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In-vitro Investigations on Ultrasonic Control of Water Chestnut

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ABSTRACT

Water chestnut (*Trapa natans* L.) is native to southern Europe and tropical Africa and Asia and was first introduced into North America in 1874. Since then, wild populations have quickly become established in many locations in the northeastern United States. *T. natans* is referred to as a noxious aquatic weed since its aggressive growth usually results in complete coverage of the water surface with floating rosettes of leaves. This study investigated the potential of the ultrasonic control of water chestnut since ultrasound has been documented to effectively damage plant cells and tissues. Various frequencies and amplitudes of ultrasound waves generated by submerged transducers were applied directly to water chestnuts. Ultrasound frequencies of 20-kHz, 100-kHz, 500-kHz, 1-MHz, and 2-MHz caused substantial damage to plant cells and penetrated petiole tissues. 20-kHz ultrasound caused the most significant cell damage after 10 seconds of ultrasound exposure. The mortality rate of water chestnut plants treated with ultrasound aimed directly at water chestnut stems was 97% with no seed production. The results of this laboratory study demonstrated that ultrasound caused severe damage and plant death by aiming 20-kHz ultrasound waves directly on water chestnut stems. In the future, development of a high-efficiency multi-transducer device is recommended for a field demonstration. Limited research has been conducted to determine the effects of 20-

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kHz ultrasound on benthic organisms, fish or wildlife, and therefore additional studies should be conducted to investigate potential impacts of ultrasound on aquatic communities prior to large-scale field application.

Key words: *Trapa natans*; aquatic plants; invasive plant management; noxious weed, ultrasound.

INTRODUCTION

Water chestnut is an annual aquatic macrophyte with floating leaves around a central stem and feathery, adventitious submersed structures (Pemberton 2002) (Figure 1). These feathery, adventitious structures have been described both as roots (Schulthorpe 1971) and as leaves (Muenscher 1944, Vasiley 1978) with their functional role as primarily nutrient absorption. Because *T. natans* has no primary root system, the submerged structures also serve to anchor the plant (Groth et al. 1996).

Water chestnut plants over winter solely by seeds/nuts. Typically, a *T. natans* nut is capable of producing three primary/first-order leaves, and as the stem of a *T. natans* plant elongates, the second-order leaves develop on the upper stems (Groth 1988). Water chestnut leaf petioles are filled with gas chambers. The spongy inflated leaf petioles provide buoyancy, allow the circulation of gases, and enable the leaves to float and perform photosynthesis.

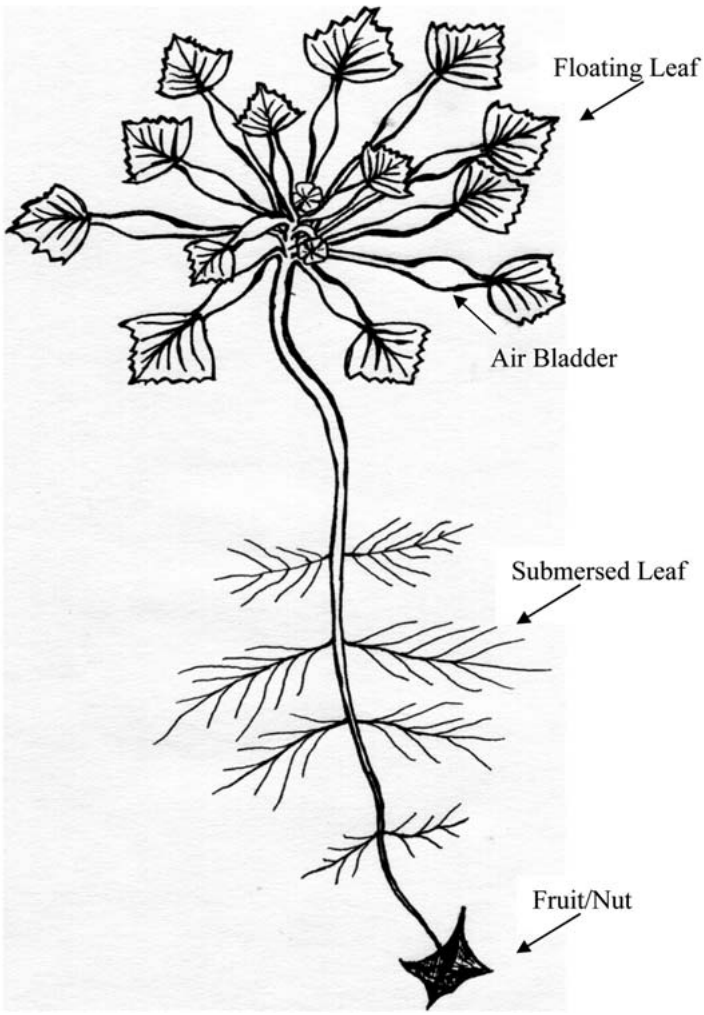


Figure 1. Illustration of water chestnut (by Andrew K. McMillan).

