

DECLARATION

STUDIES ON THE PHLEBOTOMINE SANDFLIES OF RUSINGA ISLAND AND
THEIR POTENTIAL AS VECTORS OF LEISHMANIASIS

BY

MISIANI, EUNICE ATIENO GAI
B.ED. (SCIENCE) KENYATTA UNIVERSITY

THIS THESIS IS SUBMITTED IN PARTIAL FULFILLMENT OF THE DEGREE OF
MASTER OF SCIENCE (APPLIED PARASITOLOGY), KENYATTA
UNIVERSITY


1992

KENYATTA UNIVERSITY LIBRARY

DECLARATION

This thesis is my own work and has not been presented for a degree in any other University.

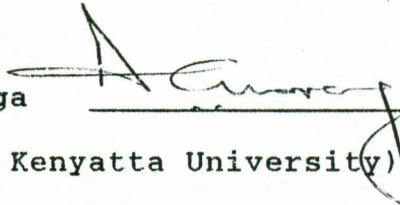
Misiani, Eunice Atieno

 17/2/92

This thesis has been submitted for examination with our approval as Supervisors.

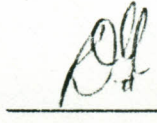
1. Dr. Charles B. Maranga

(Zoology Department, Kenyatta University)

 21/2/92

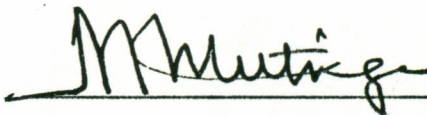
2. Dr. Romanus O. Okelo

(Zoology Department, Kenyatta University)

 21/2/92

3. Dr. Mutuku J. Mutinga

(Medical Vectors Research Programme, International Centre of Insect Science and Physiology (ICIPE))



ABSTRACT

LINKS OF THE PHLEBOTOMINE SAND FLIES OF KUSINGA ISLAND
SOUTH WYANDIA DISTRICT, KENYA, AND THEIR POTENTIAL AS

DEDICATION

This work involved a one-year study of the phlebotomine sandflies of Kusinga Island. The existence of sandflies had been reported in the vicinity by the Medical Services. I dedicate this thesis to my parents Mr. and Mrs. Dan Gal Aloo for their tireless efforts to give me a good educational foundation early in life; and to my beloved husband Sam Misiani without whose encouragement this Thesis would not have been possible.

ABSTRACT**STUDIES ON THE PHLEBOTOMINE SANDFLIES OF RUSINGA ISLAND
(SOUTH NYANZA DISTRICT, KENYA) AND THEIR POTENTIAL AS
VECTORS OF LEISHMANIASIS**

This work involved a one-year study of the phlebotomine sandflies of Rusinga Island. The existence of sandflies had been reported in the vicinity by the Medical Vectors Research Programme of the International Centre for Insect Science and Physiology (ICIPE) in 1986 (Anon. 1988) but no work had since been carried out on the flies of this region. Due to the toxicity, expense and lack of total effectiveness of drugs for treating leishmaniasis (Lainson and Shaw, 1978) control may have to depend on effective integrated management of reservoirs and/or insect vectors. It is thus important to study the sandfly population and to find out if known vectors and reservoirs of the disease do exist in places in which sandflies are known to exist and determine what potential they have as vectors and reservoirs of leishmaniasis. It is also necessary to find out if the disease has a local focus or whether it has the potential of being introduced. The objectives of the study were threefold:-

1. To identify the sandflies of Rusinga Island and determine their species incidence
2. To determine their seasonal incidence and climatic

factors affecting their abundance

3. To determine their importance as medical or veterinary vectors through dissection and blood-meal analysis.

The sticky trap (polythene sheet smeared with castor oil), mouth-suction trap and fan-suction light traps were used to trap the flies. A standard size of one metre by one metre was used for the sticky trap. The sticky trap and fan-suction traps were set in the evenings between 1730 hours and 1845 hours and left overnight. They were removed the following morning between 0600 hours and 0730 hours. The traps were set in twenty-eight different sites (approximately 2 kilometres apart) in five sectors of the Island. The flies were then washed and mounted. Identification was then done using keys developed by Lewis (1973), Abonnenc (1972), Kirk and Lewis (1951) and keys developed by Mutinga (unpublished); all based on detailed morphological characteristics. Fresh female flies (those removed from the traps and worked upon immediately) were dissected and any suspected promastigotes were cultured in Novy, Mac'Neal and Nicolle's (NNN) medium (Taylor and Baker, 1986) for five days. Fed female flies were used for blood-meal analysis. This analysis was done by Dr. C. Staak at the Robert von Ostertag Institut (Berlin, Germany). Weather data were collected daily from the Rusinga Meteorological Station.

A total of twelve sandfly species were found on the Island and eleven of them belonged to the *Sergentomyia* genus while one belonged to the *Phlebotomus* genus. When the Anova test was done the difference between the species was found to be highly significant ($F = 65.24$, $P < 0.0001$, $DF = 8$). The most abundant species was *Sergentomyia antennatus* followed by *Sergentomyia bedfordi*, then *S. serratus*, *S. schwetzi*, *S. ingrami*, *S. africanus*, *S. squamipleuris* and *S. affinis* in descending order. Only two flies of the species *S. graingeri* and *S. clydei* and one fly each, of the species, *S. inermis*, and *Phlebotomus rodhaini* were encountered. The mean number of female flies was found to be more than that of the male flies. Among the habitats from which the flies were collected, they were most abundant in tree holes, followed by termite hills, toilets, outside houses, inside houses, cow-sheds and in the vegetation and rock crevices at the lakeshores.

Statistical analysis showed that relative humidity, wind-speed and hours of sunshine had a significant correlation with the abundance of the flies. Relative humidity had a significant negative correlation ($r = -0.1009$, $P < 0.0001$, $DF = 1898$) as did wind-speed ($r = -0.0488$, $P < 0.0019$, $Df = 1898$). During the month of April there was a negative significant relationship between sandfly abundance and rainfall. The "peak season" for the flies occurred during

the months of June and October, that is, just after the rainy season and just before the onset of the short rains (November-December). There was a significant difference between the five sectors of the Island as far as the population of flies in them was concerned. Three of them (on the southern side of the Island) were different from the two sectors on the northern part. This was also reflected when correlation analysis was done for fly population and humidity in the different sectors. There was a significant negative correlation between humidity and sandfly population in three of the sectors and not in the other two, implying that the differences mentioned earlier could be due to differences in the humidity of the various sectors.

Some species of the flies did not appear throughout the year, for example, *Sergentomyia affinis* was only found during the short rains and the peak season for the sandflies (June and October). Other species were "perennial" and were caught throughout the year.

