

Original Research

Biofouling Control of Invasive Zebra Mussel (*Dreissena polymorpha*) Using Acoustic Energy

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Abstract

Effects of increasing levels of audible sound energy (500, 1000, 5000 Hz frequency) on attachment strength and mortality rates of zebra mussels were investigated in a long-term study for the control and deterrence of zebra mussel infestation. All groups exposed to sound treatments presented weight loss by 1.09, 1.44 and 2.07% in the 500 Hz, 1.000 Hz, and 5.000 Hz frequency groups, respectively, while mussels receiving no sound showed an increasing trend in weight gain by 1.37%. The mortality rate increased with both increasing levels of sound frequency, and the time-length of exposure duration within the same frequency level. Attachment strength declined with induced mortality of zebra mussel after 20 days of sound exposure, and nearly 50% of zebra mussels died by sound treatment of 5000 Hz frequency after 100-days of exposure. The findings in this study demonstrate that sound energy is a potential tool and a practical option for preventive management and control of zebra mussel biofouling in freshwater ecosystems.

Keywords: acoustic, biofouling control, sound energy, *Dreissena polymorpha*, zebra mussel

Introduction

The dispersal of zebra mussel (*Dreissena polymorpha*) in freshwaters of Eastern Europe, North America and West Asia [1], became a significant problem due to severe environmental consequences such as rapid colonization on new areas in the aquatic ecosystem [2], shifts in food web and its dynamics,

loss of zooplankton species [3], changes in feeding ecology and trophic conditions of endemic fish species [4, 5], with remarkable economic consequences [6]. Zebra mussel has been the most aggressive freshwater invader in wide areas of freshwater ecosystems around the world and are the only freshwater bivalve species that attach to hard substrates in high densities and have a planktonic larval stage [7]. Although veligers can settle upon a variety of surfaces, survival is influenced by surface selection, and hard structures are usually suitable surfaces for attachment. Once it has attached to a surface, both eggs and larvae of zebra mussels are

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capable of movement by either natural or anthropogenic means [8], relocating to more suitable locations with rapid distribution to wider areas, makes zebra mussel control extremely difficult [9]. Zebra mussels produce millions of eggs during a spawning season which showed a remarkable increase in the last decade in freshwater ecosystems of Turkey [10]. So far, several methods and control measures for mitigating biofouling and population size of zebra mussels are known with limited reports on the use of different chemicals [11], physical and mechanical treatments [12], coating [13], or biological treatments by introducing species feeding on zebra mussel [14, 15]. Further, *Pseudomonas fluorescens* bacteria were used as a potential biological control agent highly lethal against zebra mussel, due to the toxins in these bacterial cells destroying the digestive system of mussels [16]. Today, effective control strategies for zebra mussels mostly rely on chemicals such as sodium hypochlorite, copper compounds, and quaternary ammoniums [11], and potassium [17]. However chemical treatments can be expensive, limiting its use [17] and leaching of ecologically harmful residuals can further affect non-target aquatic species colonizing in the same aquatic system [18]. Aksu et al. [19] reported that divers may be employed for manual and mechanical cleaning of invasive zebra mussels, however considering the expansion of zebra mussels around the World, this type of cleaning may not be cost-effective. Further, low-voltage AC currents and acoustic vibrations have also been suggested for the prevention of attachment and colonization of zebra mussels [19]. Zebra mussels are considered intolerant of low dissolved oxygen [20], which could be a useful tool for combat against mussel attachments in closed pipe systems. From earlier investigations, it is understandable that no clear methodology has been assessed for the limitation or eradication of invasive mussel infestation in freshwater ecosystems so far. Hence, the establishment of environment friendly and low-cost treatment methods without impacts on the ecosystem balance in long-run is still open.

Effects of acoustic energy on various forms of biofouling with different frequency levels have been reported earlier for the prevention of settlements [21]. Sound waves with frequencies below 1 kHz are specified as “low frequency sound”, whereas above 20000 Hz (20 kHz - 1 GHz) are referred as ultrasonic sound which human are not able to hear [22]. Indeed, the use of sound against zebra mussel fouling dates back to the 1960s, with very limited reports from the former Soviet Union (USSR) [23], and then some reports were published in the 1990s [24], probably these practices were not cost-effective due to technological conditions those days, as no further publications were released since then. However, considering technological developments and cost-effective equipment nowadays, bringing this issue to the agenda again might encourage new attempts to compete mussel biofouling with sound as an environment friendly approach.