Water chestnut requires full sunlight and a nutrient rich, alkaline environment (Winne 1950, Papastergiadou and Babalonas 1993, Kiviat 1993). This species can grow in water up to 5 m deep but usually prefers shallow waters up to 2 m deep with muddy bottoms (Countryman 1978, Bogucki et al. 1980). In the northeastern United States, *T. natans* begins to flower in early July with four white petals born in the leaf axils of younger leaves above the water. *T. natans* nuts are woody and bear four sharply pointed horns (Kurihara and Ikusima 1991). When mature, the nuts fall from the plant and sink to the bottom of the water body (Groth 1988). The horns serve as anchors to limit the movement of the nuts and maintain them in suitable depths of water. The nuts overwinter at the bottom of the water body and start germination when the water temperature is above 8°C, with an estimated germination rate of 87% (Kurihara and Ikusima 1991). Only about 30% of the seedlings die before the floating leaves reach water surface. In most cases, the seedlings emerge and generate a bed of *T. natans* at the same site the following year. However, a small fraction of the nuts can also be carried on buoyant detached plant materials and be dispersed downstream to new sites.

Water chestnut is native to temperate and tropical Eurasia and Africa and was first introduced into North America in 1874 (Muenscher 1944, Countryman 1977, 1978, Gleason and Cronquist 1991, Crow and Hellquist 2000). Since then, wild populations have quickly become established in many locations within northeastern United States and have been reported in the Great Lakes Basin, Potomac River and Connecticut River Valley (Groth et al. 1996). Moreover, water chestnut has a potential to further spread into warmer regions of the United States since it is native to tropical and subtropical climates. This species is now listed on State Noxious Weed Lists by 35 States in the U.S. (Pemberton 2002).

T. natans is referred to as a noxious aquatic weed since its aggressive growth nature usually results in a complete coverage of water surface with floating rosettes of leaves. Dense surface mats intercept up to 95% of incident sunlight and suppress native submerged and floating plants as well as their associated microscopic flora and fauna, successfully colonizing and ultimately monopolizing aquatic habitats (Winne 1950, Kiviat 1987, 1993, Groth et al. 1996). Water chestnut plants provide low value food for wildlife, as compared to the native species it replaces. Under dense water chestnut beds, dissolved oxygen was observed to be lower, which impacts fish and invertebrate communities (Fasset 1960, Tsuchiya and Iwakuma 1993, Hummel and Kiviat 2004). Water chestnut infestation also restricts recreational water uses and navigation (Bogucki et al. 1980). In some instances, water chestnut completely chokes a waterway and makes boating impossible.

Due to its detrimental effects on the overall health of aquatic ecosystems, vigorous management efforts have begun in the Northeast U.S. Current management programs focus on two control strategies: mechanical harvesting and manual removal. Chemical treatment was not recommended because many infested water bodies serve as drinking or agriculture water supplies. Mechanical harvesting removes the

floating mass of plant materials by cutting the shoots attached to the previous years' nuts buried in the sediment floor. The plant biomass is collected and transported to shore for disposal. Manual removal or hand pulling is normally used in shallow water or areas with sparse growth. Both methods are relatively expensive, inefficient and labor-intensive; therefore, a more effective approach is urgently needed. Studies have been conducted to identify alternative control methods including biological control (Pemberton 2002). Unfortunately, to date, no cost-effective method is available to control water chestnut infestations.

