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Quantification of an Ultrasound Test Apparatus and its Potential Use to Control Nuisance Species in Commercial Aquaculture Settings

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QUANTIFICATION OF AN ULTRASOUND TEST APPARATUS AND ITS
POTENTIAL USE TO CONTROL NUISANCE SPECIES IN COMMERCIAL
AQUACULTURE SETTINGS

A Thesis
Presented in partial fulfillment for the
Master of Science
Degree
Through the Department of Physics and Astronomy
The University of Mississippi

Bradley T. Goodwiller
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ABSTRACT

As one step in the investigation of using acoustics to improve aquaculture production, work was pursued on the possible use of ultrasound to control the *Bolbophorus* trematode in commercial catfish ponds. The trematode population can be controlled by eliminating the host ram’s horn snail via exposure to high amplitude ultrasound. Initial laboratory tests indicated that a commercially available sonicator (operating at 20 kHz) is capable of killing individual snails in fish tanks. More thorough testing indicated efficiency rates of approximately 35% on batches of 10 snails. In addition to the snails killed immediately, there was evidence that the sonication technique caused mortal wounds that caused significant death a few days after the tests. The experimental setup of these initial tests provided nearly 20 dB of gain in sound levels compared to what is expected in ponds due to reverberation from the air surrounding the tank walls. Tests were run in an anechoic environment to mimic pond absorption and showed lower efficacy rates, ranging from 0% at short durations to 25% at 90 seconds. Several transducers operating between 80-500 kHz were built and calibrated to provide alternate driving frequencies but could not provide enough power to be of any benefit. The work presented here constitutes the basic research and proof of concept behind the design and development of a field deployable system capable of killing a significant percentage of a snail population.
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I. INTRODUCTION

Aquaculture, the farming of aquatic species, is a major industry in the state of Mississippi with many ancillary areas of research aimed at increasing the productivity, efficiency and environmental friendliness of the aquaculture processes. Acoustics may be implemented in several different areas of aquaculture, ranging from ultrasonic control of algae\(^1\) to improving the harvesting process of catfish farming, a $199 million dollar industry in 2010\(^2\). In cooperation with NWAC (The Thad Cochran National Warm-water Aquaculture Center in Stoneville, MS,) The National Center for Physical Acoustics (NCPA) has done work developing acoustic technologies to improve catfish production.

One application of interest is the use of audible sound to move the fish at harvest time in order to improve harvesting efficiency. Work has been done with various species of fish documenting their ability to hear and respond to sound. A group of rainbow trout in a pond were trained to come to a feeding site using a 150 Hz tone\(^3\). Smith et. al\(^4\) showed that after being trained with sound and feeding coincident in time, a group of loricariid catfish responded to sound alone without feed being present. Also under review is the use of ultrasound to aid in the delivery of vaccines into fish\(^5\). The two most common current methods are injecting individual fish, and adding the vaccine to the pond and allowing the fish to absorb it. These methods are clumsy, inefficient and unreliable. Work has been done using ultrasound to deliver drugs into grouper\(^6\), but no work to date has been done on the channel catfish, the species of interest in Mississippi aquaculture.
A third potential use of acoustics is the eradication of the parasitic trematode *Bolbophorus* by means of eliminating the intermediate host the ram’s horn snail. These trematodes have in recent years become a notable problem for U.S. catfish farmers, particularly in the south-east region of the country. When the trematodes infect fish, they form cysts under the skin and cause the fish to become lethargic. In ponds of small fish any trematode infestation is detrimental, killing large percentages of the total stock. Larger food-sized catfish are not as mortally affected by the trematodes, but they do suffer from lethargy. Since profitability in the catfish industry is based on biomass, a study was conducted by David Wise et. al\(^7\) to investigate the financial implications of trematode infestations. Several ponds with varying degrees of infestation were investigated. The ponds with medium and severe infestations (33%-66%, > 66% respectively) were shown to produce a loss to the farmers, while ponds with light infestations (< 33%) were shown to barely cover the costs of operation. Figure 1 shows a summary of these results. Even though the food-size catfish do not die, the trematodes pose a serious financial threat to the catfish industry and must be dealt with as efficiently as possible.

![Figure 1: Study showing the financial losses due to trematode infestation](image_url)
The trematodes’ life cycle consists of three phases. The cycle begins when trematode eggs are deposited into a pond by the American White Pelican. The eggs hatch and the trematode begins its second phase, which requires the host the Ram’s Horn snail. Once the trematodes mature to the third stage of their life cycle they require a fish host. The cycle is completed when infected fish are eaten by the pelicans. In order to deal with the parasites, it is most convenient to break the life cycle chain.

The American White Pelicans are a nationally protected bird meaning that there are very strict guidelines making it difficult to contain or control them. This makes the first phase of the trematodes’ cycle difficult to alter. Without an ample supply of the intermediate host, the Ram’s Horn snail, the trematodes will die out and will not mature to the third stage of their life cycle. Since the snails are an invasive species, controlling the snail population serves two purposes and is therefore the main focus of the current attempts to eliminate the trematode threat. Several methods of eliminating snails have been designed and tested, the most effective of which is the application of a solution of copper sulfate and citric acid to the borders of the pond where the snails live.

This chemical treatment is not a perfect solution to the trematode problem. The chemicals necessary are expensive and the solution must be applied seasonally as the snails repopulate. In addition, sufficient concentration of the copper sulfate is toxic to the fish. The solution is applied to the borders of the pond in an attempt to avoid direct contact with the fish. A pond cannot be treated on a windy day, as the movement of the water carries the chemical away from the shore and into contact with the fish. Any pond less than seven acres may not be treated with this solution, as the overall concentration would exceed safe levels and poison the entire pond.
Acoustics may provide an alternate mechanical solution to the problem. Exposing the snails to high amplitude ultrasound may be an effective way to kill the snails. When a high frequency, high amplitude acoustic signal is passed through non-degassed water, the micro-bubbles within the water begin to resonate; those of a certain size (based on the frequency of the sound)\textsuperscript{11} begin to grow preferentially. As they continue to resonate they eventually become unstable and collapse violently\textsuperscript{12}. Upon collapse they create shockwaves into the water; if they collapse near a surface, they do so asymmetrically and produce a high speed water jet that can damage the nearby surface (see Figure 2). While it is known that high amplitude ultrasound is capable of causing damage to the surface of metals, the mechanism of this damage is debated. Prabowo and Ohl\textsuperscript{13} demonstrated that at 16.27 kHz, bubbles attached to surfaces do indeed create inward water jets, but that the jets were not powerful enough to cause damage to the surface.

Whether by water jet, shockwave or unknown phenomenon, cavitation may be able to crack the shells of the Ram’s Horn snail. With their shells cracked, the snails would lose their ability to float to the surface for air, and would thus die.