Therefore, the present study aimed to investigate impacts of acoustic energy with increasing levels of sound frequencies ranging from 500 to 5000 Hz on deterrence potentials of invasive zebra mussel settlement as a control measure for mussel biofouling in freshwater ecosystems.

Materials and Methods

Mussel Sampling and Experimental Conditions

Zebra mussels (*Dreissena polymorpha*) were collected from Atikhisar Dam Lake (40°06'08.45"N-26°31'26.83" E) in Canakkale province, North-West of Turkey (Fig. 1).

There are no anthropogenic (human generated) entertainment activities such as extensive motor boating on the lake, or any industrial impacts that could provide an ambience for mussels adjusting to human generated sounds in the vicinity. Zebra mussels, randomly collected from different locations of Atikhisar Dam Lake were immediately transferred to the Freshwater Research Station of Canakkale Onsekiz Mart University, Faculty of Marine Science and Technology, Department of Marine Technology Engineering (Canakkale, Turkey), and randomly distributed into 12 identical rectangular shaped glass aquariums with 100 L volume each and dimensions of 70 x 40 x 40 cm. A total of 100 mussel were randomly distributed among the test aquariums.

The experimental facility was set with four-independent recirculation systems with equal water inflow (30.2 L/min) for each of the sound frequency treatment groups of no-sound treatment (control), 500, 1000, and 5000 Hz sound frequency groups. All experimental set-ups were supported with aeration using air stones and all experiments were conducted in triplicates. Freshwater was supplied from Atikhisar Dam Lake in order to provide similar water quality conditions in the experimental facility with that of the lake water conditions. Water in the experimental system was replaced with new lake water twice-a-week over the experimental period of 100-days from May to August, 2021. Introducing new lake water helped to ensure a nutrient medium for mussels allowing them to feed on life food available in the fresh lake water, and no additional food was supplied.

Acoustic System and Underwater Sound Transmission

The sound frequency sources of 500, 1000, and 5000 Hz used in this study were retrieved from <https://www.youtube.com/watch?v=GIEfshsoyZk>, <https://www.youtube.com/watch?v=TbPh0pmNjo8>, and <https://www.youtube.com/watch?v=cx1VQISKvhc>, respectively, recorded on flash memory in mp3 format. The test aquariums were equipped with waterproof hydrophones

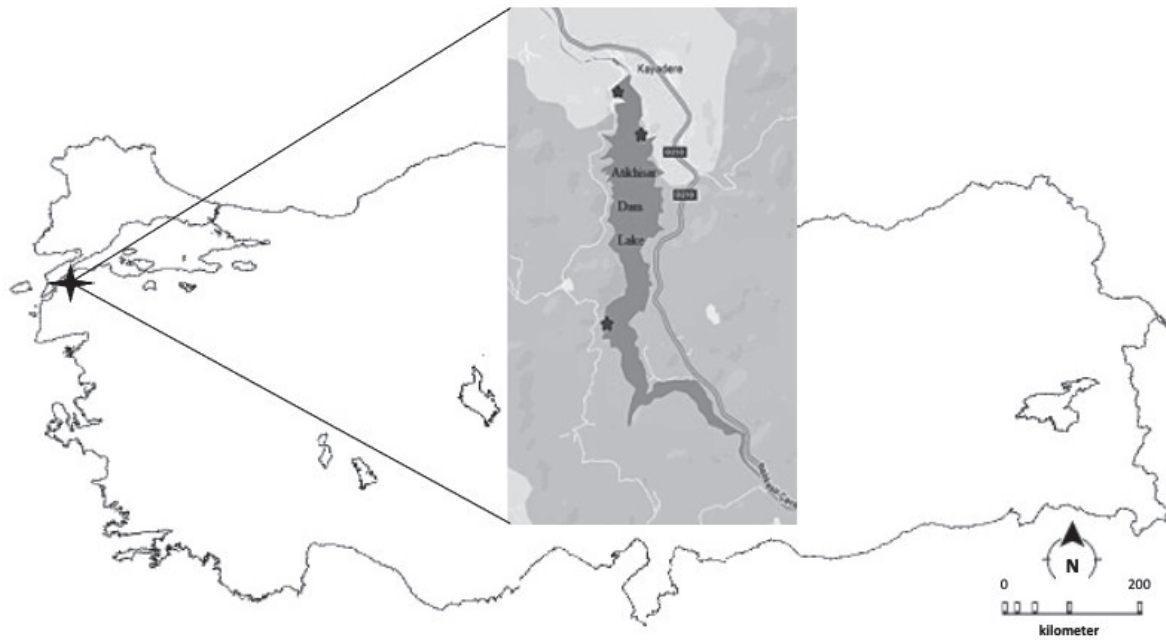


Fig. 1. Sampling area of zebra mussels (Atkhisar Dam Lake, 40°06'08.45"N-26°31'26.83"E).

