DETERMINATION OF PREDATOR ULTRASOUND PARAMETERS AND ACOUSTIC STARTLE RESPONSE IN THE AFRICAN FEMALE *Anopheles gambiae s.s.*

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DECLARATION AND RECOMMENDATION

Declaration

This thesis is my original work and has not, wholly or in part, been submitted for the award of a degree in any other University.

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Recommendation

This thesis has been submitted for examination with our approval as the candidates' official University supervisors.

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ABSTRACT

The female *Anopheles gambiae*, a malaria vector, detects ultrasound by its antenna, which can initiate an attractive or repulsive response. Modern electronic mosquito repellent devices exploit this concept in attempt to control malaria. However, they have shown only 20 % effectiveness in repellency. This work determines the transmission parameters of *Amolops tormotus*, *Coleura afra* sounds and their combination and hence their startling effects on female *Anopheles gambiae*. The sound of *C. afra* and *A. tormotus*, which have not been investigated, were recorded with the 112 Avisoft and 702 digital recorders respectively, from Kit-Mikayi caves in Kenya and Huangshan Hot Springs in China, respectively. A bioassay was set up with 3-4 day old female *A. gambiae* exposed to the three sounds at varied frequencies and the number of activities and behavioural responses noted. The fundamental frequency of the sound of *A. tormotus, C. afra* and` their combination was 5.371 kHz, 6.836 kHz and 4.883 kHz, respectively. The spectrograms of the sound of *A. tormotus*, *C. afra* and` their combination showed FM and CF components. The frequency range for significant startle of mosquitoes was 35-60 kHz for individual predator sounds whereas 10-34 kHz for the combined sound. Similarly, the amplified mean peak amplitude for the sound of *A. tormotus*, *C. afra* and` their combination was 134.08 dB SPL, 134.28 dB SPL and 133.60 dB SPL, respectively. The mean bandwidth of the combined sound was 16.4 kHz, narrowed by 3.4 kHz and 3.0 kHz from that of the sound of *C. afra* and *A. tormotus* respectively. The mean bandwidth of the combined sound was significantly narrowed from 77.24 kHz, the reported mean bandwidth of EMR sound. The sound of *A. tormotus* and *C. afra* recorded a mean peak frequency of 47.60 kHz and 45.9 kHz respectively with the combined sound recording 29.4 kHz in the optimum frequency range. The maximum acoustic energy in the optimum frequency range for the sound of *A. tormotus*, *C. afra* and` their combination were 10.84 Pa^2s, 12.32 Pa^2s and 6.08 Pa^2s, respectively. The combined predator sound and sound of *A. tormotus* evoked evasive behavioural responses in 30 % and 46 % of the mosquitoes, higher than the reported 20 % effective repulsion by EMR sound. The evasive response was characterized by 58.5^o antenna erection, physical injury, unusual rest and movement, fatigue and falls; attributed to stress on nervous system and fear of predation. This work established that ultrasound affects the female *A. gambiae* by repelling it in addition to determining the optimum acoustic transmission parameters needed for the design of an effective electronic mosquito repellent. Further investigation into the repellency of sonar sound of *C. afra* and the effective repulsive frequency band of the combined sounds is recommended.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

ABBREVIATIONS AND SYMBOLS

Ampl	Amplitude
AUSG	Avisoft Ultrasound Gate
Bandw	Bandwidth
BW	Bandwidth
CDC	Centre for Disease Control
CF	Constant Frequency
CTR	Control
DDE	Direct Data Exchange
EMR	Electronic Mosquito Repellent
FFT	Fast Fourier Transform
FIR	Finite Impulse Response
FM	Frequency Modulation
Fn	Flight state of mosquito sample n, n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
FREQ	Frequency
IRS	Indoor residual spraying
ITNs	Insecticide treated nets
Max freq	Maximum frequency
Min freq	Minimum frequency
P*f*PR	*Plasmodium falciparum* Prevalence Rate
Rn	Rest state of mosquito sample n, n = 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
USB	Universal Serial Bus

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Malaria is caused by a protozoan parasite of genius *Plasmodium* and is transmitted by at least eight *Anopheles* species of which the female mosquito, *Anopheles gambiae,* is the key cause of mortality and morbidity in Africa (Hay *et al*., 2009; Enayati *et al.*, 2010). Plasmodium parasites, the causative agents of malaria, kills over a million people per year, and another 500 million people suffer from the clinical disease (Hillyer, 2009). The symptoms of this disease are diverse among them anaemia and jaundice and may result in death if not treated in time. The female *A. gambiae*, the malaria vector, requires blood either from human or animal, for egg development. The *A. gambiae* lays 30-150 eggs in 2-3 days (Kakkilaya, 2007; Hillyer, 2009). However, the male *A. gambiae* are incapable of feeding on blood because they lack piercing mouth parts (Klowden, 1995; Ghaninia, 2007). Malaria is a life threatening disease and many control measures have been undertaken. These include: chemotherapy, chemoprophylaxis, vector control strategies and development of malaria vaccine (WHO, 2009; Enayati *et al*, 2010). Currently malaria vector control-methods preferred include the use of ITNs, IRS, destruction of mosquito breeding sites and use of mosquito repellents (WHO, 2009). 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 (Ghaninia, 2007; WHO, 2009). Effective prevention requires use of a combination of factors which address the habits of mosquitoes and their interaction with human beings (WHO, 2009).

Female mosquitoes produce familiar whining sound while searching for a blood meal (proteins). The frequency of this sound reflects the wing beat of the species. This frequency ranges from 150 -500 Hz, with maximum intensity at 380 Hz for *A. gambiae* (Maweu *et al*., 2011). The mosquito sound, which is frequency modulated, is transmitted in air activating the antennae of conspecific male besides providing directional indicators (Göpfert *et al*., 1999). These sexes are brought together by response of the males to flight sound of the females (Craig and Nijhout, 1971). There is a massive sensory organ, the Johnstone's organ, at the base of the antenna which resolves the sound (Martin and Daniel, 2000). Female mosquitoes are mated once in their lifetime and store sperms in a special organ called spermatheca (receptacula seminis). The stored sperm is used to fertilize eggs. Mated female mosquitoes, therefore, would avoid males seeking for a mate by detecting sounds produced by such males (Mohankumar, 2010).

Ultrasound from male mosquitoes and predators has been exploited as a safe and a possible effective measure to repel female mosquitoes seeking blood meals from humans. Electronic mosquito repellents which imitate the sounds of male mosquitoes, currently in use, produce 38 kHz to repel the female mosquitoes (Mohankumar, 2010). The African bat, *Coleura afra* and the Chinese frog, *Amolops tormotus* which are both insectivorous generate ultrasound, inaudible to human ear, through their vocalizations (Berry, 1966; Barlow, 2006; Feng *et al*., 2006). However, earlier experiments with functioning electronic mosquito repellents mimicking calls from bats and male *A. gambiae* in the frequency range of 125 Hz to 74.6 kHz showed 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, hence, considered insignificant to justify their use (Enayati *et al*., 2010). The repulsion of mosquitoes due to the Chinese frog, *A. tormotus* had not been investigated. Due to this low effectiveness in mosquito repulsion, the current study was undertaken to determine optimal parameters that startle female mosquitoes through combining recorded sounds of *A. tormotus* and the *C. afra* with a view to increase effectiveness. These parameters included peak frequency, peak amplitude, fundamental frequency of the call, maximum and minimum frequency, bandwidth, acoustic energy, means of the parameters and standard deviations through the automatic parameter or manual measurements for both individual and combined sound of the *C. afra* and the *A. tormotus*. Similarly, the startle effect was also observed and noted. The optimum startle frequency range was determined by recording the mosquito activity, hourly rate of activities, behavioural traits response and acoustic parameter variation through analysis of variance. The results of this study are expected to provide factual ultrasound parameters for designing an effective electronic mosquito repellent. These results also justify the use of ultrasound in startling the female *A. gambiae* hence adding to existing malaria control measures.

1.2 Statement of the Problem

Electronic mosquito repellents (EMR) that mimic ultrasonic calls from bats and male mosquitoes, *A. gambiae* have been designed and used in startling the female mosquitoes, *A. gambiae*. Earlier studies showed that the electronic mosquito repellents yielded only 20% significant repulsion on the female *A. gambiae*, due to a wide bandwidth of the sound rendering it less intense and ineffective. Hence, there was need to investigate the individual and combined natural ultrasounds from the African sheath tailed bat, *C. afra* and the Chinese torrent frog, *A. tormotus*; determine their startle effect on the female *A. gambiae* and optimal acoustic transmission parameters. Combining the predator sounds would narrow the average bandwidth and intensify the sound, thus improving on the effectiveness in the startling of the female mosquito *A. gambiae*.

1.3 Objectives

1.3.1 General objective

To determine the acoustic transmission parameters of the individual and combined ultrasound of the African sheath-tailed bat, *C. afra* and the Chinese torrent frog, *A. tormotus*; and the startling effect of the sounds on the female *A. gambiae,* a malaria vector.

1.3.2 Specific objectives

i. To determine the transmission parameters of sounds of the African bat, *C. afra* and the Chinese frog, *A. tormotus.*
ii. To determine the transmission parameters of the combined sound of *C. afra* and the *A. tormotus*.
iii. To determine the startling effect of the individual and combined sound of *C. afra* and *A. tormotus* on the female *A. gambiae.*

1.4 Justification

Electronic mosquito repellents which mimic ultrasound from animal species are currently being used to repel mosquitoes. However, these electronic mosquito repellents which generated wide bandwidth sound, yielded only 20% startle response in the female *A. gambiae* rendering them less effective. The African bat *C. afra* and the Chinese frog *A. tormotus* generated ultrasound naturally through vocalisation. It was therefore important to investigate the effect of these naturally generated ultrasounds on the behaviour of the *Anopheles* mosquito. This research was also conducted in order to establish whether there was an improvement on the 20% startle effect on mosquitoes by combining ultrasound from *C. afra* and *A. tormotus*. Combining these animal ultrasounds intensified the acoustic energy and narrowed the bandwidth. However, investigation into the ultrasound from the *A. tormotus* on its startle effect on the female *A. gambiae* had not been conducted and reported. Therefore, the startle effect and optimal acoustic transmission parameters of the sound of *A. tormotus* was determined. Given that *C. afra* and *A. tormotus* were natural predators of mosquitoes, a combination of their sounds was expected to effectively startle the female *A. gambiae* due to natural fear of predation. The behavioural startle response of the female *A. gambiae* elicited by the combined sounds of *C. afra* and *A. tormotus* was also observed and the significant startle effect noted. The optimal parameters determined from the current research are critical to electronic mosquito repellents designers since effective devices could be realized. Thus, the results provide an additional tool in mosquito control which was environment friendly.

1.5 Scope of the Study

In this study, the acoustic transmission parameters and mosquito startle response to predator sound were determined and compared. The sound analysis softwares were donated by Avisoft Bioacoustics; Germany and Pettersson Elektronik AB; Australia. The sounds of *C. afra* were recorded from Kit-Mikayi caves in Kisumu, Kenya whereas the sound of *A. tormotus* was sourced from Huangshan Hot Springs in China through Prof. Feng of Illinois University, USA. A three months period bioassay study was conducted from KEMRI laboratories in Kisumu where the female *A. gambiae* mosquitoes were bred and reared.

1.6 Limitations of the Study

The original bioassay study was to involve twenty samples of the female mosquitoes for determining the number of mosquitoes repelled from the sound source, those sustained and those that were unable to distinguish the source of sound and escape route. The mosquitoes changed position freely through the three compartments of the bioassay arena hence the need to modify the experimental set up, cage design and parameters to be observed. The sounds of *A. tormotus* could not be sourced locally, hence the need to import. Similarly, ultrasound microphones were not readily available in Kenya hence sourced from Avisoft Bioacoustics; Germany and Pettersson Elektronik AB; Australia. Guidance on the use of the devices and sound analysis was directly sought from Raimund Spetch. Lack of ultrasound speakers necessitated the amplification of the sounds under in order to intensify the vibration of the diaphragm of ordinary speakers. The sound of *C. afra* recorded from a colony of bats consisted of both sonar and social calls, compromising on the overall quality. Also direct determination of the wavelength of the sound was difficult since the carrier frequency was seldom in the predator sounds. The maximum and minimum wavelengths of pulses were determined from automatically generated data and the velocity of sound in air, 340 m /s. The actual detection distance from source of sound to position of the female *A. gambiae* could not be determined as expected since a standard bioassay cage of fixed length was used. Only the bandwidth could be compared due to limited reported acoustic parameters.

CHAPTER TWO
LITERATURE REVIEW

2.1 Mosquito Biology

The *Anopheles* mosquito completes its life cycle in 1.5-3.0 weeks; going through the egg, larva, pupae, and adult stages (Keating *et al*., 2004; Kakkilaya, 2007; Hillyer, 2009; Yau, 2011). The first three stages in the life cycle of mosquitoes are aquatic and they last for 5-14 days (Kakkilaya, 2007; Hillyer, 2009). The female *A. gambiae* acts as a malaria vector during the adult stage (Benedict *et al*., 2003; Hillyer, 2009). The mosquitoes mate during flight after which the female search for a blood meal. The blood meal is digested and ovaries develop. The female *A. gambiae* lays 30-150 raft shaped eggs within two days which then hatch within 2-3 days (Kakkilaya, 2007; Hillyer, 2009). The *Anopheles* mosquito larva has a well-developed head with mouth brushes for feeding, a large thorax, and a segmented abdomen with no legs. The larva develops through four stages, or instars, after which it metamorphoses into a pupa. At the end of each instar, the larva molts, shedding its exoskeleton, to allow for further growth (Kakkilaya, 2007; Hillyer, 2009; Yau, 2011).

The pupa is comma-shaped with the head and thorax merged into a cephalothorax and comes to the surface frequently to breathe. After a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges. The *Anopheles* mosquito develops from egg to adult within 10-14 days under tropical conditions (Keating *et al*., 2004, Kakkilaya, 2007; Hillyer, 2009). The adult has slender body divided into head, thorax and abdomen. The head contains eyes and a pair of long, many-segmented antennae. The segmented body part expands considerably when a female takes a blood meal. The *Anopheles* mosquito has palps and discrete blocks of black and white scales on the wings (Keating *et al*., 2004; Kakkilaya, 2007; Hillyer, 2009). It rests with the abdomen sticking up in the air. The adult mosquitoes mate within a few days after emerging from the pupae stage. The females will feed on sugar sources for energy requiring a blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed (Kakkilaya, 2007; Hillyer, 2009; Yau, 2011).

2.2 Mosquito Communication and the Antenna Theory

Mosquitoes communicate using their antennae which are ultrasound sensors (Martin and Daniel, 2001; Mohankumar, 2010). The hearing organ mediates the direction of sounds at particular frequencies and low intensity (Craig and Nijhout, 1971). The ventral view of the head of an adult female *A. gambiae* giving the location of the antennae is shown in Plate 1 (Martin and Daniel, 2001). A case study on mosquito communication and differences in antennal sensitivity was conducted by Avitabile *et al*., (2010) using *Toxorhynchites brevipalpis*. The pedicel at the base of the antenna has the Johnstones' organ which is a sensory organ (Martin and Daniel, 2001). It serves as a movement sound detector in male mosquitoes, sensing antennal vibrations induced by the flight sounds of conspecific females (Robert and Jackson, 2006). The antennae of the female *A. gambiae* are sexually dimorphic (Zwiebel and Pitts, 2006). The flight frequency of the male *T. brevipalpis*, shown in Table 1, was greater than that of the female *T. brevipalpis* mosquito (Martin and Daniel, 2000). The audible frequencies which are received by the male antennae range between 150 - 500 Hz and evoke sexual behaviour that involves shifting pitches of their buzzes until they synchronize. Same sex mosquitoes' sounds cannot converge (Craig and Nijhout, 1971; Maweu *et al*., 2009).

Table 1: Flight and flagella resonant frequency of *T. brevipalpis* (Martin and Daniel, 2000)

Frequency (Hz)	Males	Females
Flight sound frequency	525.0± 27.0	415.0± 33.0
Flagella resonant frequency	420.0± 5.0	244.0± 11.0

The male's auditory system is thus selectively tuned to female flight frequencies of approximately 300 – 400Hz with maximum intensity at 380 Hz for *A. gambiae* (Robert and Jackson., 2006; Maweu *et al*., 2009; Avitabile *et al*., 2010). The distal elongated flagellum of each antenna, shown in Plate 2, acts as a mechanical filter and it is resonantly tuned in response to particle oscillations as a forced damped harmonic oscillator (Göpfert *et al*., 1999; Zwiebel and Pitts, 2006; Avitabile *et al*., 2010).

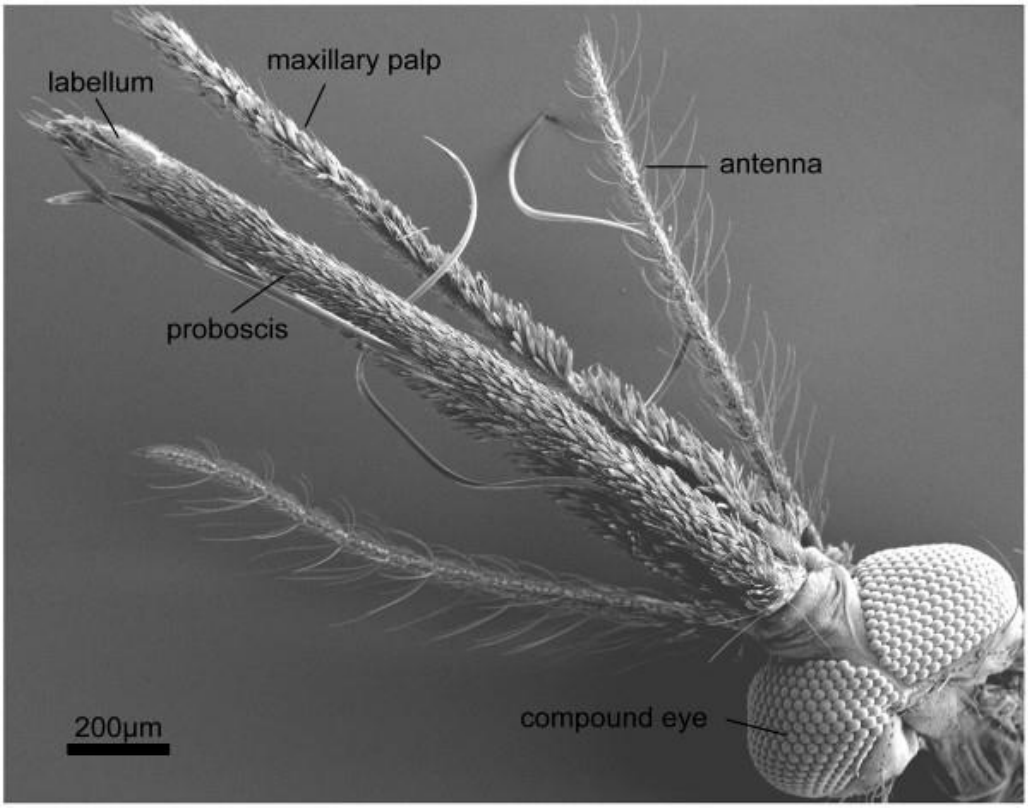

Plate 1: The ventral view of the head of the adult female *A. gambiae* (Göpfert *et al*., 1999; Zwiebel and Pitts, 2006).

Plate 2: The structure of an antenna of the *Anopheles* mosquito (Zwiebel and Pitts, 2006)

Simultaneous examination of acoustically elicited antennal vibrations and neural responses in *T. brevipalpis* demonstrates the exquisite acoustic and mechanical sensitivity of Johnston's organ in males and females (Avitabile *et al.*, 2010). The less sensitive female's Johnston's organ responds to antennal deflections of ± 0.0005° induced by ± 11 nm air particle displacements in the sound field which surpasses other insects' sensitivity (Martin and Daniel, 2000). Data indicated in Table 2 was obtained from six males and six females, measured through an input-output analysis of both the particle displacement d_p and the flagella displacement d_f. The greater sensitivity in the male *T. brevipalpis* is due to the verticillate flagella structure which increases the effective surface of the sound receiver. The data indicates that the sensitivity of the female's Johnston's organ is less compared to that of the male *T. brevipalpis* (Martin and Daniel, 2000).

Table 2: The antennal mechanics and Johnston's organ sensitivity (Martin and Daniel, 2000)

Displacement	Males	Females	Sensitivity factor
d_f at the neural threshold (nm)	7.3± 1.6	22.8± 6.4	3.1
d_p at the neural threshold (nm)	2.4± 0.3	11.2± 2.5	4.7
Mechanical sensitivity d_f/d_p	4.3 ± 0.4	2.8 ± 0.4	1.5

Mosquito sound is transmitted in air as frequency modulated wave and activates the antennae (Maweu *et al.*, 2009). The bandwidth for the medically important *A. gambiae* was found to be 781.6 Hz (Maweu *et al.*, 2011). 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 (Avitabile *et al.*, 2010; Mohankumar, 2010). The ultrasound also jams mosquitoes' own ultrasound frequency besides immobilizing them (Mohankumar, 2010). The mated female *A. gambiae* mosquitoes which detect the presence of male mosquitoes by sensing the 38 kHz ultrasound require blood meal for egg maturation (Kamau *et al.*, 2006). They keep away from males to avoid further insemination. An ultrasound generator imitating male mosquitoes produces 38 kHz that repel the female mosquitoes (Mohankumar, 2010). However, the frequency range also affected other insects such as beetles, but the minimum frequency being 30 kHz and the higher frequency being 45 kHz at thresholds of 50 ± 55 dB SPL (Yager *et al.*, 2000).

Recent research on reactive near-field of the *A. gambiae* mosquito shown in Fig. 1 indicated that the antenna theory helped in understanding the communication in mosquitoes (Maweu *et al*., 2009; Mohankumar, 2010). The hairs from the mosquito antennae serve as the dipole lengths and are equally spaced. The dipole lengths range from 0.139 to 0.05 cm for the *A. gambiae, Culex pipiens* and *Aedes aegypti*. There are 20 dipoles stemming from the 0.26 cm long transmission line (Maweu *et al*., 2009). Basically, mosquitoes use the reactive near-field in antennae communication.

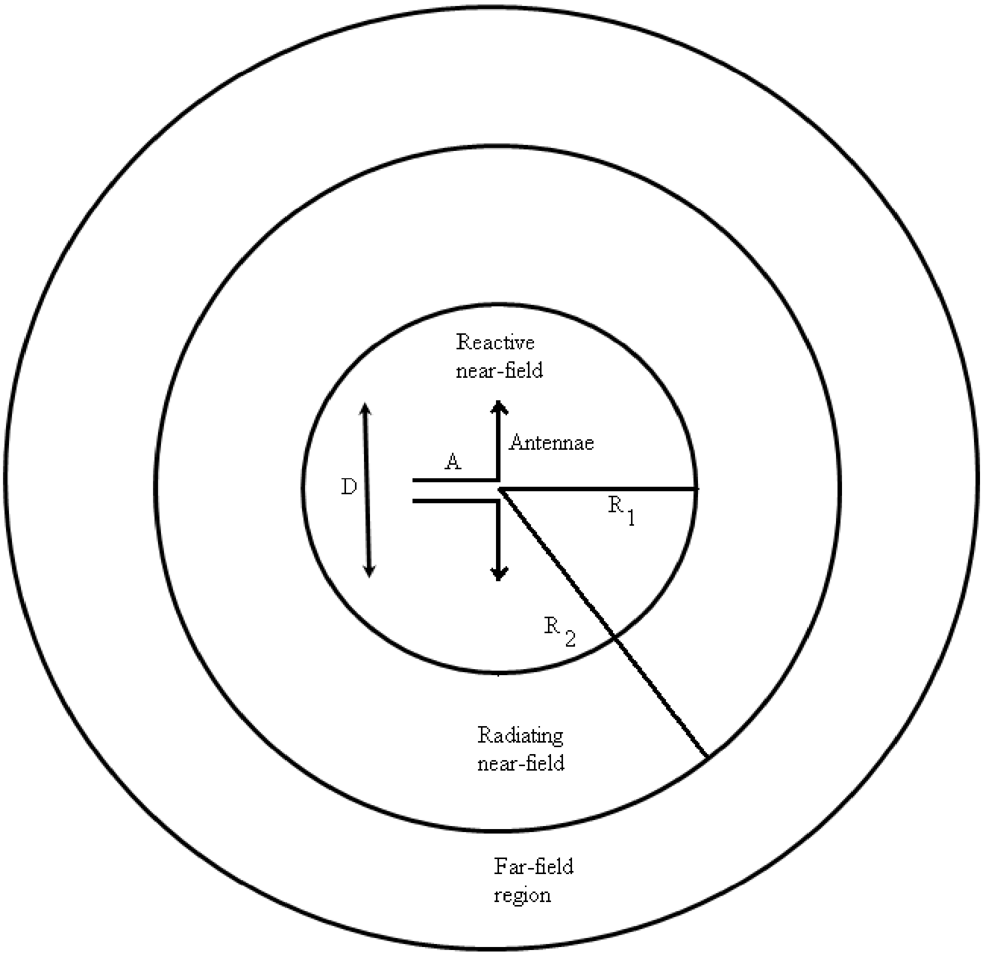

Fig. 1: The field regions of an antenna (Balanis, 1982)

These regions include the reactive near-field, the radiating near-field and the far-field region and are related to the mosquito antennae. The distances R_1 and R_2 represent the reactive near-field and the radiating near-field respectively whereas the part labeled A is the transmission line (Balanis, 1982). The reactive near-field is at distance given as $R < 0.62\sqrt{\frac{D^3}{\lambda}}$ from the antenna. The radiating near-field, which is predominant, is bound by distances

$R \geq 0.62\sqrt{\frac{D^3}{\lambda}}$ and $R < \frac{2D^2}{\lambda}$ and its angular field distribution depends on distance from the antenna. This field is called Fresnel if the antenna is focused at infinity. The far-field exists at a distance greater than $\frac{2D^2}{\lambda}$ and it is independent of the distance from the antenna. It is called Fraunhofer if the antenna is focused at infinity (Balanis, 1982; Gupta, 1989). Electromagnetic waves which transmit information through a wireless medium possess power and energy given by the Poynting vector,

$$\vec{W} = \vec{E} \, x \, \vec{H} \quad \text{…….……………...………..……….……………………..………......….........(2.1)}$$

Where $\vec{W}$ – Instantaneous Poynting vector, $\vec{E}$ – Instantaneous electric field intensity and $\vec{H}$ – Instantaneous magnetic field intensity.

The directive gain $D_g = \frac{U}{U_0} = \frac{4\pi U}{P_{rad}}$ ………….…......….….……...…………...………… (2.2)

Whereas the directivity is given as $D_o = \frac{U_{\max}}{U_o} = \frac{4\pi U_{\max}}{P_{rad}}$ ….............……….…..................(2.3)

Where P_{rad} is the total radiated power, U_o radiation intensity of the isotropic source, $U_{\max}$ maximum radiation intensity and U is the radiation intensity. The directive gain and directivity of an antenna can be expressed in decibel (dB) instead of the dimensionless quantities. Thus the directive gain, $D_g(dB) = 10 \log_{10}\{D_g(d)\}$ ……………………….........(2.4)

Similarly, the directivity is given as $D_o(dB) = 10 \log_{10}\{D_o(d)\}$ …………………..………(2.5)

The antenna efficiency is given as $e_t = e_r e_c e_d$ …………….……………………….....……....(2.6)

Where e_t is the total overall efficiency, e_r reflection efficiency, e_c conduction efficiency and e_d dielectric efficiency (Balanis, 1982; Gupta, 1989).

2.3 Malaria Situation in Africa

Malaria, which is transmitted by the female *A. gambiae*, is a major health problem in the sub-Saharan Africa (Mattingly, 1977; Ghaninia, 2007; Enayati *et al.*, 2010). The human malaria parasites include the *Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodium falciparium* of which later is common in Africa and life threatening

(Ikekpeazu *et al*., 2010). In the year 2008, an estimated 243 million malaria cases were reported worldwide, 85% of which were in Africa. Malaria accounted for an estimated 863 000 deaths in the year 2008, of which 89% were in the African Region (WHO, 2009). There are 1.4 billion people worldwide who are at risk of malaria infection (Kazuyuki *et al*., 2010). Most adults living in malaria endemic areas have partial immunity and are at risk of repeated infections. These adults are symptomatic carriers of the disease (Kakkilaya, 2002). Nearly 49% of the world's population lives in malaria risk areas (Ghaninia, 2007). Currently, malaria parasites have developed unacceptable levels of resistance to drugs and many insecticides are no longer useful against mosquitoes that transmit it (Ikekpeazu *et al*., 2010; Mnyone *et al.*, 2010). Extreme poverty and shortage of trained personnel impose severe constraints on medical services in most African states, and often a bad situation is rendered worse by tragic misuse of available resources (WHO, 1975).

The intensity of *P. falciparum* prevalence rates (*Pf*PR) in Africa for children aged between 2–10 years is pictorially represented by colour, which ranges from yellow to blue, corresponding to the probability of malaria prevalence intensity as shown in Plate 3. The stable *P. falciparum* transmission area in Africa region covered 18.17 million square kilometre, 61.10 % consisting 656.61 million people at risk and spanned a wide range in transmission intensity in the year 2007 (Hay *et al*., 2009).

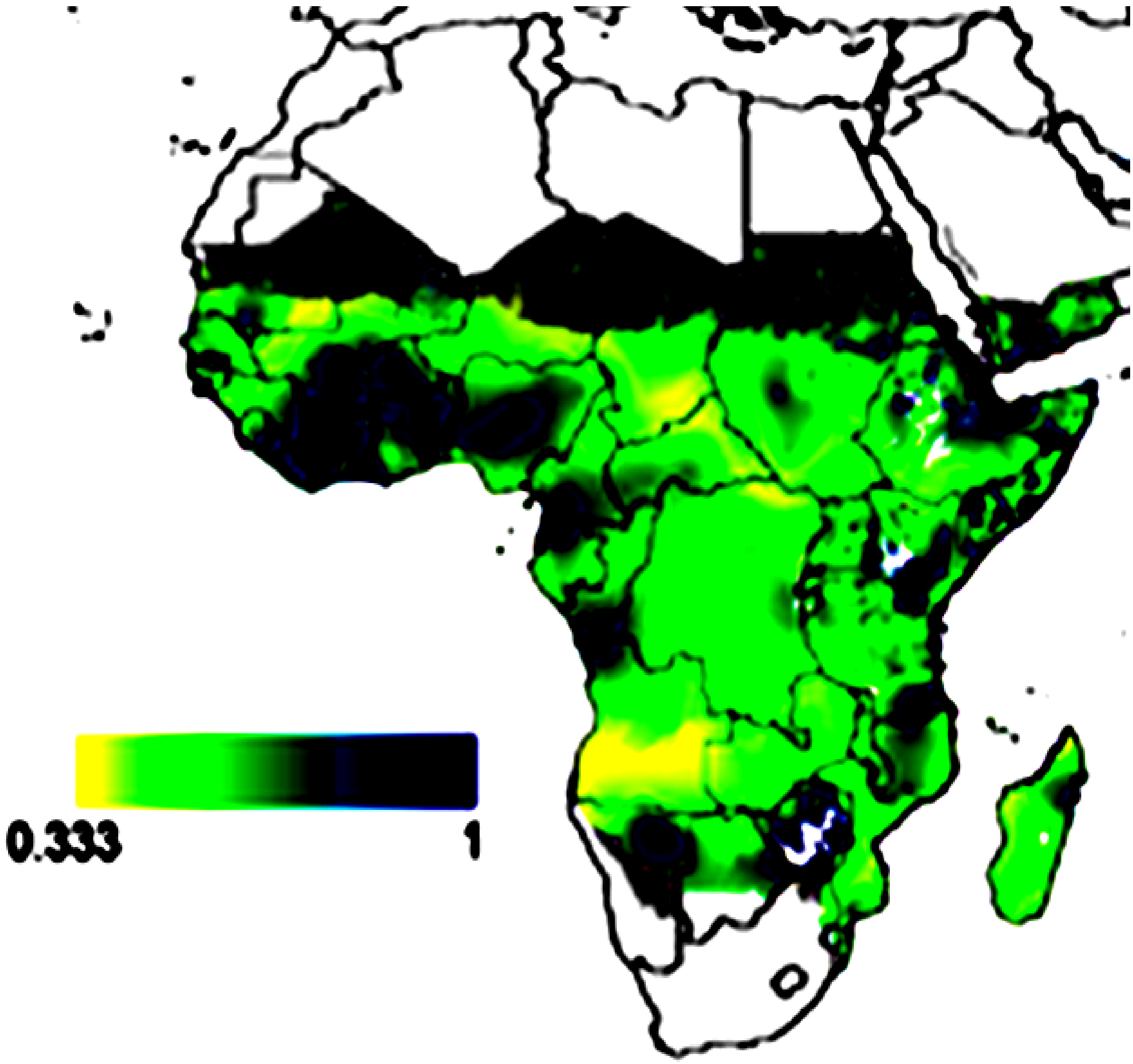

Plate 3: The intensity of P*f*PR in children aged 2-10years in Africa (Hay *et al*., 2009)

An estimated 74% of the population in Africa lives in highly endemic malaria areas and 19% in epidemic prone areas (Plate 4). Only 7% of the region’s population lives in low risk or malaria free areas (McGin, 2002).

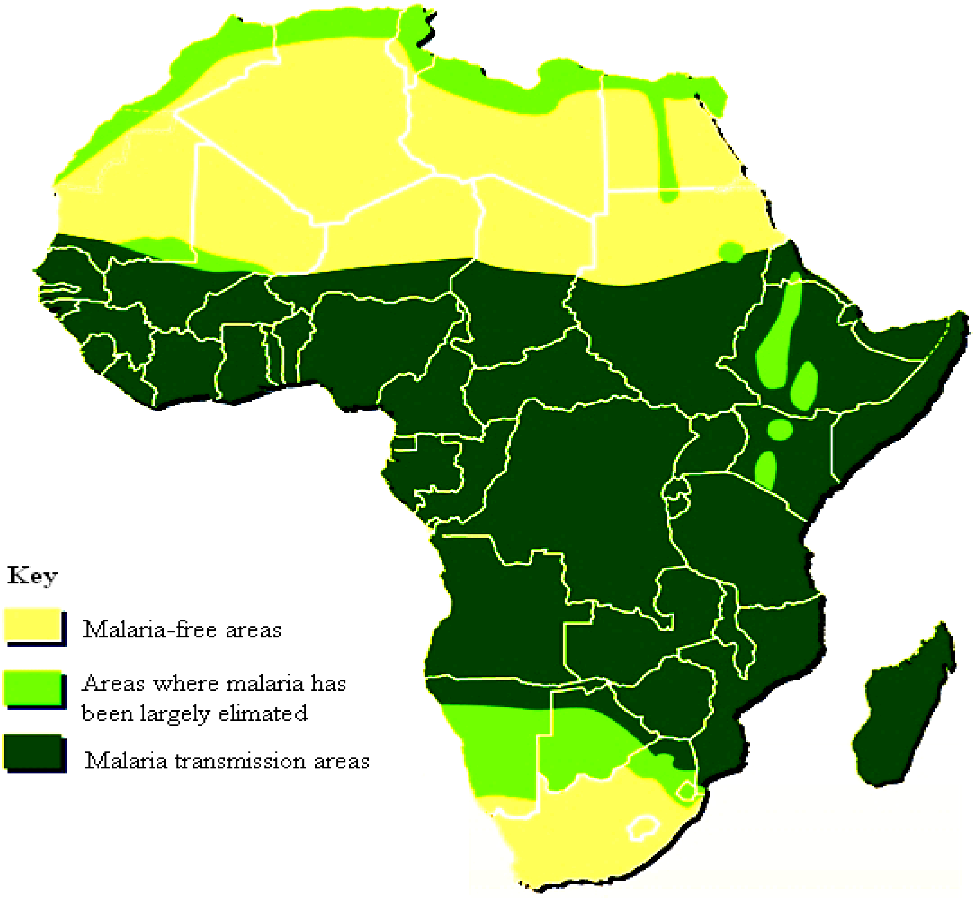

Plate 4: Malaria distribution in Africa (McGin, 2002)

2.4 Current Prevention and Control Measures for Malaria

The control measures being used against adult mosquitoes and larvae include chemical, biological, environmental and personal protection; some of which have negative environmental effects. Other strategies and approaches available for preventing mosquito bites and malaria infection include the use of repellents, insecticide treated mosquito nets (ITNs) and prophylaxis. ITNs have been proven to reduce mortality rate by approximately 20% (Okenu, 1999; Keating *et al*., 2004; WHO, 2009; Enayati *et al*., 2010; Johansson *et al*., 2010). Attempts in by use of chemical control and eradication of mosquitoes and malaria failed due to the buildup of resistance among both the mosquitoes and the disease agents (Ghaninia, 2007). Control of mosquito vectors is the mainstay in the fight against malaria, but alternatives are required in view of emerging insecticide resistance (Mnyone *et al.*, 2010). Experiments with functioning EMR yielded 12 out of 15 experiments in which the landing rates in the groups were actually higher than in the control groups; translating to 20% effective mosquito repulsion (Enayati *et al*., 2010). The reduced efficacy of current control methods, compounded by the failure to discover new drugs, insecticide replacements and effective vaccines, it became necessary to develop new control strategies. One strategy developed in recent years was to genetically manipulate insect pests such that they are unable to transmit disease-causing pathogens, and to mass release them into the environment to displace natural populations of susceptible mosquitoes (Benedict *et al.*, 2003). The challenge with the strategy was the identification of candidate mosquito genes that confer resistance to infection (Okenu, 1999).

2.5 Development, Use and Design of Sound Devices in Malaria Control

An experiment with the female, *Anopheles albimanus* recorded sound was performed and it was observed to lure male mosquitoes which were then electro-cuted (Morton and Offenhauser, 1949). Similar work done in Kenya by Maweu *et al* (2009) established that an electronic device was efficient in disrupting acoustic communication between the male and the female *A. gambiae*. Different bioassays were conducted with broadcasts from the device and taped mimics of the female wing beats at different distances and frequencies. The taped mimic sounds of the female mosquitoes in flight elicited a maximum response rate of 75% (Maweu *et al.*, 2009). An electronic arrangement for simulating animal sounds in form of warning calls, luring calls or scare-off calls. A clock pulse generator produces clock pulses which may be either integrated or differentiated for a portion of the pulse. These pulses are then applied to multivibrators of the astable type and transmitted to loud speakers through the use of power amplifiers. By partially integrating and/or differentiating the pulses, any desirable combination of audio signals may be produced (Hans *et al.*, 1971). Ultrasonic electronic devices repel mosquitoes by emitting high pitched simulates of the sound of mosquito predators (Roxanne, 2008). Mosquito electronic repellent devices (EMR) mimicking calls from single species predators are popular in developed countries though they give 20% effectiveness in repulsion (Enayati *et al*., 2010).

2.6 The Africa Bat, *Coleura afra* and the Chinese Frog, *Amolops tormotus*

2.6.1 The African sheath-tailed bat, *Coleura afra*

The bat belongs to order *Chiroptera* and inhabit in caves feeding on mosquitoes and other insects. It produces sound which falls in the frequency range of 20 – 100 kHz which is used for communication and navigation purposes (Khanna and Bedi, 1988; Hoy *et al.*, 1989; Miller and Surlykke, 2001). This sound is produced by tongue clicks or vocalization and occurs as paired clicks (Simmons *et al.*, 1984; Surlykke *et al.*, 2009). Aerially hawking bats emit these ultrasonic probes and detect flying insect prey by the echoes that return from their bodies (Pollack and Martins, 2007). The African bat points the sound beam alternately on either side of the target. Ultrasonic signals from bats are categorized as short clicks, frequency swept pulses and constant frequency pulses (Habersetzer and Marimuthu, 1986; Neuweiler *et al.,* 1987; Surlykke *et al*., 2009). The echoes of high intensity ultrasonic pulses are used to locate and track flying prey. Bats sophisticated echolocation enables them distinguish between mosquitoes and other objects (Monto, 2010). Bat signals are transmitted in the air as vibrations and its pitch is determined by frequency. Echolocating bats produce ultrasonic signals and analyze the returning signal in order to determine the distance from the object, its speed besides identifying the type of object (Miller and Surlykke, 2001). Bats alternate the production of sound and listening to the incoming sound. Bats change their echolocation based on situation (Neuweiler *et al*., 1987). This approach maximizes their ability to detect mosquitoes and other fast moving insects that serve as food (Moss *et al*., 2010). Hearing in many insects is the product of evolutionary adaptation to bat predation (Forrest *et al.,* 1995).

2.6.2 The Chinese frog, *Amolops tormotus*

The Chinese frog belongs to the sub-order *neobatrachia* of the animal kingdom and produces countless vocalizations calls of ultrasonic frequency components (Barlow, 2006). However, most amphibians do not hear sound whose frequency is greater than 12 kHz. Some frog species have special muscles in the larynx hence produce longer glottal pulses giving time for frequency modulation (FM) of the carrier frequency which is simple. Frame-by-frame video analysis of the frog's calling behaviour has suggested the presence of two pairs of vocal sacs that contributed to the remarkable call-note complexity (Feng *et al*., 2002). The *A. tormotus* are found in hill streams and the surrounding habitats, and breeds in streams and use ultrasound up to 128 kHz for communication (Penna and Rogoberto, 1998; Barlow, 2006; Feng *et al*., 2006; Steve, 2006). Ultrasonic communication was observed in the Chinese frog from Huangshan Hot Springs, China and whose males generate diverse bird-like melodic calls (Ngo *et al*., 2006; Shen, 2007). Since amphibians are a distinct evolutionary lineage from microchiropterans and cetaceans, ultrasonic perception in these animals represents a new example of independent evolution (Feng *et al*., 2006). The *A. tormotus* generated ultrasound could be useful in developing new treatment for hearing loss (Shen, 2007). They feed on a wide range of terrestrial and aquatic animals of which insects form the greater part (Berry, 1966). The noise from running water where sound of *A. tormotus* was recorded was broadband with a peak near 100 Hz decaying by 19 dB at 2 kHz and by 63 dB at 28 kHz. Investigation into sounds of *A. tormotus* were conducted under dynamic range of 90 dB (Narins *et al*., 2004).

2.6.3 Conversion of ultrasound to audible sound using bat detectors

Bat detectors are ultrasonic recorders based on the principle of heterodyning. They convert ultrasound into audible sound (Patterson, 2001). The heterodyne converter involves two mixing stages: first, the signal frequency is translated up to a high frequency where the desired signal is filtered out then signal is translated down to the audible range (Bruno *et al*., 1996). To convert ultrasonic signal down to the human audible range, the frequency division, heterodyning and time expansion are used. The frequency division bat converters divide the incoming frequency by factor which allows only the dominant frequency to be converted thereby losing the amplitude information (Grigulevich *et al*., 1969; Patterson, 2001). A mixing detector requires that the locally generated oscillator signal be multiplied with the bat signal resulting in two new frequency components; one with frequency equal to the sum of the original signal frequencies and the other with a frequency equal to the difference of the original frequency (frequency of interest). The mixing detector requires correct tuning to a certain centre frequency (Grigulevich *et al*., 1969; Pedersen *et al*., 1993; Patterson, 2001). The ultrasonic spectrum is shifted down and the signal to noise ratio is better (~13dB) due to limited bandwidth (Hall, 1987). The frequency division bat converter is inbuilt in the Avisoft SASLab Version 5.1 which is to be used in this study. The loudness or frequency levels of sound are expressed on a decibel scale. The difference between the maximum and minimum value in magnitude, X, is expressed in a deci-Bell (dB) notation (Hall, 1987; Ballou, 2002). Thus;

$$X_{dB} = 20\,LogX \quad \text{…………………………………………………………………..}(2.7)$$

Similarly,

The sound pressure level, $\mathrm{SPL} = 20\mathrm{Log}\left(\frac{\mathrm{P}}{0.00002}\right)$ ……........……………………………(2.8)

Where P is the sound pressure expressed in Pascal, and the SPL is expressed in dB referenced to 0.00002 Pa. The pressure of 20µPa is the threshold for human perception hence reference value, though acoustic pressure levels are stated in dB. When the sound pressure, P = 0.00002 Pa, the SPL= 0 dB.

Similarly, the sound power level is defined as:

$$\text{Sound Power, } L_{dB} = 10 \log 10 \left(\frac{P}{P_{ref}} \right) \ldots\ldots 2.9$$

Where P is the estimated sound power under test, in Watts and P_{ref} is the general sound power reference level, $P_{ref} = 10^{-12}$ Watts. Greater amplitude of vibrations of particles in a medium indicate high energy and more intense the sound wave (Ballou, 2002).

Bat ranging is done by measuring the time delay between the sound emitted and any echoes (Schnitzler and Kalko, 2001). Echoes at the ears arrive at different times and loudness levels depending on the position of the object generating the echoes (Nelson *et al.*, 2006). Time and loudness help perceive distance and direction by the bats. Echolocation enables bats to occupy a niche where there are many insects (Nelson *et al.*, 2006; Monto, 2010). Bats produce sound at low rate (10 – 20/s) during prey search, coupled with respiration and wing beat (Monto, 2010). On detecting prey, the pulse rate is increased ending with a terminal buzz (as high as 200/s) recording a reduction in duration of sound as it approaches the target (Nelson *et al.*, 2006; Monto, 2010). The average maximum distance that echolocation bats can hear, determined from equation 2.9 and 2.10 is seventeen metre (Hall, 1987).

The sound speed, $v = \text{Wavelength, } \lambda \text{ x Frequency, } f$

$$v = \lambda f \ldots\ldots (2.9)$$

Total distance covered by sound emitted by the bat, $D = 2d$

Sound speed, $v = 340 \text{ms}^{-1}$

$$v = \frac{2d}{t} \ldots\ldots (2.10)$$

2.7 The Transmission Parameters and Acoustic Features of Sound

Sound which is a mental sensation is due to vibratory movement of a body which may be slight or rapid. The characteristics of a musical sound are its periodicity and continuity (Khanna and Bedi, 1988). Musical sounds differ in characteristics; loudness, intensity, pitch and quality (or timbre). Loudness and intensity are related by the Weber and Fechner's law given in equation 2.11;

$$L \propto \log I \ldots\ldots (2.11)$$

(Khanna and Bedi, 1988). Where L is the sensation of loudness and I its intensity.