Ultrasound is a sound wave, the frequency of which is above the audible frequency range for humans; i.e., frequency >20,000 Hz. The relevant physical principles of ultrasound include resonance phenomena and acoustic intensity. Intensive research has been conducted on interactions between ultrasound and cells/tissues in plant leaves, seeds and roots. Mechanisms of bioeffects of ultrasound include "thermal" and "mechanical" effects (National Council on Radiation Protection and Measurements 2002). When plants absorb ultrasound waves, energy associated with ultrasound waves is converted into heat, or a thermal effect. An ultrasound wave, as it passes through a water medium, can cause bubble activities known as acoustic cavitation. Cavitation causes a wide variety of changes in plant cells, ranging from microstreaming of a cell's internal structure, to a mass disruption of cell walls (Coakley and Myborg 1978, Akopyan and Sarvazyan 1979). Acoustic cavitation, the dominant mechanism in ultrasound application, is especially evident on aquatic plants due to the presence of gas in the interconnected chambers inside plant petioles. In general, the smaller the radius of the gas size, the greater the acoustic cavitation. Documented effects of ultrasound on plant cells include chromosomal anomalies, cell death, damage to or destruction of cellular structures, reduced growth rates and mitotic indices, changes in osmotic potential of cells, and chemical changes within the liquid being cavitated (Miller 1979, Newroth and Soar 1986, Soar 1986). Ultrasound was found to cause cell structural damage and death of algae by the disruption of the connections between the plasmalemma and the algal cell walls (Center for Aquatic Plant Management 2003). Sonication can effectively remove algae, *Microcystis aeruginosa*, by collapsing of its gas vesicles and causing algae cell to loss its buoyancy (Zhang et al. 2006). Ultrasonic treatment systems have been installed in eutrophied lakes and effectively controlled cyanobacterial blooms (Lee et al. 2002).

Harvey and Looms (1928) observed the effects of ultrasound on the leaves of *Elodea canadensis* Michx, under a microscope and found increased fluid flow, stirring of intracellular contents such as chloroplasts, rotation of organelles, and cell disruption inside of the leaf during exposure to ultrasound at 400 k-Hz. The disruption of an elodea leaf cell was reported to proceed in two stages upon exposure of ultrasound (Miller 1983). At the first stage, the vacuolar membrane was disrupted with mixing of cytoplasm with the vacuolar contents. At the second stage, the plasma membrane was permanently broken and the leaf lost its viability. In another study by researchers at the British Columbia Ministry of Environment, ultrasound effectively damaged plant cells and tissues of Eurasian watermilfoil (*Myriophyllum spicatum* L.) with single exposures of only several seconds (Newroth and Soar 1986, Soar 1985). Immediate damage consisted of rupture or flooding of the aerenchyma, deterioration of plant tissues, biomass reduction, and 100% mortality after two exposures to ultrasound (Newroth and Soar 1986). Newroth and Soar (1986) compared the success of ultrasound treatment for watermilfoil with the effectiveness of other control strategies and concluded that ultrasound was "one of the most promising approaches" and has "advantages for management and high levels of effectiveness in treatment of shoot and root tissues" of watermilfoil. However, to date, no commercial ultrasound device has been made available for aquatic vascular plant management.

The objective of this study was to determine the feasibility of ultrasonic control for water chestnut. A preliminary study was first conducted to determine the optimal ultrasound wave to successfully eradicate water chestnut plants. Ultrasound waves of various frequencies and amplitudes generated by submerged transducers were applied directly to water chestnut plants to determine the optimal ultrasound waves for water chestnut management. A subsequent study was conducted to assess the effectiveness of ultrasonic control of water chestnuts using selected ultrasound waves under a controlled greenhouse environment.

MATERIALS AND METHODS

Plant Materials

Water chestnut plants were collected from South Bay of Lake Champlain in June 2004 and 2005. Plants collected in June 2004 were used to conduct the ultrasound selection study. Plants collected in June 2005 were used in the effectiveness of ultrasound study. All collected plant materials were transferred to a greenhouse located at State University of New York College at Plattsburgh, NY. The plants were washed completely clean of sediment, plankton and invertebrates and then placed in a 2800-liter tank (1 m wide, 1.21 m deep, and 2.4 m long) constructed with stainless steel frames and polyvinyl chloride liners. The tank was filled with Hoagland's solution containing 20 mg/L nitrogen as ammonium nitrate (NH_4NO_3), 5 mg/L phosphorus as monobasic sodium phosphate ($naH_2PO_4 \cdot H_2O$), 20 mg/L potassium as potassium sulfate (K_2SO_4), 20 mg/L calcium as calcium chloride ($CaCl_2 \cdot 2H_2O$), 20 mg/L magnesium as magnesium sulfate ($MgSO_4 \cdot 7H_2O$), and traces of manganese, boron, zinc, copper, and iron. The plants were kept in Hoagland's solution for at least two weeks before they were used in the experiments. Temperature in the greenhouse ranged between 25 and 30°C during the study period. Dead leaves were removed by hand, simulating the natural removal of dead leaves by waves under field conditions.

