include proteins, nucleic acid sequences, antibiotics, antifungals, steroids, vitamins, nutrients, minerals, hormones, and vaccines. Zohar et al. (2001) stated that the frequencies and intensities used to implement the molecule transfer range from 20 kHz to 10 MHz, below 3 W cm^{-2} with exposure time of a few minutes. According to LaLiberte and Haber (2014) it is possible that fish in natural systems could be at risk for disease or possibly environmental contaminant uptake if US exposure is great enough to induce epidermal permeability (e.g., 20 kHz-10 MHz; 8.5-40 W). Although, US used for algae control could be in the same frequency and power range as the US which is reported to increase fish skin permeability (Lowe 2011), the volumes of water in which fish are exposed to sonication are much different. In the experiments where fish were treated or vaccinated with US the sonication took place in small volumes of water in order to apply US directly on fish skin (the distance between the fish and the transducer was 15 cm or less), which contributed to the increased impact of US on fish. On the other hand, in nature systems treated by US, fish are exposed to long-term sonication (days or even months). Therefore, research on skin damage and thus the possible environmental contaminant uptake in fish exposed to algae control US devices is needed in order to evaluate possible long-term effects of algae control US devices on fish.

Effect of ultrasound on zooplankton

Effects of ultrasound used for counteract algal growth on zooplankton

Very few studies are available on the effect of commercially available US systems for algae-control on non-target aquatic organisms; moreover, we could identify only five studies with focus on zooplankton, namely Govaert et al. (2007), Hedge (2013), and Lürling and Tolman (2014a,b) together with our recent study Klemenčič and Krivograd Klemenčič (2021). Lürling and Toldman (2014a) tested commercially available US (Flexidal AL-10, Belgium) which is according to the manufacturer used to reduce algae growth in small ponds, aquaria and small water reservoirs (~12 kHz to ~200 kHz, acoustic power 0.7 (±0.2) W, 8.5×10^{-4} W mL^{-1}) on non-target zooplankton species *Daphnia magna* in 1 L jars (actual volume of 800 mL). After 15 minutes of sonication all *D. magna* organisms exposed to sonication died (acute lethal effect), while all *D. magna* in control groups survived. Effect of temperature (possible overheating due to sonication) was excluded. The same year Lürling and Tolman (2014b) published a similar research in which they exposed *D. magna* (~2 mm body size) in 1 L jars (actual volume of 800 mL) to the US device of the same producer (Flexidal AL-05, Belgium) supplied at 20 kHz, 28 kHz, 36 kHz or 44 kHz with acoustic power of 0.63 (±0,05) W. All animals were killed between 10 min (44 kHz) and 135 min (20 kHz) (acute lethal effect). An experiment with differently sized *Daphnia* (0.7–3.2 mm) testing the hypothesis that juveniles are more susceptible than adults showed that all animals were killed between 4 and 30 min when exposed to 44 kHz. The survival time in organisms of different body-size were lowest in animals between 1.1 and 1.7 mm and larger in the smallest and largest animals tested. Increasing water volumes up to 3.2 L and thus lowering the US intensity did not markedly increase survival of *Daphnia* exposed to 44 kHz US. A tank experiment with six 85 L tanks containing a mixture of green algae, cyanobacteria and *D. magna* was performed to study the effect of US over a longer period of time (25 days). The results showed animal densities were extremely low in the treatments compared to the controls. Higher frequencies exerted a stronger effect on *D. magna* than lower frequencies. Animals between 1 and 2 mm seemed strongly affected by 44 kHz US than smaller and larger specimens, but again survival times were very short (about 2 to 17 min only). Increasing water volume and thereby lowering the intensity of ultrasonication did not elevate the survival time significantly. Hence, even in small ponds and aquaria the tested transducers are expected to exert an effect on non-target organisms such as *Daphnia*. This finding is supported by a field study conducted by Govaert et al. (2007) (the original report is not available, data are cited from Lürling and Tolman (2014b)) in two identical ponds that were interconnected and received the same water. One of the ponds was treated with Flexidal AL-50 (Belgium) transducer (the same manufacturer as in Lürling and Tolman, 2014a,b), while the other served as a control. The authors reported an almost complete disappearance of *Daphnia* from the US treated pond, while *Daphnia* remained abundant in the non-treated control pond. Since the original report (Govaert et al. 2007) is not available, the volume of the ponds, the frequency and the output power of the US are not known.