(8.24 x 3.1 cm, mini speaker w/Wires–8 Ohm, 1.5 W Stwc), set 5 cm below water surface and in opposite direction towards the water inlet pipe. All triplicate test groups were set using mp3 amplifiers (Magic Voice) with three outputs for the transfer and even distribution of underwater sounds in the test aquariums. The ambient noise level in decibel (dB re 1 μ Pa SPL) was measured in the water ambient with a Sound Level Meter JE: 2244466 (frequency range 31.5 Hz-8000 Hz; measurement range 30-130 dB re 1 μ Pa; accuracy \pm 1.5). The schematic illustration of the experimental set-up with underwater transmission of sound and hydrophone output recording in the test aquarium is shown in Fig. 2.

Playbacks of underwater transmissions of low sound frequencies (500 and 1000 Hz), and high frequency (5000 Hz) have been initiated simultaneously in all test aquariums at the same time and continued throughout

the study period without any interruption. Photoperiod followed the natural course over the 100-days study. Special care was given to prevent any contact of vibrating pipes with the surface of the glass aquarium in order to ensure no additional or external sound penetration but the sound frequency test level only. The sound pressure levels (SPL) were measured and recorded in 10-days intervals in order to ensure similar range of sound levels throughout the study.

The average SPLs in the control group with no sound treatment at all was recorded as 43.21 \pm 1.33 dB re 1 μ Pa (range 36-47), which represented ambient noise level, whereas mean SPLs for the experimental groups exposed to 500, 1000 and 5000 Hz frequencies were recorded as 85.14 \pm 1.13 (range: 82-89), 83.31 \pm 1.51 (range: 77-89), and 86.72 \pm 1.36 (range: 80-90) dB re 1 μ Pa, respectively. SPLs in all sound treatment groups

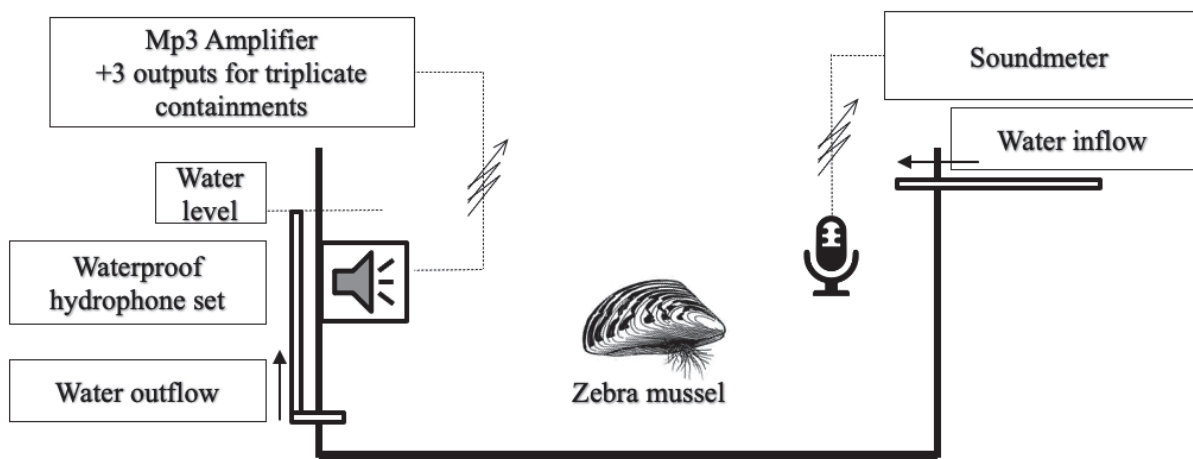


Fig. 2. Schematic illustration of sound transmission set-up and recording system in the water environment.

exceeded and overlapped the ambient noise level that was measured as 43.21±1.33 dB re 1 µPa (range 36-47).