The structure of an FM sound is a broadband signal which contains a downward sweep through a range of frequencies (Simmons *et al.*, 1984; Habersetzer and Marimuthu, 1986; Neuweiler *et al.*, 1987; Schnitzler and Kalko, 2001; Narins *et al.*, 2004; Surlykke*et al.*, 2009). An FM signal is precise in range discrimination though the distance from an FM-Bat detecting target is limited (Simmons *et al.*, 1984; Schnitzler and Kalko, 2001; Beedholm, 2005). The CF-Bat detects both target velocity and the fluttering of target wings as doppler shifted frequencies (Schuller, 1980). The FM component is excellent for hunting prey while flying in close, cluttered environment due to precise target localization (Schnitzler and Kalko, 2001; Narins *et al.*, 2004). Bats have three dimensional localization ability of the broadband signal enabling it to resolve their prey from large amounts of background noise (Schnitzler and Kalko, 2001). Short FM call is best in close cluttered environment because it enables the bat to emit many calls very rapidly without overlap. CF component is suited for bats flying in open during hunting or bats that wait on perches for their prey to appear due to excellent prey detection ability (Schnitzler and Kalko, 2001). Other acoustic features include Intensity, harmonic composition, call duration and pulse interval (Kingston *et al.*, 2003).

2.7.1 Sound synthesis and analysis using the Fourier transform

Fourier theorem analyses or synthesizes complex periodic vibrations into or from simple harmonic constituents (Khanna and Bedi, 1988). Any periodic curve, however complex, of wavelength λ may be regarded as made up of simple harmonic curves of definite amplitudes, phases and periods. The wavelengths of the components are in the ratio $\lambda : \frac{\lambda}{2} : \frac{\lambda}{3} : \frac{\lambda}{4}$...... etc while their frequencies are in the ratio $n : 2n : 3n : 4n$ .... If T is the period of the complex periodic vibrations, then the period of the component of the simple vibrations consist of numbers $T, \frac{T}{2}, \frac{T}{3}$ etc. The resultant displacement of any particle in the medium transmitting the complex periodic wave is given in equation 2.12:

$$f(\omega t) = A_o + \sum_{r=1}^{\infty} A_n \sin r\omega t + \sum_{r=1}^{\infty} B_n \cos r\omega t \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.12)$$

(Gupta, 1989; Greengard and Lee, 2004)

A_o is the measure of the mean displacement of the axis of vibration from the arbitrary time axis whereas A_1, A_2 , A_3, ……….. etc and B_1 ,B_2, ………etc are the amplitudes of various sine and cosine terms respectively. To find the presence or absence of a term, the values of A_0 and coefficients A_1, A_2,………B_1, B_2…. are determined mathematically using equations 2.13, 2.14 and 2.15 (Greengard and Lee, 2004). Hence;

$$A_o = \frac{1}{T}\int_0^T y\,dt \;\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2.13)$$

$$A_r = \frac{2}{T}\int_0^T y \sin r\omega t dt \text{ ....}(2.14)$$

$$B_r = \frac{2}{T}\int_0^T y \cos r\omega t dt \text{ ....}(2.15)$$

The harmonics generated from the Fourier Transform are determined from equation 2.16 and 2.17:

$$f(t) = \frac{a_0}{2} + a_1 \cos t + a_2 \cos 2t + a_3 \cos 3t + \text{.......... .....} + b_1 \sin t + b_2 \sin 2t + b_3 \sin 3t + \text{.......... ....}(2.16)$$

$$f(t) = \frac{a_0}{2} + (a_1 \cos t + b_1 \sin t) + (a_2 \cos 2t + b_2 \sin 2t) + (a_3 \cos 3t + b_3 \sin 3t) + \text{.......... ....}(2.17)$$

Where terms $(a_1 \cos t + b_1 \sin t)$, $(a_2 \cos t + b_2 \sin t)$, $(a_3 \cos t + b_3 \sin t)$, $(a_4 \cos t + b_4 \sin t)$, $(a_5 \cos t + b_5 \sin t)$ etc represent the fundamental frequency, second harmonic, third harmonic, fourth harmonic, fifth harmonic etc respectively (Khanna and Bedi, 1988).

2.7.2 Determination of rest frequency and side-band pairs of an FM signal

The frequency modulated wave equation which cannot be solved by algebra or trigonometric identities is given as $e_{FM}(t) = E_C \sin(\omega_C t + m_f \sin \omega_M t)$ ....(2.18)

Certain Bessel-function identities are used to solve the above equation and allow the determination of the frequency components of an FM wave (Ramabhadran, 1988). The resulting equation, given in equation 2.19, after solving the above equation itemizes the various signal components in an FM wave and their amplitudes.

$$e_{fm} = J_0(m_f)\sin \omega_c t - J_1(m_f)\{\sin(\omega_c - \omega_m)t - \sin(\omega_c + \omega_m)t\} + J_2(m_f)\{\sin(\omega_c - 2\omega_m)t + \sin(\omega_c + 2\omega_m)t\} - J_3(m_f)\{\sin(\omega_c - 3\omega_m)t - \sin(\omega_c + 3\omega_m)t\} + \text{.......... ....}2.19$$

There are an infinite number of side-band pairs for an FM wave. Each sideband pair is symmetrically located about the transmitter's rest frequency f_C, and separated from the rest frequency by integral multiples of the modulating frequency, n x f_M, where n = 1, 2, 3, …… (Ramabhadran, 1988). The magnitude of the rest frequency and side-band pairs, shown in Fig. 2, depends on the index of modulation m_f and is given by the Bessel function coefficients, $J_n(m_f)$ where n is the order of the sideband pair. $J_n(m_f)$ is one term representing the amplitude of the nth pair of sidebands with an index of modulation, m_f (Hall, 1987).

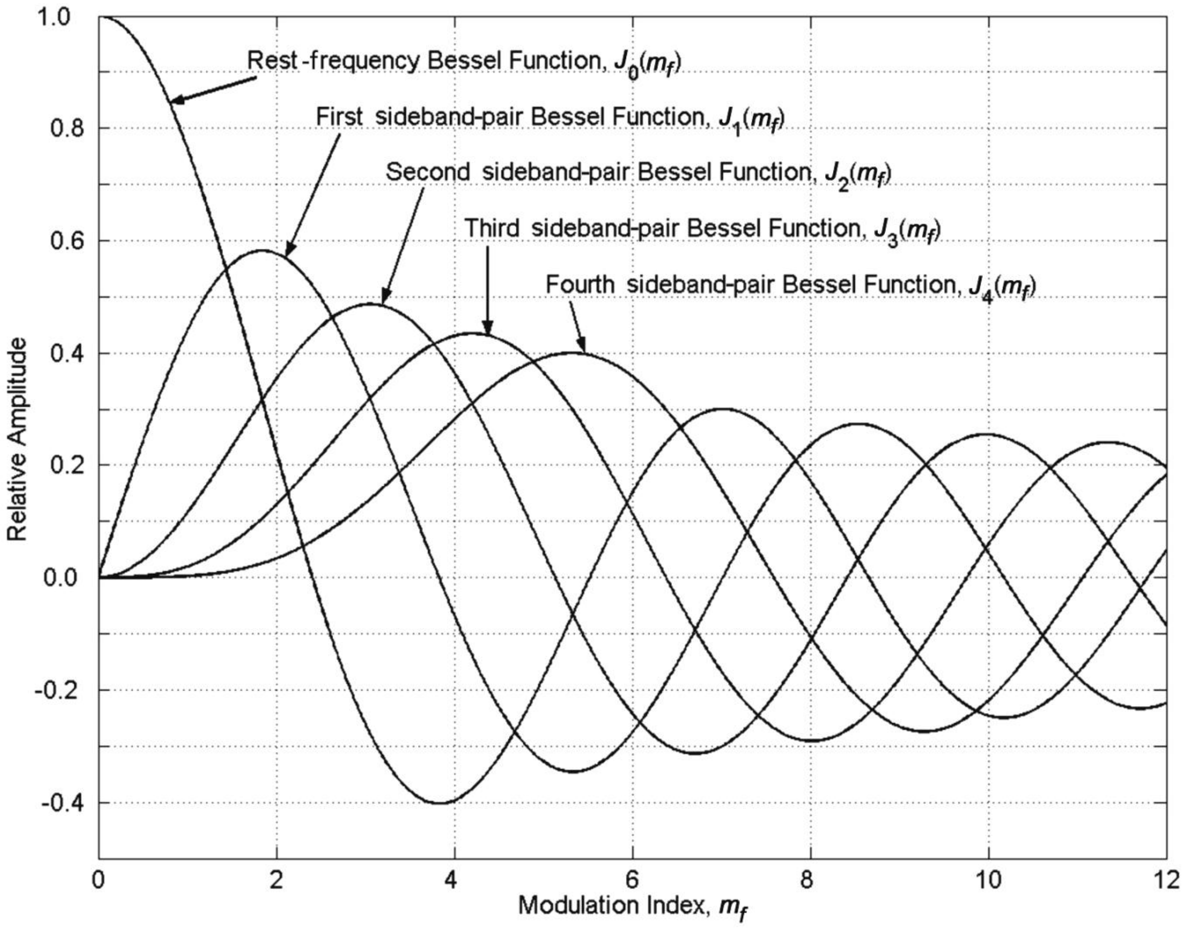

Fig. 2: Plot of the Bessel functions (Ramabhadran, 1988; Gupta, 1989)

The values of $J_0(m_f)$, $J_1(m_f)$, $J_2(m_f)$,….$J_n(m_f)$ obtained from mathematical calculations are usually plotted to give this graph (Gupta, 1989; Ramabhadran,1988). For small values of m_f, the significant amplitude are $J_0(m_f)$ and $J_1(m_f)$, while the amplitude of the higher-order ($n > 1$), the side-band pairs are very small. As m_f 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). As m_f keeps increasing, the sideband pairs are have zero amplitude until about $m_f = n$, at which point they increase in amplitude to a maximum and then decrease again. As m_f increases, each Bessel function appears to act like an exponentially decaying sine wave and the amplitudes of the higher-order sideband pairs eventually approach zero. The amplitude values with negative signs represent phase shifts of 180 degrees and that amplitude values less than 0.01 represent component frequencies with insignificant power content. These values, shown in Table 3, were determined from Fig. 2 through recording integer or fractional values of m_f (Ramabhadran, 1988).

Table 3: The Bessel function table of the first kind (Ramabhadran, 1988)

m_f	J_0	J_1	J_2	J_3	J_4	J_5	J_6	J_7	J_8	J_9
0.00	1.00	-	-	-	-	-	-	-	-	-
0.25	0.98	0.12	-	-	-	-	-	-	-	-
0.50	0.94	0.24	0.03	-	-	-	-	-	-	-
1.00	0.77	0.44	0.11	0.02	-	-	-	-	-	-
1.50	0.51	0.56	0.23	0.06	0.01	-	-	-	-	-
2.00	0.22	0.58	0.35	0.13	0.03	-	-	-	-	-
2.41	0.00	0.52	0.43	0.20	0.06	0.02	-	-	-	-
2.50	-0.05	0.50	0.45	0.22	0.07	0.02	0.01	-	-	-
3.00	-0.26	0.34	0.49	0.31	0.13	0.04	0.01	-	-	-
4.00	-0.40	-0.07	0.36	0.43	0.28	0.13	0.05	0.02	-	-
5.00	-0.18	-0.33	0.05	0.36	0.39	0.26	0.13	0.05	0.02	-
5.53	0.00	-0.34	-0.13	0.25	0.40	0.32	0.19	0.09	0.03	0.01
6.00	0.15	-0.28	-0.24	0.11	0.36	0.36	0.25	0.13	0.06	0.02

2.7.3 Frequency modulation

Frequency modulation is realized through varying the angular frequency, ω in accordance with the modulating signal or message. The carrier voltage, modulating voltage and the FM wave equations are given by equations 2.20, 2.21 and 2.22 respectively.

$$e_c(t) = E_c \sin(\omega_c t + \theta) \quad \text{......(2.20)}$$

$$e_m(t) = E_m \cos \omega_m t \quad \text{......(2.21)}$$

$$e_{FM}(t) = E_C \sin(\omega_C t + m_f \sin \omega_M t), \quad \text{......(2.22)}$$

Where E_C is the peak amplitude of the rest-frequency (carrier frequency), ω_C the rest frequency, ω_M the modulating frequency and m_f the index of modulation (Ramabhadran, 1988; Ballou, 2002).

2.7.4 The acoustic transmission parameters

a) Minimum frequency $(f_{\min})$ and maximum frequency $(f_{\max})$

The modulation sensitivity of an FM transmitter associated with equations 2.23, 2.24 and 2.25 is given by a constant, k_f, of many kHz/V. The rest frequency of a transmitter is the output frequency with no modulating signal applied. For a transmitter with linear modulation characteristics, the frequency deviation of the carrier is directly proportional to the amplitude of the applied modulating signal (Ramabhadran, 1988; Gupta, 1989). The maximum and minimum value of frequency are calculated as shown in equations 2.23 and 2.24.

The maximum value of frequency, $f_{\max} = f_c + k_f \frac{E_m}{2\pi}$ ……………..……..………...... (2.23)

The minimum value of frequency, $f_{\min} = f_c - k_f \frac{E_m}{2\pi}$ ……………..…..……………..... (2.24)

b) Frequency deviation (*f_d*)

The frequency deviation $f_d = f_{\max} - f_c = f_c - f_{\min} = k_f \frac{E_m}{2\pi}$ …….……………………..... (2.25)

These parameters were automatically determined using the sound analysis softwares.

c) The Bandwidth, *BW*

$BW = 2 \times n \times f_m$ …………...…………………..……..……………..…........................... (2.26)

Where n is the number of sidebands pairs and f_m is the modulating frequency (Ballou, 2002).

d) Wavelength, λ

$\lambda = \frac{v}{f_c}$ ……...………………............…………………….…………………….……………… (2.27)

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

3.1.1 The *A. gambiae* mosquitoes

The *A. gambiae* mosquitoes were 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. Three sets of 10 female *A. gambiae*, 3-5 day old were used in the study.

3.1.2 Sound of *A. tormotus*

Six samples of the sound of *A. tormotus* were recorded individually for varied duration ranging from 1.60 s to 2.90 s using the 702 digital recorders from the Huangshan Hot springs, Anhui Province; China at a sampling frequency of 192 kHz which was converted to 500 kHz.

3.1.3 Sounds of *C. afra*

Nine sound samples from *C. afra* were recorded from a colony in Kit-Mikayi caves, Kisumu; Kenya using the Avisoft recorder which consisted of the AUSG (Model 112) individually for a duration which varied from 45.00 s to 300.00 s at a sampling frequency of 500 kHz.

3.1.4 Combined sound of the *A. tormotus* and *C. afra*

The Avisoft SASLab Pro Version 5.1 was used to combine the appended sounds of the *A. tormotus* and *C. afra* at a sampling rate of 500 kHz.

3.1.5 Equipment

A laptop running on Windows XP and office 2007 mounted with a sound card, hardlock key and sound output ports was used together with the Avisoft recorder during the first stage of the study. The Avisoft recorder, compatible with Windows XP, consisting of the AUSG (model 112) running on specific software RECORDER USG (rec_usg.exe), ultrasound microphone with high pass filter with cut-off frequency of 10 kHz was used in the recording of ultrasounds from the African bat, *C. afra*. During the second stage of the study, 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 shown in Plate 5, 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.

Plate 5: The external amplifier and the laptop used in the study

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. Softwares which included the Avisoft-SAS LAB Pro Version 5.1 and Batsound were installed in the laptop to facilitate automatic analysis of sound signals. An aspirator was used to transfer the female *A. gambiae* from the rearing cage to the bioassay cage and also remove them from it.

3.1.6 The bioassay arena (Cage)

A standard bioassay glass cage of dimensions 0.50 m by 0.25 m by 0.25 m was fitted with untreated mosquito net on the 0.25 m by 0.25 m faces and used in the bioassay. A small hole was perforated on both nets to allow the mosquito samples in and out the cage. The cage was divided into three equal sections; A, B and C as shown in Plate 6, Plate 7 and Plate 8. Section C was the central region of the bioassay cage.

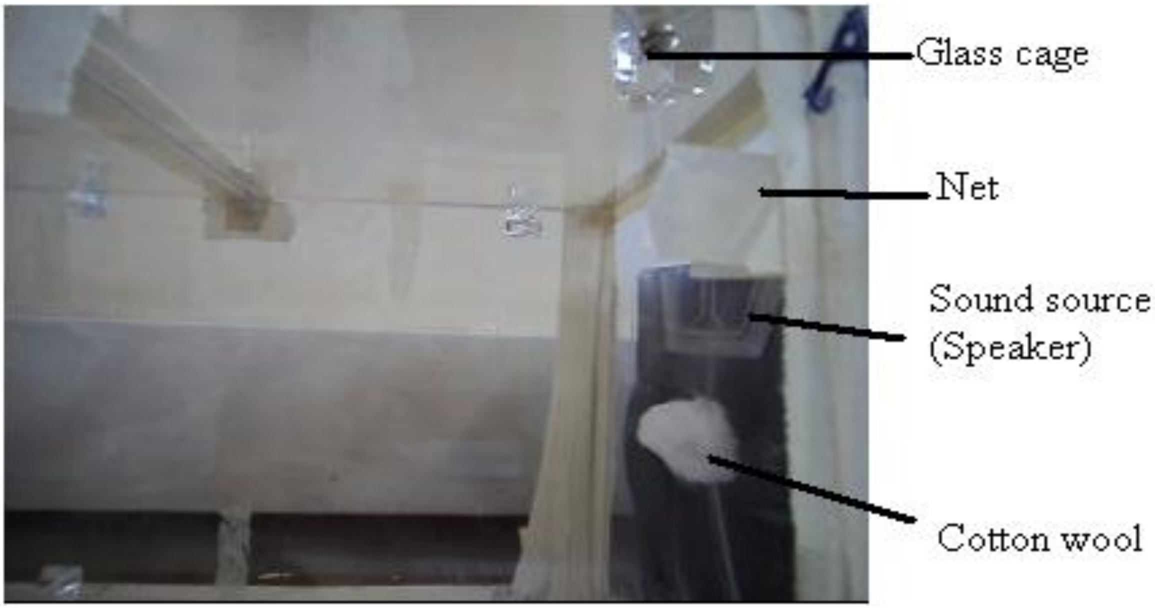

Plate 6: The bioassay cage showing the location of the speakers

Plate 7: The mosquito release point in a bioassay cage

Plate 8: A bioassay cage showing section C of the cage with a mosquito resting normally

Section A was to the right whereas section B was to the left of the cage. Both the 25 cm by 25 cm faces of the cage were covered with untreated mosquito which had a 1.0 cm diameter hole perforated in the middle of the net. The two holes were covered with cotton wool. Either holes on side A or B could be used as mosquito release points. However, the hole on side B of the net was used as the mosquito release point for consistence whereas the hole on side A was closed permanently using a piece of cotton wool. The release point (opening) was used to confine the mosquitoes hence prevent them from escaping. Two speakers were attached to side A of the cage as shown in Plate 9 and Plate 10.

3.2 Methods

3.2.1 Recording, combining and filtering of sounds

(a). Recording of the sound of *Coleura afra*

The Avisoft recorder which consists of the AUSG (model 112) was used in recording the sound from *C. afra*. The condenser microphone capsule (CM16) which consisted of a thin metalized polyester film and a metal black plate was used in the study. It was connected to the AUSG (model 112) which was then connected to the laptop through one of the universal serial bus (USB) port. The omnidirectional microphone was selected as a default microphone from the voice recording settings in the laptop. The time domain filter, Finite Impulse

Response (FIR) was set to zero for both upper cut-off frequency (f_{uco}= 0 kHz) and lower cut-off frequencies (f_{lco}= 0 kHz). The Fast Fourier transform (FFT) was also set to 512 and the Hamming window selected for the display. The temporal resolution overlap was also set to 50% with the graypal selected for the colour palette. The frame size was set to 100% for real time spectrogram parameters. The black and white box (B/W) was checked for the display option. The Avisoft-SAS LAB Pro, Version 5.1 software was open and the microphone directed to the source of sound. The gain on the AUSG (model 112) was adjusted to an appropriated level to avoid over modulation and the recording level from the computer set to 20 dB. Recording of the sound was started by pressing the record button on the AUSG. Nine sound samples from *C. afra* were recorded separately from a colony in Kit-Mikayi caves, Kisumu at a sampling frequency of 500 kHz for a duration varying from 45.00 s to 300.00 s. The sound samples were saved in the hard disc. The sound sample for the study was obtained by appending four quality sound samples and gave a 1754.07 s playback duration which was saved as "*Coleura*Sample 2.wav" hard disc.

(b). Recording of the sound of *A. tormotus*

Six samples of sounds of *A. tormotus* were recorded individually for a total duration of 12.4 s using the 702 digital recorder from the Huangshan Hot springs, Anhui Province; China at a sampling frequency of 192 kHz. The sound samples were appended with a view of increasing playback duration using the Avisoft SASLab Pro Version 5.1 installed in the laptop computer hard disc. The appended sound was further appended to ensure a uniform duration of 1754.07 s and saved as "*A. tormotus*.wav" in the hard disc and the sampling frequency converted from 192 kHz to 500 kHz using Avisoft SASLab Pro Version 5.1 for compatibility. The samples were donated from Illinois University (USA) to facilitate the study.

(c). Combination of the appended sounds of the *A. tormotus* and *C. afra*

The Avisoft SASLab Pro Version 5.1 was used to convert the sampling frequency of the sound of *A. tormotus* from 192 kHz to 500 kHz; combine the appended sounds of the *A. tormotus* and *C. afra* at a sampling rate of 500 kHz. The sound file of *A. tormotus* was open using the Avisoft SASLab analysis software and copied. The sound of *C. afra* was then open with the Avisoft SASLab analysis software and mixed with the copied sound of *A. tormotus* to give the combined sound, using the mix option in the edit menu. It was then saved as "Combined sound.wav" in the hard disc.

(d). Filtering of sounds samples

The high pass filter, band pass filter and low pass filter, inbuilt in the Avisoft SASLab analysis software, were used to segment the appended sounds into appropriate frequency ranges essential for the study. All the three sounds were subjected to a high pass filter with a cut-off frequency f_{co} = 10 kHz in order to attenuate noises and a low pass filter with a cut-off frequency f_{co} = 90 kHz. Three sound segments, namely, 10-34 kHz, 35-60 kHz and 61-90 kHz from every sound sample were required for the investigation. In order to obtain the 10-34 kHz the frequency range segment, the sounds saved as *Coleura*Sample 2.wav, *A. tormotus*.wav and Combined sound.wav were subjected to a band pass filter with an upper cut-off frequency, f_{uco}= 34 kHz and a lower cut-off frequency, f_{lco}=10 kHz. The band pass filter modified the frequency response as shown in Fig. 3. Frequencies below 10 kHz and above 34 kHz were gradually attenuated (amplitude = 0 i.e. off), allowing those in the range of 10-34 kHz (amplitude = 1, or on)

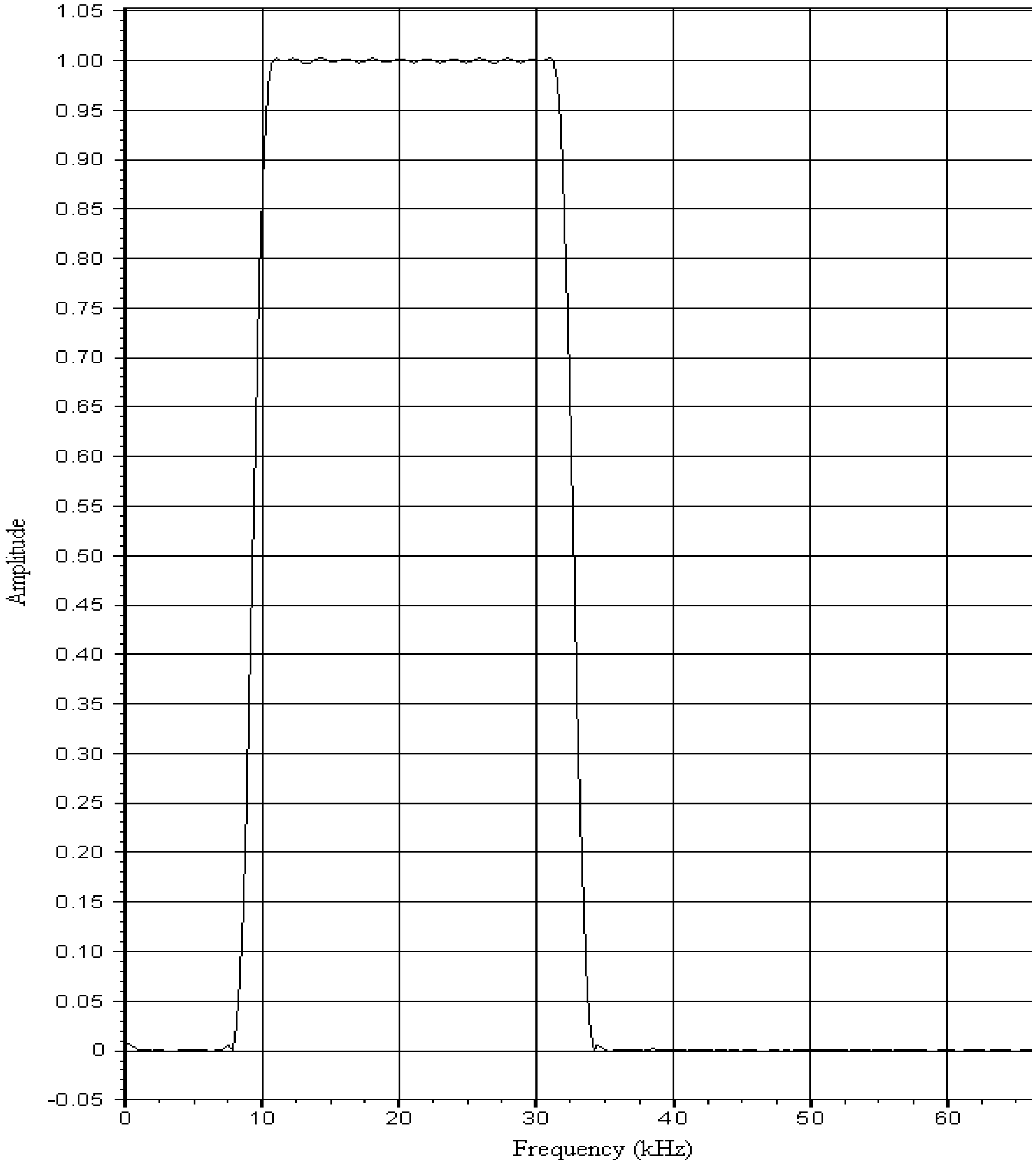

Fig. 3: Frequency response modification by the 10-34 kHz band pass filter

Similarly, the band pass filter settings that yielded the 35-60 kHz and 61-90 kHz frequency ranges were f_{lco}= 35 kHz, f_{uco}= 60 kHz and f_{lco}= 61 kHz, f_{uco}= 90 kHz respectively. The settings were made from the time domain filter (FIR). These band pass filters modified the frequency response as shown in Fig. 4 and Fig. 5. These sound segments were also saved in the hard disc.

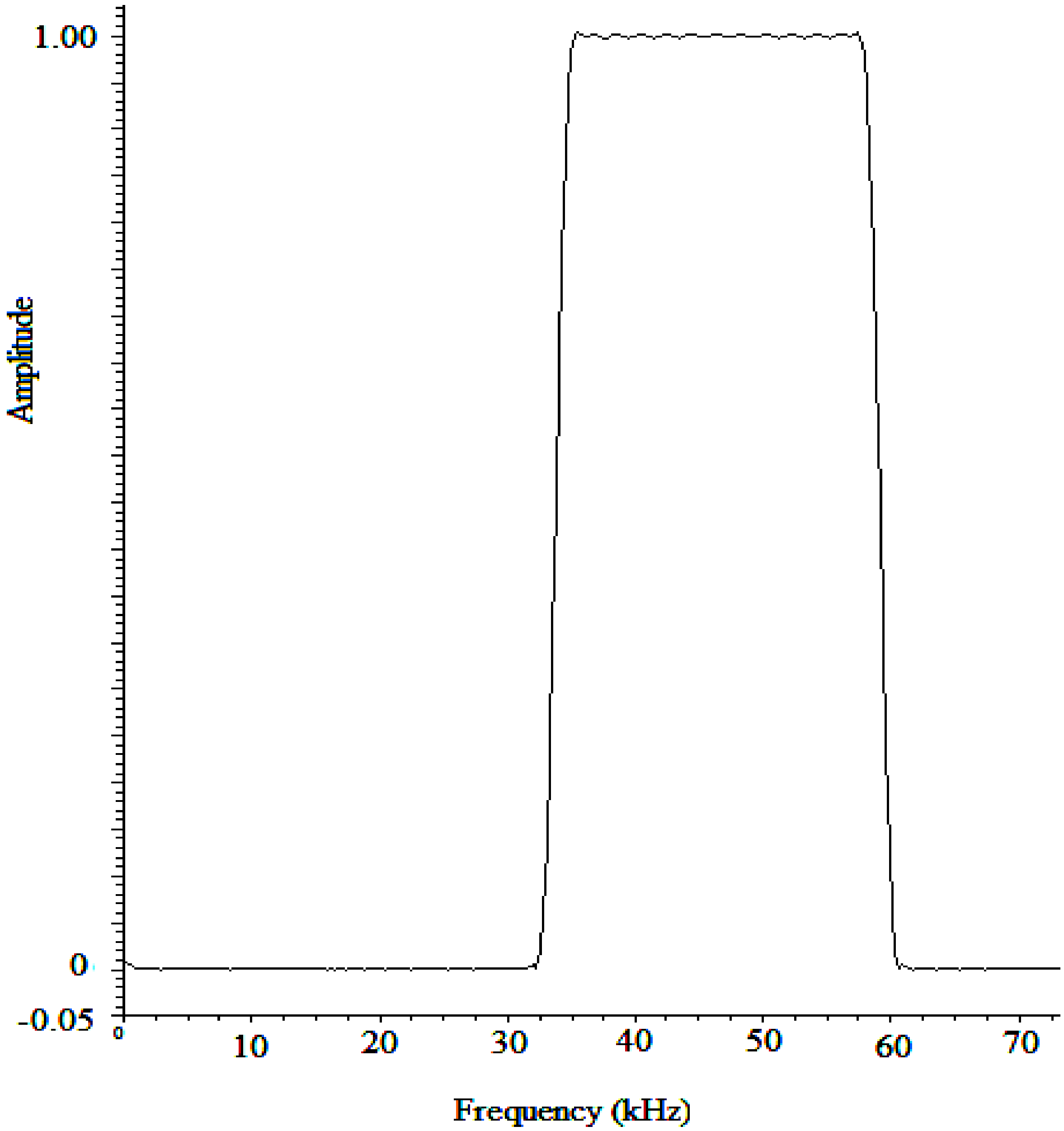

Fig. 4: Frequency response modification by the 35-60 kHz band pass filter

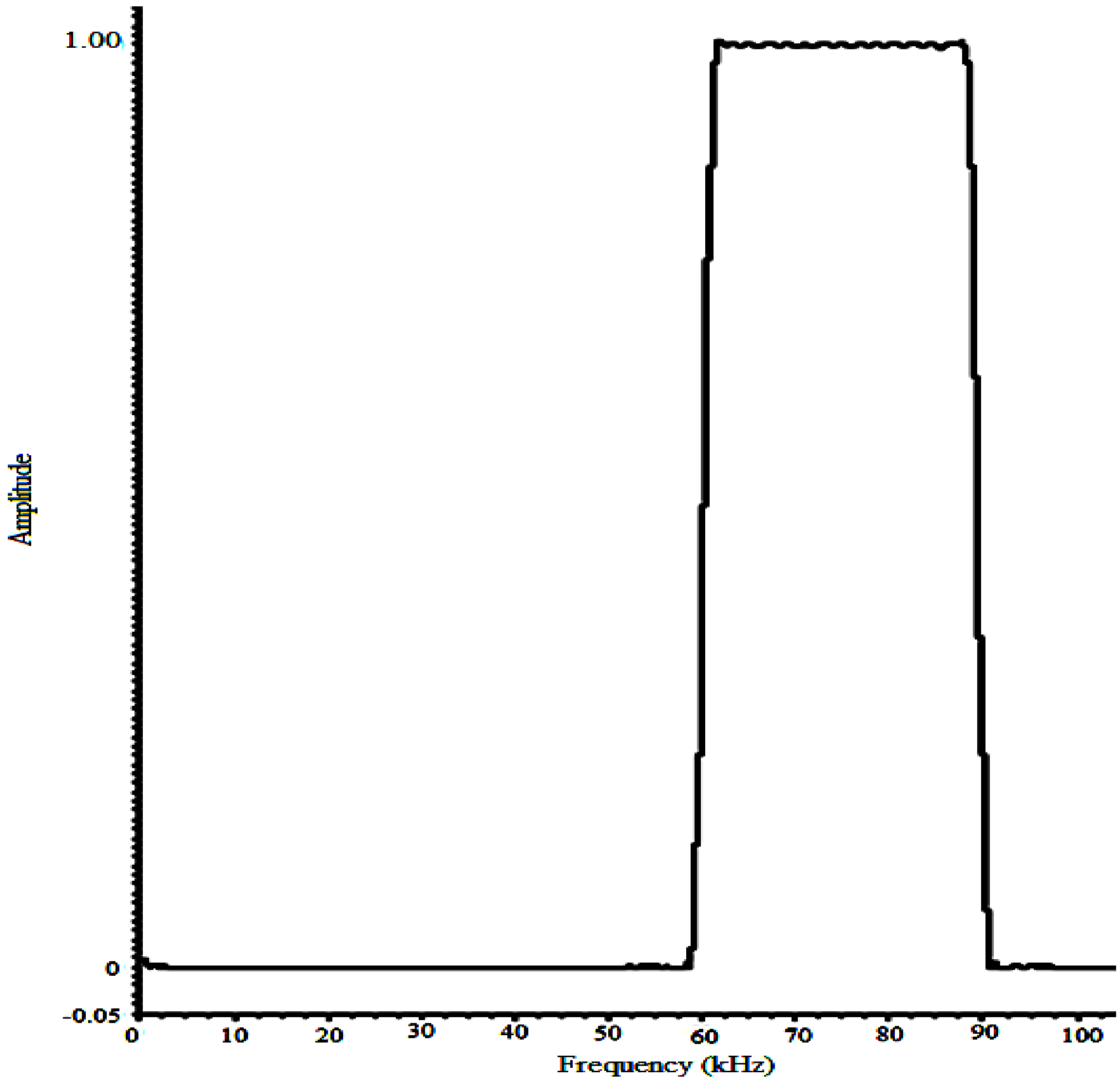

Fig. 5: Frequency response modification by the 61-90 kHz band pass filter

3.2.2 Determination of acoustic transmission parameters of predator sounds

A laptop installed with Batsound and the Avisoft SASLab Pro Version 5.1 software and fitted with hardlock key was used in the study. 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 described in 3.2.1 were analysed using the Avisoft SASLab Pro Version 5.1 and the Batsound software. The power spectrum and oscillograms of the sounds discussed in 4.1, 4.2 and 4.3 were generated using the Batsound software. The parameters whch included peak frequency, peak amplitude, fundamental frequency, maximum frequency, minimum frequency, bandwidth, acoustic energy, means of the parameters and standard deviations were automatically generated using

Avisoft SASLab Pro Version 5.1 software. Similarly, the spectrograms, oscillogrammes and filters were generated by the Avisoft SASLab Pro Version 5.1 and Batsound software. Fourier transforms were performed on the oscillograms to extract acoustic transmission parameters for each call through automatic and manual parameter measurements. 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 SASLab software under the tools menu. The acoustic pressure level was set to a reference of 20 µPa, which is the threshold. On calibration, the reference signal emerged as 1 Pa. The SI unit of acoustic energy is Pa^2s resulting from the product of the squared amplitudes by sample time, depending on calibration. The energy generated is actually the sum of the squared amplitudes multiplied by time. Similarly, 1 Pascal pressure is equal to SPL of 94 dB. The recording of the sound was at 2.5 m away from the sound source, a distance essential in determining the sound power. The data obtained was then transferred into an excel worksheet by copying and pasting data for the entire signal for formatting. 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 the cage from side A. The speaker was set to face the cage. The amplitude modulation constant of the appended sound of *A. tormotus* was set to n = 0.8 i.e. Normalize at 80% for the entire duration for the *A. tormotus* signal. Similarly, the sound of *C. afra* was internally amplified by setting the 10-34 kHz to volume = 150 %, 35-60 kHz to volume = 130 % and the 61-90 kHz was set to 600 %. The combined sound of *C. afra* and *A. tormotus* was also internally amplified by setting the 10-34 kHz to volume = 80 %, 35-60 kHz to volume = 80 % and the 61-90 kHz was set to 600 %. The sound was also 50 % amplified using an external amplifier in order to enhance diaphragm vibrations.

3.2.3 Bioassay

The bioassay study involved determination of the effect of sound on the female *A. gambiae* by varying frequency in order to establish the frequency range where startling effect was optimum. The mosquitoes were bred and reared at Kenya Medical Research Institute Centre for Global Health Research laboratories, Entomology department in Kisumu and maintained at 60-80 % humidity and 25±2 ^{o}C. Morphologically, the male mosquitoes have a blunt proboscis whereas the female have a sharp proboscis. They were fed on 10% glucose solution soaked in cotton wool. Three to five day old female *A. gambiae* were used in the bioassay experiment. The female *A. gambiae* are characterized by large body size and affinity to blood meal. The criteria for the choice of the female *A. gambiae* included the size, status (fed or unfed), activity, mouth parts, resting position and age.

The sound of the African bat *C. afra* and the Chinese frog *A. tormotus* and their combination were played separately through two external speakers attached to the cage at the end labeled A, shown in Plate 9 and Plate 10. The sound frequency ranges included 10-34 kHz, 35-60 kHz and 61-90 kHz, obtained by use of filters incooperated in the Avisoft SASLab software. The background noise, which was below 10 kHz, in the sound signals was attenuated using high pass filters. A set of ten, 3-5 day old female *A. gambiae* were released into the cage using an aspirator, one at a time, through the release hole in the net. The bioassay study, performed in two parts, involved investigation into the behaviour of one mosquito at a time exposed to 10-34 kHz, 35-60 kHz and 61-90 kHz sound frequency ranges in prose. The first part of the bioassay involved investigation into the startle effect observed from changes in mosquitoes' behaviour, and the number of mosquito samples exhibiting the traits expressed as a percentage.

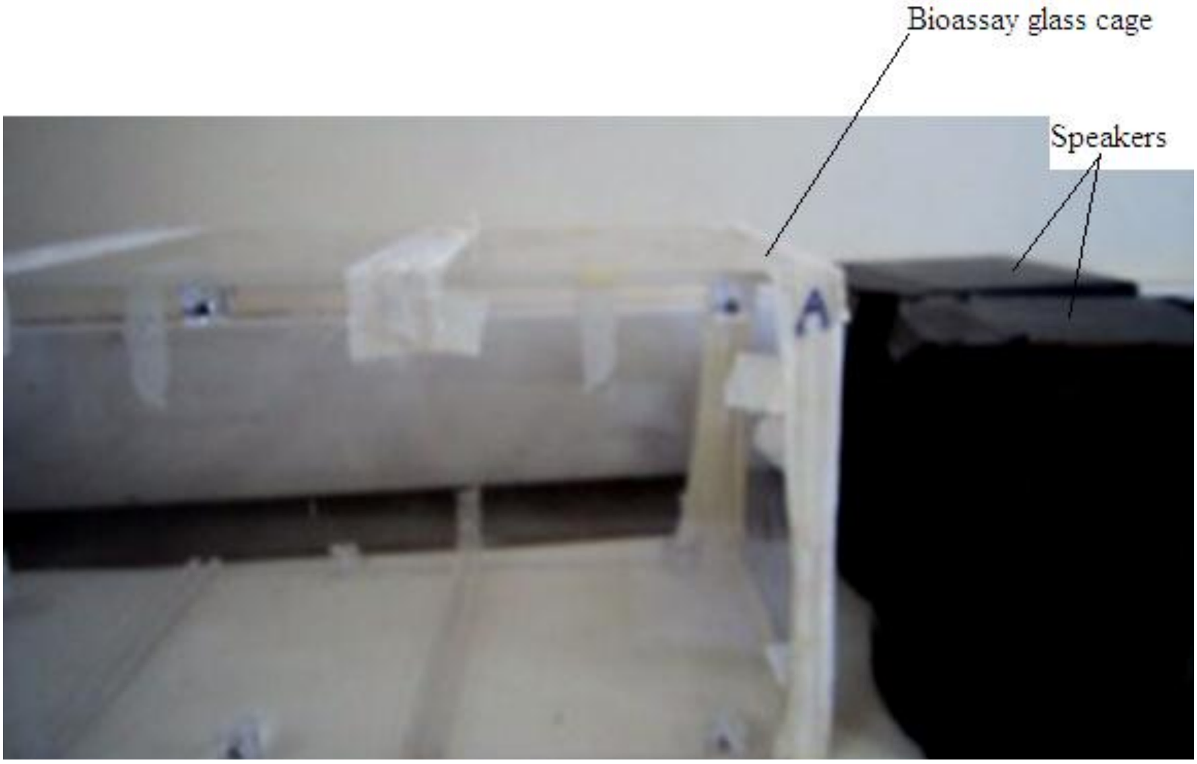

Plate 9: The experimental set-up for the bioassay study

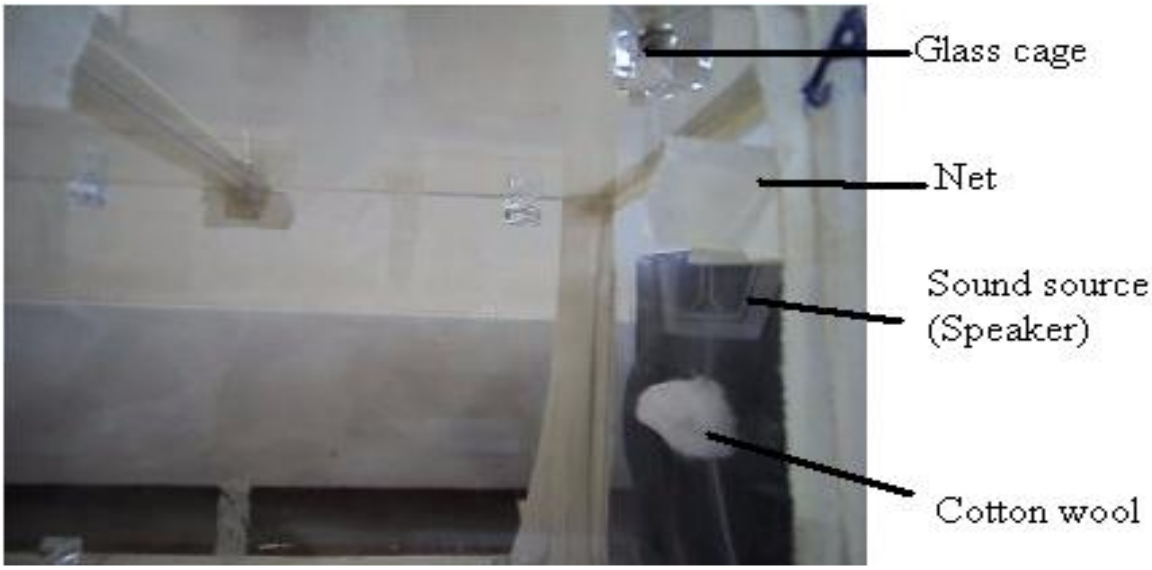

Plate 10: A bioassay cage showing section A on which the speakers are attached

The behavoural traits considered included directional body movement, jumps, hiding, raising of limbs, raising and lowering of body, body shaking associated with bending of abdomen, wing and limb rubbing, nature of body rest, mosquito movement, spreading of limbs, antennae erection, fatigue, rolling and loss of body parts. Antennae erection from the proboscis was measured from unmodified photo printout of the mosquito and the angle measured using a protractor. The antennae and proboscis were extrapolated for convenience in angle measurement by protractor. The second part of the bioassay involved playing of predator for various frequency ranges and the number of activities and time, in this case flight and rest recorded correspondingly. The predator sound was played and simultaneously starting the stopwatch, keenly observing and recording duration for flights (F) and rests (R) in the data capture tool attached in Appendix E. The observation of each mosquito took a total duration of 7016.28 s with each exposure taking 1754.07 s.

The saved sound files of *A. tormotus, C. afra* and their combination were played using a laptop. The bioassay study involved the use of ten mosquitoes, one mosquito studied at a time. An initial observation was made on the mosquito without playing any of the predator sounds. That was the control experiment whose results were compared to those obtained when sounds were played. Each mosquito was exposed to sounds of frequency ranges 10-34 kHz, 35-60 kHz and 61-90 kHz, obtained by subjecting them to appropriate filters inbuilt in the Avisoft SASLab filters. Finally, there was mass exposure of ten female mosquitoes to this sound involved and the number of grounded mosquitoes noted.

3.2.4 Statistical analysis

The data for the acoustic transmission parameters was automatically generated using the Avisoft SASLab software. The software generated the mean, maximum, minimum, and standard deviation values of the parameters. In order to determine the significance level, the data of the parameters being compared was subjected to T-test and one way ANOVA test using the SPSS software. The bandwidth (maximum entire) for all sounds under study generated from the Avisoft SASLab were subjected to T-test using SPSS version 16.0 software in which the mean bandwidths (maximum entire) were compared by 77.24 kHz, the mean of the reported bandwidths of EMR. This comparison was determined at a 95 % confidence and the significance level of 0.05 establishing a two-tailed significance. Similarly, the one way ANOVA at a significance level of 0.05 and confidence interval was used to determine the significance values (p-value) for other parameters using SPSS version 16.0. Also, the data collected from the bioassay study was also analysed using the SPSS programme. The mean total activities for the mosquitoes under exposure to varied predator sounds were compared with the mean activities under the control using one-way ANOVA.