None of the "flagellates" inoculated in the NNN medium proved positive for *Leishmania* parasites. The blood-meal analysis showed that the fed flies caught fed on chicken and monitor lizards; none was anthropophilic. They apparently preferred monitor lizards (66.67 %) to all other animals. Some of the fly species that were found are alternate zoonotic vectors elsewhere (for example *Sergentomyia ingrami*

is an alternate zoonotic vector of *Leishmania major* in Baringo District). However, at the moment the sandflies of Rusinga Island though having potential of being vectors of leishmaniasis do not pose any present danger of being vectors since they neither have the parasites nor are they anthropophilic. But if the parasites are transported from an endemic focus to the Island, there could be a possibility of the disease occurring in the area, just in the same way that visceral leishmaniasis was introduced into Kenya from Sudan (Adler, 1964).

CHAPTER TWO	
2.0. SEASONAL VARIATION IN THE INCIDENCE OF SANDFLIES IN RUSINGA ISLAND	1
2.1. INTRODUCTION	1
2.2. STUDY AREA	1
2.3. MATERIALS AND METHODS	2
2.3.1. Trapping and Collection of Sandflies	2
2.3.1.1. The Sticky Trap	2
2.3.1.2. The Mouth-Suction Trap	2
2.3.1.3. The Mechanical Light Trap	2
2.3.2. PROCESSING AND MOUNTING OF THE SANDFLIES	2
2.3.3. IDENTIFICATION	2
2.4. RESULTS	2
2.4.1. Sandfly species Recorded	2
2.4.2. Analysis of Results	2
2.4.3. Seasonal Incidence of the Species of Sandflies	2
2.5. DISCUSSION	2
CHAPTER THREE	
3.0. THE SANDFLY SPECIES COMPOSITION AND SEASONAL VARIATION IN RUSINGA ISLAND	1
3.1. INTRODUCTION	1
3.2. MATERIALS AND METHODS	2
3.2.1. Trap	2
3.2.2. Ident.	2
3.3. RESULTS	2
3.3.1. Species Composition in various Habitats	2
3.3.2. Variation in the Species Composition in the different months	2

TABLE OF CONTENTS

	Page
Title	1
DECLARATION	2
DEDICATION	3
ABSTRACT	4
TABLE OF CONTENTS	9
LIST OF TABLES	10
LIST OF FIGURES	11
ACKNOWLEDGEMENT	13
INTRODUCTION	
MATERIALS AND METHODS	
CHAPTER ONE	14
1.0. INTRODUCTION AND LITERATURE REVIEW	14
1.1. General Introduction	14
1.2. Literature Review	17
1.3. JUSTIFICATION OF THE THESIS	24
1.4. OBJECTIVES	27
CHAPTER TWO	35
2.0. SEASONAL VARIATION IN THE INCIDENCE OF SANDFLIES IN RUSINGA ISLAND	35
2.1. INTRODUCTION	35
2.2. STUDY AREA	35
2.3. MATERIALS AND METHODS	37
2.3.1. Trapping and Collection of Sandflies	37
2.3.1.1. The Sticky Trap	38
2.3.1.2. The Mouth-Suction Trap	39
2.3.1.3. The Mechanical Light Trap	41
2.3.2. PROCESSING AND MOUNTING OF THE SANDFLIES	42
2.3.3. IDENTIFICATION	42
2.4. RESULTS	43
2.4.1. Sandfly species Recorded	43
2.4.2. Analysis of Results	43
2.4.3. Seasonal Incidence of the Species of Sandflies..	53
2.5. DISCUSSION	55
LIST OF TABLES	
CHAPTER THREE	59
3.0. PHLEBOTOMINE SANDFLY SPECIES COMPOSITION AND INCIDENCE IN RUSINGA ISLAND	59
3.1. INTRODUCTION	59
3.2. MATERIALS AND METHODS	60
3.2.1. Trapping and Collection	60
3.2.2. Identification	60
3.3. RESULTS	61
3.3.1. Species Incidence in various Habitats	67
3.3.2. Variation in the Species Composition in the different Months	69

	PAGE
3.3.2.1. Sandfly Incidence in different Sectors during various Months	70
3.3.3. Species Variation within the different Sectors	72
3.3.4. Variation in the Sexes of the flies	84
3.4. Altitudinal Variation	48
3.5. DISCUSSION	88
CHAPTER FOUR	91
4.0. POTENTIAL OF THE RUSINGA PHLEBOTOMINE SANDFLIES AS VECTORS OF LEISHMANIASIS	91
4.1. INTRODUCTION	91
4.2. MATERIALS AND METHODS	92
4.2.1. DISSECTION	92
4.2.1.1. Trapping and Collection of Sandflies	92
4.2.1.2. Dissection of female flies	92
4.2.2. Parasite Culturing	93
4.2.3. Blood-meal Analysis	94
4.2.3.1. Trapping and Collection of flies	94
4.2.3.2. Analysis of the Meals	95
4.3. RESULTS	95
4.3.1. Dissection	95
4.3.2. Blood-meal Analysis	95
4.4. DISCUSSION	100
CHAPTER FIVE	103
5.0. GENERAL DISCUSSION	103
5.1. Recommendations	107
LITERATURE CITED	109
APPENDIX	123

LIST OF TABLES

Table 1. Description of the Sectors	40
Table 2. Analysis of the Various Factors that influenced Sandfly Catches during the study period (GLM procedure on Square-root transformed data	44
Table 3. Duncans' Multiple Range Test (Months)	45
Table 4. Monthly Rainfall during the Study Period (January - December 1990)	48
Table 5. Duncans' Multiple Range Test (Flies in the Sectors)	49
Table 6. Duncans' Multiple Range Test (Species)	63
Table 7. Duncans' Multiple Range Test (Flies in the Different Habitats	64

APPENDIX

Table 8.	Duncans' Multiple Range Test (Variation of flies in the Habitats in July)	65
Table 9.	Duncans' Multiple Range Test (Variation of flies in the Habitats in October)	66
Table 10.	DMRT of the Species in Sectors 4 and 5 in July	73
Table 11.	DMRT for Habitats in Sector 1 and 3 in June...	74
Table 12.	Duncans' Multiple Range Test (Species in Sector 3 in June)	75
Table 13.	DMRT (Species in Sectors 1, 3, and 4 in May) ..	76
Table 14.	GLM Results and DMRT for Species in Sector 4 in September	77
Table 15.	DMRT for Species in Sector 1 in October.....	78
Table 16.	DMRT for Species in Sector IV and V in November	79
Table 17.	DMRT for male and female flies	85
Table 18.	DMRT for Sex of the flies in January and September	86
Table 19.	Phlebotomine Sandflies that had fed on specific hosts in Rusinga Island.....	98
Table 20.	Phlebotomine Sandflies (fed) collected from various habitats	99

LIST OF FIGURES

	PAGE	
Figure 1.	The sectors in which sandflies were caught ...	32
Figure 2.	The Sampling Sites in Rusinga Island	33
Figure 3.	Mouth-Suction Trap	34
Figure 4 a and b.	Sandfly Incidence in Relation to Climatic Factors in Rusinga Island	46
Figure 5.	Altitudinal Distribution of Phlebotomine Sandflies Species in Rusinga Island	87
Figure 6.	Percent Host Preference of Phlebotomine Sandfly Species (Rusinga Island)	97

