Applying Ultrasound Technology to Control Algae and Biofilm

Presented to AALSO April 20, 2009
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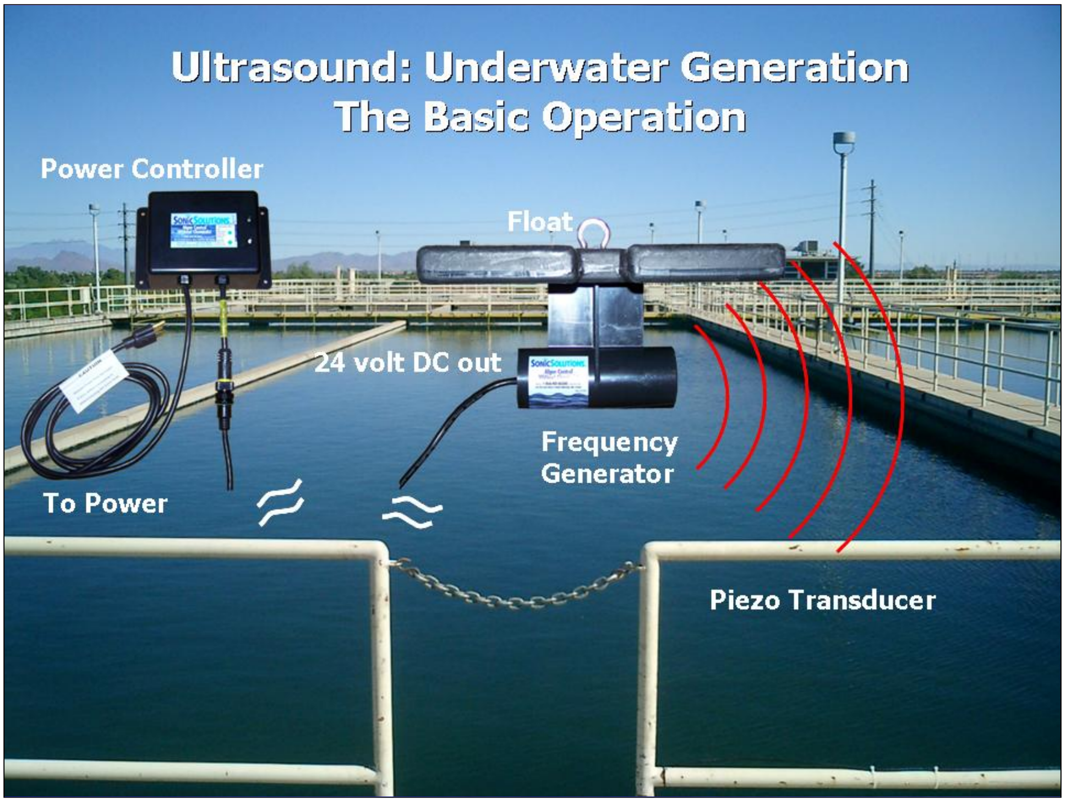
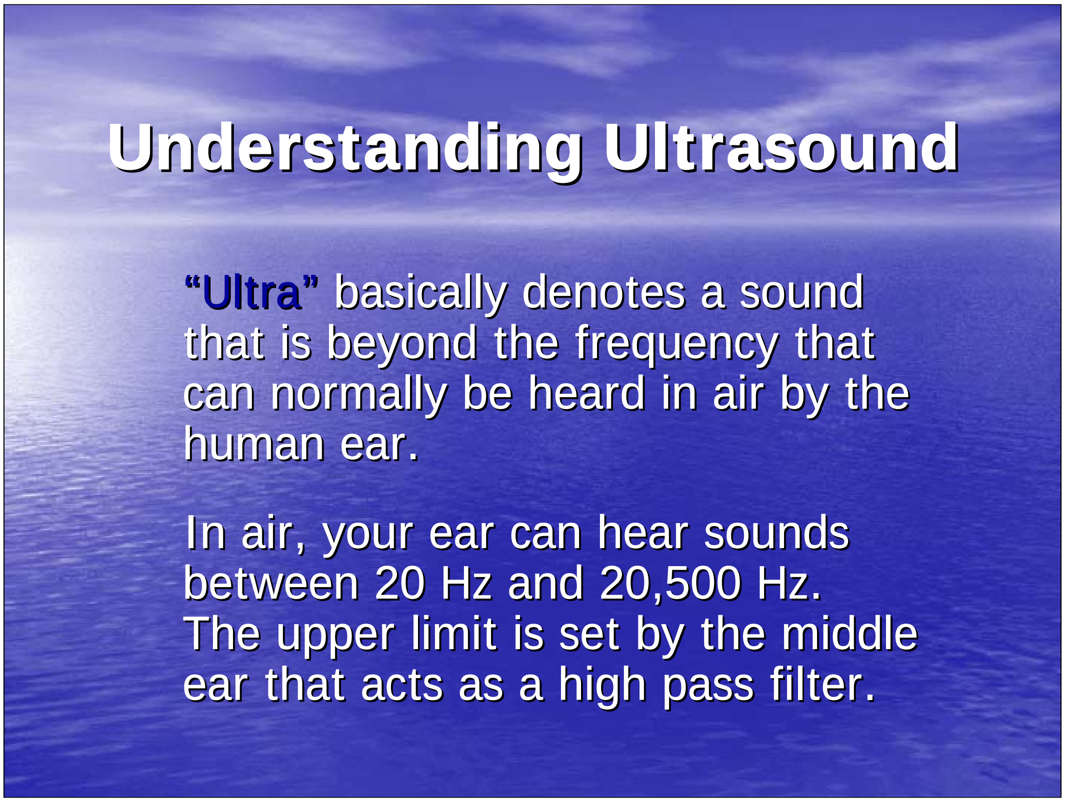
These presentation materials were prepared to inform the audience about ultrasound and how it affects algae.



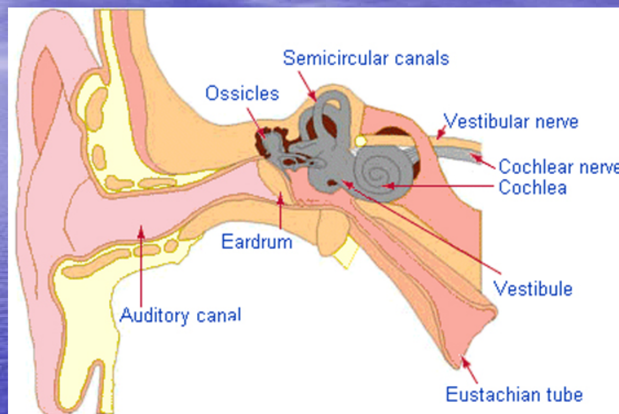
Typical device operation. The transducer can be mounted on a bracket or many times comes with a float. It is typically mounted on the edge of the water body you want to treat rather than in the middle. The power consumption is very low at less than 11 watts making solar power a good option for remote areas.



So, what is ultrasound? It is a sound that is at a frequency beyond the normal person's hearing range in air which starts around 20 Hz and ends at about 20500 Hz. Your middle ear acts as a high pass filter preventing higher frequency sound from being transferred to your inner ear.

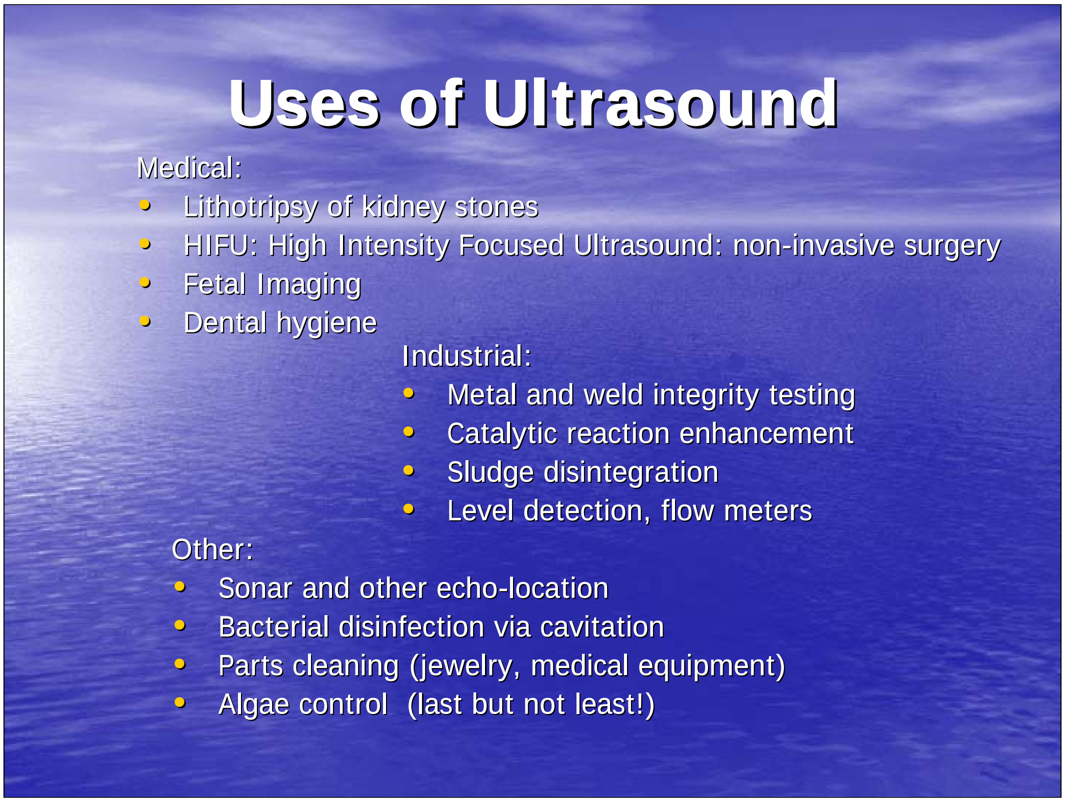
The transducer outputs a very loud sound that most people don't notice. Some people with sensitive hearing actually feel a pressure around the ears if the device is turned on in a room, for example.

