AUDITORY EVOKED POTENTIALS OF THE LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*)

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Presented to
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The College of William and Mary in Virginia

In Partial Fulfillment
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Masters of Arts

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by
Soraya E. Moein
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APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

Masters of Arts

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Approved, July 1994

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This thesis is dedicated to my parents, George and Jane Moein, who provide an immeasurable amount of guidance, patience, and love.

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ABSTRACT

Repulsion from hopper dredges using auditory stimuli is one frequently proposed solution for reducing incidental mortalities of sea turtles. However, before this tactic can be assessed, research must first be performed on the auditory mechanism of sea turtles, an area underdeveloped in the literature. In this study, threshold for response to stimuli and the effects of stimuli and white noise on the threshold were determined for the loggerhead sea turtle, Caretta caretta.

35 juvenile loggerhead turtles caught in the Chesapeake Bay were used in this study. A computer capable of delivering stimuli and receiving bioelectric activity via electrodes implanted in the loggerhead sea turtle was used. Either a low frequency broadband click or tone bursts (250, 500, 750 or 1000 Hz) were deliver by a bone vibrator to the turtle's tympanum. Intensity and frequency of stimulus was manipulated for the threshold experiment. Rate of stimulus presentation and intensity of white noise were manipulated for the rate and masking experiments, respectively.

The maximum sensitivity was in the low frequency region of at least 250 to 1000 Hz with a maximum sensitivity at 250 Hz of -24.4 dB re: 1 gravity unit. The broadband click produced clear auditory response with a mean threshold of -10.8 dB re: 1 gravity unit and 8.5 dB re: 1 dynes/cm^2. In the rate experiment, interpeak latencies for peak I and peak V were significantly dependent on rate. In the masking experiment, signal to noise ratios ranged from -3.5 to -8.5 dB ($x=-5.2 \pm 2.4$).

The broadband click stimuli elicited synchronous neural activity of the hair cells and was determined to be the most efficient stimulus to use when recording threshold from the loggerhead sea turtle. An increase in the stimulus rate resulted in the disruption of neural synchrony and thus interpeak latencies increased with rate of stimulus. Finally, loggerheads appear to be able to resolve the stimulus through a high level of white noise. These techniques of auditory evoked potentials may be utilized in two fields of applied research: the development of an acoustic repelling device and the identification of diseases of the brain of sea turtles.

AUDITORY EVOKED POTENTIALS OF THE LOGGERHEAD SEA TURTLE (*CARETTA CARETTA*)

INTRODUCTION

Hopper dredging is the most effective way of widening and deepening channels to accommodate deep draft shipping traffic. However, this procedure alters marine habitat and disrupts residing marine life. One group of marine organisms largely affected by dredging is sea turtles, animals protected by the Endangered Species Act of 1973 (Dickerson et al., 1991, Studt, 1985). Sea turtles have been found entrained and killed during dredging operations (Hopkins & Richardson, 1984). These operations may harm all five species of sea turtles found along the eastern United States coast: the loggerhead (Caretta caretta), green (Chelonia mydas), Kemp's ridley (Lepidochelys kempii), hawksbill (Eretomochelys imbricata), and the leatherback (Dermochelys coriacea). However, the National Marine Fisheries Service (NMFS) has concluded that only Kemp's ridley, loggerhead, and green sea turtles may be at risk by hopper dredging activities because of their geographic distribution and life history attributes (Grossblatt, 1990).

The concern over the mortality of sea turtles from dredging increased in 1980 at Port Canaveral Channel,

Florida, when an unusually large number of loggerheads were present. Over 77 loggerheads were killed by dredges during the removal of 1.9×10^6 m^3 sediment (Carr et al., 1981; Joyce, 1982). When mortality from dredging was first recognized, NMFS and the US Army Corps of Engineers (COE) trawled the channel and relocated approximately 1,250 loggerheads from Canaveral channel to offshore locations. This relocation project was not entirely successful, however, because many loggerheads returned to the channels immediately (Grossblatt, 1990).

Other courses of action are being explored to reduce mortalities from hopper dredging, including the appointment of observers on hopper dredges to identify turtle parts, modification of dredge dragheads to displace turtles, radio and sonic tracking in navigation channels to determine habitat utilization, and investigation of repulsion of sea turtles from dredges using auditory stimuli (Grossblatt, 1990). Repulsion via auditory stimuli may help reduce the incidental take of sea turtles by dredges, however, the feasibility of an acoustic repelling device must be evaluated.

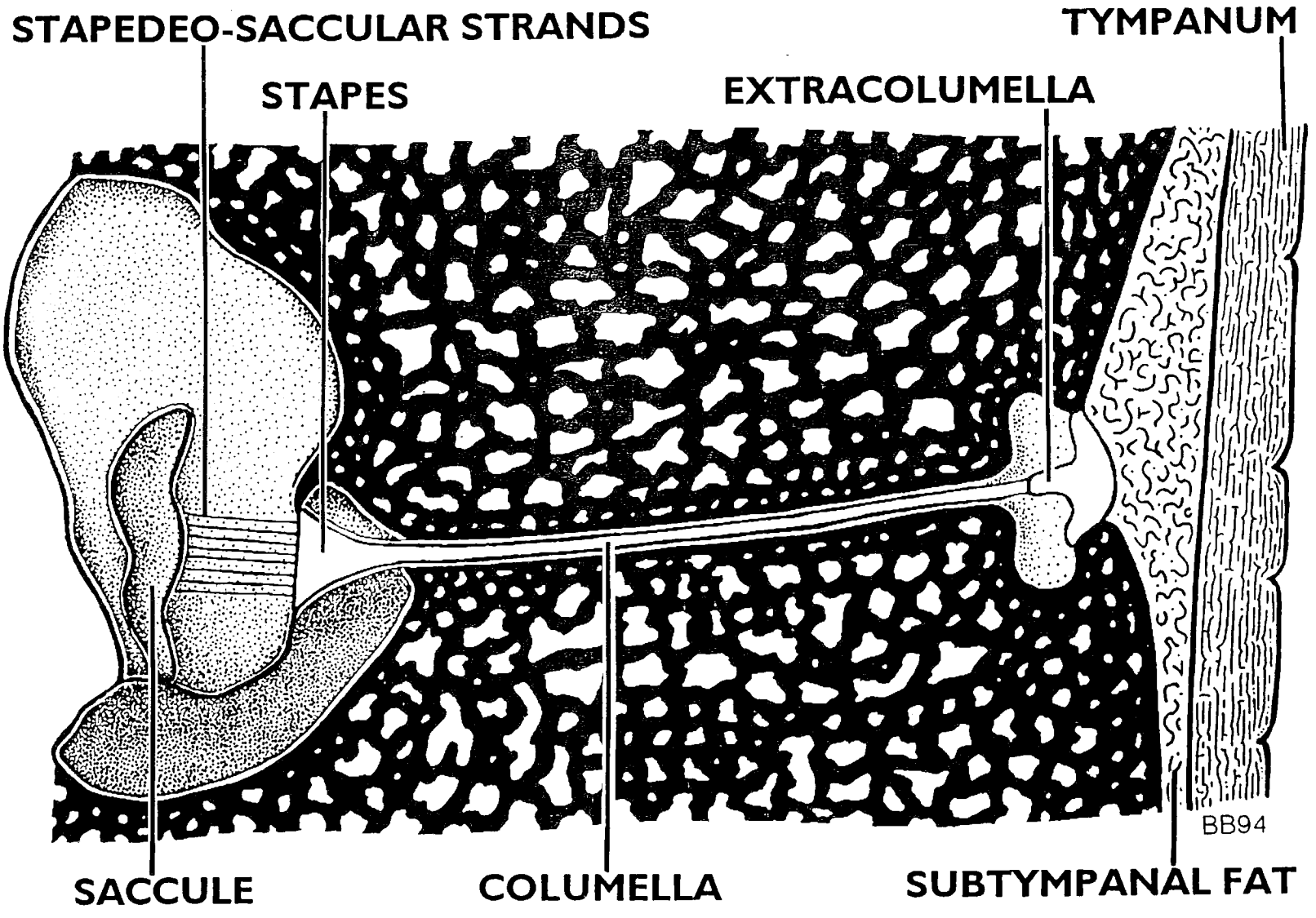
Until recently, little research has been performed on the auditory mechanism of sea turtles. Almost nothing is known about how sea turtles use hearing under natural conditions or its role in their adaptive behavior. Sea turtles have been reported to show a lack of response to even intense sounds (Wever, 1978). Thus, a number of

factors, including anatomy, behavior responses, and electrophysiological response to sound, should be considered when evaluating the hearing capabilities of sea turtles.

The anatomy of the turtle ear has been well researched (Lenhardt et al., 1985; Manley, 1970; McGill, 1960; Patterson, 1966; Wever, 1978). Sea turtles have a thick layer of subtympanal fat, a feature which distinguishes them from both terrestrial and semiaquatic species (Fig 1). There is no external ear, and the tympanum is a continuation of the facial tissue. Removal of the tympanum produces only negligible change in the displacement of the columella (middle ear bone) which suggests that the tympanum is a poor aerial receptor (Moffat and Capranica, 1978). Unlike mammals, sea turtles have no pinnae, ear canal or elongated coiled cochlea, which are associated with sensitivity, localization, and the determination of frequency range (Wever, 1978).

Sea turtles have an ossicular mechanism consisting of two elements, the columella and the extracolumella. The extracolumella is a cartilaginous disk under the tympanic membrane which is attached to the columella by ligaments. The columella is long and curved with the majority of the mass concentrated at each end. The proximal end expands within the oval window to form a funnel shaped stapes. Unique to all sea turtles are the stapedo-saccular strands; fibrous strands which connect the stapes and the oval window to the saccule. The stapedo-saccular strands presumably

Figure 1. Schematic drawing of the loggerhead middle ear.


STAPEDEO-SACCULAR STRANDS
TYMPANUM
STAPES
EXTRACOLUMELLA
BB94
SACCULE
COLUMELLA
SUBTYMPANAL FAT

relay vibrational energy to the saccule (Lenhardt et al., 1985; Wever and Vernon, 1956). The shape of the columella and its interactions with the cochlea and saccule suggest that the sea turtle's middle ear is a compromise for sound conduction through two media, bone and water. Through the utilization of bone conduction, sound flows via the bones and soft tissues of the turtle. The ear drum acts as a release mechanism rather than a sound receptor (Bekesy, 1948; Lenhardt, 1982; Lenhardt et al., 1983; Lenhardt and Harkins, 1983; Tonndorf, 1972). High frequencies are attenuated by bone which limits the range of frequency heard by sea turtles to low frequencies. Furthermore, it is believed that the thick layer of subtympanal fat functions as additional mass loading to the ear and consequently lowers the frequency sensitivity (Tonndorf, 1972).

Studies performed on the cochlear hair cells of turtles are extensive (Art et al., 1985, Crawford and Fettiplace, 1980; Fettiplace and Crawford, 1980; Manley, 1974; Paton et al., 1976). These experiments were performed on the isolated half-head of the red eared turtle (Pseudemys scripta elagans). Fettiplace and Crawford (1980) compared membrane potential changes to the frequency of the acoustic stimulation and concluded that cochlear hair cells convert the basilar membrane motion (the nerve terminals, hair cells, and supporting cells) into electrical signals. These electric signals are then received by the auditory nerve. In another study, Crawford and Fettiplace (1980) established

frequency-threshold curves of auditory nerve fibers (hair cells) for eleven red eared turtles by recording the responses of single cochlear hair cells. These threshold curves fell between 30-700 Hz with no evidence of discontinuity.