In all case, the findings were unlikely to have arisen by chance and the idea that there was no difference between the two treatments (null hypothesis) was rejected for small value of p ($p < 0.05$). With p-values small (or smaller) the results from the trial are statistically significant (unlikely to have arisen by chance). Smaller p-values ($p < 0.05$) are described as ‘highly significant’ because they indicate that the observed difference happened less than once in a hundred times if there was really no true difference (Stigler, 2008).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Determination of the Transmission Parameters of Sounds of *C. afra* and *A. tormotus*

4.1.1 Determination of the transmission parameters of unfiltered original sounds of *C. afra* and *A. tormotus*

a) Generation and modulation of sounds of *C. afra* and *A. tormotus*

The spectrograms shown in Fig. 6 and Fig. 7 revealed existence of frequency modulation and constant frequency modulation, labeled as FM and CF respectively. The spectrogram for FM calls showed variation in frequency with time. A varying degree of frequency modulation with abrupt onset and disappearance of harmonic components was noted in all the sounds studied. The frequency modulation for both the short and long duration calls prominently swept downwards and also upwards with no evidence of the carrier frequency in the predator sounds. The bats in the colony generated sound through tongue clicks or vocalization, an observation also reported in recent studies.

Generation of sound through tongue clicks or vocalization in bats and *A. tormotus* had been reported in recent studies (Berry, 1966; Barlow, 2006; Feng *et al*., 2006; Pollack and Martins, 2007). Also, the existence of the FM and CF calls was in agreement with recent findings by Feng *et al* (2002) and Narins *et al* (2004). The FM sound waves were reported to activate the mosquito antennae (Maweu *et al*., 2009). The sounds were detected by mosquito antennae and initiated avoidance response (Mohankumar, 2010). These research results therefore provide proof of the existence of both FM and CF calls in the sound of *A. tormotus* and *C. afra* as reported in recent findings (Feng *et al*., 2002; Narins *et al*., 2004; Barlow, 2006; Monto, 2010). However, the presence of CF modulation had not been reported in the sound of *A. tormotus*. The ultrasound from both *A. tormotus* and *C. afra* are used for communication purposes (Khanna and Bedi, 1988; Penna and Rogoberto, 1988; Hoy *et al*., 1989; Miller and Surlykke, 2001; Barlow, 2006; Feng *et al*., 2006; Steve, 2006; Monto, 2010). The CF components of sound in bat are used for detecting both target velocity and fluttering of target wings as doppler shifted frequencies and are suited for bats flying in open during hunting. The FM components are suited for close and cluttered environment due to precise target localization (Schuller, 1980). The production of the FM components in *A. tormotus* was generally described indicating communication as a major use of the ultrasound (Feng *et al*., 2002). The specific use of the FM in *A. tormotus* had not been reported.

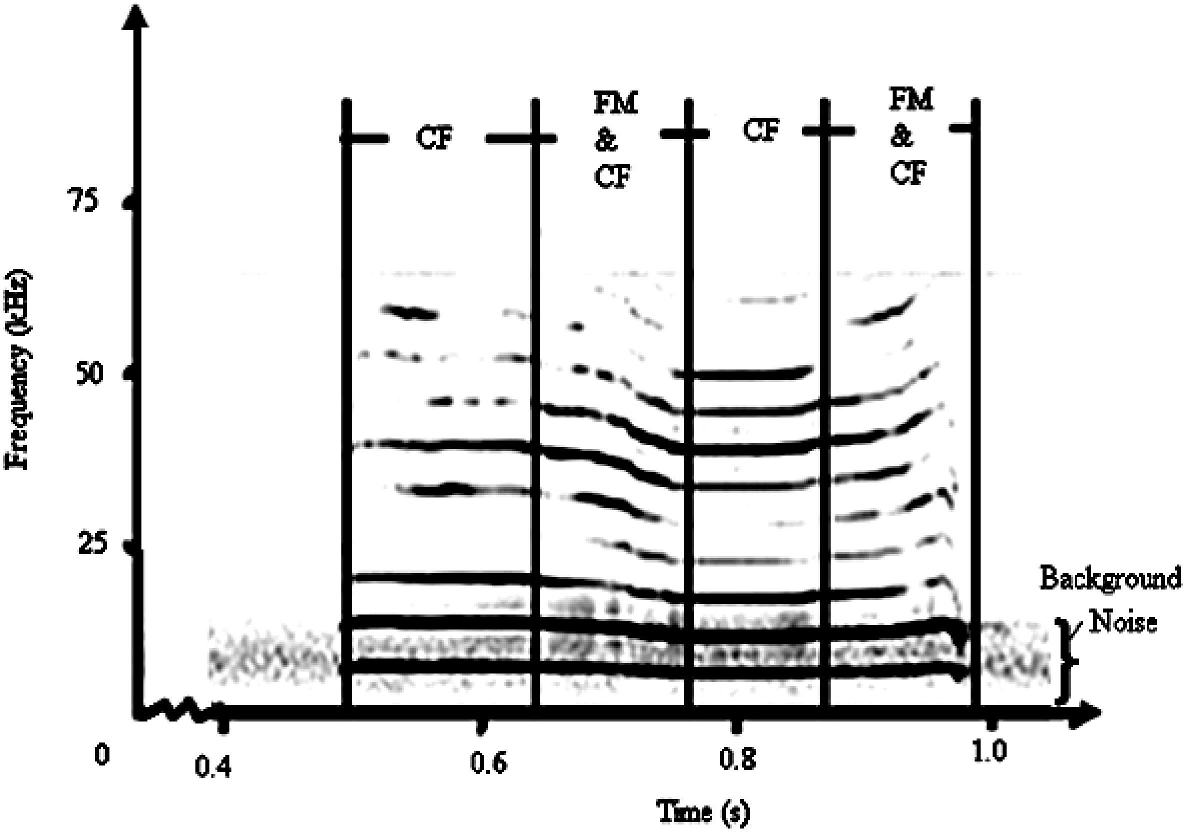

Fig. 6: A sound spectrogram for the appended sound of *A. tormotus*

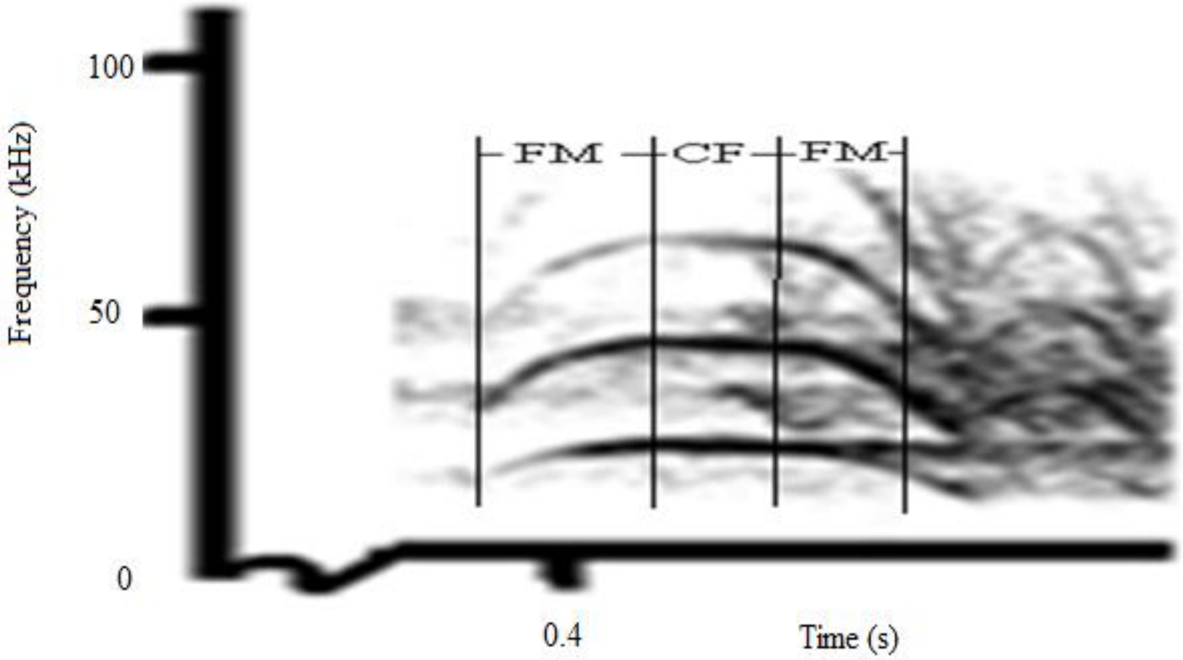

Fig. 7: A section of the spectrogram of sound calls of *C. afra*

b) Acoustic transmission parameters for the unfiltered original sounds of *C. afra* and *A. tormotus*

I. Fundamental frequency and harmonics

The spectrograms in Fig. 8 and Fig. 9 show part of the original and unfiltered appended sound signals of *A. tormotus* and *C. afra* respectively, which have been found to be major acoustic features in sound signals (Kingston *et al.*, 2003). The formants, representing harmonics in the signal of *A. tormotus* extended from 5.371 kHz to 55.34 kHz, the fundamental frequency being 5.371 kHz as shown in Fig. 8. Such formants which represent harmonics had also been reported in other animal calls (Khanna and Bedi, 1988). Recent findings by Narins *et al* (2004) showed distinct formants in the ultrasonic ranges, with harmonics of up to 54 kHz; a deviation of 1.34 kHz from the value determined in this study. The difference in harmonic frequency was attributed to sound quality, analysis system and software difference. The fundamental frequency in the sound of *C. afra*; shown in Fig. 9, was 6.836 kHz and stretched beyond 68.359 kHz in the ultrasonic range. There were several tone calls of both the sonar and social calls in the recorded sound of *C. afra.* Similar results, with slight difference had been reported by Narins *et al* (2004). There were at least eleven different frequency emissions from the sound of *A. tormotus* simultaneously, constituting the harmonics. The harmonics of the sounds, shown in Fig. 8 and Fig. 9, are multiples of the fundamental frequency (Ramabhadran, 1988). Ultrasonic and audible sounds had been reported to exist in sounds of *A. tormotus* and bats (Khanna and Bedi, 1988; Hoy *et al.*, 1989; Miller and Surlykke, 2001; Feng *et al.*, 2002; Barlow, 2006; Feng *et al.*, 2006; Ngo *et al.*, 2006; Shen, 2007). These findings show the existence ultrasonic calls in the sound of African sheath tailed bat, *C. afra.*

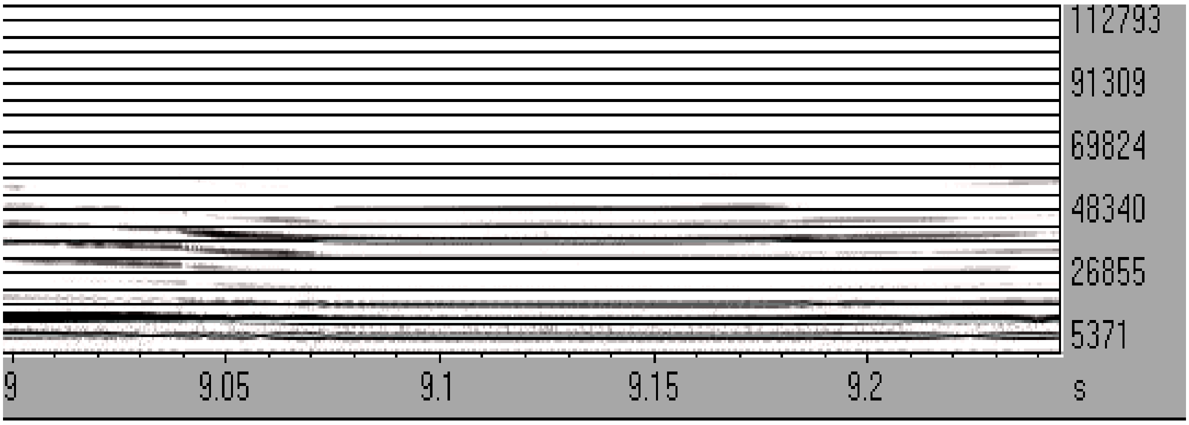

Fig. 8: Harmonics distribution in the original sound signal of *A. tormotus*

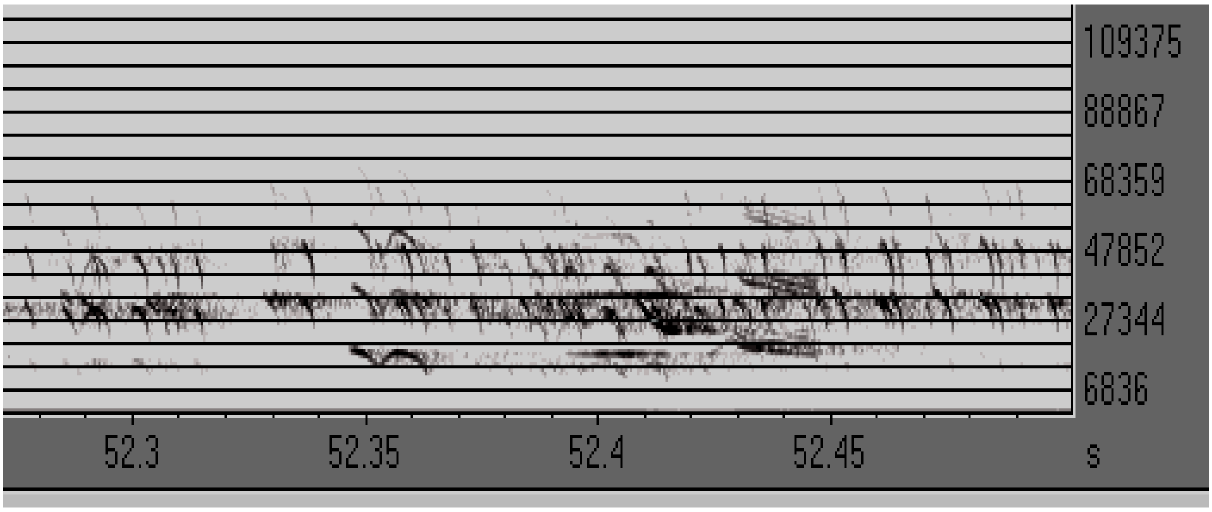

Fig. 9: Harmonics distribution in the original sound signal of *C. afra*

II. Minimum frequency and maximum frequency

The automatically generated minimum frequency for *C. afra* and *A. tormotus* were 900 Hz and 3.90 kHz respectively, recorded in Appendix I as Table I. However, these values differed from the manually determined fundamental frequencies discussed in 4.1.1 b (I) and the previously reported findings (Khanna and Bedi, 1988; Hoy *et al*., 1989; Miller and Surlykke, 2001; Ahmad *et al*., 2007., Enayati *et al*., 2010). The difference in minimum frequency was due to background noise since the sounds were reported to have been recorded near running water in the streams and wind blowing through the caves for the sounds of *A. tormotus* and *C. afra* respectively (Penna and Rogoberto, 1998; Barlow, 2006; Feng *et al*., 2006; Steve, 2006). The background noise was attenuated as described in 3.2.2. The minimum frequency (minimum entire), which is the fundamental frequnency, for *C. afra* and *A. tormotus* was 6.836 kHz and 5.371 kHz respectively. Similarly, the overall maximum frequency for the sound of *C. afra* and *A. tormotus* was 97.60 kHz and 65.40 kHz respectively. The reported EMR sound frequency ranges were 125 Hz to 74.6 kHz and 20 kHz to 100 kHz (Ahmad *et al*., 2007; Enayati *et al*., 2010). The sound of *A. tormotus* recorded slightly lower minimum frequency (minimum entire), maximum frequency (maximum entire) and mean minimum frequency (minimum entire) than the values in the sound of *C. afra*. The one-way ANOVA comparison of the maximum frequency (maximum entire) of sound of *A. tormotus* by the sound of *C. afra*, shown in Table 4, resulted to significant value, $p = 0.537 > 0.05$. Similar comparison of the maximum frequency (maximum entire) in the sound of *C. afra* by that of the sound of *A. tormotus* resulted to $p = 0.748 > 0.05$ also shown in Table 4.

Table 4: Significance values of the comparison of the maximum frequency of unfiltered predator sound

Parameter	F	p
Comparison of maximum frequency of the sound of *A. tormotus* by the maximum frequency of the sound of *C. afra*	0.961	0.537
Comparison of maximum frequency of the sound of *C. afra* by the maximum frequency of the sound of *A. tormotus*	0.705	0.748

At 5 % level of significance, there was no evidence to show significant deviation in the maximum frequency (maximum entire) for the sound of *A. tormotus* and *C. afra*.

III. Bandwidth

The mean bandwidth (maximum entire) for the sound of *A. tormotus* and *C. afra* are 23.68 kHz and 46.26 kHz respectively. The bandwidth in sounds of *A. tormotus* and *C. afra* was narrowed by 53.56 kHz and 30.98 kHz from the roported mean bandwidth of the EMR of 77.24 kHz respectively. The significance values shown in Table 5, which were equal for the bandwidth (maximum entire) of the sound of *A. tormotus* and *C. afra* compared by 77.24 kHz on a one-sample T test, was $p = 0.000 < 0.05$. Similaly, the one-way ANOVA comparison of the bandwidth (maximum entire) of *A. tormotus* by *C. afra*, shown in Table 6, resulted to significant value, $p = 0.046 < 0.05$.

Table 5: Significance values of the comparison of the bandwidth of unfiltered predator sounds compared by 77.24 kHz

Parameter	t	p
Comparison of bandwidth of sound of *A. tormotus* by 77.24 kHz	-95.924	0.000
Comparison of bandwidth of sound of *C. afra* by 77.24 kHz	-167.501	0.000

Table 6: Significance values of the comparison of the bandwidth of unfiltered predator sound

Parameter	F	p
Comparison of bandwidth for sound of *A. tormotus* by the bandwidth of the sound of *C. afra*	1.349	0.046

There was enough evidence at 0.05 significance level to suggest high significant difference in bandwidth for both sounds of *A. tormotus* and *C. afra* from the reported mean bandwidth of 77.24 kHz. The bandwidth in sounds of *A. tormotus* and *C. afra* were significantly narrowed from the reported mean bandwidth of the EMR sound. The bandwidth (maximum entire) for the sound of *A. tormotus* deviated significantly from the bandwidth (maximum entire) of the sound of *C. afra*.

IV. Peak amplitude

Acoustic energy, which is a product of the square of the amplitude with time, is therefore determined by amplitude. The maximum peak amplitude (maximum entire) for the sound of *A. tormotus* and *C. afra* are 114.91 Pa (135.19 dB SPL) and 112.07 Pa (134.97 dB SPL) respectively referenced to 20 µPa (0 dB SPL). Also, the mean peak amplitude (maximum entire) of *A. tormotus* and *C. afra* are and 108.12 Pa (134.66 dB SPL) and 97.37 Pa (133.75 dB SPL) respectively. The one-way ANOVA comparison of peak amplitude (maximum entire) of the sound of *C. afra* by that of *A. tormotus*, shown in Table 7, yielded a significance value, p = 0.507 > 0.05; whereas the comparison of peak amplitude (maximum entire) of the sound of *A. tormotus* by that of *C. afra* resulted to p = 0.884 > 0.05, as indicated in Table 7.

Table 7: Significance values of the comparison of peak amplitude of unfiltered predator sounds

Parameter	F	p
Comparison of peak amplitude of the sound of *A. tormotus* by the peak amplitude of sound of *C. afra*	0.854	0.884
Comparison of peak amplitude of the sound of *C. afra* by the peak amplitude of sound of *A. tormotus*	0.995	0.507

At a significance level of 0.05, there was no significant difference in peak amplitude (maximum entire) for the sound signals of *A. tormotus* and *C. afra*. The peak amplitude (maximum entire) for the sound of sound signals of *A. tormotus* and *C. afra* was 134±1 dB SPL, which was insignificant in deviation. This showed that the amplitude depended acoustic energy for individual predator sound was almost equal.

V. Acoustic energy

The sound of *A. tormotus* and *C. afra* provided a mean acoustic energy of 3.34 Pa^2s and 0.12 Pa^2s respectively. Similarly, the maximum energy for the sounds of *A. tormotus* and *C. afra* was 19.57 Pa^2s and 35.80 Pa^2s respectively. The one way comparison of the energy from the sound of *A. tormotus* by that of *C. afra* shown in Table 8 yielded a significance value, p = 0.831 > 0.05 whereas the comparison of the energy from the sound of *C. afra* by *A. tormotus* provided a significant value, p = 1.000 > 0.05 also indicated in Table 8.

Table 8: Significance values of the comparison of acoustic energy of unfiltered predator sounds

Parameter	F	p
Acoustic energy of the sound of *A. tormotus* by the acoustic energy of the sound of *C. afra*	0.876	0.831
Acoustic energy of the sound of *C. afra* by the acoustic energy of the sound of *A. tormotus*	0.481	1.000

At a statistical significance level of 0.05, there was high evidence for no significant deviation in acoustic energy for the predator sounds.

Acoustic energy determines the magnitude of speaker diaphragm vibration which later disturbed neighbouring air particles. On average, the acoustic energy of *A. tormotus* which initiated high disturbance to air particles is 3.22 Pa^2s greater than the energy in the sound of *C. afra*. However, the maximum acoustic energy was from the sound of *C. afra*, which was 16.23 Pa^2s above that of *A. tormotus*. The disturbance was transmitted through the air to the antennae of mosquitoes initiating resonance (Zwibel and Pitts, 2006; Maweu *et al*., 2009).

VI. Peak frequency

Peak frequencies for the sound of *A. tormotus* and *C. afra* were determined through the Avisoft SASLab analysis software provided frequencies for pulses at various amplitudes. Frequency is essential in acoustic energy determination. The maximum peak frequency (maximum entire) for the sound of *A. tormotus* by *C. afra* was 40.00 kHz and 41.19 kHz respectively. The one-way ANOVA comparison of the peak frequency (maximum entire) of the sound of *C. afra* by that of the sound of *A. tormotus*, shown in Table 9 yielded the significance value, p = 0.067 > 0.05 whereas the comparison of peak frequency (maximum entire) of the sound of *A. tormotus* by that of the sound of *C. afra* resulted to p = 0.031< 0.05 as shown in Table 9.

Table 9: Significance values of the comparison of peak frequency of the unfiltered predator sounds

Parameter	F	p
Comparison of peak frequency of the sound of *A. tormotus* by peak frequency of the sound *C. Afra*	1.520	0.031
Comparison of peak frequency of the sound of *C. afra* by peak frequency of the sound *A. tormotus*	1.841	0.067

Only the peak frequency (maximum entire) of the sound of *C. afra* differed significantly from the sound of *A. tormotus* at a statistical significance level of 0.05.

4.1.2 Determination of the transmission parameters of filtered sounds of *C. afra* and *A. tormotus*

a) Generation and modulation of sounds of *C. afra* and *A. tormotus*

The sounds in the 10-34 kHz, 35-60 kHz and 61-90 kHz frequency ranges were generated as discussed in 4.1.1 (a) and displayed similar modulation components as shown in Fig. 10 and Fig. 11.

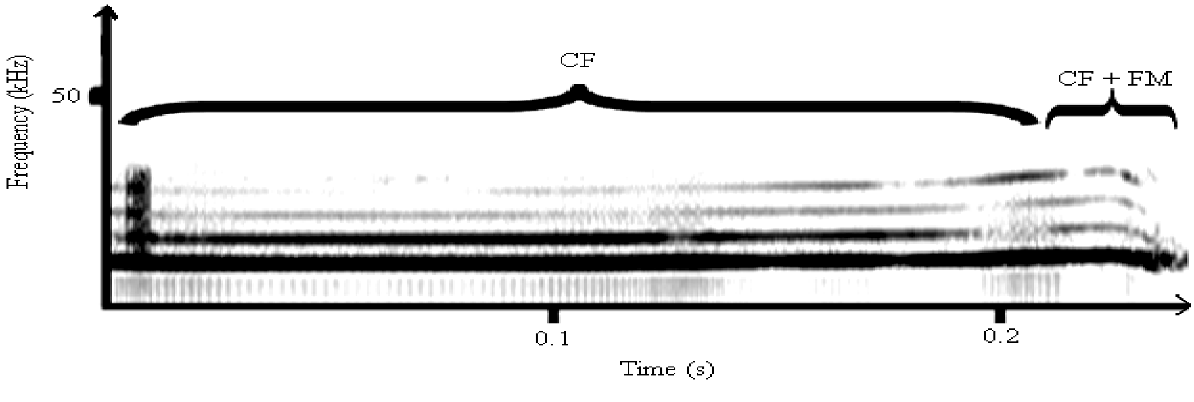

Fig. 10: The signal spectrogram for the 10-34 kHz band for the sound of *A. tormotus*

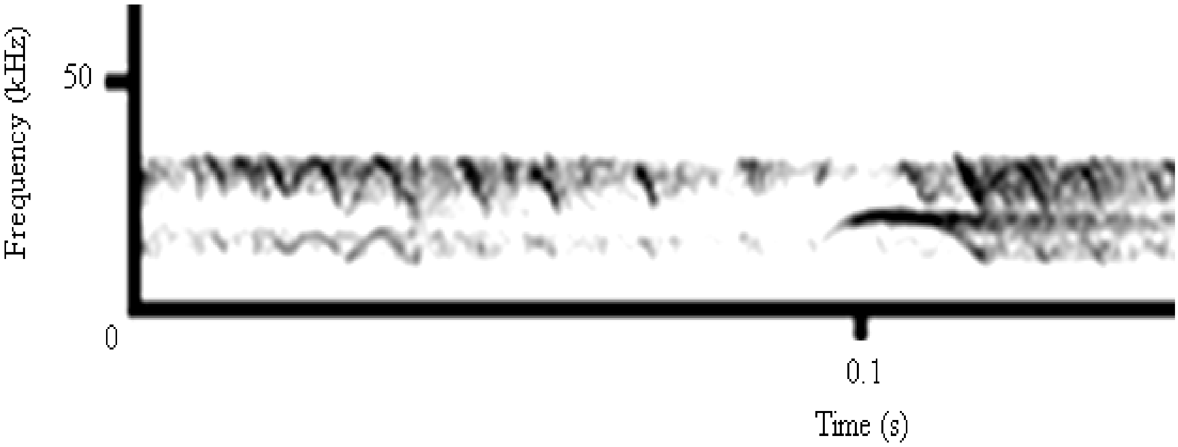

Fig. 11: A spectrogram for the sound of *C. afra* in the frequency range of 10-34 kHz

Recent studies showed similar constant frequency and frequency modulation in animal calls (Berry, 1966; Narins *et al*., 2004; Barlow, 2006; Feng *et al*., 2006; Pollack and Martins, 2007).

b) Acoustic transmission parameters of filtered sounds of *C. afra* and *A. tormotus* in the 10-34 kHz range

The software settings described in 3.2.2 generated the acoustic transmission parameters for the filtered sounds of *A. tormotus* and *C. afra* which are shown as Table A1 and Table A2 respectively in Appendix A.

I. Fundamental frequency and harmonics

The fundamental frequency being the lowest frequency sound were determined from the original unfiltered sounds as discussed in 4.1.1 (b) I (Khanna and Bedi, 1988). There existed formants within this frequency range as shown in Fig. 10 and Fig. 11indicating presence of harmonics which stretched into the ultrasonic range (Narins *et al*., 2004).

II. Minimum frequency and maximum frequency

The mean of the maximum frequency (maximum entire) for *A. tormotus* and *C. afra* was 22.57 kHz and 33.67 kHz respectively. These frequencies fall in the frequency ranges earlier investigate in EMR. However, the minimum frequencies of predator sounds differed considerably from the investigated and reported minimum frequencies due to the band pass filtration (Khanna and Bedi, 1988; Hoy *et al*., 1989; Miller and Surlykke, 2001; Ahmad *et al*., 2007., Enayati *et al.*, 2010). Similarly, the mean of the minimum frequency (minimum entire) for *A. tormotus* and *C. afra* was 10.73 kHz and 14.80 kHz respectively. These frequency values were limited by the band pass filters. The one-way ANOVA comparison of the minimum frequency (minimum entire) of the sound of *A. tormotus* by that of the sound of *C. Afra* resulted to p = 0.313 > 0.05. Similar comparison of the minimum frequency (minimum entire) in the sound of *C. afra* by that of the sound of *A. tormotus* sound resulted to p = 0.634 > 0.05.

The comparison of maximum frequency (maximum entire) for the sound of *A. tormotus* by that of the sound of *C. afra* resulted to p = 0.563 > 0.05. Similarly, the comparison of the maximum frequency (maximum entire) in the sound of *C. afra* by that of the sound of *A. tormotus* resulted to p = 0.494 > 0.05.

All the F and p values determined through one-way ANOVA comparison of the minimum frequency (minimum entire) and also maximum frequency (maximum entire) are shown in Table 10.

Table 10: Significance values of the comparison of minimum and maximum frequency for the 10-34 kHz predator sounds

Parameter	F	p
Comparison of minimum frequency for the sound of *A. tormotus* by minimum frequency for the sound of *C. afra*	1.131	0.313
Comparison of minimum frequency for the sound of *C. afra* by minimum frequency for the sound of *A. tormotus*	0.687	0.634
Comparison of maximum frequency for the sound of *A. tormotus* by minimum frequency for the sound of *C. Afra*	0.868	0.563
Comparison of maximum frequency for the sound of *C. afra* by minimum frequency for the sound of *A. tormotus*	0.935	0.494

At 5 % level of significance, there was no evidence to show that the minimum frequency (minimum entire) for *C. afra* sounds in the 10-34 kHz frequency range differed significantly from the minimum frequency (minimum entire) of *A. tormotus*. Similar results were obtained when the sound of *A. tormotus* was compared by *C. afra*. The difference in mean minimum frequency (minimum entire) for the predator sounds was 4.067 kHz, considered highly insignificant. Also, at 5 % level of significance, there was no enough evidence showing that the maximum frequency (maximum entire) in the sound of *A. tormotus* and *C. afra* differed significantly high. The maximum frequency (maximum entire) and minimum frequency (minimum entire) for *C. afra* were slightly higher than those of *A. tormotus*.

III. Bandwidth

The mean bandwidth for the sound of electronic mosquito repellents was 77.24 kHz (Ahmad *et al*., 2007; Enayati *et al.*, 2010; Mohankumar, 2010). The mean bandwidth (maximum entire) for *A. tormotus* and *C. afra* was 10.98 kHz and 17.71 kHz respectively were less compared to the average EMR bandwidth of 77.24 kHz observed by Ahmad *et al*, 2007 and Enayati *et al*, 2010. The bandwidth of the sounds of *A. tormotus* and *C. afra* was highly narrowed from the reported mean bandwidth of EMR by 66.26 kHz and 59.53 kHz respectively. The mean bandwidth (maximum entire) for the sound of *C. afra* and that of the sound of *A. tormotus* differed by only 6.726 kHz. The significance values for the bandwidth (maximum entire) of the sound of *A. tormotus* and *C. afra* compared by 77240 Hz as a test value on one-sample T test were p = 0.000 < 0.05 and p = 0.000 < 0.05 respectively as shown in Table 11.

Table 11: Significance values of the comparison of the bandwidth for the 10-34 kHz predator sounds by 77.24 kHz

Parameter	t	p
Comparison of bandwidth for the sound of *A. tormotus* by 77.24 kHz	-296.287	0.000
Comparison of bandwidth for the sound of *C. afra* by 77.24 kHz	-471.783	0.000

Table 12: Significance values of the comparison of the bandwidth for the 10-34 kHz predator sounds

Parameter	F	p
Comparison of bandwidth for the sound of *A. tormotus* by the bandwidth for the sound *C. afra*	0.902	0.593
Comparison of bandwidth for the sound of *C. afra* by the bandwidth for the sound *A. tormotus*	0.621	0.825

The bandwidth (maximum entire) in sounds of *A. tormotus* and *C. afra* in this frequency range yielded a highly significant deviation from the reported EMR mean bandwidth of 77.24

kHz. However, there was no significant difference in bandwidth (maximum entire) of the individual predator sounds when subjected to one-way ANOVA, yielding significant values greater than 0.05 shown in Table 12.

IV.Peak amplitude

The sound of *A. tormotus* and *C. afra* yielded a mean peak amplitude (maximum entire) of103.244 Pa (134.26 dB SPL) and 99.825 Pa (133.96 dB SPL) respectively. The maximum amplitudes for the sound pulses in both predators are illustrated in Fig. 12 and Fig. 13, with amplitudes expressed as percentage, referenced to 0 dB SPL (20 µPa). The maximum peak amplitude (maximum entire) for the sounds of *A. tormotus* and *C. afra* was 112.739 Pa (135.02 dB SPL) and 113.129 Pa (135.05 dB SPL). The maximum peak amplitude (maximum entire) for the sounds of *A. tormotus* was higher in the filtered sound by 2.171 Pa compared to the results for unfiltered sound discussed in 4.1.1 (b) IV. However, the maximum amplitude in the sounds of *C. afra* reduced in the filtered sound. Investigation into insects startle response to ultrasounds of 50±55 dB SPL yielded effective response in beetles (Yager *et al*., 2000). Comparison of the peak amplitude (maximum entire) of the sound of *A. tormotus* by that of the sound of *C. afra* yielded significance value as $p = 0.398 > 0.05$ whereas the comparison of the sound of *C. afra* by that of the sound of *A. tormotus* yielded significance value as $p = 0.996 > 0.05$ respectively as shown in Table 13.

Table 13: Significance values of the comparison of peak amplitude of the predator sounds in the 10-34 kHz range

Parameter	F	p
Comparison of the peak amplitude for the sound of *A. tormotus* by the peak amplitude for the sound *C. afra*	1.052	0.398
Comparison of peak amplitude for the sound of *C. afra* by the peak amplitude for the sound *A. tormotus*	0.684	0.996

These results show no evidence for significant deviation in the maximum peak amplitude (maximum entire) in the sounds *C. afra* and *A. tormotus*.

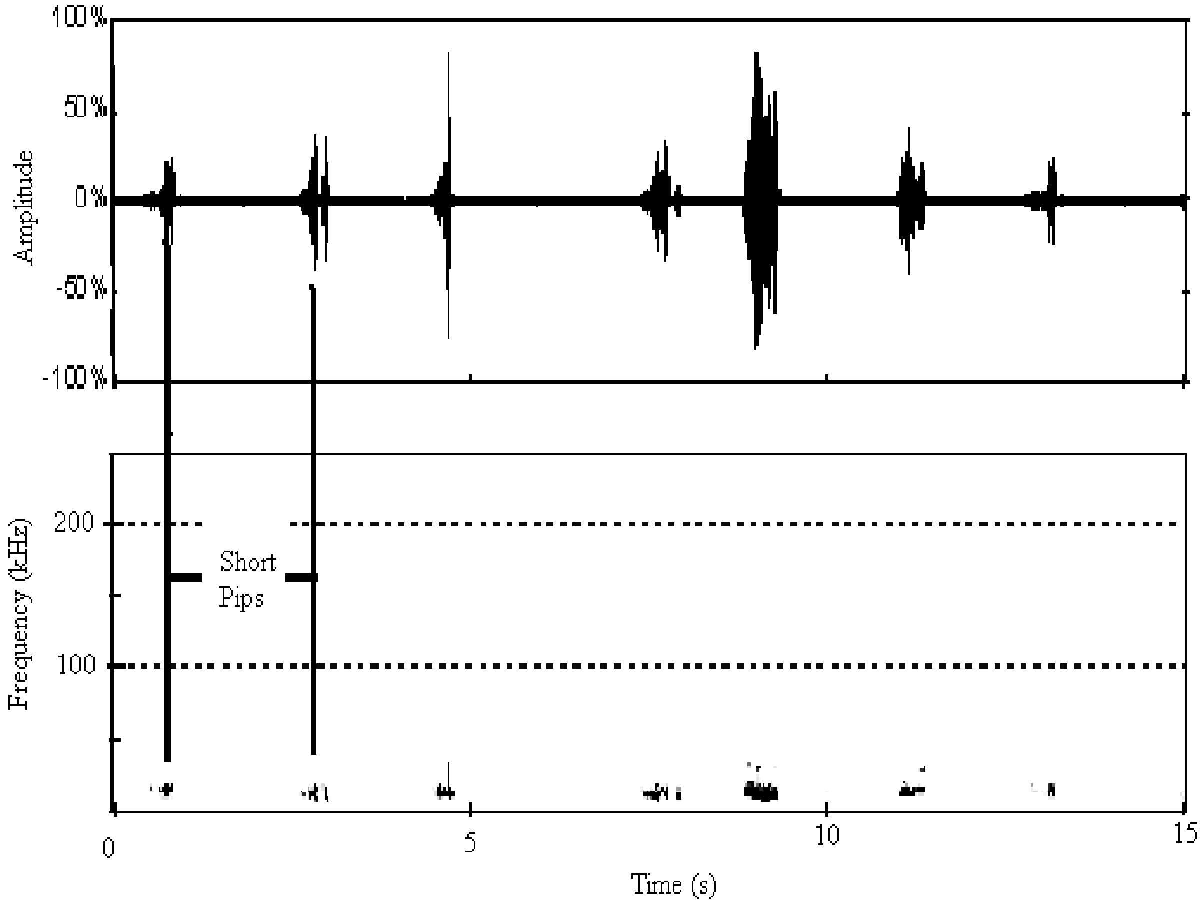

Fig. 12: The sound spectrogram and oscillogram for *A. tormotus* in the range of 10-34 kHz

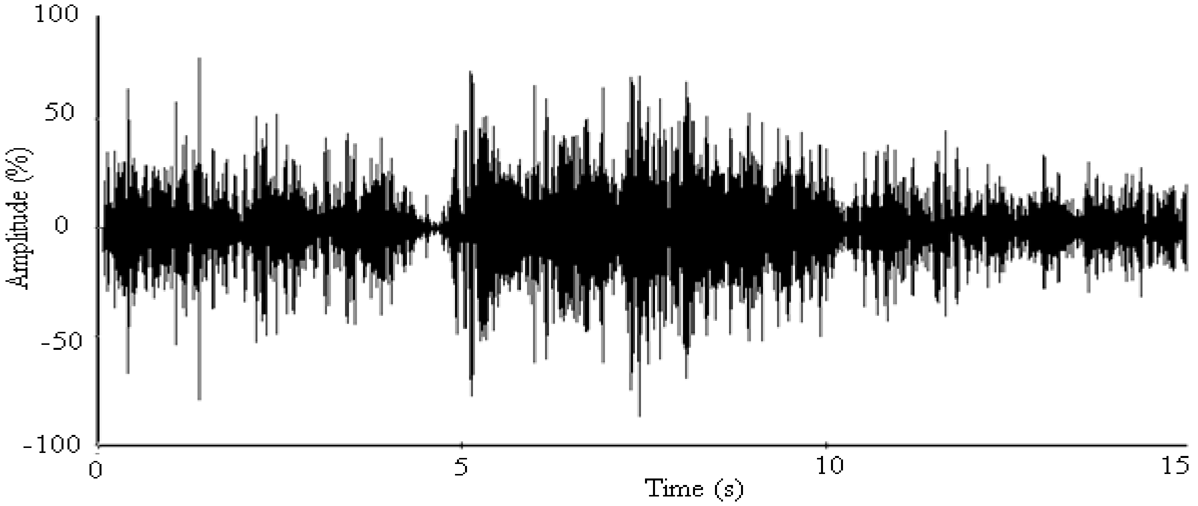

Fig. 13: The sound oscillogram of *C. afra* in the 10-34 kHz range

This research established that the peak amplitude for the predator sounds was 134±1 dB SPL. The peak amplitude for the sound signal of *C. afra* therefore did not differ considerably from that of *A. tormotus*. The peak amplitude varied slightly by the nature of call duration, loudness and energy as recently reported (Khanna and Bedi, 1988).

V. Acoustic energy and power

The maximum total energy for the sound of *A. tormotus* and *C. afra* was 8.857 Pa^2s and 14.857 Pa^2s respectively. Similarly, the mean total acoustic energy for sounds of *A. tormotus* and *C. afra* was 1.220 Pa^2s and 0.103 Pa^2s respectively. The difference in the maximum acoustic energy in the predator sounds is 6.000 Pa^2s whereas the mean energy is 1.117 Pa^2s. Acoustic energy is determined by the sound frequency and amplitude as discussed in 4.2.1 b (I - IV) (Ramabhadran, 1988; Gupta, 1989; Ballou, 2002). The sound of *C. afra* recorded the greatest acoustic energy due to maximum peak amplitude as discussed in 4.2.1 (c). Ultrasound, which is used to repel insects, had been reported in recent findings to have significant energy (Narins *et al*., 2004; Monto, 2010). The comparison of the total energy of the sound of *A. tormotus* by the sound of *C. afra* yielded a significance value as $p = 0.163 > 0.05$. Similarly, comparison of total energy of the sound of *C. afra* by that of the sound of *A. tormotus* yielded a significance value as $p = 0.876 > 0.05$. The F and p values in the ANOVA comparison of acoustic energy are shown in Table 14.

Table 14: Significance values of the comparison of acoustic energy for the 10-34 kHz predator sounds

Parameter	F	p
Comparison of the acoustic energy for the sound of *A. tormotus* by the acoustic energy of the sound of *C. Afra*	1.217	0.163
Comparison of acoustic energy for the sound of *C. afra* by the acoustic energy of the sound of *A. tormotus*	0.683	0.876

These results showed that there existed no significant difference in acoustic energy between the sound of *A. tormotus* and *C. afra* at a significance level of 5 %.

The power spectrums in Fig. 14 and Fig. 15 were generated at a threshold of -20 dB. Fig. 15 shows that the entire signal power for *A. tormotus* varied from -97 dB to -135 dB, declined from -97 dB to -118 dB within this range. The signal power, which is a measure of sonic energy per unit time, was maintained at -118 dB with slight dips at 22 kHz, 24 kHz and 30 kHz. The power spectrum for *C. afra*, shown in Fig. 14, maintained an average signal power of about -53 dB with a maximum of -35 dB between 30-34 kHz (Ramabhadran, 1988; Gupta, 1989; Ballou, 2002). The signal power in *C. afra* intensified with increase in frequency up to about 32.5 kHz and then declined drastically to -55 dB. The sound of *C. afra* had more signal power within this range than the sound of *A. tormotus* due to the increasing trend with frequency, unlike the fluctuating power trend in the sound of *A. tormotus*. It had been reported that high intensity pulses are used, particularly in *C. afra* to locate and track flying prey (Monto, 2010).

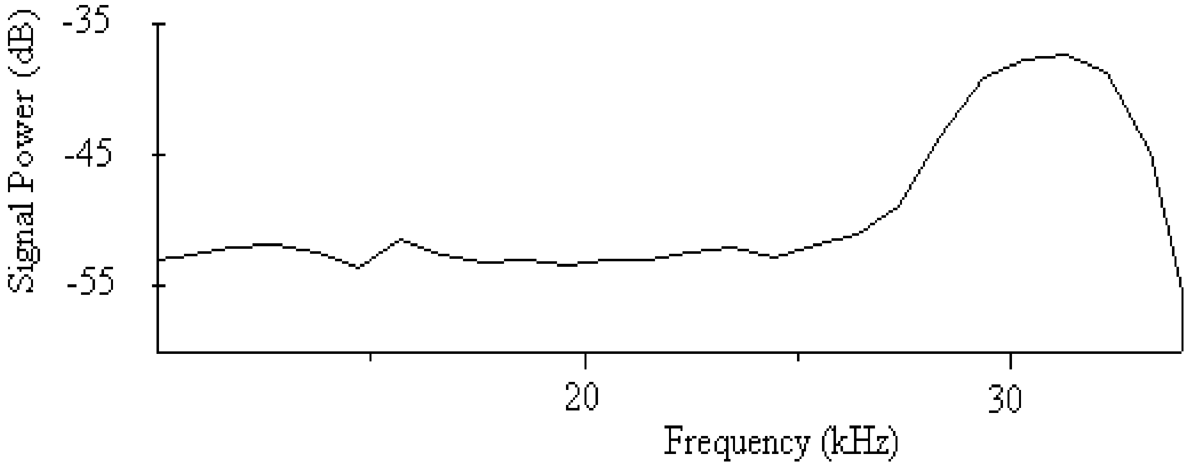

Fig. 14: The power spectrum for the sound signal of *C. afra* in the range of 10-34 kHz

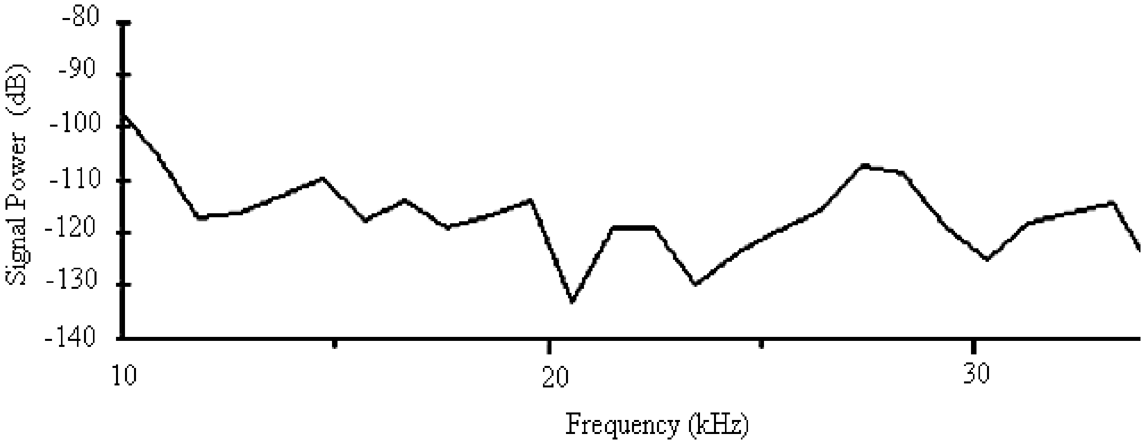

Fig. 15: The power spectrum for the sound of *A. tormotus* in the 10-34 kHz range

VI. Peak frequency

The mean peak frequency (maximum entire) for the sound of *A. tormotus* and *C. afra* was 16.18 kHz and 30.846 kHz respectively. The maximum frequency of the signal peaks for all predator sounds was considered. The peak frequency (maximum entire) in the sound of *C.*

afra differed by 14.666 kHz from the peak frequency (maximum entire) of the sound from *A. tormotus*. The one-way ANOVA comparison of the peak frequency (maximum entire) for the sound of *C. afra* by that of the sound of *A. tormotus* at a significance level of 5 % yielded significance value, $p = 0.041 < 0.05$, shown in Table 15.

