ing waveforms from the hydrophone and playing signals back through a sound emitter. We have successfully implemented our active echo-cancellation procedure with a signal-processing system (model: System II) from Tucker Davis Technologies (Alachua, Florida, USA).

Conducting hearing studies within a specialized anechoic environment also generally presupposes working with species that can be transported to the laboratory. The ability to access hearing abilities of a broader range of aquatic animals than fit this criteria is becoming increasingly important because of the need to understand and predict the disruptive effects of anthropogenic noise, especially in the marine environment [14,15]. For example, the proposed expansion of wind- generated electrical power (*i.e*., "wind farms") to continental shelf areas appears likely to increase ambient noise to levels that could diminish significantly the range of effective communication in soniferous fishes [16].

With the auditory brain response (ABR) technique, neural activity is monitored electrically in response to a series of specified exposures to sounds of unique single frequency, duration, and amplitude in order to construct a hearing response curve. Our active echo cancellation procedure makes ABR experiments more readily doable outside of an anechoic environment. It therefore permits a wider range of organisms to be investigated, including aquatic organisms that are difficult (or impossible) to transport to shore side tanks, maintain in captivity, or both.

2. THEORETICAL CONSTRUCT

Every chamber or aquarium used for hearing studies has a unique system response to acoustic perturbations that must be characterized prior to generating a stimulus that will be perceived at a particular location as echo-free. Sounds emitted by the stimulus source will reflect from the walls and floor. Thus, at a particular location in the chamber, the sound heard will consist of the sum of the emitted waveform plus reflections. Reflections that involve multiple surfaces are sometimes termed reverberation, but for the purposes of this paper (and in the literature, generally), these reflected waveforms are called "echoes".

Echo-cancellation was invented at Bell Labs in the 1960's. In its most basic form, a time-delayed sample of the emitted signal is subtracted from the received signal. The Least Mean Squares [17] and the Normalized Least Mean Squares algorithms that followed are now implemented in software or hardware in a host of modern devices including faxes and telephones to reduce echoes generating by reflections from impedance mismatching in the communications channel, and other effects. The net result of echo-cancellation is an improvement in the signal-to-noise ratio of the received signal. More recently, nonlinear echo cancellation [18] can adaptively cancel noise in a dynamic environment where the echoes are constantly changing. For example, these algorithms are used in noise-canceling headphones used in noisy environments. Although there is some evidence that marine mammals like dolphins have neural circuitry able to cancel noise from unwanted reflections from their own emitted sound signals [19], most organisms will perceive the sum of all arriving waveforms with their sensory appartus.

Most hearing studies are conducted over a range of frequencies; thus it is appropriate to characterize the system response of the test chamber using a white-noise source. By measuring the system response to white noise at a specific location in the chamber, it is possible to then create an acoustic inverse waveform that nulls reverberations at that location through destructive interference. This "inverse wave" is convolved with a desired stimulus wave (in frequency space), to produce a pre-compensated stimulus waveform. When transformed into a time domain signal and played into the chamber, an echo-free stimulus wave will be perceived at the location where the acoustic system response was measured.

3. IMPLEMENTATION USING MATLAB SCRIPTS

A set of four interconnected MATLAB scripts: BBTEST.m, MAKESTIMULUSFILE.m, ACOUSTICINV.m, and SEGMENT.m are used to produce a pre-compensated stimulus for location-specific echo cancellation. Each script is described below in chronological order of operation. Copies of the scripts are available for download at: "http://www.vims.edu/about/directory/faculty/brill_rw.php" as "MATLAB scripts.zip".

3.1. BBTEST.m

The BBTEST.m script makes a broadband noise file in signed 16 bit integer format that is then played through the sound emitter to measure the system acoustics (*i.e.*, the acoustic transfer function of the tank and the room). This script must be run prior to running the MAKESTIMULUSFILE.m script described immediately below. The parameters settable by the user, are: the system frequency (in Hz, given by sampersec/2), the lowest and highest frequencies of the "broadband" stimulus to be used for a given set of experiments (lowfreqcps and hifreqcps, respectively, in Hz), the length of the test noise (samlensec, in seconds), and the peak amplitude of the generated signal in volts DC (peakvolts), where –10 to +10 volts DC is assumed to correspond to values of 32,768 to 32,767 in the 16 bit generated noise file, which is named "bb.16". The BBTEST.m script checks for violations of the Nyquist sampling criterion (sampling

must be at least twice the desired highest frequency for testing) when setting the lowest and highest frequencies, and the system frequency, and advises the user appropriately. It also checks that the peak voltage amplitude is set between 0 and 10 volts DC.