You Can "Hear" Ultrasound



If the sound vibrates the skull, the cochlear nerve will allow you to "hear" up to 200,000 Hz, an effect discovered by divers who were able to hear sonar pings at about 50,000 Hz.

Here is a diagram showing our ear mechanism. The first time I heard of using the cochlear nerve to "hear" was a deaf young man who was a Boy Scout in my troop. They have a vibrational transmitter that is surgically implanted in their skull that helps them hear sound via the cochlear nerve, rather than the vestibular nerve.



There are so many useful tools offered by ultrasonic technology. After having had the kidney stone lithotripsy (twice) I have developed a greater sense of sympathy for algae. Well…not that much sympathy.

The HIFU is a technique in practice in Europe and undergoing testing here in the states that offers a unique way to destroy tumors without having to do internal surgery.

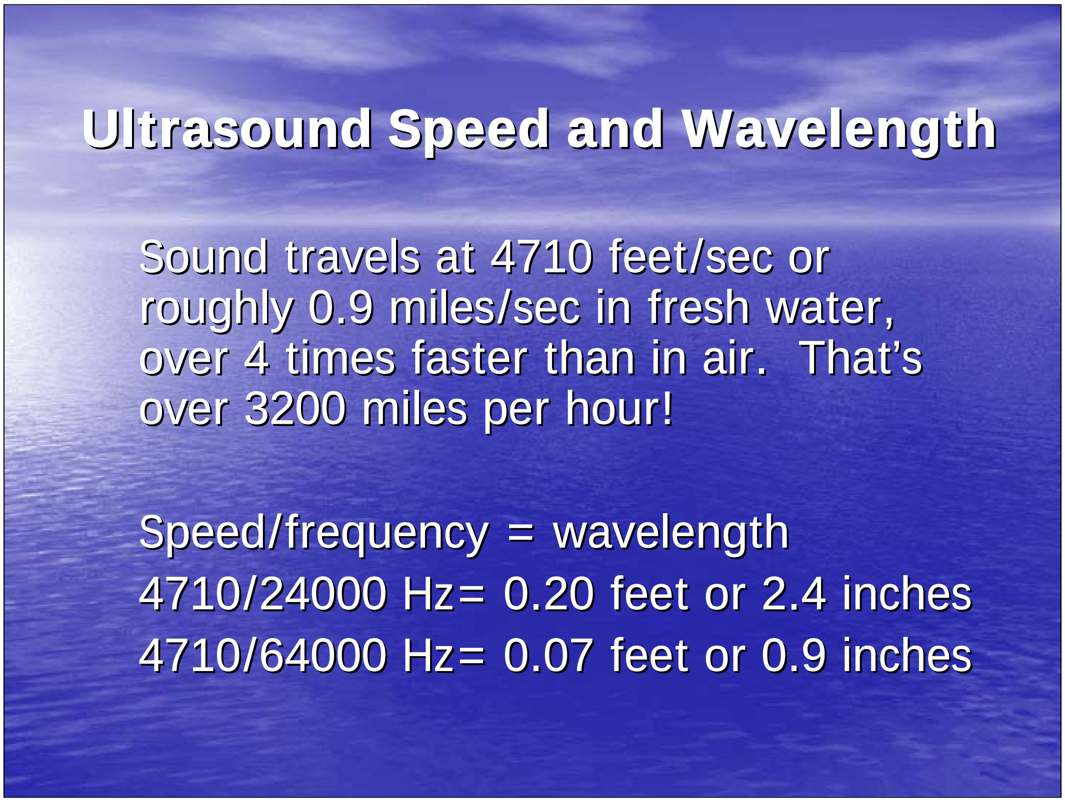
We've all heard of fetal imaging and the dentist will use ultrasound for cleaning and other useful dental procedures.

Industry use ultrasound to test metal weld integrity, enhance catalytic reactions, disintegrate sludge for further processing, and also use ultrasound to detect levels and flow rates.

Today's navy could not operate the same way without sonar.

Cavitation can offer a means to disinfect bacteria from surgical tools and implants. Ultrasound can help clean at the molecular surface level for jewelry and medical equipment.

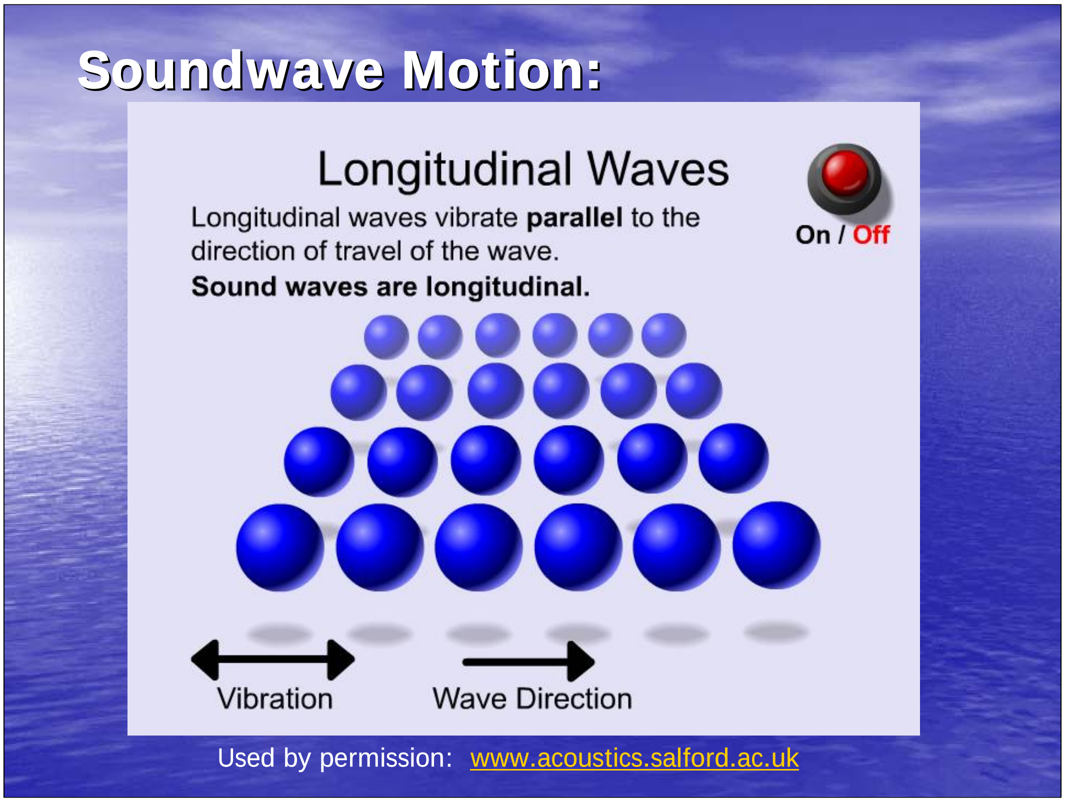
And now last but not least, algae control.



So let's do the math. Sound travels about 4 times faster in water than in air at nearly 0.9 miles per second or 3200 miles per hour.

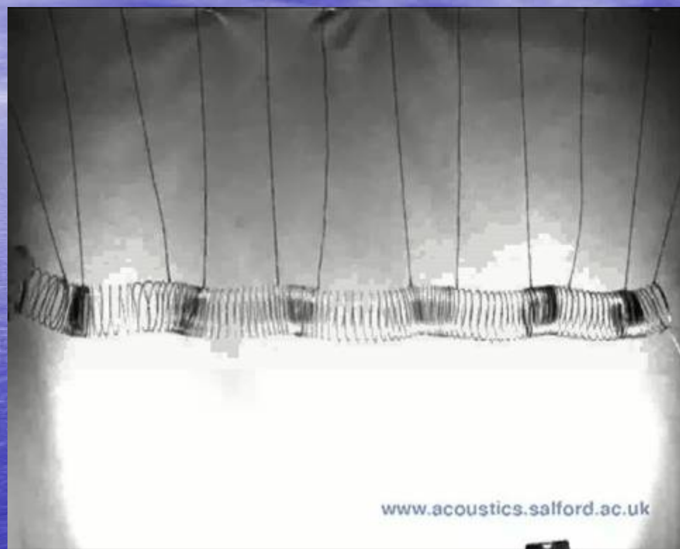
Speed over frequency yields wavelength, so at the frequency range employed to kill algae the wave length is between 1 and 2 inches.

Now imagine you are an algae being pounded 56000 times per second by a wavelength of 1 inch! Sounds like it might be a rough ride.



Let's see what that looks like. Sound travels as a longitudinal wave. The device producing the sound is typically a plate at the end of an ultrasonic transducer where the vibration occurs. It is moving back and forth at the speed of the ultrasonic driving frequency. The water molecules and anything in its path that are in this wave are being vibrated by this back and forth movement.