Table 15: Significance values of the comparison of peak frequency for the 10-34 kHz predator sounds

Parameter	F	p
Comparison of peak frequency for the sound of *C. afra* by the peak frequency for the sound of *A. tormotus*	2.340	0.041

There was enough evidence to show that the peak frequency (maximum entire) in the sound of *C. afra* differed significantly from the peak frequency (maximum entire) of the sound from *A. tormotus*.

c) Acoustic transmission parameters of filtered sounds of *C. afra* and *A. tormotus* in the 35-60 kHz range

I. Fundamental frequency and harmonics

The fundamental frequency was determined from the original unfiltered sounds as discussed in 4.1.1 (b) I. There existed formants within this frequency range as shown in Fig. 16 and Fig. 17 indicating presence of harmonics which stretched above 50 kHz in the ultrasonic range, as reported in recent findings (Khanna and Bedi, 1988; Narins *et al.*, 2004). Layers of harmonics were observed between 0.1 s to 0.2 s in Fig. 16, which range from 35 kHz up to 50 kHz. The fundamental frequency was determined from the unfiltered sound described in 4.1.1 (b) I. Fig. 17 shows formants in the same range of frequency. However, the calls of *C. afra* take short duration than those of *A. tormotus*. The FM calls are pronounced due to changes in frequency. Also, the CF exists in this range for both predator sounds.

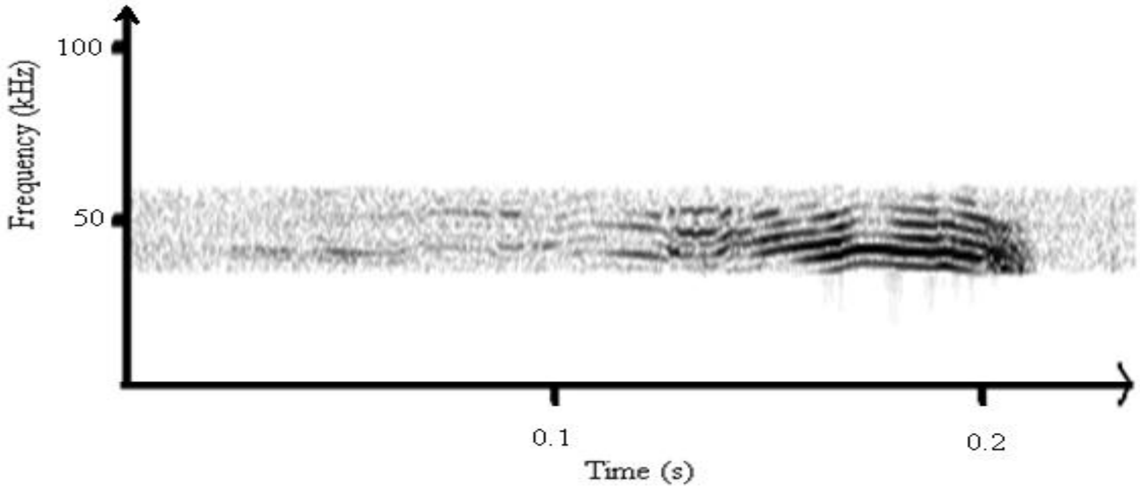

Fig. 16: A spectrogram for the sound of *A. tormotus* in the range of 35-60 kHz

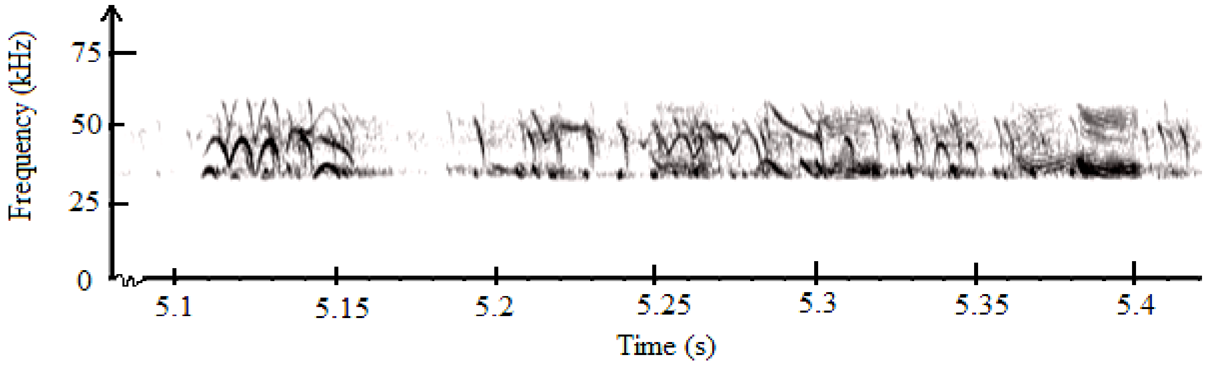

Fig. 17: A spectrogram for the sound of *C. afra* in the frequency range of 35-60 kHz

II. Peak amplitude

The oscillograms for the sound signals of the *A. tormotus* and *C. afra* are shown in Fig. 18 and Fig. 19 respectively. The mean peak amplitude (maximum entire) for *A. tormotus* and *C. afra* was 103.475 Pa (134.28 dB SPL) and 101.141 Pa (134.08 dB SPL) respectively. The peak amplitudes for both sound signals exceeded 50 % of the maximum amplitude at -20 dB threshold as indicated in Fig. 18 and Fig. 19.

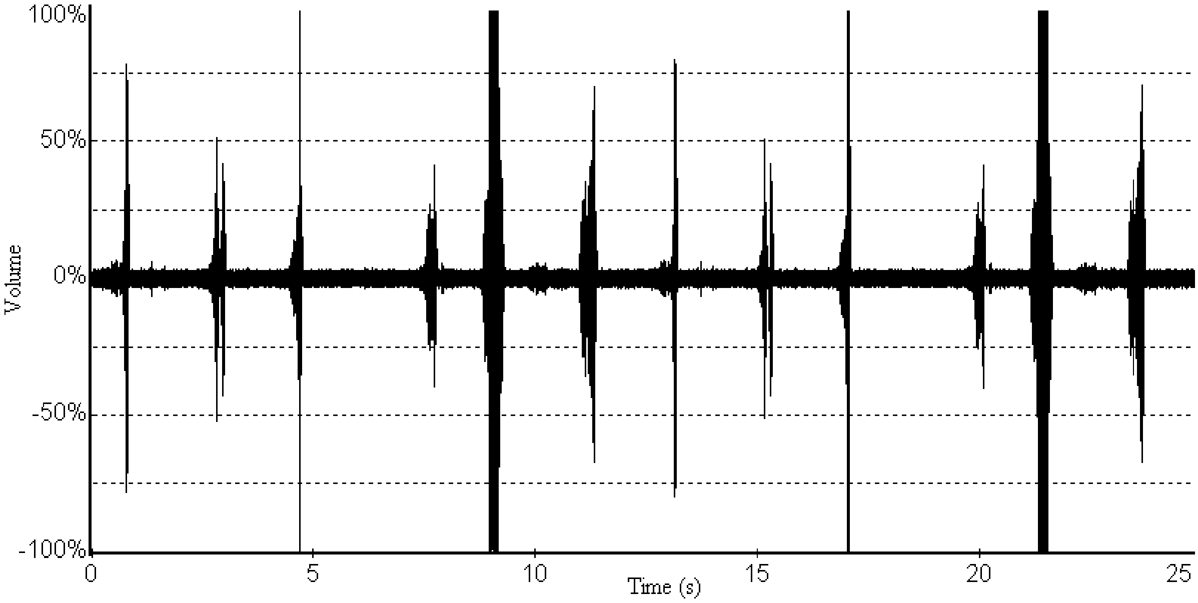

Fig. 18: A section of the sound oscillogram for *A. tormotus* for the range 35-60 kHz

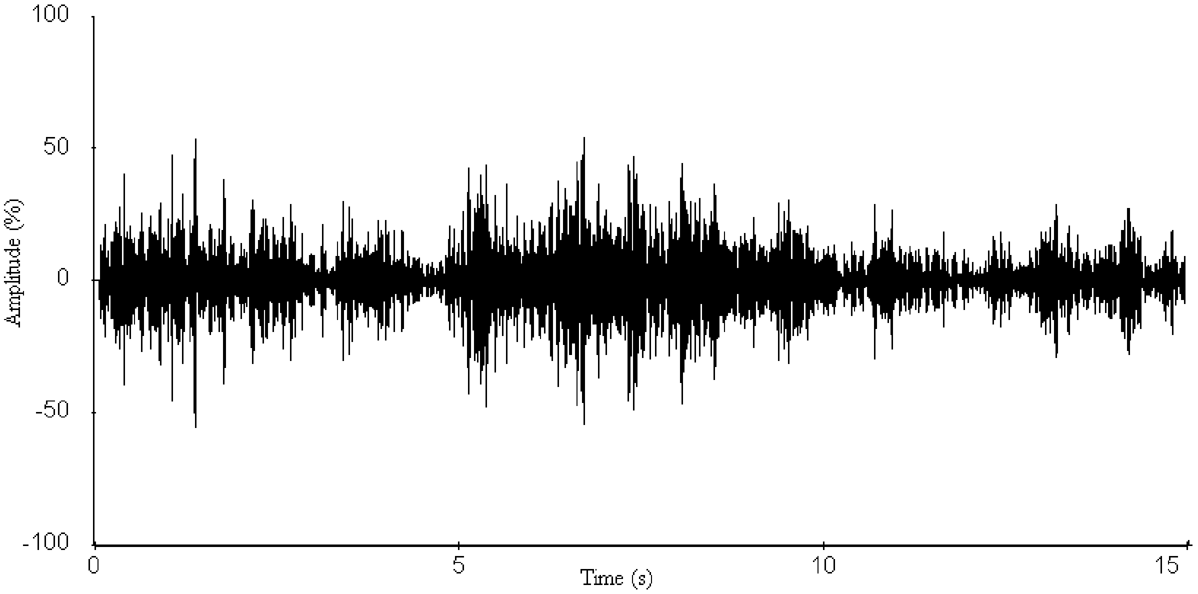

Fig. 19: A section of the sound oscillogram for *C. afra* for the range 35-60 kHz

The minimum peak amplitude (mean) for the entire sound was found to be 80.14 Pa (132.06 dB SPL) and 92.12 Pa (133.27 dB SPL) for the sound of *A. tormotus* and *C. afra* respectively. The peak amplitude (maximum entire) and peak amplitude (minimum entire) of the sound of *A. tormotus* by the sound of *C. afra* differed by 0.20 dB SPL and 1.21dB SPL respectively.

Comparison of the peak amplitude (maximum entire) of the sound of *A. tormotus* by *C. afra* yielded significance value as $p = 0.904 > 0.05$ and the comparison of the sound of *C.*

afra by *A. tormotus* yielded significance value as p = 0.743 > 0.05 respectively as shown in Table 16.

Table 16: Significance values of the comparison of peak amplitude of the predator sounds

Parameter	F	p
Comparison of peak amplitude for the sound of *A. tormotus* by the peak amplitude for the sound of *C. Afra*	0.840	0.904
Comparison of peak amplitude for the sound of *C. afra* by the peak amplitude for the sound of *A. Tormotus*	0.921	0.743

At a statistical significance level of 5 %, results show no evidence of significant deviation in the maximum peak amplitude (maximum entire) for the sounds *C. afra* by *A. tormotus*.

III. Minimum frequency and maximum frequency

The mean maximum frequency (maximum entire) and mean minimum frequency (minimum entire) for the sound of *A. tormotus* was 55.1353 kHz and 34.6588 kHz respectively. Similarly, the mean maximum frequency (maximum entire) and the mean minimum frequency (minimum entire) for the sound of *C. afra* was 55.4766 kHz and 44.2581 kHz respectively. The minimum frequencies (minimum entire) fell slightly outside the 38-44 kHz effective startling frequency range produced by EMR (Mohankumar, 2010). The mean minimum frequency (minimum entire) and the mean maximum frequency (maximum entire) in the predator sounds differed by 9.5993 kHz and 0.3413 kHz respectively. The one-way ANOVA comparison of the minimum frequency (minimum entire) of *A. tormotus* sound by the sound of *C. afra* resulted to p = 0.807 > 0.05. Similar comparison of the minimum frequency (minimum entire) in the sound of *C. afra* by *A. tormotus* sound resulted to p = 0.659 > 0.05. The comparison of maximum frequency (maximum entire) for the sound of *A. tormotus* by *C. afra* resulted to p = 0.919 > 0.05. Similar comparison of the maximum frequency (maximum entire) in the sound of *C. afra* by the sound of *A. tormotus* resulted to p = 0.832 > 0.05. These comparison results are given in Table 17.

Table 17: Significance values of the comparison of the minimum and maximum frequency of the predator sounds

Parameter	F	p
Comparison of minimum frequency for the sound of *A. tormotus* by the of minimum frequency for the sound of *C. afra*	0.655	0.807
Comparison of minimum frequency for the sound of *C. afra* by the of minimum frequency for the sound of *A. tormotus*	0.653	0.659
Comparison of maximum frequency for the sound of *A. tormotus* by the of maximum frequency for the sound of *C. afra*	0.609	0.919
Comparison of maximum frequency for the sound of *C. afra* by the of maximum frequency for the sound of *A. tormotus*	0.597	0.832

At 5 % level of significance, there was no evidence to show that the minimum frequency (minimum entire) for the sound of *C. afra* in the 35-60 kHz frequency range differed significantly from the minimum frequency (minimum entire) of the sound of *A. tormotus*. Similarly, no significant difference was established when the sound of *A. tormotus* was compared by the sound of *C. afra*. Also, there was no evidence that the maximum frequency (maximum entire) in the sound of *A. tormotus* and *C. afra* differed significantly high with each other.

IV. Bandwidth

The mean bandwidth (maximum entire) for *A. tormotus* and *C. afra* was 19.396 kHz and 19.8482 kHz respectively. The mean bandwidth in sound of *C. afra* exceeded the mean bandwidth of the sound from *A. tormotus*. The mean bandwidth (maximum entire) deviated in the sounds of *C. afra* and *A. tormotus* by 0.4522 kHz. Similarly, the bandwidth (maximum entire) of the predators was highly narrowed from the reported mean bandwidth of EMR sounds.

The significance values for the bandwidth (maximum entire) of the sound of *A. tormotus* and *C. afra* compared by 77240 Hz as a test value on one-sample T test were equal to $p = 0.000 < 0.05$ shown in Table 18. The one-way ANOVA comparison of the bandwidth (maximum entire) of the sound of *A. tormotus* by *C. afra* yielded a significance value, $p = 0.593 > 0.05$ whereas the comparison of the bandwidth (maximum entire) of the sound of *C. afra* by *A. tormotus* yielded a significance value, $p = 0.570 > 0.05$ as indicated in Table 19.

Table 18: Significance values of the comparison of bandwidth of the predator sounds by 77.24 kHz

Parameter	t	p
Comparison of bandwidth of the sound of *A. tormotus* by 77.24 kHz	-283.838	0.000
Comparison of bandwidth of the sound of *C. afra* by 77.24 kHz	-650.243	0.000

Table 19: Significance values of the bandwidth for the comparison of predator sounds

Parameter	F	p
Comparison of bandwidth of the sound of *A. tormotus* by the bandwidth of the sound of *C. afra*	0.902	0.593
Comparison of bandwidth of the sound of *C. afra* by the bandwidth of the sound of *A. tormotus*	0.877	0.570

At 5 % significance level, the bandwidth in sounds of *A. tormotus* and *C. afra* in this frequency range yielded a highly significant deviation from the reported EMR mean bandwidth of 77.24 kHz. Similarly, there was no significant difference in the bandwidth (maximum entire) of individual predator sound.

V. Acoustic energy and power

The minimum total energy for the sound of *A. tormotus* and *C. afra* was 0.00521 Pa^2s and 0.00033 Pa^2s whereas the maximum total energy was 10.8434 Pa^2s and 12.3229 Pa^2s respectively. Though the greatest energy recorded in this frequency range was from the sound of *C. afra*, there was a decline in energy recorded in the range of 10-34 kHz. Similarly, the minimum energy was less than that of *A. tormotus*. The sound of *A. tormotus* recorded a progressive increase in energy from 8.85674 Pa^2s to 10.8434 Pa^2s though the minimum energy reduced from 0.01463 Pa^2s to the current value. The mean total energy of the sound of *A. tormotus* and *C. afra* was 1.3391 Pa^2s and 0.0591 Pa^2s respectively. The mean acoustic energy for *A. tormotus* increased by 0.1194 Pa^2s whereas that of *C. afra* reduced by 0.0532 Pa^2s from the energy in the 10-34 kHz. The mean and maximum acoustic energy in predator sounds differed by 1.2800 Pa^2s and 1.5795Pa^2s respectively.

The comparison of the total energy of the sound of *A. tormotus* by *C. afra* yielded a significance value as p = 0.934 > 0.05 as indicated in Table 20. Similarly, comparison of the total energy of the sound of *C. afra* by *A. tormotus* yielded a significance value as p = 1.000 > 0.05 also shown in Table 20.

Table 20: Significance values of the comparison of acoustic energy in the 35-60 kHz predator sounds

Parameter	F	p
Comparison of coustic energy for the sound of *A. tormotus* by of coustic energy for the sound of *C. Afra*	0.790	0.934
Comparison of acoustic energy for the sound of *C. afra* by of coustic energy for the sound of *A. tormotus*	0.459	1.000

At 5 % significance level, there was no significance difference in acoustic energy in the two predator sounds.

The signal power of *A. tormotus* fluctuated between -100 dB and -120 dB as shown in Fig. 20. However, it was maintained at -110 dB between 37 kHz and 57 kHz, with a slight dip to -120 dB at the 50 kHz frequency as shown in Fig. 20. The signal intensity for *A. tormotus* in 35-60 kHz was therefore greater than that of the 10-34 kHz frequency range. The signal power for *C. afra* declined uniformly from -55 dB and -59 dB as indicated in Fig. 21 rendering it weak compared to the constant power of *A. tormotus*. The sound signal of *A. tormotus* in the 35-60 kHz was considered to have the greatest intensity compared to that of *C. afra* in the same frequency range due to the steadiness in signal power.

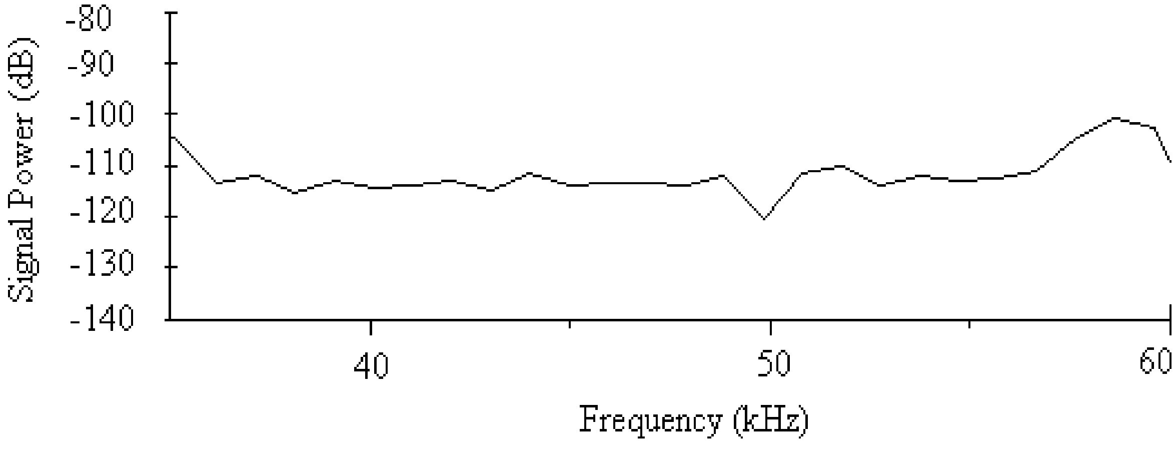

Fig. 20: The power spectrum for the sound of *A. tormotus* in the 35-60 kHz range

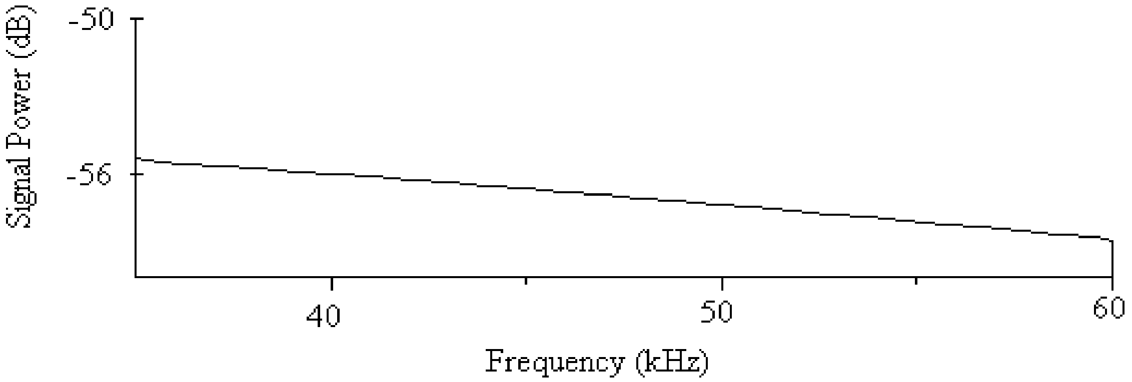

Fig. 21: The power spectrum for the sound of *C. afra* in the 35-60 kHz range

VI. Peak frequency

The sounds of *A. tormotus* and *C. afra* recorded equal maximum peak frequency (maximum entire) of 58.50 kHz. The mean peak frequency (maximum entire) for sounds of *A. tormotus* and *C. afra* was 47.626 kHz and 45.923 kHz respectively. The difference in the maximum peak frequency (maximum entire) in the predator sounds was 0.000 kHz whereas the difference in the mean peak frequency was 1.703 kHz.

The one-way ANOVA comparison of the peak frequency (maximum entire) for the sound of *A. tormotus* by the sound of *C. afra* yielded significance value, $p = 0.940 > 0.05$ whereas the comparison of by *C. afra* the sound *A. tormotus* resulted to $p = 0.845 > 0.05$, shown in Table 21.

Table 21: Significance values of the comparison of peak frequency of the predator sounds

Parameter	F	p
Comparison of peak frequency of the sound of *A. tormotus* by the peak frequency of the sound of *C. afra*	0.584	0.940
Comparison of peak frequency of the sound of *C. afra* by the peak frequency of the sound of *A. tormotus*	0.625	0.845

At a statistical significance level of 0.05, the deviation in peak frequency (maximum entire) in the two predator sounds was highly insignificant.

d) Acoustic transmission parameters of filtered sounds of *C. afra* and *A. tormotus* in the 61-90 kHz range

I. Fundamental frequency and harmonics

The spectrograms for both *C. afra* and *A. tormotus* in the frequency range of 61-90 kHz showed presence of formants at various times indicating existence of harmonics, an evidence of FM-CF calls as described in 4.1.1(Khanna and Bedi, 1988; Narins *et al.*, 2004).

II. Peak amplitude

The oscillograms for calls of *A. tormotus* and *C. afra* shown in Fig. 22 and Fig. 23 respectively represent amplitude variation over 11.00 s and 15.00 s duration respectively. Most pulses in *A. tormotus* and *C. afra* recorded amplitudes less than 25 % and 50 % respectively referenced to -20 dB in both oscillograms. The amplified mean peak amplitude (maximum entire) for *A. tormotus* and *C. afra* was 96.503 Pa (133.67 dB SPL) and 99.07 Pa (133.90 dB SPL) respectively referenced to 0 dB SPL (20µPa). Comparison of peak amplitude (maximum entire) of the sound of *A. tormotus* by *C. afra* yielded significance value, $p = 0.574 > 0.05$ and the comparison of the sound of *C. afra* by *A. tormotus* yielded significance value, $p = 0.869 > 0.05$ respectively as shown in Table 22.

Table 22: Significance values of the comparison of peak amplitude for the predator sounds

Parameter	F	p
Comparison of peak amplitude for the sound of *A. tormotus* by the peak amplitude for the sound of *C. afra*	0.989	0.574
Comparison of peak amplitude for the sound of *C. afra* by the peak amplitude for the sound of *A. tormotus*	0.923	0.869

These results show no evidence for significant difference in the maximum peak amplitude (maximum entire) in the sounds of the two predators. The amplified maximum peak amplitude (maximum entire) fell in the range of 133.785±0.115 dB SPL, differing insignificantly.

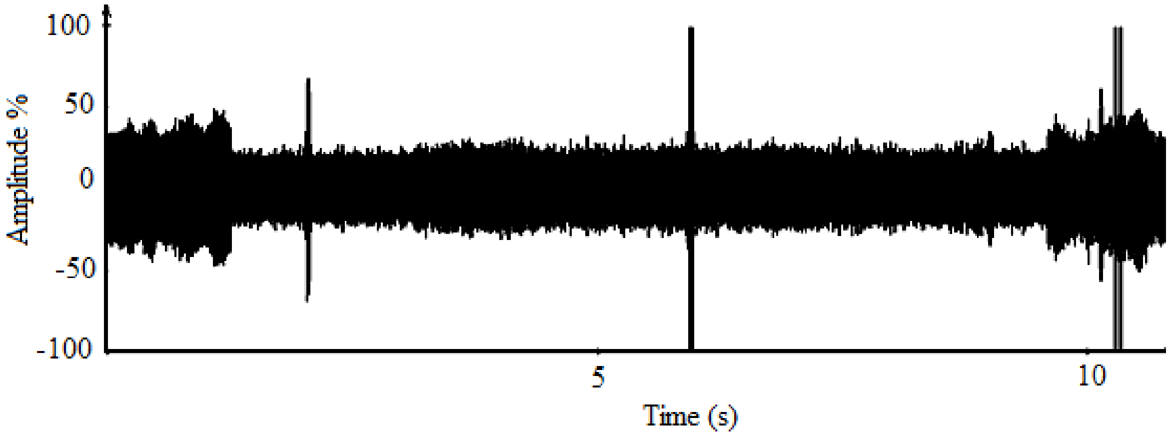

Fig. 22: A section of the oscillogram for *A. tormotus* for the range 61-90 kHz

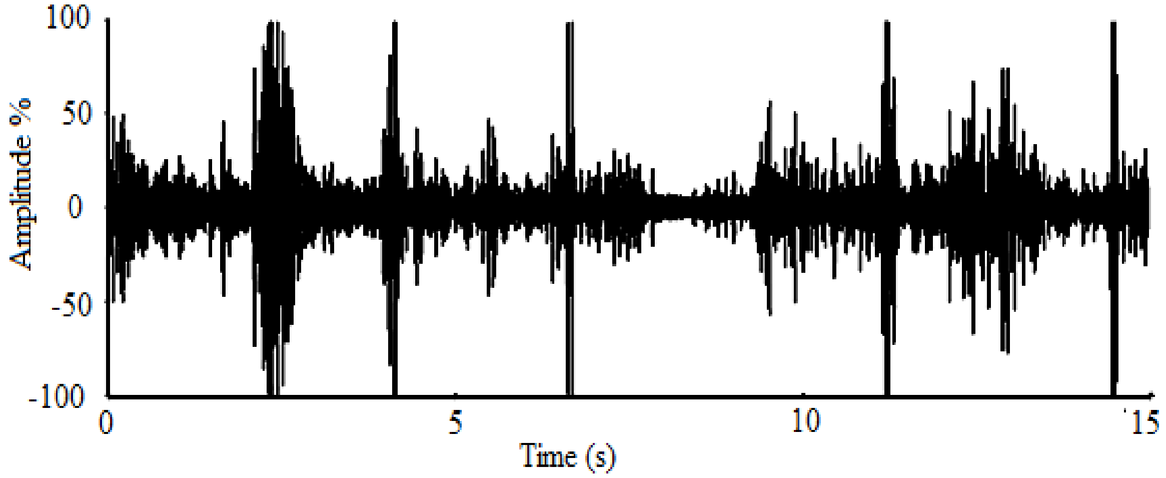

Fig. 23: A section of the sound oscillogram for *C. afra* for the range of 61-90 kHz

III. Minimum and maximum frequency

The difference in the mean of the maximum frequency (maximum entire) for the sound of *A. tormotus* from that of the sound of *C. afra* was 15.473 kHz. Similarly, the difference in the mean of the minimum frequency (minimum entire) in the predator sounds was 0.053 kHz. The mean of the maximum frequency (maximum entire) and mean of the minimum frequency (minimum entire) in this frequency range were above the reported optimal startle range for insects. However, sound in this range had been reported to substantially startle lizards and rats (Mohankumar, 2010).

The one-way ANOVA comparison of the minimum frequency (minimum entire) of the sound of *C. afra* by sound of *A. tormotus* resulted to p = 0.763 > 0.05. The one-way ANOVA comparison of the minimum frequency (minimum entire) of the sound of *A. tormotus* by sound of *C. afra* resulted to $p = 0.003 < 0.05$. The comparison of maximum frequency (maximum entire) for the sound of *A. tormotus* by *C. afra* resulted to $p = 0.064 > 0.05$ whereas the comparison of maximum frequency (maximum entire) for the sound of *C. afra* by *A. tormotus* resulted to $p = 0.909 > 0.05$. All the F and p values determined through ANOVA comparison are shown in Table 23.

Table 23: Significance values of the comparison of minimum and maximum frequency for the predator sounds

Parameter	F	p
Comparison of minimum frequency for the sound of *A. tormotus* by the minimum frequency for the sound of *C. Afra*	2.155	0.003
Comparison of minimum frequency for the sound of *C. afra* by the minimum frequency for the sound of *A. tormotus*	0.767	0.763
Comparison of maximum frequency for the sound of *A. tormotus* by *C. afra*	1.328	0.064
Comparison of maximum frequency for the sound of *C. afra* by the minimum frequency for the sound of *A. tormotus*	0.640	0.909

There was sufficient evidence to show that the minimum frequency (minimum entire) for *C. afra* sounds in the 61-90 kHz frequency range differed insignificantly from the minimum frequency (minimum entire) of the sound of *A. tormotus* at 5 % significance level. However, comparison of the minimum frequency (minimum entire) of the sound of *A. tormotus* by that of sound of *C. afra* yielded highly significant deviation at 5 % significance level. The sound of *A. tormotus* recorded the greatest mean maximum frequency (maximum entire) whereas the sound of *C. afra* recorded the greatest mean minimum frequency (minimum entire). Similarly, at 5 % level of significance, there was no evidence to indicate significant deviation in the maximum frequency (maximum entire) of the sound of *A. tormotus* from that of the sound of *C. afra*.

IV. Acoustic energy and power

The sound signal of *A. tormotus* in this range recorded minimum, maximum and mean energy of 0.00044 Pa^2s, 7.6989 Pa^2s and 0.44193 Pa^2s respectively. Similarly, the minimum, maximum and mean energy for *C. afra* was 0.0002 Pa^2s, 5.59491 Pa^2s and 0.05331 Pa^2s respectively. The difference in mean acoustic energy in the predator sounds was 0.38862

Pa^2s, which was significantly high. Similarly, the total maximum energy of the predator sounds differed by 2.4040 Pa^2s. The sound signal, being in the ultrasonic range it possessed a lot of energy for both predators (Khanna and Bedi, 1988; Hoy *et al*., 1989; Miller and Surlykke, 2001; Feng *et al*., 2002; Barlow, 2006; Feng *et al*., 2006; Ngo *et al*., 2006; Shen, 2007; Narins *et al*., 2004).

The comparison of the total energy of the sound of *A. tormotus* by *C. afra* yielded a significance value, $p = 0.001 < 0.05$ whereas the comparison of the total energy of the sound of *C. afra* by *A. tormotus* yielded a significance value, $p = 0.000 < 0.05$ as indicated in Table 24.

Table 24: Significance values of the comparison of acoustic energy for the predator sounds

Parameter	F	p
Comparison of acoustic energy for the sound of *A. tormotus* by the acoustic energy for the sound of *C. afra*	1.184	0.001
Comparison of Acoustic energy for the sound of *C. afra* by the acoustic energy for the sound of *A. tormotus*	1.302	0.000

In both cases, there was a remarkably high significant difference in acoustic energy between the two predator sounds.

The power spectrum for shown in Fig. 24 represents the sound signal of *C. afra* with a signal power between -35 dB and -80 dB. The signal power for this signal fluctuated about -60 dB in the frequency range of 61-90 kHz with prominent dip of -80 dB in power was observed at the 82.5 kHz frequency. The power spectrum for the sound of *A. tormotus* in Fig. 25 shows power variation from -130 dB and -100 dB. Most pulses within the frequency range had an average signal power of -118 dB with dips at 62.5 kHz, 72.5 kHz and 85.0 kHz. This power was less compared to that in the 35-60 kHz frequency range. The sound of *A. tormotus* recorded the least power compared to the sound of *C. afra*. The fluctuations in the signal of *A. tormotus*, as indicated in the power spectrum in Fig. 25 rendered it less intense compared to the slightly steady power signal of *C. afra*.

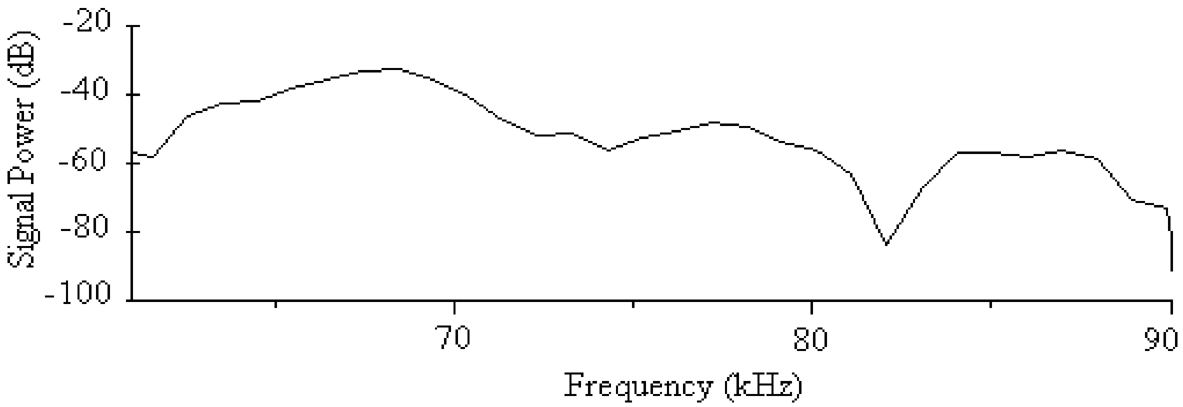

Fig. 24: The power spectrum for sound of *C. afra* in the 61-90 kHz range

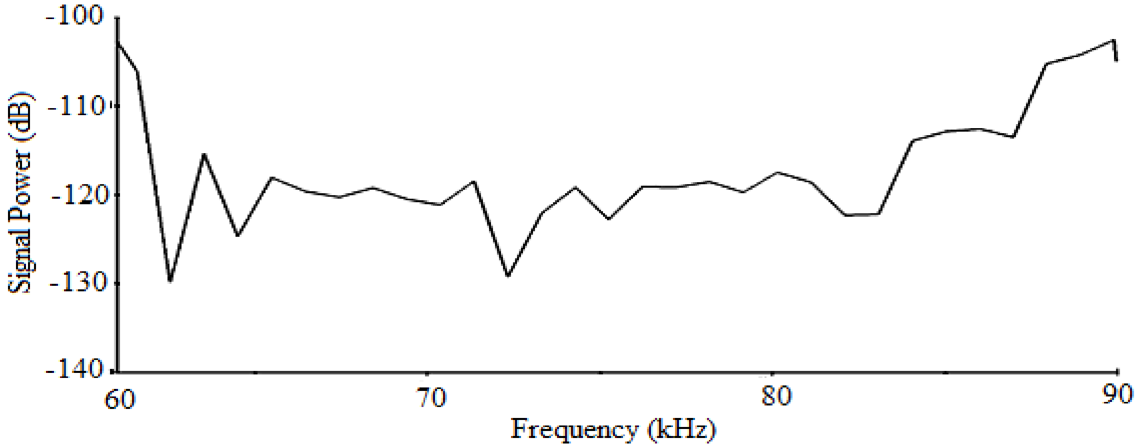

Fig. 25: The power spectrum for the sound of *A. tormotus* in the 61-90 kHz range

V. Bandwidth

There was a mean bandwidth (maximum entire) for the sound of *A. tormotus* and *C. afra* of 37.169 kHz and 21.584 kHz respectively, narrower than the reported EMR ranges (Ahmad *et al*., 2007; Enayati *et al*., 2010). The mean bandwidth (maximum entire) in sound of *A. tormotus* exceeded the mean bandwidth (maximum entire) for the sound from *C. afra*.

The significance values for the bandwidth (maximum entire) of the sound of *A. tormotus* and *C. afra* compared by 77.24 kHz as a test value on one-sample T test were both equal to $p = 0.000 < 0.05$ as shown in Table 25. Similarly, the significance values in the one-way ANOVA comparison of the bandwidth (maximum entire) for the sound of *A. tormotus* by *C. afra;* and the sound of *C. afra* by *A. tormotus* were both equal to $p = 0.000 < 0.05$ shown in Table 26.

Table 25: Significance values of the comparison of bandwidth of predator sounds by 77.24 kHz

Parameter	t	p
Comparison of bandwidth for the sound of *A. tormotus* by 77.24 kHz	-54.449	0.000
Comparison of bandwidth for the sound of *C. afra* by 77.24 kHz	-83.886	0.000

Table 26: Significance values of the comparison of bandwidth of predator sounds

Parameter	F	p
Comparison of bandwidth for the sound of *A. tormotus* by the bandwidth for the sound of *C. Afra*	2.267	0.000
Comparison of bandwidth for the sound of *C. afra* by the bandwidth for the sound of *A. tormotus*	0.660	0.917

The bandwidth (maximum entire) in sounds of *A. tormotus* and *C. afra* in this frequency range yielded a highly significant deviation from the reported mean bandwidth of the EMR sound. The bandwidths of the predators were very narrow than the reported mean bandwidth of EMR. Similarly, there was high significant difference in bandwidth (maximum entire) for the sound of *A. tormotus* compared by *C. afra*. The mean bandwidth (maximum entire) in sounds of *A. tormotus* was 15.585 kHz greater than that of the sound of *C. afra*. However, the mean bandwidth (maximum entire) in the sound of *C. afra* compared by sound of *A. tormotus* was insignificantly reduced by 15.585 kHz.

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VI. Peak frequency

The mean of the peak frequency (maximum entire) for the sound of *A. tormotus* and *C. afra* are 73.607 kHz and 72.433 kHz respectively. The one-way ANOVA comparison of the peak frequency (maximum entire) for the sound of *C. afra* by the sound of *A. tormotus* at a significance level of 5 % yielded significance value, $p = 0.909 > 0.05$ whereas the comparison of *A. tormotus* by the sound *C. afra* resulted to $p = 0.064 > 0.05$, shown in Table 27.

Table 27: Significance values of the comparison of peak frequency for predator sounds

Parameter	F	p
Comparison of peak frequency for the sound of *A. tormotus* by the peak frequency for the sound of *C. afra*	1.328	0.064
Comparison of peak frequency for the sound of *C. afra* by the peak frequency for the sound of *A. tormotus*	0.640	0.909

At 95 % confidence, there was no evidence to show that the peak frequency (maximum entire) in the sound of *C. afra* differed significantly with that of *A. tormotus*. The mean peak frequency for the predators' sound was 73.02±0.59 kHz.

Other acoustic transmission parameters for the sounds of *A. tormotus* and *C. afra* were determined automatically for various frequency ranges using the Avisoft SASLab software and attached in Appendix A as Table A1 and Table A2 respectively. The greatest standard deviation of frequency for the sound of *A. tormotus*, shown in Appendix C as C1, was 30.47 kHz in the 61-90 kHz range whereas the minimum standard deviation of the frequency was 1.40 in the 10-34 kHz range. The standard deviation in the minimum frequency and maximum frequency for *C. afra* in the 10-34 kHz range, shown in Appendix C as Table C2, was 5.28 kHz and 7.65 kHz respectively.

4.2 Determination of Acoustic Transmission Parameters of the Combined Sound of *A. tormotus* and *C. afra*

4.2.1 Determination of the transmission parameters of unfiltered combined sounds of *C. afra* and *A. tormotus*

a) Modulation of sounds of *C. afra* and *A. tormotus*

The spectrogram for the combined sounds of *A. tormotus* and *C. afra* shown in Fig. 26 revealed existence of frequency modulation and constant frequency modulation. The ultrasound in the combined sound was composed of ultrasonic components of individual predator sound whose use was discussed in 4.1.1 (a). The calls from both predators maintained a constant frequency that was almost linear as reported in recent findings (Narins *et al*., 2004). A varying degree of frequency modulation with abrupt onset and disappearance of harmonic components was noted, as reported in earlier findings (Feng *et al*., 2002).

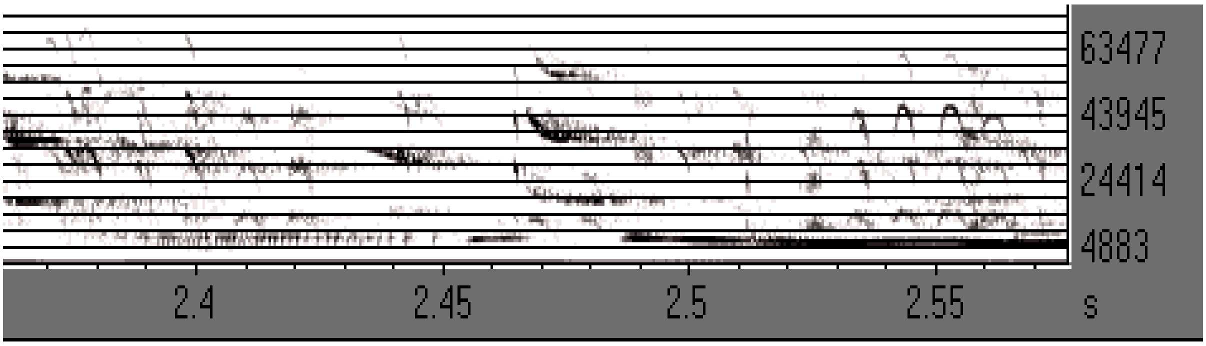

Fig. 26: Harmonics in the original combined sound signal of *A. tormotus* and *C. afra*

b) Acoustic transmission parameters for the unfiltered combined sound of *C. afra* and *A. tormotus* (Comparative Analysis)

I. Fundamental frequency and harmonics

The fundamental frequency in the combined sound was 4.883 kHz, manually obtained from the spectrogram in Fig. 26, which was less than the fundamental frequency of individual predator sound. This was due to interference of sound waves. Harmonics extended in the ultrasonic range up to 63.477 kHz, with some formants exceeding this value. The ultrasonic range was in agreement with recent studies (Khanna and Bedi, 1988). Ultrasonic and audible sounds had been reported to exist in sounds of *A. tormotus* and *C. afra*, hence their presence in the combined sound (Khanna and Bedi, 1988; Hoy *et al*., 1989; Miller and Surlykke, 2001; Feng *et al*., 2002; Barlow, 2006; 2006; Feng *et al*., 2006; Ngo *et al*., 2006; Shen, 2007). Combining the sounds of *A. tormotus* and *C. afra* lowered the fundamental frequency of the individual predator sounds.

II. Minimum and maximum frequency

The minimum frequency (minimum entire), which is also the fundamental frequency, was obtained manually as 4.883 kHz, which was slightly above the automatically generated mean minimum frequency (minimum entire) value by 0.869 kHz. The difference was due to background noise recorded from running water in the streams and blowing wind through the caves for the sounds of *A. tormotus* and *C. afra* respectively. Running water in the streams and blowing wind through the caves for the sounds of *A. tormotus* and bats respectively were reported to clutter in animal sounds affecting its quality (Penna and Rogoberto, 1998; Barlow, 2006; Feng *et al*., 2006; Steve, 2006). The background noise was attenuated as described in 3.2.2. The acoustic intensity of the predator sound was determined by the sound frequencies (Ramabhadran, 1988; Gupta, 1989; Ballou, 2002). The mean of the maximum frequency for the combined sound was equal to 60.929 kHz. The reported EMR sound frequency ranges were 125 Hz to 74.6 kHz and 20–100 kHz (Khanna and Bedi, 1988; Hoy *et al.*, 1989; Miller and Surlykke, 2001; Ahmad *et al.*, 2007., Enayati *et al.*, 2010).

The one-way ANOVA comparison of the minimum frequency (minimum entire) of combined sound by *A. tormotus* resulted to significant value, p = 0.088 > 0.05. On the other hand, the comparison of the minimum frequency (minimum entire) in the sound of *C. afra* by combined sound resulted to p = 0.868 > 0.05. Similar comparison of the maximum frequency (maximum entire) of combined sound by that of *A. tormotus* resulted to p = 0.932 > 0.05 whereas the comparison of the maximum frequency (maximum entire) of the combined sound by that of *C. afra* resulted to significant value, p = 0.998 > 0.05. These values are shown in Table 28.

Table 28: Significance values of the comparison of the minimum and maximum frequency of the unfiltered combined sound by individual predator sounds

Parameter	F	p
Comparison of minimum frequency for combined sound by the minimum frequency of the sound of *A. tormotus*	2.189	0.088
Comparison of minimum frequency for combined sound by the minimum frequency of the sound of *C. afra*	0.710	0.868
Comparison of maximum frequency for combined sound by the maximum frequency of the sound of *A. tormotus*	0.470	0.932
Comparison of maximum frequency for combined sound by of the sound of *C. afra*	0.522	0.998

At a statistical significance level of 0.05, there was no significant difference in the minimum frequency (minimum entire) by combining the individual sounds of *C. afra* and *A. tormotus*. Similarly, at 5 % level of significance, there was no evidence to show that the maximum frequency (maximum entire) in the sound of *A. tormotus* and *C. afra* differed significantly with that of the combined sound.

III. Bandwidth

Combining the sound of *A. tormotus* and *C. afra* was expected to yield a remarkable reduction in bandwidth leading to intensified sound. The mean bandwidth (maximum entire) for the combined sound was 54.88 kHz. The bandwidth (maximum entire) of the combined sound was narrowed by 22.34 kHz from the reported mean bandwidth of the EMR sounds reported by Ahmad *et al* (2007) and Enayati *et al* (2010).