Hedge (2013) performed a lab-scale experiment (5 days) in 65 L tanks with low- frequency US (Pool Tec 10", Hughes Sonic Systems, 45-60 kHz, 110-240 V, output power not reported), while long-term (2.5 months) field-scale experiment was performed with high-frequency US (~580 kHz, producer and output power not reported) in an artificial lake with the approximate area of 2.4 km^2 being partitioned into four sections. Separation allowed for two sections of the lake to be subjected to the US while the other two were controls. As model organisms different species from genera *Daphnia* were used. According to the results there was no negative effect of ultrasonication found on *Daphnia* in lab and field-scale experiments. High frequency ultrasonication did not reduce reproduction, increase mortality rates or negatively alter the environment in a way that decreases its suitability for zooplankton. Moreover, ultrasonication did not influence the dispersion of *Daphnia* within the tank in lab-scale conditions. Similarly, Klemenčič and Krivograd Klemenčič (2021) reported that commercially available US for algae control of the Dutch producer LG Sonic (LG Sonic e-line, 25 W, 20–100 kHz) had no acute effect on the mobility of juvenile and adult *D. magna* specimens in lab-scale experiment with up to 48 h exposure to ultrasonication. They concluded that US devices from different manufacturers can have different effects on target- and non-target organisms.

Effect of ultrasound used for ballast water treatment on zooplankton

The US has been investigated not only for algae control but also as a control for zooplankton in ballast waters (e.g., Sassi et al. 2005, Laliberte and Haber 2014). However, usage of acoustic cavitation in ballast water treatment is relatively new and remains insufficiently researched (Gregg et al. 2009, Lloyd's Register 2014). Sassi et al. (2005) performed laboratory and onshore test trials for ballast water treatment with US unit specially designed for disintegration (e.g., cell disruption, emulsifying, homogenising), thermoplastic molding, coating-lacquer removal, intensive surface cleaning, wire cleaning, cutting, drilling, lapping and compressing, used in industry or sonochemistry laboratories. The operating frequency of US unit used was 20 kHz with output power of 2000 W. Laboratory tests showed total reduction rates of 84-100% for Artemia salina with the best results obtained with a flow rate of 200 L h^{-1} and a maximum transducer amplitude of 50%. For test organisms Nereis virens, Acartia tonsa, Tisbe battagliai and Alexandrium tamarense the mortality attained was always below 40% for all tests. In the onshore trials, the mortality rates achieved were 94-99% for copepods, 86-99% for copepod nauplii, 95-98% for cladocerans, 80% for rotifers and 97% for barnacle nauplii. Holm et al. (2008) tested the effect of high-power US (19 kHz) on a cladoceran (*Ceriodaphnia dubia*), rotifers (*Brachionus plicatilis, B. calyciflorus*, and *Philodina sp*.), and brine shrimp (*Artemia* sp.) in a flow-through system. Ultrasonic intensities were 13.5-25.5 W cm^{-2}. The results showed that most effective treatment against zooplankton larger than 100 μm were exposure times below 10 seconds and energy densities less than 20 J mL^{-1}resulted in 90% mortality. Microjets within the zooplankton caused by the collapse of cavitation bubbles were the hypothesized cause of zooplankton mortality in the experiments.

Effects of ultrasound used for other applications on zooplankton

Wells (1968) studied the effect of ultrasonication on *Daphnia magna* (~0.2 cm length). He used the US transducer with diagnostic frequency (~3 MHz) and diameter 0.95 cm specially constructed to permit the irradiation of single specimens of *Daphnia*. The length of the water jacket in front of the transducer was 4–7 cm. The animal was placed in a small irradiation chamber filled with water. The results showed that exposure of *D. magna* to the US with power 10 W corresponding to 29 W cm^{-2} and frequency of 3 MHz caused the death of all exposed animals in 2 minutes (acute lethality). However, at lower power (below 8 W corresponding to 23 W cm^{-2}) and the same frequency all animals survived. There was a threshold level at about 5 W at the experimental conditions below which ultrasonic irradiation had no significant effect on *D. magna* survival. Kamenskii (1970) studied the influence of the US on eggs and larvae of some fish trematodes together with their intermediate hosts *Cyclops*, *Daphnia* and *Lymnaea stagnalis* (shell height 0.8–1.2 cm, width 0.3–0.6 cm). The animals were treated with ultrasonic waves 50, 500 or 1,000 kHz in standing water for 3 seconds or in flowing water for 10 seconds. In still water, all organisms except *Lymnaea* were killed, even at the lowest rate of frequency. *Lymnaea* was not killed at any frequency. Analogous results were obtained in flowing water. Unfortunately, only frequency and no output power of the US unit used is reported. However, from acute lethal effect in very short contact time (up to 10 seconds) also at low frequencies we can assume that author tested high-power US.