Ultrasound Selection Study

A laboratory study was first performed to determine the optimal frequency, acoustic pressure amplitude, and minimum ultrasound exposure duration required to successfully damage water chestnuts. A computer-controlled measurement system (NTR Systems, Seattle, Washington, USA), including

three linear position manipulators and a digital oscilloscope as a digitizer (Model 9310, LeCroy, Inc., Chestnut, NY, USA), was used to measure a two-dimensional cross-axis sound field. A calibrated pvdf membrane hydrophone with a 0.2 mm diameter electrode (Sonic Consulting, Inc. Wyndmoor, PA, USA) was used as a sound-wave sensor for all mega hertz frequencies. A calibrated 6 mm diameter pvdf hydrophone (Model 8103, Brüel & Kjær, Nærum, Denmark) and a charge amplifier (Model 2635, Brüel & Kjær, Nærum, Denmark) were used for 20-kHz and submega hertz sound fields. The three dimensional position of a hydrophone was controlled by a computer *via* 3 linear manipulators (NTR Systems, Seattle, Washington, USA). A transducer was electronically connected to HP 3314A function generator (Hewlett Packard, CA) and an ENI A-300 RF power amplifier (ENI, Rochester, NY, USA) (Table 1). A 20-kHz sound field was generated by a 20-kHz horn driven by a power supply from a sonicator (Model 450, Branson Inc., Danbury, Connecticut, USA). When a non-focusing transducer was used, a hydrophone was scanned at a plane that was perpendicular to the acoustic axis of the sound field with a distance of 1 cm from the surface of the transducer. When a focusing transducer was used, a hydrophone-sensing element was scanned at the focal plane of the sound field. The *in situ* spatial-peak pulse-average intensity, I_{SPPA} (National Council on Radiation Protection and Measurements 1983), was also calculated post-measurements.

After the sound field mapping, a portable single ultrasound transducer of known resonance frequency was then submerged in a 30-gallon tank. A water chestnut leaf and petiole freshly dissected from a healthy plant were mounted on a plastic holder. When a non-focusing transducer was used, the sample/sample holder was positioned at 1 cm from the transducer. Consequently, the petiole was exposed to a near field ultrasound field generated by the transducer. When a focusing transducer was used, the plant leaf was placed within the ultrasound's focal region (Figure 2). After the ultrasound exposure, the treated petiole was dissected horizontally. Each dissected cross section (approximately 1 mm thick) of the petiole was then examined under a microscope to examine the impacts of ultrasonic treatment on the water chestnut plant tissue.

Effectiveness of Ultrasound Study

The laboratory-scale effectiveness of ultrasound was conducted using 15 tanks measuring 55 cm in diameter and 68 cm in height under a controlled greenhouse environment. Temperature in the greenhouse ranged between 25 and 30°C during the study period. Each tank was filled with 90 liter of Hoagland's solution. Six water chestnut plants with an average number of 18.3 leaves per plant were placed into each tank one week before the beginning of the experiment (Table 2). Five tanks of plants were treated with ultrasound aimed directly on petioles for approximately 2 second per petiole; this was designated the "petiole" treatment. Another five tanks of plants were treated with ultrasound aimed directly at one target spot on each plant stem for 10 seconds; this was designated the "stem" treatment. No ultrasound treatment was performed on the control group. Ultrasound transducers were submerged in water and aimed directly at target plants from underneath. Plant mortality, number of leaves per plant and seed production was investigated daily as well as water temperature and pH. Once a plant lost all its leaves and buoyancy, a plant was pronounced dead. Dead leaves were removed by hand, simulating the natural removal of dead leaves by waves under field conditions. Water temperature ranged between 22 and 26°C and water pH between 6.8 and 7.7 during the study period. Statistical analysis was performed using SPSS 14.0, and analysis of covariance (ANCOVA) was used to control for the potentially confounding effect of the days in analysis. Follow-up test of significant ANCOVA effects were compared using the Turkey's "honestly significant difference" (HSD) post hoc test.

TABLE 1. SUMMARY OF CHARACTERISTICS OF SOUND SOURCES.