Growth Performance and Mortality Rates of Zebra Mussels

During the course of the study, counts of dead individuals were performed using the following classifications for alive and non-alive mussels; (a) mussels were defined as “alive”, when shell gaping was noticed and attached on surface; (b) mussels were accepted as “non-alive”, when shell was open and detached from their byssus without visible activity, or did not respond to prodding (tactile stimuli) in individuals with closed shell. Through this identification method, it was ensured that alive mussels attached on glass wall were not disturbed and none of the attached mussels were detached from their byssus to avoid additional stress or damage to the mussels. Only those showing non-alive signs have been withdrawn from the test aquariums and counted individually every 20-days intervals. These counts were then subtracted from the total number of mussels introduced that was 100 at initial for each test aquarium, in order determine the number of alive mussels attached on surface. The remaining mussels then presented the mortality encountered over a certain time after exposure to underwater sound frequencies.

Throughout the study, growth performance of zebra mussels was assessed in terms of wet weights at initial (day 0) and final (day-100), using a Shimadzu electronic balance (type BL-3200H; range 3200 g; accuracy ±0.01 g). At initial set-up, zebra mussels were bulk weighed to the nearest 0.01 g, and gently distributed into the test aquariums in order to avoid stress conditions. At final, all alive mussels were again bulk weighed to nearest 0.01 g and growth performance

and mortality rates were calculated using following formulations:

$$\text{Weight gain (g)} = \text{final weight (g)} - \text{initial weight (g)}$$

$$\text{Percent weight increase (\%)} = ((\text{final weight (g)} - \text{initial weight (g)}) / \text{initial weight (g)}) \times 100$$

$$\text{Specific growth rate (\%/day)} = ((\ln \text{ final weight (g)} - \ln \text{ initial weight (g)}) / \text{days}) \times 100$$

$$\text{Mortality rate (\%)} = 100 - ((\text{number of mussels at final} / \text{number of mussels at initial}) \times 100)$$

Statistical Analyses

All measured data in this study were given as means±SD. When homogeneity and normal distribution of data was observed, Tukey Multiple Range Test was used to evaluate the growth performance and mortality data. In case of homogeneity but no normally distributed data, Kruskal-Wallis test was used, while the Tamhane test was applied for any data with no homogeneity, via SPSS 19 (IBM SPSS Statistics 19) Statistical Software. Critical limits of significance were set at P<0.05.

Results and Discussion

The number of alive mussel attachments after exposure to underwater sound frequencies, irrespective to the level, showed a gradual decline with exposure time (Fig. 3). By the end of the 100-days experimentation, the highest attachment rate was

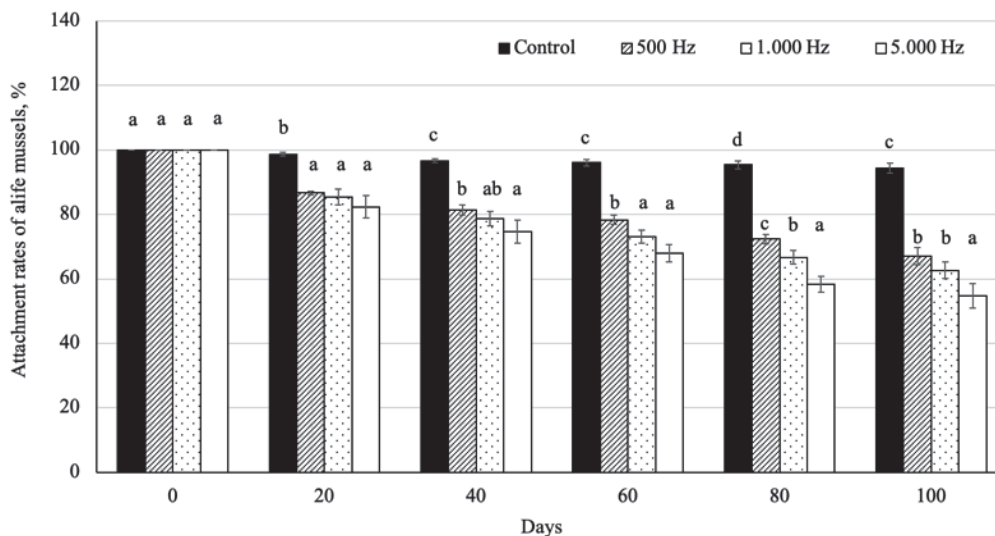


Fig. 3. Attachments of zebra mussels exposed to different sound-frequency levels for 100 days. Different letters above bars in each time period represents significant differences at 0.05 level.