While the range of frequencies turtles may hear has been established through the study of the turtle anatomy and physiology, the appropriate presentation of these frequencies to turtles has not. Low frequencies may be presented to the loggerhead as tones, clicks, or modulated frequencies presented pulsed or continuously. The ability of the turtle to analyze sound can depend on how sound stimuli are presented (Wever, 1949). One method of examining this ability to analyze sound is by performing conditioning or localization experiments. Early studies used an aerial sound source only a few centimeters away (Andrews, 1915; Chernomidikov, 1958; Karimova; 1958; Kuroda, 1923; Kuroda, 1925; Poliakov 1930). However, these behavioral studies could not be replicated. For instance, Andrews (1915) trained turtles of the genus Chrysemys to approach food at the sound of a bell but not to approach food at the sound of a whistle. Kuroda tried to repeat this study, using the same methodology, in both 1923 and 1925, without success. Poliakov (1930) conditioned the european pond turtle (Emys orbicularis) to withdraw its head using a variety of sounds, bells, noises, and pure tones, Chernomidikov (1958) and Karimova (1958) attempted to

replicate this experiment, but were unsuccessful. Furthermore, there are no published underwater localization studies for sea turtles. Thus, the appropriate presentation of the frequencies, whether tones or clicks presented continuously, pulsed or intermingled, has not yet been established.

A few attempts have been made to collect electrophysiological responses to the aerial stimulation of the turtle's hearing apparatus. Wever and Vernon (1931) were successful in attaining synchronized responses from the eighth cranial nerve (the auditory nerve) of the painted turtle (Chrysemys picta) with responses occurring below 500 Hz. Adrian et al. (1938) reported a response of the eighth cranial nerve of the eastern box turtle (Terapene carolina) and the spur-thighed tortoise (Testudo graeca) using tones of 400 Hz. Finally, Wever (1978) measured the sensitivity of cochlear potentials of 14 species of turtles using an aerial sound source.

Electrophysiological research on sea turtles, however, has been less promising. Foa and Peroni (1930) applied an electrode to the eighth cranial nerve of the loggerhead sea turtle and used organ pipe tones between 16.5 and 132 Hz as the aerial stimulus. However, the resulting potentials did not appear to relate to the stimulus. The only other attempt to collect electrophysiological data from sea turtles was one study performed on the green sea turtle (Ridgeway et al., 1969). The frequencies tested on these

turtles ranged from 50 to 2000 Hz. The results revealed that green sea turtles detected limited sound frequencies (200-700 Hz) and displayed a high level of sensitivity at the low tone region of about 400 Hz. Moreover, with an increase in frequencies, their range of sensitivity declined by a rate of 40 dB per octave.

Threshold levels also play an important role in evaluating turtle hearing responses. Threshold of hearing is the lowest stimulus intensity below which the stimulus ceases to be heard (Gibson, 1982). It appears that the use of feeding/conditioning response, though adequate for generalized studies, is not a reliable method in determination of thresholds (Tavolga, 1963). There has yet to be established a clear cut criterion of response behavior to determine threshold. A standard behavior has not been identified because thresholds are a statistically determined point around which there exists a probability of positive responses both above and below the determined threshold. Consequently, as the researcher approaches the subject's threshold level, behavior of the test subject can become variable (Tavolga, 1963). In order to obtain a more reliable measurement of a threshold level to a stimulus, auditory evoked potentials can be measured.

Auditory evoked potentials are electric responses to the stimulation of the nervous system; they are the sum of the action potentials of the initial discharge of many neurons firing in synchrony due to stimulation. These

potentials consist of a series of waves identified by amplitude and latency. However, a problems occurs when measuring single auditory evoked responses. Excessive biological noise of ongoing neural and muscular electrical activity introduces components unrelated to the stimulus (Spehlmann, 1985). This problem can be solved by summing and averaging single auditory responses. In the absence of stimulation, the electroencephalogram (EEG) is random at any one moment, thus there are as many positive as negative values at any point. When these random values are averaged, the EEG should appear as a flat line. Alternatively, if a neural discharge occurs at a certain time (latency) as the stimulus is presented, then the summation and averaging of many response times locked to the stimulus will produce an exaggeration of the single response (Gelfand, 1990).

Auditory evoked responses to stimuli can be described by their response latency. The earliest brainstem responses occur within the first eight milliseconds and have been coined the "Jewett bumps" (Chiappa et al., 1979; Jewett, 1970; Jewett et al., 1970). Studies on humans and cats have led to the mapping of these peaks as follows: peak I, auditory nerve; peak II, cochlear nuclei; peak III, superior olivary complex; peak IV, midbrain; and peak V, inferior colliculus (Buchwald and Huang, 1975; Chiappa et al., 1979; Markand, 1994; Rowe, 1978). The absolute locations of the peaks in the sea turtle are not conclusive, in fact it is thought that the peaks beyond peak I are the

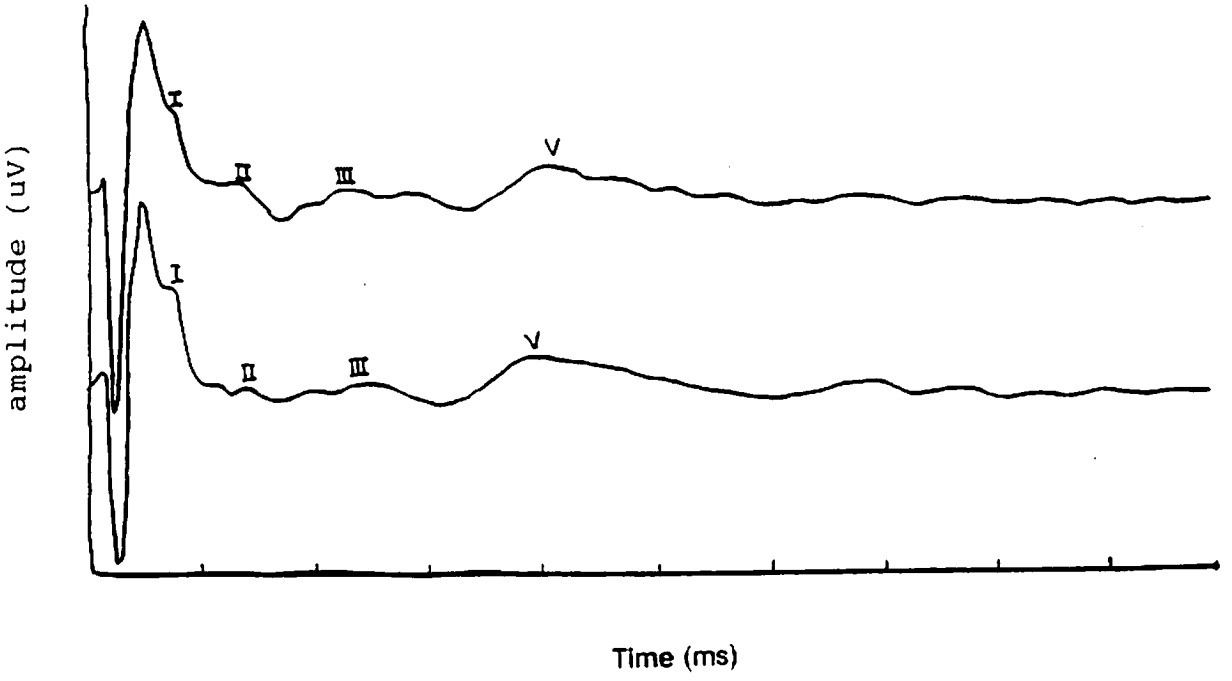
result of the summation of multiple sources. Of the peaks found in the first 8 ms, peak V is the largest and most predictable (Gelfand, 1990), and thus can be used as the index peak to establish threshold (Fig 2).

Two variations of the threshold test parameters can be examined to test their effect on the synchrony of the neural response collected by auditory evoked potentials. The rate of the stimulus can be tested to determine its effect on the conduction time of peak I and V. Secondly, the ability of the loggerhead to distinguish a stimulus through ambient noise can be investigated using a masker of white noise.

In humans, increasing the click stimulus rate prolongs all the peaks, but the latency of peak I appears to be the least affected (Chiappa et al., 1979; Markand, 1994). It is possible to examine the effectiveness of the turtle's ability to analyze sound at different rates of presentation by examining the I-V interpeak conduction time, the time taken for the stimulus to travel between the origins of these two peaks. This analysis can be accomplished by examining the auditory evoked potentials and their peak's latencies at various clicks per second.

Masking transpires when the threshold of audibility of the stimulus is raised by the introduction of another sound (noise) (Green, 1976; Yost and Neilsen, 1977). By incorporating white noise with the stimulus, the signal and noise levels at which the masker wipes out the synchrony of the stimulus and the threshold for the stimulus is canceled,

Figure 2. Auditory evoked potentials collected from the loggerhead sea turtle. The two waves represent the output from the left and right ear. Peak I, II, III, and V are the earliest brainstem responses which occur within the first 10 ms of stimulation.


amplitude (uV)
I
II
III
V
I
II
III
V
Time (ms)

can be determined.

The objectives of this project were threefold: a) collect auditory evoked potentials from loggerhead sea turtles to determine threshold of response for both tone bursts and click stimuli, b) test the stimulus rate as presented to the loggerhead for its effect on the I-V interpeak conduction time, and c) test white noise for its ability to mask the stimulus and render the stimulus inaudible. These goals were achieved by laying out a methodology for collecting evoked potentials from sea turtles.

MATERIALS AND METHODS

Thirty-five healthy loggerhead turtles were used for this study (Table 1). The turtles were caught by poundnets in the Chesapeake Bay: at the mouth of the Potomac River and in Mobjack Bay, at the mouth of the York River. The animals were housed in tanks in a greenhouse facility prior to testing.

Bioelectric measurement

Auditory evoked potentials may be measured from sea turtles. Turtles were placed in a box to reduce extraneous vibrations. Subdermal electrodes were implanted on either side of the fronto-parietal plate on the dorsal surface of the head. A reference electrode was inserted in the skin immediately behind the skull over the extension of the supraoccipital. Finally, a ground electrode was placed in the inactive skin of the lateral neck (Fig 3).

A computer capable of delivering stimuli and receiving bioelectric activity (Nicolet Spirit Portable) was used to measure evoked potentials. This computer contains an

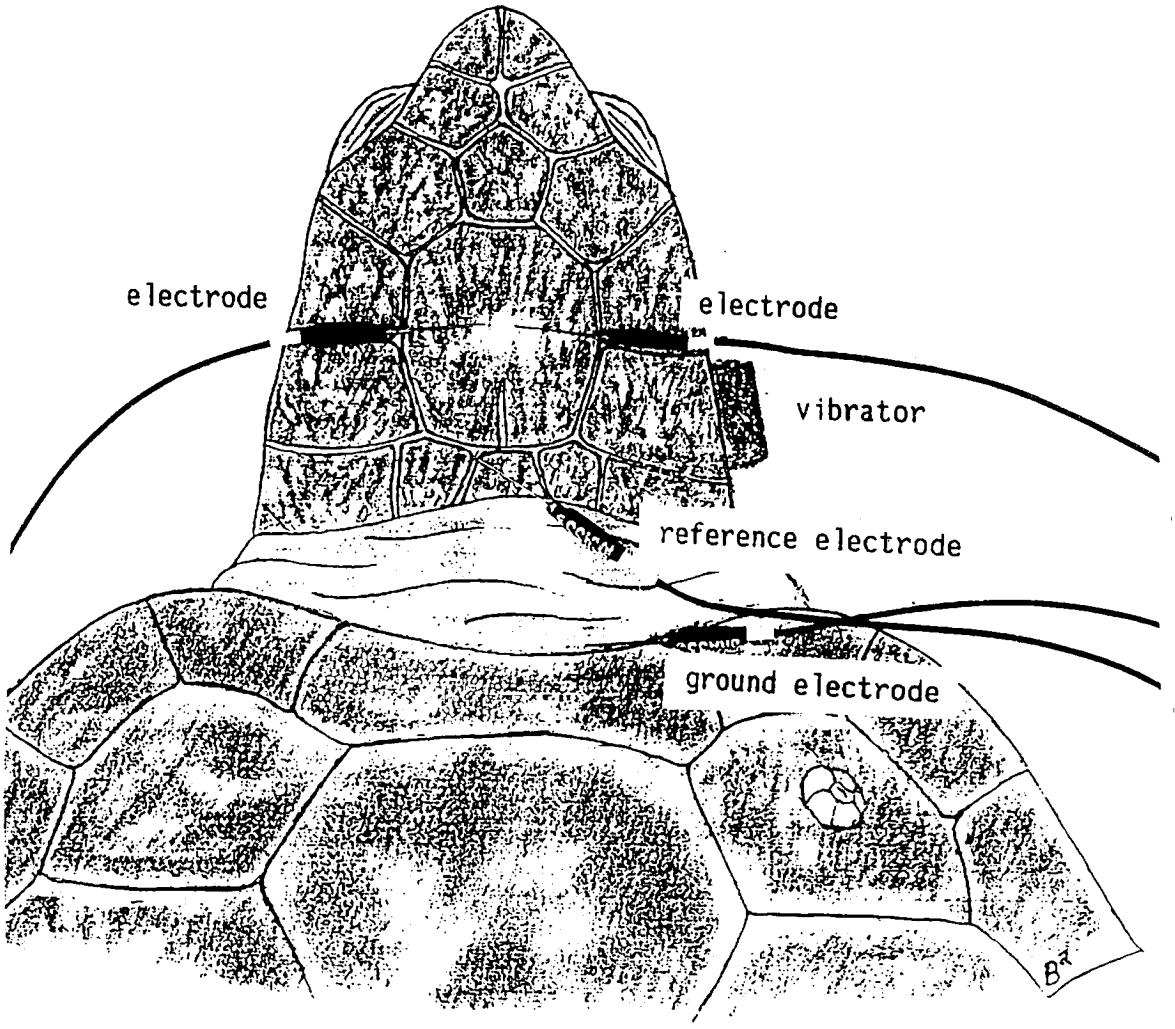
Table 1. Tag numbers, dates captured and released, weight, and length of the 35 loggerhead turtles used in the three phases of the hearing study.