Frequency (Hz)	-6 dB beam diameter	Highest acoustic pressure amplitude	I_{SPPA}	$F(P, f)$
20 k	12 mm	1.9 MPa	860W/cm²	13.4
200 k	12 mm	1.2 MPa	340 W/cm²	2.7
500 k	3 mm	2.8 MPa	1.9 kW/cm²	4.0
1 M	12 mm	1.3 MPa	400 W/cm²	1.3
2 M	12 mm	1.3 MPa	400 W/cm²	0.9

RESULTS AND DISCUSSION

Ultrasound Selection Study

The effects of ultrasound on plants include thermal effect and acoustic cavitations. Since the acoustic attenuation of plants was relatively low in the frequency-range tested, 20-kHz, 200-kHz, 500-kHz, 1-MHz, and 2-MHz, as well as the short duration (less than 10 seconds), the thermal effect is considered to be minimal (Fukazawa 2002). Acoustic cavitations (bubble activities under ultrasound) presumably played a primary role in damaging treated plants. Among all the above tested frequencies, 20-kHz ultrasound of 1.8 Map acoustic pressure amplitude demonstrated the most sever damage to treated water chestnut (Figure 3). Ruptures of water chestnut petioles were observed immediately after 10 seconds of ultrasound treatment. Treated plants lost all leaves, buoyancy and viability within 24 hours. Under a microscope, cell membrane disruption was observed on treated plants (Figure 4). Similar damage was caused by the other submegahertz and megahertz frequencies (200-kHz, 500-kHz, 1-MHz, and 2-MHz), but longer exposure duration, up to 2 minutes, was needed to produce similar damages on water chestnut plants. A mechanical index (MI) developed as an indicator for the potential of non-thermal damage caused by acoustic cavitation was further used to verify the results. The MI index is defined as

$$MI = \frac{P_r(MPa)}{\sqrt{f(MHz)}},$$

where P_r is the *in situ* peak negative acoustic pressure amplitude expressed in MPa and f is the central frequency in MHz (National Council on Radiation Protection and Measure-

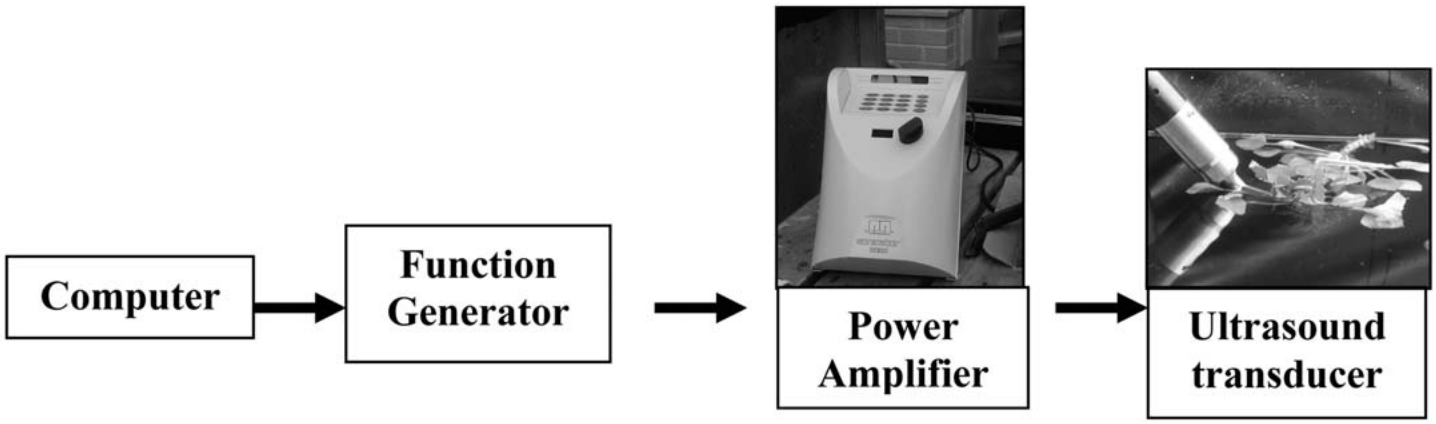


Figure 2. A portable ultrasound transducer was aimed directly at water chestnut from beneath. A computer that was electronically connected to a power amplifier and a function generator controlled the transducer.

ments 2002). The results showed that the low frequencies used in this study were much below the 1-MHz limit of diagnostic imaging applications. Nevertheless, $F(P, f)$ that is related to MI may still be a good indicator for the plant destruction due to acoustic cavitation. This is consistent with our observation; the 20-kHz sound source caused the most severe damage to the plant. Although the 500-kHz-focused sound field has the highest acoustic pressure amplitude at its focal region, its $F(P, f)$, is still lower than that of 20-kHz, as its frequency is much higher (Table 1). The MI index suggested that 20-kHz ultrasound of 1.8 MPa acoustic pressure amplitude has the highest MI value and can cause the severest damage to plants among the tested ultrasound waves. Another disadvantage of the 500-kHz-focused sound field is that it is critical to place the plant at its focus to get maximum acoustic pressure amplitude. Since the 500-kHz focal zone is relatively small, it is time-consuming and impractical to use a focused sound field in a large-scale management practice. A 20-kHz, non-focused sound field may provide a more effective management strategy.