The significance value in one-sample T test for the bandwidth (maximum entire) of the combined sound by 77.24 kHz wasp = 0.000 < 0.05 as shown in Table 29. Similarly, the one-way ANOVA comparison of the bandwidth (maximum entire) of the combined sound by the sound of *A. tormotus* and *C. afra* resulted to significance values, p = 0.652 > 0.05 and p = 0.540 > 0.05 respectively which are given in Table 30.

Table 29: Significance values of the comparison of the bandwidth of the unfiltered combined sound by 77.24 kHz

Parameter	t	p
Comparison of bandwidth of combined sound by 77.24 kHz	-70.469	0.000

Table 30: Significance values of the comparison of the bandwidth of the unfiltered combined sound by individual predator sounds

Parameter	F	p
Comparison of bandwidth for the combined sound by the bandwidth of the sound of *A. tormotus*	0.824	0.652
Comparison of bandwidth for the combined sound by the bandwidth of the sound of *C. afra*	0.973	0.540

Combining the sounds of *A. tormotus* and *C. afra* yielded highly significant deviation in bandwidth (maximum entire) from the reported EMR mean bandwidth of 77.24 kHz. The bandwidth was highly narrowed from the reported mean bandwidth of EMR. However, there was no significant difference in bandwidth (maximum entire) of the combined sound from the bandwidth (maximum entire) of individual predator sounds.

IV. Peak amplitude

Amplitude in sound determines the acoustic energy as discussed in 4.1.1. The maximum peak amplitude (maximum entire) for the combined sound of *A. tormotus* and *C. afra* was 113.18 Pa (135.05 dB SPL) with a mean peak amplitude (maximum entire) value of 97.6414

Pa (133.7721 dB SPL) referenced to 20 µPa (0 dB SPL), higher than the maximum peak amplitude (maximum entire) of individual predator sounds. The maximum peak amplitude (maximum entire) for the combined sound was 0.14 dB SPL less than the maximum peak amplitude (maximum entire) for the sound of *A. tormotus*. However, the mean peak amplitude (maximum entire) for the combined sound was 0.4243 dB SPL greater than the mean peak amplitude (maximum entire) for the sound of *A. tormotus*. The maximum peak amplitude and the mean peak amplitude for the combined sound differed by 0.08 dB SPL and 0.0246 dB SPL respectively from those of the sound of *A. tormotus*. The one-way ANOVA comparison of the peak amplitude (maximum entire) for the combined sound by the sound of *A. tormotus* yielded a significance value, $p = 0.482 > 0.05$ whereas the comparison of combined sound by the sounds of *C. afra* resulted to $p = 0.038 < 0.05$ and shown in Table 31.

Table 31: Significance values of the comparison of the peak amplitude of the unfiltered combined sound by individual predator sounds

Parameter	F	p
Comparison of peak amplitude of the combined sound by the peak amplitude of the sound of *A. tormotus*	1.003	0.482
Comparison of peak amplitude of the combined sound by the peak amplitude of the sound of *C. afra*	1.078	0.038

There was no evidence to show any significant difference in peak amplitude (maximum entire) in the combined sound from the peak amplitude (maximum entire) of the sounds of *A. tormotus.* However, there existed a significant deviation in the peak amplitude (maximum entire) of the combined sound from that in the sound of *C. afra.*

V. Acoustic energy

Combining the sounds of *A. tormotus* and *C. afra* yielded a maximum and mean acoustic energy of 31.361 Pa^2s and 0.351 Pa^2s respectively. The combined sound recorded a greater mean of acoustic energy by 2.985 Pa^2s and 0.234 Pa^2s from the sound of *A. tormotus* and *C. afra* respectively. The Combined sound also yielded greater maximum acoustic energy from that of the sound of *A. tormotus* by 11.792 Pa^2s. However, the maximum acoustic energy of the combined sound was 4.440 Pa^2s less than the acoustic energy of the sound of *C. afra*. Acoustic energy determined the level of diaphragm vibration in speakers, which later caused a disturbance in neighbouring air particles. The disturbance was transmitted through the air to the mosquito antennae initiating resonance (Zwibel and Pitts, 2006; Maweu *et al*., 2009). The one way comparison of the combined sound energy by the energy of the sound of *A. tormotus* yielded a significance value, p = 1.000 > 0.05 whereas the comparison of the energy from the combined sound and the energy from the sound of *C. afra* a significant value, p = 0.022 < 0.05 and shown in Table 32.

Table 32: Significance values of the comparison of the acoustic energy of the unfiltered combined sound by the energy of individual predator sounds

Parameter	F	p
Comparison of acoustic energy of the combined sound by energy of the sound of *A.tormotus*	0.392	1.000
Comparison of acoustic energy of the combined sound by energy of the sound of *C. afra*	1.086	0.022

At 5 % level of significance, there was no significant difference between the energy of the combined from the acoustic energy of the sound of *A. tormotus* except in the energy of the sound of *C. afra*. But, there was sufficient evidence to show significant deviation in acoustic energy of the combined sound from that of the sound of *C. afra*.

VI. Peak frequency

The mean peak frequency (maximum entire) of the unfiltered combined sound of *A. tormotus* and *C. afra* was 29.95 kHz, reduced by 11.24 kHz from the mean peak frequency (maximum entire) of the sound of *A. tormotus*. However, it increased by 17.06 kHz from the mean peak frequency (maximum entire) of the sound of *C. afra*.

The one-way ANOVA comparison of the peak frequency (maximum entire) of combined sound by that of the sound of *A. tormotus* yielded the significance value, p = 0.852 > 0.05. Also, the comparison of the peak frequency (maximum entire) of the combined sound by that of sound of *C. afra* resulted to p = 0.498 > 0.05. The significance values of the comparison of the peak frequency of the combined sound by the individual predator sounds are indicated in Table 33.

Table 33: Significance values of the comparison of the peak frequency of the unfiltered combined sound by the individual predator sounds

Parameter	F	p
Comparison of peak frequency for the combined sound by the peak frequency for the sound of *A. tormotus*	0.506	0.852
Comparison of peak frequency for the combined sound by peak frequency for the sound of *C. Afra*	0.989	0.498

It was established that the peak frequency (maximum entire) of the combined sound did not differ significantly from that of the individual predator sounds at 5 % significance level.

4.2.2 Determination of the transmission parameters of filtered combined sounds of *C. afra* and *A. tormotus* (Comparative Analysis)

a) Generation and modulation of sounds of *C. afra* and *A. tormotus*

The sounds of *C. afra* and *A. tormotus,* which constituted the combined sound, were generated as described in 4.1.1 (a). The prominent hook and doom like formants, shown in Fig. 27 and Fig. 28 revealed the existence of CF-FM calls in the combined sound, whose nature and role was discussed in 4.1.1. These components were characterized by occasional upward and downward sweeps of frequency variations, as reported in recent findings as reported by Gerlach (2007).

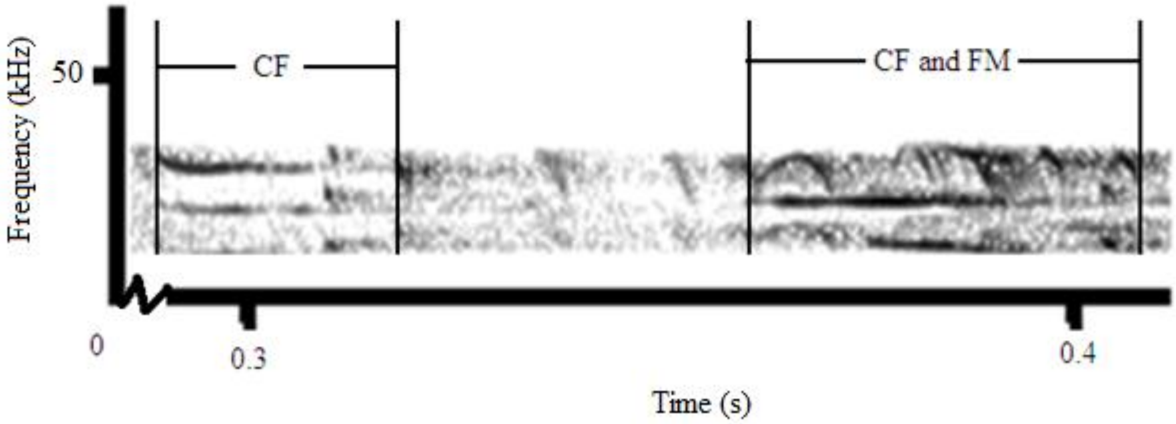

Fig. 27: The spectrogram for the appended combined sound of *C. afra* and *A. tormotus*

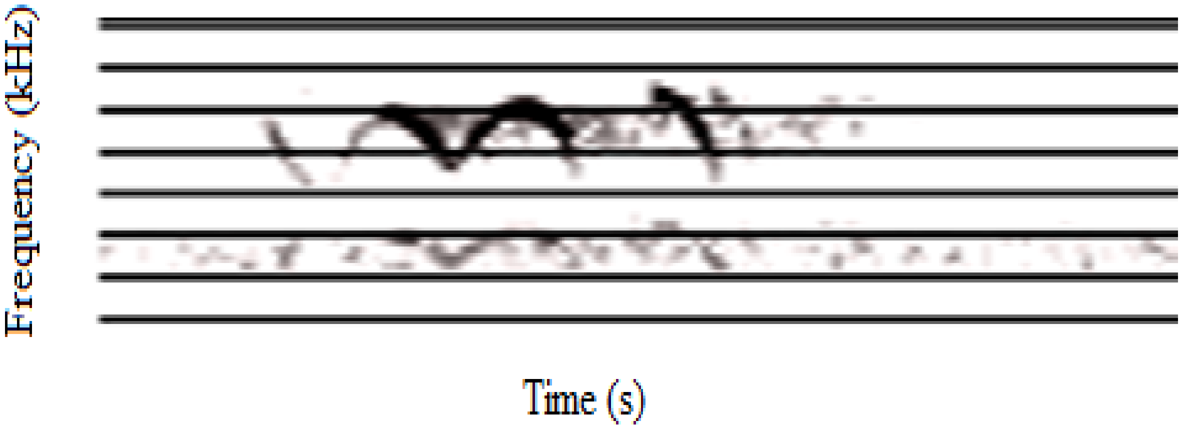

Fig. 28: The 10-34 kHz spectrogram for the combined sound of *C. afra* and *A. tormotus*

Combining the sounds of *C. afra* and *A. tormotus* did not alter the modulation components and harmonic composition though cluttering of background sound was intense. The background chaotic noise shown in Fig. 26 but not present in Fig. 27 and Fig. 28 and stretching up to about 10 kHz was attenuated by a high pass filter, in cooperated in the analysis software (Penna and Rogoberto, 1998; Barlow, 2006; Feng *et al*., 2006; Steve, 2006). The cluttered background was due to presence of both sonar and social calls captured during the recording of the sound of *C. afra* besides interference of sounds during sound mixing *(*Narins *et al*., 2004).

b) Acoustic transmission parameters for the filtered combined sound of *C. afra* and *A. tormotus* in the 10-34 kHz range

I. Fundamental frequency and harmonics

The spectrograms in Fig. 27 and Fig. 28 were composed of formants for the combined sound signals of *C. afra* and *A. tormotus* signifying presence of harmonics, similar to that of individual predator calls as discussed in 4.1.1 (b). The call pips were nonlinear with the onset and offset of harmonics as acoustic features as reported in recent studies (Kingston *et al*., 2003). However, the fundamental frequency of the combined sound of *C. afra* and *A. tormotus*, determined from the spectrogram in Fig. 26 was 4.883 kHz, falling in the audible range. It was noted that the fundamental frequency of the combined sound was less than that of the component predator sounds due to interference. The fundamental frequency of the combined was 0.488 kHz and 1.953 kHz less than the fundamental frequencies of the sound of *C. afra* and *A. tormotus* respectively. However, all the fundamental frequencies were attenuated from the sound samples under investigation.

II. Peak amplitude

The oscillogram in Fig. 29 shows sound intensity variation over time. The maximum and mean peak amplitude (maximum entire) for the combined sound in this frequency range was111.01 Pa (134.89 dB SPL) and 95.72 Pa (133.60 dB SPL) respectively, all referenced to 20 µPa (0 dB SPL). The maximum peak amplitude (maximum entire) for the combined sound differed from that of the sound of *A. tormotus* and *C. afra* by 0.13 dB SPL and 0.16 dB SPL respectively. Similarly, the mean peak amplitude (maximum entire) for the combined sound deviated from that of the sound of *A. tormotus* and *C. afra* by 0.66 dB SPL and 0.36 dB SPL. The maximum and mean peak amplitude (maximum entire) for the combined sound was reduced considerably compared to the individual predator sound amplitudes.

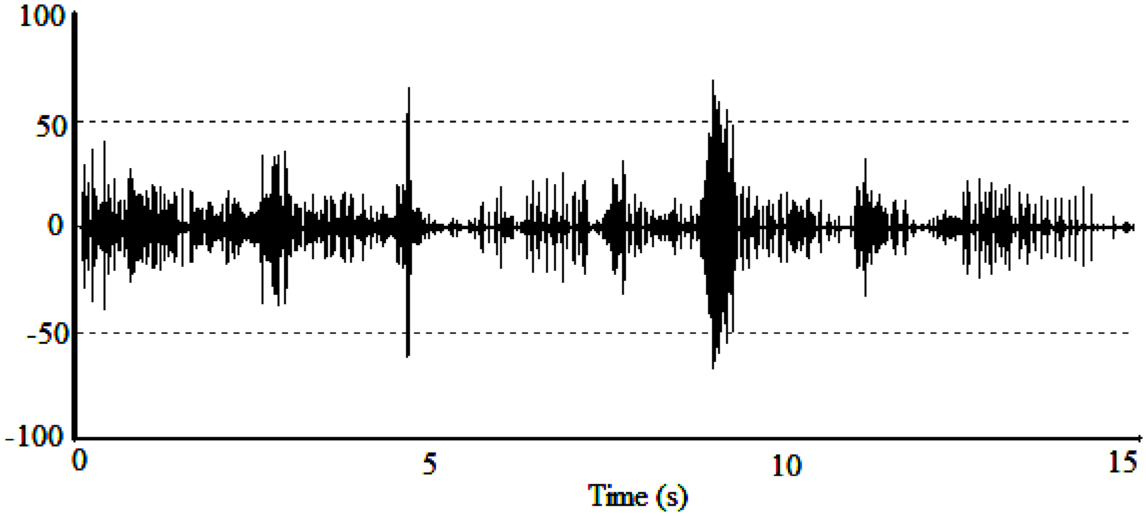

Fig. 29: The oscillogram in the 10-34 kHz range for the combined sound of *C. afra* and *A. tormotus*

The one-way ANOVA comparison of the peak amplitude (maximum entire) for the combined sound by the peak amplitude (maximum entire) for the sound of *C. afra* and *A. tormotus* separately, shown in Table 34, yielded significance values, $p = 0.903 > 0.05$ and $p = 0.914 > 0.05$ respectively.

Table 34: Significance values of the comparison of the peak amplitude of 10-34 kHz combined sound by individual predator sounds

Parameter	F	p
Comparison of peak amplitude of the combined sound by the peak amplitude of the sound of *A. tormotus*	0.830	0.903
Comparison of peak amplitude of the combined sound by the peak amplitude of the sound of *C. afra*	0.942	0.914

At 5 % significance level, there was no evidence to indicate significant deviation in peak amplitude (maximum entire) of the combined sound from the sound of *A. tormotus* and *C. afra*.

III. Acoustic energy and power

The minimum, maximum and mean acoustic energy for the combined sound in this frequency range was 0.00008 Pa^2s, 6.08173 Pa^2s and 0.09480 Pa^2s respectively. The acoustic energy in the combined sound was slightly reduced from the individual predator sounds. The one way ANOVA comparison of acoustic energy of the combined sound by the sound of *A. tormotus* yielded a significance value, $p = 0.982 > 0.05$ yet the comparison of acoustic energy from the combined sound by the sound of *C. afra* resulted to a significance value, $p = 0.635 > 0.05$, shown in Table 35.

Table 35: Significance values of the acoustic energy of the 10-34 kHz combined sound compared by the individual predator sounds

Parameter	F	p
Comparison of acoustic energy for the combined sound by the sound of *A. tormotus*	0.493	0.982
Comparison of acoustic energy for the combined sound by the sound of *C. afra*	0.986	0.635

At a statistical significance level of 0.05, it was highly evident that there was no significant difference in the acoustic energy for the combined sound from predator sounds.

The power spectrum, shown in Fig. 30, yielded a signal power which fluctuated between -98 dB and -136.67 dB respectively between 10.0 kHz to 27.5 kHz. The signal power weakened with increase in frequency which increased to -110 dB at about 29 kHz.

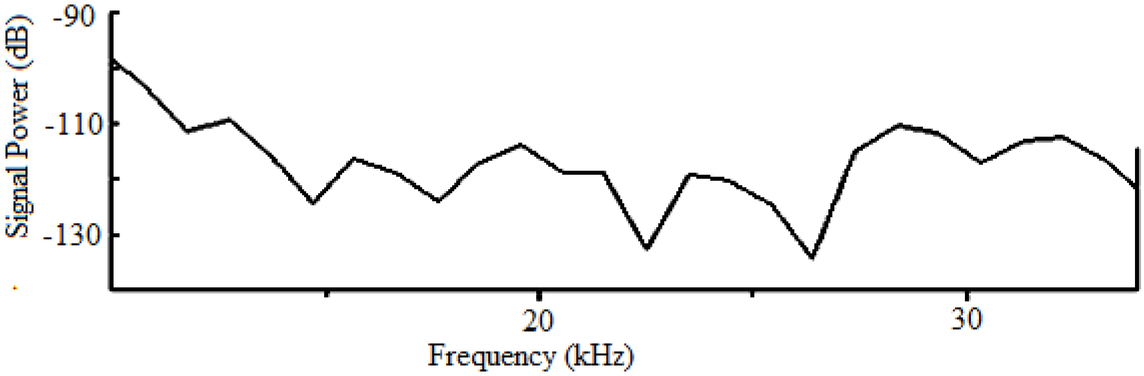

Fig. 30: The power spectrum for the combined sound signal in the range of 10-34 kHz

The signal power of the combined sound was slightly greater than that of the sound of *A. tormotus* but less than that of the sound of *C. afra*. Combining the two predator sounds did not yield a signal of great acoustic energy and power. The combined sound was rendered less intense in the 10-34 kHz frequency range compared to energy of individual predator sounds.

IV. Bandwidth

The minimum, maximum and mean bandwidth (maximum entire) for the combined sound was 2.90 kHz, 27.30 kHz and 16.42 kHz respectively. These bandwidth values were less than the reported bandwidth in EMR sounds (Ahmad *et al.*, 2007; Enayati *et al.*, 2010). The maximum bandwidth of the combined sound was less than the bandwidth of the individual predator sounds. However, the mean bandwidth (maximum entire) of the combined sound was greater than that of the individual predator sound.

The comparison of the bandwidth (maximum entire) of the combined sound by 77.24 kHz on one-sample T test was yielded significance value, $p = 0.000 < 0.05$ shown in Table 36. Similarly, the comparison of the bandwidth (maximum entire) for the combined sound by the bandwidth (maximum entire) of the sound of *A. tormotus* and *C. afra*, shown in Table 37, using the one-way ANOVA yielded significance values; $p = 0.428 > 0.05$ and $p = 0.350 > 0.05$ respectively.

Table 36: Significance values of the bandwidth for the 10-34 kHz combined sound by 77.24 kHz

Parameter	t	p
Comparison of bandwidth (maximum entire) for combined sound by 77.24 kHz	-673.534	0.000

Table 37: Significance values of the bandwidth for the 10-34 kHz combined sound by the individual predator sounds

Parameter	F	p
Comparison of bandwidth for combined sound by the bandwidth of the sound of *A. tormotus*	1.021	0.428
Comparison of bandwidth for combined sound by the bandwidth of the sound of *C. afra*	1.074	0.350

At 5 % significance level, there was sufficient evidence to show significant deviation in the bandwidth (maximum entire) of the combined predator sound from the reported bandwidth of the EMR sound. The bandwidth (maximum entire) of the combined predator sound was narrowed by 49.94 kHz from the reported EMR bandwidth. However, the bandwidth (maximum entire) in the combined sound did not deviate significantly from that of the sound of *A. tormotus* and *C. afra*. Combining the two predator sounds did not yield significant narrowing in bandwidth (maximum entire) from the sounds of *A. tormotus* and *C. afra*.

V. Peak frequency

The mean peak frequency (maximum entire) of the combined sound was 29.40 kHz. Combining the sounds of *A. tormotus* and *C. afra* yielded a peak frequency which was 5.89 kHz above the average of the peak frequencies of the two predator sounds. However, the peak frequency for the combined sound was less than the peak frequency of the sound of *C. afra* but less than that of the sound of *A. tormotus*. The one-way ANOVA comparison of the combined sound of the sound of *A. tormotus* and *C. afra* separately, shown in Table 38, yielded significance values, $p = 0.967 > 0.05$ and $p = 0.426 > 0.05$ respectively.

Table 38: Significance values of the peak frequency of the10-34 kHz combined sound compared by the individual predator sounds

Parameter	F	p
Comparison of peak frequency of the combined sound by the peak frequency of the sound of *A. tormotus*	0.189	0.967
Comparison of peak frequency of the combined sound by the peak frequency of the sound of *C. afra*	1.027	0.426

It was established that at a significance level of 0.05, the peak frequency (maximum entire) for the combined sound differed insignificantly with that of the sound of *C. afra* and *A. tormotus*.

VI. Minimum and maximum frequency

The minimum frequency (minimum entire) for the sound of *C. afra* and *A. tormotus* was 6.836 kHz and 5.371 kHz respectively. These frequency components were attenuated from the 10-34 kHz frequency range. However, combining the two sounds yielded a lowered fundamental frequency of 4.883 kHz. The mean minimum frequency (minimum entire) for the combined sound was 15.27 kHz, which was greater than that of the sound of *C. afra* and *A. tormotus*. The mean maximum frequency (maximum entire) for the combined sound was 22.57 kHz which was equal to the mean maximum frequency (maximum entire) for *A. tormotus* but less than that of *C. afra*. The acoustic intensity of the predator sound was determined by the sound frequencies (Ramabhadran, 1988; Gupta, 1989; Ballou, 2002).

The one-way ANOVA comparison of the minimum frequency (minimum entire) of combined sound by that of the sound of *A. tormotus* and *C. afra* resulted to significant values, $p = 0.958 > 0.05$ and $p = 0.122 > 0.05$. The comparison of the maximum frequency (maximum entire) of combined sound by that of the sound of *A. tormotus* resulted to $p = 0.668 > 0.05$. Similar comparison of the maximum frequency (maximum entire) in the combined sound by that of the sound of *C. afra* resulted to significance value, $p = 0.149 > 0.05$. The p and F values determined from the comparison of the minimum frequency (minimum entire) and maximum frequency (maximum entire) of the 10-34 kHz combined sound by individual predator sounds are shown in Table 39.

Table 39: Significance values of the minimum and maximum frequency of the10-34 kHz combined sound compared by individual predator sounds

Parameter	F	p
Comparison of minimum frequency of the combined sound by the minimum frequency of the sound of *A. tormotus*	0.210	0.958
Comparison of minimum frequency of the combined sound by the minimum frequency of the sound of *C. afra*	1.331	0.122
Comparison of maximum frequency of the combined sound by the maximum frequency of the sound of *A. tormotus*	0.745	0.668
Comparison of maximum frequency for combined sound by the maximum frequency of the sound of *C. afra*	1.278	0.149

It was noted that there was no significant difference in the minimum frequency (minimum entire) in the combined sound from that of the individual sound of *C. afra* and *A. tormotus*. Similarly, at significance level of 5 %, there was no evidence to show that the maximum frequency (maximum entire) in the combined sound differed significantly with that of the individual predator sounds.

c) The acoustic transmission parameters for the combined sound of *C. afra* and *A. tormotus* in the 35-60 kHz range

I. Fundamental frequency and harmonics

The fundamental frequency of the combined sound of *C. afra* and *A. tormotus* was 4.883 kHz. The formants between 33.20 s and 33.22 s in the spectrogram shown in Fig. 31 was an evidence for the existence of harmonics which were characterized by both frequency modulation and constant frequency, as described in 4.1.1 (Gerlach, 2007). The harmonics reflected in the formants from time t_1= 33.19 s and t_2 = 33.22 s stretched from 34.18 kHz to 59.814 kHz, which were in the ultrasonic levels (Narins *et al*., 2004; Gerlach, 2007). There was either one or more tone calls as seen in the formants in the individual predator sounds.

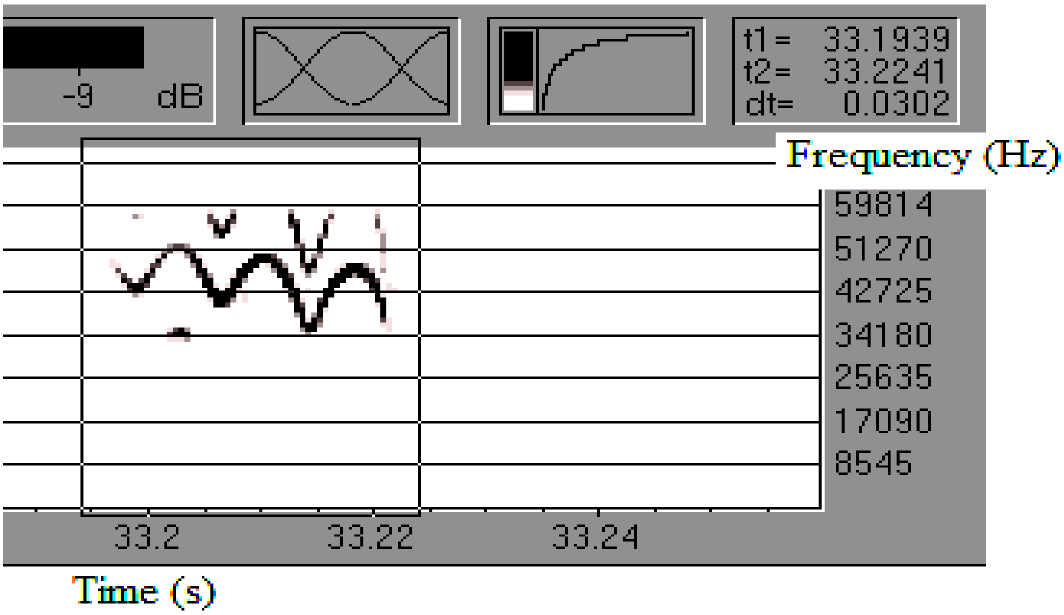

Fig. 31: Harmonic components in the 35-60 kHz range for the combined sound signal

II. Peak amplitude

The maximum and mean peak amplitude (maximum entire) in the combined sound was 112.93 Pa (135.04 dB SPL) and 98.20 Pa (133.82 dB SPL) respectively. The maximum and mean peak amplitudes (maximum entire) in the combined sound and that of *C. afra* were equal. However, the combined sound recorded reduced peak amplitude (maximum entire) by 0.04 dB SPL from the sound of *A. tormotus*. Similarly, the mean peak amplitude (maximum entire) of the combined sound was reduced by 0.46 dB SPL from the mean peak amplitude (maximum entire) of the sound of *A. tormotus*. With an amplitude threshold of -20 dB, the combined predator sound maintained peak frequencies below 50 % of the maximum peak amplitude (maximum entire) as shown in Fig. 32.

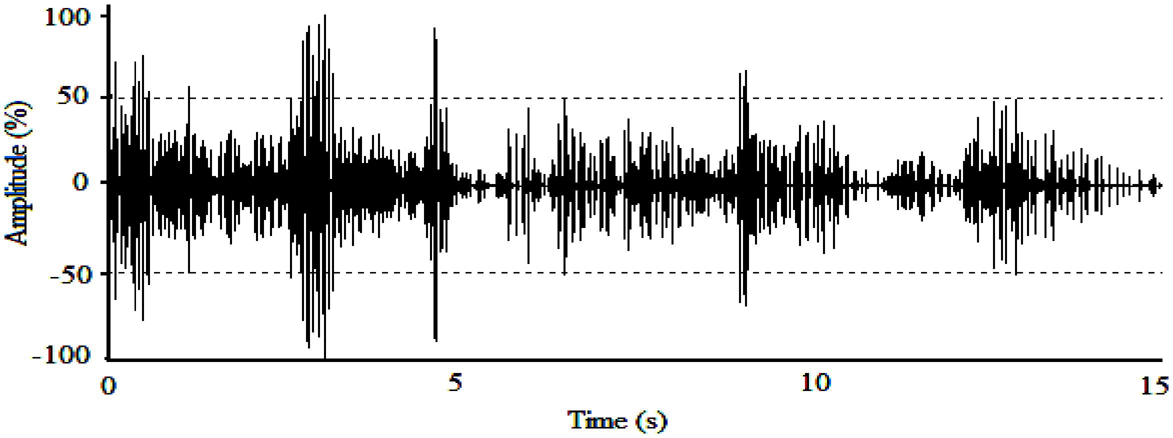

Fig. 32: The oscillogram for the combined sound signal in the range 35-60 kHz range

The one-way ANOVA comparison of the peak amplitude (maximum entire) of the combined sound in this frequency range by the peak amplitude (maximum entire) for the sound of *A. tormotus* produced a significance value $p = 0.634 > 0.05$. Further comparison of the peak amplitude (maximum entire) of the combined sound by the peak amplitude (maximum entire) for the sound of *C. afra* produced a significance value, $p = 0.268 > 0.05$, given in Table 40.

Table 40: Significance values of the peak amplitude of the 35-60 kHz combined sound compared by individual predator sounds

Parameter	F	p
Comparison of peak amplitude of the combined sound by the peak amplitude of the sound of *A. tormotus*	0.957	0.634
Comparison of peak amplitude of the combined sound by the peak amplitude of the sound of *C. afra*	0.935	0.933

These results showed no evidence of significant difference in peak amplitude (maximum entire) for the combined sound from the sound of *A. tormotus* and *C. afra* at 5 % significance level.

III. Acoustic energy and power

The maximum, minimum and mean acoustic energy for the combined sound in this frequency range was 10.3425 Pa^2s, 0.0002 Pa^2s and 0.0521 Pa^2s respectively. The maximum and minimum acoustic energy of the combined sound was greater than the acoustic energy in the 10-34 kHz frequency range. The energy of the combined sound increased from 6.08173 Pa^2s to 10.3425 Pa^2s, hence enhancement. The acoustic energy for the combined sound was slightly less than that of the sound of *A. tormotus* and *C. afra* by 0.5 Pa^2s and 1.98 Pa^2s respectively. Combining the sounds of *A. tormotus* and *C. afra* resulted in a slight decline in acoustic energy.

The one way ANOVA comparison of the energy of the combined sound by that of the sound of *A. tormotus*, shown in Table 41 yielded a significance value, $p = 0.316 > 0.05$. Similarly, the one way comparison of acoustic energy of the combined sound by that of the sound of *C. afra*, also shown in Table 41, yielded a significance value, $p = 0.993 > 0.05$.

Table 41: Significance values of the acoustic energy of the 35-60 kHz combined sound compared by the individual predator sounds

Parameter	F	p
Comparison of acoustic energy of the combined sound by the acoustic energy of the sound of *A. tormotus*	1.075	0.316
Comparison of acoustic energy for the combined sound by the acoustic energy of the sound of *C. afra*	0.905	0.993

At a statistical significance level of 0.05, there was no evidence showing significant deviation in the acoustic energy for the combined sound from individual predator sounds.

The power spectrum for the combined sound, shown in Fig. 33, indicated unsteady power which fluctuated with great margins. The acoustic power declined from 35 kHz, recording a decline of -40 dB at 40 kHz. The acoustic power fluctuated between -100 dB and -155 dB sustaining a rise in power trend from 54 kHz to 58 kHz beyond which the power declined.

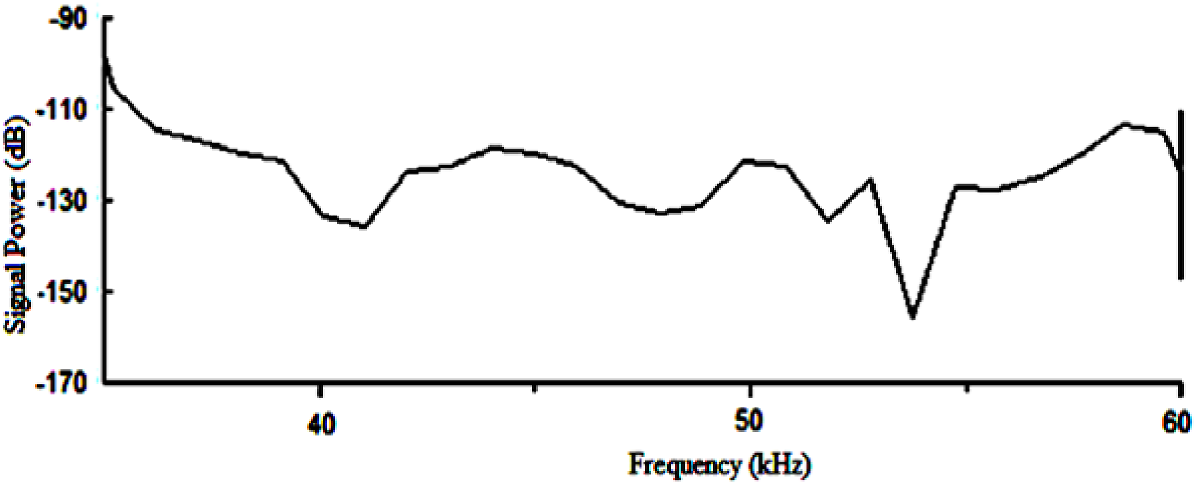

Fig. 33: The power spectrum for the 35-60 kHz frequency range for combined sound

IV. Minimum and maximum frequency

The mean of the minimum frequency (minimum entire) for the combined sound in this frequency range was 34.754 kHz. Combining the two sounds yielded a slightly higher mean of the minimum frequency (minimum entire) by 0.4960 kHz and 0.0950 kHz from that of the sounds of *C. afra* and *A. tormotus* respectively. The mean of the maximum frequency (maximum entire) for the combined sound in this range was 55.418 kHz which was higher than the mean of the maximum frequency (maximum entire) for *A. tormotus* by 0.283 kHz but less than that of *C. afra* by 0.059 kHz.

The one-way ANOVA comparison of the minimum frequency (minimum entire) of combined sound by that of the sound of *A. tormotus* yielded a significance value, $p = 0.951 > 0.05$ and a similar comparison of the minimum frequency (minimum entire) in the sound of *C. afra* by combined sound also resulted to $p = 0.805 > 0.05$. Similar comparison of the maximum frequency (maximum entire) of combined sound by that of the sound of *A. tormotus* resulted to significant value, $p = 0.645 > 0.05$ whereas the comparison of the maximum frequency (maximum entire) of the combined sound by that of the sound of *C. afra* resulted to, $p = 0.005 < 0.05$. Table 42 shows p and F values determined through comparison of the minimum frequency (minimum entire) and maximum frequency (maximum entire) of the 35-60 kHz combined sound by the individual predator sound.

Table 42: Significance values of the minimum and maximum frequency of the 35-60 kHz combined sound compared by individual predator sound

Parameter	F	p
Comparison of minimum frequency of the combined sound by the minimum frequency of the sound of *A. tormotus*	0.226	0.951
Comparison of minimum frequency for the combined sound by the minimum frequency of the sound of *C. afra*	0.701	0.805
Comparison of maximum frequency of the combined sound by the maximum frequency of the sound of *A. tormotus*	0.795	0.645
Comparison of maximum frequency for the combined sound by the maximum frequency of the sound of *C. afra*	1.824	0.005

Combining the sounds of *C. afra* and *A. tormotus* did not yield any significant difference in the minimum frequency (minimum entire) from that of the individual predator sounds at 5 % significance level. Similarly, there was no evidence to show that the maximum frequency (maximum entire) of the combined sound differed significantly with that of the sound of *A. tormotus*. However, the maximum frequency (maximum entire) of the combined sound deviated significantly high from that of the sound of *C. afra*.

V. Bandwidth

The mean bandwidth (maximum entire) for the combined sound of *A. tormotus* and *C. afra* was 19.32 kHz in the 35-60 kHz range, was less than the reported EMR bandwidth and that of individual predator sound (Ahmad *et al*., 2007; Enayati *et al*., 2010). The difference in the minimum bandwidth (maximum entire) of the combined sound from that of the sound of *A. tormotus* and *C. afra* were 6.80 kHz and 0.00 kHz respectively. The mean bandwidth (maximum entire) of the combined sound was narrowed from that of the sounds of *A. tormotus* and *C. afra* by 78.70 Hz and 530.90 Hz respectively.

A comparison of the bandwidth (maximum entire) for the combined sound compared by 77.24 kHz as a test value on one-sample T test yielded a significance value, $p = 0.000 < 0.05$ as shown in Table 43. Similarly, the significance values for the bandwidth for the combined sound compared by the sounds of *A. tormotus* and *C. afra,* shown in Table 44, using the one-way ANOVA yielded significance values $p = 0.879 > 0.05$ and $p = 0.130 > 0.05$ respectively.

Table 43: Significance values of the comparison of the bandwidth of the 35-60 kHz combined sound by 77.24 kHz

Parameter	t	p
Comparison of the bandwidth of the combined sound by 77.24 kHz	-888.487	0.000

Table 44: Significance values of the comparison of the bandwidth of the 35-60 kHz combined sound by individual predator sound

Parameter	F	p
Comparison of bandwidth for the combined sound by the bandwidth of the sound of *A. tormotus*	0.554	0.879
Comparison of bandwidth for the combined sound by the bandwidth of the sound of *C. afra*	1.300	0.130

The bandwidth of the combined predator sound was significantly narrowed from the reported bandwidth of the EMR sound at 5 % significance level in the 35-60 kHz range. However, the bandwidth (maximum entire) of the combined sound in this frequency range did not deviate significantly from the sounds of *A. tormotus* and *C. afra,* separately compared.

VI. Peak frequency

The mean peak frequency (maximum entire) of the combined sound in this frequency range was 46.329 kHz. However, the mean peak frequency (maximum entire) of the sound of *A. tormotus* and *C. afra* in this frequency range was 47.626 kHz and 45.923 kHz respectively. It was observed that the peak frequency (maximum entire) of the combined sound was between that of the sounds of *A. tormotus* and *C. afra*. Combining the sounds yielded a mean peak frequency (maximum entire) which was slightly less than the average of the mean peak frequency (maximum entire) of individual predator sounds by 0.446 kHz. Similarly, combining the sounds of *A. tormotus* and *C. afra* yielded no deviation from the individual predators' maximum and minimum values of the mean peak frequency (maximum entire). All the sounds recorded a minimum and maximum mean peak frequency (maximum entire) of 34.100 kHz and 58.500 kHz respectively.

The one-way ANOVA comparison of the peak frequency (maximum entire) of the combined sound by that of the sound *A. tormotus* yielded significance values, p = 0.063 > 0.05 whereas the comparison of the combined sound by that of *C. afra* resulted to p = 0.268 > 0.05 and shown in Table 45.

Table 45: Significance values of the peak frequency of the 35-60 kHz combined sound compared by the individual predator sounds

Parameter	F	p
Comparison of peak frequency of the combined sound by the peak frequency of the sound of *A. tormotus*	1.646	0.063
Comparison of peak frequency for combined sound by the peak frequency of the sound of *C. afra*	1.157	0.268

It was established that at a statistical significance level of 0.05, the peak frequency (maximum entire) of the combined sound did not differ significantly with that of *C. afra* and *A. tormotus*. Combining the sounds of *A. tormotus* and *C. afra* did not yield any significant deviation in peak frequencies from the peak frequency (maximum entire) of the two individual predator sounds.

d) The acoustic transmission parameters for the combined sound of *C. afra* and *A. tormotus* in the range 61-90 kHz

I. Fundamental frequency and harmonics

The fundamental frequency for the combined sound is 4.883 kHz as discussed in 4.2.1 (b) I. The sound in this frequency range was composed of harmonics as shown in Fig. 34. Frequencies below 61 kHz have been attenuated and do not appear in Fig. 34. The harmonics represented by the formants indicated the presence of acoustic energy as reported in recent findings (Narins *et al*., 2004).

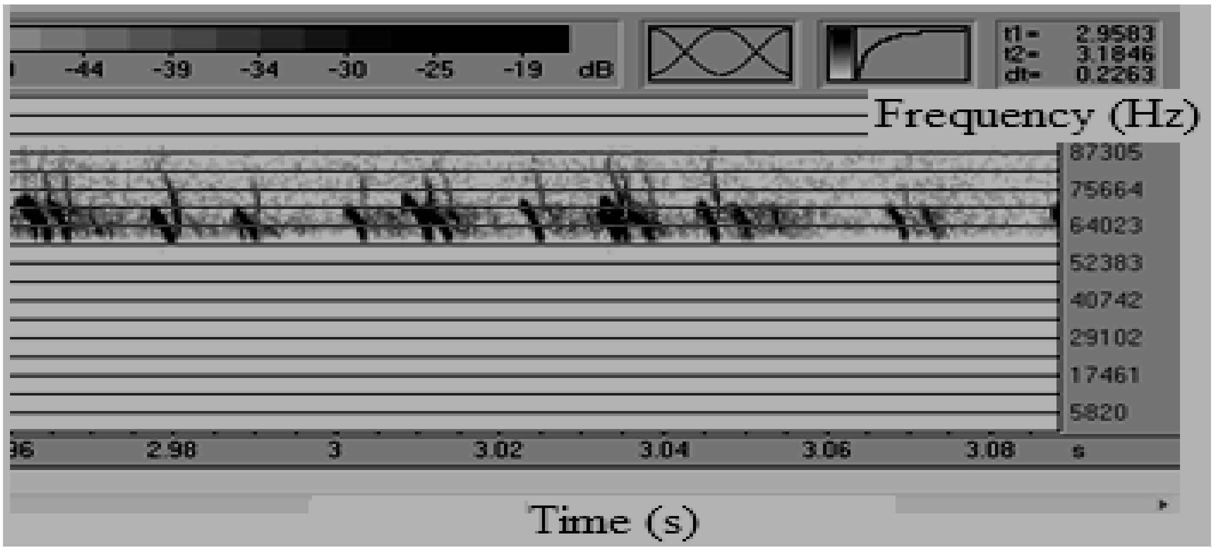

Fig. 34: The sound spectrogram of the 61-90 kHz combined sound

II. Peak amplitude

The oscillogram in Fig. 35 shows amplitude variation over 15 s duration. The maximum and mean peak amplitude (maximum entire) in this range was 113.85 Pa (135.11 dB SPL) and 98.80 Pa (133.87 dB SPL) respectively. The maximum peak amplitude (maximum entire) in the combined sound was 0.03 dB SPL less than the maximum peak amplitude (maximum entire) of the sound of *A. tormotus* but 0.03 dB SPL above that of the sound of and *C. afra*. The deviation in mean peak amplitude (maximum entire) in the combined sound was 0.20 dB SPL above the mean peak amplitude (maximum entire) of the sound of *A. tormotus* and 0.03 dB SPL less than that of the sound of *C. afra*. The deviation in the overall maximum and mean peak amplitude (maximum entire) of the combined sound was attributed to interference. The waveform, step type, indicated that most pulses had amplitudes less than 50% of the maximum amplitude at a -20 dB threshold.

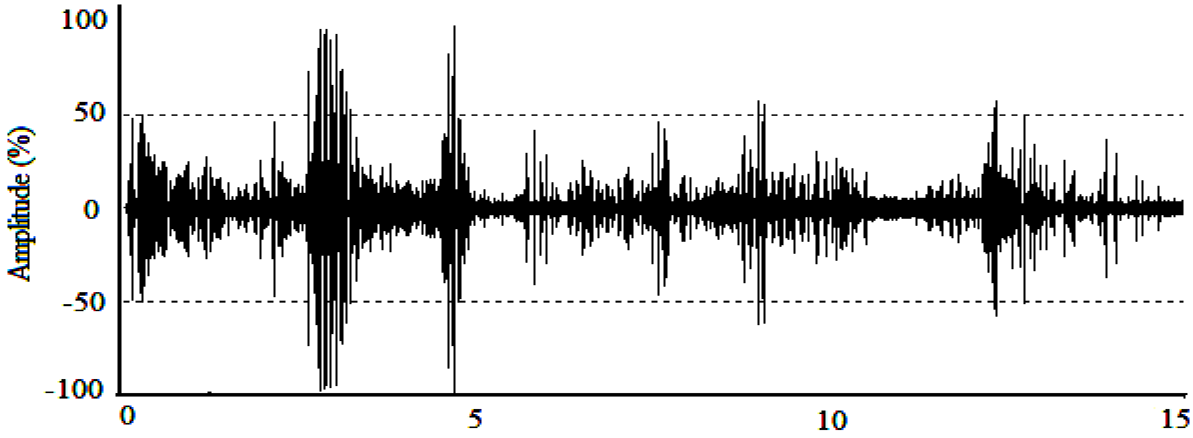

Fig. 35: The oscillogram for the combined sound in the 61-90 kHz frequency range

The one-way ANOVA comparison of the peak amplitude (maximum entire) of the combined sound in this frequency range by that of the sound of *A. tormotus* produced a significance value, p = 0.610 > 0.05. Similarly, the comparison of the peak amplitude (maximum entire) of the combined sound in this frequency range by that of the sound of *C. afra* produced a significance value, p = 0.467 > 0.05. These values, determined through one way ANOVA comparison of the peak amplitude (maximum entire) of the combined sound by the individual predator sound, are indicated in Table 46.

Table 46: Significance values of the peak amplitude of the 61-90 kHz combined sound compared by individual predator sound

Parameter	F	p
Comparison of peak amplitude of the combined sound by the peak amplitude of the sound of *A. tormotus*	0.980	0.610
Comparison of peak amplitude of the combined sound by the peak amplitude of the sound of *C. afra*	1.004	0.467

At 95 % confidence level, there was no significant deviation in peak amplitude (maximum entire) between the combined the sounds of *A. tormotus* and *C. afra* from the peak amplitude (maximum entire) of the individual predator sound.