The generated noise file has all frequencies represented with equal amplitude. The script achieves this by generating a flat spectrum (in frequency space), with frequencies outside the desired window truncated to zero, with zero DC offset. The resulting spectrum is translated back to the time domain using an inverse discrete Fourier transform, implemented using a fast Fourier transform, and the real part is scaled appropriately by both the peak voltage amplitude, followed by the signed sixteen bit integer format.

3.2. MAKESTIMULUSFILE.m

The MAKESTIMULUSFILE.m script writes the acoustically pre-compensated stimulus files. This script creates the following three subdirectories and a parent file directory labeled "directory":

1) "bbplayfile" — a subdirectory that holds the stochastic differential broadband noise file used to characterize the system acoustics. The BBTEST.m script (see above) is used to produce a broadband noise file in 16 bit signed integer format that is stored in this subdirectory, under the name "bb.16",

2) "bbrecvfile" — a subdirectory that contains the received signal caused by playing "bbplayfile". It is equal in length to the file "bbplayfile", and is stored under the name "bb.txt",

3) "stimulus" — a subdirectory where the generated stimulus file is placed along with two other files: "ones 0.16" and "ones1.16", which are files filled with -1 and +1 values, respectively. These two files are used to invert the generated sound stimulus signals during presentation to the test subject during an experiment, when the inverted pre-compensated stimulus file is sent to the sound emitter. (Their purpose is to allow mechanical artifacts arising from the sound stimuli themselves to be later removed from the ABR.)

The script allows the user to set the following:

1) system frequency (sampersec/2, in Hz),

2) stimulus duration (stimdurms, in ms),

3) a vector containing the stimulus frequencies to be generated (stimfreqs, in Hz),

4) the phase at the center of the stimulus window (stimcentphasedeg, in degrees),

5) the type of window function to be applied to the stimulus [Blackman, Hann, Hamming, None, Rectangular (also called "Rectangle") windows are available], and

6) whether the resulting stimulus file will be acoustically pre-compensated based on the acoustic characterization of the system contained in the file "bbrecvfile".

3.3. ACOUSTICINV.m

The ACOUSTICINV.m script does not directly interact with the user, unless the transmitted and received broad noise files are not of the same length, in which case the user is notified of the error. The ACOUSTICINV.m script creates the acoustic inverse response of the system at the location of the test subject. This script opens the file of signed 16 bit integers ("playfile"), the broadband noise file previously played through the sound emitter, as well as a file of equal length that was received at the hydrophone ("recvfile"). The script then reads the data into two signal matrices, and scales the received signal such that its maximum value is equal to that in the played signal. The discrete Fourier transform of the played signal is then computed using a fast Fourier transform.

The resulting transform is subsequently masked (through convolution) to deal with the zeroes that result from the band-limiting of the played signal. The mask has ones where the discrete transform component was not too small (normalized coefficient > 1/200). A mask is also applied to the received signal to zero out any frequency components that were weakly received (normalized coefficient < 1/50). The generated inverse signal has a DC component equal to zero, is symmetric in time around the origin, and is inverted. If this pre-compensated signal were played at the same time that the original sound source was played, no sound would be detected at the hydrophone (or microphone). This waveform is thus noise-canceling via destructive interference for the system at the location of the hydrophone (or microphone).

After making an acoustic inverse file using the ACOUSTICINV.m script, the MAKESTIMULUSFILE.m script checks that the length of the desired stimulus is not longer in time than the acoustic pre-compensation signal used to characterize the system. It then runs the SEGMENT.m script described below. Note that the SEGMENT.m script is called automatically and uses parameters previously supplied by the user in the MAKESTIMULUSFILE.m script, such as the frequency of the stimulus to be generated, the length in ms, etc.

3.4. SEGMENT.m

The SEGMENT.m script generates sine wave signals that are windowed by various apodization functions. This script can handle signal lengths that are even or odd in size and produces waves either with no windowing; or with Rectangula(r), Hann, Hamming, or Blackman windowing. It only notifies the user if an unknown windowing argument is used in the MAKESTIMULUSFILE.m script. The SEGMENT.m script scales the signal appro-

priately for a 16 bit D/A converter with a range of -10 to 10 volts DC.