PLATES

Plate 1.	A Sticky trap set outside a house in Rusinga island	28
Plate 2.	Mechanical light trap inside a toilet in Rusinga	29
Plate 3.	Flies being picked from a Sticky trap in a cowshed in Rusinga.....	30
Plate 4.	A Sticky trap set by the lakeside (Rusinga Island)	31

APPENDIX

Appendix I	Pearsons Correlation Coefficients (Relationship between Climatic Factors and Sandfly Catch in different Sectors and Months).....	123
Appendix II	Summary of the Weather Conditions during the Study Period	124
Appendix III	Relationships between the Climatic Factors (Correlation Coefficients)	125
Appendix IV	DMRT for the months when species varied significantly	126
Appendix V	Months when the sectors were significant (GLM)	128
Appendix VI	DMRT for the months during which the fly numbers in the sectors varied significantly	129
Appendix VII	Percentage Composition of Monthly Sandfly Catches in Rusinga Island from January to December 1990.....	130
Appendix VIII	Blood-meal analysis Results	131
Appendix IX	Anova Results for Various Factors in January and September.....	132
Appendix X	Anova Results for Months when Species and Habitats showed significant results.....	133
Appendix XI	GLM results showing relationships between species, habitats and sex in sector IV in July and December	134
Appendix XII	GLM table showing interaction between habitat, sex and species for sector 3 in June	135
Appendix XIII	GLM for Sectors I, III, and IV in May	136

ACKNOWLEDGEMENT

I am grateful to the Director of ICIPE, Professor Thomas R. Odhiambo for allowing this work to be jointly done through ICIPE and Kenyatta University. I am indebted to Dr. Mutuku Mutinga (Programme leader, MVRP of the ICIPE) for the great help he offered as far as resources are concerned and for the guidance (as a supervisor) and training he gave me in sandfly work. I'm thankful to his technical staff such as David Omogo and Francis Kyai who helped in preparation of some materials and in the identification of some flies.

I am especially appreciative to Dr. Hassan Shawgi (Unit Head, LTRP, Mbita Research Station, ICIPE) and his staff for the cooperation when I was doing the field-work. On the same note, I also extend my gratefulness to Dr. Mohammed of the Tse-Tse Research Programme.

Many thanks are due to Kenyatta University for offering the research funds for this project. I am mostly thankful to my supervisors in Zoology Department, Dr. Charles Maranga and Dr. Romanus Okelo for their guidance which enabled me to involve in more constructive thinking. As the Chairman of the Department Dr. Okelo ensured that I had the necessary materials when I needed them and I thank him for his unceasing willingness to help. A lot of gratitude is also extended to my colleagues in the department for the moral support they gave me, especially Syprine Akinyi who was always passing on information to me when I was in Mbita.

Appreciation is also extended to my sisters Betty and Juddy Gai and other colleagues such as Japheth Ngoya, Zack Ngalo, Mr. and Mrs. Annan, Mrs. P. Oriwa, David Munyinyi (analyzed the data) and many others who were a constant source of encouragement and understanding. I can not forget to thank my assistants Elly Otieno and Ken Misioni who did a tremendous job in the field work and in the laboratory.

Finally and most important of all, gratitude goes to my dear husband Samuel Otieno Misioni for the great understanding shown when I had to leave home and stay in Mbita for the field-work. His constant prayers and encouragement kept me going at times when I would have given up. I am thankful for his financial support when my research funds ran out.

because ... are vectors of leishmaniasis which is a protozoal ... brings about a lot of human suffering ... caused diseases ... in animals and in man, represents a considerable medical and economic burden in

CHAPTER ONE

1.0. INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL INTRODUCTION

Leishmaniasis is a disease caused by parasites of the genus Leishmania and transmitted by phlebotomine sandflies belonging to the sub-family Phlebotominae of the Psychodidae family (Service, 1986a). According to Kirk and Lewis (1946) the sandfly species of the Ethiopian region (Kenya included) are divided into 3 subgenera, viz. Phlebotomus Rondani, Sergentomyia Franca and Parrot and Sintonius Nitzulesci. Leishmaniasis probably ranks next to malaria in terms of human suffering and economic importance among the protozoal diseases, although it is not as common as malaria and sleeping sickness (Molyneux and Ashford, 1983). Infection in man ranges from mild, self-curing disease such as oriental sore to severe fatal disease, kala azar (Zuckermann and Lainson, 1977).

Phlebotomine sandflies are of great economic importance because they are vectors of leishmaniasis which is a protozoal disease that brings about a lot of human suffering. Parasite-induced diseases, both in animals and in man, represent a considerable medical and economic burden in

many countries. An important problem is that of increasing resistance of vectors and parasites against both successfully used and newly developed drugs. For leishmaniasis in particular, there is no non-living vaccine against it (Lainson and Shaw, 1978) and thus control strategies should be geared on management of the vectors and reservoirs of the parasites. Chemotherapeutic drugs are toxic, expensive and not 100% effective. This and the absence of non-living vaccine against the disease (Lainson and Shaw, 1978) means that control may have to depend on effective integrated management of reservoirs and/or insect vectors. It is thus important to know which species of sandflies could be the potential vectors in any given place. Equally important is the isolation and characterization of parasites by dissecting naturally infected wild-caught sandflies which could be carrying disease-causing Leishmania in nature and to contribute to the understanding of the ecology of sandflies in a country like Kenya.

Studies on the phlebotomine sandflies in Kenya have mostly concentrated on the established foci of the disease such as Kitui, Machakos, Baringo, Meru and West Pokot districts (Heisch et al. 1956, Mutinga et al. 1984 and 1989; Mutinga, 1986, Mutinga and Odhiambo, 1986). Other areas in Kenya need to be studied as well in order to establish whether they

have disease-transmitting sandflies or not and hence their potential for disease outbreaks. This is important in the epidemiology of leishmaniasis in this country. It is believed that visceral leishmaniasis was brought into Kenya through migrants from Sudan (Adler, 1964), where it was first reported by Neave in 1904. Thus movement from one district which is an endemic focus to another where vectors thrive within Kenya, could easily establish the disease in new areas due to the presence of the potential vectors. Moreover, factors such as overpopulation and man-made inventories such as dams, irrigation schemes and roads help in the spread of parasites and parasitic diseases (Marquardt and Demaree, 1985). In view of the above mentioned points it is necessary to study the sandfly population and reservoirs of the disease in places in which sandflies are known to exist such as Rusinga Island and determine what potential they have and whether or not reservoirs of leishmaniasis do exist or known ones have potential of being introduced.