Front Flipper Tag#	Date Captured	Date Released	Weight (Kg.)	Length (Curved Notch to Notch) (cm)
QQM791 QQM794	20 JUL 92	27 MAY 93	26.0	57.7
PPX804 PPX817	30 JUL 92	7 JUN 93	69.0	83.1
QQM800 QQM785	3 AUG 92	27 MAY 93	25.0	56.4
QQM700 QQM701	17 AUG 92	7 JUN 93	21.0	57.2
QQM797 QQM798	31 AUG 92	7 JUN 93	35.0	67.0
QQM792 QQM775	11 SEP 92	21 JUL 93	27.0	61.1
QQM605 QQM606	15 SEP 92	21 JUL 93	33.0	60.1
QQM791 PPX807	21 SEP 92	7 JUN 93	24.0	56.5
QQZ417 QQZ401	2 NOV 92	2 JUN 93	32.0	63.4
QQZ418 QQZ414	5 NOV 92	7 JUN 93	48.3	75.7
QQZ407 QQZ406	26 MAY 93	3 AUG 93	19.5	54.7
QQZ409 QQZ408	26 MAY 93	16 JUN 93	31.0	63.5
QQZ426 QQZ427	4 JUN 93	21 JUL 93	N/A	N/A

Front Flipper Tag#	Date Captured	Date Released	Weight (Kg.)	Length (Curved Notch to Notch) (cm)
QQZ429 QQZ430	8 JUN 93	3 AUG 93	14.0	48.7
QQZ437 QQZ438	15 JUN 93	10 AUG 93	27.0	62.0
QQZ441 QQC530	16 JUN 93	24 JUL 93	24.5	55.5
QQZ442 QQZ443	19 JUN 93	10 JUL 93	55.0	78.6
QQZ451 QQZ452	21 JUN 93	24 JUL 93	28.5	61.4
QQZ455 QQZ456	22 JUN 93	27 JUL 93	99.3	97.0
QQZ476 QQZ477	24 JUN 93	28 JUL 93	N/A	69.0
QQZ482 QQZ483	2 JUL 93	8 JUL 93	34.8	64.3
QQZ486 QQZ487	6 JUL 93	2 AUG 93	23.0	53.8
QQZ492 QQZ493	13 JUL 93	10 AUG 93	N/A	69.0
QQZ496 QQZ497	16 JUL 93	4 SEP 93	26.0	58.8
QQZ500 QQZ353	21 JUL 93	4 SEP 93	23.8	54.0
QQZ354 QQZ355	22 JUL 93	3 NOV 93	35.0	64.2
QQZ425 QQZ424	23 JUL 93	13 SEP 93	19.0	53.2
QQZ360 QQZ361	27 JUL 93	13 SEP 93	21.5	56.0
QQZ362 QQZ363	30 JUL 93	3 NOV 93	32.0	63.0
QQZ364 QQZ380	3 AUG 93	3 NOV 93	30.0	58.5

Front Flipper Tag#	Date Captured	Date Released	Weight (Kg.)	Length (Curved Notch to Notch) (cm)
QQZ368 QQZ369	10 AUG 93	12 NOV 93	55.0	77.1
QQM764 QQM772	10 AUG 93	3 NOV 93	32.5	64.9
SSB801 SSB802	18 MAY 94	7 JUN 94	27.0	58.3
SSB805 SSB806	19 MAY 94	7 JUN 94	14.0	49.7
SSB827 SSB818	25 MAY 94	7 JUN 94	23.0	54.8

Figure 3. Placement of the electrodes and mechanical vibrator on the head of the loggerhead sea turtle when collecting auditory evoked potentials.


electrode
electrode
vibrator
reference electrode
ground electrode

interface for the electrodes. Two channels, left and right, of electroencephalographic (EEG) activity were amplified (x20k) and filtered (5-3000 Hz) by the computer. Bioelectric activity was time-locked to the delivery of the stimulus (mechanical vibrator) secured over the eardrum and thus recorded by the computer at the same rate as the stimulus. Evoked potentials were extracted from the EEG by repeating and averaging single responses. At least 500 responses were averaged for each trial. Averaging reduces the components of the EEG unrelated to the stimulus (such as muscle contractions and other extraneous biological activity) so that responses can be clearly distinguished (Spehlmann, 1985). A time window of 10 milliseconds for collecting EEG activity was set on the computer. The stimulus used was either a broadband click composed of a frequency spectrum from 250-1250 Hz or tone bursts with a central frequency at 250, 500, 750, or 1000 Hz. The actual frequency was obtained by coupling the vibrator to a piezoelectric film sensor and measuring the energy with a real time spectral analyzer (Appendix).

Threshold measurements

All turtles were used in the threshold experiments. Stimuli of either clicks composed of a broadband frequency or tone bursts were delivered through the bone vibrator strapped to the tympanum. The intensity of the stimulus was manipulated, ranging from -36 to 7 dB [re: one gravity

unit (g)]. An accelerometer was used to measure the intensity of the stimulus, and acceleration of the mechanical vibrator was obtained.

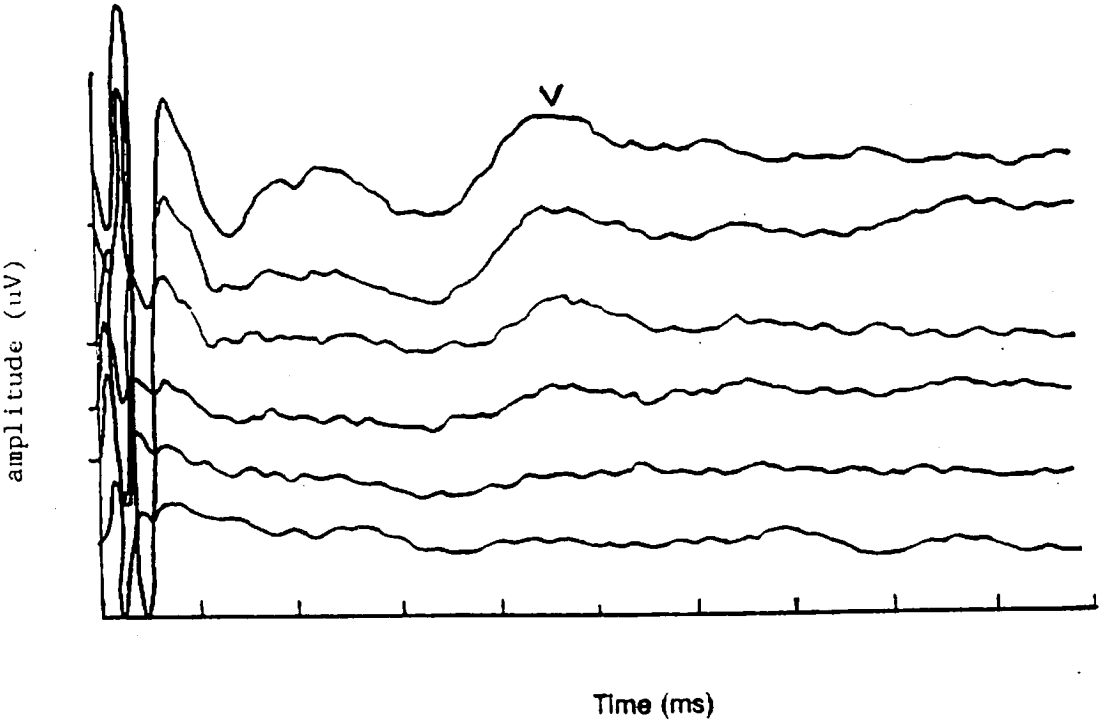
Measurement of the stimulation of the auditory nervous system with the use of this bone vibrator was through the examination of the EEG readouts produced by the computer. A positive wave at about 4.5 milliseconds (peak V) was used as an index for determining threshold. This wave decreased in amplitude and increased in latency as the stimulus intensity decreased. The lowest intensity at which peak V was identifiable by subjective observation was termed the threshold (Fig 4).

Measurements of threshold of hearing for clicks were also converted to sound pressure level (SPL), a reference level commonly used by researchers. Evoked potentials were measured in the manner described above. However, for this test, the stimulus was presented by a loudspeaker positioned above the turtle's head. The click intensity was measured with an SPL meter held between the loudspeaker and the turtle's head. Threshold was measured and compared to the threshold obtained from the bone vibrator.

Repetition rate

Nineteen of the 35 loggerheads were used in the repetition rate experiments. The broadband click used in the threshold study was utilized as the stimulus (with a fixed intensity of 6 dB re: 1 gravity unit) to examine the

Figure 4. Representative drawing of the EEG waves measured from a loggerhead sea turtle while testing for hearing threshold. Peak V is the index peak used to determine threshold.


V
amplitude (uV)
Time (ms)

response to the change in the repetition rate. The rate of the click was then systematically varied from 1.1 to 90.1 clicks per seconds. Interpeak latency between peak I and peak V was computed as the conduction time between the peaks (Fig 5). Interpeak latencies were examined for dependence on the rate of the stimulus by performing a regression for each of the 19 turtles (Zar, 1984).

Masking Experiment

Fifteen of the 35 loggerheads were used in the stimulus masking experiments. White noise was incorporated into the stimulus so that both noise and click were delivered to the same ear. The click intensity remained constant at a superthreshold level and was determined for each turtle individually. Repetition rate of the stimulus was fixed at 10.1 clicks/s. The white noise was varied from 20 dB below the click intensity to 10 dB above the click intensity (Fig 6). Signal and noise levels for the last point at which the turtle could distinguish the stimulus were measured and signal to noise ratios were determined. In decibels, the signal to noise ratio is equal to the signal energy minus the white noise energy (Yost and Nielsen, 1977).

Figure 5. An example of waves collected from a loggerhead sea turtle while examining for the effect of stimulus rate on the latencies of peak I and V. Rates tested were 1.1, 10.1, 20.1, 30.1, 40.1, 50.1, 60.1, 70.1, 80.1, and 90.1 respectively.