Simulation of a Longitudinal Sound Wave

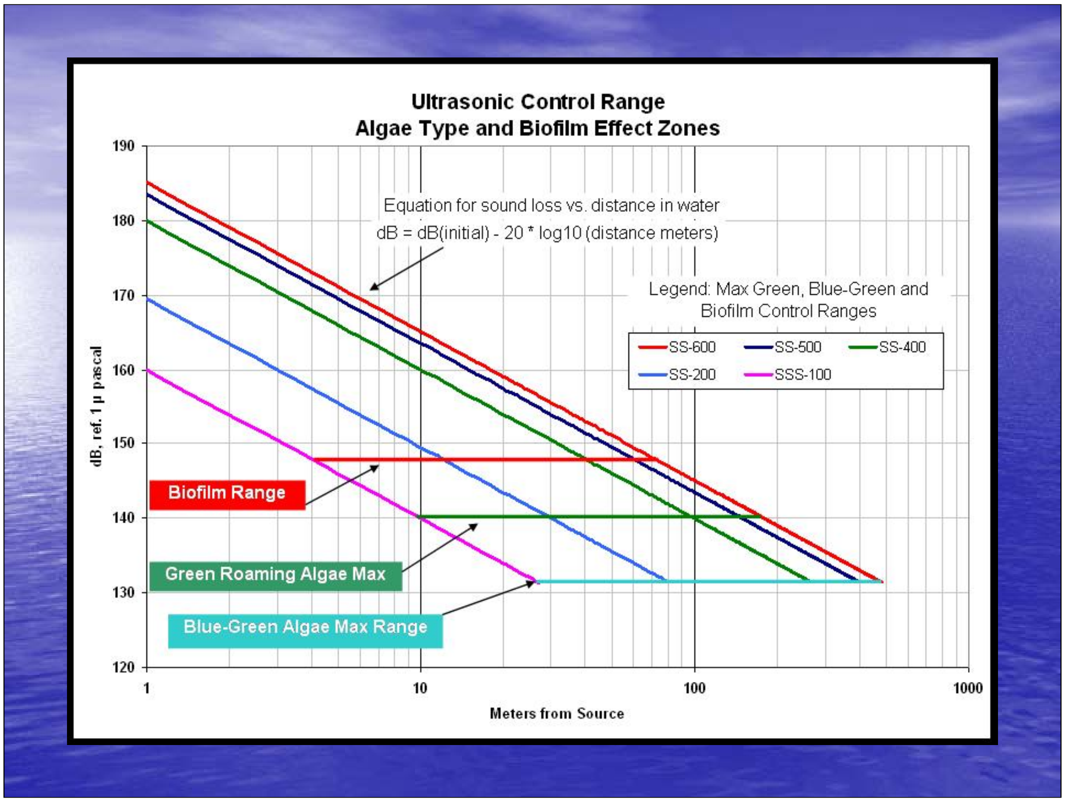


Used by permission: www.acoustics.salford.ac.uk

Here’s a simulation of a longitudinal sound wave using a suspended slinky. Imagine how this might look in the water with the pulses separated by their wavelength of 1 to 2 inches at 28000 to 56000 times per second.

Sound is a force that will lose it's intensity as the sound travels away from the source. Here is an illustration of that effect. Note the loss of color as the balloon expands.

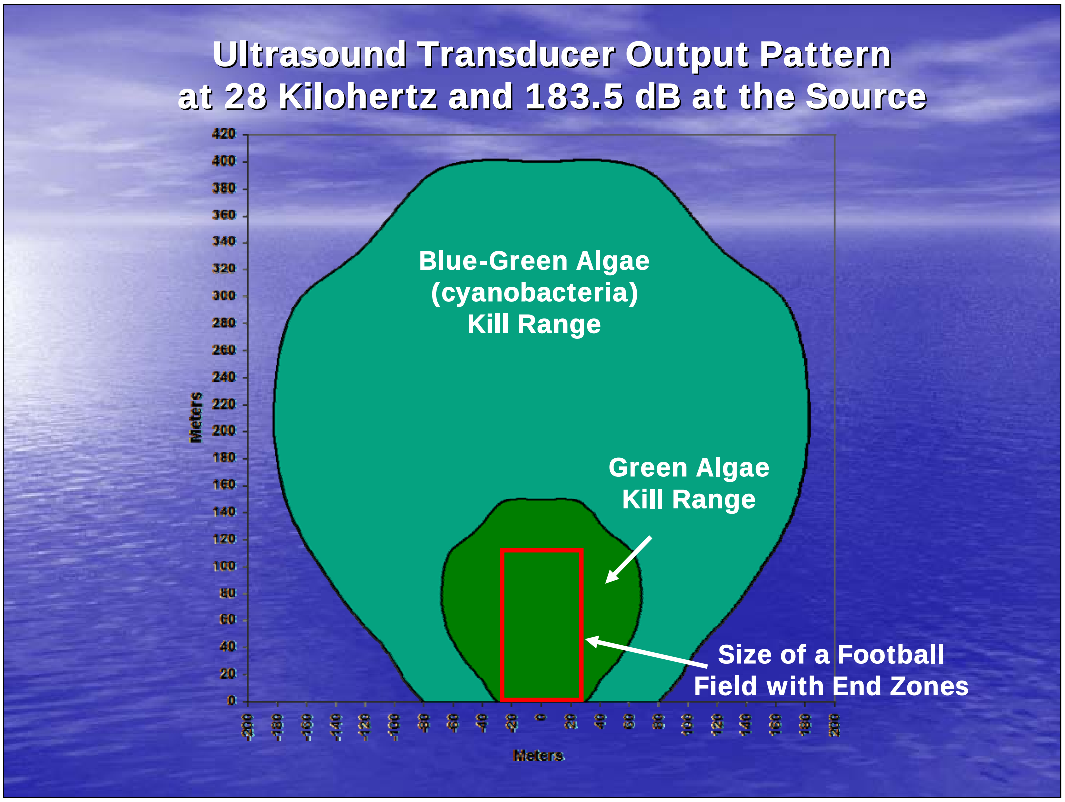
This illustration is from the Salford, UK school of acoustics. They have a very good course on sound basics that I would highly recommend. The link is shown on the slide.



Sound is a force that will lose it's intensity as the sound travels away from the source. Here is an illustration of that effect in terms of the algae type kill zones.

Sound loses dB level at 20*Log10(Distance from source in Meters) with initial reference point at 1 meter from the source. So log10(1) = 0, log10(10)=1, log10(100)=2, log10(1000)=3, etc.

So at 10 meters, the dB level will drop about 20. At 100 meters it will drop about 40. At 1000 meters it will drop by 60 dB.



So what does the sound intensity profile look like? Here is a bird's eye view of a transducer output. The technology is similar to a transducer you would mount under your boat for a fish finder or depth gauge. The pattern shown here is at 28 kilohertz. The shape narrows somewhat at higher frequencies. The red rectangle is the size of a football field with the end zones to give the drawing some perspective.

Here is one of the first ponds where we tested the technology. It still looks like that today.

Who Discovered This?

Over 80 years ago, French scientist Paul Langevin (1872-1946) discovered by accident that ultrasonic sound waves killed algae while he was developing submarine sonar.

He saw that micro-organisms like algae cells hit by the powerful ultrasonic waves died. The powerful transducers used at the time created an effect called cavitation that caused the algae to die.

The early pioneer in this technology was Paul Langevin a famous French scientist and is considered the father of underwater sonar technology. He noted that algae were killed in the path of his experimental ultrasound transducers in the early 1920’s. His inventions to creating ultrasonic pulses using piezo crystal technology are the basis for most designs today.

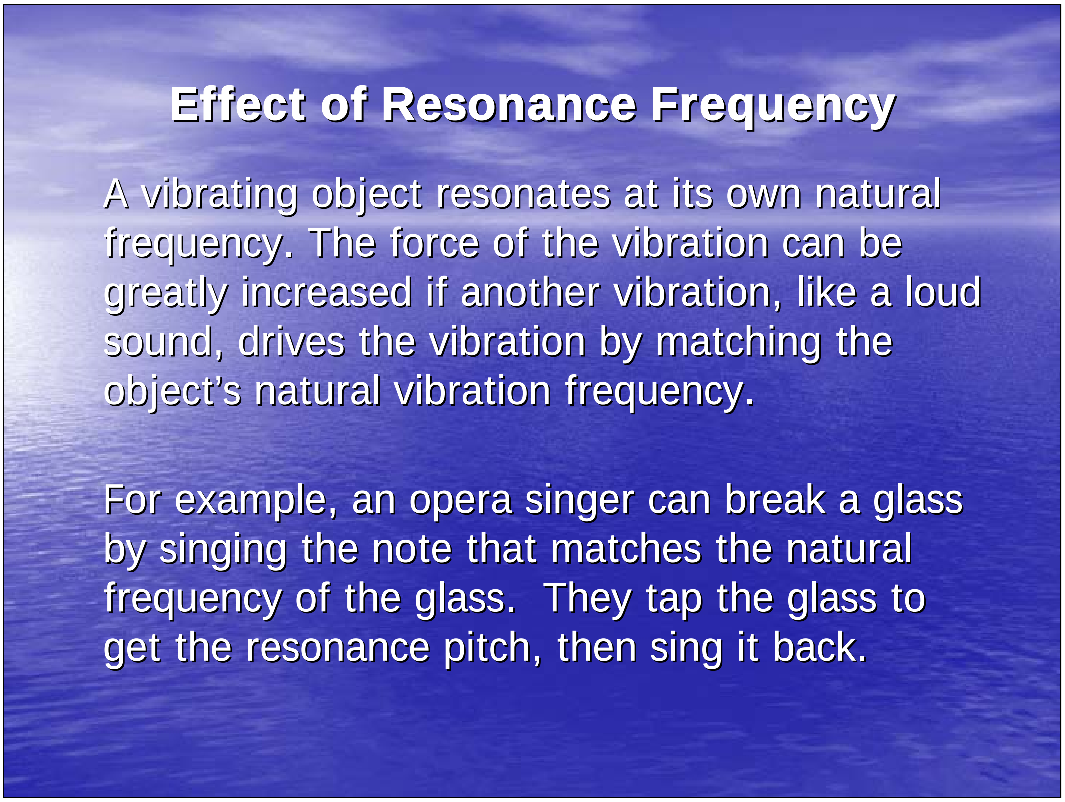
The New Discovery: Ultrasound Can Kill Algae Without Cavitation

Most ultrasonic algae control devices do not produce appreciable levels of cavitation at the piezo head. It was discovered that certain ultrasonic sound vibrations in water, match **critical resonance frequencies** of algae gas vesicles, vacuoles and plasmalemma cell linings.

Exposure to these sounds cause the cell membranes of these critical life functions to break or tear. Once torn, the life cycle of the algae cell is broken and it dies.

So for many years it was assumed that you needed a cavitational ultrasonic sound source to kill algae. These devices require about 150 to 400 times more power than low intensity ultrasonic devices. Also the cavitation effect is only produced in close proximity to the transducer. So it was assumed that the technology would not be useful for large water systems because of the power required and the limited range of the cavitation effect.

Low intensity ultrasound affects the algae gas vesicles and plasmalemma cell linings. The cyanobacteria have gas vesicles that are broken by the vibrational resonance with the sound. Green algae do not have these vesicles, but their contractual vacuole connected with the function of the plasmalemma are damaged preventing the algae to get fluids, nutrients and to control it’s internal pressure. Without these functions, these single celled algae die.