After the windowed sine wave is created, its fast Fourier transform is computed and convolved with the acoustic inverse file. The resulting waveform is inverse Fourier transformed. The real part is taken and stored as the pre-compensated stimulus waveform in signed 16 bit integer form as a binary file. Finally, the script writes "ones 0.16" and "ones1.16" files, which are files filled with -1 and +1 values, respectively, into the same directory as the pre-compensated stimulus files. These files are used to create in-phase and out-of-phase stimulus signals to allow for elimination of electrode movement artifacts caused by the sound stimulus itself through subtraction.

To summarize, the scripts must be executed in the following order:

1) Use the BBTEST.m script to produce a broadband noise file in .16 binary format with the user-supplied bandpass, sample rate, sound duration, and sound amplitude. The file produced is called "bb.16" and resides in a user-specified directory.

2) Play the "bb.16" file into the test chamber by sending the data within file to the sound emitter, taking care to set the D/A output rate to the same sample rate that was used to create "bb.16". (Note by the Nyquist sampling criterion, the sample rate needs to be twice the highest frequency to be presented, and preferably at least ten times. For example, a sample rate of 20,000 Hz gives a system maximum frequency of 10,000 Hz, so it would be prudent to output a maximum frequency of 1,000 Hz.) Record the received wave form concurrently from the hydrophone and name it "bb.txt". Place it in the same directory as "bb.16".

3) Run the MAKESTIMULUSFILE.m script, specifying the directory where the "bb.txt" and "bb.16" are now located, the stimulus duration, the type of windowing, the peak stimulus amplitude, the set of discrete frequencies desired in the stimulus files created (each tone will be in its own file), the sample rate to be output, and the phase in degrees at the center of the stimulus. Note that this script also makes two waveforms, each equal in length to the stimulus file, filled either with -1's or 1's. The stimulus waveform can be multiplied by these files to create waveforms that are 180 degrees out of phase (*i.e.*, of opposite polarity) which can be successively presented to the test subject. This procedure can be used to eliminate electrode movement artifacts caused by the sound stimuli. These files are created solely for convenience of the experimenter and are not used in creating the pre-compensated stimulus waveforms.

4. USING THE ACTIVE ECO CANCELLATION TECHNIQUE

Our procedures are easily executed by end-users with only modest familiarity with MATLAB, once the system is fully implemented. Alternatively, script execution can be automated, thereby requiring only minimal input from the end-user. The software also allows for the use of Blackman, Hanning, Hamming, and Rectangular window functions to be applied to the stimulus. These window functions have differing effects on the minimum stopband attenuation (dB) and the width of the transition bandwidth window (Hz). For example the Rectangular window has the poorest minimum stopband attenuation (21 dB), but the best (smallest) transition bandwidth based on order of filtering, approximately 1.84 (system frequency in Hz) / (order of the filter). By contrast, the Blackman filter has the best attenuation (74 Hz) and for the same order filter and system frequency, a transition bandwidth 605% greater than the Rectangular window (11.13 x system frequency/filter order). (The Rectangular window is also known as a Rectangle window and a Dirichlet window.) All of the included window functions in the code presented are examples of high to moderate resolution windows, with lower dynamic range than some other filters such as the Nuttall or Blackman-Harris windows. This flexibility in windowing the acoustic stimulus allows users to tailor the stimulus to the sensory ability of the organism under study [20], and has the advantage that both spectral sidelobes and speaker transients are reduced [21].

The effectiveness of the active echo cancellation procedure (using the Tucker Davis Technologies model System II described in [13]) is illustrated in **Figure 1**. In the absence of an anechoic chamber, windowed electronic sinusoidal waveforms delivered to the speaker (A) result in highly distorted acoustic waveforms at the hydrorophone (B) due to reverberations. In contrast, when precompensated electronic waveforms are delivered to the speaker (C), the resultant acoustic waveforms received by the hydrophone (D) are excellent representations of the desired windowed sinusoidal waveforms (A).