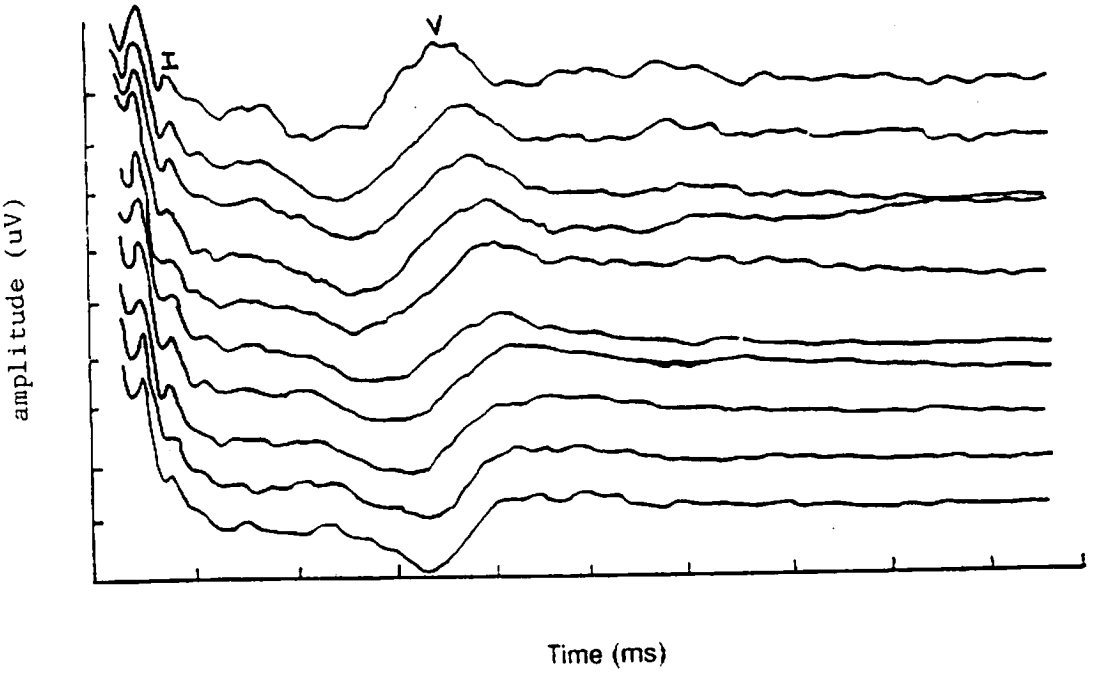
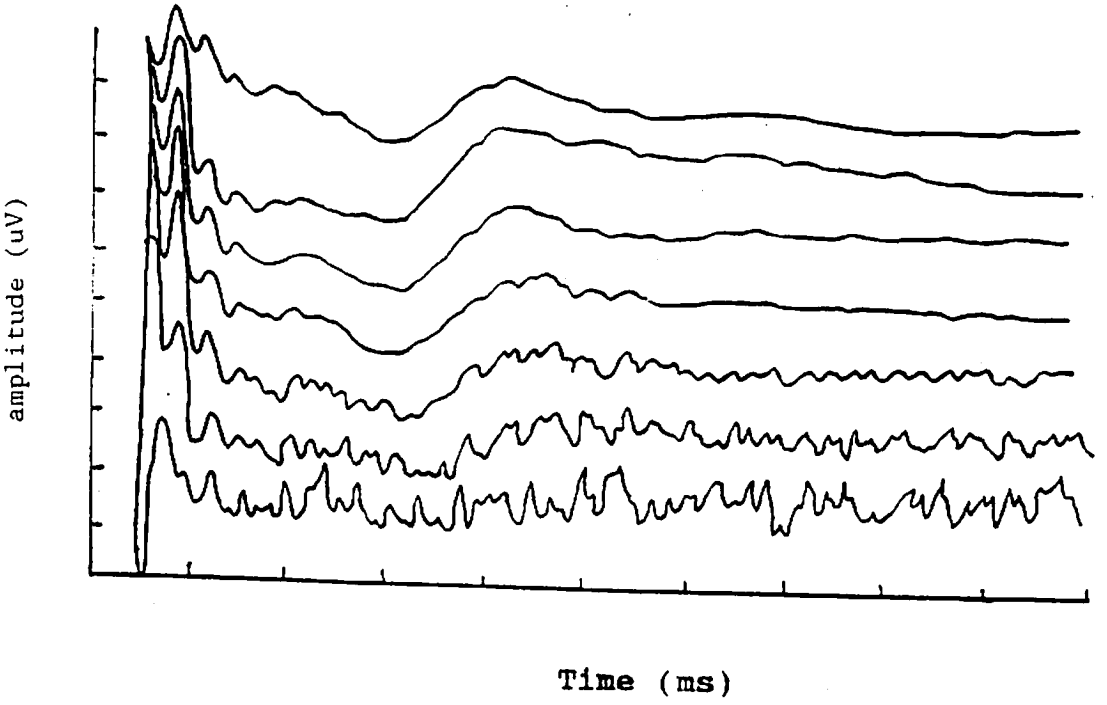

V
I
amplitude (uV)
Time (ms)

Figure 6. Representative evoked potential waves collected when white noise and broadband click were used as the stimuli for a loggerhead sea turtle. The click intensity remained constant and noise intensity varied from 20 dB below to 10 dB above the click intensity.


amplitude (uV)
Time (ms)

RESULTS

The broadband click produced very clear and repeatable auditory responses. The mean intensity threshold for the 35 turtles was -10.8 dB re: 1 gravity unit with a standard deviation of 4.6 dB. It was possible to convert these data into sound pressure level with a resulting mean of 8.5 dB re: 1 dyne/cm^2 with a standard deviation of 5.5 dB (Table 2).

There were several difficulties in recording the auditory evoked potentials from tone burst stimuli. Readable and repeatable responses were extracted from only six of the turtles tested. Furthermore, it was impossible, with the available equipment, to convert the decibel levels of tones into sound pressure levels. Thus, the evoked potentials (Figures 7-12) were due to vibratory stimulation and calibrated in decibels relative to acceleration (gravity). The maximum sensitivity was in the low frequency region of 250-1000 Hz. The decline in sensitivity was great after 1000 Hz and beyond the recording capabilities of the equipment. The most sensitive threshold for these five turtles was found to be at 250 Hz with a mean intensity

Table 2. Threshold level of hearing with a click stimulus for loggerhead sea turtles calibrated in both acceleration and sound pressure level.

Front Flipper Tag Numbers	Threshold dB re: 1 gravity	Threshold dB: 1 dyne/cm^2
QQM700/QQM701	-16.5	1.7
QQM791/QQM794	-16.5	1.7
QQM792/QQM775	-12.5	6.5
QQM791/PPX807	-17.5	0.5
QQM797/QQM798	-9.5	10.1
QQM605/QQM606	-14.5	4.1
QQM800/QQM785	-16.5	1.7
QQZ417/QQZ401	-12.5	6.5
QQZ418/QQZ414	-7.5	12.5
PPX804/PPX817	-1.5	19.6
QQZ409/QQZ408	-7.5	12.5
QQZ426/QQZ427	-5.5	14.9
QQZ407/QQZ406	-10.5	8.9
QQZ429/QQZ430	-14.5	4.1
QQZ441/QQC530	-7.5	12.5
QQZ437/QQZ438	-14.5	4.4
QQZ442/QQZ443	-7.5	12.5
QQZ451/QQZ452	-6.5	14.9
QQZ455/QQZ456	-1.5	19.5
QQZ476/QQZ477	-7.5	12.5
QQZ482/QQZ483	-9.5	10.1
QQZ486/QQZ487	-9.5	10.1

Front Flipper Tag Numbers	Threshold dB re: 1 gravity	Threshold dB: 1 dyne/cm^2
QQZ492/QQZ493	-12.5	6.5
QQZ500/QQZ353	-19.5	-1.8
QQZ496/QQZ497	-7.5	12.5
QQZ360/QQZ361	-15.5	2.9
QQZ362/QQZ363	-12.5	6.5
QQZ425/QQZ424	-12.5	6.5
QQZ364/QQZ380	-9.5	10.1
QQZ354/QQZ355	-7.5	12.5
QQM764/QQM772	-12.5	6.5
QQZ368/QQZ369	-9.5	10.1
SSB801/SSB802	-2.5	18.4
SSB805/SSB806	-17.5	0.5
SSB827/SSB818	-12.5	6.5
Mean $\pm$ Standard deviation	-10.8 $\pm$ 4.6	8.5 $\pm$ 5.5

Figure 7. Threshold levels collected from turtle QQM800/QQM785 for 250, 500, 750, and 1000 Hz frequency levels. The intensity level is due to vibratory stimulation and is calibrated in decibels relative to acceleration.

Threshold Levels for Turtle QQM800/QQM785

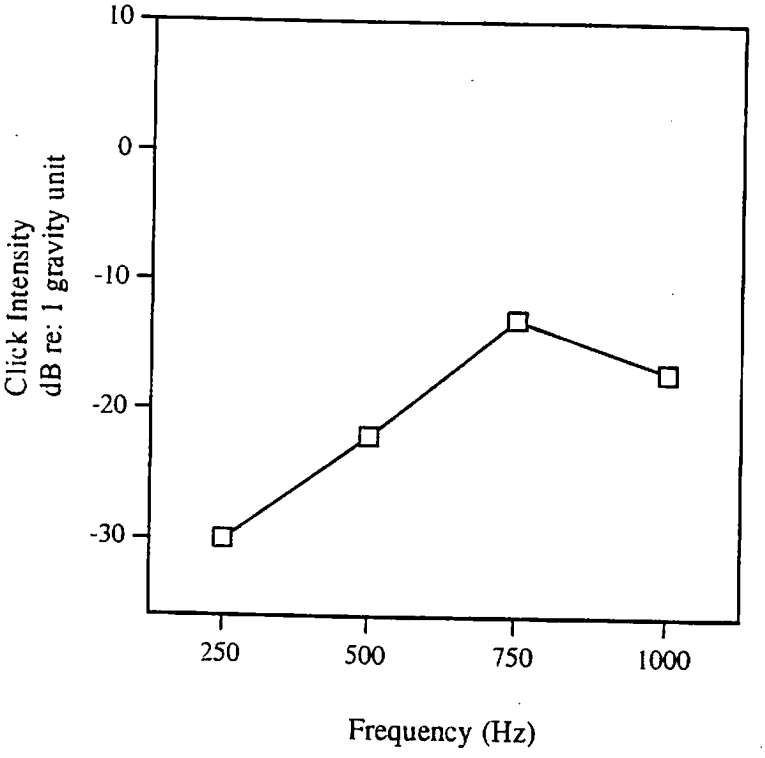


Figure 8. Threshold levels collected from turtle QQZ418/QQZ414 for 250, 500, 750, and 1000 Hz frequency levels. The intensity level is due to vibratory stimulation and is calibrated in decibels relative to acceleration.

Threshold Levels for Turtle QQZ418/QQZ414

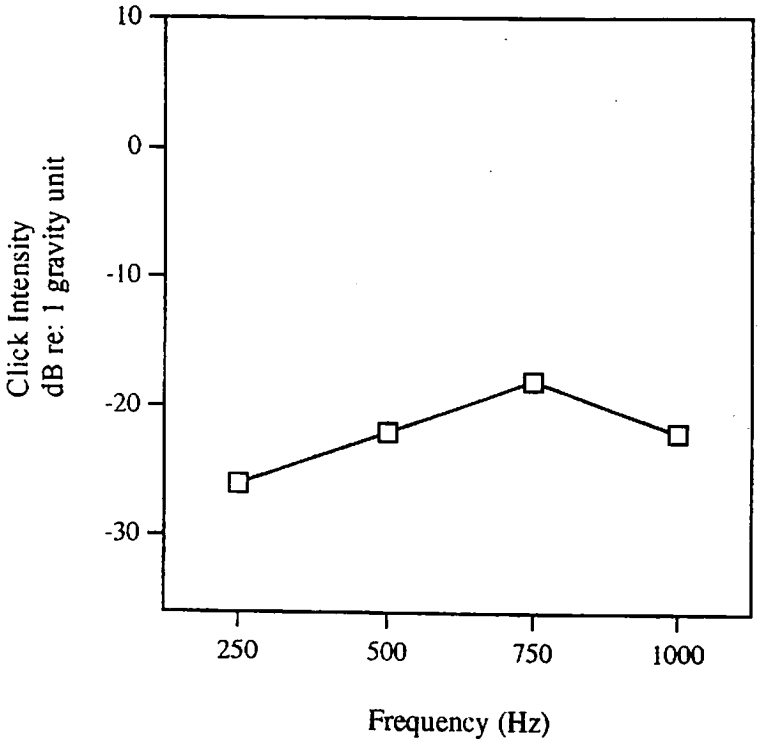


Figure 9. Threshold levels collected from turtle SSB805/SSB806 for 250, 500, 750, and 1000 Hz frequency levels. The intensity level is due to vibratory stimulation and is calibrated in decibels relative to acceleration.