How many of you remember the "Is it live or is it Memorex?" commercial? Most kids today don't even know what a cassette tape is, let alone an 8-track. But I'm dating myself.

A vibrating object resonates at its own natural frequency. The force of the vibration can be greatly increased if another vibration, like a loud sound, drives the vibration by matching the object's natural vibration frequency.

For example, an opera singer can break a glass by singing the note that matches the natural frequency of the glass. They tap the glass to get the resonance pitch, then sing it back.

Have you ever had a tire that would vibrate at one speed, but if you were going slower or faster it would not? The rotation at that speed matched the natural vibration frequency of the tire or it's critical frequency. This is an important function to understand in rotating equipment.

The next slide shows a crystal glass beginning to resonate at its critical frequency.

Crystal Glass Reaching Critical Resonance

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Here is a glass being taken to its critical frequency by a speaker. The video is in slow motion.

Crystal Glass Reaching Critical Resonance

Used by permission: www.acoustics.salford.ac.uk

Here is the same glass as seen from above being taken to its critical frequency by a speaker. Again, the video is in slow motion. Most of these vibrations happen so quickly that you can't really see them with the naked eye in real time.

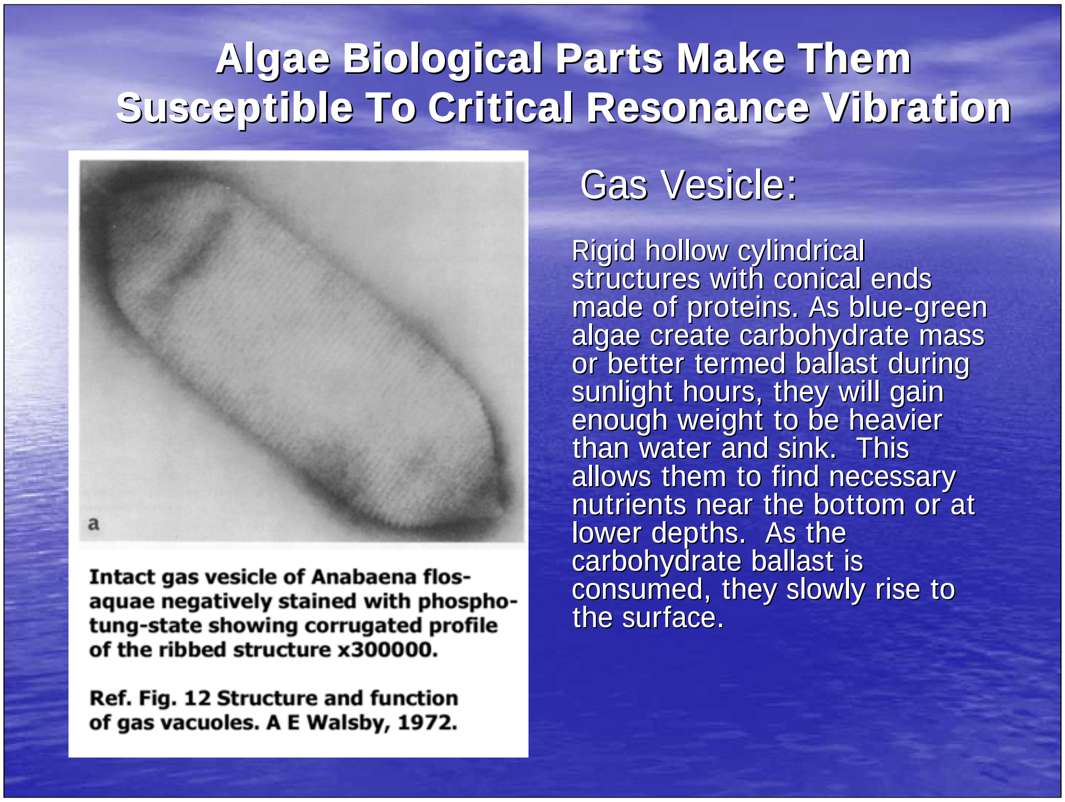
Algae Biological Parts Make Them Susceptible To Critical Resonance Vibration

Blue-green Algae (cyanobacteria) Gas Vacuole:

A cellular organelle with many tiny cylindrical vesicles about 75 x 300 nm in size, often in clusters. The wall of the gas vacuole, which is permeable to gases but not to water and is about 2 nm thick. Gas vacuoles are found mainly in planktonic cyanobacteria and their primary purpose is to make them buoyant. Some fungi and archaea bacteria have gas vesicles and will be affected.

http://www.biology-online.org/

Algae parts make them susceptible to vibrational damage when vibrated at their critical resonance frequency. The easiest types to kill are the blue-green types that are more a bacteria than an algae and generally known as cyanobacteria. Its gas vacuole contains many tiny cylindrical vesicles about 75 x 300 nm in size, often in clusters. The wall of the gas vacuole, which is permeable to gases but not to water and is about 2 nm thick. Gas vacuoles are found mainly in planktonic cyanobacteria and their primary purpose is to make them float or sink. Some fungi and archaea bacteria have gas vesicles and will also be affected by ultrasound.



Here is an example of the a gas vesicle a tiny hollow structure like a cylinder with a cone shape on its ends. The blue-green algae use these to change their buoyancy to rise and sink as needed for light and nutrients. When they are broken, its life functions begin to stop because of lack of nutrients and sunlight. They cannot regenerate the gas vesicles as fast as the ultrasound can break them. Note the magnification at 300000. They are very small devices. Dr. Walsby was able to get this picture in 1972 and realized from his work that they may be the key to controlling these algae.

Algae Gas Vesicle Rigidity Makes Them Susceptible To Critical Resonance Vibration

Gas vesicles are the exception to the rule that all bacterial cells have one contiguous membrane. Gas vesicles are found in *Cyanobacteria*, which are photosynthetic and live in aquatic systems. In these lakes and oceans, the *blue-green algae* control their position in the water column to obtain the optimum amount of light and nutrients.

Ref: http://lecturer.ukdw.ac.id/dhira/BacterialStructure/Inclusions.html

Note how the stacked configuration adds some rigidity to this area of the algae cell. Gas vesicles are the exception to the rule that all bacterial cells have one contiguous membrane.

Blue-Green Algae Can Have Thousands of Gas Vesicles

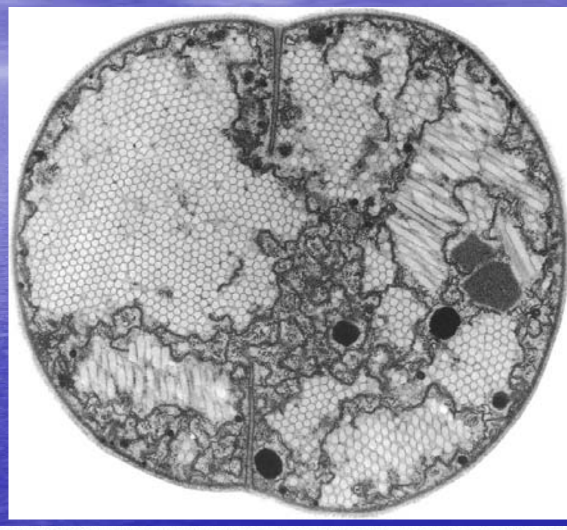
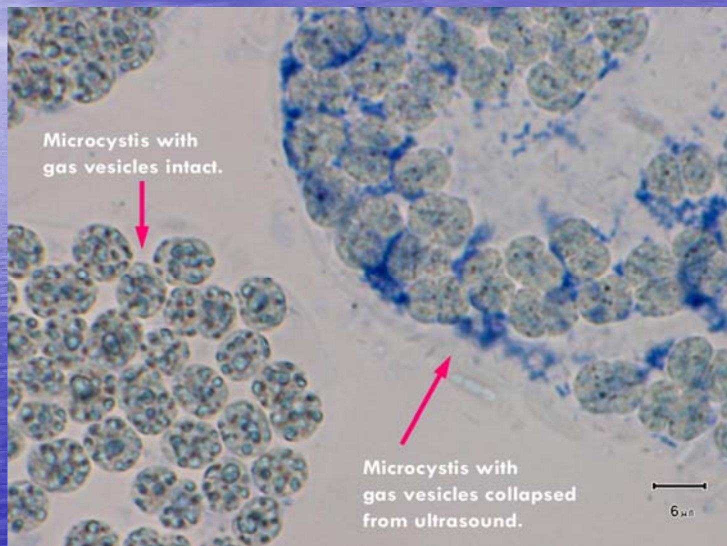


FIG. 1. Transverse section of a dividing cell of the cyanobacterium *Microcystis sp.* showing hexagonal stacking of the cylindrical gas vesicles. (Micrograph by H. S. Pankratz.) Magnification, x31,500.

Ref: "Gas Vesicles", Anthony E. Walsby, Microbiological Reviews, March, 1994

There are many of these structures in a cell. Here is a Microcystis cell in the process of dividing. Note the stacked structure of these gas vesicles.

