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Analysis of the 35khz - 60khz Ultrasound of *O. Tormota* Evoking Optimal Startle Response in the African *A. Gambiae S. S.*

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Abstract: *The Chinese frog, Odorrana tormota generates ultrasound naturally through vocal apparati. This ultrasound detectable by the female Anopheles gambiae antennae evoked evasive response due to neural stress and fear for predation. The mated female A. gambiae are the Malaria vectors seeking blood meal from humans for egg nourishment through bites. Many Malaria preventive and control measures currently in use have had minimal impact in Africa. Mosquitoes and malaria causing organisms have also developed resistance to chemicals in use. The use of EMR in mosquito repellency has been reported to be ineffective. Recent studies have shown that ultrasound of O. tormota evoked significant evasive responses in the female A. gambiae, due to its pulsatile nature. In view of this, this study examines and analyses the 30-60 kHz frequency band of the recorded sound of O. tormota reported to have the highest repellency to the female A. gambiae. In this study, the 30-60 kHz frequency band was filtered from the recorded sound of O. tormotus, acoustic transmission parameters determined and analysed using Avisoft-SASLab Pro version 5.1 software and Raven Pro. 1.4. The activity and the behavioural response of the female A. gambiae to the ultrasound in the optimal frequency range were also dtermined and analysed. A bioassay study involving 3-4 day old female A. gambiae exposed to the 35 kHz-60 kHz frequency range of the sound of O. tormota was conducted and the rate of mosquito activity and behavioral responses noted. It was established the 35-60 kHz sound of O. tormotus was composed of 583 pulsate calls with call duration ranging from 0.003s to 0.4167s. The maximum and minimum mean peak amplititudes were 85.42 Pa and 102.15 Pa respectively; with most calls between 90 - 99 Pa. The signal power of the sound of O. tormotus varied between 40.5 dB and 73.0 dB, characterized by dips and peaks. The behavioral response of female A. gambiae to the ultrasound was characterized by excitation and immobility due to the pulsate nature of the acoustic energy. These responses included weak movement, exhaustion, collapsing, unusual rest on the floor, antennae erection, low flights which was a manifestation of stress on nervous system and fear for predation. There was sufficient evidence for significant relationship between acoustic energy with amplitude, bandwidth and frequency. The comparison between mean mosquito activity and acoustic energy using a paired samples T-test at 95% confidence was highly significant at $p = 0.0000487$, with a low positive correlant ($r = 0.156$). The mean of the acoustic energy and mosquito activity in this frequency range was 1.429 Pa^2s and 59.333 respectively. The mosquito activities under the influence of 35-60 kHz sound differed significantly ($p = 0.0032$) from their activities under the control and were highly correlated ($r = 0.773$). The rate of mosquito activities under the 35-60 kHz sound of O. tormotus increased 4.617 times of the activities under the control experiment. The change in the rate of mosquito activities, attributed to nervous system besides fear of predation, was statistically significant at $p = 0.013$ and $r = -0.356$. Also, the mean rate of mosquito activities under the influence of the 35-60 kHz sound of O. tormotus varied significantly with the peak amplitude, bandwidth and frequency. This study provide insight into the acoustic transmission parameters of the sound of O. tormotus which affect the rate of activity and behavioural response in mosquitoes. The female A. gambiae, a Malaria vector can therefore be effectively be repelled using the 35-60 kHz sound of O. tormotus besides other control measures.*

Keywords: *Vocal Apparati, Evasive Response, Optimal Frequency, Electronic Mosquito Repellents (EMR), Insecticide treated nets (ITNs), Indoor residual spraying (IRS)*.

1. INTRODUCTION

A rare Chinese frog, *O. tormotus*, belonging to the order *Anura* of the animal kingdom, was reported to exhibit communication by means of ultrasonic Sound [1, 5, 14, 15, 18, 17]. This is the only amphibian known to make use of ultrasonic communication; males produce diverse bird-like melodic calls with pronounced frequency modulations that often contain spectral energy in the ultrasonic range [5]. It was observed that some frog species had special muscles in the larynx for producing longer glottal pulses giving time for frequency modulation (FM) of the carrier frequency which is simple. A frame-by-frame video analysis of the frog's calling behavior suggested the presence of two pairs of vocal sacs that contributed to the remarkable call-note complexity [6]. This Chinese frog, *O. tormotus,* feeds on a wide range of terrestrial and aquatic animals of which insects form the greater part [2], hence mosquito predators. Ecologically, *O. tormotus* occurs in hill streams and the surrounding habitats, and breeds in streams. The existence of tadpoles has not been recorded for this species [5].

Malaria which is caused by a protozoan parasite of genius *Plasmodium* and transmitted by the female *Anopheles gambiae,* through mosquito bites is the key cause of mortality and morbidity in Africa; and many control measures which include: chemotherapy, chemoprophylaxis, vector control strategies and development of malaria vaccine have been undertaken [4, 20]. These measures have had negligible effect in malaria control. Malaria results in 300-500 million clinical cases and causes more than 1 million deaths annually. Children under the age of five in sub-Saharan Africa are most affected, dying at the rate of nearly 3,000 every day. Malaria causes approximately 20 per cent of all child deaths in Africa. It also leads to acute attack of cerebral malaria that quickly leads to coma and death; severe anemia, or to the consequences of low birth-weight caused by malaria infection in the mother's womb [4, 8, 19].

Currently, the malaria vector control-methods preferred include the use of ITNs, IRS, destruction of mosquito breeding sites and use of mosquito repellents [20]. However, the use of insecticides to control malaria vectors and drugs to control malaria parasites have previously failed due to build up of resistance in mosquitoes and the disease agent [7, 19, 20]. Effective prevention requires use of a combination of factors, one of them being addressing the habits of mosquitoes and their interaction with human beings [20]. The mated female *A. gambiae* mosquitoes require blood meal from humans and animals for egg maturation [9]. In this view, ultrasound has been seen to be an alternative breakthrough in Malaria vector control which is environment friendly [4, 10]. Mosquitoes communicate using their antennae which are ultrasound sensors [12]. The distal elongated flagellum of each antenna , shown in Figure 1 below, acts as a mechanical filter and it is resonantly tuned in response to particle oscillations as a forced damped harmonic oscillator [22].

Fig1.1. *Antennal sensilla of the Anopheles mosquito [22]*

Source: *Malaria Journal* (2006)

Mosquitoes detect ultrasound in the range of 38 - 44 kHz, regardless of the source, initiating avoidance response since it creates--stress on their nervous system. This ultrasound also jams mosquitoes' own ultrasound frequency besides immobilizing them [10, 13].

Recent research with recorded ultrasound of *O. tormotus*, yielded greatest repellency in the female *A. gambiae* compared to other sounds, giving the maximum rate of activity in the 35-60 kHz frequency range [10,11]. Further, the optimum frequency range of this sound, 35-60 kHz, evoked evasive responses in an average of 46 % of the mosquitoes [10, 11], higher than the reported 20 % effective repulsion of EMR sound [4]. It is therefore important to critically analyse the 35-60 kHz ultrasound of *O. tormotus* with a view of adopting it as an environment friendly means of malaria vector control.