Threshold Levels for Turtle SSB805/SSB806

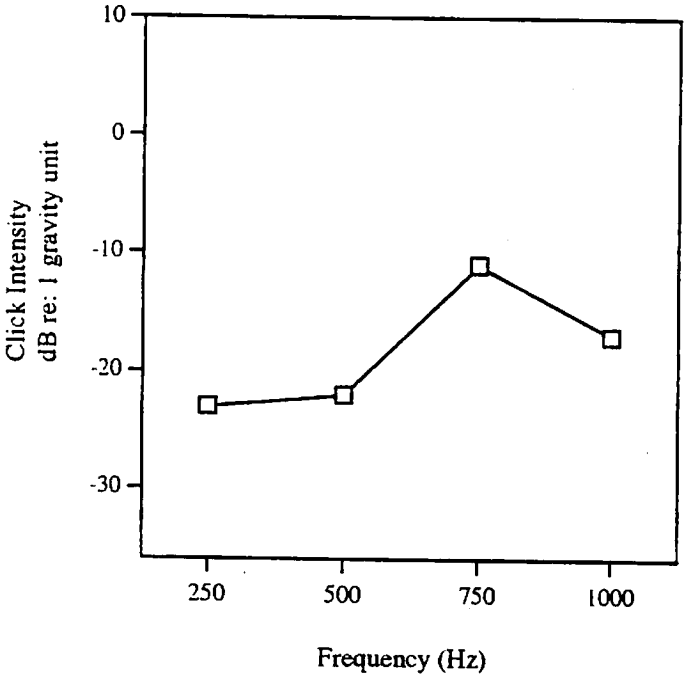


Figure 10. Threshold levels collected from turtle PPX804/PPX817 for 250, 500, 750, and 1000 Hz frequency levels. The intensity level is due to vibratory stimulation and is calibrated in decibels relative to acceleration.

Threshold Levels for Turtle PPX804/PPX817

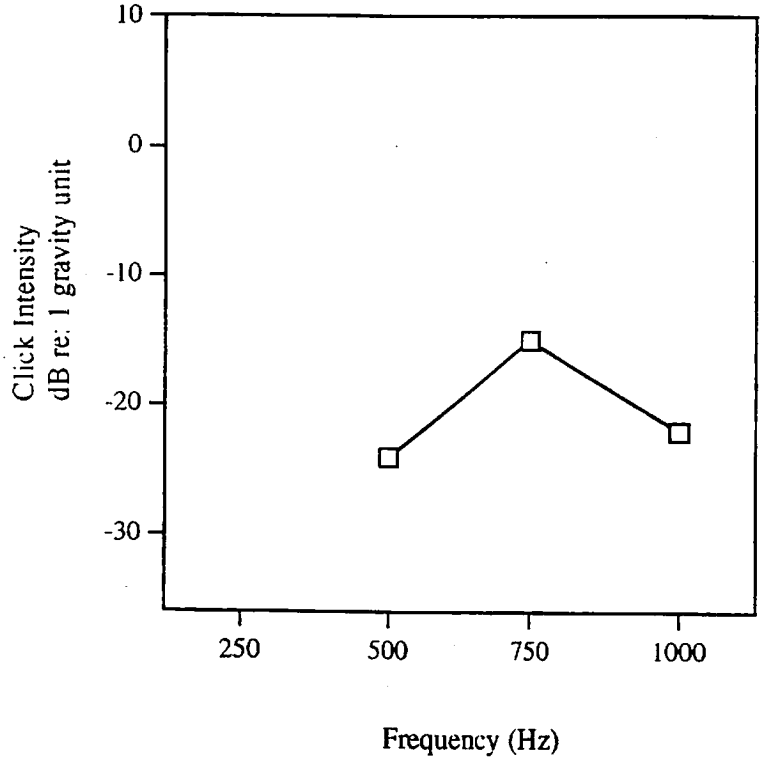


Figure 11. Threshold levels collected from turtle QQM791/PPX 807 for 250, 500, 750, and 1000 Hz frequency levels. The intensity level is due to vibratory stimulation and is calibrated in decibels relative to acceleration.

Threshold Levels for Turtle QQM791/PPX807

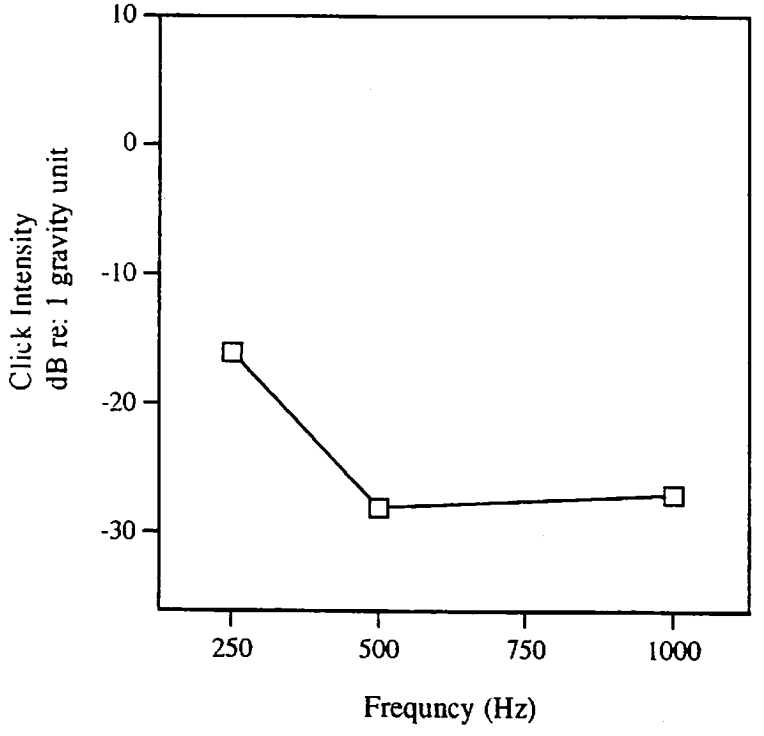
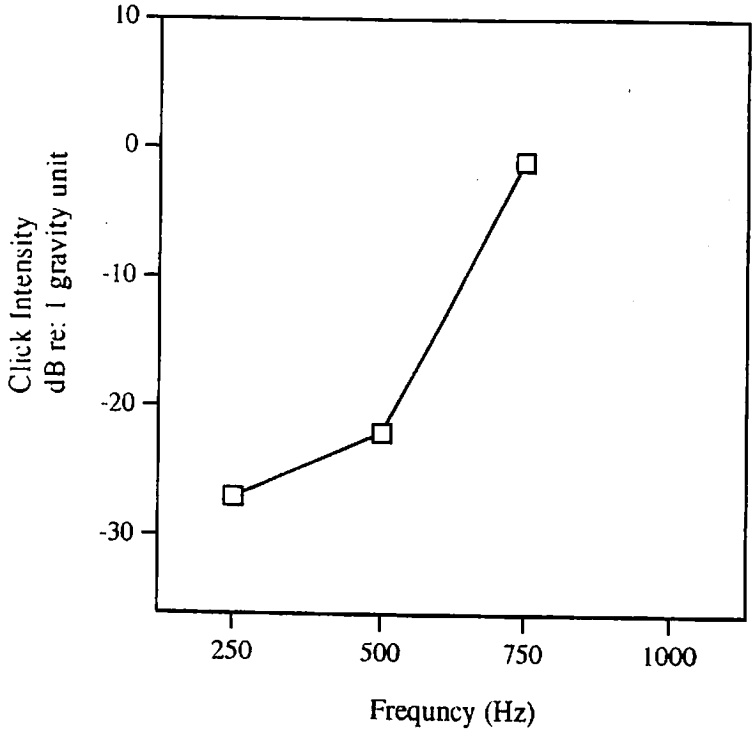


Figure 12. Threshold levels collected from turtle QQZ417/QQZ401 for 250, 500, 750, and 1000 Hz frequency levels. The intensity level is due to vibratory stimulation and is calibrated in decibels relative to acceleration.

Threshold Levels for Turtle QQZ417/QQZ401



threshold of -24.4 dB (Table 3).

In the repetition rate experiments, interpeak latencies for peak I and peak V were significantly dependant on rate (Table 4 and 5). An increase in latency was observed with the increase of stimulus rate.

In the masking experiment, stimulus intensity ranged from -2.5 to 7.5 dBs. White noise required to mask these stimuli ranged from 6 to 16 dBs. Stimulus to noise ratios ranged from -3.5 to -8.5 dB($x = -5.2 \pm 2.4$)(Table 6).

Table 3. Threshold data for six loggerhead turtles using tone burst with frequencies centered around 250 Hz, 500 Hz, 750 Hz, and 1000 Hz.

Front Flipper Tag#	250 Hz	500 Hz	750 Hz	1000 Hz
QQM800 QQM785	-30	-22	-13	-17
QQZ418 QQZ414	-26	-22	-18	-22
SSB805 SSB806	-23	-22	-11	-17
PPX804 PPX817	N/A	-24	-15	-22
QQM791 PPX807	-16	-28	N/A	-27
QQZ417 QQZ401	-27	-22	-1	N/A
Mean ± standard deviation	-24.4 ± 5.3	-23.3 ± 2.4	-11.6 ± 6.5	-21 ± 4.2

Table 4. Latencies between peak I and peak V collected from the auditory evoked potentials of 19 loggerhead sea turtles. The stimulus was a broadband click and the stimulus rate varied from 1.1-90.1 clicks per second.

Front Flipper Tag Numbers	Rate (clicks/sec)	Latency (ms)
QQM792/QQM775	1.1	3.02
	10.1	3.22
	20.1	3.36
	30.1	3.56
	40.1	3.64
	50.1	3.76
	60.1	3.76
	70.1	4.16
	80.1	4.18
	90.1	4.22
QQM791/QQM794	1.1	2.76
	10.1	2.82
	20.1	2.96
	30.1	2.94
	40.1	3.08
	50.1	3.14
	60.1	3.40
	70.1	3.74
	80.1	4.84

Front Flipper Tag Numbers	Rate (clicks/sec)	Latency (ms)
	90.1	3.94
QQM797/QQM798	1.1	3.36
	10.1	3.68
	20.1	3.88
	30.1	3.98
	40.1	4.34
	50.1	4.62
	60.1	4.88
	70.1	4.9
	80.1	4.98
	90.1	5.16
QQM605/QQM606	1.1	2.92
	10.1	3.16
	20.1	3.26
	30.1	3.48
	40.1	3.36
	50.1	3.72
	60.1	3.74
	70.1	3.70
	80.1	3.94
	90.1	4.06
QQM800/QQM785	1.1	3.16
	10.1	3.46
	20.1	3.56
	30.1	3.66
	40.1	3.78
	50.1	3.86
	60.1	4.20
	70.1	4.30
	80.1	4.38

Front Flipper Tag Numbers	Rate (clicks/sec)	Latency (ms)
	90.1	4.44
QQZ417/QQZ401	1.1	3.04
	10.1	3.28
	20.1	3.44
	30.1	3.72
	40.1	3.88
	50.1	3.92
	60.1	4.08
	70.1	4.16
	80.1	4.12
	90.1	4.12
QQZ409/QQZ408	1.1	3.70
	10.1	3.72
	20.1	3.96
	30.1	4.04
	40.1	4.10
	50.1	4.18
	60.1	4.22
	70.1	4.46
	80.1	5.02
	90.1	4.82
QQZ426/QQZ427	1.1	3.08
	10.1	3.46
	20.1	3.60
	30.1	3.74
	40.1	3.70
	50.1	3.76
	60.1	3.78
	70.1	3.94
	80.1	4.08