1.2. LITERATURE REVIEW

Sandflies are small brownish hairy flies with nearly erect, rather narrow wings and slender bodies (Lewis, 1973). The optimum physical conditions for sandflies are still air, darkness, a constant temperature of approximately 28 degrees centigrade and high relative humidity (Kirk and Lewis, 1951). Sandflies are nocturnal and during the daytime they rest in dark corners especially where there is moisture such as behind clothes, cupboards, pictures, in the interior of rubble and stone walls; in tree crevices, drains, caves, dug-outs and the banks of streams; amongst heaps of damp stone, bricks, tiles, clods of earth; in animal burrows and in cracks and fissures in the soil (Adler 1964; Minter, 1964a). Thus their habitats range from natural to artificial sites.

Sandflies are attracted to specific animals for their blood-meals and this has been the basis of performing blood meal analyses to determine upon which hosts a particular sandfly species feeds (Leeuwenburg and Lawyer, 1987). Specific sandfly vectors are different in different endemic areas. "They however can be recognized by one or more of the following attributes: (i) their distribution agrees with that of leishmaniasis, (ii) they are comparatively easy to infect with the local leishmania; (iii) the infection in the sandflies extends forwards into the head (pharynx and pre-

pharynx) (iv) the infection persists for the remainder of the life of the sandfly; and (v) the sandflies are sufficiently prevalent to maintain the cycle of infection" (Kirk and Lewis, 1951).

The life cycle of Leishmania parasites depends on an insect vector (the sandfly) and on vertebrate animal hosts, thus different parasite species are associated with specific environmental conditions. Sandflies of the genera Phlebotomus Rondani and Berte, Sergentomyia Franca and Parrot, and Lutzomyia Franca transmit Leishmania parasites to man and other animals in the Old and New Worlds (Molyneux and Ashford, 1983). Leishmania aethiopica Bray, Ashford and Bray, Leishmania donovani Laveran and Mansil, Leishmania major Yakimov and Schoklav, Leishmania tropica Wright, are the species of Leishmania parasitic in man in the Old World (Molyneux and Ashford, 1983). Leishmania braziliensis Vianna and Leishmania mexicana Biagi and L. donovani are parasitic in man in the New World (Molyneux and Ashford, 1983).

Distribution of Leishmaniasis is confined to tropical and sub-tropical regions and is often remarkably localized and patchy (Lainson and Shaw, 1978). The Old World countries which have cases of Leishmaniasis reported include countries in Europe (France, Greece, Italy, Portugal, Rumania, Spain, Turkey, USSR, Yugoslavia, Albania and Bulgaria); in North Africa (Algeria, Morocco, and Tunisia); and in the Middle East (both Mediterranean and Non-Mediterranean - Cyprus,

Egypt, Israel, Lebanon, Libya, Malta, Syria, Afghanistan, Iran, Iraq, Jordan, Kuwait, Saudi Arabia, Yemen, South Yemen). In the Asian/Pacific region leishmaniasis is endemic in Pakistan, India and Bangladesh and used to be endemic in China between the 1950's and sixties after which control measures were taken and it reduced (Anon. 1980). In Japan and Nepal there have been sporadic cases reported. In tropical Africa the disease has been reported in Eastern Africa (Djibouti, Ethiopia, Kenya, Somalia, Sudan, Tanzania, and Mozambique); in Central Africa (Chad and Central African Republic); in West and Southern Africa (Cameroon, Mali, Namibia, Republic of South Africa, Senegal, Upper Volta, Angola, Benin, Gabon, Gambia, Guinea and Ivory Coast). In the New World the disease is distributed in South America (especially the Northern countries) Central and some sporadic cases have also been reported in North America (Molyneux and Ashford, 1983).

Thirty-eight species of sandflies occur in Kenya. Three species, namely Phlebotomus pedifer, P. martini Parrot and P. duboscqi have been confirmed as carrying Leishmania aethiopica, L. donovani and L. major respectively (Kaddu, 1986). Some species, namely Phlebotomus rodhaini Parrot, Sergentomyia garnhami, S. squamipleuris Newstead, S. africanus Newstead, S. kirki Parrot, S. ingrami Newstead, S. antennatus Newstead, S. graingeri Heisch, Guggisberg and Teesdale and S. clydei Sinton have been shown to carry

various flagellates whose characters have not been carried out (Minter, 1964a and b; Mutinga and Kamau, 1986; Mutinga et al. 1986b).

Though sandflies are small in size, their bites cause severe irritating papules some of which develop into blisters and may become septic due to scratching (Kirk and Lewis, 1951). The human victims are generally bitten on the face, hands, arms, ankles and legs, that is, the parts of the body most exposed when sitting out in the verandas of tropical houses or sitting near termite hills (Kirk and Lewis, 1951). Thus even if the anthropophilic sandfly species do not have the disease-causing parasites, their general geographical distribution should be established and the people made aware of the potential harm the flies could cause.

In natural vertebrate hosts few or no pathological effects are produced by the parasites but in certain hosts including man, there are violent host-cell reactions to the parasites resulting in skin lesions or severe pathological changes in the internal organs such as in Kala-azar (visceral leishmaniasis) (Lainson and Shaw, 1978). In the vertebrate host the parasites have an ovoid form, that is, the amastigotes, which measure 1.5 - 3.0 by 3.0 - 6.5 micrometers in size, depending on the species (Cheng, 1986). They are tissue parasites of the reticuloendothelial system

and are thus found within the macrophages of the hosts. Amastigotes are ingested from the skin or peripheral blood when a female sandfly feeds upon a host. Within three days they are transformed into the promastigote form (an elongated form that has a flagellum) which ultimately migrate to the anterior parts of the sandfly gut (Adler, 1964).

According to Abonnenc (1972) and Lainson and Shaw (1978), there are three clinical forms of human leishmaniasis, namely:- visceral leishmaniasis, cutaneous leishmaniasis, and muco-cutaneous leishmaniasis. Leishmania donovani which is responsible for visceral and mucosal leishmaniasis (Abdalla, 1982) is transmitted by sandflies belonging to the genus Phlebotomus. In East Africa, visceral leishmaniasis is transmitted to man by the P. martini, a species of the Synphlebotomus Theodor group which may also infect other animals, for example, wild canidae and dogs (Lewis and Ward, 1987). In some parts of Kenya S. garnhami has been shown to be a potential vector of Kala-azar (Mutinga and Odhiambo, 1982). The most notable landscape feature associated with epidemic visceral leishmaniasis in Kenya is the presence of eroded termite hills produced by Macrotermes bellicosus Smeathman (Heisch et al. 1956). Termite hills are breeding and resting sites for many sandfly species, for example, P. martini, P. celiae, P. vansomeranae and the Synphlebotomus complex suspected to be the major vector of Leishmania

donovani in the Eastern Province of Kenya (Heisch, 1954; Molyneux and Ashford, 1983; Mutinga and Kamau, 1986; Mutinga et al. 1989).