III. Bandwidth

The mean bandwidth (maximum entire) for the combined sound of *A. tormotus* and *C. afra* was 13.23 kHz in the 61-90 kHz range which was 64.01 kHz less than the reported bandwidth of the EMR sound (Ahmad *et al.*, 2007; Enayati *et al.*, 2010). Combining the sounds of *A. tormotus* and *C. afra* narrowed the mean bandwidth (maximum entire) from the bandwidth of the sound of *A. tormotus* and *C. afra* by 23.94 kHz and 8.36 kHz respectively. A comparison of the bandwidth (maximum entire) of the combined sound by 77.24 kHz as a test value on one-sample T test yielded a significance value, $p = 0.000 < 0.05$ as shown in Table 47. Similarly, the significance values for the bandwidth for the combined sound compared by that of the sound of *A. tormotus* and *C. afra*, shown in Table 48, using the one-way ANOVA yielded significance values, $p = 0.749 > 0.05$ and $p = 0.542 > 0.05$ respectively.

Table 47: Significance values of the bandwidth in the 61-90 kHz combined sound compared by 77.24 kHz

Parameter	t	p
Comparison of bandwidth of combined sound by 77.24 kHz	-568.341	0.000

Table 48: Significance values of the bandwidth of the 61-90 kHz combined sound compared by individual predator sounds

Parameter	F	p
Comparison of bandwidth of the combined sound by the bandwidth of the sound of *A. tormotus*	0.813	0.749
Comparison of bandwidth of the combined sound by the bandwidth of the sound of *C. afra*	0.962	0.542

The bandwidth of the combined predator sound was significantly narrowed from the reported bandwidth EMR sound at 5 % significance level in the 61-90 kHz range. However, the bandwidth (maximum entire) of the combined sound did not deviated significantly from the bandwidth (maximum entire) of the sound of *A. tormotus* and *C. afra*. The bandwidth (maximum entire) of the combined sound was insignificantly narrower than that of the individual predator sounds.

IV. Acoustic energy and power

The maximum and mean acoustic energy for the combined sound in the 61-90 kHz range was 5.5174 Pa^2s and 0.0328 Pa^2s respectively. Similarly, the maximum and mean acoustic energy for the sound of *A. tormotus* in the 61-90 kHz range was 7.6989 Pa^2s and 0.4419 Pa^2s respectively as given in Fig. 36, Fig. 37; Table A1, Table A2 and Table A3 in Appendix A. The maximum and mean acoustic energy for the sound of *C. afra* in the 61-90 kHz range was 5.5949 Pa^2s and 0.0533 Pa^2s respectively. Combining the sounds of *A. tormotus* and *C. afra* lowered the maximum and mean acoustic energy from that of individual predator sounds. The acoustic energy in the combined sound declined significantly from the energy in the individual predator sounds.

The one way comparison of the combined sound energy by the sound *A. tormotus* yielded a significance value, $p = 1.000 > 0.05$ whereas the comparison of acoustic energy of the combined sound by the acoustic energy of the sound of *C. afra* resulted a significance value, $p = 0.000 < 0.05$, as shown in Table 49.

Table 49: Significance values of the acoustic energy of the 61-90 kHz combined sound compared by individual predator sounds

Parameter	F	p
Comparison of acoustic energy for the combined sound by the acoustic energy of the sound of *A. tormotus*	0.715	1.000
Comparison of acoustic energy for the combined sound by the acoustic energy of the sound of *C. afra*	5.180	0.000

At a statistical significance level of 0.05, there existed highly insignificant difference in the acoustic energy for the combined sound from sound of *A. tormotus*. The deviation in acoustic energy between the combined sound and the sound of *C. afra* was highly significant at 5 % significance level.

In summary, combining the sounds of *A. tormotus* and *C. afra* in the 10-34 kHz sound range resulted to a reduction in the maximum acoustic energy by 2775.00 Pa^2s and 8775.27 Pa^2s fom the sound of *A. tormotus* and *C. afra* respectively. Reduction in the maximum acoustic energy of the combined sound from the individual predator sound was also observed in the 35-60 kHz and 61-90 kHz frequency ranges. The variation in acoustic energy with frequency for every individual predator sound and their combination is shown in Fig. 36.

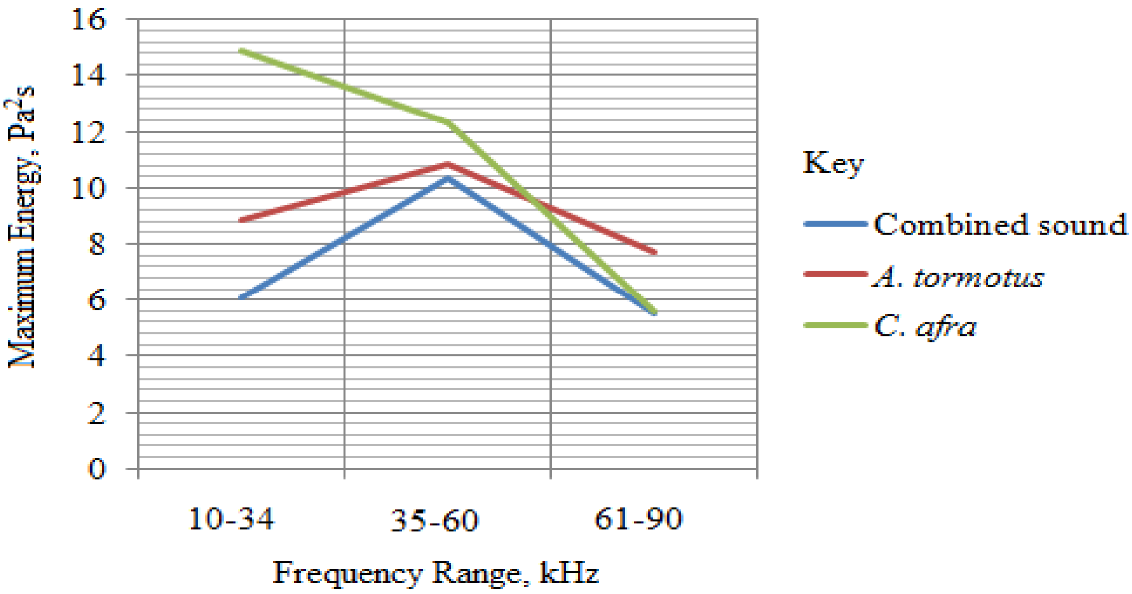

Fig. 36: Maximum energy variation in predator sound with frequency

Similarly the mean acoustic energy for the combined sound declined from the individual predator sound in all frequency ranges except for the sound of *C. afra* in the 35-60 kHz. Combining the sound of *A. tormotus* and *C. afra* increased the mean acoustic energy by 1.96 Pa^{2}s from the sound of *C. afra* in the 35-60 kHz frequency range as shown in Fig.37. The mean and maximum energy for the combined sound in the 61-90 kHz was less than the individual predator sound declining to 0.03279 Pa^{2}s and 5.51744 Pa^{2}s respectively.

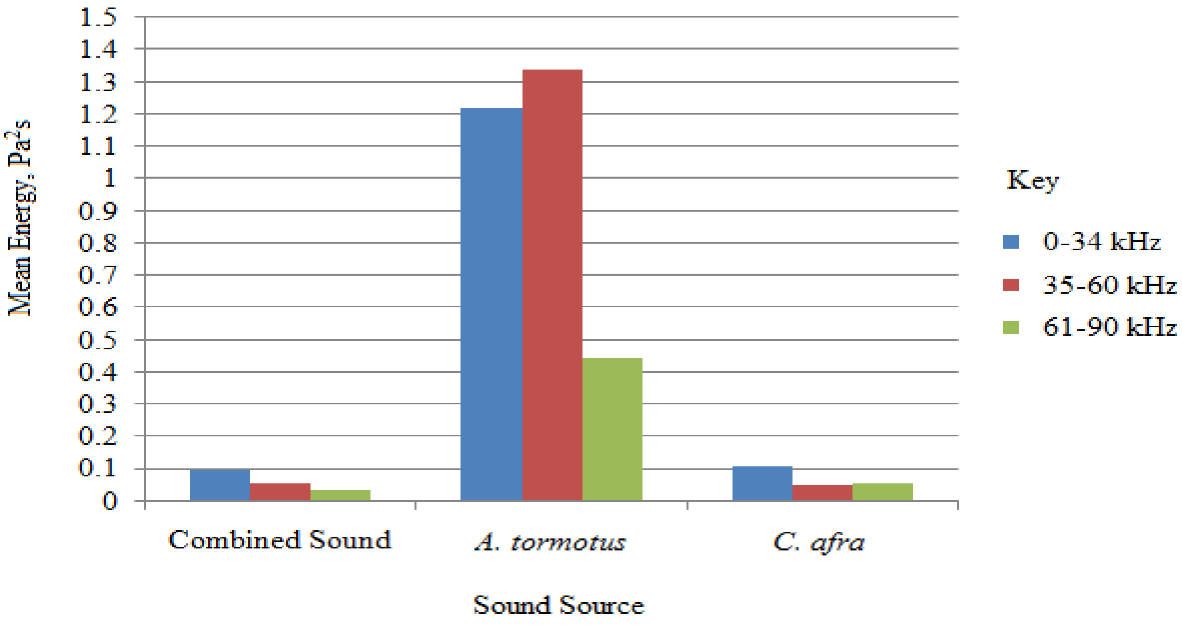

Fig. 37: Mean acoustic energy variation in predator sound with frequency

The signal power for the combined sound varied almost steadily between -108.75 dB and -130 dB at a -20 dB threshold as shown in Fig. 38. The signal power dropped from -112.5 dB to -123.75 dB between 60 kHz and 65 kHz, thereafter, maintaining an average of -123.75 dB up to 80.91 kHz, fluctuating thereabout. The power then increased from -123.75 dB to a maximum of -108.75 dB at 90 kHz.

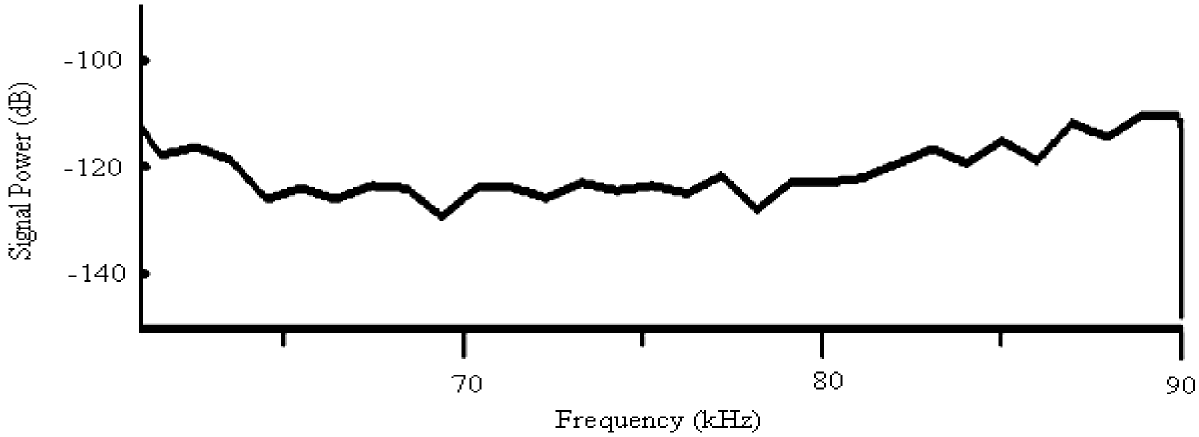

Fig. 38: The power spectrum for the 61-90 kHz frequency range for combined sound

V. Peak frequency

The mean peak frequency (maximum entire) of the combined sound in this frequency range was 69.563 kHz. The mean peak frequency (maximum entire) of the combined sound was less than that of the sound of *A. tormotus* and *C. afra* in this frequency range by 4.044 kHz and 2.870 kHz respectively. The reduction in mean peak frequency contributed to reduced acoustic energy in this frequency range as discussed in 4.2.2 (d) IV above.

The one-way ANOVA comparison of the peak frequency (maximum entire) of the combined sound by that of the sound of *A. tormotus* and later by the sound of *C. afra* yielded a significance value, $p = 0.903 > 0.05$. Similar comparison of the peak frequency (maximum entire) of the combined sound by that of the sound of *C. afra* yielded a significance value, $p = 0.850 > 0.05$. The comparison of the peak frequency (maximum entire) in the 61-90 kHz combined sound by the individual predator sounds yielded the significance values shown in Table 50.

Table 50: Significance values of the peak frequency of the 61-90 kHz combined sound compared by individual predator sounds

Parameter	F	p
Comparison of peak frequency of the combined sound by the peak frequency of the sound of *A. tormotus*	0.648	0.903
Comparison of peak frequency for of the combined sound by the peak frequency of the sound of *C. afra*	0.807	0.850

It was established that the peak frequency (maximum entire) of the combined sound did not differ significantly from that of *C. afra* and *A. tormotus*. Combining the sounds of *A. tormotus* and *C. afra* yielded highly in significant reduction in peak frequencies from the peak frequency (maximum entire) of the two individual predator sounds due to interference.

VI. Minimum frequency and maximum frequency

The mean and maximum values of the minimum frequency (minimum entire) for the combined sound in this frequency range were 60.504 kHz and 69.300 kHz respectively. Similarly, the mean and maximum of the minimum frequency (minimum entire) of the sound of *A. tormotus* was 58.188 kHz and 62.500 kHz respectively; whereas the mean and maximum of the minimum frequency (minimum entire) of the sound of *C. afra* was 58.135 kHz and 68.300 kHz respectively. The mean value of the maximum frequency (maximum entire) for the combined sound was 74.675 kHz; which was 21.525 kHz less than the mean value for the maximum frequency (maximum entire) of the sound of *A. tormotus* and less by 6.052 kHz from the sound of *C. afra*. The mean and maximum values of the minimum frequency (minimum entire) for the combined sound was greater than that of individual predator sounds.

The one-way ANOVA comparison of the minimum frequency (minimum entire) of the combined sound by that of the sound of *A. tormotus* yielded a significance value, $p = 0.498 > 0.05$ and a similar comparison of the minimum frequency (minimum entire) in the combined sound by that of the sound of *C. afra* also resulted to $p = 0.010 < 0.05$. Also, the one-way ANOVA comparison of the maximum frequency (maximum entire) of combined sound by *A. tormotus* resulted to $p = 0.297 > 0.05$ whereas the comparison of the maximum frequency (maximum entire) for the combined sound by that of the sound of *C. afra* yielded a significance value, $p = 0.447 > 0.05$. The output from the comparison of the minimum frequency (minimum entire) and maximum frequency (maximum entire) of the 61-90 kHz combined sound by the individual predator sound is given in Table 51.

Table 51: Significance values of the minimum and maximum frequency of the 61-90 kHz combined sound compared by individual predator sound

Parameter	F	p
Comparison of minimum frequency of the combined sound by the minimum frequency of the sound of *A. tormotus*	0.970	0.498
Comparison of minimum frequency for the combined sound by the minimum frequency of the sound of *C. afra*	1.765	0.010
Comparison of maximum frequency for the combined sound by the maximum frequency of the sound of *A. tormotus*	1.177	0.297
Comparison of maximum frequency for the combined sound by the maximum frequency of the sound of *C. afra*	1.013	0.447

At a statistical significance level of 0.05, there was no significant difference in the minimum frequency (minimum entire) by combining the individual sounds of *C. afra* and *A. tormotus* from the minimum frequency (minimum entire) of the sound of *A. tormotus*. But the difference in the minimum frequency (minimum entire) for the combined sound from the sound of *C. afra* was highly significant. At 95 % confidence level, there was no evidence to show that the maximum frequency (maximum entire) in the combined sound differed significantly from that of the sounds of *A. tormotus* and *C. afra*.

The combined sound was composed of 4869 pulses, 5664 pulses and 4650 pulses for the frequency range of 10-34 kHz, 35-60 kHz and 61-90 kHz respectively with formants stretching into the ultrasonic range as observed in previous findings. The other acoustic transmission parameters for the combined sound were automatically determined and values shown in Appendix A as Table A3. The standard deviation in the maximum and minimum frequency for the entire signal was 2.446 kHz and 5.767 kHz respectively, which is given in Appendix C as Table C3.

4.3 Determination of the Startling Effect of Individual and Combined Sounds of *C. afra* and *A. tormotus* on the Female *A. gambiae.*

4.3.1 The effect of ultrasound from *A. tormotus* and *C. afra* and their combination on the female *A. gambiae*

(a). Initial behavioural observations in the female *A. gambiae*

(i) Initial behavioural observations in the female *A. gambiae* elicited by the sounds of *A. tormotus* and *C. afra*

In all the control experiment 60 % and 70 % of the female *A. gambiae* exhibited normal flight and moved freely within the cage respectively. Only 10 % of the mosquito samples rested behind barriers under the control experiment. For the control experiments under the sounds of *A. tormotus* and *C. afra*, 80 % and 70 % of the mosquito samples maintained body rest at 45^o from surface with wings laid along the abdomen as shown in Plate 11 and Plate 12 respectively. Normal body movement within the cage was noted in 70 % and 80 % of the mosquito samples in the control experiment with sounds of *A. tormotus* and *C. afra* respectively. However, 10 % of the mosquito samples showed no body movement at all under the control experiment that preceded the bioassay study with the sound of *A. tormotus* and none for *C. afra* sound. Rubbing of limbs and wings was observed in 20 % of the mosquito samples under the control experiments for both predator sounds. Raising of limbs was occasionally observed in 10 % of the mosquito samples under the control experiment of the sound of *A. tormotus* only. Similarly, an equal number of mosquitoes were observed to rest their limbs and proboscis on the net surface under the control experiment that preceded investigations with the sound of *C. afra*. It was also noted that in all mosquito samples, the proboscis was maintained almost collinear with the antennae under the control experiment that preceded both predator sounds. These were the normal and natural behavioural traits in mosquitoes under normal conditions. These behavioural responses of the mosquito samples at the control were an evidence of the use of active mosquito samples in the bioassay study. Detailed data of the behavioural response in the mosquitoes is shown in Appendix J as Table J1.

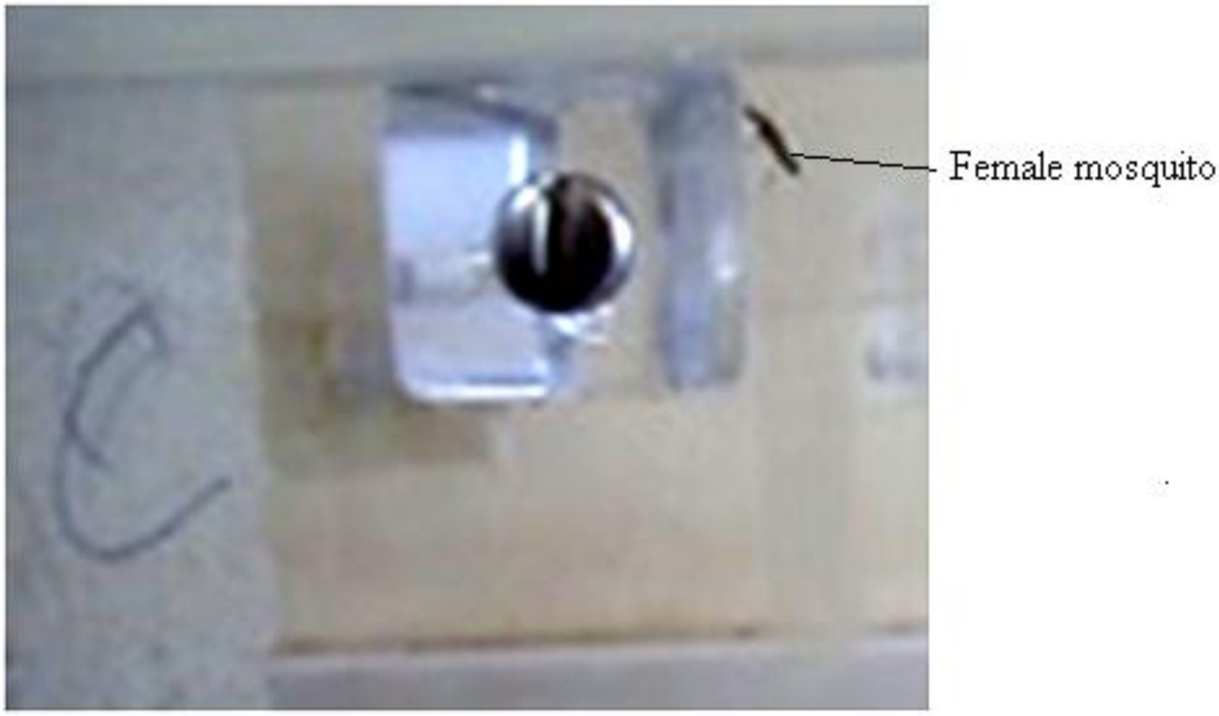

Plate 11: The female *A. gambiae* at normal rest

Plate 12: The female *A. gambiae* resting at angle β = 45^{o}

The sound in the 10-34 kHz frequency range had not been reported to have any effective startle effect on the female *A. gambiae* (Mohankumar, 2010). The effect of the sound of *A.*

tormotus on *A. gambiae* had also not been reported. However, the 10-34 kHz sound of *A. tormotus* and *C. afra* elicited rubbing of hind limbs, fore limbs and wings in the female *A. gambiae*. The effect was also observed in 30 % of the mosquito samples at this frequency range which increased from 20 % at the control experiment. It was also observed that 30 % of the mosquito samples did not exhibit remarkable body movement on exposure to both sounds of *A. tormotus* and *C. afra* separately. Recent findings with ultrasound from EMR reported immobilization in mosquitoes, an effect also observed in this research (Mohankumar, 2010). However, the EMR were noted to generate ultrasound that had low degree of repellency on mosquitoes (Ahmad *et al*., 2007; Enayati *et al.*, 2010). It was clearly observed that 30 % and 60 % of the mosquitoes exposed to the sounds of *C. afra* and *A. tormotus* respectively moved away from the source of the sounds, an evasive response also reported in earlier findings (Mohankumar, 2010). This was attributed to greater signal power in the sound of *C. afra* at this frequency range, hence initiating such responses in the female *A. gambiae*. Though the maximum acoustic energy of the sound of *C. afra* in this frequency range was 6.00 Pa^2s above that of *A. tormotus*, the later recorded a mean sound energy which was 11.81 times greater than that of *C. afra*. In both cases, the bandwidth was narrowed. The sound of *A. tormotus* evoked jumps and bounces; raising and lowering of bodies from surface in 50 % of the mosquito samples. Only 20 % of the mosquito samples were seen raising and lowering their bodies on exposure to sound of *C. afra* which was less by 30 % from that due to sound of *A. tormotus* despite the high power and energy.

The sound of *A. tormotus* elicited flapping and opening of wings; weak or exaggerated flights, falls and eventual escape in 30 % of the female *A. gambiae*. Similarly, flapping or opening of wings while resting was observed in 10 % of the sample mosquitoes but 40 % of the mosquitoes displayed weak or exaggerated flights, falls and escape when the sound of *C. afra* was played. The mosquitoes which exhibited flights, falls with some even escaping from the cage due to the powerful sounds of *C. afra* were 10 % more than those observed under the influence of the sound of *A. tormotus*. Raised limbs which were occasionally folded over the body was observed in 60 % of mosquitoes which were exposed to the sounds of *A. tormotus* and the sound of *C. afra*, with the later recording 20 % less. Another evasive behaviour was observed in 90 % and 30 % of sample mosquitoes which were exposed to sounds of *A. tormotus* and *C. afra* respectively; which included squeezing of body and proboscis through barriers and surfaces besides exhibiting hiding tendencies as shown in Plate 13. Body shaking associated with abdomen curving towards thorax, a behaviour not observed at the control study, was observed in 60 % and 30 % mosquito samples under the influence of the sound of

A. tormotus and *C. afra* respectively. Unusual forward and backward or sideways body movement was observed in 10 % of mosquito samples on exposure to both predator sounds in this frequency range. Due to the exhaustion, fear of predation and stress, 40 % and 20 % of sample mosquitoes exposed to sounds of *A. tormotus* and *C. afra* respectively rested by their abdomen and thorax (Forrest *et al*., 1995; Roxanne, 2008; Mohankumar, 2010).

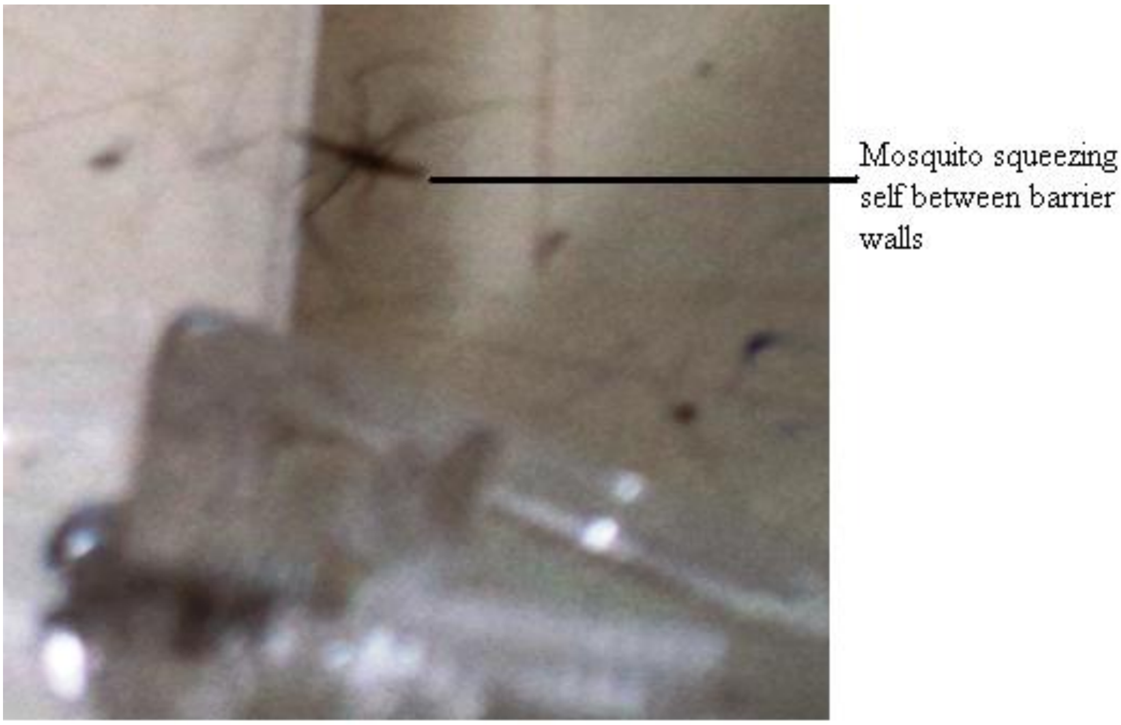

Plate 13: The female *A. gambiae* squeezing through barrier

Spreading of limbs on the resting surface, shown in Plated 14; and antennae erection was observed in 30% and10 % of the mosquito samples respectively on exposure to the sound of *A. tormotus* only. The mosquito antennae erection angle increased to 18.5^{o} from its normal rest position as shown in Plate 14. The antennae which are ultrasound sensors responded to the 10-34 kHz sound by erecting above normal, a verification of its essentiality in communication through oscillations as earlier reported (Morton and Offenhauser, 1949; Craig and Nijhout, 1971; Balanis, 1982; Martin and Daniel, 2000; Martin and Daniel, 2001; Robert and Jackson, 2006; Zwibel and Pitts, 2006; Maweu *et al*., 2009). Such intense response was not observed with the sound of *C. afra* partly because the sound consisted of both sonar and social calls (Narins *et al*., 2004; Gerlach, 2007).

Plate 14: Mosquito antennae erection at 18.5^o due to the 10-34 kHz sound of *A. tormotus*

The 35-60 kHz ultrasound from EMR had been reported to startle the female *A. gambiae* in recent research findings, yielding only 20 % effective repellency (Ahmad *et al*., 2007; Enayati *et al*., 2010; Mohankumar, 2010). However, the repellency due to the sound of *A. tormotus* on *A. gambiae* had not been reported. This research established that the sound of *A. tormotus* and *C. afra* elicited rubbing of hind and fore limbs, and wings in 20 % and 10 % of the mosquitoes respectively. However, the effect was observed to decline by 10 % for the sound of *A. tormotus* and 20 % in the sound of *C. afra* in the 10-34 kHz frequency range. There was a 20 % and 70 % of the mosquito samples which did not exhibit remarkable body movement on exposure to both sounds of *A. tormotus* and *C. afra* respectively. There was a remarkable increase in the number of mosquitoes by 40 % on exposure to the sound of *C. afra* with that of *A. tormotus* declining by 10 % which did not display remarkable body movement. This immobilization in mosquitoes due to neural stress and fear of predation, observed in recent research findings, was greatest with the sound of *C. afra* (Forrest *et al*., 1995; Roxanne, 2008; Mohankumar, 2010). The percentage of the mosquitoes which avoided the source of sounds on exposure to the sounds of *C. afra* remained constant at 30 %. However, the percentage of the mosquitoes that avoided the source reduced by 10 % on exposure to the sounds of *A. tormotus*. The mosquito samples were observed moving away from the sound source. The sound of *A. tormotus* evoked jumps and bounces; raising and lowering of bodies from surface of rest in 50 % of the mosquito samples, a value similar to that in 10-34 kHz range. The percentage of mosquitoes raising and lowering their bodies on exposure to sound of *C. afra* increased by 20 %. The sound of *C. afra* did not evoke jumps and bounces at all in any mosquito samples under study, behaviour similar to that in 10-34 kHz range. The number of mosquito samples which exhibited antennae erection increased by 70 % in response to the sound of *A. tormotus*, a response not observed with the sound from *C. afra*. The antennae erection shown in Plate 15, increased gradually by 40.0^{o} above the erection elicited under the 10-34 kHz sound range. The pronounced behavioural change in this frequency range had also been reported (Mohankumar, 2010).

Plate 15: The female *A. gambiae* resting by side with antennae erection at 58.5^{o}

The percentage of mosquitoes which were seen to rest on their abdomen with limbs on surface was 40 % and 20 % under exposure to the sound from *A. tormotus* and *C. afra* respectively. The sound of *A. tormotus* elicited flapping and opening of wings which increased remarkably in 40 % of the female *A. gambiae* and 60 % displayed weak or exaggerated flights, falls and eventual escape from cage. Similarly, flapping or opening of wings when resting was noted in 20 % of the mosquito samples and 30 % of the mosquitoes, reduced from the number in 10-34 kHz, displayed weak or exaggerated flights, falls and escape when the sound of *C. afra* was played. There was a reduction in the number of mosquito samples which exhibited raised limbs when exposed to the sound of *A. tormotus* and *C. afra* by 20 % and 10 % respectively from the number under the 10-34 kHz frequency range. The number of mosquito samples which squeezed their bodies and proboscis through barriers and surfaces besides exhibiting hiding tendencies were also reduced by 20 % from the number reported in the previous frequency range when they were exposed to both predator sounds. There was a 20 % and 30 % increase in the number of mosquito samples under the influence of the sound of *A. tormotus* and *C. afra* respectively which exhibited body shaking which was associated with abdomen that curving towards thorax as shown in Plate 17. The forward and backward movement, or sideways body movement increased from 10 % of mosquitoes under the 10-34 kHz range for both predator sounds to 40 % on exposure to the sound of *A. tormotus*, with none for the sound of *C. afra*. This was mainly due to the decline in the mean acoustic energy by 0.05 Pa^2s of the sound of *C. afra* besides the presents of both sonar and social calls (Narins *et al.*, 2004; Gerlach, 2007). The mean acoustic energy of the sound of *A. tormotus* increased progressively from the energy in the 10-34 kHz by 0.12 Pa^2s. It was this frequency range which evoked new behavioural traits which included resting by back or side as shown in Plate 16, and rolling on surfaces. These new behavioural traits were observed in 60 % and 20 % of the mosquito samples exposed to sounds of *A. tormotus* and *C. afra* respectively. Other behavioural responses such as spreading of limbs when resting on surface was observed in 50 % and 10 % of the mosquito samples exposed to the sounds of *A. tormotus* and *C. afra* respectively. Severe secondary responses which entailed exhaustion and loss of limbs was observed in 20 % and 10 % of mosquito samples which were exposed to the sound of *A. tormotus* only. These physical injuries were caused by mosquitoes knocking themselves on cage walls and net hence resulting to loss of limbs. The difference in response to predator ultrasound in this frequency range was attributed to slightly broadened bandwidth than that in the 10-34 kHz range in both predator ultrasounds.

Plate 16: The female *A. gambiae* rests by side with erected antennae

There was a progressive increase in the maximum value of acoustic energy in the sound from *A. tormotus* by 1.99 Pa^{2}s. However, the sound of *C. afra* recorded a progressive decline in this acoustic energy, though remaining it above the energy in the sound from *A. tormotus*. The power also declined significantly in both predator sounds.

The 61-90 kHz frequency range had not been reported to repel mosquitoes in recent findings by Mohankumar, 2010 yet this study observed its repulsive effect on mosquitoes. The number of mosquito samples that exhibited tiredness, weakness, loss of limbs, rested by side or back, rolled on surface, flapped wings, occasionally collapsing and opening wings on exposure to sound of *A. tormotus* increased by 10 % in this frequency range. Similar sound evoked rest by abdomen with limbs on surface in 70 % of the mosquito samples, an increase by 30 % from the number in the 35-60 kHz range. For the sounds of *A. tormotus*, the number of mosquitoes which raised their limbs and sometimes folded them over the body, shook their bodies and curved their abdomen towards the thorax as shown in Plate 17 were $\geq$ 40 %, a number maintained from the 35-60 kHz frequency range. However, the number of mosquitoes exhibiting similar behaviour was reduced by 40 % when subjected to the sound of

C. afra. The intensified evasive response to this sound frequency range was attributed to variation in call bandwidth, acoustic energy and power variation; and presence of both predation and neural stress causing calls (Forrest *et al*., 1995; Roxanne, 2008; Mohankumar, 2010). The minimum total energy for *C. afra* and *A. tormotus* was reduced by 1.3 x 10^{-4} Pa^2s and 4.77 x 10^{-03} Pa^2s respectively. Similarly, the sound signal of *A. tormotus* and *C. afra* in this range recorded a reduced maximum total energy by 3.1445 Pa^2s and 6.73 Pa^2s respectively from the energy in the 35-60 kHz frequency range. The maximum and minimum acoustic energy of *C. afra* was greatly reduced compared to that of *A. tormotus*. Due to this change in energy, the number of mosquito samples which did not show any body movement on exposure to the sound of *A. tormotus* and *C. afra* reduced by 10 % and 50 % respectively. The mosquito samples earlier immobilized recovered by 50 % on exposure to the sound of *C. afra*. The 61-90 kHz sound of *C. afra* did not evoke raising and folding limbs over the body, raising and lowering body, resting by abdomen, flapping or opening wings, spreading limbs, resting by back or side or rolling on surface in any mosquitoes. The deviation in the number of mosquito samples was between 10 % and 40 % from the number of mosquito samples in the 35-60 kHz range.

The mosquito samples observed rubbing their limbs and wings on exposure to the sound of *A. tormotus* was reduced by 20 % from the number recorded in the 35-60 kHz frequency range. In this frequency range, the number of mosquito samples jumping, bouncing and squeezing of bodies and proboscis in barriers were reduced by 50 % on exposure to the sound of *A. tormotus*. Similarly, the number of mosquitoes exhibiting erection of antennae, spreading of limbs and moving away from the sound of *A. tormotus* reduced by 50 %, 20 % and 10 % respectively; though the movement away from the sound of *C. afra* was unchanged. The antennae erection in mosquitoes was maintained at 58.5^o as indicated in Plate 15. The total number of mosquito samples that had previously shown weak or exaggerated flights, falls and directional body movement was reduced by 30 %. The significant reduction in the number of mosquito sample was attributed to the maximum signal power which was greatest in the sound of *C. afra* than that of *A. tormotus* which has a wide energy range compared to its power. This response was also due to the mosquitoes continuously being subjected to high energy ultrasonic sounds which evoked fear of predation and stress on neural system (Forrest *et al*., 1995; Narins *et al*., 2004; Roxanne, 2008; Mohankumar, 2010). Similarly, there was a widened mean bandwidth (maximum entire) for the sound of *A. tormotus* and *C. afra* than the bandwidth for the sounds in the 35-60 kHz, though narrower than the reported EMR bandwidths (Ahmad *et al*., 2007; Enayati *et al*., 2010).

(ii) Initial behavioural observations in the female *A. gambiae* elicited by the combined sound of *A. tormotus* and *C. afra*

The percentage of mosquito samples that rested with their bodies inclined at 45^o from surface of rest with wings laid along their bodies and moved normally within the cage was 90 % and 60 % respectively. In all the mosquito samples, the antennae and proboscis were almost collinear at the control. None of the mosquito samples under study squeezed themselves between barriers, hid behind barriers, raised their limbs, nor rested the limbs and proboscis on net or cage surface in the control experiment; responses observed in individual predator sounds. Only 10 % of the mosquito samples displayed normal flight within the cage. An equal number of mosquito samples were seen to rub their legs, wings or both under similar conditions and shown in Appendix J as Table J1.

The effect of the combined sound of *A. tormotus* and *C. afra* on the *A. gambiae* had not been reported in recent findings for all sound frequency ranges. Contrary to recent findings involving the 10-34 kHz ultrasound generated by EMR by Mohankumar (2010) the current study observed that combined natural sound from predators evoked behavioural response. New responses not observed with individual predator ultrasound included; rest by back or side or rolling on surface; and exhaustion or collapsing in mosquitoes which were noted in 30 % and 10 % of the mosquito samples studied respectively. In this frequency range, 50 % of the mosquito samples studied exhibited spread of limbs on rest surface and erection of antennae which was an increase from the number of mosquitoes affected by individual predator sound. The number of mosquitoes that did not show any body movement under the influence of the combined sound was reduced significantly by 30 % from the number recorded in individual predator sounds. However, the number of mosquitoes that displayed jumping or bouncing due to exposure to the combined sound was 50 %, a number maintained for the sound of *A. tormotus* but above that of *C. afra* by 50 %. These behavioural traits confirmed the startling of mosquitoes by ultrasound as reported in recent studies (Roxanne, 2008; Mohankumar, 2010).

There was a considerable reduction in the number of mosquitoes that squeezed their bodies and proboscis in barriers when subjected to combined sound by 50 % from those under the sound of *A. tormotus*. However, the number of mosquitoes that squeezed their bodies and proboscis in barriers when subjected to combined sound increased by 10 % from the number noted under influence of *C. afra*. However, the number of mosquitoes raising or folding of limbs, or both, was reduced by 10 % from the number under the sound of *A. tormotus* but increased by an equal margin with the sound of *C. afra*. Raising and lowering of mosquito body from the resting surface was observed in 20 % of the mosquitoes, a number that was maintained for the sound of *C. afra* but reduced by 30 % from the number under *A. tormotus* sound. Directional mosquito movement was not observed in this frequency range for the combined sound, a reduction of 10 % from the number observed under the sound of *A. tormotus* and *C. afra*. There was a 10 % increase in the number of mosquitoes that rubbed their hind limbs or wings, above the number recorded under the influence of individual predator ultrasound. The number of mosquito samples exhibiting body shaking and abdomen curving, illustrated in Plate 17, were 70 %, an increase by 10 % and 40 % from the number of mosquito samples under *A. tormotus* and *C. afra* respectively.

Plate 17: Mosquito resting on spread limbs with abdomen curved towards thorax

Out of the ten *A. gambiae* mosquitoes studied, 40 % exhibited weak or exaggerated flights associated with occasional falls when exposed to the combined predator sound. There was a decrease by 10 % of the mosquitoes exhibiting similar behaviour under the influence of the sound of *C. afra*. However, the number of mosquitoes with similar behavior under the influence of the combined sound and the sound of *A. tormotus* was maintained. The number of mosquitoes moving away from the combined sound source was10 %, a decline by 50 % and 20 % from the number of mosquitoes recorded under the influence of *A. tormotus* and *C. afra* respectively. In the 10-34 kHz frequency range, 10 % of the mosquitoes seemed to move away from the sound source, rested most in corners or behind barriers, an evasive response observed in higher frequencies emitted from EMR and currently in both individual and combined predator sound (Mohankumar, 2010). Rest by abdomen with limbs on surface was observed in 30 % of the sample mosquitoes, a decline by 10 % from the number of mosquitoes under the sound of *A. tormotus* and increased by 10 % from that of the sound of *C. afra*.

Flapping of wings was prominent in 30 % of the sample mosquitoes when the samples were exposed to combined ultrasound. A percentage mean of 30 % of the total mosquito samples studied in this frequency range was startled by the combined sound which was greater of than the mean percentage affected by the sound of *C. afra* by 12.94 %, but less than that due to the sound from *A. tormotus* by 4.12 %. The difference in response in mosquitoes to predator ultrasound was due to reduced maximum and mean acoustic energy in this frequency range. This is evidenced in a reduction of maximum acoustic energy by 2.78 kPa^2s and 8.78 kPa^2s in sounds of *A. tormotus* and *C. afra* respectively. Similar reduction was observed in the mean acoustic energy by 1.13 kPa^2s and 0.0085 kPa^2s in the sounds of *A. tormotus* and *C. afra* respectively. More so, the maximum and a minimum signal power of the combined sound fluctuated between -98 dB and -136.67 dB respectively referenced to -20 dB, an equal range to the power in the sound of *A. tormotus*, which weakened with increase in frequency. The signal power in *C. afra* was the greatest of all. The closeness in mosquito behavioural response to the sound of *A. tormotus* and the combined sound was attributed to equal power range with minimum deviation in acoustic energy. Similarly, the difference in mosquito response to combined sound from individual predator sound was because the mean bandwidth for the combined sounds in this range was broader than that of *A. tormotus* but narrowed from that of *C. afra* by 5.44 kHz and 1.29 kHz respectively.

The one-way ANOVA comparison of the percentage of mosquito samples affected on exposure to the combined sound by the percentage of mosquito samples affected on exposure to sound of *A. tormotus* resulted to $p = 0.000 < 0.05$. The comparison of the percentage of mosquito samples affected by the combined sound by the percentage of mosquito samples affected by sound of *C. afra* yielded significance value, $p = 0.099 > 0.05$. The p and F values obtained from the comparison of the percentage of mosquito samples affected by the 10-34 kHz combined sound by the number under exposure to the individual predator sounds are shown in Table 52.

Table 52: Significance values of the number of mosquitoes affected by the 10-34 kHz combined sound compared by the number exposed to individual predator sound

Parameter	F	p
Comparison of the number of mosquitoes affected by combined sound compared to the number affected by sound of *A. tormotus*	1883	0.000
Comparison of the number of mosquitoes affected by combined sound compared to the number affected by sound of *C. afra*	5.232	0.099

At 5 % significance level, there was adequate evidence to show that the percentage of mosquito samples affected by the combined sound differed significantly from that of the individual sounds of *A. tormotus*. However, the percentage of mosquito samples affected by the combined sound did not differ significantly from the number of mosquitoes under the sound of *C. afra*.

In the 35-60 kHz frequency range, fatigue, loss of limbs and collapsing was seldom in any mosquito samples studied. However, this frequency range had been reported to startle mosquitoes optimally in recent findings (Ahmad *et al*., 2007; Enayati *et al.*, 2010; Mohankumar, 2010). Fifteen out of seventeen (88.23 %) behavioural traits were observed in mosquito samples which were exposed to ultrasound from the combined sound as indicated in Appendix J as Table J2. However, the sound of *C. afra* evoked 70.59 % behavioural traits while the sound of *A. tormotus* evoked 100 % of the behavioural traits considered. All the sounds had substantial startle effect in this range. This frequency range elicited the least number of mosquito samples, 10 %, which rubbed their limbs or showed no body movement at all, compared to 20 % and 70 % under the influence of *A. tormotus* and *C. afra* respectively. Antennal erection up to 58.5^{o}, was observed in 70 % of the mosquito samples, was also elicited by the combined sound. This number was 10 % less than that observed in the sound of *A. tormotus*. The combined sound elicited spreading of limbs, movement away from sound source, flapping and opening of wings in 30 % of the sample mosquitoes, a number less than that observed in the sound of *A. tormotus* in the same frequency range. The number of mosquitoes spreading of limbs, movement away from sound source, flapping and opening of wings in the combined sound was higher or equal to the number under the influence of *C. afra*. In this frequency range, 90 % of the sample mosquitoes were noted to squeeze their bodies and proboscis between barriers, 20 % and 40 % above the number noted under the sounds of *A. tormotus* and *C. afra* respectively.

Jumping and/or bouncing was observed in 50 % of the mosquito samples, a number equal to that recorded under the sounds of *A. tormotus*. This frequency range recorded 70 % of the sample mosquitoes which raised and folded their limbs and also rested by their abdomen, a value which was 30 % and 40 % above the number of mosquito samples recorded under the sound of *A. tormotus* and *C. afra* respectively. The number of mosquito samples showing shaken body, abdomen curved towards thorax as shown in Plate 17; rested by side, back or rolled on surface reduced to 50 % compared by the number in individual predator sounds. There were 40 % of the mosquito samples which displayed either weakened or exaggerated flights and falls on exposure to the combined sound. Combining the sound of *A. tormotus* and *C. afra* yielded a percentage of mosquito samples greater than the percentage affected by the sound of *C. afra*, but was significantly less than that of the sound from *A. tormotus*. The response in mosquitoes when they were exposed to the combined sound in the 35-60 kHz range was not optimum, save for the response due to the sound of *A. tormotus*.

The average percentage of the mosquitoes affected by sound of *A. tormotus*, *C. afra* and their combination in the 35-60 kHz frequency range was 45.88 %, 22.94 % and 38.82 % respectively. The high response in the sound of *A. tormotus* was due to progressive increase in maximum and mean acoustic energy from the 10-34 kHz frequency range. Similarly, the signal intensity for *A. tormotus* in 35-60 kHz was greater than that of the 10-34 kHz frequency range. However, the energy and signal power for *C. afra* declined from their value in 10-34 kHz rendering it weak compared to the constant power of *A. tormotus*. The decline in the energy and power in *C. afra* yielded a reduced number of the mosquitoes affected by the ultrasound in this range. The maximum acoustic energy for the combined sound, which progressively increased from the energy in the 10-34 kHz, was 0.5009 Pa^2s and 1.9804 Pa^2s less the maximum acoustic energy in the sound of *A. tormotus* and *C. afra* respectively. However, the mean acoustic energy in the combined sound, which was higher than that for the sound of *C. afra* in this frequency range reduced by 0.0427 Pa^2s from its energy recorded in 10-34 kHz. The signal power for the combined sound sustained a constant power trend with a narrowed mean bandwidth (maximum entire) from both sounds of *A. tormotus* and *C. afra* by 0.079 kHz and 0.5312 kHz respectively.