1.1. Statement of the Problem

Malaria ranks top in the cause of morbidity and mortality in Africa despite many control measures and treatment recently adopted. Currently, the use of insecticide-treated nets provide the highest degree of protection against Malaria, expected to reduce child mortality by 20 %. However, this realization is hampered by low household use, constrained by regular and timely re-treatment of the nets. Also, the use of insecticides to control malaria vectors and drugs to control malaria parasites have previously failed due to build up of resistance in mosquitoes and the disease agent. Studies with EMR mimicking ultrasound from male mosquito and bat in the range of 125 Hz to 74.6 kHz and used to repel mosquitoes have shown that 12 out of 15 field experiments yielded higher landing rate on the human bare body parts than the control experiments, translating to 20 % effectiveness. Further studies with natural recorded ultrasound identified the ultrasound from *O. tormotus*, from all sounds studies, yielded the greatest repellency in mosquitoes in the 35-60 kHz frequency range. This optimal frequency range for the sound of *O. tormotus* has not been analysed fully yet it is the most effective malaria vector control measure, thus malaria control. This study therefore filters the sound of *O. tormotus* using the Avisoft-SASLab Pro version 5.1 software; analyses both the acoustic transmission parameters and the mosquito behavioural responses to the sound of *O. tormotus* in the 35-60 kHz frequency range. Mated female *A. gambiae* were used in the bioassay study due to their human biting capability in search for blood meal for egg nourishment. The understanding of the acoustic transmission parameters and mosquito behavioural response to ultrasound in this range provides proper grounding in using the 35-60 kHz sound of *O. tormotus* in mosquito repellency.

1.2. Objectives

1.2.1. General Objectives

To filter the 30 kHz-60 kHz frequency band, determine the acoustic transmission parameters of the sound of *O. tormota*; hence analyse the acoustic parameters, activity and the behavioural response of the female *A. gambiae* to the ultrasound in the optimal frequency range.

1.2.2. Specific Objectives

- Determine the 35 kHz-60 kHz frequency band from the recorded sound of *O. tormota.*
- Determine and analyse the acoustic transmission parameters of the sound of *O. tormota* in the 35 kHz-60 kHz frequency range
- Analyse the behavioural response and activity of the female *A. gambiae* in the optimal frequency range.

1.3. Justification

The female *A. gambiae* mosquitoes are the main vectors of malaria in Africa. After mating, they seek blood meal from humans and animals through bites, hence transitiing malaria which is caused by a protozoan parasite of genius *Plasmodium.* A vector control measure effective on mosquitoes would relieve the world of the economic burden and also lower the mortality and morbidity due to malaria. Recent efforts in malaria treatment and vector control have yielded negligible impact due to build up of resistance in vectors and pathogens to the drugs and chemicals used. Ultrasound, having been recently explored as a malaria vector control measure in

EMR, have been observed to give only 20% startle response by female *A. gambiae* rendering them less effective. However, the 35-60 kHz recorded sound of the Chinese frog, *A. tormotus,* naturally generated through vocal apparati, has evoked evasive responses in an average of 46 % of the mosquitoes studied. It is therefore important to filter and analyse this optimal frequency range of ultrasound in order to relate the behavioural response to the acoustic transmission parameters, essential in providing an additional tool in mosquito control which is environment friendly.

1.4. Hypotheses

- There exist no significant difference between the acoustic energy and: amplititude, frequency and bandwidth of the sound of *O. tormotus*.
- The acoustic energy of sound of *O. tormotus* did not affect the mosquito activity significantly.
- There exist no significant difference between the mean mosquito activities under control and the mean mosquito activities under the 35-60 kHz sound of *O. tormotus*.
- The mean rate of mosquito activities was not significantly affected by the peak amplititude, frequency and bandwidth (mean) of the sound of *O. tormotus*.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. The Anopheles Gambiae Mosquitoes

Three sets of ten female *A. gambiae*, 3-5 day old, bred and reared at the Kenya Medical Research Institute Centre for Global Health Research laboratories, Entomology department at 60-80 % humidity, 25±2 ^{o}C temperature and light-day cycle of 12L: 12D hours were used in the study.

2.1.2. Sound of Odorrana Tormotus

Six samples of the sound of *Odorrana tormotus* recorded using the 702 digital recorders from the Huangshan Hot springs, Anhui Province; China at a sampling frequency of 192 kHz and converted to 500 kHz were used for this study. The sound was saved as *Odorrana tormotus.wav*.

2.1.3. Equipment

A laptop running on Windows XP and office 2007 mounted with a sound card, hardlock key and sound output ports were used together with the Avisoft-SASLab Pro version 5.1 software (besides Raven Pro. 1.4). Two Panasonic 8.0 Ω ordinary external speakers were used to play sound from a single source directed to the bioassay cage housing the female *A. gambiae*. The sound was amplified externally using the amplifier, with output power = 18 W, impedance = 4.0 Ω with separation ≥ 45.0 dB. The stopwatch option in the Samsung cell phone was used to capture activity duration.

A bioassay study involving the recorded sounds was conducted in a glass cage, covered at the two ends with a mosquito netting whose dimensions were 50 cm long, 25 cm width and 25 cm in height.

2.2. Methods

2.2.1. Filtering of Sounds Samples

The band pass filter inbuilt in the Avisoft SAS Lab analysis software was used to segment the appended sounds into the 35-60 kHz frequency range essential for the study. The sound of *O. tormotus* was subjected to a band pass filter with an upper cut-off frequency, f_{uco}= 60 kHz and a lower cut-off frequency, f_{lco}=35 kHz. The filtered sound of *O. tormotus* was saved in the hard disc as *35-60 kHz Odorrana tormotus.wav*.

2.2.2. Determination of Acoustic Transmission Parameters of Predator Sounds

A computer installed with Raven Pro. Version 1.5 and Avisoft SAS Lab Pro version 5.1 software and fitted with hard lock key was used in determining the acoustic transmission parameters of the sound, *35-60 kHz Odorrana tormotus.wav*. The Raven Pro 1.5 software was used to generate spectrograms and oscillographs. This study dwelt on acoustic transmission parameters that influenced the signal power and energy essential in mosquito repellency. The parameters included:

- Number of calls in the sound
- Duration of each call
- Amplitude of the pulses
- Frequency of the calls
- Signal bandwidth
- Signal Energy and Power

In order to generate the transmission parameters, the spectrogram parameters were set to FFT: 512, Window: Hamming, Frame size: 100% and Overlap: 50%. The sound card was set to a sampling frequency of 500 kHz at 16 bits with a down sampling of 1. The saved predator sounds was analysed using Avisoft SAS Lab Pro version 5.1 and Raven Pro. version 1.5 software. The parameters were automatically generated. In order to generate these parameters which included amplitude and energy, the calibration method was set to SPL with reference sound for Channel 1and at a /gain (dB) set to zero. These calibration settings were made in the Avisoft SAS Lab software under the tools menu. The acoustic pressure level was set to a reference of 20 µPa, which is the threshold for human perception. On calibration, the reference signal emerged as 94 dB. The envelope was set to original waveform whereas the pulse detection was set to gate function. The predator sounds could not be played by ordinary moving coil speakers hence the need to amplify them. The predator signal was internally amplified and then externally amplified before getting into the external speakers, placed 5 cm from one side of the cage. The speaker was set to face the cage. The amplitude modulation constant of 35-60 kHz appended sound of *A. tormotus* was set to n = 0.9 i.e. Normalize at 80% for the entire duration for the *A. tormotus* signal.