Cutaneous leishmaniasis is caused by Leishmania tropica and L. major which are transmitted by members of the genus Phlebotomus. Leishmania tropica affects man and dogs. "In man L. tropica causes chronic oriental sore of slow incubation and long duration (one year or more). Lesions are usually single and occur principally on the face. The lesions are dry and ulcerate only after several months. Leishmania major infects man and rodents. In man Leishmania major causes cutaneous leishmaniasis of rapid incubation and short duration" (Zuckermann and Lainson, 1977). Lesions occur principally on the lower limbs. They are moist and ulcerate within one to three weeks. (Ashford and Bettini, 1987). In Ethiopia and some parts of Kenya, especially Mount Elgon, L. aethiopica, the causative agent of human highland cutaneous leishmaniasis is transmitted by P. pedifer (Mutinga, 1971; Ashford, 1974 and Mutinga, 1975). Leishmania aethiopica causes oriental sore of long incubation period and of a long duration. Mutinga (1986) and Mutinga et al. (1986c) showed that Phlebotomus duboscqi is the major zoonotic vector of L. major in Baringo district, with S. ingrami and P. martini being alternate zoonotic vectors. It should be noted that sometimes the parasite causing some known form of leishmanial infection in one place may cause

another infection that it does not "normally" cause in a different region, for example, in Sudan the dermal lesions in endemic visceral leishmaniasis areas are due to L. donovani and not L. tropica (Hoogstral and Heyneman, 1969), hence clinical manifestation of the disease must be accompanied by parasite characterization.

There are other diseases transmitted by sandflies apart from the leishmaniasis and other parasites infecting sandflies. The gregarines of the species Lankestria mackieii were found to infect sandflies in California (Ayala, 1971). Some sandfly species transmit Bartonella bacilliformis Barton the causative agent of bartonellosis or Carrion's disease (Herrer and Christensen, 1975). Some Phlebotomus species, primarily Phlebotomus papatasi Gabbi and Visentini transmit viral strains that cause sandfly fever (Service, 1986 b). Chaniotis et al. (1988) reported that sandflies are potential vectors of arboviruses for example, vesicular stomatitis virus in Panama. Phlebotomus vexator occidentalis has been incriminated as the vector of a trypanosome species that infects the toad Bufo boreas halophilus (Anderson and Ayala, 1968).

The leishmaniasis diseases such as Kala-azar attack mostly the young age group of about 7-14 years. Kala azar is characterized by fever, anemia, reduced white cell count, wasting, splenomegally, and serious imbalance of serum

proteins (Zuckerman and Lainson, 1977). Kala-azar is fatal unless adequately treated; whereas, Oriental sore (cutaneous leishmaniasis) is less fatal but can be very disfiguring (Lainson and Shaw, 1978).

1.3. JUSTIFICATION OF THE STUDY

Leishmaniasis was rare in Kenya before World War II (Adler, 1964) and it is therefore likely that some areas which have not had outbreaks of the disease may harbor them undetected or be invaded and new foci established. This has been the case for example, in Israel where the distribution of leishmaniasis is unstable (Schlein et al. 1984). The disease had disappeared from old foci such as Haifa in the north (Sternfeld, 1944) and other foci. The focus in Revivim was inactive for many years, but later on there were new reports from this focus as well as new foci on the same area (Katzenllenbogen, 1947). The main known endemic foci of leishmaniasis in Kenya and where there are various species of sandflies include Machakos, Kitui, Baringo, and Meru districts (Mutinga et al. 1989; Mutinga, 1986). Masinga focus which lies between Kitui and Machakos is believed to be a bridge between Kitui and Machakos Kala-azar foci (Mutinga, 1987). In addition sandflies have been reported in West Pokot, Mount Elgon and Bungoma District (Mutinga et al. 1984; Mutinga and Odhiambo, 1986 and Kaddu et al. 1988). Minter (1964 a) reporting on the distribution of Kenyan sandflies noted that around Lake Victoria region, the

following sandfly species exist; Phlebotomus rodhaini, Sergentomyia antennatus, S. schwetzi, S. africanus and S. durenii.

Host blood-meal analysis in sandflies is useful in determining the reservoir hosts of the leishmanial parasite species and whether the flies are anthropophilic or not. Rodents, dogs and goats and sheep (Mutinga et al. 1988) have been found to be naturally infected with Leishmania parasites. In the Mediterranean region, the dog is considered a major reservoir of visceral leishmaniasis and seemingly canine infections are mainly responsible for human visceral leishmaniasis (Bettini and Gradoni, 1986). Man gets in touch with these animals in one way or the other, for example, rodents invading houses, goats sleeping inside huts and the dog always near it's owner; so transmission can easily take place from these reservoirs to the human hosts. Leishmania parasites which have been repeatedly isolated from wild-caught sandflies have been shown to carry the same parasites found in patients suffering from leishmaniasis (Killick-Kendrick, 1979). Thus it is important to isolate parasites from sandflies identified in any given area. Some of the authors cited in the review did not carry out any dissections on the sandflies that they studied. Examples of leishmanial parasites isolated from wild-caught sandflies include leishmanial parasites in the guts and malphigian tubules of Sergentomyia garnhami Heisch, Guggisberg and

Teesdale and S. antennatus Newstead (Kaddu and Mutinga, 1984) and Leishmania aethiopica in the oesophagus of P. pedifer Lewis, Mutinga and Ashford (Kaddu et al. 1988).

Control strategies for leishmaniasis should be carried out especially in areas which have a potential for development and tourism. Development would be deterred in such a place as a result of people fearing the risk of contracting the disease. Surveys should be carried out to determine the risk of infection before any development projects are brought to such an area (Anon. 1984). It is therefore important that research on sandflies be intensified just as it is done with other parasitic vectors and diseases which have been detrimental to mans' entire development. This study was done bearing in mind that the distribution of a vector is normally greater than that of the disease; and the lack of documented reports on leishmaniasis in Mbita area, necessitated this study.

1.4 OBJECTIVES

The overall aims of this study were therefore, to:-

1. Identify the phlebotomine sandflies of Rusinga Island and determine their species incidence
2. Determine their seasonal incidence and climatic factors affecting their abundance
3. Determine their medical or veterinary importance as vectors (by dissection and blood-meal analysis).

PLATE 1



A STICKY TRAP SET OUTSIDE A HOUSE IN RUSINGA ISLAND

PLATE 2



MECHANICAL LIGHT TRAP INSIDE A TOILET IN RUSINGA

PLATE 3



FLIES BEING PICKED FROM A STICKY TRAP IN A COWSHED IN RUSINGA.