Blue-Green Algae With Ultrasonically Damaged Gas Vesicles – Before / After



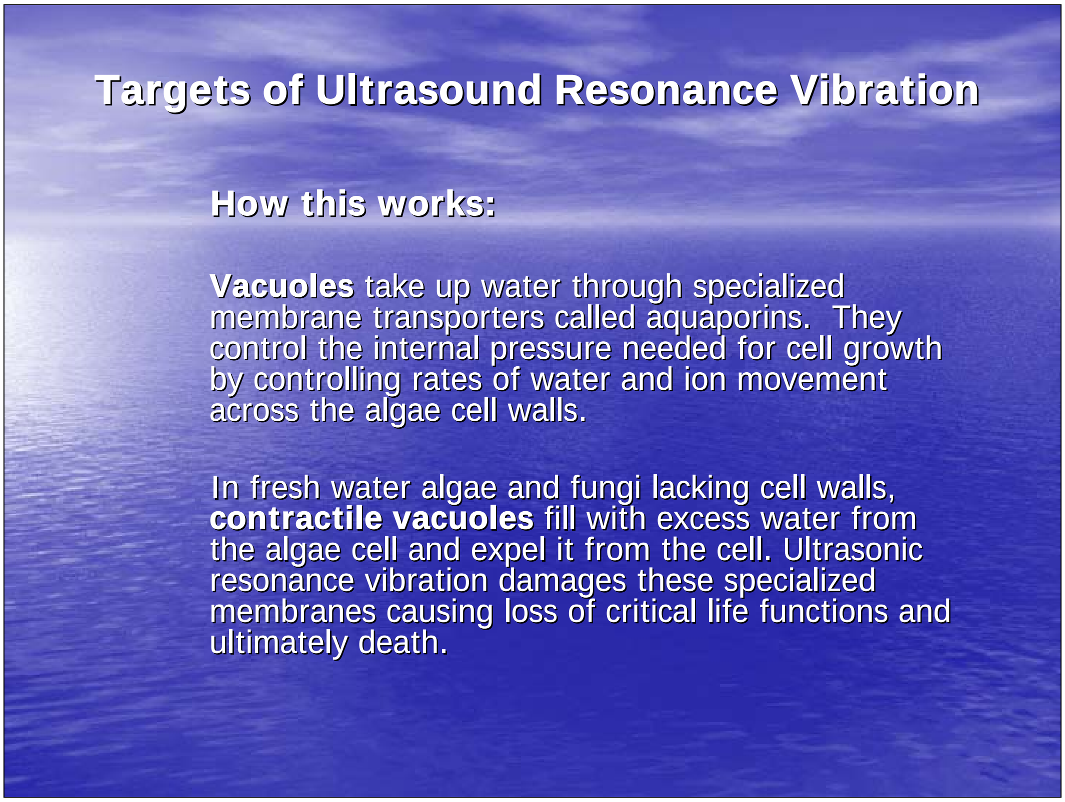
These pictures show how Microcystis cells look under a light microscope before and after the gas vesicle structures are broken. The individual gas vesicles are too small to be seen individually. However the intact gas vesicles give the cell a shiny appearance while the broken ones look optically flat.

Other Targets of Ultrasound Resonance Vibration

Contractile vacuoles are osmoregulatory organelles on the algae outer sheath surface and allow water and nutrients to flow into and out of the cell through specialized membrane transporters called aquaporins.

They are connected to the plasmalemma or inner cell wall that lies beneath the outer sheath. The ultrasound causes the plasmalemma to detach from the outer wall and the contractile vacuole. When this occurs, the internal cell begins to shrink as it can no longer control its internal pressure, receive nutrients, expel waste, or protect itself from external bacterial attack.

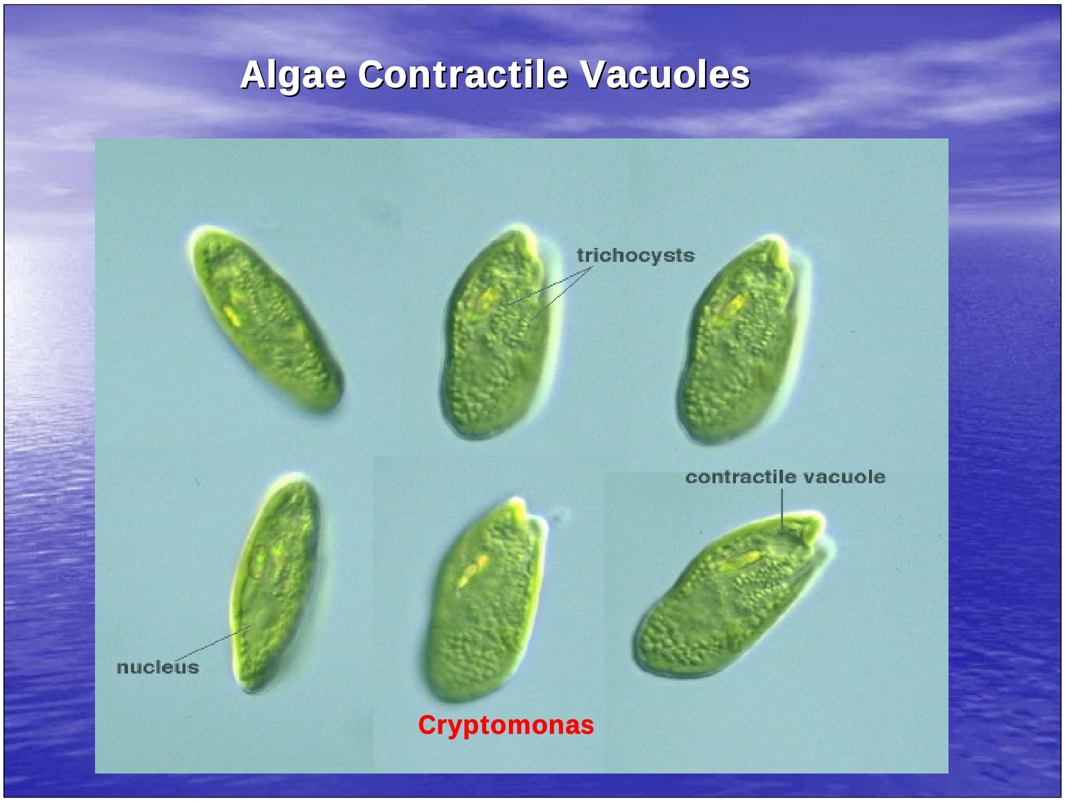
Green algae can also be targeted, but the range of effectiveness is much smaller than for the blue-green types. In this case, the contractile vacuoles connected with the plasmalemma are the primary target of the low intensity ultrasound. These parts are slower to break or tear than the gas vesicles. It can sometimes take more than a week of exposure to kill or render the algae cell unviable. When broken, the cells quickly become unviable and cannot repair themselves.



How this works:

Vacuoles take up water through specialized membrane transporters called **aquaporins**. They control the internal pressure needed for cell growth by controlling rates of water and ion movement across the algae cell walls.

In fresh water algae and fungi lacking cell walls, **contractile vacuoles** fill with excess water from the algae cell and expel it from the cell. Ultrasonic resonance vibration damages these specialized membranes causing loss of critical life functions and ultimately death.



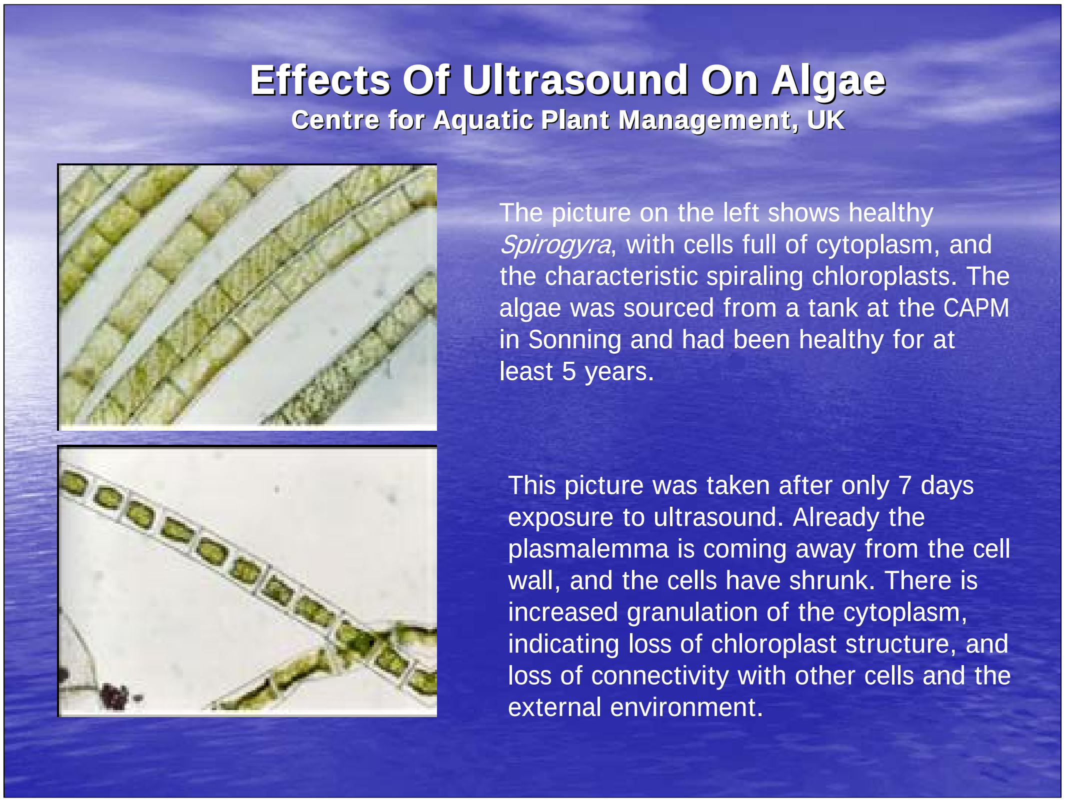
Here is a typical examples of a contractile vacuole in Cryptomonas algae. The dimpled area is the water/nutrient flow area and is attached to the plasmalemma. Once detached internally, the cell will die.

Effects Of Ultrasound On Algae

Centre for Aquatic Plant Management, UK

The following pictures of *Spirogyra* were taken over a three week period from a tank experiment done in controlled glasshouse conditions. The mode of action appears to be by disruption of the connections between the plasmalemma and the algal cell walls causing loss of membrane integrity, probable leakage of cytoplasm and a collapse of the cell into a dense brown mass. The cells remain buoyant for at least 4-5 weeks after exposure, although they are no longer viable.

The following pictures are of *Spirogyra, a* filamentous green algae taken over a three week period from a tank experiment done in controlled glasshouse conditions. They noted that the disruption of the connections between the plasmalemma and the algal cell walls caused a loss of membrane integrity, probable leakage of cytoplasm and a collapse of the cell into a dense brown mass. The cells remain buoyant for at least 4-5 weeks after exposure, although they are no longer viable. Some of the buoyancy comes from bacterial gases collecting in the cells as the bacteria invade the cell and begin consuming the dying material inside.