The Pearson's product moment correlation coefficient that established the relationships between the acoustic transmission parameters with the signal energy was determined statistically using the SPSS software.

2.2.3. Determination of the A. Gambiae Behavioural Response and Rate of Activity Evoked by 35-60 Khz Sound of O. Tormotus

A bioassay study involving the 35-60 kHz recorded sound of *O. tormotus* and the female *A. gambiae* was conducted using a glass cage, covered at opposite ends with a mosquito netting whose dimensions were 50 cm long, 25 cm width and 25 cm in height. An aspirator was used to transfer the female *A. gambiae* from the rearing cage to the bioassay cage and also remove them from it. During the bioassay study, observations were based on behavioural response of the female *A. gambiae* to ultrasound. Also, the rate of landing rate activity in terms of flight and rest were noted. The stopwatch option in the Samsung cell phone was used to determine the landing rate in terms of flight and rest. The effect of the acoustic transmission parameters (acoustic energy, mean peak amplitude, mean maximum frequency entire, and mean bandwidth) on the activity of the female *A. gambiae* was established statistically using the Paired Samples T-test.

3. Results and Discussion

3.1. Filtering of the 35-60 KHz Frequency Range from the Recorded Sound of *O. Tormota.*

The sound of *O. tormotus* appended using Avisoft SAS Lab Pro version 5.1 software was subjected to a band pass filter. The band pass filter modified the frequency response as shown in Figure 3.1 below. The frequencies below 35 kHz and above 60 kHz were gradually attenuated (amplitude = 0 i.e. off), allowing those in the range of 35-60 kHz (amplitude = 1, i.e. on). The resultant sound from the appended sound of *O. tormotus* was saved as *35-60 kHz Odorrana tormotus.wav* in the computer hard disc.

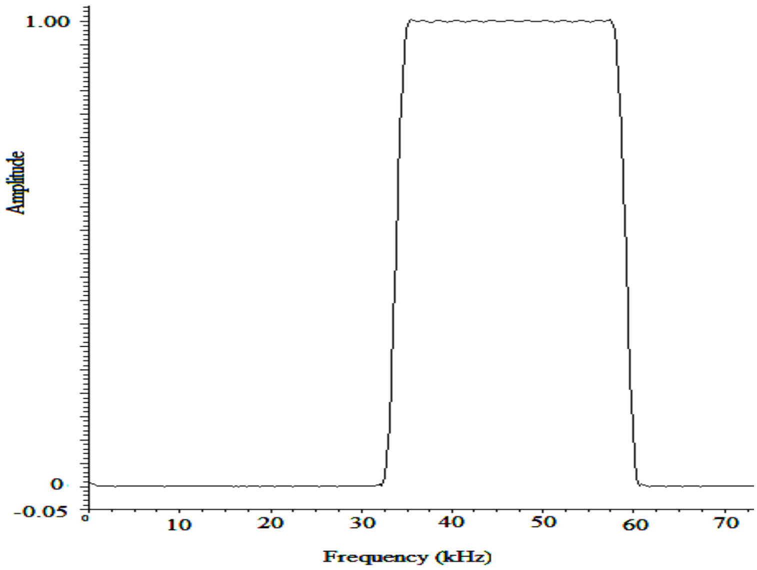

Fig3.1. *Frequency response modification by the 35-60 kHz band pass filter*

3.2. Determination and Analysis of the Acoustic Transmission Parameters of the Sound of O. Tormota in the 30 Khz-60 KHz Frequency Range

The study determined the following acoustics transmission parameters that affected signal acoustic energy and power; essential in mosquito repellency:

- Number of calls in the sound of *O. tormota.*
- Duration of each pulse
- Amplitude of the signal
- Frequency of the signal
- Signal bandwidth

A total of 583 calls were studied in this optimal frequency range for the sound of *O. tormota*. It was observed that 54 calls recorded the minimum duration of 0.003 second, with 16 calls taking the maximum duration of 0.4167 second. Figure 3.2 below, generated using the SPSS software, shows how the 583 calls are distributed over the Mean Peak amplitudes.

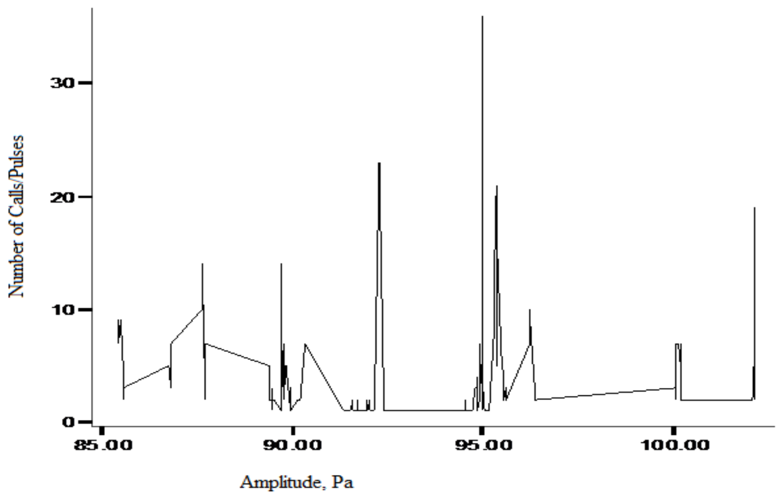

Fig3.2. *Call Distribution over Call Amplitudes*

It can be observed that most calls were at mean peak amplitudes above 90 Pa with 94.96 Pa recording 36 calls. The sound of *O. tormota* being pulsate in nature, the minimum and maximum mean peak amplitudes were 85.42 Pa in nine calls and 102.15 Pa in two calls respectively. It was noted that only two calls recorded the maximum mean peak amplitude of 102.15 Pa, translating to 0.34 % of the entire sound studied. With the acoustic pressure level set to a reference of 20 µPa (threshold for human perception) and volume set to 0.9, a total of 36 calls, which were the majority calls recorded 133.53 dB. This pressure level corresponded to 2.46 x 10^{-1} Pa^2s. The sound of *O. tormota* was dominated by mean peak amplitudes in the 90.00 - 99.00 Pa range as shown in Table 3.1.

Table3.1. *Distribution of Calls of O. tormotus per Mean Peak Amplitude Range*

Mean Peak Amplitude Range, Pa	Total Pulses or Calls
80.00 - 89.00	170
90.00 - 99.00	305
100.00 - 110.00	108

This being an FM-CF signal, as index of modulation increases, the amplitude of the rest frequency decreases and the amplitude of the higher-order sidebands increases, indicating an increasing signal bandwidth (Dattoli *et al.*, 2002; Feng *et al.*, 2002; Mang'are *et al.*, 2012). The Oscillogram in Figure 3.3 below was generated by amplifying the signal using a multiplying factor of 3 in the Raven Pro. version 1.5 software.