Another possible mechanism of killing the snails involves the vibratory motion of high amplitude pressure waves passing through the snails. By shaking the snails sufficiently hard on a scale comparable to their physical size, it is possible that the snails may simply hemorrhage and die.
Figure 2: Liquid jet production during the collapse of a pulsating bubble driven at 60Hz\textsuperscript{14}
II. PROOF OF CONCEPT

A test batch of approximately 150 snails was collected from some of the catfish ponds at the facility in Stoneville. Pond water was collected with the snails in order to maintain conditions in the field. The snails were transported to temperature controlled fish tanks at NCPA, filled with the pond water. A biology student was tasked with maintaining the snails as well as with finding the most effective method of determining whether a snail is alive or dead. Snails have a vital response to external stimuli to their muscle. The muscles of several snails (known to be alive) were ‘poked’ and the response to the prick was noticeable. When stimulated, the muscle flexes and retracts toward the back of its shell. This prick test was the primary metric used throughout this research.

A proof of concept experiment was designed using a 20 kHz Branson Sonicator [see Appendix II] as the acoustic source. An extra fish tank was filled with pond water and a plastic cylinder was placed in the center in order to constrain the test snail. The sonicator was submerged in the center of the cylinder as shown in Figure 3. The sonicator was turned on, and a snail was introduced into the container. Within a few seconds the snail sank to the bottom and was determined to be dead. This test was repeated several times with the same results.

Expanding upon this test, groups of ten snails were placed in the cylinder with the sonicator turned off. They were allowed to acclimate before the sonicator was activated. This test was run several times with various durations of exposure to the ultrasound. The results of this test are shown in Table 1.
It is important to note that no snails died during the 5 second exposure which seems to contradict the earlier tests. The only apparent difference between these two tests is that in the initial tests (where the snails died almost instantly) the snails were dropped into the tank with the sonicator already running. Since the scenario of the snails being acclimated and attached to the side is more pond-like, the snails were allowed to acclimate for all future tests. Also of note is that the
snails were constrained to be very close to the sonicator, within 4 cm. In the catfish ponds, the snails congregate on the banks, thus 4 cm is not unrealistic. As an observation, the snails did not appear to die due to broken shells as was an initial premise. There were no visible signs of holes or significant damage. However, the cavitation did clean the shells of several of the snails, as seen in Figure 4. Another immediately obvious effect of the sonicator was the pattern of bubble clouds created, as shown in Figure 5. There was a significant amount of gross water movement that may have caused internal injuries, contributing to the death of the snails.
These preliminary experiments demonstrated the potential for the sonicator to be a useful tool for controlling snail populations. However, these tests were very lab-specific. In order to develop an apparatus that will prove successful in a pond, the output of the sonicator and the optimum duration of exposure must be determined.
III. QUANTIFICATION OF THE TEST APPARATUS

A. Free-field Quantification of the Sonicator

Quantifying the acoustic field generated by the sonicator is problematic. The sonicator produces cavitation-level ultrasound, which can cause serious damage to scientific equipment such as hydrophones which could otherwise be used for direct measurements. Also, the pattern of bubble clouds observed during the proof of concept tests indicate that the acoustic field is not spherically symmetric (although this could just be a factor of being in a rectangular fish tank. This lack of symmetry suggests that a spot measurement is likely to be non-representative of the entire acoustic field.

When quantifying an acoustic device, the location of the measuring device is of extreme importance. For any acoustic source there is a near field and a far field. In the near field, the acoustic waves are not well behaved and measurements are subject to interference from the edges of the source. In the far field, the acoustic field generated by the source is well behaved, and the source itself can be treated as a point source. When this is the case, several characteristics and assumptions are valid. The most important of these is the assumption that the acoustic pressure amplitude from a given source decays as \( \frac{1}{r} \), or *spherical spreading*. In terms of sound pressure level, halving the distance between the measuring device and the source should result in a 6 dB gain:
\[ L_p = 20 \log_{10} \left( \frac{P_{rms}}{P_{ref}} \right) \]

\[ \Delta L_p = 20 \log_{10} \left( \frac{2 P_{rms}}{P_{rms}} \right) = 20 \log_{10}(2) \approx 6 \]

If the measuring device is not in the far field, this relationship will not hold. Likewise, it will not hold if the environment is plagued by reverberation or if there are any non-linear effects caused by the pressure waves. When quantifying the sonicator, it is important to make sure that the hydrophone is in the far field of the sonicator, but also not located in a reverberant field or else the measurement is not accurate.

In order to get meaningful data, an experiment was designed in a large water tank (seven feet squared by six feet deep.) A mounting bracket was made for the sonicator that allowed it to be hung from a metal bar clamped across the diagonal of the water tank. Also hung from this crossbar was a piece of aluminum with a pivot point that was level with the center of the horn of the sonicator (below the water line.) Attached to the pivot point was a one meter lever arm with a hydrophone mounted at the end. By lowering the lever arm known lengths at a time (17.5 cm corresponding to ten degree increments at one meter), the acoustic pressure produced by the sonicator was measured at known angles. See Figure 6 for a schematic of the experimental setup.

To ensure that the measurements were taken in the free-field of the sonicator, several preliminary data points were taken. The pivot arm with the hydrophone was made so that measurements could be taken at distances of either 1 meter, 50 centimeters, 25 centimeters and 12.5 centimeters. Measurements were taken at three angles, 0 degrees, 45 degrees and 90 degrees, and the data is presented in Table 2.
Figure 6: Sonicator Quantification Setup

Table 2: Determination of Free-Field

<table>
<thead>
<tr>
<th>Degrees Below Horizontal</th>
<th>Pressure (kPa) at 0.25 m</th>
<th>Pressure (kPa) at 0.5 m</th>
<th>Pressure (kPa) at 1.0 m</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>21.0</td>
<td>9.98</td>
<td>6.44</td>
</tr>
<tr>
<td>45</td>
<td>21.8</td>
<td>11.0</td>
<td>10.8</td>
</tr>
<tr>
<td>90</td>
<td>21.5</td>
<td>10.0</td>
<td>4.04</td>
</tr>
</tbody>
</table>
This preliminary experiment shows that 1 meter is too close to the walls of the tank; the measurements are affected by the reverberation from the water to air interface. The measurements taken at 50 centimeters, however, are very nearly half the amplitude of those taken at 25 centimeters. From this it can be taken that 50 centimeters is in the free field of the sonicator. Thus for the quantification experiment, the hydrophone was positioned 50 centimeters from the sonicator.

From the voltage output by the hydrophone, the sound pressure level was calculated using one volt as the reference in the equation below, with $R_x$ denoting the sensitivity of the receiver.

$$SPL = 20 \times \log_{10} \left( \frac{V_{rms}}{V_{ref}} \right) - R_x$$

The receiver was calibrated and showed to have a sensitivity of -209 db. Sound pressure levels are typically written as the sound pressure level one meter from the source. Thus the calculated sound pressure level was adjusted to fit standard practice. As previously mentioned, the transmission loss is:

$$Tl = 20 \times \log_{10} \left( \frac{r}{r_{ref}} \right)$$

So finally;

$$SPL = 20 \times \log_{10} \left( \frac{V_{rms}}{V_{ref}} \right) - R_x - 20 \times \log_{10}(0.5)$$

The results show that at the highest power setting (the setting used during the initial experiments), the sonicator has a maximum acoustic output of 191 dB rel. 1 μPa.
Using the calculated sound pressure level at ten degree increments, the total acoustic power output by the sonicator was calculated. See Appendix II for details of this experiment as well as the calculated data at the individual steps. The total power output by the sonicator at the power settings on the power supply is shown in Figure 7. For this experiment, the maximum error was calculated by calculating the appropriate values of acoustic power given the worst case scenario of the error. Thus the error bars represent the most extreme possible error in the experiment.