PLATE 4



A STICKY TRAP SET BY THE LAKESIDE (RUSINGA ISLAND)

The sectors in which moflies are caught

Fig. 1

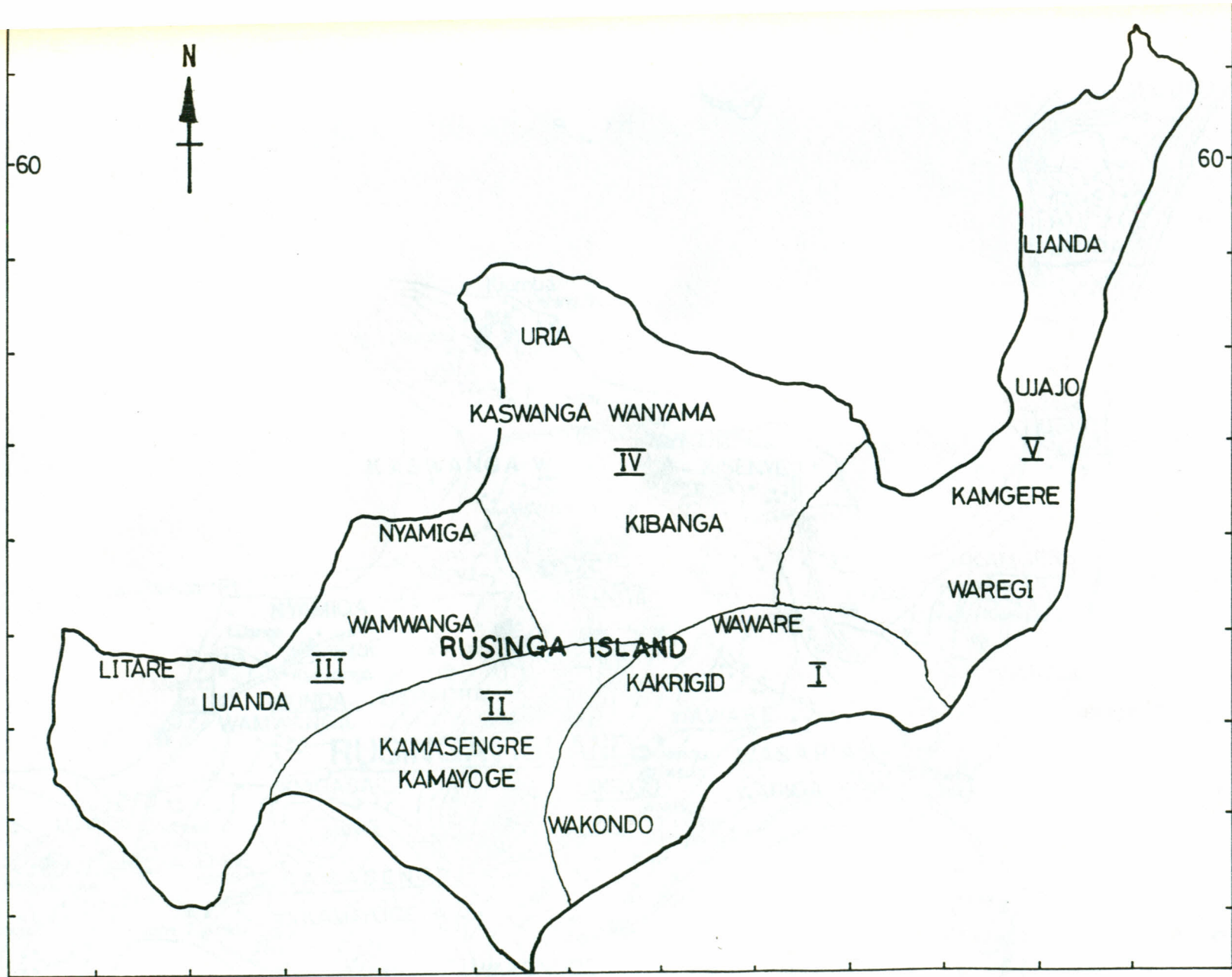


Fig: 1

The sectors in which sandflies were caught

● SAMPLING SITE

NGODHE ISLAND

60



60

ULUGI
ULUGI HILLS
LIANDA
Akanana Pt

Kiumba Fishing Centre
Wauma
URIA

Malwa 115 T 63
sch
Utajo
UTAJO
Luoma

KASWANGA WANYAMA KISENYE

WAYANDO
Wikondjek

KAMGERE
Kiwegi sch

Kinua Pt
NYAMIGA
Luanga

Tom Mboya sch
Shops
KIBANGA
Niyamatati

KOLO
sch

WAREGI
sch

Limande Pt

Meteor. st.