The one-way ANOVA comparison of the percentage of mosquito samples affected under the combined sound by the percentage of mosquito samples affected under the sound of *A.*

tormotus resulted to p = 0.000 < 0.05. Similarly, the comparison of the percentage of mosquito samples affected by the combined sound by the percentage of mosquito samples affected by sound of *C. afra* yielded significance value, p = 0.065 > 0.05. These values are shown in Table 53.

Table 53: Significance values of the number of mosquitoes affected by the 35-60 kHz combined sound compared by the number exposed to the individual predator sound

Parameter	F	p
Comparison of the number of mosquitoes affected by combined sound compared to the number affected by sound of *A. tormotus*	830.907	0.000
Comparison of the number of mosquitoes affected by combined sound compared to the number affected by sound of *C. afra*	5.027	0.065

At a statistical significance level of 0.05, the percentage of mosquito samples affected by the combined sound differed significantly from the sound of *A. tormotus*. However, the percentage of mosquito samples affected by the combined sound did not differ significantly from that of the sound of *C. afra*.

The study observed that the 61-90 kHz frequency range startled the female *A. gambiae* considerably. The mosquitoes erected and opened their antennae and sustained it at 58.5^o in 80 % of the mosquito samples studied in this combined sound frequency range. The number of mosquitoes exhibiting this antennal behaviour was 50 % more than those noted in *A. tormotus*. Shaking in mosquito bodies, rest by back or side or rolled, weak flights with several falls and resting by abdomen or side was observed in 40 %, 90 %, 40 % and 50 % of the mosquito samples respectively on exposure to the combined sound. Only 50 % of the mosquitoes investigated rested by the abdomen on the net and also 10 % of the mosquito samples rubbed wings and legs. Occasionally the mosquitoes hang on the net weakly with their abdomen curved towards thorax. The mosquitoes had either one or both wings open. There were loses of limbs, five remaining in 10 % of the sample mosquitoes, a drop of 10 % from the number observed with the sound of *A. tormotus*. The 20 % of mosquito samples which rested by abdomen also spread their limbs. An equal number tended to move away from the source of the combined sound. Bouncing and jumping on the surface was also observed in 40 % of the sample mosquitoes, occasionally, raising their legs and wings simultaneously. The mosquitoes appeared weak and displayed no body movement in 20 %

and 10 % of the sample mosquitoes respectively, as observed in the 35-60 kHz frequency range. The limbs in 70 % of mosquito samples were occasionally raised and folded backwards while resting by the abdomen. The 20 % of the sample mosquitoes appeared exhausted though they flew about weakly. The 10 % of mosquito samples were observed moving up and down from the rest surface as they jumped and rolled severally. The combined sound of *C. afra* and *A. tormotus* evoked squeezing of their bodies and proboscis in barriers in 30 % of the mosquitoes. Similarly, flapping and opening of wings was observed in 40 % of the sample mosquitoes. The percentage of mosquitoes startled by both the sound of *A. tormotus* and the combined sound was 34.12 %. However, the sound of *C. afra* recorded the lowest mean of 8.24 % of the mosquito samples which were startled. The increase in the number of mosquitoes disturbed by the combined sound was due to the steady signal power that stretched between -108.75 dB and -130 dB at a -20 dB threshold and referenced to 1 pW. It was also noted that the mean bandwidth (maximum entire) for the combined sound was greatly narrowed from that of the sound of *A. tormotus* and *C. afra* by 23.9415 kHz and 8.3566 kHz respectively. However, combining the sounds of *A. tormotus* and *C. afra* lowered the maximum acoustic energy from that of individual predator sounds by 2.104 Pa^2s and 0.0775 Pa^2s respectively. Similarly, the combined sound energy was less than the energy in the 35-60 kHz by 4.8251 Pa^2s.

The one-way ANOVA comparison of the percentage of mosquito samples affected by the combined sound by the percentage of mosquito samples affected by sound of *A. tormotus* resulted to $p = 0.000 < 0.05$ and the comparison of the percentage of mosquito samples affected by the combined sound by the percentage of mosquito samples affected by sound of *C. afra* yielded significance value, $p = 0.055 > 0.05$ as shown in Table 54.

Table 54: Significance values of the number of mosquitoes affected by the 61-90 kHz combined sound compared by the number exposed to the individual predator sound

Parameter	F	p
Comparison of the number of mosquitoes affected by the combined sound compared to the number of mosquitoes affected by the sound of *A. tormotus*	1228	0.000
Comparison of the number of mosquitoes affected by the combined sound by the number of mosquitoes affected by the sound of *C. afra*	3.802	0.055

The percentage of mosquito samples affected by the sound of *A. tormotus* differed significantly high with that of the combined sound. However, the percentage of mosquito samples affected by the sound of and *C. afra* did not differ significantly from that of the combined sound.

(b). Mosquito activity under the influence of the predator sounds

(i) Mosquito activity under the influence of filtered sounds of *A. tormotus* and *C. afra*

Recent findings based on mosquito landing rates on bare human body parts with ultrasound from functioning EMR yielded 20 % effective repellency (Ahmad *et al*., 2007; Enayati *et al.*, 2010). The mosquito activities in this section of the research was limited to flight and rest besides the behavioural response discussed in 4.3.1(i). All the mosquitoes exposed to predator sounds separately, had their flight time above the control. However, 6.7 % and 33.3 % of these mosquito samples displayed their flight time below the control when exposed to the sounds of *A. tormotus* and *C. afra* respectively. Fig. 39, Fig.40, Fig.41, Fig.42, Table 55, Table 56; Table B1 and Table B2 in Appendix B show the relationship between flight duration with frequency for ten mosquitoes exposed to the sounds of *A. tormotus* and *C. afra*. When the mosquitoes were exposed to 10-34 kHz, 35-60 kHz and 61-90 kHz sound frequencies of *A. tormotus*, the flight duration increased by an average duration of 433.52 s, 352.52 s and 654.88 s respectively, above the control experiment as shown in Table 55. Similarly, the rest time for ten mosquitoes was investigated and presented for different frequencies of predator sounds in Appendix D as Table D1 and Table D2. The mosquitoes, under the influence of the 35-60 kHz sound of *A. tormotus* and *C. afra* spent most time in air as shown in Table L1 and Table L2 in Appendix L. The mosquitoes exposed to sound of *A. tormotus* also yielded remarkable suspension time in the 10-34 kHz sound range. The mosquito samples exhibited a decline in the average flight duration by 18.249 s from the control in the 10-34 kHz of the sound of *C. afra* as shown in Table 56. This response was because of acoustic energy for the sound of *C. afra* declining by 2.534 Pa^{2}s and the signal power also declining uniformly from -55 dB to -59 dB in the 35-60 kHz frequency range. Ultrasound in the 35-60 kHz range, yielded significant acoustic energy as earlier reported with the sound from bats (Narins *et al*., 2004). The sound of *A. tormotus* recorded progressive increased in energy from 10-34 kHz to 35-60 kHz. However, the energy declined as the frequency changed from 35-60 kHz to 61-90 kHz by 3.1445 Pa^{2}s, recording an energy of 7.699 Pa^{2}s, which was still higher than that of *C. afra*. The signal power maintained the maximum value at -100 dB with the minimum value less by -10 dB from the minimum power in the 35-60 kHz frequency range. Energy and power variation in predator sounds enhanced both flight and rest activities above the control for sample mosquitoes investigated. The highest rest time was recorded in the frequency ranges of 10-34 kHz and 35-60 kHz with the sound from *C. afra* and *A. tormotus* respectively as shown in Table K1 and Table K2 in Appendix K. All frequency ranges under the sound of *A. tormotus* recorded a total rest time below the control experiment, with a slight increase in the 35-60 kHz and a decline towards the 61-90 kHz. Long rest time besides the many collapses of the mosquitoes was attributed to the search for safe conditions within the cage, reported as immobilization in recent findings (Mohankumar, 2010). The mosquitoes' behaviour in the 10-34 kHz and 35-60 kHz under the sound from *C. afra* and *A. tormotus* respectively was attributed to search for safe conditions within the cage. The mosquitoes were characterized by immobilization though they struggled to escape; occasionally taking long flights. The mosquitoes' evasive behaviour was attributed to the stress caused on the nervous system and fear of predation (Forrest *et al.*, 1995; Roxanne, 2008; Mohankumar, 2010).

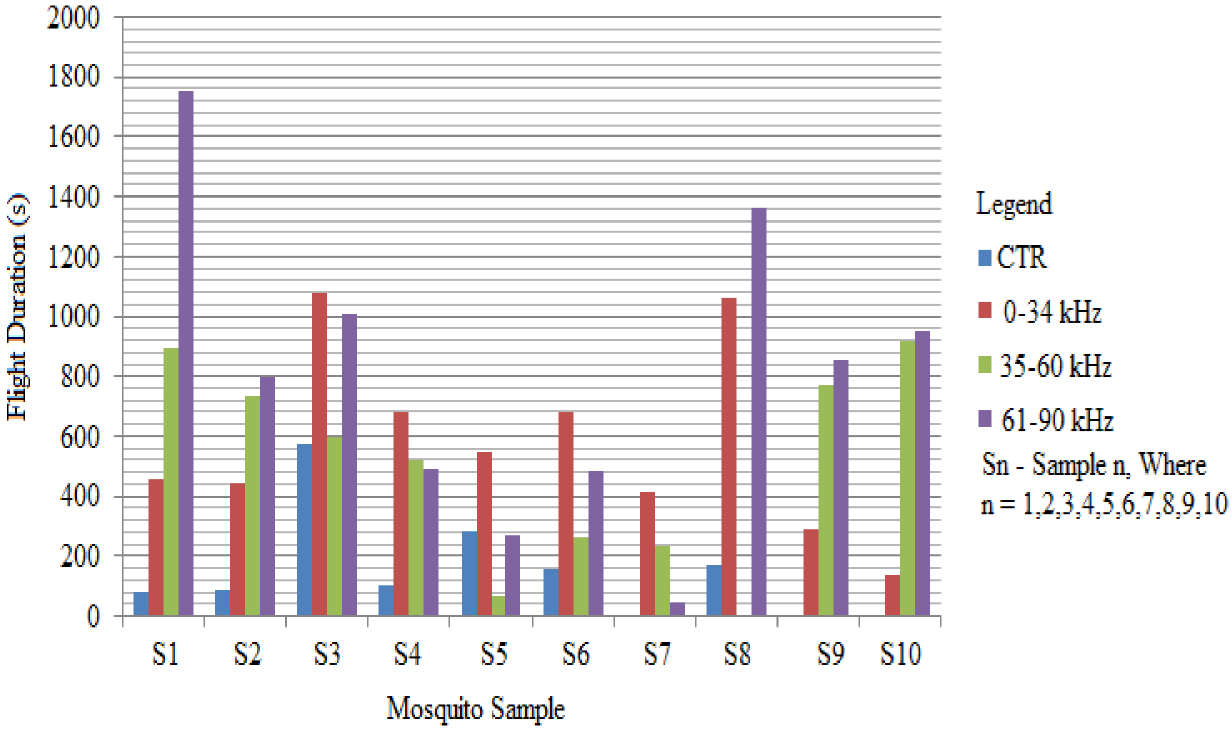

Fig. 39: The relationship between mosquito flight duration with frequencies of *A. tormotus*

Table 55: Average flight duration per mosquito due to sound frequencies of *A. tormotus*

Frequency Range	Flight Duration (s)
CTR	146.408
0-34 kHz	579.932
35-60 kHz	498.931
61-90 kHz	801.283

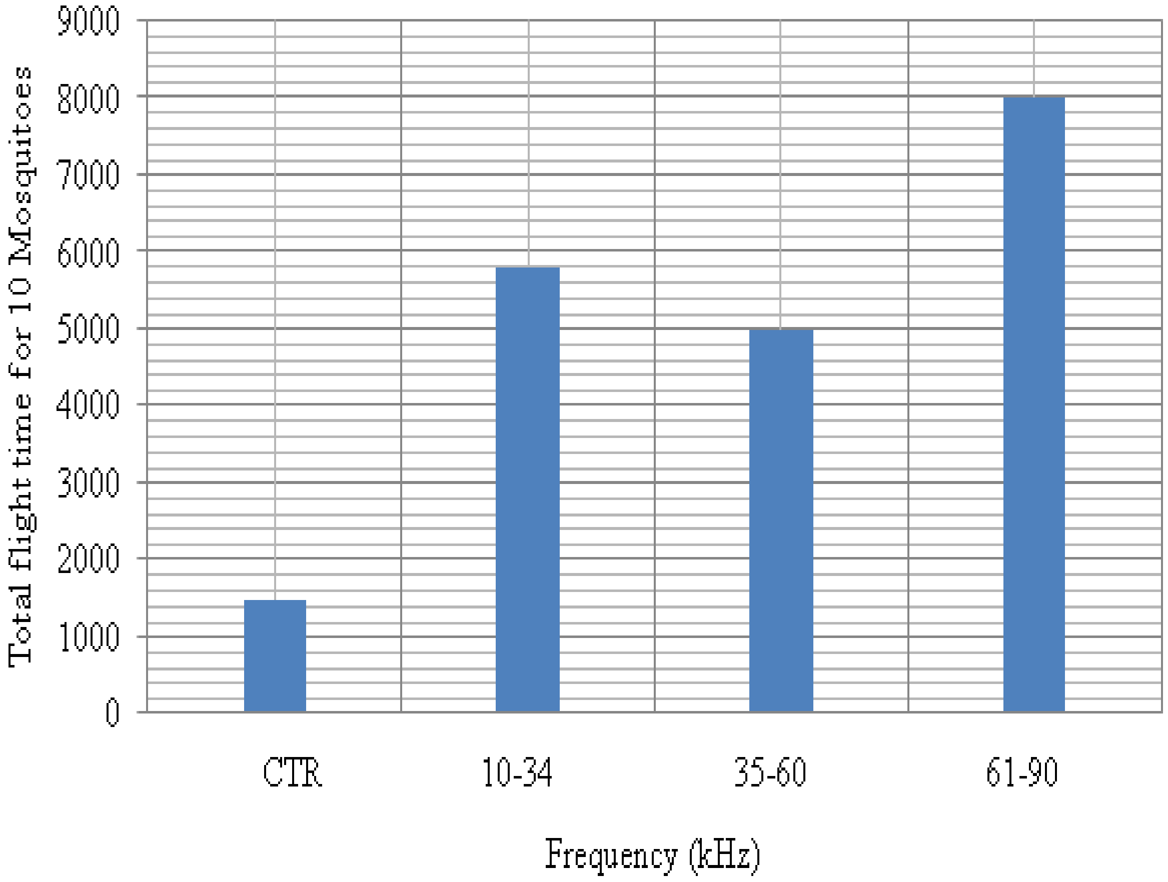

Fig. 40: The mosquitoes' total flight time under varied sound frequencies of *A. tormotus*

Table 56: Average flight duration per mosquito due to sound frequencies of *C. afra*

Frequency Range	Flight Duration (s)
CTR	357.773
0-34 kHz	339.524
35-60 kHz	381.798
61-90 kHz	333.803

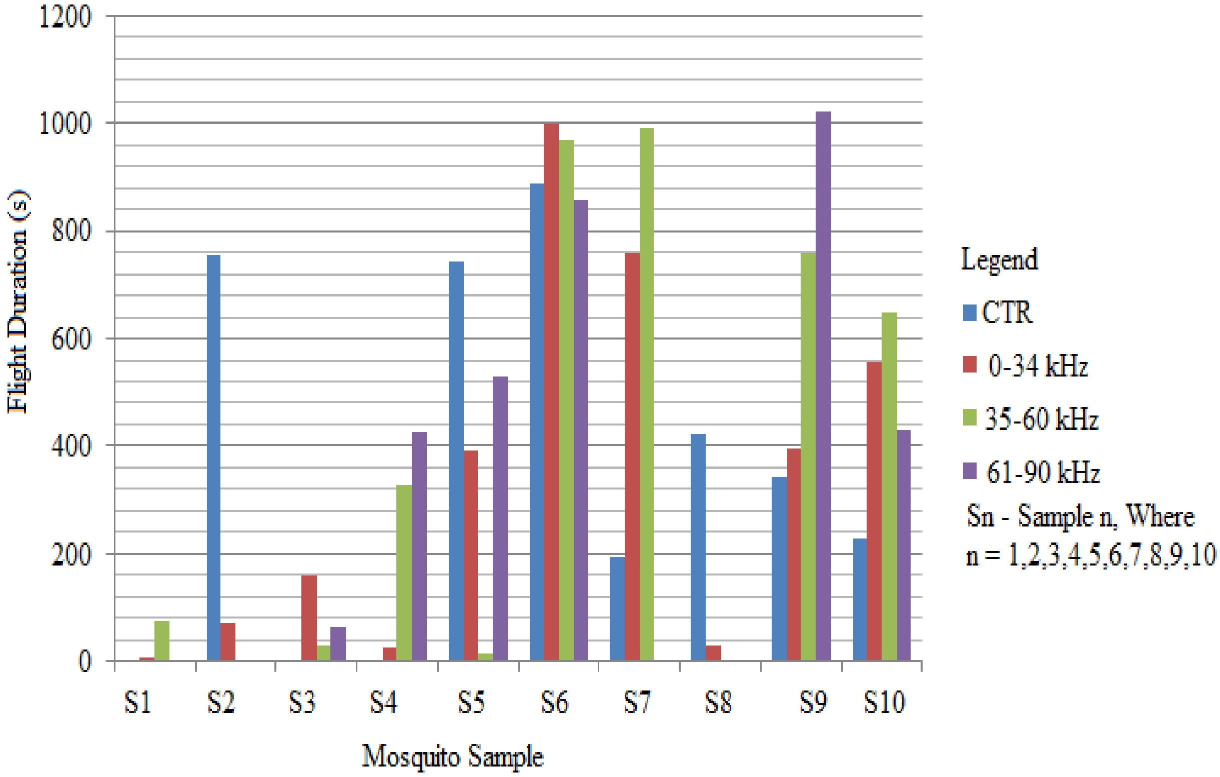

Fig. 41: The relationship between mosquito flight duration with frequencies of *C. afra*

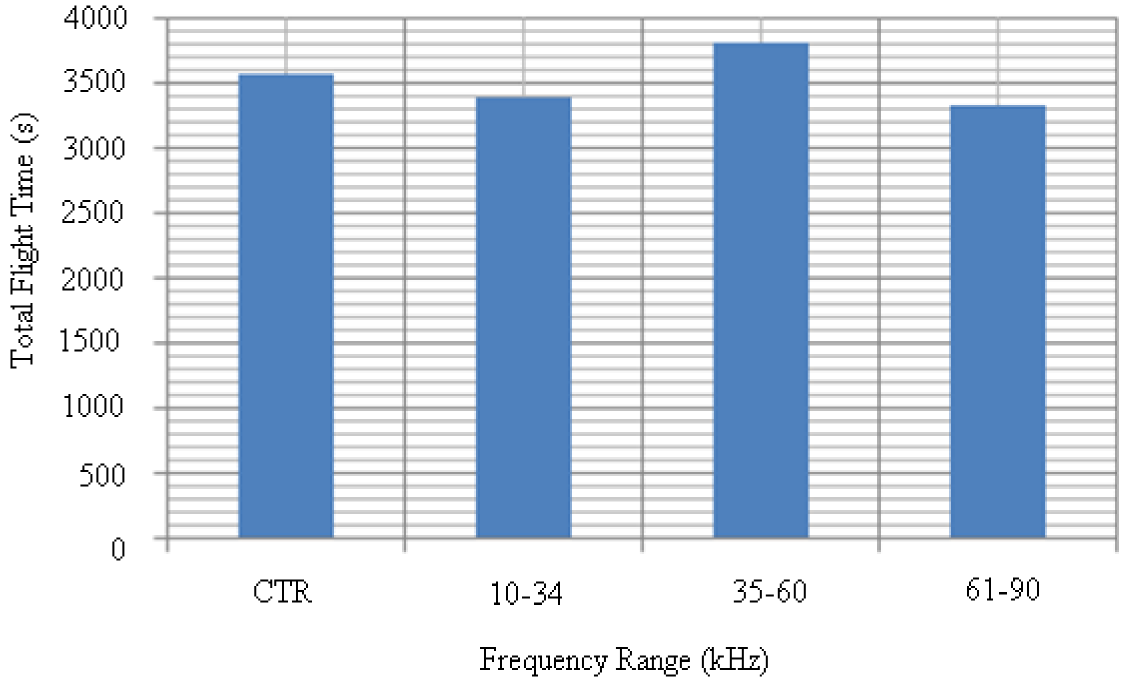

Fig. 42: The total flight time of *A. gambiae* under varied sound frequency of *C. afra*

Table F1 and Table F2 in Appendix F shows the distribution of flight and rest activities of the female mosquitoes under the influence of the sound of *A. tormotus* and *C. afra* respectively. The mosquitoes displayed normal activity under the control experiment. All the frequency ranges in the sounds of *A. tormotus* and *C. afra* initiated activities above the control. On exposure to energetic ultrasound, this research revealed that the mosquitoes exhibited startle response in all frequency ranges. The female *A. gambiae* were excited on exposure to the sound of the *A. tormotus* with an increase of 583 activities under the 10-34 kHz frequency range from the control as shown in Fig. 44 and Table H2 in Appendix H. The number of mosquito activities increased further at higher frequency band of 35-60 kHz, which then remained almost constant at 61-90 kHz, vividly shown in Fig. 43, Fig. 44 and Appendix F under Table F1. The average mosquito activity at 10-34 kHz sound of *A. tormotus* was 3.52 times the average activities under the control experiment, shown in Table 57. However, the sound of *C. afra* initiated 4.48 times the average activities under the control experiment, shown in Table 58. This was due to high onset maximum acoustic energy of the sound of *A. tormotus* which was 8.8568 Pa^2s and signal power which was steady and maintained at -118 dB. The acoustic energy of the sound of *C. afra* was greater than the acoustic energy of *A. tormotus,* though it declined greatly as shown in Fig. 45 with increase in frequency. These findings were in agreement with the results from previous studies (Yager *et al.*, 2000; Narins *et al.*, 2004; Ahmad *et al.*, 2007; Enayati *et al.*, 2010; Monto, 2010). The onset frequency range of 10-34 kHz initiated the greatest number of activity under the influence of ultrasound from *C. afra*, clearly shown in Fig. 45 and Fig. 46 and Table H2 in Appendix H. The 61-90 kHz frequency range of the sound of *C. afra* initiated almost an equal amount of activity with the 35-60 kHz, with a lag of twelve due to exhaustion. Therefore, the female *A. gambiae* were startled by the sound of *C. afra*, with maximum number of activities occurring in the 10-34 kHz range. The mosquito activities were sustained below 18 for all the ten mosquito samples under the influence of the sounds of *C. afra* as shown in Fig. 45, Fig. 46 and Table F2 in Appendix F.

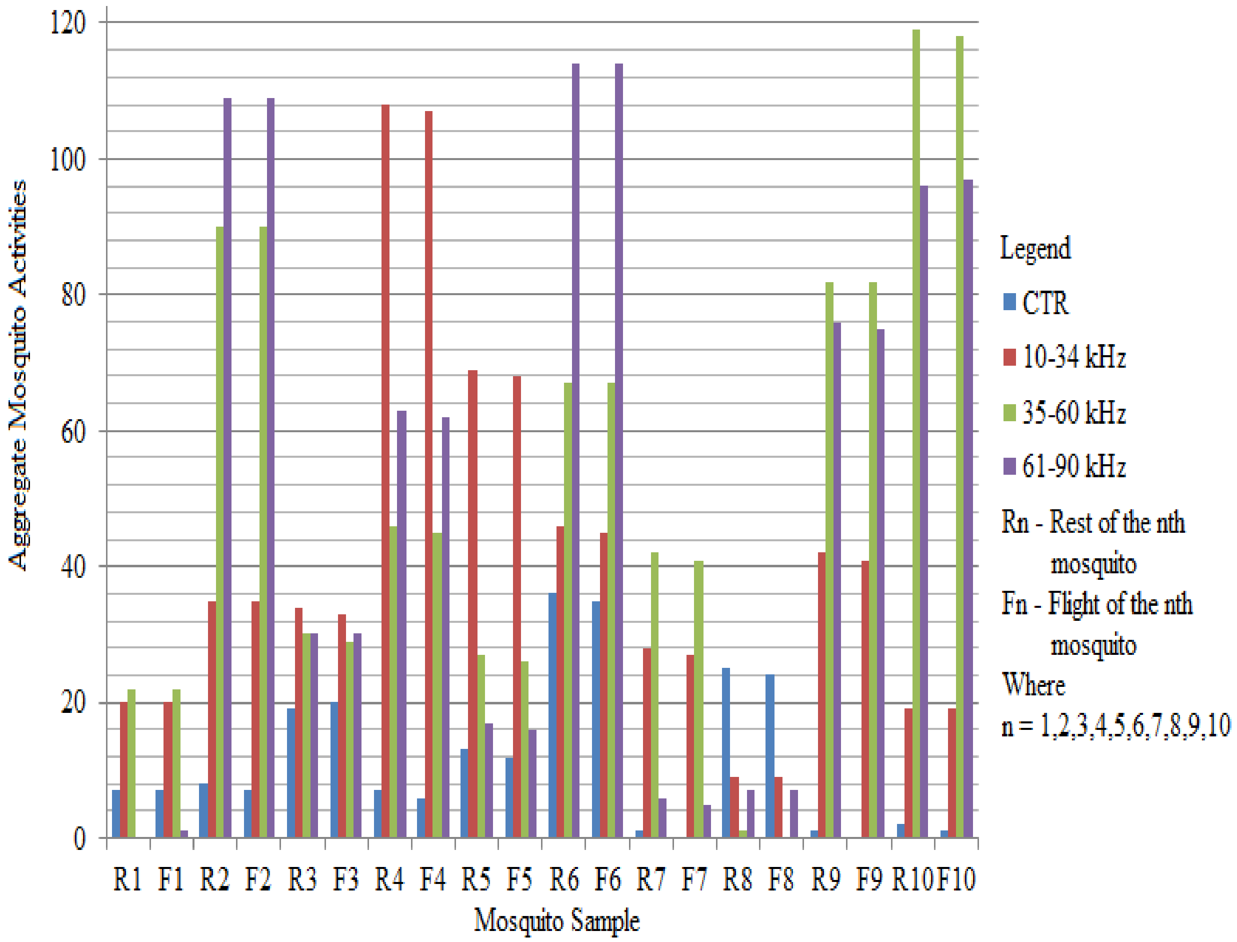

Fig. 43: The number of mosquito activities under varied sound frequencies of *A. tormotus*

Table 57: The average mosquito activities elicited by varied sounds of *A. tormotus*

Frequency range	Average activities per Mosquito
CTR	11.55
0-34 kHz	40.7
35-60 kHz	52.3
61-90 kHz	51.7

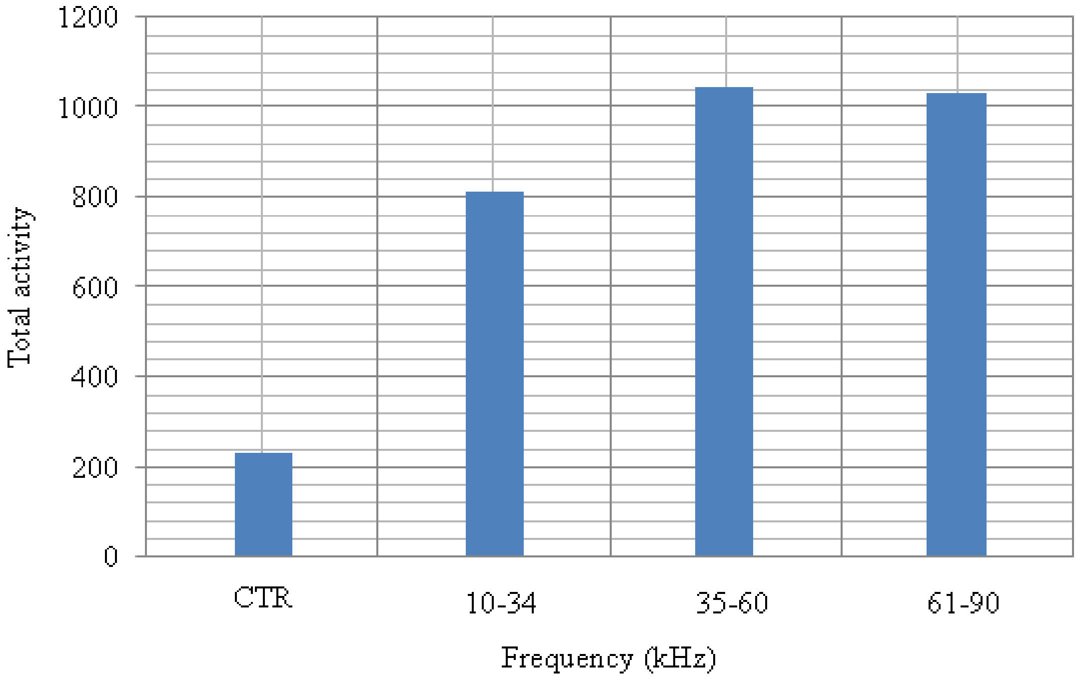

Fig. 44: The total mosquito activity under varied sound frequencies of *A. tormotus*

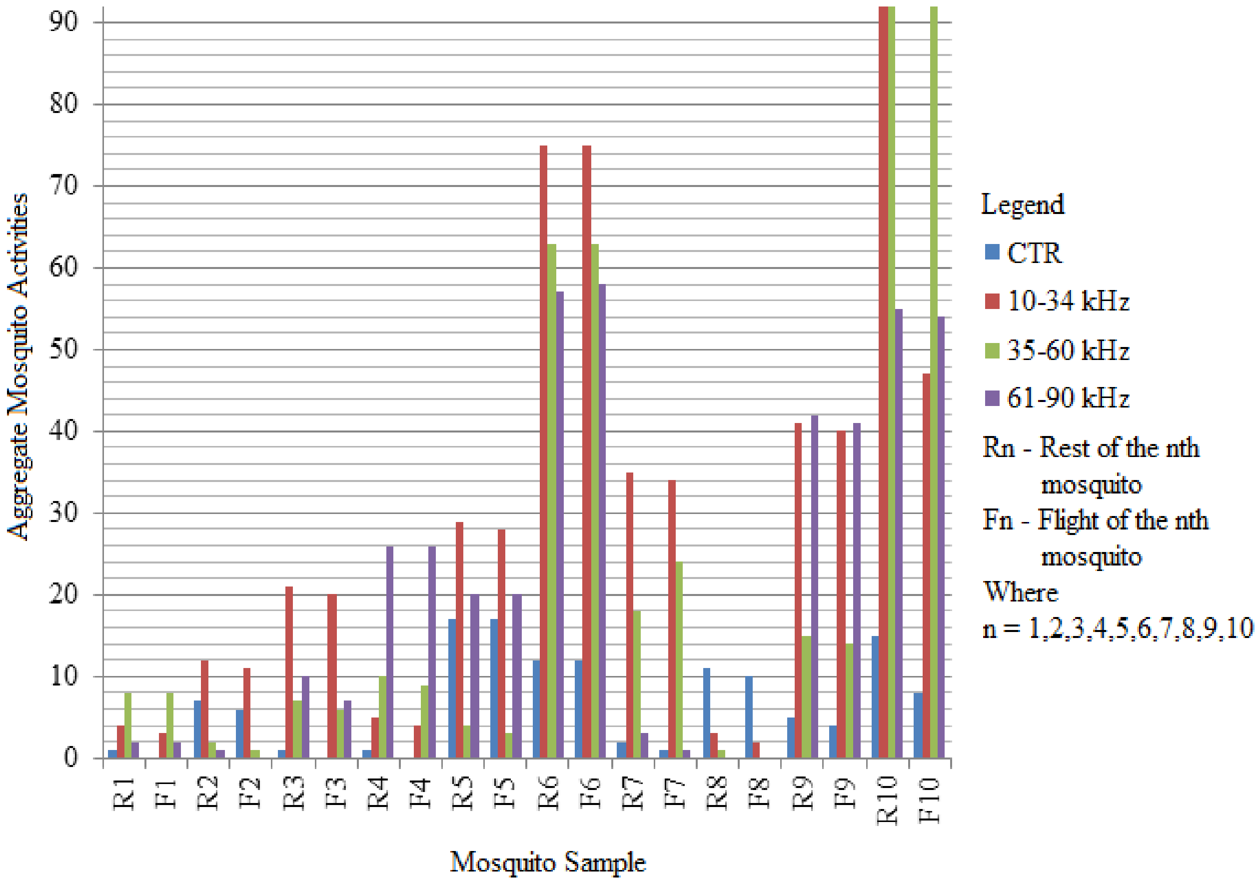

Fig. 45: The number of mosquito activities under varied sound frequencies of *C. afra*

Table 58: The average mosquito activities elicited by varied sounds of *C. afra*

Frequency range	Average activities per Mosquito
CTR	6.5
0-34 kHz	29.1
35-60 kHz	22.3
61-90 kHz	21.25

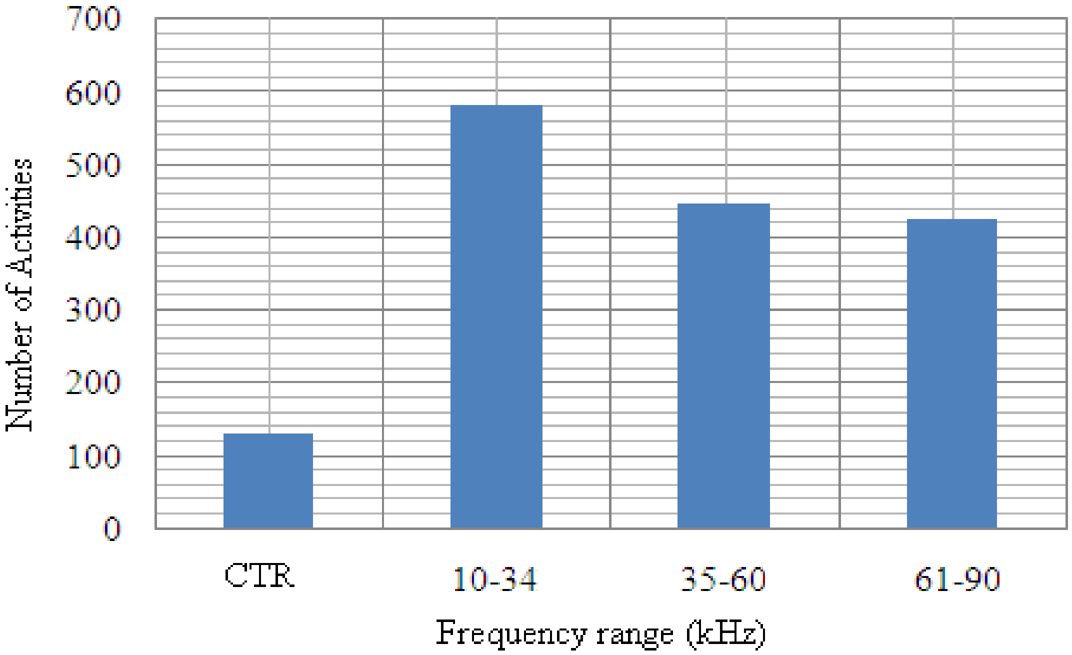

Fig. 46: The total mosquito activity under varied sound frequencies of *C. afra*

Exposure of the female *A. gambiae* to the 10-34 kHz sound of *C. afra* yielded a decline in mosquito activity as shown in Fig. 44 and Fig. 46. However, the decline in mosquito activity on exposure to the sound of *A. tormotus* began at 35-60 kHz, a frequency slightly higher than that in *C. afra*. The mosquito activities were sustained below 18 for all the ten mosquito samples under the influence of the sounds of *C. afra* as shown in Fig. 45 and Table F2 in Appendix F. The decline in maximum energy of the sound of *C. afra*, shown in Fig. 47, and the uniform decline in acoustic power resulted to progressive decline in mosquito activity as shown in Fig. 46. However, the maximum energy of the sound of *A. tormotus* increased slightly resulting to increased activity in mosquito samples up to 35-60 kHz frequency range. A decline in acoustic energy in the 61-90 kHz frequency range of the sound of sound of *A. tormotus*, shown in Fig. 47 resulted to a corresponding decline in activity as shown in Fig.43 and Fig.44 and Appendix F under Table F1. Though low, the power in the sound of *A. tormotus* remained almost constant in this frequency range.

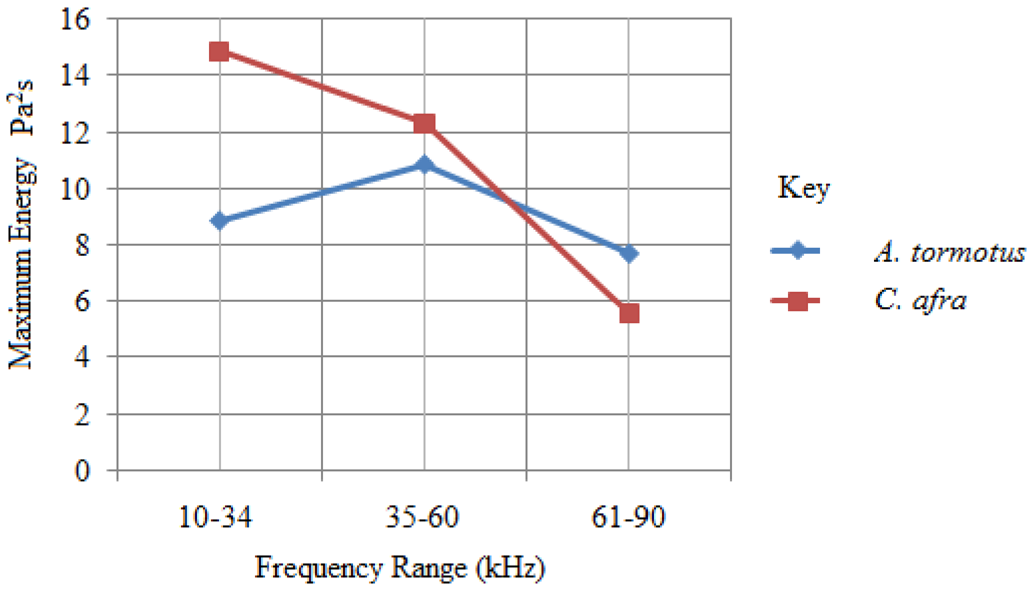

Fig. 47: The variation of predator maximum energy with frequency

The sound of *A. tormotus* and *C. afra* is detected by the antennae of which the ultrasound component causes neural stress on *A. gambiae*. The sounds of *A. tormotus* and *C. afra* which predate on *A. gambiae* also evoke natural fear of the animals emitting it. The sounds of *A. tormotus* and *C. afra* initiate avoidance response in mosquitoes, as reported in recent findings (Forrest *et al*., 1995; Roxanne, 2008; Mohankumar, 2010).

The rate of mosquito activity caused by the sounds of *A. tormotus* and *C. afra* which was based on a total time of 4.87 hr and shown in Appendix G as Table G1 and Table G2 respectively was greatest in the 35-60 kHz and 10-34 kHz frequency respectively. The rate of activities in the sampled mosquitoes rose to a maximum value of 92.62 activities per hour above the control on exposure to the 10-34 kHz sound frequency range of *C. afra*. The greatest rate of activity in mosquitoes due to the sound of *C. afra* was recorded in the 10-34 kHz sound frequency range with a slight decline of 27.83 activities per hour as it tended towards the 35-60 kHz and then 4.46 activities per hour towards 61-90 kHz. There was a slight decline by 2.45 rate of activity per hour as the frequency increased towards the 60-91 kHz for *A. tormotus*. The maximum rate of mosquito activity in *A. tormotus* was recorded in the 35-60 kHz frequency range. The trend line in Fig. 48 showed that the rate of activity per hour of the female mosquito increased at the rate of 54.20 Activities/hr under the sound of *A. tormotus*. However, the rate of mosquito activities per hour for *C. afra* in the same frequency range, shown in Fig. 49, declined on exposure to higher frequencies.

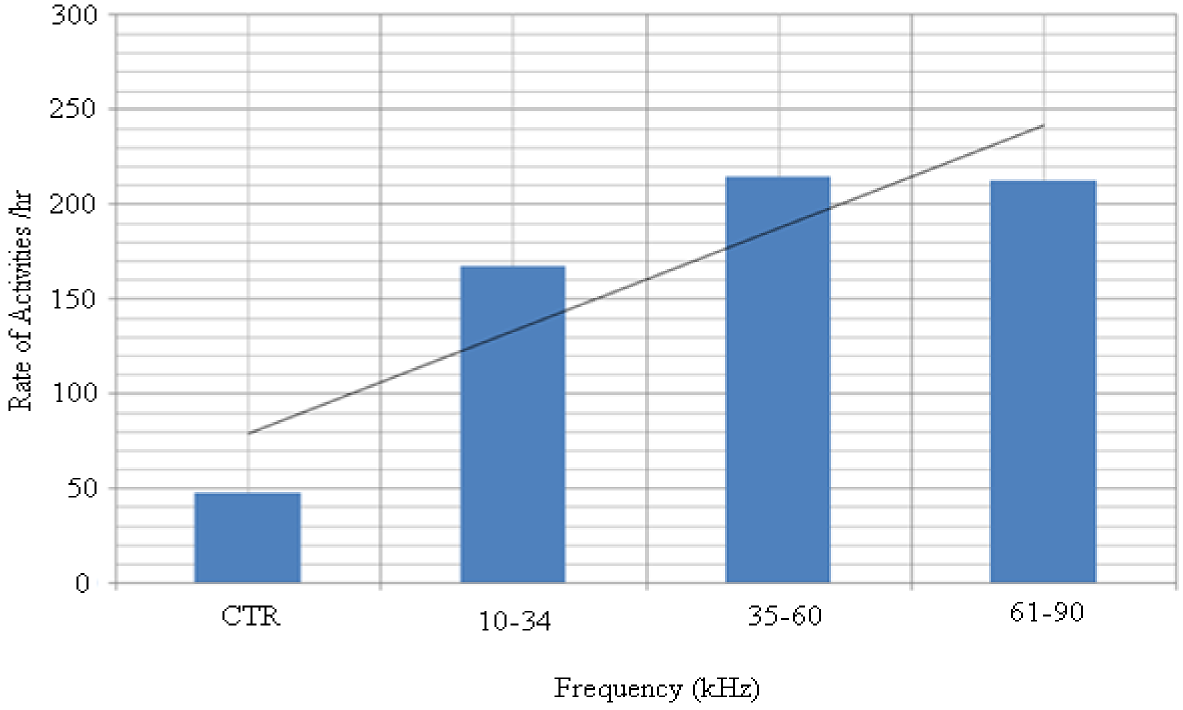

Fig. 48: The trend of rate of activity per hour with sound frequency ranges of *A. tormotus*

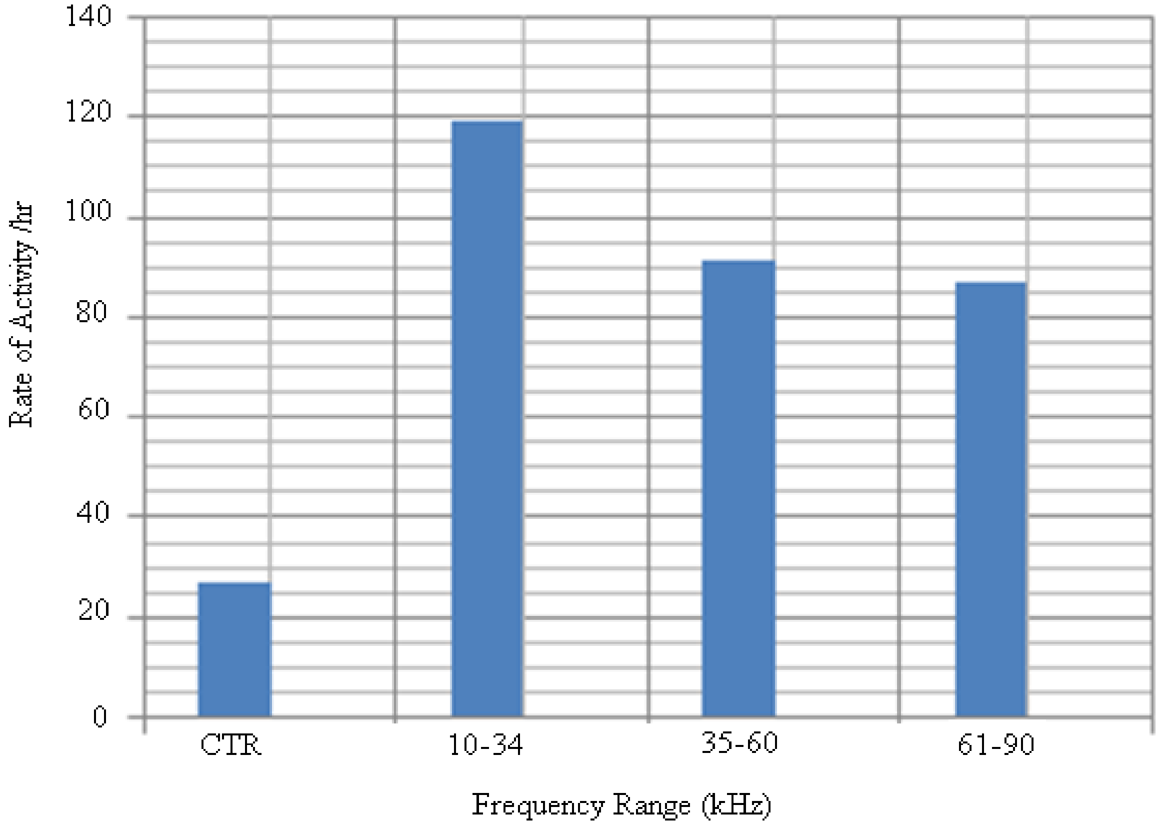

Fig. 49: The trend of rate of activity per hour with sound frequency ranges of *C. afra*

The one-way ANOVA comparison of the mosquito activities elicited by the 10-34 kHz, 35-60 and 61-90 kHz sound of *A. tormotus* by the mosquito activities under control was determined. The significance values obtained in this comparison was greater than 0.05 ($p > 0.05$) in all the frequency ranges. On the other hand, the comparison of the mosquito activities elicited by 10-34 kHz, 35-60 and 61-90 kHz sound of *C. afra* by the activities under the control yielded significance values less than 0.05 ($p < 0.05$). In comparison of mosquito activities in varied frequency ranges of individual predator sounds by the control, the significance values determined are shown in Table 59.