![Acoustic Power Generated by the Sonicator at its Different Power Settings](image)

Figure 7: Total Acoustic Power Produced by the Sonicator at its Various Power Settings
B. Determining the Effects of the Reverberant Field

The original ‘proof of concept’ tests were conducted in a small container surrounded by air. When a pressure wave in a medium encounters an interface with another medium, a portion of the wave is reflected and a portion is transmitted, the degree of which is based largely on the impedance difference of the two media. Since the impedances of water and air differ by close to a factor of 3000, it is likely that the original tests benefited from a significant amount of reverberation. If a field-deployable system is to be created, the effect of this reverberation must be determined so that it can be replicated if necessary. There are several methods for quantifying this reverberation, three of which are presented here; theoretical calculation, calculation by analogy with architectural acoustics and direct measurement. The last two of these methods rely on being able to compare data to measurements taken in an anechoic environment. Therefore the first step in quantifying the reverberation is to create an anechoic environment in the lab. To this end, an anechoic sink was designed and built. Three staggered layers of redwood (porous and absorbent at high frequencies) were constructed to fit inside of a stainless steel sink at the NCPA.

In order to determine the effectiveness of this construction, several measurements were made using a custom built transducer operating at 20 kHz, and a Reson TC4013 piezoelectric hydrophone. Figure 8 shows two screen shots of an oscilloscope trace. The left trace is a single 20 kHz burst in the stainless steel sink without the addition of the redwood lattice. The right trace is the same burst but with the redwood lattice in place. As a first order indication of the benefit provided by the redwood lattice, signal from a single ping was measureable as long as 150 ms without the lattice. Addition of the lattice reduced this to only 1.17 ms. With this anechoic environment available, the three methods for quantifying reverberation can be explored.
i. Theoretical Calculation

The first method of quantifying the reverberation is to calculate the reflection and transmission coefficients for the appropriate boundaries at the operating parameters (20 kHz, 20 degrees Celsius). Figure 9 gives a graphical representation of the situation. An incident wave traveling through medium one (with acoustic impedance, \( r = \rho \times c \)) is incident on the boundary with medium two. A portion of the wave is reflected back into medium one, and a portion is transmitted through to medium two. The transmitted wave then propagates through medium two until it becomes incident on the boundary with medium 3. Again, some of the wave is transmitted and some is reflected. The reflected wave passes back through medium two until it reaches medium one, where again some of it is transmitted etc. The goal is to determine the total amount of the initial pressure wave that is reflected back into medium one, and the total amount that is transmitted all the way through to medium three.
Starting with the equation for the pressure reflection coefficient:\(^{15}\):

\[
R_P = \frac{\left(1 - \frac{r_1}{r_3}\right) \cos(k_2L) + i \left(\frac{r_2}{r_3} - \frac{r_1}{r_2}\right) \sin(k_2L)}{\left(1 + \frac{r_1}{r_3}\right) \cos(k_2L) + i \left(\frac{r_2}{r_3} + \frac{r_1}{r_2}\right) \sin(k_2L)}
\]

The power reflection coefficient is:

\[
R_{\Pi} = |R_P|^2
\]

There are two scenarios that need to be calculated. The first case is the situation of the preliminary experiment: the sonicator in a fish tank surrounded by air. The second case is the ‘anechoic’ environment with the sonicator in a fish tank surrounded by water in the anechoic sink. The interface for case two is water to glass to water. The reflection and transmission coefficients of the first case can be compared to those of the second case, thus determining the amount of gain provided by the experimental setup.
In order to calculate the coefficients, the acoustic impedances of water glass and air must be determined. Each of the impedances is calculated as the product of the density and the speed of sound through the material.

For air:

$$r_{air} = \left(1.21 \frac{kg}{m^3}\right) \times \left(343 \frac{m}{s}\right) = 415 \frac{kg}{m^3s}$$

Water:

$$r_{water} = \left(1000 \frac{kg}{m^3}\right) \times \left(1500 \frac{m}{s}\right) = 1.50 \times 10^6 \frac{kg}{m^3s}$$

Glass:

$$r_{glass} = \left(2505 \frac{kg}{m^3}\right) \times \left(5360 \frac{m}{s}\right) = 1.34 \times 10^7 \frac{kg}{m^3s}$$

For all three cases, medium 2 is glass. Thus $k_2L$ is the same for all three cases; $L$ is the thickness of the glass and $k$ is the wave number,

$$k = \frac{\omega}{c} = \frac{2\pi f}{c}$$

It is important to note that in the event of medium 2 being a solid, the speed of sound used must be the bulk speed. For tempered glass, $c = 5360 \frac{m}{s}$. Since the frequency of interest is 20 kHz

$$k = 23.4$$

$L$, the thickness of the fish tank glass, is 1/8 in or $3.20 \times 10^{-4}$ m.

$$k_2L = (23.4) \times (3.20 \times 10^{-4} m) = 7.49 \times 10^{-3}$$
Using the small angle approximation simplifies the calculations.

\[
\sin(k_2L) \approx k_2L
\]

\[
\cos(k_2L) \approx 1
\]

For Case 1 (water - glass - air):

\[
R_{p1} = \frac{\left(1 - \frac{r_{water}}{r_{air}}\right) + i \left(\frac{r_{glass}}{r_{air}} - \frac{r_{water}}{r_{glass}}\right) * (k_2L)}{\left(1 + \frac{r_{water}}{r_{air}}\right) + i \left(\frac{r_{glass}}{r_{air}} + \frac{r_{water}}{r_{glass}}\right) * (k_2L)}
\]

Taking the modulus squared yields the power reflection coefficient for case 1:

\[
R_{\Pi 1} = \left|R_{p1}\right|^2 = Re^2\{R_{p1}\} + Im^2\{R_{p1}\}
\]

\[
R_{\Pi 1} = \frac{13115726.69}{13130185.42} = 0.999
\]

For Case 2 (water - glass - water):

\[
R_{p2} = \frac{\left(1 - \frac{r_{water}}{r_{water}}\right) + i \left(\frac{r_{glass}}{r_{water}} - \frac{r_{water}}{r_{glass}}\right) * (k_2L)}{\left(1 + \frac{r_{water}}{r_{water}}\right) + i \left(\frac{r_{glass}}{r_{water}} + \frac{r_{water}}{r_{glass}}\right) * (k_2L)}
\]

Again, calculating the power reflection coefficient:

\[
R_{\Pi 2} = \frac{0.004365}{4.004590} = 0.011
\]

To summarize, the fish tank reflects 99.9% of the power of an incident 20 kHz wave when it is surrounded by air, but only 1.10% when surrounded by water.
In order to determine the amount of pressure reflected in the echoic environment compared to the anechoic, the sound power levels can be compared.

\[ \Delta L_W = L_{W1} - L_{W2} \]

\( L_W \) is the sound power level, defined in terms of the acoustic power \( W \) and a reference power \( W_{ref} \) as:

\[ L_W = 10 \cdot \log_{10} \left( \frac{W}{W_{ref}} \right) \]

Using the properties of the log,

\[ \Delta L_W = 10 \cdot \log_{10} \left( \frac{W_1}{W_2} \right) \]