Rusinga
Kaswanga sch

DIERAORA
Hosp

WAWARE
sch

Luanga Pt

WAMWANGA

RUSINGA ISLAND
Kakrigu sch

WASARIA
WARIKA-WAYAGI
Chief's Office

LITARE

LUKOWE

LUANDA
Kidiwa Air strip
WAHEMBE
sch

KIAGASA
Klahera

KAKRIGU
Wakiaga village
Polytechnic

KIGODA

WAMBA

Nyawangwe
GUMBA
Kiakanga

KAMASENGRE
Luore
Mkt

KAMAYOGE
Kasiwe

WAKONDO
Kamayoge
sch
Nyamkini

Health centre
PO
Mkt
115 T 57

Mbita DO
Agricultural Office

CHAMAUNGA ISLANDS

Ligumba Pt

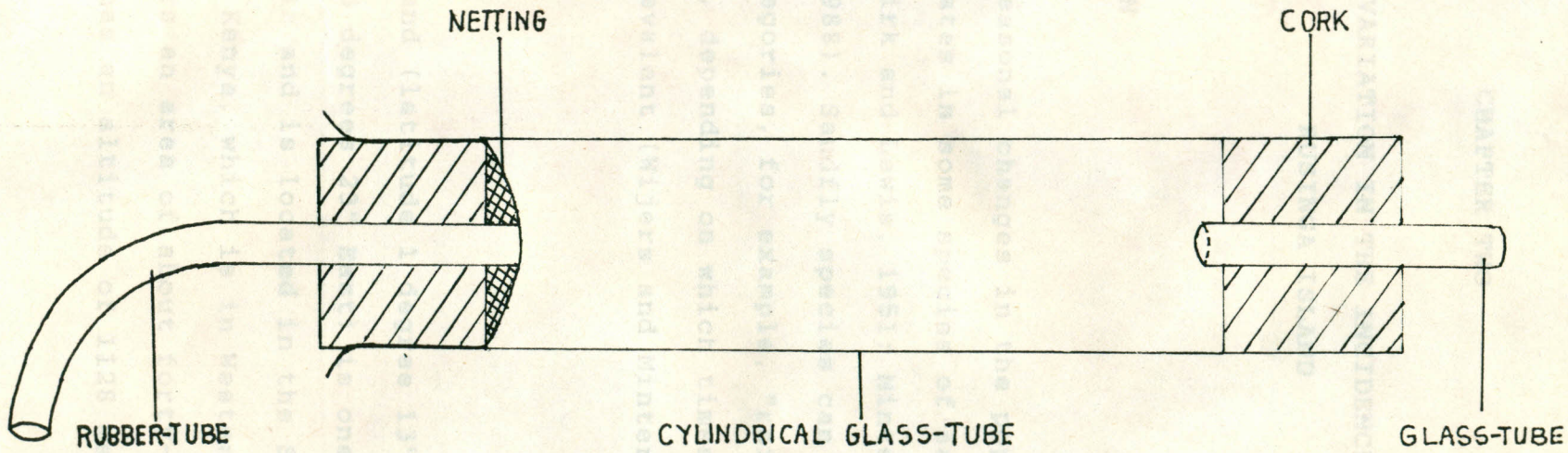
SIENGATTO
115 T 65

Tambasa

Kariatia Pt

33

FIG.3. MOUTH-SUCTION TRAP



34

CHAPTER TWO

2.0. SEASONAL VARIATION IN THE INCIDENCE OF SANDFLIES IN RUSINGA ISLAND

2.1. INTRODUCTION

There are seasonal changes in the population and infection rates in some species of sandflies (Heisch et al. 1956; Kirk and Lewis, 1951; Minter, 1964 b and Basimike, 1988). Sandfly species can be divided into various categories, for example, "rainy-season" or "perennial", depending on which times of the year they are most prevalent (Wijers and Minter, 1962).

2.2. STUDY AREA

Rusinga Island (latitude 1 degree 13" South and longitude 34 degrees 23" East) is one of the islands in Lake Victoria and is located in the South Nyanza District of Kenya, which is in Western Kenya (Figure 1). It covers an area of about forty-eight kilometres square. It has an altitude of 1128 metres and the

highest point is the Lugongo Hill peak at an altitude of 1433 metres above sea-level (Punyua, 1988). The vegetation is mostly bushland with a few trees and it falls under ecological zones IV/V following the classification of Pratt et al. (1966). Geologically it consists of tertiary sediments. Most of the island is made up of loam soils often with gravel or stone surface. Another part is made of well-drained grayish brown, sandy, clay loam soils (Jaetzold and Schmidt, 1982). The soil is the black-cotton type and is quite sticky when it rains. The crops grown are maize, millet, some pulses, for example, cow-peas and cassava. The most common natural vegetation comprises plants of the Lantana species.

Situated on the South-East of Rusinga Island is Mbita which is the nearest mainland. To the North-East is Siaya District (Uyoma) and to the West is Mfangano Island. The annual rainfall varies between 760 - 1015 millimetres, and the maximum and minimum temperatures are 30-34 degrees centigrade and 14-18 degrees centigrade respectively (Anon. 1970). The rainy season is between February and May and the dry season is between August and October. The short rains fall in November and December.

2.3 MATERIALS AND METHODS

2.3.1 Trapping and Collection of Sandflies

The whole island was divided into five major sectors (Figure 1), which had some randomly selected sites (Figure 2). The sectors varied in vegetation type and even soils and altitude (Table 1). The trapping sites included houses (inside and outside), cow-sheds, tree-holes, termite-hills, latrines, rock-crevices and bushy areas near the lake-shore. Although the choice of the sites was random, some logistical considerations were made in regard to places that were not very accessible and that would consume a lot of travelling time since reaching them would mean walking very long distances. There were twenty-eight different sites (in the whole island) which were located at various altitudes. The sites ranged from low-lying areas near the lake shores to hilly areas. The following traps were used: the sticky-trap (Mutinga, 1981), the mechanical light trap and mouth-aspirator trap. The traps were set at each site once a week for one year beginning January 1990 to December 1990 except during the month of August when trapping was interrupted. The sticky trap and light trap were set in the evenings between 1730 hours and 1845 hours and left overnight. The flies caught were removed the following morning between 0600 hours and

0730 hours. The sticky trap and aspirator traps do not use attractants and thus they give less biased data and sample all species of flies more or less equally (Service, 1976). The sticky trap was used in all the habitats, whereas the light trap was only set in sheltered places where it could not be stolen.

2.3.1.1. The Sticky Trap

The sticky trap is a very useful method of trapping flies especially when the sandfly population is low, for example during the dry season (Heisch et al. 1956). It consists of polythene sheeting (gauge 1000) smeared with castor oil on both sides (Plate 1). The polythene sheeting makes this trap advantageous because it is hardy enough to withstand harsh environmental conditions such as strong winds and rainfall; furthermore it can also be cleaned and re-used. Nevertheless it was problematic in the sense that it was stolen easily and at times the traps would be missing the next day. Initial surveys that were carried out during the months of November and December 1989, showed that the sticky trap was more favourable compared to the other two traps, and it caught more sandflies. After these surveys, the sites that were prone to easy theft were abandoned and the Administrative Chiefs and Headmasters of the primary

schools were contacted to alert the people about the experiments going on. A standard size of polythene sheeting of one metre by one metre was used. It was used for both indoor and outdoor trapping. The trap was set vertically and pegs or strings were used to hold it in place depending on the place at which it was being set. This trap was used for the collections made throughout the research work and the overall statistical analysis was done based on data obtained from the sticky trap. The flies caught on the sticky trap were initially removed using spines of the thorn-tree (Acacia species), but later on it was found that a split smooth piece of a twig of the Lantana species was more effective in picking the flies without destroying them.

2.3.1.2. The Mouth-suction Trap

This consisted of a cylindrical glass tube which had a width of 2.5 centimetres. Both ends of it were closed with rubber corks that had holes through which a rubber tube passed on one end and a glass tube passed on the other end (Figure 3). A small piece of netting material covered the cork (from inside) on the end that had the rubber tubing. This was to prevent the inhalation of any dust particles by the person collecting the flies.

Table 1

Description of the Sectors

Sector	Vegetation structure	Soil Type
1	Bushland with shrubs and a few scattered trees	Black cotton, muddy
2	Bushland	Some parts rocky, others black cotton type
3	Grassland	Rocky especially some parts near the lake
4	Bushland	Black cotton
5	Bushland	Places near the lake fertile, others stony

Flies were caught by sucking on the rubber tube and they got in through the other end.

This method was favourable for catching live flies but it was not used for the long term collections, as the man-power and time it consumed made it inconvenient, and so it was not used frequently.

2.3.1.3. The Mechanical Light Trap

This was a modified CDC (designed by Communicable Disease Centres, United States of America) trap. It consisted of a battery-driven light trap which had a fan that sucked in air (Plate 2). It was attached to a plastic cage that measured 15 cms by 15 cms. The cage had a net on one side of it and another piece of net surrounding a circular hole on the top of the cage, through which insects could get into the cage after being sucked in. Four batteries each of 1.5 volts were used on this trap at any one setting. This trap was also set in the evenings and flies collected from it at dawn. It was especially used for the collection of live flies needed for blood-meal analysis.

2.3.2. PROCESSING AND MOUNTING OF THE SANDFLIES

After removal of the flies from the traps, they were washed in a dilute solution of "Teepol" detergent and preserved in 70 % alcohol. During the process of mounting, the flies were placed in saline solution and the head were cut off and then turned upside down. (The head is very useful especially in the identification of female flies). The fly was then mounted using a drop of gum chloral (solution having 50 ml of distilled water, 30 grams of gum acacia, 20 ml of glycerin, and 50 grams of chloral hydrate; Minter, 1962) and examined under the microscope.