Front Flipper Tag Numbers	Rate (clicks/sec)	Latency (ms)
	90.1	4.44
QQZ407/QQZ406	1.1	3.44
	10.1	3.54
	20.1	3.82
	30.1	3.96
	40.1	4.04
	50.1	3.96
	60.1	4.04
	70.1	4.04
	80.1	4.18
	90.1	4.52
QQZ429/QQZ430	1.1	2.92
	10.1	3.10
	20.1	3.20
	30.1	3.36
	40.1	3.36
	50.1	3.56
	60.1	3.58
	70.1	3.72
	80.1	3.78
	90.1	3.94
QQZ441/QQC530	1.1	3.50
	10.1	3.52
	20.1	3.70
	30.1	3.88
	40.1	4.04
	50.1	4.10
	60.1	4.24
	70.1	4.28
	80.1	4.24

Front Flipper Tag Numbers	Rate (clicks/sec)	Latency (ms)
	90.1	4.28
QQZ437/QQZ438	1.1	3.00
	10.1	3.42
	20.1	3.42
	30.1	3.94
	40.1	4.00
	50.1	4.14
	60.1	4.18
	70.1	4.32
	80.1	4.36
	90.1	4.58
QQZ442/QQZ443	1.1	3.04
	10.1	3.10
	20.1	3.16
	30.1	3.22
	40.1	3.26
	50.1	3.40
	60.1	3.46
	70.1	3.58
	80.1	4.16
	90.1	4.22
QQZ482/QQZ483	1.1	2.88
	10.1	2.74
	20.1	2.90
	30.1	2.94
	40.1	3.00
	50.1	2.96
	60.1	3.04
	70.1	3.12
	80.1	3.16

Front Flipper Tag Numbers	Rate (clicks/sec)	Latency (ms)
	90.1	3.12
QQZ486/QQZ487	1.1	2.28
	10.1	2.34
	20.1	2.54
	30.1	2.66
	40.1	2.64
	50.1	2.64
	60.1	2.78
	70.1	3.02
	80.1	2.94
	90.1	3.00
QQZ500/QQZ353	1.1	2.78
	10.1	2.82
	20.1	2.94
	30.1	2.98
	40.1	3.64
	50.1	3.74
	60.1	3.80
	70.1	3.96
	80.1	4.06
	90.1	4.16
QQM764/QQM772	1.1	2.72
	10.1	2.82
	20.1	2.82
	30.1	3.00
	40.1	3.04
	50.1	3.20
	60.1	3.20
	70.1	3.28
	80.1	3.30

Front Flipper Tag Numbers	Rate (clicks/sec)	Latency (ms)
	90.1	3.32
QQZ425/QQZ424	1.1	3.26
	10.1	3.70
	20.1	3.86
	30.1	3.92
	40.1	3.96
	50.1	4.28
	60.1	4.18
	70.1	4.32
	80.1	4.40
	90.1	4.08
SSB827/SSB818	1.1	3.98
	10.1	4.44
	20.1	4.48
	30.1	4.32
	40.1	3.92
	50.1	4.06
	60.1	4.16
	70.1	4.46
	80.1	5.12
	90.1	5.36

Table 5. R^2 and p-values for the regression analysis, performed on 19 loggerhead sea turtles, to determined if a dependency existed between interpeak latencies and stimulus rate. All p-values were significant at $\propto = 0.05$.

Front Flipper Tag Numbers	R^2	P-value
QQM792/QQM775	.966	<.0001
QQM791/QQM794	.727	.0017
QQM797/QQM798	.967	<.0001
QQM605/QQM606	.938	<.0001
QQM800/QQM785	.972	<.0001
QQZ417/QQZ401	.883	<.0001
QQZ409/QQZ408	.891	<.0001
QQZ426/QQZ427	.872	<.0001
QQZ407/QQZ406	.853	.0001
QQZ429/QQZ430	.982	<.0001
QQZ441/QQC530	.909	<.0001
QQZ437/QQZ438	.916	<.0001
QQZ442/QQZ443	.849	.0002
QQZ482/QQZ483	.840	.0002
QQZ500/QQZ353	.932	<.0001
QQZ486/QQZ487	.920	<.0001
QQM764/QQM772	.947	<.0001
QQZ425/QQZ424	.708	.0023
SSB827/SSB818	.419	.0432

Table 6. Measurements of click intensity and white noise intensity levels for the last point at which the loggerhead sea turtle could distinguish the click.

Front Flipper Tag Numbers	Click Intensity (dB re: 1 gravity unit)	White Noise Intensity (dB re: 1 gravity unit)	Signal-to-Noise Ratio (dB re: 1 gravity unit)
QQM605/QQM606	2.5	6	-3.5
QQM797/QQM798	2.5	6	-3.5
QQM792/QQM775	2.5	6	-3.5
QQM791/QQM794	-2.5	6	-8.5
QQZ417/QQZ401	-2.5	6	-8.5
QQZ409/QQZ408	2.5	6	-3.5
QQZ426/QQZ427	7.5	11	-3.5
QQZ429/QQZ430	7.5	16	-8.5
QQZ442/QQZ443	7.5	16	-8.5
QQZ451/QQZ452	7.5	11	-3.5
QQZ482/QQZ483	2.5	6	-3.5
QQZ486/QQZ487	2.5	6	-3.5
QQZ362/QQZ363	2.5	6	-3.5
QQZ425/QQZ424	2.5	6	-3.5
SSB805/SSB806	-2.5	6	-8.5
		Mean $\pm$ Standard deviation	-5.2 $\pm$ 2.4

DISCUSSION

Threshold

The recording of the auditory evoked potentials for tones became very difficult due to the inability of attaining discernable and repeatable responses and only data from six turtles could be recorded. However, the click, a composite of all of the individual tones tested, produced consistently clear responses. This lack of agreement among the tone and click data is thought to be a result of the nature of the stimuli as well as the recording techniques used to attain responses. The responses recorded in this project are reflections of the synchronous discharge of neural fibers found at the base of the hair cells. Hair cells are the sensory receptor cells responsible for converting the motion of the basilar membrane into an electric signal which is then received by the auditory nerve (Yost & Nielsen, 1977). Each hair cell contains a filter and thus the cell is tuned selectively to a narrow band of frequencies (Crawford & Fettiplace, 1980; Fettiplace & Crawford, 1980). A transient stimulus, such as the broadband click, initially stimulates the basal end of the

cochlea, the site of synchronous activity of neural fibers. The low frequency tone burst, however, appears to stimulate the apical end of the cochlea and thus elicits an asynchronous response of the neurons. If this is the case, and the techniques for auditory evoked potentials record the synchrony of the neural discharge, then the efficiency of the click over the tone burst is apparent.

Another possible problem in recording tone burst data could be related to the volume of the neural response. This problem becomes evident when examining the placement of the electrodes. The loggerhead skull is composed of many layers of thick bone. By stimulating a small portion of the hair cell population with the tone bursts (only those hair cells tuned to the central frequency of the tone), it is possible that the resulting electrical signals were not strong enough in all cases to travel through the bone to the electrodes. Yet by stimulating a larger set of hair cells with the broadband click (a composite of five frequencies), I was able to collect a clear peak V that was trackable in nearly every turtle tested. Due to the loggerheads protected status, however, I was unable to place the electrodes anywhere but unintrusively on top of the skull.

The frequency range of response found in this project can be compared to a study by Ridgeway et al. (1969) in which he examined the threshold levels of the green sea turtle. Ridgeway tested tones on the green sea turtle from 30 to 700 Hz and found the maximum sensitivity to fall

between the 300-500 Hz frequency range. I found similar results with the tone burst data. Using a variety of stimuli, the maximum sensitivity fell between 250-1000 Hz. The computer was unable to test below 250 Hz so I am unable to speculate on the low end of the loggerhead's sensitivity. However, I was able to test up to 8 kHz and found that over 1000 Hz the sensitivity fell off drastically.

Comparing the sound pressure data from the green sea turtle (Ridgeway et al., 1969) to loggerhead sea turtles, a larger discrepancy is found. Ridgeway (1969), using tones, found the sound pressure in dynes/cm^2 to range from -5 to -35 dB for the 100-700 Hz range. I could only record the sound pressure level successfully for the click, a stimuli which encompassed approximately the same frequency range, and found the mean threshold to be 8.5 dB re: 1 dyne/cm^2. This dissimilarity of results can possibly be explained by a difference in recording techniques. Ridgeway collected cochlear potentials with electrodes surgically inserted into the paralymphic spaces. This technique would allow for greater detection by the electrodes. This disparity of results could also be explained by a dissimilarity between species. However, I do not believe that recordings using sound pressure levels in air as a reference are appropriate when collecting data from sea turtles. I ran this calibration in the laboratory so that my results could be compared to the limited published research on turtle hearing sensitivity. However, there is convincing research which

strongly suggests that sea turtle auditory perception is through bone rather than air conduction. The tympanum appears to be a poor aerial receptor, and displacement of the columella was not significantly changed by the removal of the tympanum (Moffat and Capranica, 1978). Furthermore, except for females nesting on the beach and green sea turtles basking in the Pacific, sea turtles spend the majority of their time underwater (Keinath, 1993) and thus it would be unlikely that the sea turtle would have a developed and functional air conduction hearing mechanism. The bones of the shell and skull, much denser than sea water, could serve as a receptor for vibrations in underwater sound fields (Lenhardt et al., 1983). In this scenario the tympanum is displaced outward as a mechanism for the release of the columella rather than inward as an air conductive sound receptor. Consequently, the use of vibratory stimuli, placing a vibrator against the turtle skull and relaying stimuli through the bone, is a more appropriate technique and likely to result in a more accurate measure of the sensitivity of the sea turtle hearing mechanism. Ideally, recording of auditory evoked potentials in an underwater environment large enough to eliminate the harmonics due to reflection of sound would result in thresholds more representative of the turtle's true hearing ability.

Loggerhead's ability to detect low frequency sounds has been theorized to be involved in natal beach homing behavior

(Dodd, 1988). Tagging data reveals that adult females repeatedly return to the same nesting beach, and possibly the same beach from which they hatched. Furthermore, it has been recorded that surf waves have a signature sound distinct to each beach (Bowen et al., 1993). The sounds of the beach may be distinct enough to serve as a cue for loggerheads when nesting. However, this theory implies that the turtle is able to discriminate between frequencies, a feature of sea turtle hearing that has not yet been investigated.

Repetition Rate

Auditory evoked potentials reflect synchronous electrical activity and thus, as found in the threshold section of this study, clicks represent the best stimulus for evoking the synchronized response. Of all of the peaks (Jewett bumps) found in these recordings, I was most interested in peak I and peak V. Latencies of these peaks are a convenient and useful measurement for evaluating auditory evoked potentials. Absolute latencies are variable depending on a number of factors, including temperature and stimulus intensity. However the interpeak latencies, the time between the firing of two peaks, is a consistent and reliable response among individuals.

The direct dependency of latency on rate reflects the reduction in efficacy of the stimulus with an increase in click rate to activate a synchronous progression of the

signal down the auditory pathway. After the neuron discharges, it remains in a refractory period, a period of no activity. This refractory period limits the number of times the neuron can discharge in a second. With an increasingly high rate of the stimulus, the neurons were unable to respond in a synchronized fashion and thus the signal required a longer period of time to activate the path.

An application for the interpeak latencies could be the identification of brain lesions. In the medical field, auditory evoked potentials have been used extensively in human diagnostic techniques to identify brainstem disorders and lesions (Markand, 1994). In patients who show no clinical symptoms, auditory evoked potentials have been capable of detecting lesions of the brainstem in one third of the cases. A common abnormality observed is the prolongation of the interpeak latency of peaks I and V.