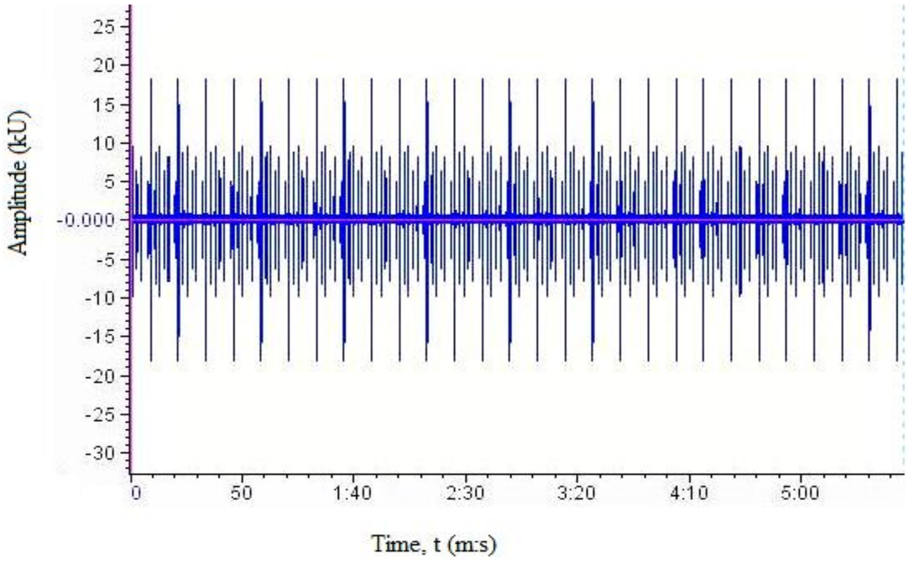

Fig3.3. *An oscillogram of the pulsate sound of O. tormotus (Mang'are et al., 2012)*

The sound of *O. tormotus* which was observed to be in bursts as shown in Figure 3.3 and Figure 3.4 due to varied amplitude and frequency yielded to energies of varied intensity. High energy yielded greatest repellency of the female *A. gambiae* in recent studies (Mang'are *et al.*, 2012). For longitudinal waves, change in energy $\Delta E = \frac{1}{2}\Delta m\omega^2 A^2$ (Young *et al.*, 2012; Serway and Jewett, 2014)

But $\omega^2 = 4\pi^2 f^2$

Hence the relationships between energy E, amplitude A and frequency f are given as $E \propto A^2$ and $E \propto f^2$.

A Pearson's product moment correlation coefficient, ***r*** was determined statistically using SPSS statistical software to establish the relationship between the acoustic energy for the sound of *O. tormotus* and the amplitude. This comparison was determined at a 99 % confidence and significance level of 0.01. The results are shown in Table 3.2 below:

Table3.2. *The Correlation and Significant level between the Acoustic energy and Mean Peak Amplitude*

		Acoustic Energy	Mean PeakAmplitude
Acoustic Energy	Pearson Correlation	1	0.5848
	Sig. (2-tailed)		9.07×10^{-55}
	N	583	583
Mean Peak Amplitude	Pearson Correlation	0.5848	1
	Sig. (2-tailed)	9.07×10^{-55}	
	N	583	583

The acoustics energy and the amplitude were highly related with r = 0.5848. Also, at 1 % level of significance, there was great evidence to show significant relationship between the acoustic energy for the sound of *O. tormotus* and the amplitude

A Pearson's product moment correlation coefficient, ***r*** was determined statistically using SPSS statistical software to establish the relationship between the acoustic energy for the sound of *O. tormotus* and the mean peak frequency. The results are shown in Table 3.3 below:

Table3.3. *The Correlation and Significant level between the Acoustic energy and Mean Peak Frequency*

		Acoustic Energy	**Mean Peak Frequency**
Acoustic Energy	Pearson Correlation	1	-0.2523
	Sig. (2-tailed)		6.4345×10^{-10}
	N	583	583
Mean Peak Frequency	Pearson Correlation	-0.2523	1
	Sig. (2-tailed)	6.434×10^{-10}	
	N	583	583

The acoustics energy and the mean peak frequency indicated a weak negative correlation at r = -0.2523. Also, at 1 % level of significance, there was great evidence to show relationship between the acoustic energy for the sound of *O. tormotus* and the mean peak frequency.

Out of the 583 calls analysed, the maximum fundamental frequency (mean) determined was 44,900 Hz. The frequencies were limited to 35 kHz and 60 kHz, using a band pass filter. The lowest mean maximum frequency recorded was 43.90 kHz. The minimum and maximum mean bandwidth of the sound were 9.70 kHz and 28.30 kHz. Similarly, a Pearson's product moment correlation coefficient, ***r*** was determined statistically using SPSS statistical software to establish the relationship between the acoustic energy for the sound of *O. tormotus* and the mean bandwidth. Table 3.4 below shows the results:

Table3.4. *The Correlation and Significant level between the Acoustic energy and Mean Bandwidth*

		Acoustic Energy	**Mean Bandwidth**
Acoustic Energy	Pearson Correlation	1	0.0148
	Sig. (2-tailed)		0.7218
	N	583	583
Mean Bandwidth	Pearson Correlation	0.0148	1
	Sig. (2-tailed)	0.7218	
	N	583	583

There exists a weak positive correlation between the acoustic energy and the Mean Bandwidth of the sound of *O. tormotus*. The relationship between the acoustic energy and the Mean Bandwidth of the sound of *O. tormotus* is therefore slightly significant (p = 0.7218).

The acoustic signal power in this frequency range recorded a maximum power of 73.0 dB whereas the minimum power was 40.5 dB declining with increase in frequency as shown in Figure 3.4 below.

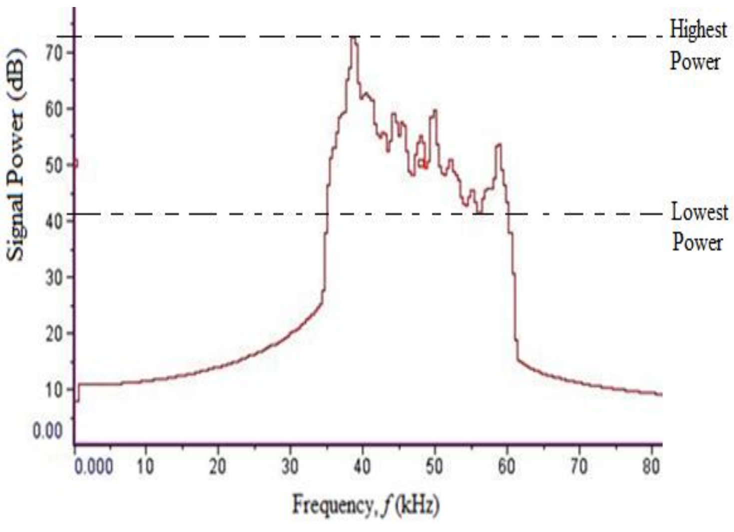

Fig3.4. *Signal Power Variation with Frequency*

It can be noted that every signal power dip is followed with an abrupt increase. Within the 35-60 kHz frequency range, the signal power dips eight times each being followed by a rise in value. This is explained by the pulsate nature of the signal which was characterised by bursts as shown in Figure 3.3. The distal elongated flagellum of the mosquito antenna mechanically filters and resonantly tunes itself in response to particle oscillations as a forced damped harmonic oscillator dependent on signal energy (Zwiebel and Pitts, 2006). The entire maximum peak frequency (max) and peak amplitude (max) of the signal were 58.5 kHz and 113.56 Pa respectively. The acoustic transmission parameters were automatically generated and analysed using the Avisoft SAS Lab Pro version 5.1 software. The results of the parameters are summarised in Table 3.5.