The power in each case is the product of the power reflection coefficient and the acoustic power of the source.

\[ W_i = R_{\Pi i} \cdot W_{source} \]

Since the source is the same, the ratio \( \frac{W_1}{W_2} \) becomes the ratio of the reflection coefficients:

\[ \Delta L_W = 10 \cdot \log_{10} \left( \frac{R_{\Pi1}}{R_{\Pi2}} \right) = 10 \cdot \log_{10} \left( \frac{0.999}{0.011} \right) = 19.6 \text{ dB} \]

The sound pressure level is related to the sound power level by a factor which accounts for the position of the measurement.

\[ L_p = L_W + 10 \cdot \log_{10} \left( \frac{S_0}{4\pi r^2} \right) \]
When comparing two situations, as long as the measurements are made at the same distance from the source \( S_0 \), the second terms cancel themselves.

\[
\Delta L_p = \Delta L_w = 19.6 \text{ dB}
\]

Thus the echoic environment provides **19.6 dB** of acoustic gain over the anechoic environment.

ii. Calculation by Analogy with Architectural Acoustics

The second method of quantifying this reverberation is by analogy. In architectural acoustics, there is a method of predicting the sound pressure level \( L_p \) in a reverberant environment based on the sound power level of the source \( L_w \) and the acoustic properties of the room:

\[
L_p = L_w + 10 \log_{10} \left[ \frac{Q}{4\pi r^2} + \frac{4}{A} \right]
\]

In this equation, \( L_p \) is the sound pressure level at the point of the receiver, and \( L_w \) is the sound power level of the source. \( Q \) is a parameter that is used to account for reflective surfaces that affect the percentage of the power that is propagated toward the source. For \( n \) walls immediately adjacent to the source,

\[ Q = 2^n \]

In both cases presented here, the source is not touching any of the walls; thus \( n = 0 \), and \( Q = 1 \).

The parameter \( A \) is defined as the absorptive surface area. This is the quantity that is affected by placing the redwood lattice in the sink. \( A \) can be directly calculated if the acoustic absorption \( \alpha \) is known for all surfaces (with surface area \( S \)) within the volume of interest.
\[ A = \sum_{\text{all surfaces}} \alpha_i \cdot S_i \]

Calculating \( A \) this way is rather tedious for any complicated environment and is plagued by error if the alphas are not precisely known and if each element in the room is not meticulously measured. Fortunately, \( A \) appears in the equation for a parameter known as the reverberation time.

\[ t_{60} = 4 \cdot \frac{\ln(10^6)}{c} \cdot \frac{V}{A} \]

\( t_{60} \) is the time required for the sound pressure level of sound of an instantaneous source to drop by 60 dB (or a factor of 1000). This ‘reverb time’ is easily measured by creating a sound and recording the amplitude of the resulting pressure wave on an oscilloscope or a digital sound level meter. It is often the case that the sound source is not of sufficient amplitude to drop 60 dB and still be above the noise. Thus it is standard practice to extrapolate the decay of the signal and estimate the reverberation time.

In order to determine \( A \) for the echoic and anechoic environments, a transducer operating at 20 kHz was used as the sound source, and a Reson TC4013 hydrophone was used to monitor the pressure level inside the fish tank. For the anechoic case, the fish tank was placed inside the stainless steel sink which was filled with water. In the echoic case, the sink was drained. See Figure 10 for a schematic and Figure 11 for a photograph.
Figure 10: Top View of the Setup Used for the Anechoic vs. Echoic Tests

Figure 11: Anechoic Sink with Sonicator in Place
Using the extrapolation technique, the reverberation times were determined to be:

\[ t_{60}^{\text{echoic}} = 178 \, ms \quad \text{and} \quad t_{60}^{\text{anechoic}} = 6.06 \, ms \]

By solving the \( T_{60} \) equation for \( A \), the absorptive area can be calculated for both cases.

\[ A = \frac{24 \times \ln(10)}{c} \times V \times t_{60} \]

Evaluating the constants, with \( c = 1500 \, m/s \) yields

\[ A = 0.0368 \times V \times t_{60} \]

For the echoic case, the volume \( V \) is the volume of the fish tank: \( V_{\text{echoic}} = 0.041 \, m^3 \). For the anechoic case, the volume of the entire sink is used: \( V_{\text{anechoic}} = 0.14 \, m^3 \). Using these and the measured reverberation times,

\[ A_{\text{echoic}} = 0.0085 \, m^2 \quad , \quad A_{\text{anechoic}} = 0.855 \, m^2 \]

As expected, the absorptive surface area of the anechoic setup is much greater than that of the water to air interface. The pieces are now all in place to calculate the predicted difference in sound pressure levels between the echoic and anechoic environments.

\[ \Delta L_p = L_{p_{\text{echoic}}} - L_{p_{\text{anechoic}}} = L_{w_{\text{echoic}}} - L_{w_{\text{anechoic}}} + 10 \times \log_{10} \left( \frac{\frac{1}{4\pi r^2} + \frac{4}{A_{\text{echoic}}}}{\frac{1}{4\pi r^2} + \frac{4}{A_{\text{anechoic}}}} \right) \]

Since \( L_w \) is a property of the source, it is the same in both the echoic and anechoic cases so the terms cancel each other. Using the reference distance (\( r = 1 \, m \)) and the appropriate values of \( A \):
\[
\Delta L_p = 10 \times \log_{10} \left[ \frac{470.7}{4.76} \right] = 20.0 \text{ dB}
\]

This means that sound pressure level generated by a source operating at 20 kHz should be 20.0 dB higher in the fish tank surrounded by air than in the anechoic environment due to the water-to-air interface.

iii. Direct Measurement

The third and final method is a simple experimental check. Several measurements using the same configuration of transducer and hydrophone as the second method were taken within the fish tank. The locations of the hydrophone and transducer were changed with each measurement in order to account for modes within the tank. Half of the measurements were taken with the sink drained and the other half with the sink full. The data are in Table 3. The average received voltage in the echoic environment was 602 mV. The average received voltage in the anechoic environment was 59.2 mV. The ratio of the average received voltage for echoic to anechoic is 10.2, or 20.1 dB. All three methods yield very similar answers; taking their average, it can be concluded that the gain due to reverberation is **19.9 dB**

<table>
<thead>
<tr>
<th>Table 3: Received Voltages (mV&lt;sub&gt;pp&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Echoic</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td>560</td>
</tr>
<tr>
<td>580</td>
</tr>
<tr>
<td>590</td>
</tr>
<tr>
<td>610</td>
</tr>
<tr>
<td>670</td>
</tr>
</tbody>
</table>
In order to determine if this gain from reverb is necessary to kill snails, several tests with the sonicator and groups of snails were conducted in the anechoic environment. Figure 11 shows the anechoic setup with the sonicator in place. Groups of twenty snails and the sonicator were placed into a fish tank (within the anechoic sink) and the snails were allowed to acclimate. After a fixed amount of acclimation time, the sonicator was turned on for a predetermined amount of time. The snails were then moved to a partition (marked according to their exposure time) within another fish tank and observed over a period of one week. A control group of twenty snails was given the same treatment, but with the sonicator never turned on. This control provided a "background" in case any unknown variables such as handling, feeding, oxygen levels or water chemistry were introduced. The test consisted of six different times of exposure: 0s (control), 5 s, 20 s, 45 s, 60 s, 90s. The results of this experiment are shown in Table 4.
Table 4: Data from Duration Experiment in Anechoic Environment