The top left picture shows healthy *Spirogyra*, with cells full of cytoplasm, and the characteristic spiraling chloroplasts. The algae was sourced from a tank at the CAPM in Sonning and had been healthy for at least 5 years.

The lower picture was taken after only 7 days exposure to ultrasound. Already the plasmalemma is coming away from the cell wall, and the cells have shrunk. The granulation of the cytoplasm, indicates loss of chloroplast structure, and loss of connectivity with other cells and the external environment. In essence, it is already beginning to die.

Effects Of Ultrasound On Algae

Centre for Aquatic Plant Management, UK

This picture was taken after 14 days exposure. The cells have continued to shrink, with some forming denser circular brown agglomerations in the center of the cell. There is some evidence of cytoplasm leakage from the cells, indicating further damage to the cell walls.

The picture on the right was taken after 21 days, and shows complete breakdown of cell structure.

After 14 days exposure, the cells have continued to shrink, with some forming a denser circular brown mass in the center of the cell. There is some evidence of cytoplasm leakage from the cells, indicating further damage to the cell walls.

The bottom right picture shows the effect after 21 days that caused complete breakdown of cell structure.

Effects Of Ultrasound On Algae

"In summary, exposure of *Spirogyra* and *Selenastrum* to ultrasound waves causes irreversible structural damage to the cells, loss of chlorophyll and loss of viability. "

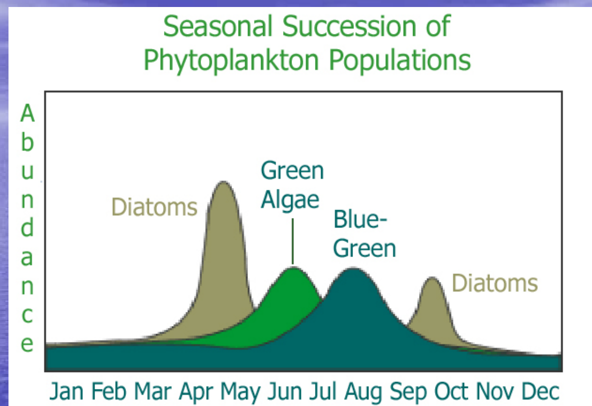
Ref. CAPM 2003 Annual Report

AlgaeControl.US reached similar conclusions based on ponds and waterways treated with ultrasonic technology.

Microscopic results recorded by AlgaeControl.US during ultrasonic treatment test work and performance reviews.

Summary confirmed by our own observations.

Seasonal Shift in Algal Population



http://www.waterontheweb.org

Current ultrasonic effective kill range:

Diatoms and Green algae:	150 meters
Blue-green or cyanobacteria:	400 meters

Seasonal succession of algae types. Kill range noted.

Ultrasound Side Benefit: Biofilm Impact

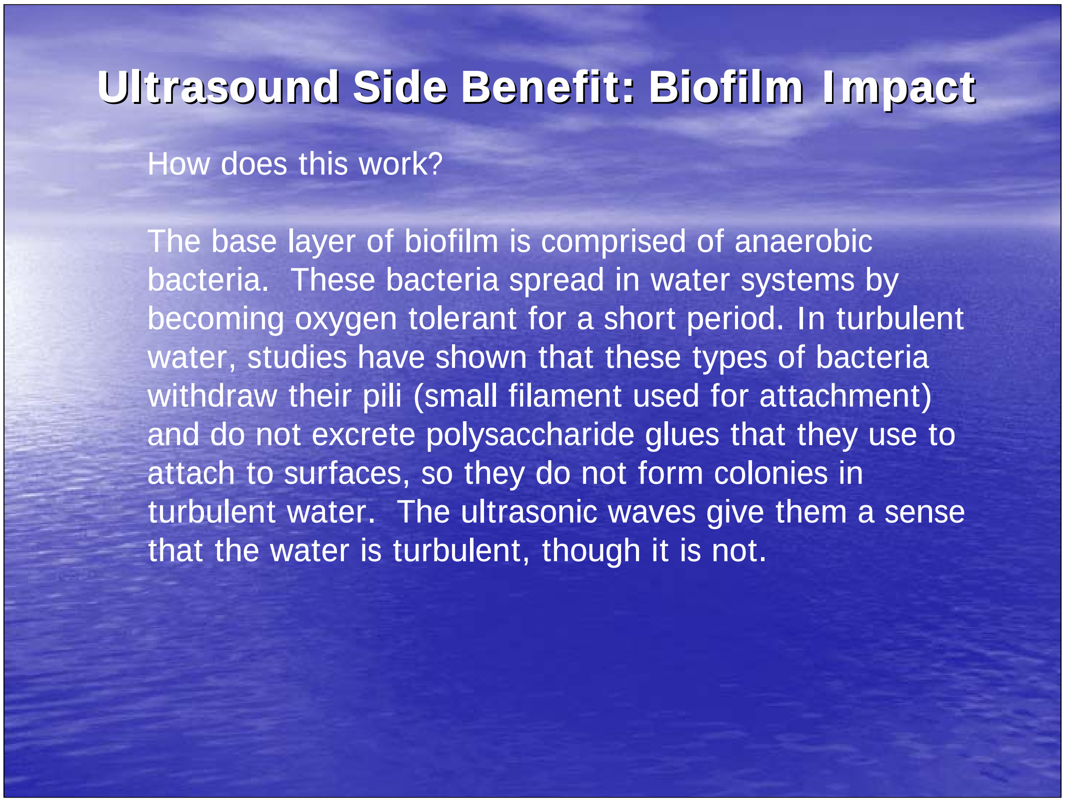
Biofilm formation, made primarily from initial bacterial layers and then colonized by algae and other organisms, is greatly reduced in the presence of ultrasonic waves.

In cooling towers, interruption of the biofilm colony by ultrasound has been shown to reduce Legionella count.

Numerous water facilities have reported a stark drop in biofilm formation after ultrasound is introduced.

Low intensity ultrasound with enough exposure time can kill coccoid bacteria. Tests have shown that biofilm formation, made primarily from initial bacterial layers and then colonized by algae and other organisms, is greatly reduced in the presence of ultrasonic waves. Starting with a clean surface, biofilm growth will be substantially limited in the ultrasonic field and what does grow will be lightly attached and easily removed.

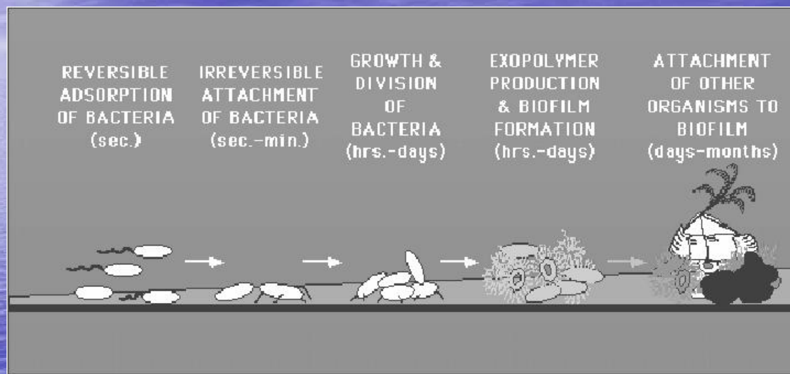
In cooling towers, interruption of the biofilm colony by ultrasound has been shown to reduce Legionella count.



How does this work?

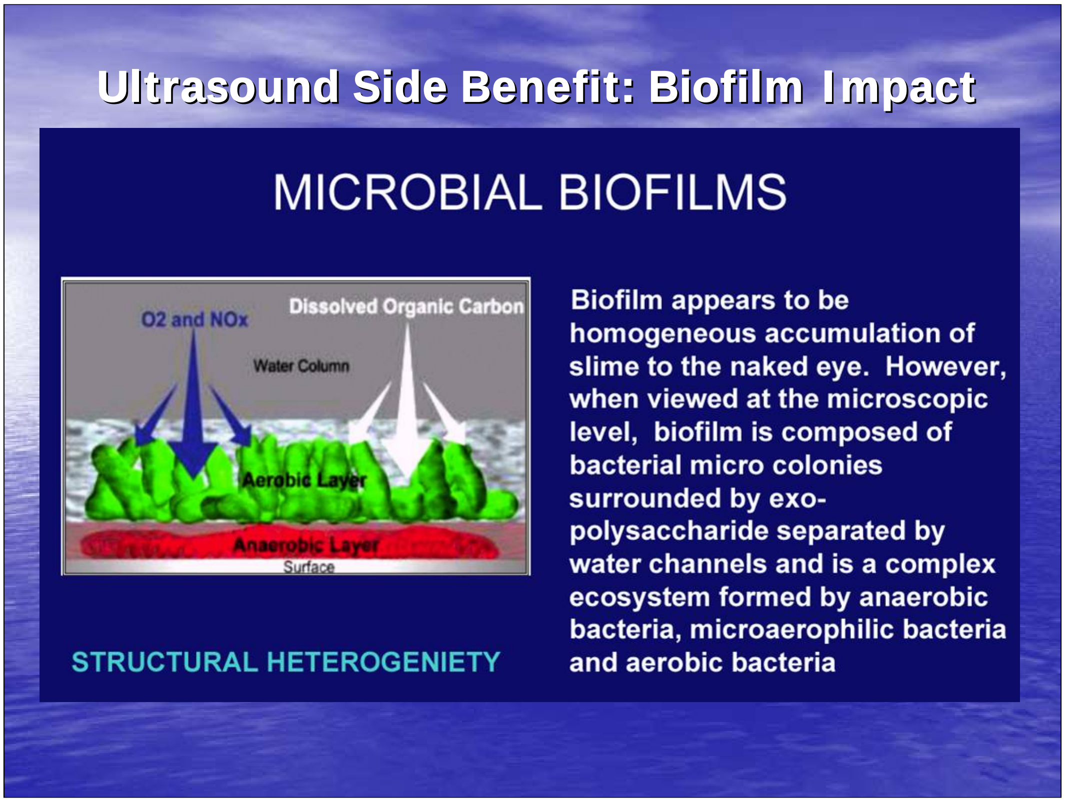
Our working theory is that the ultrasonic waves give the bacteria a sense that the water is turbulent, though it is not. In turbulent water, studies have shown that bacteria withdraw their pili (small filament used for attachment) and do not excrete polysaccharide glues that they use to attach to surfaces. Recent studies with the ultrasonic devices has shown that coccoid bacteria in controlled experiments were reduced in count by 60% in 4 days.