The sound pressure and particle acceleration thresholds of six sciaenid fishes commonly found in Chesapeake Bay (eastern USA) have been successfully assessed using the ABR and the system described above [13]. Likewise, the system has been used successfully at sea aboard a large oceanographic research ship (the NOAA research vessel "Oscar Elton Sette") to assess hearing in yellowfin tuna (*Thunnus albacares*) with the ABR technique (R. Brill, unpublished). Although the steel superstructure of the ship presented a highly echoic environment, the active echo cancellation procedures successfully produced clear sound pulse stimuli required by the
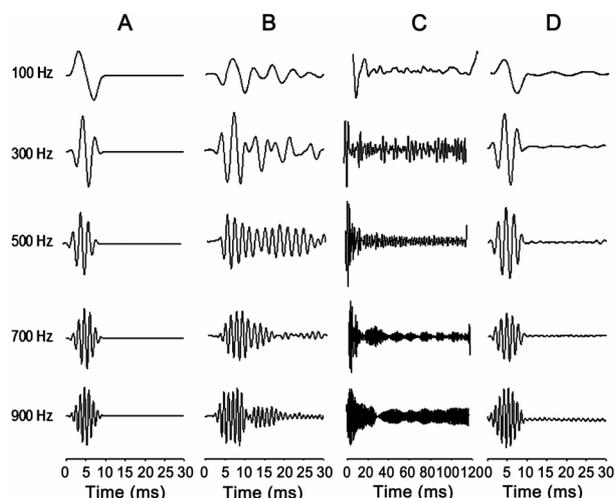
Figure 1. Waveforms generated by the scripts, resulting in echo-cancellation at an omnidirectional hydrophone submerged in a small test tank used to study fish hearing. A = windowed (Blackman filter) sinusoid electronic signal delivered to the speaker. B = windowed non-pre-compensated acoustic signal received at the hydrophone. Note that although the tone burst was set to 10 ms, echoes continue well past the end of the signal and the fundamental frequency is corrupted by additive effects of reverberations. C = pre-compensated electronic signal delivered to the speaker for echo cancellation (the x-axis is 120 ms, instead of 30 ms, because the source sounds produced long after the tone burst are required to cancel tank reverberations). D. Echo-cancelled acoustic signal received at the hydrophone.

ABR technique, allowing the delineation of hearing threshold at specific frequencies (**Figure 2**).

5. LIMITATIONS OF THE ACTIVE ECHO CANCELLATION TECNIQUE

There are two limitations to the active echo cancellation technique. The first is that the juxtaposition of the sound source and all echoic surfaces must remain fixed once the pre-compensated audio files have been generated. Echoic surfaces typically include the water surface (for experiments with aquatic organisms in tanks), major hard surfaces, personnel (if they were near the sound source or test subject when the "bb.16" broadband noise file was played), and the test subject itself. Any changes in the juxtaposition of reflective surfaces will cause the precompensated waveform file to fail to produce an echocancelled signal. Failure of active echo cancellation is easily detected however, if the hydrophone or microphone used to create the pre-compensated waveforms is left in place and the acoustic stimulus waveforms observed during their presentation to the test subject. Should such deviations occur, the sequence of files described above can be quickly re-run. In principle, the pre-compensated files could be created, and the subject introduced into the experimental set-up at the same position as the hydrophone

or microphone. In practice, however, this is impractical because of the likelihood of affecting the juxtaposition of the echoic surfaces. We therefore recommend that compensation be done with subject and hydrophone (or microphone) in their final positions. In our experience, this requirement is not problematic as subjects aregenerally anesthetized, paralyzed, and restrained for ABR studies [13,20].

The second limitation of the procedure is that the most effective echo cancellation occurs at the position of the hydrophone or microphone used to receive the original broadband signal. The effectiveness of echo cancellation diminishes with distance from this position (**Figure 3**). Therefore, the distance between test subject and hydrophone (or microphone) used to create the pre-compensated signal must be minimized and should preferably be less than 5 cm. The spectral composition of the sound signal
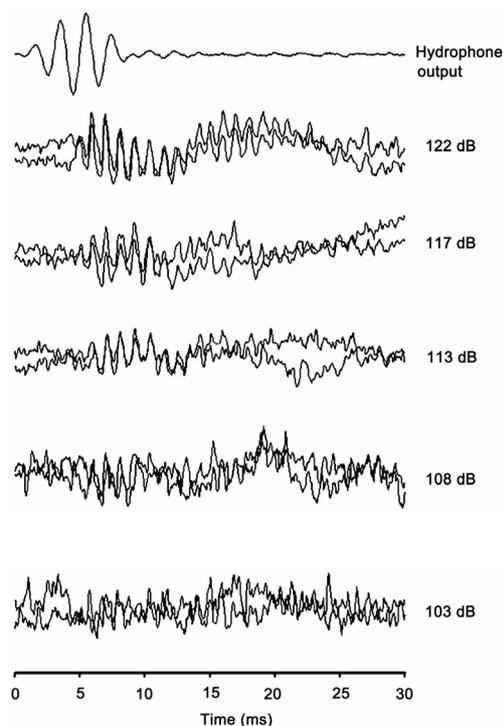