<table>
<thead>
<tr>
<th>Date / Time</th>
<th>Test Number</th>
<th>Number Alive</th>
<th>Date / Time</th>
<th>Test Number</th>
<th>Number Alive</th>
</tr>
</thead>
<tbody>
<tr>
<td>11/02/10 18:30</td>
<td>Control</td>
<td>20</td>
<td>Control</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/06/10 16:00</td>
<td>5 s</td>
<td>20</td>
<td>5 s</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/02/10 18:30</td>
<td>20 s</td>
<td>20</td>
<td>20 s</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/06/10 16:00</td>
<td>45 s</td>
<td>20</td>
<td>45 s</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/02/10 18:30</td>
<td>60 s</td>
<td>20</td>
<td>60 s</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/06/10 16:00</td>
<td>90 s</td>
<td>15</td>
<td>90 s</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>11/04/10 18:30</td>
<td>Control</td>
<td>20</td>
<td>Control</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/07/10 16:00</td>
<td>5 s</td>
<td>20</td>
<td>5 s</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/04/10 18:30</td>
<td>20 s</td>
<td>20</td>
<td>20 s</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/07/10 16:00</td>
<td>45 s</td>
<td>20</td>
<td>45 s</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/04/10 18:30</td>
<td>60 s</td>
<td>20</td>
<td>60 s</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>11/07/10 16:00</td>
<td>90 s</td>
<td>15</td>
<td>90 s</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>11/05/10 14:00</td>
<td>Control</td>
<td>20</td>
<td>Control</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>11/09/10 13:30</td>
<td>5 s</td>
<td>20</td>
<td>5 s</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>11/05/10 14:00</td>
<td>20 s</td>
<td>20</td>
<td>20 s</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>11/09/10 13:30</td>
<td>45 s</td>
<td>20</td>
<td>45 s</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>11/05/10 14:00</td>
<td>60 s</td>
<td>20</td>
<td>60 s</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>11/09/10 13:30</td>
<td>90 s</td>
<td>15</td>
<td>90 s</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

From this experiment, it is obvious that the reverberation present in the initial experiment is necessary in order to kill a large number of snails. The 25% killed during the 90 second duration is not terrible, but it is far from the desired result. This gain will need to be recreated in the eventual field system. Adding the gain from the reverb to the maximum output of the sonicator yields a maximum acoustic output of 211 dB in the reverberant tank. This level is now the design goal for the project.
IV. TESTS WITH REVERBERATION

Since the anechoic experiment proved that the reverberation is necessary, the rest of the experiments presented here were conducted in the echoic environment. In an attempt to determine the optimum duration of sonication, an experiment was designed consisting of groups of ten snails being exposed to sonication for varying amounts of time. The results shown in Table 5 are the average over three runs of the experiment.

<table>
<thead>
<tr>
<th>Sonication Duration</th>
<th>Number of Snails Killed</th>
<th>Sonication Duration</th>
<th>Number of Snails Killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 s</td>
<td>0</td>
<td>45 s</td>
<td>3.33</td>
</tr>
<tr>
<td>10 s</td>
<td>0.33</td>
<td>60 s</td>
<td>3</td>
</tr>
<tr>
<td>20 s</td>
<td>1.66</td>
<td>90 s</td>
<td>4.66</td>
</tr>
<tr>
<td>30 s</td>
<td>2.66</td>
<td>120 s</td>
<td>5.66</td>
</tr>
</tbody>
</table>

Based on the results of this experiment, another was conceived with the goal of gathering some statistically significant data. Twenty-two groups of ten snails were put through the exact same procedure (two control groups). They were gathered and placed into a fish tank and allowed to
acclimate. After five minutes of acclimation time, the sonicator was turned on for 90 seconds.

After another two minutes, the snails were checked for mortality. The number killed for each test is displayed in Figure 12. The average number of snails killed is 3.5, or 35%. The standard deviation is 1.6. This data brings to light a few potential problems; notably that on a trial by trial basis, this acoustic method is not very reliable. However, statistically speaking, it appears as though this method is capable of killing 35% of a snail population. Also of note is that, although the survivors from each trial were not separated from the control groups of snails, the snails experimented upon were placed in a different fish tank than the ‘stock’ of snails. Four days after the experiment was conducted, an extra 42% of the snails experimented upon were dead, whereas only 9% of the ‘stock’ snails were dead. Thus all told, the sonication appears to have killed ~65% of the snails exposed to the sonication. Further experimentation is needed to explore the extent and applicability of this observation.

![Figure 12 Number of Snails Killed per Trial](image)

Figure 12 Number of Snails Killed per Trial
V. TESTS WITH CUSTOM BUILT TRANSDUCERS

All of the tests to this point used the Branson sonicator as the source of high amplitude ultrasound. Preliminary echoic tests were repeated with a different sonicator, a Misonix CL5 which also operates at 20 kHz. The Misonix sonicator seemed to churn the water more, and the snails underwent considerably more gross motion, but in a churning motion, not a vibratory motion. After several trials no snails were killed. It should be noted that this sonicator appears to focus much more of its energy downward. Since the water churned so much, the sonicator simply pushed the snails out of its way, thus the snails were not exposed to the powerful sonication for very long.

In order to explore the possibility of using different ultrasonic frequencies to achieve elimination, several piezoelectric transducers were built in-house. Three transducers were built to have a central frequency around 84 kHz, three around 112 kHz, and another three around 460 kHz. A problem with using custom transducers is that they are un-calibrated. Since these transducers may potentially produce high enough amplitude pressure waves to damage the sensitive hydrophone, a method of calibrating the transducers without the use of a hydrophone is required. The parameters of interest are $M_{db}$ and $S_{db}$, the sensitivities of the transducers both receiving and transmitting. When a voltage is applied across a piezoelectric material, it deforms and creates a pressure wave; likewise when a pressure wave is incident on the material it generates a voltage. The receive and transmit sensitivities tell how much voltage is created for a given pressure and vice versa. These parameters are necessary in order to know the sound pressure level generated by
a transducer. For a given transducer with transmit sensitivity $S_{db}$ (in dB rel. $1 \mu Pa/V$), the sound pressure level at 1 meter is

$$Sl = V_{db\text{transmit}} + S_{db}$$

where

$$V_{db\text{transmit}} = 20 \times \log_{10} \left( \frac{V_{rms}}{V_{ref}} \right)$$

Typically, $V_{ref}$ is one volt. $V_{rms}$ is the root mean squared voltage across the transmitting transducer. On the receiving end, for a given sound level $El$ incident on a transducer, the voltage generated by the transducer with receive sensitivity $M_{db}$ (dB rel. $1 V/\mu Pa$) is

$$V_{db\text{received}} = El + M_{db}$$

In underwater acoustics, it is standard practice to measure the sound pressure level of the source, $Sl$ at a distance of one meter from the source. Thus, when the receiving transducer is placed exactly one meter from the transmitting transducer, $El = Sl$. For the case where this is not practical, it is possible to account for the spherical spreading of acoustic waves by adding a term called the transmission loss, $Tl$.

$$Tl = 20 \times \log_{10} \left( \frac{r}{r_{ref}} \right) \quad \text{with} \quad r_{ref} = 1 \text{ m}$$

At a distance $r$, the source level and echo level are related by the following equation:

$$El = Sl - Tl$$
In a pitch-catch configuration using two piezoelectric devices, the sound pressure level at any point in the water can be determined by measuring the voltage applied across the transmitting transducer and the voltage generated by the receiving transducer and using these in the relevant equations above.