recorded in the control group without sound exposure ($94.33 \pm 1.53\%$). Significantly lower ($P < 0.05$) attachment rates were observed for all sound exposure treatments compared to the control, and attachment rates presented a declining trend from $67.0 \pm 2.65\%$ to $54.67 \pm 3.79\%$ with increasing frequency levels from 500 to 5000 Hz. No significance was found between the 500 and 1000 Hz frequency groups ($P > 0.05$), while the 5000 Hz sound frequency resulted in significantly lower attachment compared to the other treatment groups. Lowest attachment rate of $5.67 \pm 1.53\%$ was recorded in the control group without sound exposure at all.

The findings from the present study provides significant evidence that zebra mussels biofouling could be controlled through acoustic applications. Despite the fact that some research has focused on the use of sound energy in mitigating zebra mussel expansion of colonies [24], there are still inconsistent data that need wider investigations.

In the present study, impacts of low (500 and 1000 Hz), and high frequency (5000 Hz) underwater sound transmissions on growth and mortality rate of zebra mussels at post-attachment stage have been evaluated. Donskoy [23] tested continuous sound waves with frequencies of 78, 156, 685, and 1000 Hz and sound pressure level (SPL) of 182-192 dB re 1 μ Pa for six hours, however, the author did not observe significant impacts on mortality rates in zebra mussels at these frequency levels after six hours of exposure. The sound frequency levels applied in the present study were comparable and within the range of those used earlier [23], with the difference in SPLs, which ranged between 77 and 89 dB re 1 μ Pa in the present work. Further, Donskoy [23] tested ultrasonic treatment with 20 kHz frequency, which is over the limits of human hearing ability [22], and again no remarkable effect was observed on a zebra mussel colony after 15 minutes of sound exposure. Different than Donskoy [23], a long-term course was followed in the present study and significant detachment rates of zebra mussels were recorded after 20 to 40 days of exposure. The findings in this study indicate that impacts of both low or high frequency sounds on mussel detachment strength are

time-dependent, namely, increasing exposure time resulted in increased detachment and mortality rate depending on frequency level.

Weight gain of zebra mussels in the control group with no sound treatment showed an increasing trend over the course of the study. In contrast however, all sound-treatment groups presented a declining trend throughout the study period. By the end of the study (day-100), highest final weight was recorded in the control group (0.0855 ± 0.00012 g), followed by the 500 Hz (0.0857 ± 0.00006 g), 1000 Hz (0.0856 ± 0.00006 g), and the 5000 Hz (0.0854 ± 0.00021 g) group. Final weight of zebra mussels in the control group was significantly higher than the sound treatment groups ($P < 0.05$). No significance was found between the 500 Hz and 1000 Hz exposure groups ($P > 0.05$), in terms of final weight, weight gain, percent weight-increase or specific growth rate. However, zebra mussels exposed to 5000 Hz sound frequency presented significantly lower weight gain ($p < 0.05$) compared to the other test groups. All groups, except the control with no sound treatment, presented weight loss, that was expressed as negative weight gain (Table 1). The negative growth or weight loss of mussels exposed to underwater sound energy in the present study might be attributed to the increase of stress generated by the unfavorable vibrating conditions that mussels were exposed to, however, stress conditions were not evaluated in this study, and needs further clarification. Low frequency continuous sound of 58 Hz (170 dB re 1 μ Pa) generated by vibration for 12 hours provided remarkable impact on translocation of zebra mussels, however, after termination of sound exposure, mussels showed increasing numbers of resettlement, showing that most of the mussels were alive, and only translocating ability of mussels were suppressed during the sound treatment [23]. Low frequency vibration levels between 70 and 445 Hz were investigated on biofouling control of barnacles [21], where no influence was noted on barnacles when frequencies below 200 Hz were applied. However, sound frequencies above 200 Hz supported with vibration increased deterrence strength in barnacle settlements in a study conducted for 98 days [21], similar to the experimentation in this study

Table 1. Weight increase and mortality rate of zebra mussels exposed to different sound frequencies for 100 days. Data given as means \pm standard deviation, and different superscript letters in a row show significant differences at 0.05 level.