Ultrasound Side Benefit: Biofilm Impact



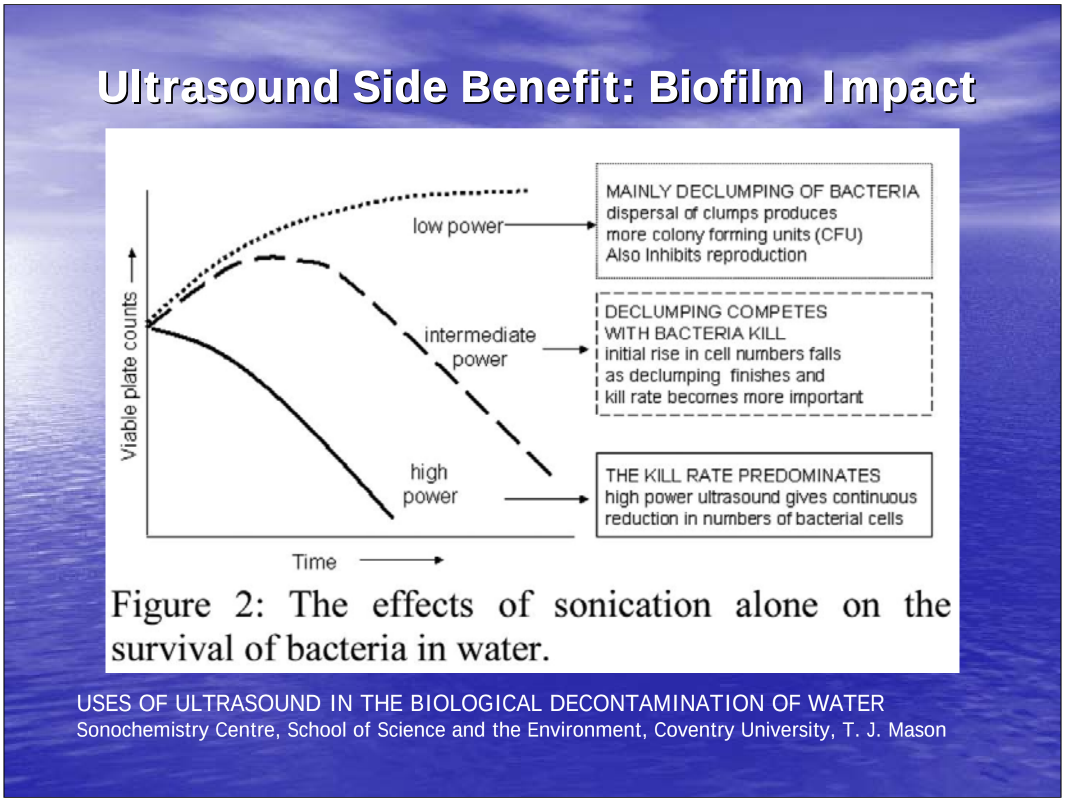
Ultrasound keeps the bacteria in the reversible absorption stage by producing a sensation of turbulence.

Initial anaerobic colony formation occurs quickly, sometimes in a matter of seconds. It can become irreversible in a matter of minutes. After days, the biofilm forms after sufficient exopolymer has been deposited. Algae and other organisms join the colony for food, nutrients and protection.



Biofilm is a complex accumulation of different types of bacteria, algae, and higher order organisms. The most important layer is the layer connected to the surface and it primarily made up of anaerobic bacteria. They attract the aerobic and aerophilic bacteria by excreting a chemical that attracts them to the colony. This phenomenon is sometimes known as quorum sensing. Once the aerobic bacteria have coated over the anaerobic layer, they are then shield from oxygen in the water.

The anaerobic layer will slough off new colony forming units that are oxygen tolerant, but do not reproduce until they find a suitable surface to colonize. From our experience, this reproduction inhibition lasts for a short period, usually long enough to prevent colonization during the transition from one tank or clarifier to the next.



Various power levels of ultrasound can affect bacteria differently. Low power can keep the cells dispersed and in a non-reproductive state. Power level increases in ultrasonic output can also cause order of magnitude changes in the cost of the device.

Ultrasound Side Benefit: Biofilm Impact

Will it remove existing biofilm?

Typically, existing biofilm will brown out on the edges, but will not always completely go away.

Again, mother nature has provided bacteria a means of “quorum sensing”, so an existing colony will give out a chemical that tells other bacteria of like genetics, that a compatible colony exists. Despite the ultrasonic vibrations, new bacteria will attach.

For best results, starting with a mechanically or chemically cleaned surface will prevent reattachment of strongly attached biofilm.

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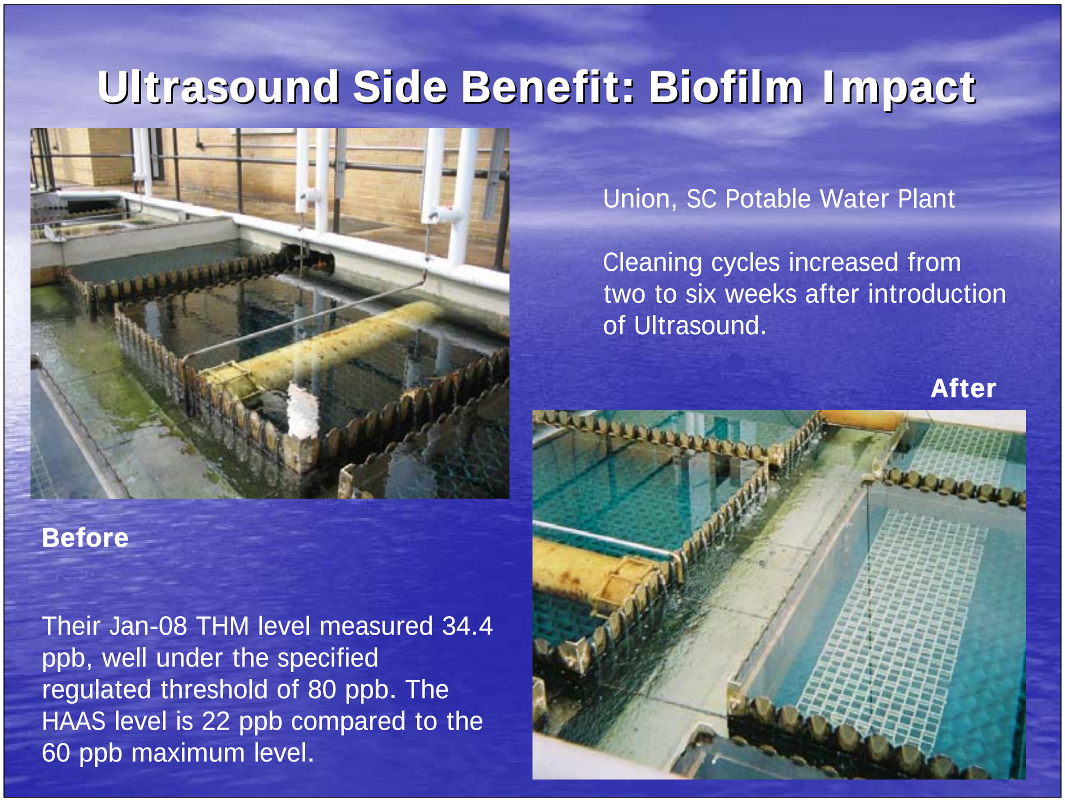
Pressure washing or use of a chemical bio-removing agent (eg. Floran Fit) will help remove the biofilm from the surface.



Biofilm growth was substantially reduced at Union, SC Potable Water Plant allowing the plant to maintain its normal cleaning cycle after hypochlorite addition was halted. Cleaning is now easier and a 50% reduction in hypochlorite was recorded at the plant. THM and HAAS levels are well within EPA health requirements.

USDA Ultrasound Test Work Underway

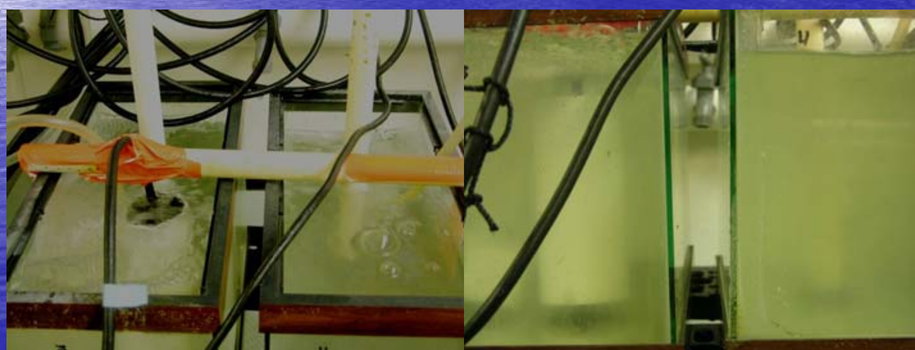
Catfish “off-flavor” results from many chemical compounds produced by algae. Here is one of the test sites under study by the USDA SE Regional Aquaculture Div. in Stoneville, MS. Microcystis and anabaena blooms were controlled vs. side-by-side tests with untreated ponds. Now a new tool is available to prevent that earthy, musty taste in the catfish.