Table3.5. *The Acoustic Transmission Parameters of the 35-60 kHz Sound of O. tormotus*

Parameter	Minimum	Maximum	Mean	Standard Deviation	Total Pulses
Duration (s)	0.003	0.417	0.102	0.113	583
Energy, Pa^2s	0.005	10.844	1.339	3.055	583
Peak Frequency (mean), Hz	34,100.000	49,800.000	40,902.200	4,200.110	583
Peak Amplitude (mean), Pa	85.420	102.150	93.943	4.773	583
Minimum Frequency (mean), Hz	33,200.000	39,000.000	34,727.900	1,373.780	583
Maximum Frequency (mean), Hz	43,900.000	61,500.000	54,765.300	4,797.900	583
Bandwidth (mean), Hz	9,700.000	28,300.000	19,989.500	5,169.880	583
Peak frequency (minimum entire), Pa	34,100.000	40,000.000	36,189.000	1,994.270	583
Minimum frequency (minimum entire), Hz	33,200.000	39,000.000	34,658.800	1,451.020	583
Maximum frequency (minimum entire), Hz	900.000	47,800.000	20,448.700	21,233.800	583
Bandwidth (minimum entire), Hz	900.000	9,700.000	2,933.440	3,236.670	583
Peak frequency (maximum entire), Hz	34,100.000	58,500.000	47,625.700	7,399.520	583
Peak amplitude (maximum entire), Pa	95.690	113.559	103.475	5.294	583
Minimum frequency (maximum entire), Hz	33,200.000	247,000.000	151,658.000	103,147.000	583
Maximum frequency (maximum entire), Hz	42,900.000	60,500.000	55,135.300	4,737.430	583
Bandwidth (maximum entire), Hz	9,700.000	27,300.000	19,396.000	4,920.640	583
Peak frequency (mean entire), Hz	34,100.000	45,800.000	40,983.700	3,393.540	583
Peak amplitude (mean entire), Pa	92.170	105.500	97.604	3.889	583
Minimum frequency (mean entire) , Hz	33,200.000	53,800.000	40,681.100	5,359.230	583
Maximum frequency (mean entire), Hz	42,900.000	52,300.000	47,774.000	2,725.990	583

3.3.

(i). Analysis of the acoustic transmission parameters and the behavioural response of the female *A. gambiae* in the 35-60 kHz sound of *O. tormotus*.

The filtered sound of *O. tormotus* which was saved in the computer hard disc as *35-60 kHz Odorrana tormotus.wav* was played using Avisoft SAS Lab Pro version 5.1software. The female *A. gambiae* were placed in the bioassay cage using an aspirator one at a time and observations made. In recent studies the behaviour of the female *A. gambiae* initiated by the sound of *O. tormotus* was compared to its behaviour under the control experiment. The behaviour under the control was obtained without playing any of the sounds (Mang'are *et al*., 2012). Table 3.6 shows the behaviour of the female *A. gambiae* under the control and under the influence of the 35-60 kHz sound of *O. tormotus*. The behavioural responses observed as the 35-60 kHz sound of *O. tormotus* progressed, compared to the control indicate excitation and occasional immobility in the mosquito. The pulsate incoming sound associated with acoustic energy that also varied as the amplitude evoked the antenna to resonate accordingly. This in turn immobilizesd the female *A. gambiae* as in the case of mosquito 3, 5, 6-10 (Mohankumar, 2010, Mang'are *et al.*, 2012). Immobility was characterised by weak movements, exhaustion, collapsing, laying on floor, rolling, low flights, limbs curved on abdomen. Recent studies showed that Mosquitoes detected ultrasound in the range of 38 - 44 kHz, regardless of the source, initiating avoidance response since it creates stress on their nervous system (Mohankumar, 2010, Mang'are *et al.*, 2012). Nerve stress was responsible for elevated antennae and other behavioural responses indicated in Table 3.6.

Table3.6. *The Chronological Behaviour of the female A. gambiae Elicited by the 35-60 kHz Sound of O. tormotus*

Mosquito Number.	**Control Study (No sound)**	**Mosquito Behaviour under 35-60 kHz Sound of *O. tormotus***
1.	- One hind leg raised	- Escaped through a gap between net and cage.
2.	- Raised two hind limbs	- Squeezed through net but did not get out
		- Proboscis squeezed through net
		- Movement along net was noted
		- Shaking body noted
		- Rested above sound source.
		- Shaken and moved along net.
		- Rubbed hind limbs
		- Moved sideways and shaking and Raised body
		- Body moved up and down at a fast rate
		- Antennae was raised
3.	- Normal movement	- Preferred corners and barriers
		- Right wing opened
		- Appeared exhausted
		- Collapsed and rested by back, wings down and limbs up. Recovered
		- Squeezed through net
		- Head rested on surface
		- Moved forward and backwards
		- Appeared Weakened
		- Lay with abdomen on surface and very weak.
		- Antennae raised
4.	- Rubbed hind limbs while resting on net	- Movement along net
Mosquito Number.	**Control Study**	**Mosquito Behaviour under 35-60 kHz Sound of *O. tormotus***
		- Squeezed proboscis through net
		- Shaken severally accompanied with backward movement.
		- Hid in corners and barriers
		- Squeezed on floor surface
		- Hopping movement
		- Limbs widened
		- Thorax rested on the floor.
		- Jumps noted
		- Antennae raised
5.	- Flew about	- One wing opened
	- Rested behind barrier	- Shaky and lay on floor
		- Lay by back
		- Abdomen down with raised limbs
		- Lay sideways severally
		- Rolled on surface severally
		- Jumps noted
		- Raised body
		- Moved backwards and rests behind barrier
		- Limbs spread backward.
		- Rolled and lay sideways
		- Lay by abdomen
		- Exhibited low flight
		- Tried to raise up when at barrier
		- Limbs coiled over the back