A method known as ‘reciprocity’ is the standard procedure for calibrating acoustic devices. This method is standardized by the *American National Standards Institute*\textsuperscript{17}. The standard procedure requires some manipulation in order to fit the circumstances used here. See Appendix I for a detailed derivation from the ANSI standards to the applicable procedures.

Using the method described in Appendix I, the 84 kHz, 112 kHz and 460 kHz transducers were calibrated; the receive and transmit sensitivities for all six transducers are shown in Table 6.

### Table 6: Transmit and Receive Sensitivities of nine in-house transducers

<table>
<thead>
<tr>
<th>Frequency = 84 kHz</th>
<th>Transducer</th>
<th>Receive Sensitivity (dB rel. 1 V/µPa)</th>
<th>Transmit Sensitivity (dB rel. 1 µPa/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>-183</td>
<td>141</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-183</td>
<td>143</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-183</td>
<td>142</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency = 112 kHz</th>
<th>Transducer</th>
<th>Receive Sensitivity (dB rel. 1 V/µPa)</th>
<th>Transmit Sensitivity (dB rel. 1 µPa/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>-198</td>
<td>129</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-206</td>
<td>121</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-189</td>
<td>136</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Frequency = 460 kHz</th>
<th>Transducer</th>
<th>Receive Sensitivity (dB rel. 1 V/µPa)</th>
<th>Transmit Sensitivity (dB rel. 1 µPa/V)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>-192</td>
<td>161</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>-196</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-193</td>
<td>161</td>
</tr>
</tbody>
</table>
With the transducers calibrated, their maximum output can be determined. An experiment was designed using the transducers as both the transmitting and receiving elements. A signal of the appropriate frequency was generated using an HP 3314 function generator. It was then run through a ENI 1040L power amplifier which provides a 55 dB gain. The magnitude of the input signal was systematically adjusted, and the signal received was monitored. When the received signal became noticeably distorted, the transducer had reached its maximum useable output. This test was run on one of each of the three sets of the in-house transducers. The maximum outputs shown in Table 7 are significantly less than the 211 dB required at 20 kHz. It was deemed unlikely that the in-house transducers would kill snails, but nevertheless the initial proof of concept tests were repeated using the 84, 112 and 460 kHz transducers. The results from these two experiments are also shown in Table 7. As suspected, the transducers were incapable of producing high enough amplitude ultrasound to cause any significant damage to the snails. However, the transducers may be sufficient for other uses in the field of aquaculture such as the aforementioned vaccine delivery, and as such it is important to document and quantify their maximum output.

<table>
<thead>
<tr>
<th>Transducer</th>
<th>Maximum Output (dB rel. 1 μPa)</th>
<th>Snails Killed (out of ten)</th>
</tr>
</thead>
<tbody>
<tr>
<td>84 kHz</td>
<td>178</td>
<td>0</td>
</tr>
<tr>
<td>112 kHz</td>
<td>156</td>
<td>0</td>
</tr>
<tr>
<td>460 kHz</td>
<td>175</td>
<td>0</td>
</tr>
</tbody>
</table>
VI. CONCLUSIONS AND FUTURE WORK

With trematode infestations becoming a major problem to U.S. catfish farmers, an efficient mechanical method of eliminating the intermediate host the Ram’s Horn Snail is greatly sought. High amplitude acoustics has the potential of fulfilling this need. Early tests showed that exposure to a 20 kHz sonicator can in fact kill individual snails. Further testing on groups of snails proved less efficient, indicating that around 35% of the snail population could be killed instantly. The tests also indicated however that the sonication may mortally wound a significant portion of the snails, causing them to die a few days after sonication.

An experiment was designed to quantify the pressure field generated by the sonicator so that it could be replicated. The details of this experiment are in Appendix II. In addition to the field generated by the sonicator, the effects due to the reverberation present in the initial experimental setup were quantified. This required the design of an underwater anechoic environment, which was achieved by lining the interior of a stainless steel sink with three layers of redwood lattice. The difference in acoustic pressure with and without reverberation for the test environment was 19.9 dB. It was determined that this gain from reverberation is necessary to kill the snails.

There are still several problems with scaling the experiment to a pond. The gain from the reverberation still needs to be achieved in the field. This could be done any combination of ways. Multiple sources would provide some of the gain needed, and the design of the mounting apparatus may be used to provide some gain as well. A conical shaped mounting bracket, filled with air or vacuum, would provide a fair amount of gain as well as some focusing effects. Note that the size of
such a system poses some issues. An array of 6 sonicators for example, would require a mounting bracket approximately three feet wide. Requiring exposure for just 45 seconds, this limits the speed of travel around the edge of the pond to about .0666 ft/s. At this speed, it would take approximately 11 hours to traverse the edges of a ten-acre pond. Obviously this is an unfeasible length of time, so something must be changed. These design issues will be more thoroughly examined as the project moves forward.

The basics of the work presented here can be adapted to other potential uses for high amplitude acoustics in aquaculture. As previously mentioned, there are potential uses for algae control as well as for vaccination of catfish en masse. The pursuit of these applications requires collaboration between biologists and acousticians, and this work should provide the basis for that collaboration.
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10 Jeffery S. Terhune, "Infestations of the trematode bolbophorus sp. in channel catfish [electronic resource] / Jeffery S. Terhune ... [et al.]", in SRAC publication ; no. 1801 ([Stonerville, Miss.?] : Southern Regional Aquaculture Center, [2003], 2003).
APPENDIX I

RECIROCITY CALIBRATION OF THREE TRANSDUCERS
There are two properties of the in-house transducers that need to be determined so that they can be used accurately. These are the magnitudes of the free-field receiving voltage sensitivity (M) and the transmitting voltage sensitivity (S). These values are important characteristics of any transducer and are essential when determining the sound level of an underwater source. The standard for this procedure is the American National Standard Procedures for Calibration of Underwater Electro-Acoustic Transducers. These ANSI standards are written for the case of calibrating a reciprocal transducer with a pre-calibrated ‘projector’ and ‘hydrophone’. With a little manipulation and thought, they can be applied to the case of having three reciprocal transducers. The experimental setup presented here is slightly different than in the ANSI standards due to the fact that the goal is to calibrate three transducers simultaneously instead of one transducer at a time.