This same diagnostic technique may be applicable to sea turtles. Recently, a new disease of the brain of loggerheads has been identified as Giant Cell Meningoencephalitis (GME) (George et al., in press). GME has been identified by necropsies performed on loggerheads who exhibited signs of central nervous system disorders: lethargy, inactivity, and uncoordinated movement. The lesions were found in the regions of the medulla, optic lobe, and cerebellum. This disease goes undetected until symptoms are severe (George et al., in press). However, it

may be possible to test clinically for this brain lesion in loggerheads before the lesion becomes symptomatic. From the rate experiment we know that the interpeak latencies are convenient to measure and consistently increase with the rate of the stimulus. By developing a baseline for conduction time for peaks I and V in normal turtles, abnormalities in the interpeak latencies may allow researchers to examine the occurrence and possible treatments for GME brain disease.

Masking Experiment

Signal detection for marine species can be masked by the often high level of background noise found in the oceans. Ambient noise in the oceans can arise from a number of sources, including: surface waves, seismic activity shipping, and biological activity. The frequency range of ambient noise is often localized in the low frequency end of the spectrum (Hawkins and Myrberg, 1983), the range at which loggerheads hear. Thus it is possible that ambient noise actually designates the limit at which loggerheads can detect an acoustic signal.

This masking experiment investigated the limits at which the loggerhead can distinguish a signal through ambient noise by examining the point at which the noise disrupts the synchrony of the neural response. The white noise used in the study was composed of a similar spectrum as that found in the click. Masking is most effective in

concealing a signal which contains the same frequencies and thus this scenario was constructed to produce the highest level of masking.

These results, a signal to noise ratio of -5.2 dB re: 1 gravity unit, may prove to be misleading. The click stimulus is a broadband spectrum of energy as is the white noise. The difference between the two, however, is that white noise is steady with all possible frequencies represented equally (Gelfand, 1990) while the click, when activated by the bone vibrator, has a transient character. This transience, an abrupt on and off sound, can cause the vibrator to resonate around a single frequency (Green, 1976). Consequently, the overall click decibel levels, as calculated by the accelerometer, may be an underestimate of the actual amount of intensity at a particular frequency, the resonant frequency.

Even with this apparent exaggeration of the signal to noise ratio, these results do confirm that the loggerhead has the ability to distinguish a signal through ambient noise, possibly at a relatively high level of noise. An adaptation of the hearing mechanism to reduce interference from noise would certainly be advantageous for the sea turtle. Due to the high and variable level of ambient noise centered around the low frequency range in the oceans, signal detection would only be possible if the sea turtle were able to discriminate sound through an elevated level of noise.

Conclusions

This study represents one of the first steps in understanding the loggerhead's hearing mechanism. The methodology for collecting auditory evoked potentials from loggerhead sea turtles was developed and threshold levels were measured. Auditory responses for loggerheads were most sensitive from at least 250 to 1000 Hz. Secondly, the latencies of peak I and peak V were dependent on the rate and thus the interpeak latency increased with the increase in stimulus rate. Finally, loggerhead sea turtles appear to be able to distinguish signals through a relatively high level of ambient noise.

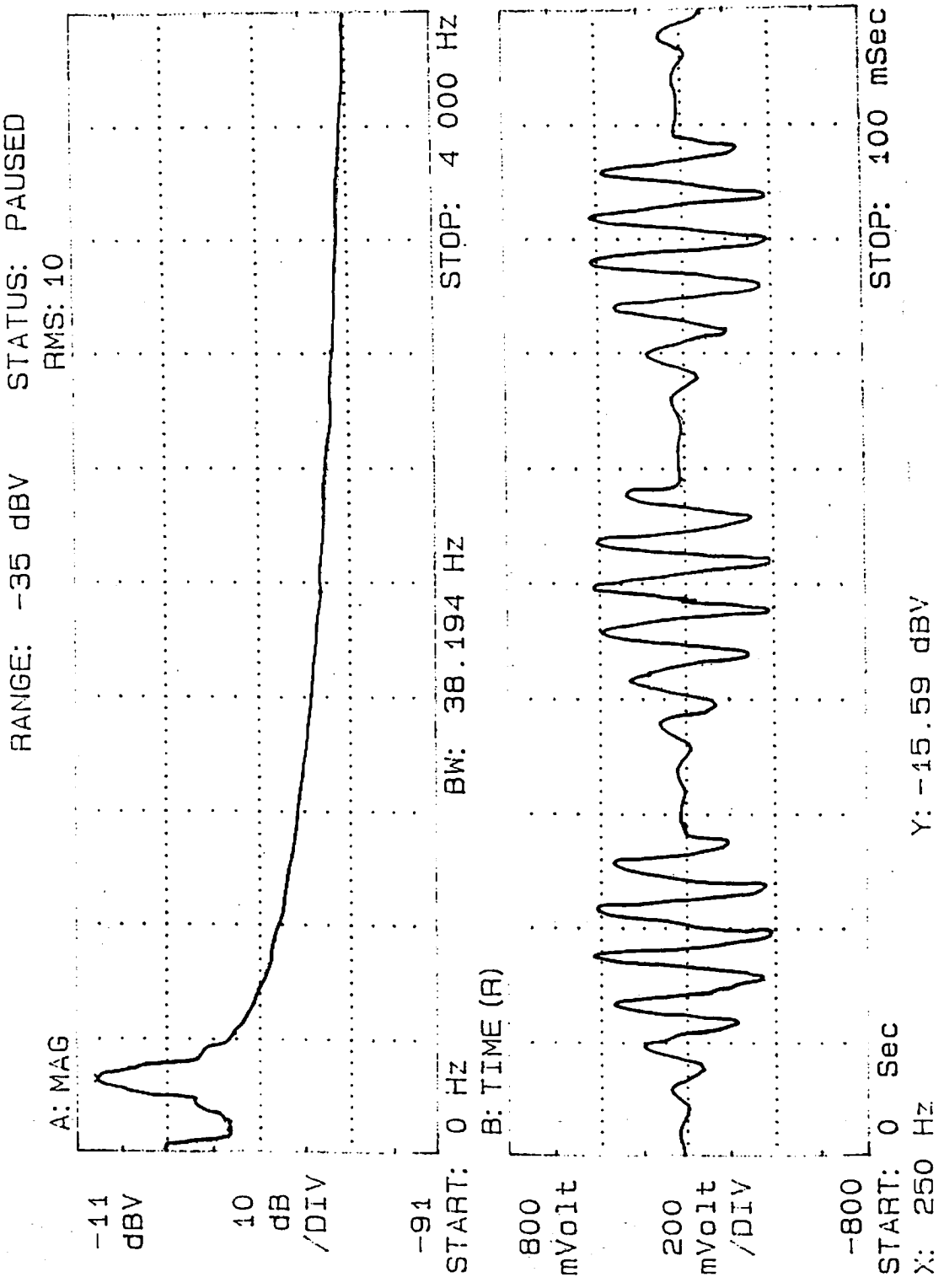
At present, evoked potential methods may be utilized in two fields of applied research: in the development of repelling devices and the identification of diseases. To return to the initial catalyst of this study, repelling devices are being developed to repel turtles away from areas where human activities place them in danger. The conclusions of this research can certainly define the frequencies and intensity for a possible repelling device. Moreover, the methods of evoked potentials laid out by this project can be used as a tool to protect the sea turtle during the development of repelling devices. Researchers have an obligation to conduct their studies unintrusively and to insure that damage is not being caused to the species they are trying to protect. By examining the threshold levels of an individual before and after testing a potential

repelling device, the researcher can take precautions to avoid damage to the turtle's hearing mechanism. These methods may also prove beneficial to the further identification of brain diseases, such as Giant Cell Meningoencephalitis. If able to detect GME before the onset of symptoms, it might be possible to record the progression of the disease as well as test possible drugs as curative agents.

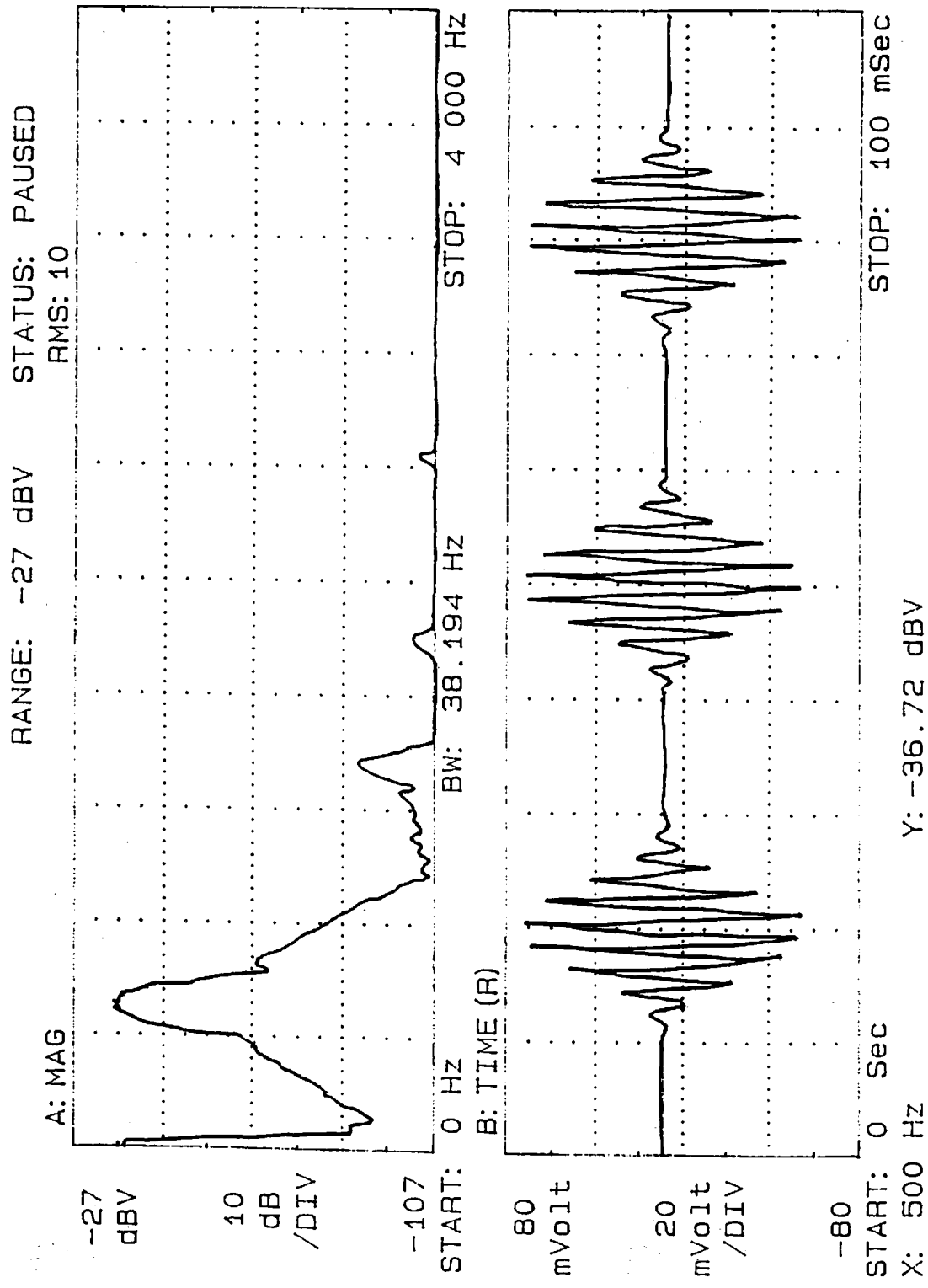
There are, however, many questions about sea turtle hearing yet to answer. Does the threshold to vibratory stimulus change when the turtle is submerged? The first step is to perform electrophysiological trials in a tank, one which is large enough to prevent the reflection of low frequencies. The second question which arises from this research is whether the loggerhead uses hearing in nature and why. Is the loggerhead ear a useless vestige or does hearing play a role in the turtle's life history? The use of hearing by sea turtles can be investigated by performing underwater localization experiments to examine whether sea turtles can be conditioned to sound stimuli. Finally, do all sea turtles hear by similar methods, specifically bone conduction? How does the leatherback, a species which has exchanged its hard shell for a leathery one, hear? All of these questions may be answerable in the very near future.

Appendix. Calibration graphs for tones and click stimuli.