	Control	Sound Treatments		
		500 Hz	1.000 Hz	5.000 Hz
Initial weight (g)	0.0855 ± 0.00012^a	0.0857 ± 0.00006^a	0.0856 ± 0.00006^a	0.0854 ± 0.00021^a
Final weight (g)	0.0866 ± 0.00015^c	0.0847 ± 0.00015^b	0.0844 ± 0.00036^b	0.0837 ± 0.00012^a
Weight gain (g)	0.0012 ± 0.00006^c	-0.0009 ± 0.00021^b	-0.0012 ± 0.00031^b	-0.0018 ± 0.00015^a
Percent weight increase (%)	1.37 ± 0.07^c	-1.09 ± 0.24^b	-1.44 ± 0.36^b	-2.07 ± 0.17^a
Specific growth rate (%/day)	0.014 ± 0.0007^c	-0.011 ± 0.0024^b	-0.015 ± 0.0036^b	-0.021 ± 0.0018^a
Mortality rate (%)	5.67 ± 1.53^a	33.0 ± 2.65^b	37.33 ± 2.52^b	45.33 ± 3.79^c

conducted for 100 days. Considering that other forms of biofouling, such as tubeworms, bryozoans, ascidians, and algae, were almost unaffected by the frequency levels supported with vibration, it can be underlined that acoustic energy levels may be species-specific and needs evaluation based on target specimens, which in fact could be an advantage to focus on target animals without impacts on non-target fellows in the same aquatic ecosystem.

The counts of non-alive (detached) zebra mussels gave a significantly lower mortality rate in the control group ($5.67 \pm 1.53\%$) compared to the sound treatment groups ($P < 0.05$), which showed an increasing trend with the increase of frequency level from 500 to 5000 Hz. The mortality rates of mussels recorded in the 500 ($33.0 \pm 2.65\%$) and 1000 Hz ($37.33 \pm 2.52\%$) exposure groups were not significantly different ($P > 0.05$), whereas the 5000 Hz group presented significantly higher mortality ($45.33 \pm 3.79\%$) compared to the other treatment groups ($P < 0.05$) by the end of the 100 days period (Table 1), an indication of time-dependent increase of mortality rate in zebra mussel exposed to sound frequencies.

In an earlier study, immobilization of zebra mussel veligers by using low frequency sound levels was succeeded, and when veligers were treated with sound energy in combination with vibration, higher mortality rates were reported in comparison to the sound treatment only [25]. The authors [25] indicated that veligers exposed to sound energy lost their swimming ability and sinking to the bottom thereafter. As a control strategy it was reported that the highest impact was observed in low-frequency range below 200 Hz, and overall sound treatments with low frequency below 200 Hz and between 10 and 100 kHz were effective against zebra mussel veligers, and vibration treatments were effective below 200 Hz and between 4 and 100 kHz against zebra mussel juveniles [25]. Further, the authors reported that ultrasonic cavitation at frequencies between 10 and 380 kHz presented high mortalities in the veliger stage, juveniles and also adult zebra mussels.

Overall, the findings in the present study are in agreement with most of earlier reports in terms of the effectiveness of sound frequencies on detachment strength of zebra mussels, but some other reports disagree with the results in this work or with those of earlier reports. The discrepancies among various studies might be attributed due to several factors of treatment types, frequency levels and range, type of organisms, growth stage of the specimen, treatment durations (time of exposure), or a combination of all these factors together.

The findings in the present study show high mortalities over a long-term sound exposure, namely, mortality rate between 30 to 40% was observed when zebra mussels were treated with 500 or 1000 Hz sound frequency, and nearly 50 % mortality was recorded when mussels were exposed to high frequency of

5000 Hz after 100 days of exposure. Compared to the findings in this study, higher mortality rates of 75-95% were recorded in zebra mussels treated with high frequencies ranging from 3000 to 18000 Hz generated by vibration [26].

Conclusions

In conclusion, the findings in this study provide evidence that sound frequencies could be used as a practical method and control strategy in preventive and destructive struggle against settled colonies in a lake environment. The highest destructive effect was obtained with sound frequency at 5000 Hz level, where nearly 50% of the mussels died after 100-days of exposure period. Considering the long-term application period necessary to reach effective results, cost evaluations corresponding to unit application for biofouling control are encouraged in future investigations.

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Conflict of Interest

The authors declare no conflict of interest.

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