USDA Ultrasound Test Work Underway

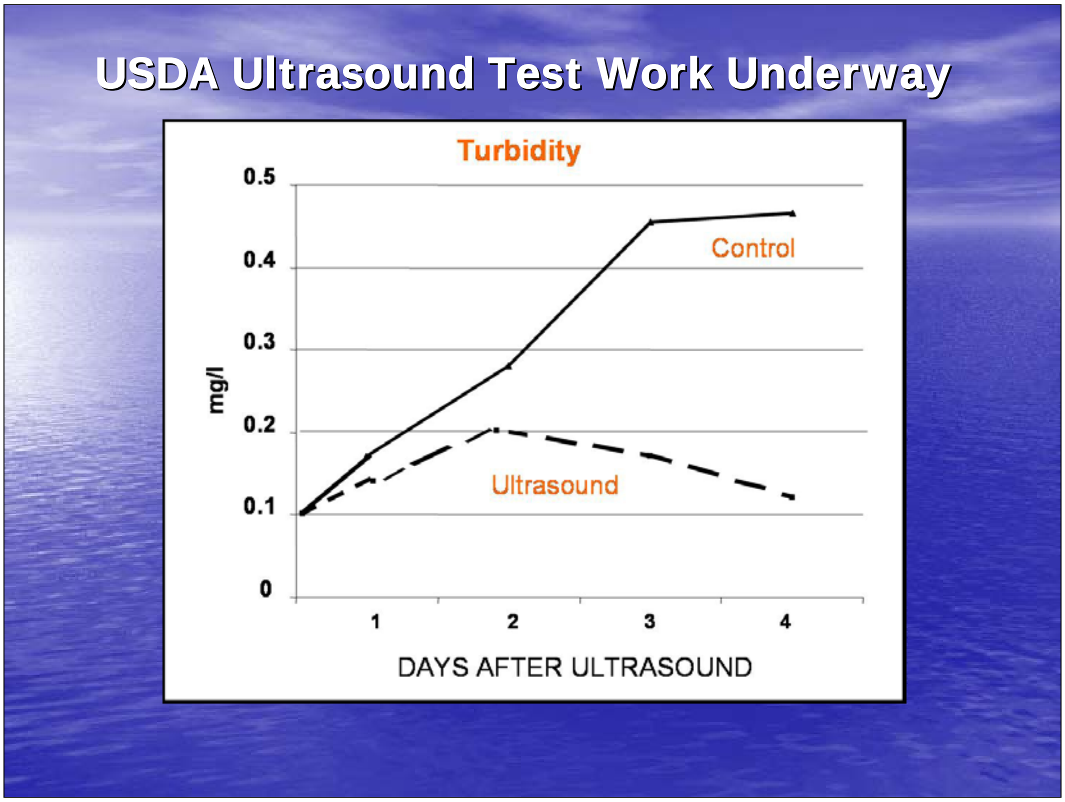
Question: What effect does ultrasound have on bacterial abundance and composition?

Design: 8-replicate 8L tanks filled with pond-water. Tanks were then "seeded" with fungi, ESC and columnaris cultures (1x10E4 addition). Ultrasound probes were added to 4 random tanks and samples were collected daily for PCR and microscopic counts. Plate counts were also made on the final day.

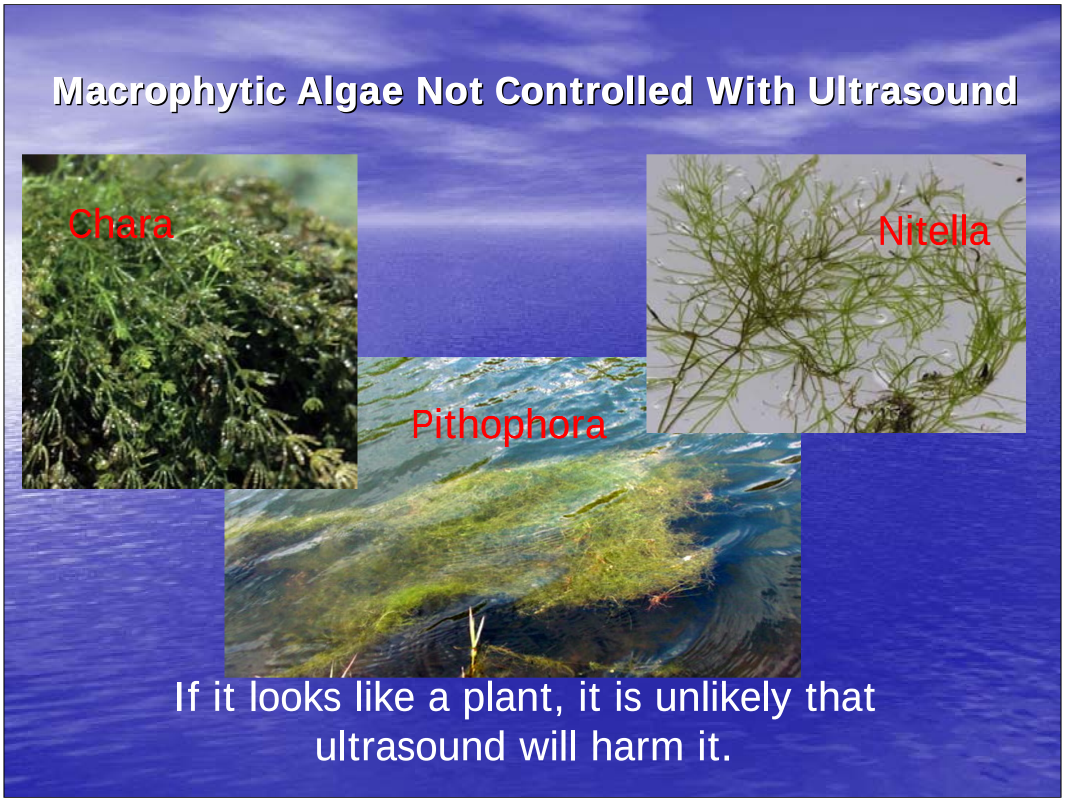
RESULTS: Total bacteria declined by 60% within 4-day treatment. Other results in progress.



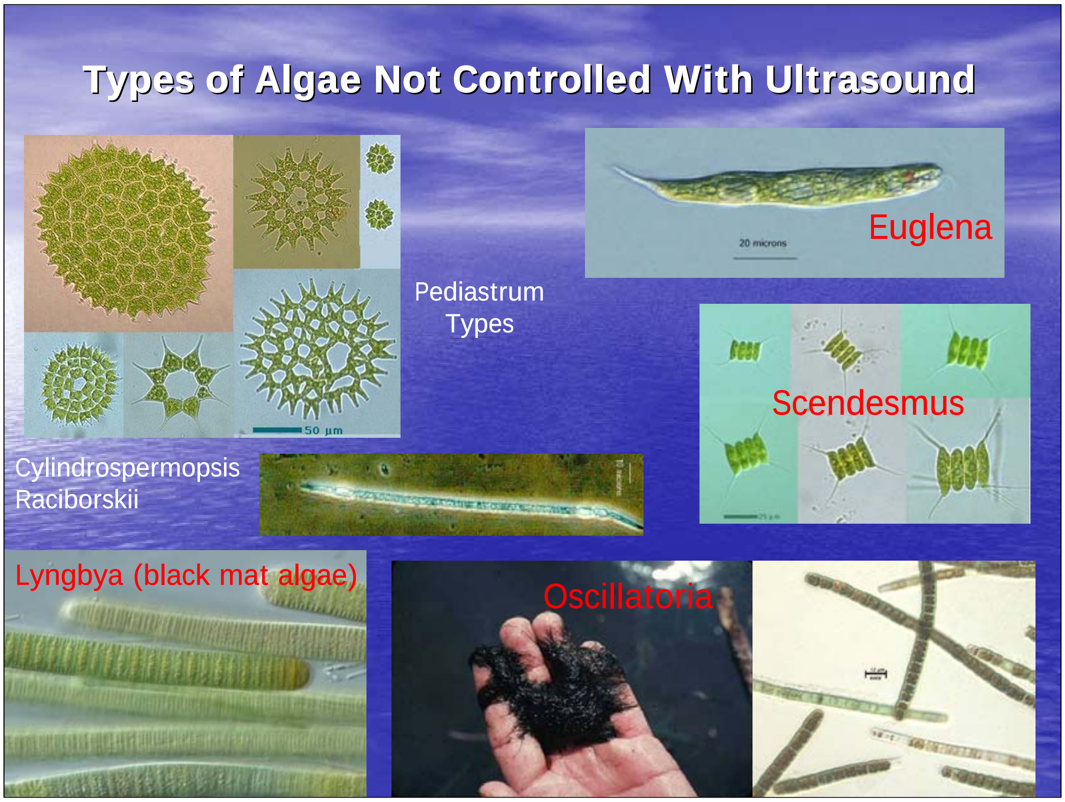
Ultrasound side-by-side controlled experiments show algae and bacteria reductions.



Turbidity levels were greatly reduced in the ultrasound treated samples versus the control samples.



Sorry, the technology does not work on macrophytic algae. If it looks like a plant then it is unlikely that ultrasound will harm it.



Some other types of algae are also resistant such as Euglena, Pediastrum, Scendesmus, Cylindrospermopsis Raciborskii and Oscillatoria. Out of 27000 to 36000 different species, that's not a bad average.


Advantages of Ultrasonic Algae Control
100% environmentally friendly.
No generation of by-products.
No genetic mutation of survivors.
Simple to install.
Very little maintenance is required.
No chemicals or additives needed.
Very low energy consumption.
Relatively inexpensive to purchase.
Large water bodies are no problem.
Wall biofilm where significant quantities of bacteria are produced can be significantly reduced or eliminated.

Advantages.

Disadvantages of Ultrasonic Algae Control

- Macrophytic or plantlike algae are more difficult to eliminate or are unaffected.
- Thick plants or these plantlike macrophytic algae structures can prevent ultrasound vibration to reach other parts of the water to be treated.
- A “line of sight” to the algae must be available for the transducer output to be effective.
- A few algae types will not be affected by ultrasound (euglenoids, oscillatoria, scendesmus, macrophytic, and some colonical types like pediastrum are resistant).
- Patience is required as weeks are sometimes needed for full effectiveness.

Disadvantages.

Summary:

- Ultrasonic algae control is now a viable and cost effective way to control most nuisance algae types.
- This technology offers an environmentally friendly approach, eliminating many chemicals and chemical handling issues.
- Other than a few fungi that have gas vesicles and certain bacteria that will stop propagating in the near field zone, the ultrasound technology using resonance frequency has not been shown to affect life forms other than algae.

Summary.

Questions?