Figure 2. Auditory brain response (ABR) recorded from a yellowfin tuna (*Thunnus albacares*) to a 500 Hz pre-compensated tone burst using a signal processing system (model System II) from Tucker Davis Technologies (Alachua, Florida, USA), and procedures described in [13]. Experiments were conducted at sea, in an interior compartment of a large steel-hulled oceanographic vessel (a highly echoic environment). Sound pressure levels were attenuated in 5 dB steps, and repeated twice at each sound pressure level. Congruence of the ABRs disappears at a sound pressure of less than 108 dB (referenced to 1 µPa at 1 m), indicating that this is minimal detectable sound pressure level.
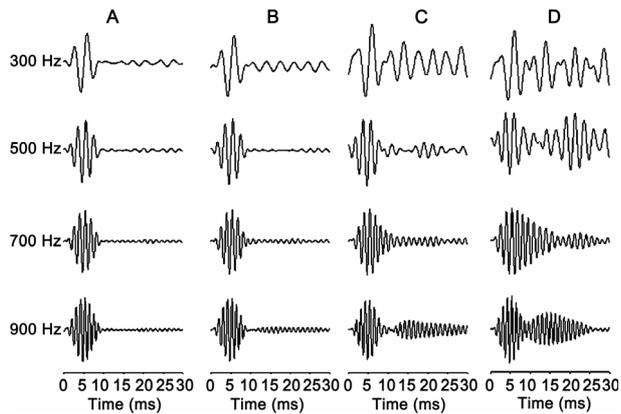

Figure 3. The degradation of the active-echo acoustic signals recorded in the same situation as those in **Figure 1**, but with the hydrophone displaced from its original position. These traces demonstrate how the distance between the hydrophone (or mi- crophone) used during creation of the pre-compensated audio files and the test subject can influence the acoustic signal rec- eived by the latter. A = hydrophone in the original position wh- en pre-compensation acoustic files were created. B = hydrophone moved 1 cm from its original position. C = hydro- phone moved 5 cm from its original position. D = hydrophone moved 10 cm from its original position.
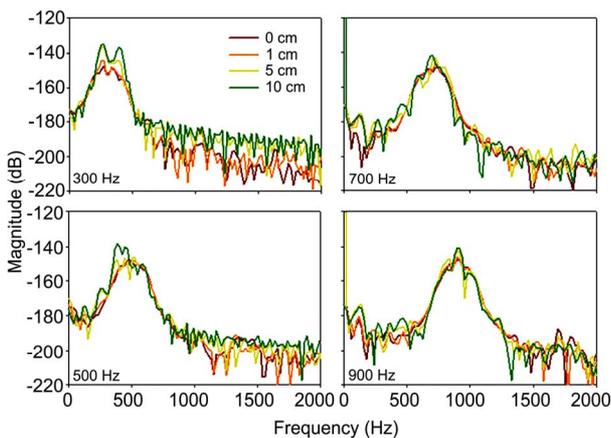

Figure 4. The frequency composition of the active-echo acous- tic signals recorded in the same situation as those in **Figure 1**, but with the hydrophone displaced from its original position. The frequency composition of the stimulus signal is only mod- erately influenced by distance from the hydrophone (or micro- phone) used to create pre-compensated stimulus files, even up to distances of 10 cm. A = hydrophone in the original position when pre-compensation acoustic files were created. B = hyd- rophone moved 1 cm from its original position. C = hydrophone moved 5 cm from its original position. D = hydrophone moved 10 cm from its original position.

is not greatly affected however (**Figure 4**). The decrease in effectiveness of active echo cancellation with distance from the hydrophone or microphone used during the initial development of the pre-compensated audio files is an important limitation that must be taken into consid- eration when this procedure is employed. It is possible that with large fishes the acoustic signal received di- rectly by the otolithic organs and signals resulting from vibrations of the swim bladder [22,23] could be different because the signal would be most effectively echo-cancelled at the structure nearest to the hydrophone used when making the pre-compensated files. Thus our technique, although highly effective at minimizing signal rever- berations, should be carefully applied and investigators fully cognizant of its limitations.

6. ACKNOWLEDGEMENTS

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