A. Deriving Equations from the ANSI Standards for Reciprocity

Before beginning the derivation, a note regarding notation is in order. The subscripts on voltage and current are used to denote the role of projector and receiver. The first number is the designation of the transducer that is acting as the projector, and the second number is the transducer that is receiving. Thus $v_{1-2}$ denotes the rms voltage when transducer number one is the projector and transducer number two is the receiver.
The equation for the free-field receiving sensitivity according to the ANSI standards is:

\[ M_2 = \left[ \frac{v_{1-2} \cdot v_{3-2}}{v_{1-3} \cdot l_{3-2} \cdot J} \right]^{1/2} \]

Where:

- \( v_{1-2} \) is the voltage (rms) output by the receiving transducer
- \( l_{3-2} \) is the current across the transmitting transducer
- \( J \) is the spherical reciprocity parameter

The reciprocity parameter is a calculated parameter that is defined as the ratio of the receive sensitivity to the transmit sensitivity.

\[ J = \frac{M}{S} \]

Since the hydrophones are in the far field of the transducers, the reciprocity parameter may be written as

\[ J = \frac{2 \cdot d \ [m]}{\rho \ \frac{kg}{m^3} \cdot f \ [Hz]} \]

Or in terms of more measurable units;

\[ J = \frac{2 \cdot d [m] \cdot 10^{-6}}{\rho \ \frac{g}{cm^2} \cdot f [kHz]} \]
The receive sensitivity is normally written in terms of decibels referenced to $1 \frac{V}{\mu Pa}$. The units on $M$ in the definition are $\frac{V}{Pa}$, dividing by $10^6$ accounts for this difference. Bringing this factor into the square root and combining it with $J$ yields

$$M_2 = \left[ \frac{v_{1-2} \cdot v_{3-2} \cdot J}{v_{1-3} \cdot i_{3-2}} \right]^{1/2}, \text{ but with}$$

$$J = \frac{2 \cdot d[m] \cdot 10^{-18}}{\rho \left[ \frac{g}{cm^3} \right] \cdot f[kHz]}$$

In the derivation of $M$ in the ANSI standards the ratio $\frac{i_{1-3}}{i_{1-2}}$ appears, is assumed to be equal to one and therefore does not appear in the final equation for $M$. This is due to the fact that the ANSI experiment deals only with one reciprocal transducer, one transmitter and one hydrophone. Thus there is no need to swap transmitting transducers during the experiment. Since the transmitting and receiving devices are not electrically linked, these currents should indeed be identically the same. In the case of three reciprocal transducers however, the physical transducers are constantly swapped. Thus the input voltages change for a given run of the experiment, and these two currents are no longer necessarily the same. Therefore, the ratio must be added back to the equation.

$$M_2 = \left[ \frac{v_{1-2} \cdot v_{3-2} \cdot \frac{i_{1-3}}{i_{1-2}} \cdot \frac{i_{1-3}}{i_{3-2}} \cdot J}{v_{1-3}} \right]^{1/2}$$

These are all measured quantities, and it is thus an easy matter to calculate the sensitivity and then write it as a decibel.

$$M_{db} = 10 \cdot \log_{10}(M)$$
If desired, taking the log of $M$ yields a more spreadsheet friendly equation.

$$ 2 \cdot M_{db2} = V_{db1-2} + V_{db3-2} - V_{db1-3} + i_{db1-3} - i_{db1-2} - i_{db3-2} + 20 \log_{10} J $$

Taking $d$ out of the equation for $J$ allows each of the voltages to be written as range-corrected voltages. Thus the receive sensitivity is in terms of useful numbers. Either equation will work, it depends on the situation and which numbers are easier to obtain.

Permuting the indices and taking the logarithm yields the equation for receive sensitivities in decibels referenced to one volt per micro-pascal:

$$ M_{db1} = 5 \cdot \log_{10} \left[ \frac{v_{2-1} \cdot v_{3-1}}{v_{2-3} \cdot i_{2-1} \cdot i_{3-1}} \cdot J \right] $$

$$ M_{db2} = 5 \cdot \log_{10} \left[ \frac{v_{1-2} \cdot v_{3-2}}{v_{1-3} \cdot i_{1-2} \cdot i_{3-2}} \cdot J \right] $$

$$ M_{db3} = 5 \cdot \log_{10} \left[ \frac{v_{1-3} \cdot v_{2-3}}{v_{1-2} \cdot i_{1-3} \cdot i_{2-3}} \cdot J \right] $$

These are the receive sensitivities in terms of measured quantities; now to do the same for the transmitting sensitivity for the transducers. According to the ANSI standards

$$ S_1 = \left[ \frac{I_{r1-3} \cdot I_{r1-2}}{I_{r3-2} \cdot J} \cdot \frac{V_{T_{3-2}}}{V_{T_{1-2}}} \right]^{1/2} \cdot \frac{1}{V_{T_{1-2}}} $$

As in the case of the receive sensitivity, the factor $\frac{V_{T_{1-3}}}{V_{T_{1-2}}}$ appears and is assumed to be one. Again, this factor must be added back due to differences in experimental procedures. Thus, the equation for transmit sensitivity becomes
In this experiment, the received current is not measured. Thus a little manipulation is required.

The electrical resistance of a transducer is a property of the transducer, and is unaffected by the role of the transducer. In equation form:

\[
\frac{V_{r_{1-2}}}{I_{r_{1-3}}} = \frac{V_{r_{3-1}}}{I_{r_{3-2}}} = \frac{V_{r_{3-2}}}{I_{r_{3-2}}}
\]

This holds true for each transducer. Using this relationship, the transmit sensitivity can be re-written in terms of measured quantities.

\[
S_1 = \left[ \frac{V_{r_{1-2}} \cdot V_{r_{1-3}} \cdot I_{r_{1-2}} \cdot I_{r_{3-2}} \cdot \frac{1}{J}}{V_{r_{3-2}} \cdot I_{r_{3-2}}} \right]^{1/2} \cdot \frac{1}{V_{r_{1-2}}}
\]

Permuting the indices and taking the logarithm again yields the equation for transmit sensitivity in decibels referenced to one micro-pascal meter per volt.

\[
S_{db_1} = 5 \cdot \log_{10} \left[ \frac{V_{r_{1-2}} \cdot V_{r_{1-3}} \cdot I_{r_{1-2}} \cdot I_{r_{3-2}} \cdot \frac{1}{J}}{V_{r_{3-2}} \cdot I_{r_{3-2}}} \right] - 10 \cdot \log_{10}[V_{r_{1-2}}]
\]

\[
S_{db_2} = 5 \cdot \log_{10} \left[ \frac{V_{r_{2-1}} \cdot V_{r_{2-3}} \cdot I_{r_{2-1}} \cdot I_{r_{3-1}} \cdot \frac{1}{J}}{V_{r_{1-3}} \cdot I_{r_{2-3}}} \right] - 10 \cdot \log_{10}[V_{r_{2-3}}]
\]

\[
S_{db_3} = 5 \cdot \log_{10} \left[ \frac{V_{r_{1-2}} \cdot V_{r_{1-3}} \cdot I_{r_{1-2}} \cdot I_{r_{3-2}} \cdot \frac{1}{J}}{V_{r_{3-2}} \cdot I_{r_{1-3}}} \right] - 10 \cdot \log_{10}[V_{r_{1-2}}]
\]
Thus the receive and transmit sensitivities are both expressed as decibels in terms of measured quantities. A spreadsheet can easily be written to do the simply math, and thus three reciprocal transducers can be calibrated in terms of their receive and transmit voltage sensitivities.