Conclusions

According to known data, fish species are not producing communication sounds within the ultrasonic frequency range and also cannot hear sounds above 3–4 kHz, although very few hearing studies have tested this ability. The only known exception are marine fish species from family Clupeidae (subfamily Alosinae) which are able to detect sound up to 180 kHz as an anti-predatory response against echolocating toothed whales.

Therefore, caution should be taken when installing ultrasonic devices in marine locations since they may interfere with communication between sea mammals or may cause adverse effects on fish from subfamily Alosinae. There are only few publically-available studies on the effects of ultrasound (US) on fish. Furthermore, not all of them report information on wavelengths and intensities of the used US devices, because that usually remains proprietary information, making the comparison between the studies very difficult. Nevertheless, based on the available per-reviewed studies, we can conclude that non-cavitation US devices used to control algae (cyanobacteria) and to reduce biofouling had no known adverse health effects on the studied fish species with no feeding and behaviour changes noticed. US is used in aquaculture for immersion vaccination or antibiotic treatment because it can make fish skin permeable. Although, research on long-term exposure of fish to low-power algae control US devices show no adverse effects on fish, it is possible that fish exposed to US in natural systems could be at risk for diseases or contaminant uptake because of increased skin permeability. We should also be aware that the studies reviewed in this paper are not considering the possible effects of the ultrasonication on molecular or genetic levels in fish.

We identified identify only four studies dealing with the effects of US used for algae control on non-target zooplankton with conflicting results. According to some authors low-frequency and low-power US (Flexidal) have acute lethal effect on *Daphnia* in lab and field conditions. On the other hand, low-frequency US of different producer had no negative effect on *Daphnia* productivity and mortality. Therefore, caution should be taken when using US for counteract algal growth in ponds or lakes especially in terms of zooplankton and natural balance maintenance. In the last decades US has been investigated together with ultraviolet irradiation, ozone and hydrodynamic cavitation as a control for zooplankton in ballast waters. The research of sonication as ballast water treatment is promising with mortality rates for zooplankton up to 100%. In contrast with algae-control systems for ballast water treatment high-power US units are used in order to achieve high mortality rates. Acute lethal effect on zooplankton can be achieved also by the use of low-power and diagnostic frequency US.

Povzetek

Obstajajo različne metode za zaviranje rasti alg in cianobakterij v vodnih telesih, kot so dodajanje kemijsko in biološko aktivnih snovi, ozonacija, različni tipi filtracije in uporaba ultrazvoka (UZ). Glavni problem dodajanja kemijsko in biološko aktivnih snovi je tvorba različnih stranskih produktov in povečano sproščanje cianotoksinov iz cianobakterijskih celic ob njihovi poškodbi oz. odmrtju, kar običajno problem še poslabša. UZ naprave, ki so prosto dostopne na trgu za namen nadzora rasti alg, delujejo v razponu od 20 do 200 kHz in so nizko energijske, kar pomeni, da ne povzročajo učinka kavitacije. Te UZ napave po trditvah proizvajalcev nimajo negativnih vplivov na netarčne organizme, kot so ribe in zooplankton. V naravnih vodnih ekosistemih, kot so na primer jezera in ribniki, je bistvenega pomena, da z uporabo UZ ne poškodujemo ostalih, t. i., netarčnih organizmov, ter s tem ne porušimo naravnega ravnovesja v vodnem telesu.

O vplivu UZ na netarčne organizme obstaja le malo raziskav in še to z nepopolnimi informacijami o karakteristikah testiranih UZ. Kljub temu raziskave kažejo, da nizko energijski UZ, ki se uporabljajo za zatiranje alg nimajo škodljivih vplivov na preučevane vrste rib. Kljub temu se priporoča previdnost pri uporabi UZ v morskem okolju, saj lahko ultrazvočni valovi ovirajo komunikacijo med morskimi sesalci ali škodijo ribam iz poddružine Alosinae - edine znane ribe s sposobnostjo zaznavanja UZ. Raziskave o vplivu nizko energijskih UZ na netarčne zooplanktonske organizme pa kažejo nasprotujoče si rezultate, od akutnih letalnih učinkov do odsotnosti vpliva. Zato je potrebna previdnost pri uporabi UZ za zmanjševanje rasti alg v ribnikih ali jezerih, zlasti z vidika varovanja zooplanktona in s tem ohranjanja naravnega ravnovesja.

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