Mosquito Number.	Control Study	Mosquito Behaviour under 35-60 kHz Sound of *O. tormotus*
		- Lacked equilibrium (balance) and tried to rise when lying by side.
		- Jumped up and down trying to raise from surface
		- Rolled on surface.
		- Appeared shaken
		- Antennae raised
6.	- Rested at 45° to surface	- Lay by thorax and abdomen
		- Widened limbs with body down
		- Jumps observed
		- Shaken and stretched hind limbs
		- Body raised and lowered from surface severally.
		- Jumped and fell by body
		- Flapped wings
		- Antennae raised
7.	- No body movement	- Lowered body
		- Forced proboscis through barrier walls with abdomen raised.
		- Widened limbs with raised antennae
		- Raised head with abdomen on floor surface.
		- Only five limbs noted
		- Entire body down
		- Shaken body which moved forward and backwards
		- All limbs curved above the back with abdomen down
		- Lay by side
		- One wing opened
8.	- Normal rest	- Folded front left leg
Mosquito Number.	**Control Study**	**Mosquito Behaviour under 35-60 kHz Sound of *O. tormotus***
		- Body moved left and right
		- Remained still for a long time
		- Body shaken
		- Raised right hind leg severally
		- Abdomen raised while rubbing hind limbs
		- Two fore limbs raised above head
		- Proboscis rested on surface
9.	- Normal rest	- Hops along net seen
	- Rubbing hind limbs	- Shaken
		- Opened wings
		- Low flight
		- Shaken and knocked itself on cage walls and net
		- Fell severally
		- Widened limbs
		- Bouncing movement observed
		- Erected antennae
		- Right hind leg raised
		- Body lowered to surface
		- Raised and separated antennae
		- Squeezed through walls
		- Jumps observed severally
10.	- Normal posture, body at 45° from surface of rest with wings along body.	- Shaken body
Mosquito Number.	**Control Study**	**Mosquito Behaviour under 35-60 kHz Sound of *O. tormotus***
	- Normal flight	- Jumped while walking along net
		- Flew for a long time as if looking for exit
		- Bounced on net surface
		- Moved along net
		- Body moved sideways while at rest

(ii). Analysis of the acoustic transmission parameters and the Activity of the female *A. gambiae* in the optimal frequency range.

The behaviour of the female *A. gambiae* discussed in 3.3 (i) was associated with movements (Flights - F) and Rests (or Landings - R), considered as mosquito activity. This section critically considers the mean activity for the ten mosquitoes under various time durations as shown in Table 3.17 and compares to the acoustics Energy, Bandwidth (mean) and Mean Peak amplitude in the 35-60 kHz sound of *O. tormotus*; and also compares with the mean mosquito activities under control, given in Table 3.16 in order to establish the existence of significant effect of the sound of *O. tormotus* on mosquito behaviour. A paired samples T-test at 95 % confidence level for the comparison between the mean activity for the ten mosquitoes under various time durations and the acoustics energy established that there was mean difference between the mean mosquito activity and acoustic energy which was statistically significant at t = -5.64005, df =8, p = 0.000487 as shown in Table 3.9. The null hypothesis is thus not accepted and instead accept alternative. In conclusion, the acoustic energy significantly affected the mosquito activity under the 35-60 kHz sound of *O. tormotus*. In this range of sound frequency, the mean acoustic energy was 1.429 Pa^2s and the mean of the mean mosquito activity was 59.333 as given in Table 3.7. It is vividly clear that there existed a low positive correlation at a Pearson's product moment correlation coefficient, ***r*** = 0.156 between the Acoustic energy and the Mean Mosquito Activity as shown in Table 3.8 due to the pulsate nature of the signal. An increase in acoustic energy sometimes led to a corresponding increase in activity. However, in some cases, immobility was observed as exhibited in mosquito sample 8 whose rate of activity under control was **1.68** per minute (Mohankumar, 2010, Mang'are *et al.*, 2012). The comparison of the rate of activities of the mosquito under the control and the 35-60 kHz sound is given in Table 3.13.

Table3.7. *Paired Samples Statistics of the Acoustic energy and Mean Mosquito Activity*

	Mean	N	Std. Deviation	Std. Error Mean
Acoustic energy	1.429	9	3.526	1.175
Mean Mosquito Activity	59.333	9	32.465	10.822

Table3.8. *Paired Samples Correlations of the Acoustic energy-Mean Mosquito Activity*

	N	Correlation	Sig.
Acoustic energy - Mean Mosquito Activity	9	0.515	0.156

Table3.9. *Paired Samples T-test of the Acoustic Energy Compared with Mean Mosquito Activity*

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Acoustic energy - Mean Mosquito Activity	-57.904	30.800	10.267	-81.5786	-34.229	-5.640	8	0.000487

Also, a paired samples T-test at 95 % confidence level for the comparison between the mean activity for ten mosquitoes under the Control experiment and the 35-60 kHz sound showed that there existed a mean difference between the mean mosquito activities under control experiment and the 35-60 kHz sound of *O. tormota* which was statistically significant at ***t = -4.142, df =8, p = 0.0032*** as shown in Table 3.10. Hence the null hypothesis is not accepted and instead accept alternative. In conclusion, there was a significant change in mosquito activity under the 35-60 kHz sound of *O. tormotus*. Also, there existed high positive correlation at a Pearson's product moment correlation coefficient, ***r*** = 0.773 between the Mean Mosquito Activity under control experiment and under the 35-60 kHz sound of *O. tormotus*; which was highly significant. The mosquitoes noted to be very active at control experiment displayed more activity under the influence of the 35-60 kHz sound of *O. tormotus*. The 35-60 kHz sound of *O. tormotus* significantly increased the activity of the female *A. gambiae* due to its excitation of nerve stress and fear for predation (Mohankumar, 2010).

Table3.10. *Paired Samples Correlations of the Mean Mosquito Activity Under Control and 35-60 kHz Sound of O. tormota*

Paired Samples Correlations			
	N	Correlation	Sig.
Control and 35-60 kHz Sound	9	0.773	0.0145

Table3.11. *Paired Samples T-test of the Mean Mosquito Activity under Control and the 35-60 kHz sound of O. tormota*

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Control and 35-60 kHz Sound	-39.333	28.491	9.497	-61.234	-17.433	-4.142	8	0.0032

A paired samples T-test of the individual mosquito sample activity under control and the 35-60 kHz sound of *O. tormota* showed significant ($p < 0.05$) change in Mosquito activities in 80 % of samples of mosquitoes observed as given in Table 3.15 and Table 3.17. The female *A. gambiae* exhibited a mean rate of activities of 3.643 /minute when exposed to the 35-60 kHz sound of *O. tormotus* which was above control results rated at 0.789 activities/minute. It is evidently clear that the mean rate of mosquito activities under the influence of the sound of *O. tormotus* was 4.617 times greater than the activities under the control. The statistics for this comparison are given in Table 3.12. A further comparative study on individual mosquito sample Rate of activities per Minute under the influence of 35-60 kHz sound of *O. tormota* and under control experiment using paired samples T-test showed a high significance, at $p = 0.013$ in the change of the rate of mosquito activity at 95 % confidence interval, shown in Table 3.18. The comparison showed insignificantly low negative correlation ($r = -0.356$) as given in Table 3.14, due to the pulsate nature of the 35-60 kHz sound of *O. tormota*. The mosquito antennae which resonated at the frequency of incoming wave receives pulsate energy which initiates increased activity and sometimes immobility due to nervous stress and fear for predation (Berry, 1966; Martin and Daniel, 2000; Zwiebel and Pitts, 2006; Enayati *et al*., 2010, Mohankumar, 2010, Mang'are *et al*., 2012).