B. Experimental Procedure

In parallel terminology with the ANSI standard, for each step of the calibration process one transducer acts as the ‘projector’ while another acts as the ‘hydrophone’. Before any measurements are taken, ensure that the transducers are placed in deep enough water. If the transducers are too shallow (or too close to the bottom), the time difference between the direct signal and the signal reflected off of the top of the water will be too small to notice, and the two signals will interfere, causing a bad data point. The equation for proper depth can be calculated from a simple time of flight argument. Accounting for variables like wavelength, length of cycle, etc., the equation for minimum depth is:

\[ h^2 > \frac{1}{4} (\lambda^2 n^2 + 2d\lambda n) \]

Here \( n \) is the number of bursts per cycle, and \( d \) is the distance in meters between the transducers. Using this minimum depth will ensure that the direct signal arrives in its entirety before the signal from the top of the water arrives at the receiver.

**Step 1: Alignment of the Transducers**

The first step is to align the projector and receiver. This is done by mounting the transducers and attaching them to the appropriate equipment (function generator, current probe...
and oscilloscope for the projector, and oscilloscope for the receiver.) Taking turns, each 
transducer is swept across its available degrees of freedom until the maximum received voltage 
is observed. Note that this takes several iterations for each transducer. This is also the most 
important step. If not done properly, none of the calculations are any good.

**Step 2: Take the Measurements**

There are four measurements to take for each run: the voltage across the projector, the 
current going into the projector, the voltage generated by the receiver and the distance between 
the transducers.

**Step 3: Switch Projecting and Receiving**

Without moving the transducers (so that they do not have to be re-aligned), swap which 
one is the transmitter and which is the receiver. Then repeat Step 2.

**Step 4: Swapping Transducers**

Physically remove one of the transducers and exchange it for the third transducer. Note 
that they must now be aligned again. Repeat Steps 1-3.

**Step 5: Repeat Step 4**

Exchange the transducer that has been unmoved so far for the first transducer removed. 
Repeat Steps 1-3.

There should be six total runs of the experiment, thus 24 data points.

**Step 6: Calculate the Desired Parameters using the Equations Presented Above**
APPENDIX II

DESCRIPTION AND QUANTIFICATION OF THE SONICATOR
Simply stated, a sonicator is a device that produces cavitation level ultrasound via mechanical oscillations. They are used in a wide variety of scientific applications across disciplines, from degassing liquids to disrupting intermolecular bonds. The sonicator used for the majority of this work is a Branson plastic welder. Little was initially known about the sonicator, as the included owner’s manual deals exclusively with the assembly and operation of the entire plastic welding system; an automated machine of which the sonicator is a small part. While the Branson website seems to have long since forgotten about this particular model, they still deal in ultrasonic equipment, so there was still some useful information.

There are four main parts to the sonicator. See Figure 13 for a labeled picture of the sonicator. The converter takes the electrical signal from the power supply and converts it to mechanical motion. The booster is an amplifying device. Assuming that the Branson color code methods have not changed in twenty years, the booster currently in use is a 1:1 amplifier. The horn is the part that actually oscillates generating the ultrasound. The tip appears to be a device to apply pressure to the plastic pieces to be welded. The tip was removed for all of the data taking experiments.
One important thing to note about the sonicator is that while its main operating frequency is 20 kHz (above most human hearing), there is a very loud audible noise generated by the sonicator. This noise measures around 95 dBA at a few feet away. This level of noise is above the threshold of pain, and according to the OSHA standards, a worker can only work in that noise level for four hours a day. Hearing protection is required when working with the sonicator.

In order to better understand what the sonicator produces, a method of testing the acoustic production of the sonicator was designed. There is a tank at NCPA that is seven feet squared and six feet deep. Across the diagonal on top of this tank is mounted a track system that allows equipment to be mounted adjusted with three degrees of freedom. This mounting system
did not meet the needs of the sonicator, as the sonicator cannot be fully submerged, and a full 180 degree view of the sonicator was necessary. A simple lever arm that mounts to the track system already in place was built and installed. The arm extended down into the water, and at the end of the arm is a pivot point to which another arm was attached. At the end of the pivot arm is a hydrophone mount and a string that allows controlled movement of the arm. A protractor was attached to the pivot point and fixed in place. This allowed the experimenters to determine the angle between the lever arm and the horizontal.

The experiment itself consisted of two people working together. One person was in charge of lowering the pivot arm to the appropriate angle. The second person recorded the data. It was decided to mark the string and a point on the track system. The string was marked to correspond to a ten degree change, 17.5 cm at a one meter radius. Using the marks on the string and the marked reference point, the experiment went much more quickly and accurately. The power supply that accompanies the sonicator has power settings from 20 to 100 in increments of 5. The experiment was run for the power settings using increments of 10. The pressures determined from this experiment are in Table 8. The decibel results for power setting 100 are shown in Figure 14.
Table 8: Acoustic Pressure Generated by the Sonicator (in kPa)

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<th>Setting 50</th>
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Figure 14: Results at Power Setting 100; Radial Axis is dB rel. 1 μPa
In addition to the maximum sound pressure level, the total power generated by the sonicator was of interest. The equation for acoustic power is:

\[ W = \iiint_S \vec{I} \cdot \vec{n} \, dS \]

and

\[ |\vec{I}| = \frac{p_{\text{rms}}^2}{\rho_0 \cdot c} \]

Recalling that the sound pressure level measured is defined as:

\[ SPL = 10 \cdot \log_{10} \left[ \frac{p_{\text{rms}}^2}{p_{\text{ref}}^2} \right] \]

The standard reference pressure for water is one micro-pascal. Thus the rms pressure in terms of the measured sound pressure level is:

\[ p_{\text{rms}}^2 = 10^{\left(\frac{SPL}{10}\right)} \cdot p_{\text{ref}}^2 \]

Plugging this into the intensity, and then into the equation for power:

\[ W = \iiint_S \frac{10^{\left(\frac{SPL}{10}\right)} \cdot p_{\text{ref}}^2}{\rho_0 \cdot c} \, dS \]

Since the area of interest is a hemisphere, \( dS \) becomes \( r^2 \sin(\theta) \, d\theta \, d\phi \). At a one meter radius, the power becomes:

\[ W = \int_{\phi=0}^{\pi} \int_{\theta=0}^{\pi} \frac{10^{\left(\frac{SPL}{10}\right)} \cdot p_{\text{ref}}^2}{\rho_0 \cdot c} \, \sin\theta \, d\theta \, d\phi \]
At this point, it can be assumed that the sonicator is symmetric with $\phi$, and data was taken that confirms this. The phi integral therefore yields a factor of pi. The power is not constant over the hemisphere, meaning that the integral must be done piecewise. Instead, since the data was taken at ten degree intervals, the integral must be broken into a sum of integrals and then integrated, evaluated at the endpoints (the value at each end of the 10 degrees), and then the results must be summed back together. This method assumes that the SPL is constant between the ten degree increments. A simple Matlab script was written to do the evaluation. The results of this calculation are displayed in Figure 7. This method is highly repeatable and should work for anything that needs to be measured in 180 degrees.
VITA

Bradley T. Goodwiller was born May 27, 1986 in Richmond, VA. He graduated from Oxford High School in Oxford, MS in May 2004. He received a Bachelor of Science degree in Physics from the University of Mississippi in May 2008. As of May 2011, he is employed as an Associate Research and Development Engineer at the National Center for Physical Acoustics.