Effectiveness of Ultrasound Study

After successfully selecting the optimal ultrasound wave, a study was conducted to determine the optimal aiming location on water chestnuts and to assess the effectiveness of ultrasound to control water chestnuts. Two potential aiming locations on water chestnut, the petiole and the stem, were examined.

Aiming ultrasound directly on the central stem of water chestnut plants caused immediate significant damages. The stems ruptured and the leaves gradually detached from the stems. No new leaf production was observed during the 14-day post treatment observation period (Figure 5). Once a water chestnut plant lost all its leaves and buoyancy, a plant was pronounced dead. Fourteen days after ultrasound treatment, the mortality rate of stem treatment reached 97%. Only one out of 30 treated plants were still alive, with only two leaves attached to its central stem (Figure 6 and Table 2). This treated plant was observed for two months. Although it did not lose its viability or all the leaves, it was never able to successfully produce seeds, which is the only means of reproduction by this annual plant species. Thus, the results suggest that ultrasound can cause high mortality of water chestnuts by aiming directly on plant stems for 10 seconds under a controlled greenhouse environment.

Table 2. Measurements for average number of leaves per water chestnut plant (n = 30) of petiole treatment, stem treatment and control (no treatment).

	Initial Mean ± S.E.	Final Mean ± S.E.
Petiole treatment	18.60 ± 0.26	0.37 ± 0.20
Stem treatment	18.00 ± 0.27	0.07 ± 0.07
Control (no treatment)	18.40 ± 0.28	22.40 ± 0.40

In the petiole treatment, the treated areas were damaged immediately and turned brown. Leaves broke off from the treated spots or detached from the central stems. Twenty-six of the thirty treated plants lost all their leaves and were not able to produce new leaves by day 14 (Figure 5). Those plants were considered dead; the mortality rate of the petiole treatment was observed to be 86.7% at day 14. The remaining four plants lost the majority of leaves with only a total of 11 leaves left among four plants (Figure 6). Ten of the 11 remaining leaves were new growth from the central stems (Table 2). Although the petiole treatment successfully damaged water chestnut petioles, interrupted gas, nutrient and water transport, and resulted in loss of plant leaves, four of the 30 treated plants were able to produce new leaves from the upper portion of the central stems. The damage on plant petioles caused by ultrasound did not successfully stop the growth of water chestnuts; the upper portion of the central stems might still be viable. Although no seeds were produced during the 2-month post treatment observation, petiole treatment alone might not be the best treatment strategy.

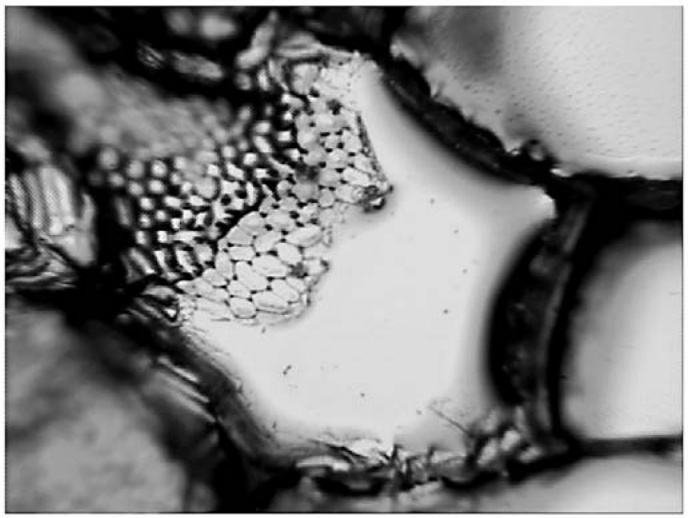
Other drawbacks of petiole treatment include treatment duration and difficulty in aiming. It takes two seconds to effectively damage one water chestnut petiole. On average, each water chestnut included in this study developed approximately 18 leaves. A total of 36 seconds of ultrasound exposure was required to treat one plant. However, several seconds were required to reposition the ultrasound transducer to aim directly on each plant petiole. Therefore, approxi-

a.

b.