A similar comparison of the between the mosquito mean rate of activities under the 35-60 kHz Sound of *O. tormotus* with Bandwidth and Amplitude yielded highly significant values as given in Table 3.19. As earlier observed, acoustic energy $E \alpha A^2$, an increase in amplitude leads to a corresponding increase in acoustic energy at which the antennae of the mosquito resonates (Martin and Daniel, 2000; Young *et al.*,2012; Serway and Jewett, 2014). The comparison between the mean mosquito activities and the Bandwidth (mean) showed insignificantly low positive correlation ($r = 0.044473$) as given in Table 3.20. Also, the comparison between the mean mosquito activities and Peak Amplitude (mean) showed a slightly higher positive correlation ($r = 0.191364$) compared to that under bandwidth. The comparison of the mean mosquito rate activities under the 35-60 kHz sound of *O. tormotus* with Peak Frequency (mean), shown in Table 3.21, was highly significant; implying that frequency which is directly proportional to acoustic energy greatly affected the mosquito behaviour and rate of activity.

Table3.12. *Paired Samples Statistics of the Rate of Mosquito Activity under Control and the 35-60 kHz sound of O. tormota*

	Mean	**N**	**Std. Deviation**	**Std. Error Mean**
Rate of Activities Under Control	0.789	10	0.801	0.253
Rate of Activities Under 30-60 kHz Sound	3.643	10	2.561	0.810

Table3.13. *Paired Samples T-test of the Rate Mosquito Activity under Control and the 35-60 kHz sound of O. tormota*

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Rate of Activities under Control- Rate of Activities under 35-60 kHz Sound	-2.854	2.943	0.931	-4.959	-0.749	-3.067	9	0.013

Table3.14. *Paired Samples Correlations of the Rate Mosquito Activity under Control and the 35-60 kHz sound of O. tormota*

	N	Correlation	Sig.
Rate of Activities under Control and Rate of Activities under 35-60 kHz Sound	10	-0.356	0.313

Table3.15. *Paired Samples T-test of the Individual Mosquito Sample Activity under Control and the 35-60 kHz sound of O. tormota*

	Paired Differences					T	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Control Mosquito Sample 1 -Ultrasound Mosquito sample 1	-29.8889	6.234	2.078	-34.681	-25.097	-14.384	8	5.332×10^{-7}
Control Mosquito Sample 2 -Ultrasound Mosquito sample 2	-96.1111	51.326	17.109	-135.564	-56.658	-5.618	8	0.0005
Control Mosquito Sample 3 -Ultrasound Mosquito sample 3	-3.33333	9.407	3.136	-10.5645	3.898	-1.063	8	0.319
Control Mosquito Sample 4 -Ultrasound Mosquito sample 4	-37.2222	26.668	8.889	-57.7212	-16.723	-4.187	8	0.003
Control Mosquito Sample 5 -Ultrasound Mosquito sample 5	-8.66667	17.826	5.942	-22.3686	5.0353	-1.459	8	0.183
Control Mosquito Sample 6 -Ultrasound Mosquito sample 6	-8.44444	36.576	12.192	-36.559	19.670	-0.693	8	0.508
Control Mosquito Sample 7 -Ultrasound Mosquito sample 7	-47.1111	29.608	9.869	-69.8695	-24.353	-4.774	8	0.0014
Control Mosquito Sample 8 -Ultrasound Mosquito sample 8	43.33333	10.087	3.362	35.57969	51.087	12.888	8	1.242×10^{-6}
Control Mosquito Sample 9 -Ultrasound Mosquito sample 9	-66.6667	51.493	17.164	-106.247	-27.086	-3.884	8	0.0046
Control Mosquito Sample 10 -Ultrasound Mosquito sample 10	-136.778	74.836	24.945	-194.302	-79.254	-5.483	8	0.000585

Table3.16. *Total Mosquito Sample Activities under Control for various Time Duration*

Mosquito Sample	Mosquito Activity Duration (s)								
	200.00	400.00	600.00	800.00	1000.00	1200.00	1400.00	1600.00	1800.00
Mosquito 1	2	4	10	11	12	14	14	14	14
Mosquito 2	1	14	14	14	15	15	15	15	15
Mosquito 3	17	19	24	39	39	39	39	39	39
Mosquito 4	12	12	12	13	13	13	13	13	13
Mosquito 5	13	20	24	25	25	25	25	25	25
Mosquito 6	16	32	67	72	72	72	72	72	72
Mosquito 7	1	1	1	1	1	1	1	1	1
Mosquito 8	20	36	49	49	49	49	49	49	49
Mosquito 9	1	1	1	1	1	1	1	1	1
Mosquito 10	1	1	1	2	3	3	3	3	3
Mean Activity	8	14	20	23	23	23	23	23	23

Table3.17. *Total Mosquito Sample Activities under influence of 35-60 kHz Sound of O. tormotus for various Time Duration*

Mosquito Sample	Mosquito Activity Duration (s)								
	200.00	400.00	600.00	800.00	1000.00	1200.00	1400.00	1600.00	1800.00
Mosquito 1	16	33	45	45	45	45	45	45	45
Mosquito 2	30	49	72	90	104	132	150	174	182
Mosquito 3	10	17	24	33	38	43	45	54	60
Mosquito 4	14	18	28	39	56	56	67	80	91
Mosquito 5	0	10	12	24	36	46	52	52	53
Mosquito 6	10	28	30	38	62	83	108	127	137
Mosquito 7	10	16	21	29	51	66	75	80	85
Mosquito 8	1	1	1	1	1	1	1	1	1
Mosquito 9	11	19	28	45	59	70	86	123	168
Mosquito 10	24	52	89	114	146	164	195	222	245
Mean Activity	13	24	35	46	60	71	82	96	107

Table3.18. *The Rate Activities of Mosquito Samples under Control and the 35-60 kHz Sound of O. tormotus*

Mosquito Sample	1	2	3	4	5	6	7	8	9	10
Number of Activities per Mosquito Sample Under Control	14	15	38	13	25	72	1	49	1	3
Number of Activities per Mosquito Sample Under the 35-60 kHz	44	182	60	91	53	137	84	1	168	245
Rate of activity per Minute under Control	0.48	0.51	1.30	0.44	0.86	2.46	0.03	1.68	0.03	0.10
Rate of activity per Minute under 35-60 kHz Sound	1.51	6.23	2.05	3.11	1.81	4.69	2.87	0.03	5.75	8.38
Activity Factor relative to Control	3.14	12.13	1.58	7.00	2.12	1.9	84.00	0.02	168.00	81.67

Table3.19. *Comparison of the Mean Mosquito Rate Activities under the 35-60 kHz Sound of O. tormotus with Bandwidth and Amplitude*

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Bandwidth (mean) - Mean Mosquito Rate Activities	19851.78	6497.569	2165.856	14857.3	24846.25	9.166	8	1.62×10^{-05}
Peak Amplitude (mean) - Mean Mosquito Rate Activities	34.096	31.876	10.625	9.594	58.597	3.209	8	0.012444