Table 59: Significance values of the comparison of mosquito activities in varied frequency ranges of individual predator sounds by the control

Sound source	Frequency Range	F	p
A. tormotus	Comparison of mosquito activities under the 10-34 kHz by the activities under the control	0.744	0.693
	Comparison of mosquito activities under the 35-60 kHz by the activities under the control	1.144	0.461
	Comparison of mosquito activities under the 61-90 kHz by the activities under the control	0.639	0.766
C. afra	Comparison of mosquito activities under the 10-34 kHz by the activities under the control	9.409	0.003
	Comparison of mosquito activities under the 35-60 kHz by the activities under the control	52.927	0.000
	Comparison of mosquito activities under the 61-90 kHz by the activities under the control	6.921	0.008

At 5 % significance level, there was no significant deviation in mosquito activities elicited by the 10-34 kHz. 35-60 kHz and 61-90 kHz sound of *A. tormotus* from the mosquito activities under the control. However, the deviation in mosquito activities elicited by 10-34 kHz, 35-60 kHz and 61-90 kHz sound of *C. afra* was significant from that of the mosquito activities under the control. Hence, the startle response, based on mosquito activity differed significantly in the 35-60 kHz for the sound of *C. afra* from the activities under the control.

(ii) The influence of combined sound of *A. tormotus* and *C. afra* on mosquito activities

The female *A. gambiae* remained suspended in air in the 10-34 kHz and 35-60 kHz frequency range; with their flight time distinctly above the control as shown in Table B3 in Appendix B, Table L3 in Appendix L and Fig. 50. There was an increase in total flight time from the control by 1926.10 s, an indication of the excitation due to ultrasound. However, there was a progressive decline in the total time of flight by 306.84 s as the frequency changed from 10-34 kHz to 35-60 kHz, and by 1740.89 s as it changed from 35-60 kHz to 61-90 kHz, shown in Appendix D as Table D3. The mosquitoes, therefore, rested for longer duration in the 61-90 kHz frequency range which was above the control; shown in Table K3 in Appendix K. In 60 % of mosquito samples exposed to the 10-34 kHz combined sound, the total flight time was distinctly above all the total flight time recorded in 36-60 kHz, 61-90 kHz and the control as shown in Fig. 51 and Table B3 in Appendix B. The samples displayed a docile behaviour in the range of 61-90 kHz with the total flight time for 40 % of the mosquito samples being least due to drastic drop in energy as shown in Fig. 36 and 37. Earlier studies reported that the mosquitoes' evasive behaviour was due to the stress caused on the nervous system and fear of predation (Forrest *et al.*, 1995; Roxanne, 2008; Mohankumar, 2010).

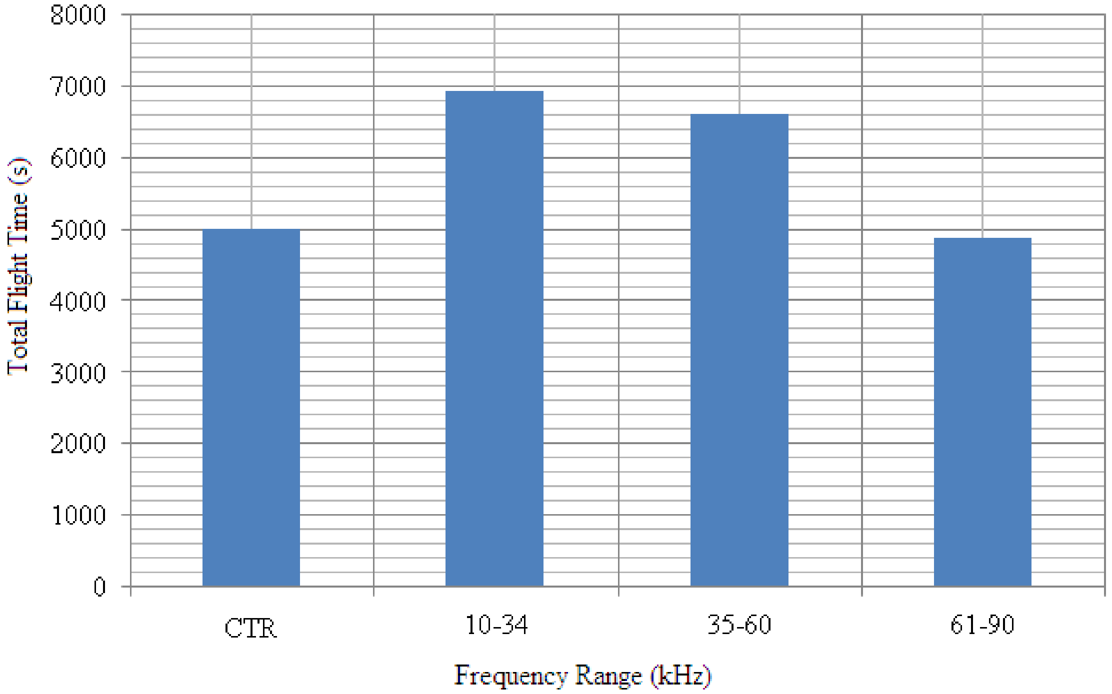

Fig. 50: The total mosquito flight time in relation to varied combined sound frequency

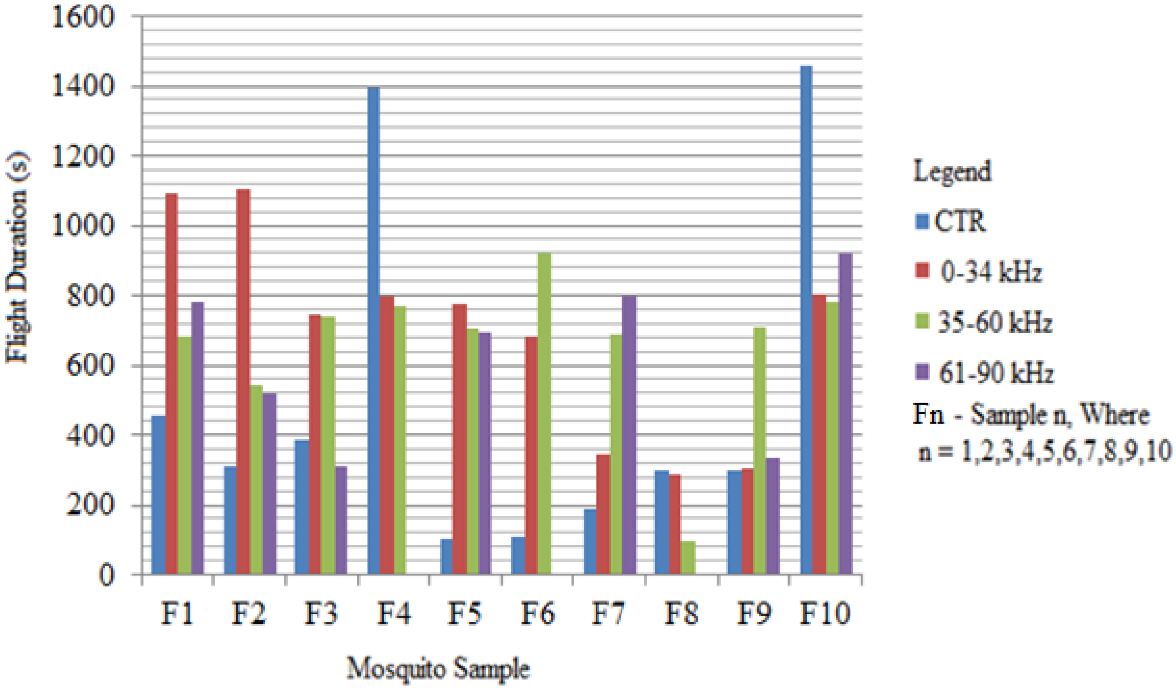

Fig. 51: Variation of mosquito flight duration with varied combined sound frequency

The rest time for the mosquitoes exposed to combined sound in the 10-34 kHz and 35-60 kHz frequency range was below the control. The mosquitoes were disturbed with the onset of the 10-34 kHz of the combined sound as noted in the sudden increase in flight time which was above the control. Other studies had reported that insects became dormant with some getting immobilized due to fatigue and stress (Forrest *et al.*, 1995; Roxanne, 2008; Mohankumar, 2010).

(iii) Mosquito activities under the influence of different frequencies of combined sound

The number of activities of the mosquitoes under the influence of different frequencies of the combined sound was critical in establishing the frequency range that evoked effective response in mosquitoes. The mosquitoes were considered to exhibit normal activity under the control experiment as shown in Table F3 in Appendix F, Fig. 52 and Fig. 53. It was noted that 60 % of the sample mosquito activities in the 10-34 kHz range were above all the rest time in other frequency ranges. All frequency ranges in the combined sound elicited activities in mosquitoes which were above the control experiment. More activities were exhibited by the female *A. gambiae* in the 10-34 kHz frequency range with minimum activities being exhibited at the control. The combination of the sound of *C. afra* and *A. tormotus* elicited increased activities in the female *A. gambiae* which were above the control as shown in

Fig.53. All the total mosquito activities in 10-34 kHz, 35-60 kHz and 61-90 kHz frequency ranges were above the control, an evidence for the startle effect of the combined sound on the female *A. gambiae*. The activities increased greatly from the control by 451, above the control. There was a slight decline by 181 activities as the frequency range changed from 10-34 kHz to 35-60 kHz. However, the activities increased to 755 in the 61-90 kHz, though still less than the activities in the 10-34 kHz frequency range illustrated in Fig. 54. The sampled mosquitoes displayed 92.81 activities per hour at the control experiment as shown in Fig. 54, which drastically rose by 185.42 activities per hour when the first sound of 10-34 kHz was played. The rate of activities per hour declined as the mosquitoes were exposed to 35-90 kHz, later increasing slightly to 155.05 activities per hour in the 61-90 kHz frequency range as shown in Appendix G as Table G3. The mean rate of activities for all in mosquitoes due to all predator sound is indicated in Table H1 in Appendix H. The activities exhibited in various frequency ranges were associated with respective behavioural responses discussed in 4.3.2 (i).

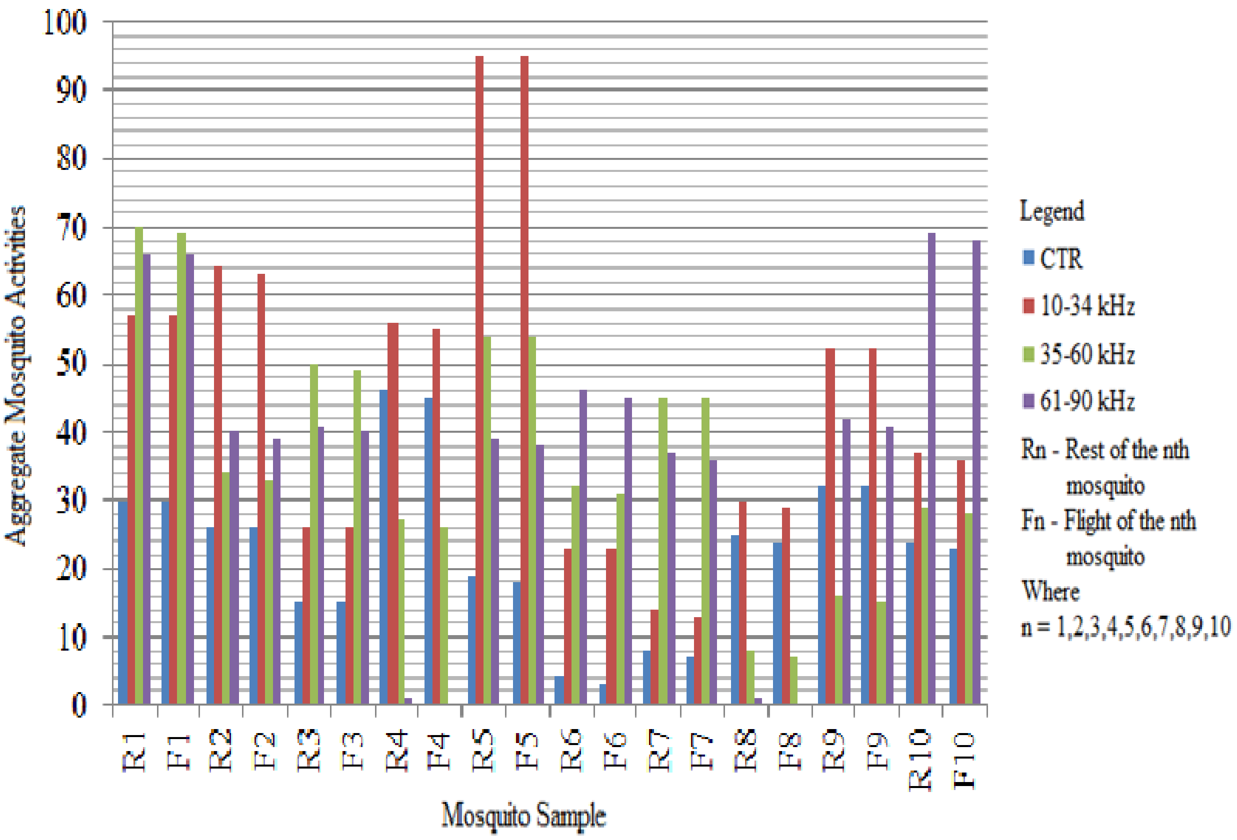

Fig. 52: Distribution of aggregate mosquito activities with varied combined sound frequencies

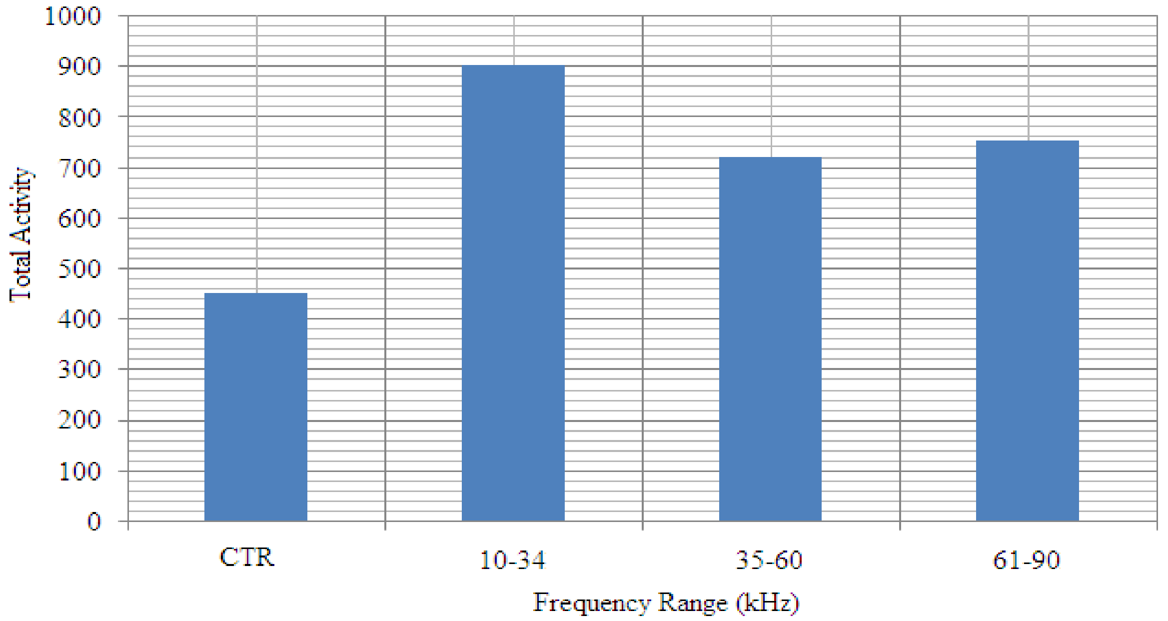

Fig. 53: Distribution of the total mosquito activity over different combined sound frequency ranges.

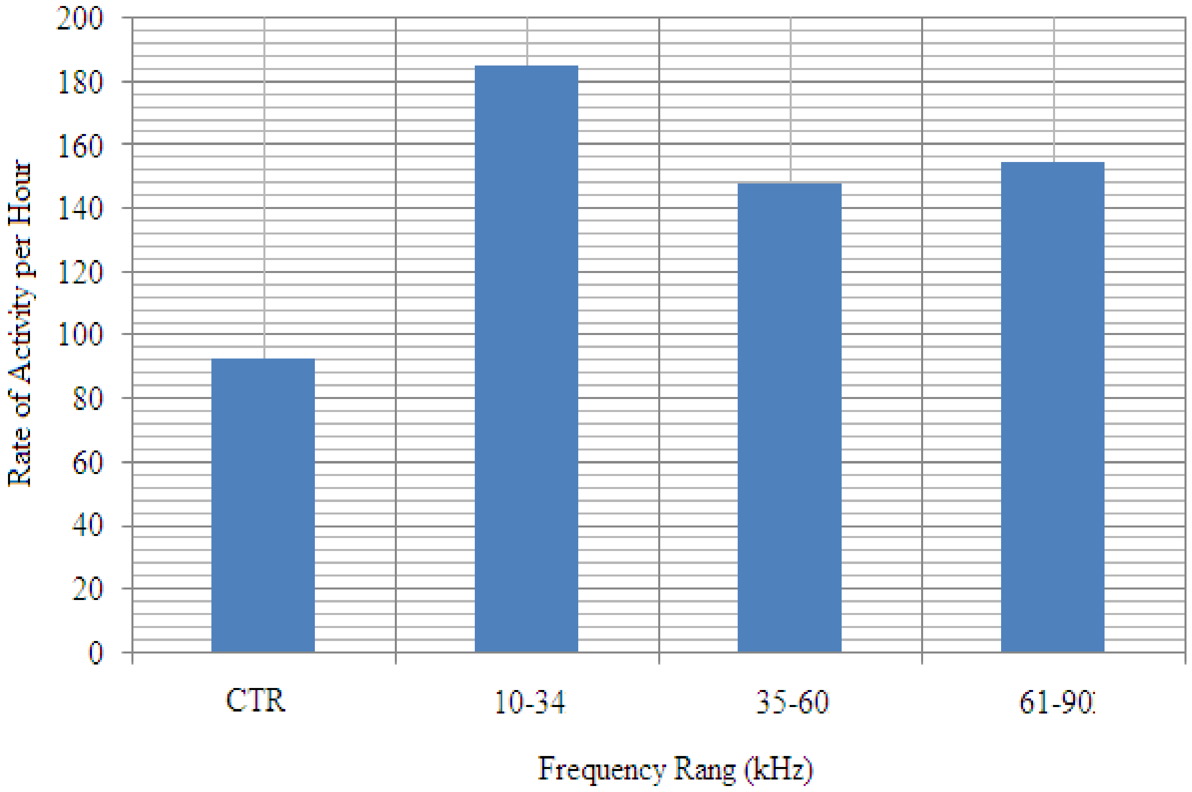

Fig. 54: Variation of the rate of mosquito activities with frequencies of combined sound

Though there was a decline in acoustic energy, the steady signal power of the combined sound in the 61-90 kHz frequency range yielded increased activity in mosquitoes. However, the signal power in the sound of *A. tormotus* fluctuated over time whereas the signal power of the sound of *C. afra* declined; lowering the mosquito activities.

The comparison of the mosquito activities in the10-34 kHz, 35-60 and 61-90 kHz combined sound ranges by activities under the control was determined and shown in Table 60. The combined sound in the 61-90 kHz frequency range yielded significance value, $p = 0.5343 > 0.05$. The combined sound under 10-34 kHz and 35-60 kHz yielded significance values, $p = 2.5657 \times 10^{-5} < 0.05$ and $0.0128 < 0.05$ respectively.

Table 60: Significance values of the comparison of mosquito activities in varied frequency ranges of the combined sound by the mosquitoes activity under the control

Parameter	F	p
Comparison of mosquito activities in 10-34 kHz of the combined sound by the mosquito activity under the control	116.045	2.5657×10^{-5}
Comparison of mosquito activities in 35-60 kHz of the combined sound by the mosquito activity under the control	8.767	0.0128
Comparison of mosquito activities in 61-90 kHz of the combined sound by the mosquito activity under the control	1.025	0.5343

There was sufficient evidence at 5 % significance level to show that the activities exhibited by mosquitoes due to the combined sound in the 10-34 kHz frequency range differed significantly from that at the control. The mosquito activities in 35-60 kHz frequency range also differed significantly from the control though the difference was less compared to that in 10-34 kHz. However, the mosquito activities in the 61-90 kHz did not differ significantly from the activities at the control.

The greatest deviation in one-way ANOVA comparison of the mosquito behavioural activities in the sound of *A. tormotus* and *C. afra* by the mosquito activities under their respective control was in the 35-60 kHz range, yielding a significance value $p = 0.461 > 0.05$ and $0.000 < 0.05$ respectively. The 10-34 kHz frequency range combined predator sound compared by the activities under the control yielded significance value, $p = 2.5657 \times 10^{-5} < 0.05$ in mosquito activities under one-way ANOVA comparison. Based on these optimum frequency ranges, further one-way ANOVA comparison of activities due to 10-34 kHz combined sound by the activities in the 35-60 kHz frequency range of the individual predator sound and the significance values indicated in Table 61. Further, the one-way ANOVA comparison of activities under the influence of the 10-34 kHz of the combined sound by the activities in the 35-60 kHz of the sound of *A. tormotus* yielded a significance value, $p = 0.000 < 0.05$. Similarly, comparison of activities due to 10-34 kHz combined sound by the activities in the 35-60 kHz of the sound of *C. afra* yielded a significance value, $p = 0.067 > 0.05$.

Table 61: Significance values of the comparison of mosquito activities due to 10-34 kHz combined sound by the mosquito activities in the 35-60 kHz individual predator sounds

Parameter	F	p
Comparison of mosquito activities due to 10-34 kHz combined sound by the mosquito activities in the 35-60 kHz sound of *A. tormotus*	5649.093	0.000
Comparison of mosquito activities due to 10-34 kHz combined sound by the mosquito activities in the 35-60 kHz sound of *C. afra*	4.916	0.067

At 5 % significance level, there was no significant deviation in mosquito activities elicited by the 10-34 kHz frequency range for the combined sound from the mosquito activities elicited under the 35-60 kHz range for sound of *C. afra*. However, the deviation in mosquito activities elicited by 10-34 kHz for the combined sound from the mosquito activities elicited under the 35-60 kHz frequency range for *A. tormotus* was highly significant. The two sounds; 10-34 kHz frequency of combined sound and 35-60 kHz frequency range for *A. tormotus* showed great variation in both behavioural response and rates of activities. The 10-34 kHz combined sound and 35-60 kHz sound of *A. tormotus* evoked behavioural response with mean percentage of 30.00 % and 45.88 % respectively, characterised by traits discussed in 4.3.1 a (i) and 4.3.1 a (ii). Similarly, the total mosquito activities for the 10-34 kHz combined sound and 35-60 kHz sound of *A. tormotus* were 452 and 1046 with their corresponding rates of activities shown in Appendix H as Table H2. The total mosquito activities and the number of mosquitoes affected under initial behavioural response reduced considerably by combining the sounds of *A. tormotus* and *C. afra*. The 10-34 kHz combined sound was characterised by reduced maximum acoustic energy by 2.77507 Pa^2s from the energy in this range for *A. tormotus*. The maximum and a minimum signal power of the combined sound fluctuated between -98 dB and -136.67 dB respectively which were equal to the power in the sound of *A. tormotus*. The sound of *A. tormotus* recorded progressive increase in acoustic energy by 1.9867 Pa^2s. The mean acoustic energy also increased correspondingly. The sound of *C. afra* recorded the greatest maximum acoustic energy in the 10-34 kHz and 35-60 kHz frequency range, above the combined sound and the sound of *A. tormotus*. However, the mean acoustic energy in the sound of *A. tormotus* was above that of the sound of *C. afra*. The sound of *A. tormotus* significantly startled the female *A. gambiae* compared to the combined sound in the 35-60 kHz frequency range. In general, the sound of *A. tormotus* had the greatest acoustic energy in the 10-34 kHz, 35-60 kHz and 61-90 kHz frequency ranges. In combining the sound of *A. tormotus* and *C. afra*, the mean acoustic energy reduced significantly from the energy of individual predator sounds. However, the startle effect on the mosquitoes for the combined sound was greater than that of the sound of *C. afra*, but very close to that of the sound of *A. tormotus*. Combining the sound of *A. tormotus* and *C. afra* did not yield any significant startle effect to the female *A. gambiae* compared to single animal species sound.

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

1. The acoustic transmission parameters included the fundamental frequency, bandwidth, peak amplitude, acoustic energy and peak frequency. The fundamental frequency of the sound of *A. tormotus* and *C. afra* was 5.371 kHz and 6.836 kHz respectively. The predator sounds showed presence of FM and CF modulation components with formants indicating harmonics. Similarly, the amplified mean peak amplitude for the sound of *A. tormotus* and *C. afra* was 134.08 dB SPL and 134.28 dB SPL respectively. The sound of *A. tormotus* and *C. afra* recorded a mean peak frequency of 47.60 kHz and 45.9 kHz respectively in the optimum frequency range. The maximum acoustic energy in the optimum frequency range for the sound of *A. tormotus* and *C. afra* was 10.84 Pa^2s and 12.32 Pa^2s respectively. The signal power for *A. tormotus* fluctuated between -100 dB and -120 dB maintaining whereas the power for *C. afra* uniformly declined from -55 dB to -59 dB. The mean bandwidth (maximum entire) of the sound *C. afra* and *A. tormotus* were 19.85 kHz and 19.40 kHz respectively. The sound of *C. afra* and *A. tormotus* recorded an equal maximum peak frequency (maximum entire) of 58.50 kHz.

2. The fundamental frequency of the combined sound of *A. tormotus* and *C. afra* was 4.883 kHz. The spectrograms of the combined sound revealed presence of FM and CF modulation components with formants indicating harmonics, just like the individual predator sound. The amplified mean peak amplitude for the combined sound of *A. tormotus* and *C. afra* was 133.60 dB SPL which is less than the mean peak amplitude of individual predator sound. The mean bandwidth of the combined sound was 16.40 kHz, narrowed by 3.40 kHz and 3.00 kHz from that of the sound of *C. afra* and *A. tormotus* respectively. The mean bandwidth of the combined sound was also significantly narrowed from 77.24 kHz, the reported mean bandwidth of EMR sound. Combining the sound of *C. afra* and *A. tormotus* yielded a mean peak frequency of 29.4 kHz in the optimum frequency range. Similarly, the combined predator sound recorded a maximum acoustic energy of 6.08 Pa2s.

3. Combining the sound of *A. tormotus* and *C. afra* did not yield significant startle effect to the female *A. gambiae* compared to single animal species sound. The combined predator sound and sound of *A. tormotus* evoked evasive behavioural responses in 30.0 % and 45.9 %

of the mosquitoes, higher than the reported 20.0 % effective repulsion by EMR sound. The sound of *A. tormotus* significantly startled the female *A. gambiae* compared to the combined sound. The startle response in the female *A. gambiae* due to the individual and combined sound of *A. tormotus* and *C. afra* was predominantly evasive, characterized by 58.5^{o} antenna erection, unusual rest and movement, attributed to stress on nervous system and fear of predation. Secondary effects of the ultrasound on the mosquitoes included physical injury, fatigue and falls. The optimum startle frequency for the individual sound of *A. tormotus* and *C. afra* on the female *A. gambiae* was 35-60 kHz. Combining the sound of individual predators yielded optimum startle response in the 10-34 kHz frequency range.

5.2 Recommendations for Further Research

Further investigation should be conducted in order to:

1. Determine the actual frequency within the optimal ranges that evokes repellency in female *A. gambiae*.
2. Determine the effectiveness of the sonar calls of *C. afra* in the repulsion of the female *A. gambiae*. The sound of *C. afra* used in the study included the sonar and social calls which seem to have lowered the gravity of its effect on the female *A. gambiae.*
3. Determine the relationship between the room size and the number of startled mosquitoes at a fixed frequency range.
4. Investigate the possibility of using the sound of *A. tormotus* to repel household insects such as cockroaches and ants.

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APPENDICES

APPENDIX A

Table A1: Acoustic transmission parameters for the sound of *A. tormotus*

	Frequency Range and Parameter Values								
	10-34 kHz			35-60 kHz			61-90 kHz		
Parameters	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Energy x 10^{-3} ($Pa^{2}s$)	14.63	8856.8	1219.86	5.21	10843.5	1339.24	0.44	7698.9	441.93
Peak ampl(maximum entire), Pa	93.50	112.74	103.24	95.69	113.56	103.48	94.33	114.33	96.50
Min freq (minimum entire), kHz	8.70	13.60	10.73	33.20	39.00	34.66	0.90	62.50	58.19
Max freq (maximum entire), kHz	16.60	33.20	22.57	42.90	60.50	55.14	84.90	247.00	96.20
Bandw (maximum entire), kHz	3.90	21.40	10.98	9.70	27.30	19.40	23.40	241.20	37.17
Peak frequency (maximum entire), kHz	12.60	20.50	16.18	34.10	58.50	47.63	61.50	88.80	73.61

Table A2: Acoustic transmission parameters for the sound of *C. afra*

	Frequency Range and Parameter Values								
	10-34 kHz			35-60 kHz			61-90 kHz		
Parameters	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Energy x 10^{-3} (Pa^2s)	0.16	14857.00	103.27	0.17	12322.90	50.10	0.20	5594.91	53.31
Peak ampl (maximum entire), Pa	93.13	113.13	99.83	93.03	113.03	98.21	94.08	114.07	99.07
Min freq (minimum entire), kHz	0.90	30.20	14.80	0.90	46.80	34.26	0.90	68.30	58.14
Max freq (maximum entire), kHz	19.50	243.10	33.67	37.10	194.30	55.48	63.40	247.00	80.73
Bandw (maximum entire), kHz	2.90	228.50	17.7055	2.90	181.60	19.85	2.90	246.00	21.58
Peak frequency (maximum entire), kHz	10.70	89.80	30.85	34.10	58.50	45.92	60.50	246.00	72.43

Table A3: Acoustic transmission parameters for the combined sound of *C. afra* and *A. tormotus*

	Frequency Range and Parameter Values								
	10-34 kHz			35-60 kHz			61-90 kHz		
Parameters	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Energy x 10^{-3} (Pa^2s)	0.08	6081.73	94.80	0.15	10342.50	52.06	0.23	5517.44	32.79
Peak ampl (maximum entire), Pa	91.02	111.01	95.72	92.93	112.93	98.20	93.85	113.85	98.80
Min freq (minimum entire), kHz	8.70	31.20	15.27	33.20	46.80	34.75	41.00	69.30	60.50
Max freq (maximum entire), kHz	13.60	34.10	32.68	37.10	59.50	55.42	63.40	240.20	74.67
Bandw (maximum entire), kHz	2.90	25.30	16.42	2.90	26.30	19.32	2.90	199.20	13.23
Peak frequency (maximum entire), kHz	10.70	33.20	29.40	34.10	58.50	46.33	60.50	87.80	69.56

APPENDIX B

Table B1: Flight duration for ten mosquitoes under different frequencies of *A. tormotus*

FREQ (kHz)	Mosquito sample number and total flight duration ±0.005s									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
CTR	77.46	89.93	577.40	98.83	285.90	156.88	0.00	172.01	0.00	5.67
10-34	459.03	442.09	1079.87	680.97	548.16	677.43	415.93	1063.95	292.66	139.23
35-60	892.92	739.02	595.70	518.48	65.85	259.61	232.82	0.00	769.35	915.56
61-90	1754.07	797.56	1003.96	492.05	267.73	481.71	43.16	1362.13	857.11	953.35

Table B2: Flight duration for ten mosquitoes under different frequencies of *C. afra*

FREQ (kHz)	Mosquito sample number and total flight duration ±0.005s									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
CTR	0.00	756.60	0.00	0.00	745.33	888.86	195.01	422.06	341.03	228.84
10-34	6.71	72.57	157.18	26.07	392.06	999.88	758.35	29.81	396.43	556.18
35-60	76.18	0.47	28.98	326.59	15.29	968.74	992.95	0.00	759.94	648.84
61-90	0.95	0.00	62.10	425.97	530.92	859.07	3.25	0.00	1023.87	431.90

Table B3: Flight duration for ten mosquitoes under different frequencies for the combined sound of *A. tormotus* and *C. afra*

FREQ (kHz)	Mosquito sample number and state duration ±0.005s									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
CTR	457.50	311.70	383.80	1396.00	101.30	106.80	191.60	300.70	299.90	1461.00
10-34	1093.00	1103.00	743.00	795.30	773.90	683.10	344.80	287.60	307.30	805.30
35-60	681.60	540.80	742.20	768.40	703.00	919.50	684.60	97.25	710.20	781.80
61-90	782.10	521.00	308.10	0.00	692.00	536.6.00	796.50	0.00	335.20	917.10

APPENDIX C

Table C1: The standard deviation for maximum and minimum frequencies of *A. tormotus*

Frequency Range, (kHz)	Minimum frequency, (minimum the entire), (kHz)	Maximum frequency, (kHz) (maximum entire), (kHz)
10-34	1.3994	5.5374
35-60	1.4510	4.7374
61-90	8.4804	30.4739

Table C2: The standard deviation for maximum and minimum frequencies of *C. afra*

Frequency Range, (kHz)	Minimum frequency, (minimum the entire), (kHz)	Maximum frequency (maximum entire), (kHz)
10-34	5.2810	7.6539
35-60	2.4064	5.1100
61-90	10.8450	32.7716

Table C3: The standard deviation for maximum and minimum frequencies of combined sound of *A. tormotus* and *C. afra*

Frequency Range, (kHz)	Minimum frequency (minimum entire), (kHz)	Maximum frequency (maximum entire),(kHz)
10-34	5.7668	2.4462
35-60	2.3670	3.9772
61-90	0.9670	6.8380

APPENDIX D

Table D1: Rest duration for ten mosquitoes under different frequencies for the sound of *A. tormotus*

Frequency (kHz)	Mosquito sample number and total rest duration ±0.005s									
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
CTR	1676.61	1664.14	1176.67	1655.24	1468.17	1597.19	1754.07	1562.06	1754.07	1748.40
10-34	1295.04	1311.98	674.20	1073.10	1205.91	1076.64	1338.14	690.12	1461.41	1614.39
35-60	861.15	1015.05	1158.37	1235.59	1688.22	1492.46	1521.25	1754.07	984.72	838.51
61-90	0.00	956.51	750.11	1262.02	1486.34	1272.36	1710.91	391.94	842.96	800.72

Table D2: Rest duration for ten mosquitoes under different frequencies of *C. afra*

Frequency (kHz)	Mosquito sample number and total rest duration ±0.005s									
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
CTR	1754.07	997.47	1754.07	1754.07	1008.74	865.21	1559.06	1332.01	1413.04	1525.23
10-34	1747.36	1681.5	1596.89	1728	1362.01	754.19	995.72	1724.26	1357.64	1197.89
35-60	1677.89	1753.6	1725.09	1427.48	1738.78	785.33	761.12	1754.07	994.13	1105.23
61-90	1753.12	1754.07	1691.97	1328.1	1223.15	895	1750.82	0.00	730.2	1322.17

Table D3: Rest duration for ten mosquitoes under different frequencies of the combined sound of *A. tormotus* and *C. afra*

Frequency (kHz)	Mosquito sample number and total rest duration ±0.005s									
	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
CTR	1297.00	1442.00	1370.00	358.00	1653.00	1547.00	1562.00	1553.00	1454.00	293.20
10-34	661.10	651.00	898.60	958.80	980.20	1071.00	1409.00	1466.00	1447.00	948.80
35-60	1072.00	1213.00	957.90	985.70	1051.00	834.60	1069.00	1657.00	1044.00	972.20
61-90	991.90	1233.00	1446.00	1754.00	1062.00	1218.00	957.60	1754.00	1419.00	837.00

APPENDIX E: DATA CAPTURE TOOL

DETERMINATION OF PREDATOR ULTRASOUND PARAMETERS AND ACOUSTIC STARTLE RESPONSE IN THE AFRICAN FEMALE *A. gambiae ss*

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SCORE SHEET (DATA CAPTURE) – LANDING RATES IN THE CAGE

Sound type: CTR ☐ *A. tormotus* ☐ *C. afra* ☐ Mixed *A. tormotus* & *C. afra* ☐

Frequency range (kHz): 10 - 34 ☐, 35 – 60 ☐, 61 – 90 ☐ 91 above☐

Sample No: 1 ☐ 2 ☐ 3 ☐ 4☐ 5 ☐ 6 ☐ 7 ☐ 8☐ 9☐ 10☐

(Tick appropriately)

Duration: 1754.07 s

Key: R: Rest State of female *A. gambiae*,
F: Flight/ Motion State of female *A. gambiae*
CTR : Control (No sound played)

Mosq. State	Duration (s)		Mosq. State	Duration (s)
1R			6R	
1F			6F	
2R			7R	
2F			7F	
3R			8R	
3F			8F	
4R			9R	
4F			9F	
5R			10R	
5F			10F	

11R			27R	
11F			27F	
12R			28R	
12F			28F	
13R			29R	
13F			29F	
14R			30R	
14F			30F	
15R			31R	
15F			31F	
16R			32R	
16F			32F	
17R			33R	
17F			33F	
18R			34R	
18F			34F	
19R			35R	
19F			35F	
20R			36R	
20F			36F	
21R			37R	
21F			37F	
22R			38R	
22F			38F	
23R			39R	
23F			39F	
24R			40R	
24F			40F	
25R			41R	
25F			41F	
26R			42R	
26F			42F	

43R			59R	
43F			59F	
44R			60R	
44F			60F	
45R			61R	
45F			61F	
46R			62R	
46F			62F	
47R			63R	
47F			63F	
48R			64R	
48F			64F	
49R			65R	
49F			65F	
50R			66R	
50F			66F	
51R			67R	
51F			67F	
52R			68R	
52F			68F	
53R			69R	
53F			69F	
54R			70R	
54F			70F	
55R			71R	
55F			71F	
56R			72R	
56F			72F	
57R			73R	
57F			73F	
58R			74R	
58F			74F	

APPENDIX F

Table F1: Mosquito activities due to the influence of varied sound frequencies of *A. tormotus*

Mosquito Sample Activity	CTR	10-34 kHz	35-60 kHz	61-90 kHz
R1	7	20	22	0
F1	7	20	22	1
R2	8	35	90	109
F2	7	35	90	109
R3	19	34	30	30
F3	20	33	29	30
R4	7	108	46	63
F4	6	107	45	62
R5	13	69	27	17
F5	12	68	26	16
R6	36	46	67	114
F6	35	45	67	114
R7	1	28	42	6
F7	0	27	41	5
R8	25	9	1	7
F8	24	9	0	7
R9	1	42	82	76
F9	0	41	82	75
R10	2	19	119	96
F10	1	19	118	97

Table F2: Mosquito activities due to the influence of varied sound frequencies of *C. afra*

Mosquito Sample Activity	CTR	10-34 kHz	35-60 kHz	61-90 kHz
R1	1	4	8	2
F1	0	3	8	2
R2	7	12	2	1
F2	6	11	1	0
R3	1	21	7	10
F3	0	20	6	7
R4	1	5	10	26
F4	0	4	9	26
R5	17	29	4	20
F5	17	28	3	20
R6	12	75	63	57
F6	12	75	63	58
R7	2	35	18	3
F7	1	34	24	1
R8	11	3	1	0
F8	10	2	0	0
R9	5	41	15	42
F9	4	40	14	41
R10	15	93	95	55
F10	8	47	95	54

Table F3: Mosquito activities due to the combined sound of *A. tormotus* and *C. afra*

Mosquito Sample Activity	CTR	10-34 kHz	35-60 kHz	61-90 kHz
R1	30	57	70	66
F1	30	57	69	66
R2	26	64	34	40
F2	26	63	33	39
R3	15	26	50	41
F3	15	26	49	40
R4	46	56	27	1
F4	45	55	26	0
R5	19	95	54	39
F5	18	95	54	38
R6	4	23	32	46
F6	3	23	31	45
R7	8	14	45	37
F7	7	13	45	36
R8	25	30	8	1
F8	24	29	7	0
R9	32	52	16	42
F9	32	52	15	41
R10	24	37	29	69
F10	23	36	28	68

APPENDIX G

Table G1: Rate of mosquito activity per hour under the sound frequencies of *A. tormotus*

Frequency	Rate of Activity/hr
CTR	47.41
10-34kHz	167.06
35-60kHz	214.68
61-90kHz	212.22

Table G2: Rate of mosquito activity per hour under the sound frequencies of *C. afra*

Frequency	Rate of Activity/hr
CTR	26.78
10-34	119.4
35-60	91.57
61-90	87.11

Table G3: Rate of mosquito activity per hour under the combined predator sound

Frequency	Rate of Activity/hr
CTR	92.81
10-34	185.42
35-60	148.25
61-90	155.03

APPENDIX H

Table H1: Rate of mosquito activity per hour under the different sound frequencies of *A. tormotus*, *C. afra* and their combination

	Rate of activity per mosquito (mean rate of activities)		
Frequency Range (kHz)	Combined Sound (*C. afra* and *A. tormotus*)	*C. afra*	*A. tormotus*
CTR	9.281	2.678	4.741
10 -34	18.542	11.94	16.706
35 – 60	14.825	9.157	21.468
61 – 90	15.503	8.711	21.222

Table H2: Total mosquito activity under the different sound frequencies of *A. tormotus*, *C. afra* and their Combination

	Total mosquito activity		
Frequency Range (kHz)	Combined Sound (*C. afra* and *A. tormotus*)	*C. afra*	*A. tormotus*
CTR	452	130	231
10 -34	903	581	814
35 – 60	722	446	1046
61 – 90	755	424	1034

APPENDIX I

Table I: The acoustic transmission parameters for the original sound of *C. afra*

Parameters	Parameter Values		
	Minimum	Maximum	Mean
Peak amplitude (maximum entire), Pa	85.62	112.07	97.3658
Maximum frequency (maximum entire), Hz	19500	97600	59992.1
Bandwidth (maximum entire), Hz	3900	96600	46262.1
Minimum frequency (minimum entire), Hz	900	33200	9834.81

APPENDIX J

Table J1: Percentage of mosquito samples under the control experiment for sounds of *A. tormotus, C. afra* and their combination

	Percentage of mosquito samples under the control experiment		
Mosquito behavior	*A. tormotus*	*C. afra*	Combined
No body movement	10	0	0
Squeezing/ hiding in barriers	10	0	0
Raised limbs	10	0	0
Normal movement in the cage	70	80	60
Rubbing of legs and/or wings	20	20	10
Normal flight about in the cage	60	70	10
Rest at 45^o from rest surface ; wings along body	80	70	90
Limbs and proboscis resting on net or cage	0	10	0
Antennae and proboscis almost collinear	100	100	100

Table J2: Percentage of mosquito samples under varied sound frequency ranges for *A. tormotus*, *C. afra* and their combination

Observable mosquito behavioural traits	*A. tormotus* frequency (kHz)			*C. afra* frequency (kHz)			Combined sound frequency (kHz)		
	0-34	35-60	61-90	0-34	35-60	61-90	0-34	35-60	61-90
No body movement	30	20	10	30	70	20	0	10	10
Jumping and/or Bouncing	50	50	30	0	0	0	50	50	40
Squeezing body and proboscis/ hiding in barriers	90	70	30	30	50	10	40	90	30
Raised limbs/ folded limbs	60	40	40	40	30	0	50	70	70
Raising and lowering of body	50	50	40	20	40	0	20	30	10
Forward/ backwards or sideways body movement	10	40	10	10	0	0	0	30	0
Body shaken/ Abdomen curving thorax	60	80	80	30	60	20	70	50	40
Rubbing of limbs or wings	30	20	0	30	10	20	40	10	10
Rest by abdomen/ thorax with limbs on surface	40	40	70	20	20	0	30	70	50
Flapping or opening of wings	30	40	50	10	20	0	30	30	40
Weak or exaggerated flights, falls and escape	30	60	30	40	30	40	30	40	40
Movement away from sound source	60	50	30	30	30	30	10	30	20
Spreading of limbs when resting	30	50	10	0	10	0	50	30	20
Erect antennae	10	80	30	0	0	0	50	70	80
Tired or weak or collapsed mosquito	0	20	30	0	0	0	10	0	20
Rest by back/ Sideways rest / Rolling on surfaces	0	60	70	0	20	0	30	50	90
Loss of limbs	0	10	20	0	0	0	0	0	10

APPENDIX K

Table K1: Total rest time of mosquito under the sound frequencies of *A. tormotus*

Frequency (kHz)	Total Rest time (s)
Control	16056.62
10-34	11740.93
35-60	12549.39
61-90	9473.87

Table K2: Total rest time of mosquito under the sound frequencies of *C. afra*

Frequency (kHz)	Total Rest time (s)
Control	13962.97
10-34	14145.46
35-60	13722.72
61-90	12448.60

Table K3: Total rest time of mosquito under the sound frequencies of combined predator

Frequency (kHz)	Total Rest time (s)
Control	12530.55
10-34	10492.05
35-60	10857.29
61-90	12672.18

APPENDIX L

Table L1: Total flight time of mosquito under the sound frequencies of *A. tormotus*

Frequency (kHz)	Total Flight time (s)
Control	1464.08
10-34	5799.32
35-60	4989.31
61-90	8012.83

Table L2: Total flight time of mosquito under the sound frequencies of *C. afra*

Frequency (kHz)	Total Flight time (s)
Control	3577.73
10-34	3395.24
35-60	3817.98
61-90	3338.03

Table L3: Total flight time of mosquito under the sound frequencies of combined predator

Frequency (kHz)	Total Flight time (s)
Control	5010.15
10-34	6936.25
35-60	6629.41
61-90	4888.52