Figure 3. Comparison of a treated water chestnut petiole (a) with an untreated petiole (b).

a.



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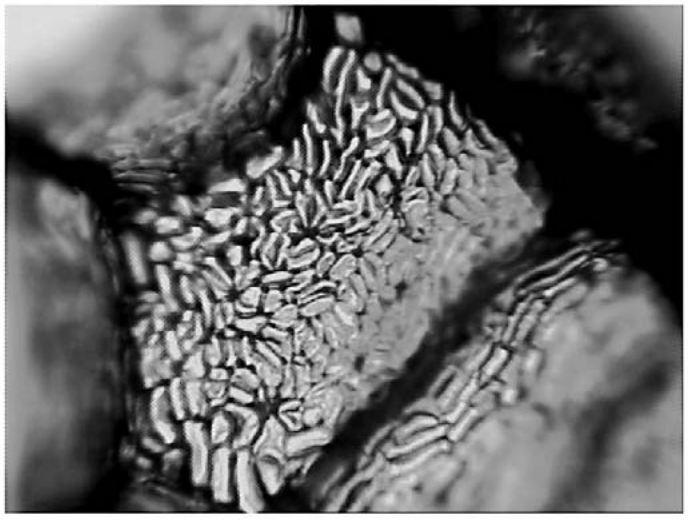

Figure 4. Comparison of a cross-section of a treated water chestnut petiole (a) with that of an untreated petiole (b).

mately 2 minutes were needed to apply ultrasound to a single plant, which is much longer than the 10-second exposure duration of the stem treatment. Second, new water chestnut leaves developed from the top of the central stem. A submerged transducer might not be able to successfully transmit ultrasound wave to new petioles, which sometimes are located above the water surface. The longer treatment time and difficulty in aiming suggest that petiole treatment might not be feasible in a large-scale field application. On the other hand, only 10 seconds is needed to finish treating one water chestnut stem. Compared to the petiole treatment, stem treatment demonstrated greater potential and greater treat-

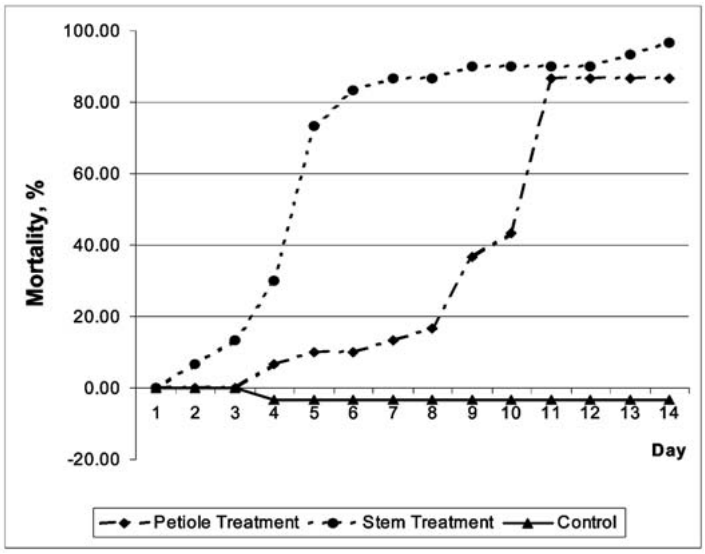

Figure 5. Average mortality rates (n = 30) of water chestnuts after petiole treatment, stem treatment and control (no treatment). Once a plant lost all leaves and buoyancy, a plant was pronounced dead.

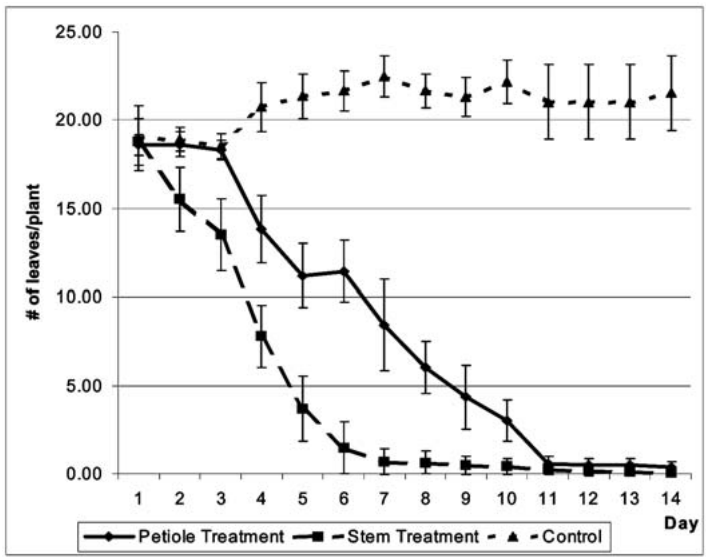

Figure 6. Average numbers of leaves per water chestnut plant (n = 30) after petiole treatment, stem treatment and control (no treatment).