Table3.20. *Paired Samples Correlations of the Mean Mosquito Rate Activities under the 35-60 kHz Sound of O. tormotus with Bandwidth and Amplitude*

	N	Correlation	Sig.
Bandwidth (mean) and Mean Mosquito Rate Activities	9	0.044473	0.90955
Peak Amplitude (mean) and Mean Mosquito Rate Activities	9	0.191364	0.621858

Table3.21. *Comparison of the Mean Mosquito Rate Activities under the 35-60 kHz Sound of O. tormotus with Peak Frequency (mean)*

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Peak Frequency (mean) and Mean Mosquito Rate Activities	41885.11	3967.366	1322.455	38835.52	44934.7	31.67223	8	1.07×10^{-09}

4. Conclusion

The 583 calls studied were pulsate in nature had varied call durations and the signal power of the sound of *O. tormotus* varied between 40.5 dB and 73.0 dB. The mean peak amplititude varied between 85.42 Pa and 102.15 Pa with calls dominating between 90 Pa and 99 Pa peak amplitudes.

There was sufficient evidence for significant relationship between acoustic energy and amplitude, bandwidth and frequency. The behavior of female *A. gambiae* was characterized by excitation and immobility responses due to the pulsate acoustic energy.

A comparison between the mean mosquito activity and acoustic energy using a paired samples T-test at 95% confidence was highly significant at $p = 0.0000487$, with a low positive correlant ($r = 0.156$). The mean of the acoustic energy and mosquito activity in this range were 1.429 Pa^2s and 59.333 respectively.

The activities of the female *A. gambiae* under the influence of 35-60 kHz sound differed significantly ($p = 0.0032$) from their activities at the control with a highly positive correlation ($r = 0.773$). The rate of mosquito activities under the 35-60 kHz sound of *O. tormotus* increased by 4.617 times greater than the activities under the control experiment. The change in the rate of mosquito activities was statistically significant at $p = 0.013$ and $r = -0.356$; attributed to stress experienced on the nervous system besides fear of predation. Also, the mean rate of mosquito activities under the influence of the 35-60 kHz sound of *O. tormotus* varied significantly with the peak amplitude, bandwidth and frequency.

The female *A. gambiae,* a Malaria vector can therefore be effectively repelled using the 35-60 kHz sound of *O. tormotus* besides other control measures whose transmission parameters have been determined and analysed.

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References

[1] Ballou, M.G. (2002). *Handbook for Sound Enginneers.* (3rd Edition). Focal Press Publisher, Burlington. Pp 1 – 25.

[2] Berry, P. Y. (1966). The food and feeding habits of the torrent frog, *Amolops larutensis*. *Journal of Zoology*, **149**. Pp 204–214.

[3] Dattoli, G., Cesarano, C. and Sacchetti, D. (2002). Pseudo-Bessel functions and applications. *Georgian Mathematical Journal.* **9**. Pp 473 – 480.

[4] Enayati, A., Hemingway, J. and Garner, P. (2010). Electronic mosquito repellents for preventing mosquito bites and malaria infection. *The Cochrane Library Journal* .**3**. pp 1 – 16.

[5] Feng, A.S., Narins, P.M., Xu, C.H., Lin, W.Y., Yu, Z.L., Qiu, Q., Xu, Z.M. and Shen, J.X. (2006). Ultrasonic communication in frogs. *Nature, International Weekly Journal of Science*. **440**. Pp 333 - 336.

[6] Feng, A.S., Narins, P.M. and Xu, C.H. (2002). Vocal acrobatics in a Chinese frog, *Amolops tormotus*. *Naturwissenshaften Journal*. **89**. Pp 352 - 356.

[7] Ghaninia, M. (2007). Olfaction in mosquitoes: Neuroanatomy and electrophysiology of the olfactory system. *Doctoral dissertation.* Swedish University of Agricultural Sciences.

[8] Hay, S. I., Guerra, C. A., Gething, P. W., Patil, A. P., Tatem, A. J., Noor, A. M., Kabaria, C. W., Manh, B. H., Elyazar, I. R. F., Brooker, S., Smith, D. L., Moyeed, R. A. and Snow, R. W. (2009). A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Medicine Journal*. **6**. Pp 281 - 291.

[9] Kamau, L., Lehmann, T., Hawley, W.A., Orago, A.S. and Collins, F.H. (2006). Microgeneographic genetic of *Anopheles gambiae* mosquitoes from Asembo Bay, Western Kenya: A comparison with Kilifi in Coastal Kenya. **103**. Pp 16619 - 16620.

[10] Mang'are, P. A., Maweu, O. M., Ndiritu F. G. and Vulule, J. M. The Startling Effect of the Sound of C. afra and A. tormotus on the Female A. gambiae. International Journal of Biophysics. 2(3): Pp 40-52. (2012).

[11] Mang'are, P. A., Maweu, O. M., Ndiritu F. G. and Vulule, J. M. A Comparative Study of the Transmission Parameters of the Sound of Mosquito Predators and EMR.

[12] Martin, C.G. and Daniel, R. (2001). Active auditory mechanics in mosquitoes. *The Royal Society Journal*. **268**. Pp 333-339.

[13] Mohankumar, D. (2010). Ultrasound and insects. *Electronics and Animal Science.* http://electroschematics.com/. Accessed on 14-June-10 10:00 AM.

[14] Ngo, A., Murphy, R. W., Liu, W., Lathrop, A. and Orlov, W. L. (2006). The phylogenetic relationships of the Chinese and Vietnamese waterfall frogs of genus *Amolops*. *Amphibian – Reptilia Journal.* **27**. Pp 81 – 92.

[15] Penna, M. and Rogoberto, S. (1998). Frog call intensities and sound propagation in the South American temperate forest region. http://www.jstor.org. Accessed on 11-July-10 2:00 PM.

[16] Serway, R. A. And Jewett, J. W. Jr. (2014). Physics for Scientists and Engineers with Modern Physics. 9th Edition. Mary Finch Publisher, Physics and Astronomy: Charlie Hartford

[17] Shen, J. (2007). New progress on acoustic communication in the concave-eared torrent frog and its revelation. *Science Foundation in China.* **15**. Pp 1-3.

[18] Steve, C. (2006). Chinese frog that squeaks like a mouse. The Independent. http://nature.net/forums/load/reptiles/msg0312334716764.html?1. Accessed on 25-April-10 11:20 AM.

[19] UNICEF. (2014). MALARIAA MAJOR CAUSE OF CHILD DEATH AND POVERTY IN AFRICA. New York

[20] WHO. (2009).World malaria report 2009: Impact of malaria control. P39. http://wholibdoc.who.int. Accessed on 26-May-10 3:18 PM.

[21] Young, H. D., Freedman, R. A. and Ford, A. L. (2012). University Physics with Modern Physics. 13th Ed. Pearson Education, Inc., publishing as Addison-Wesley.

[22] Zwiebel, J. L. and Pitts, R. J. (2006). Antennal sensilla of two female Anopheline sibling species with differing host ranges. *Malaria Journal*. **5**. Pp 1 – 12.

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