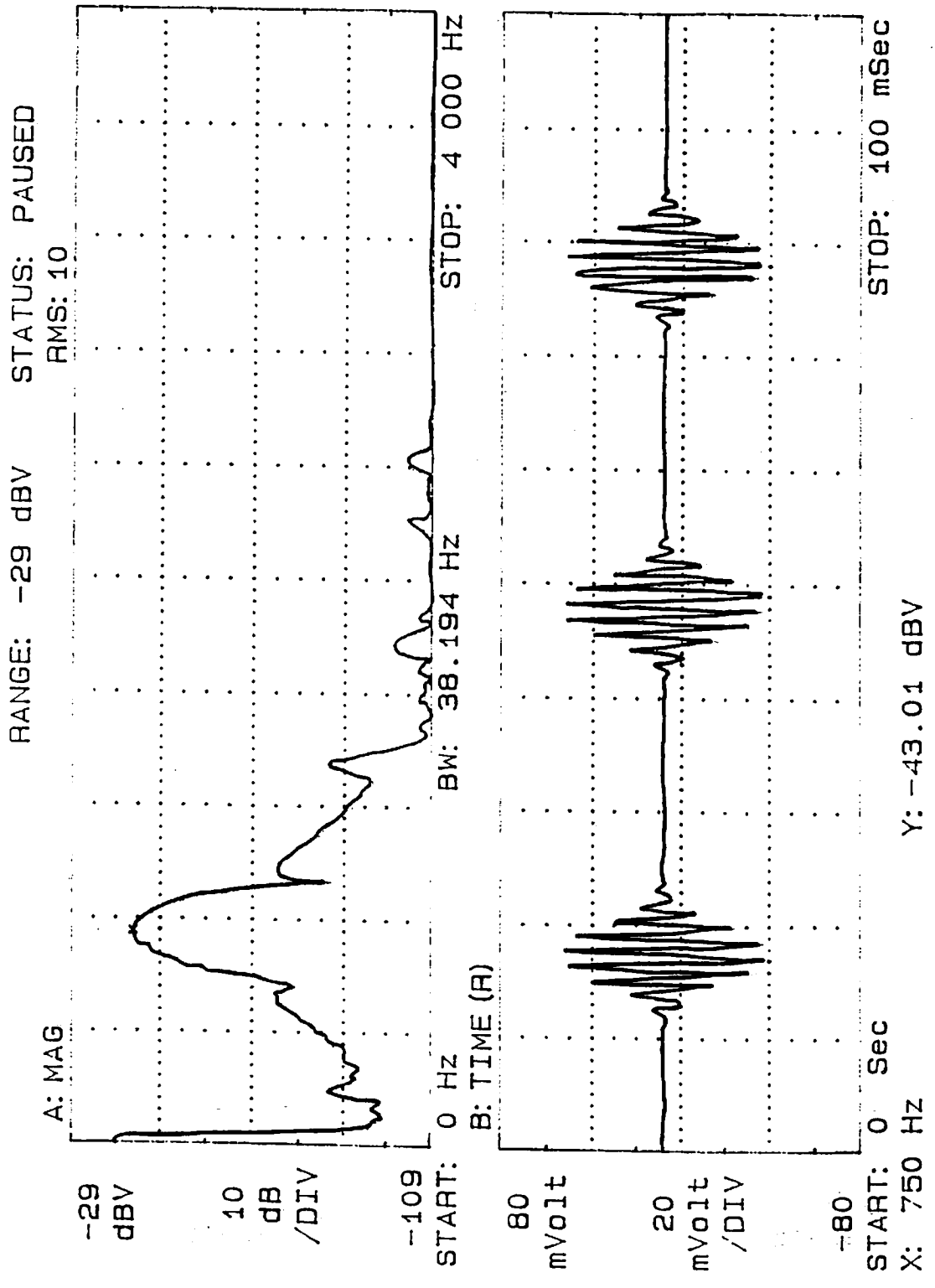
A-1. Frequency output of the bone vibrator, as measured by a real time spectral analyzer, for 250 Hz.



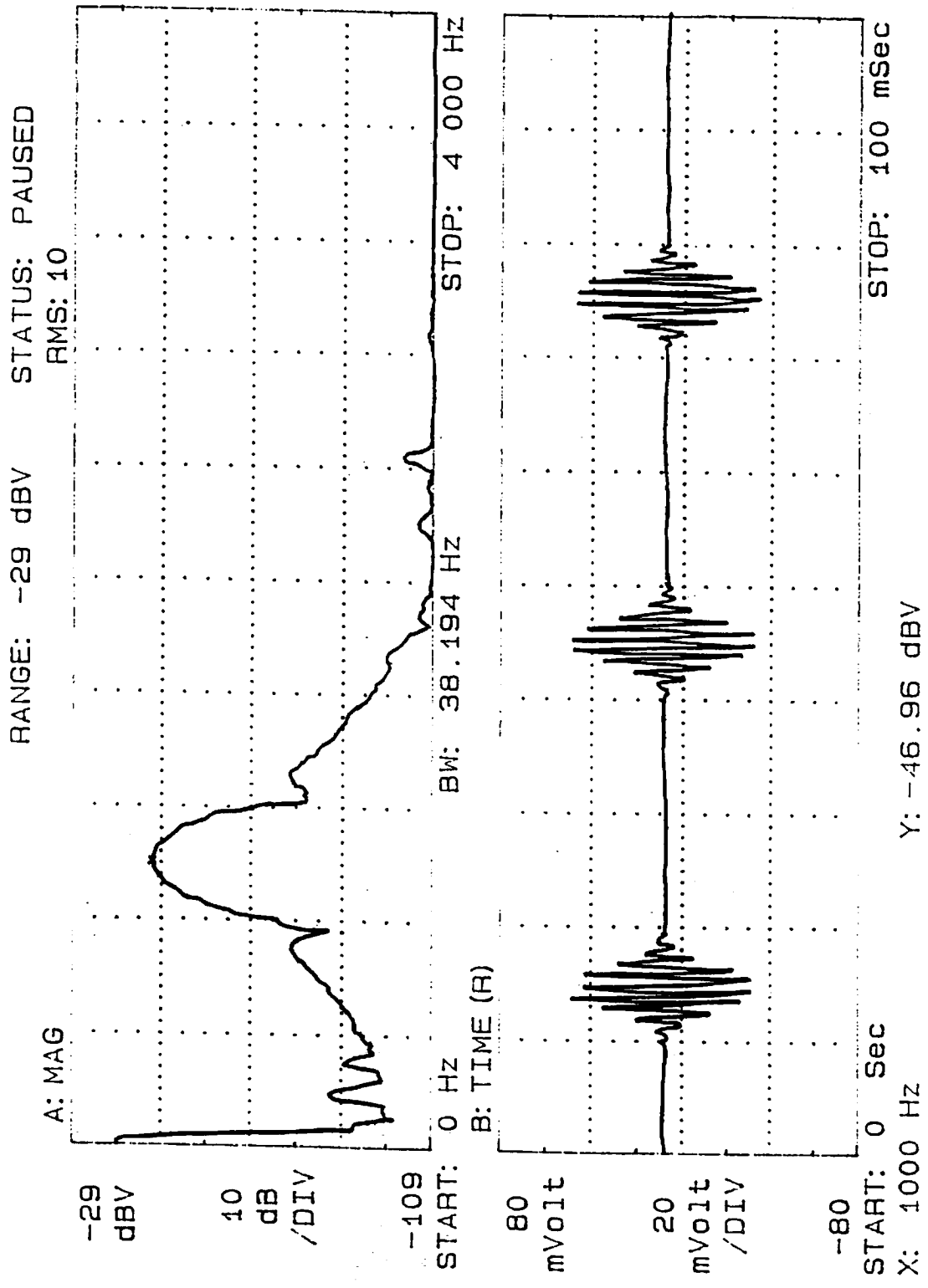
A-2. Frequency output of the bone vibrator, as measured by a real time spectral analyzer, for 500 Hz.



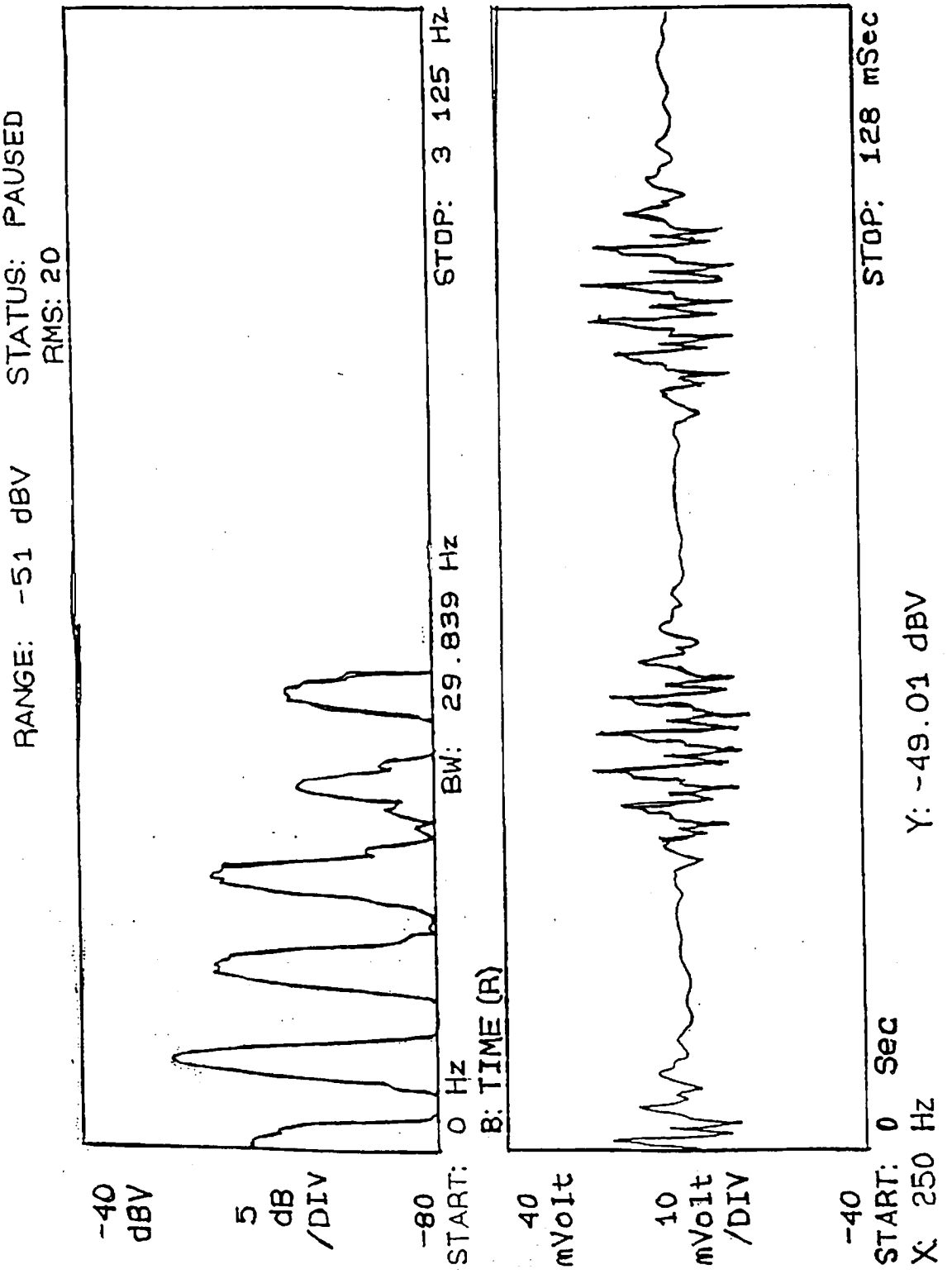
A-3. Frequency output of the bone vibrator, as measured by a real time spectral analyzer, for 750 Hz.



A-4. Frequency output of the bone vibrator, as measured by a real time spectral analyzer, for 1000 Hz.



A-5. Frequency output of the bone vibrator, as measured by a real time spectral analyzer, for the click.



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