ment efficiency because 1) the stem is easier to locate by submersed transducer, 2) no repositioning of the transducer is necessary while treating a water chestnut plant, and 3) less time is needed.

Water chestnuts in the control (no treatment) group grew during the 14-day observation period. The number of leaves in the control group increased from 18.4 leaves/plant to 22.4 leaves/plant (Figure 6 and Table 2), and one additional plant was observed in the control group via vegetative growth (Figure 5). An analysis of co-variance (ANCOVA) was performed to detect significant differences on numbers of leaves of water chestnuts after stem treatment, petiole treatment and control (no treatment) during the study period. The numbers of leaves of water chestnuts was significantly different, $F(2,38) = 63.231$, $p = 0.000$ (Table 3). Tukey's HSD test indicated that the numbers of leaves of water chestnuts in both stem and petiole treatments are significantly different from the number of leaves of water chestnuts in control (no treatment) (Table 4). A significant reduction in the number of leaves was found on both the stem treatment (0.07 ± 0.067) and petiole treatment (0.37 ± 0.195) compared to the control (no treatment) (22.4 ± 0.403) at the end of the observation period (Table 2). Although the average number of leaves of the stem treatment was significantly less than that of the petiole treatment, water chestnuts in both stem and petiole treatments were significantly damaged by ultrasound. The control group produced a total of 133 seeds by the end of the 2-month post treatment observation period. Water chestnut's high seed production rate can rapidly increase its populations when a successful management program is lacking.

Since 1982, over $6 million has been spent to control the advance of water chestnut and to prevent the lake-wide spread of water chestnut in Lake Champlain with limited success (Bove and Hunt 1997). The potential for the continued northward advance of the infestation demonstrates an urgent need for a more effective approach. The results of this study demonstrated ultrasound application might have a potential to be an alternative approach for water chestnut management. Ultrasound application has limited environmental impacts compared to other means of control such as: 1) no foreign substances are added during treatment; ultrasound treatment does not adversely affect drinking or irrigation water quality, 2) treatment can start as soon as ice breaks before a large biomass develops, 3) collection of plant biomass is not necessary; there is no need to transport or dispose plant materials, and 4) ultrasound electronic equipment including transducer, function generator and power amplifier has a long life expectancy (approximately 30 years). A low equipment cost makes ultrasound technology practical and affordable (Newroth and Soar 1986, Taylor 1992). The results of this study suggested that an estimate of 10 seconds is re-

Table 3. Analysis of variance table for effects of ultrasound on water chestnuts.

Source	Degrees of freedom (df)	Sum of squares (SS)	Mean square (MS)	*F*-ratio	*p*-value
Time	1	512.171	512.171	29.304	0.000
Treatment	2	2210.252	1105.126	63.231	0.000
Residual	38	664.153	17.478		
Total	42	8417.373			

TABLE 4. MEAN DIFFERENCES BETWEEN ALL POSSIBLE PAIRS OF TREATMENTS IN THE FEASIBILITY STUDY OF ULTRASONIC TREATMENT OF WATER CHESTNUTS.

	Control (no treatment)	Petiole treatment	Stem treatment
Control (no treatment)	0.000		
Petiole treatment	13.083 (0.000)	0.000	
Stem treatment	16.955 (0.000)	3.871 (0.000)	0.000

quired to cause detrimental damage to a water chestnut plant with one single transducer. One set of high power ultrasound electronic equipment can support and operate multiple transducers simultaneously. Thus, a multi-transducer ultrasound device, such as a device including twenty transducers, is preferred in order to improve the operating efficiency and reduce the management cost. Limited research has been conducted to determine the effects of ultrasound on benthic organisms, fish or wildlife (Wood and Loomis 1927), and additional studies should be conducted to investigate potential impacts of ultrasound on aquatic communities prior to large-scale field application.

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