2.3.3 IDENTIFICATION

The identification of the flies was done using keys developed by Lewis (1973), Abonnenc (1972), Kirk and Lewis (1951) and keys developed by Mutinga (unpublished); all based on detailed morphological characteristics.

2.4. RESULTS

2.4.1. Sandfly Species Recorded

A total of 12 phlebotomine sandfly species were found on Rusinga Island namely:- *Sergentomyia affinis* Theodor, *Sergentomyia africanus* Newstead, *S. antennatus* Newstead, *S. bedfordi* Newstead, *S. ingrami* Newstead, *S. schwetzi* Adler, Theodor and Parrot, *S. serratus* Parrot and Malbrant, *S. squamipleuris* Newstead, *S. clydei* Sinton, *S. inermis* Theodor, *S. graingeri* Heisch, Guggisberg and Teesdale and *Phlebotomus rodhaini* Parrot.

2.4.2. Analysis of Results

The overall results of the sandflies caught during the 11 months of study are shown in Appendix VII. The square-root transformation ($X = \text{square-root} [\log_{10} (\text{count} + 1) + 0.5]$) (Zar, 1974) was used in the data analysis and in Figure four. When the general linear models procedure (GLM) was carried out on the overall data set, including the month, sector, location (site), species and sex, there was a significant difference between the means of these groups.

Table (2) shows that there were highly significant differences in the means of the counts of the sandflies for

Table 2

Analysis of the Various factors that influenced Sandfly
Catches during the study period

SOURCE	DF	SS (I)	MS	F	PR > F
MONTH	10	2.3698	0.2369	13.71	0.0001****
SECTOR	4	2.6796	0.6699	38.74	0.0001****
LOCATION	6	0.6421	0.1070	6.19	0.0001****
SPECIES	8	9.0248	1.1281	65.24	0.0001****
SEX	1	0.0033	0.0033	0.19	0.6644 NS
ERROR	1868	32.2991	0.0173		

**** = Significance level of 0.0001

NS = Non significant

Table 3

Duncans' Multiple Range Test (Months)

Means with the same letter are not significantly different

Month	Mean	Duncan Grouping		
JUNE	1.0710	A		
JULY	1.0544	A	B	
OCTOBER	1.0534	A	B	
MAY	1.0408	C	A	B
NOVEMBER	1.0266	C		B
SEPTEMBER	1.0126	C	D	
APRIL	1.0060	C	D	
DECEMBER	0.9833		D	E
JANUARY	0.9664			E
FEBRUARY	0.9640			E
MARCH	0.9480			E

p < 0.05

Df = 1868

MSE = 0.017291

Fig. 4a: SANDFLY INCIDENCE IN RELATION TO CLIMATIC FACTORS IN RUSINGA ISLAND.

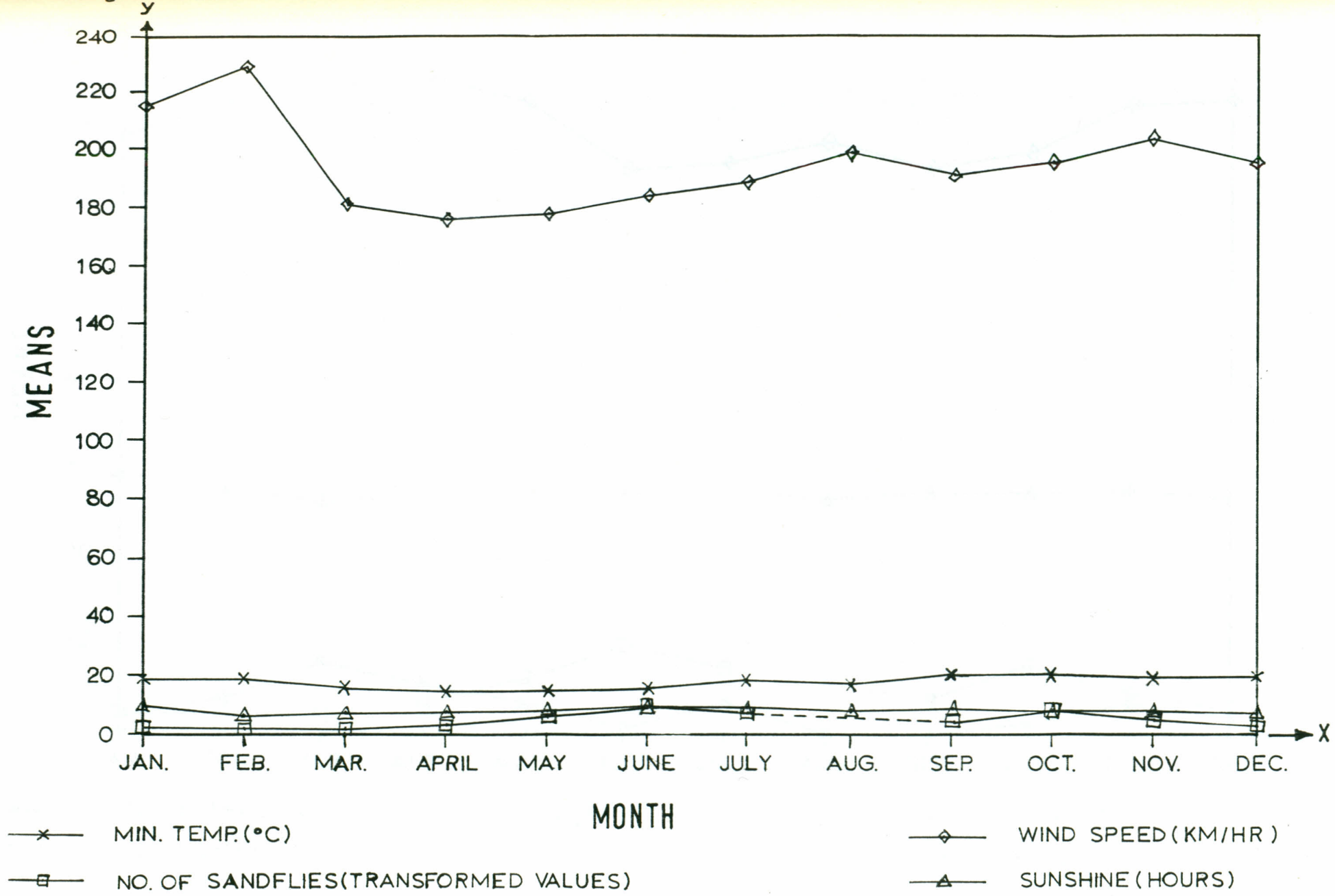


Fig. 4b: SANDFLY INCIDENCE IN RELATION TO CLIMATIC FACTORS IN RUSINGA ISLAND.

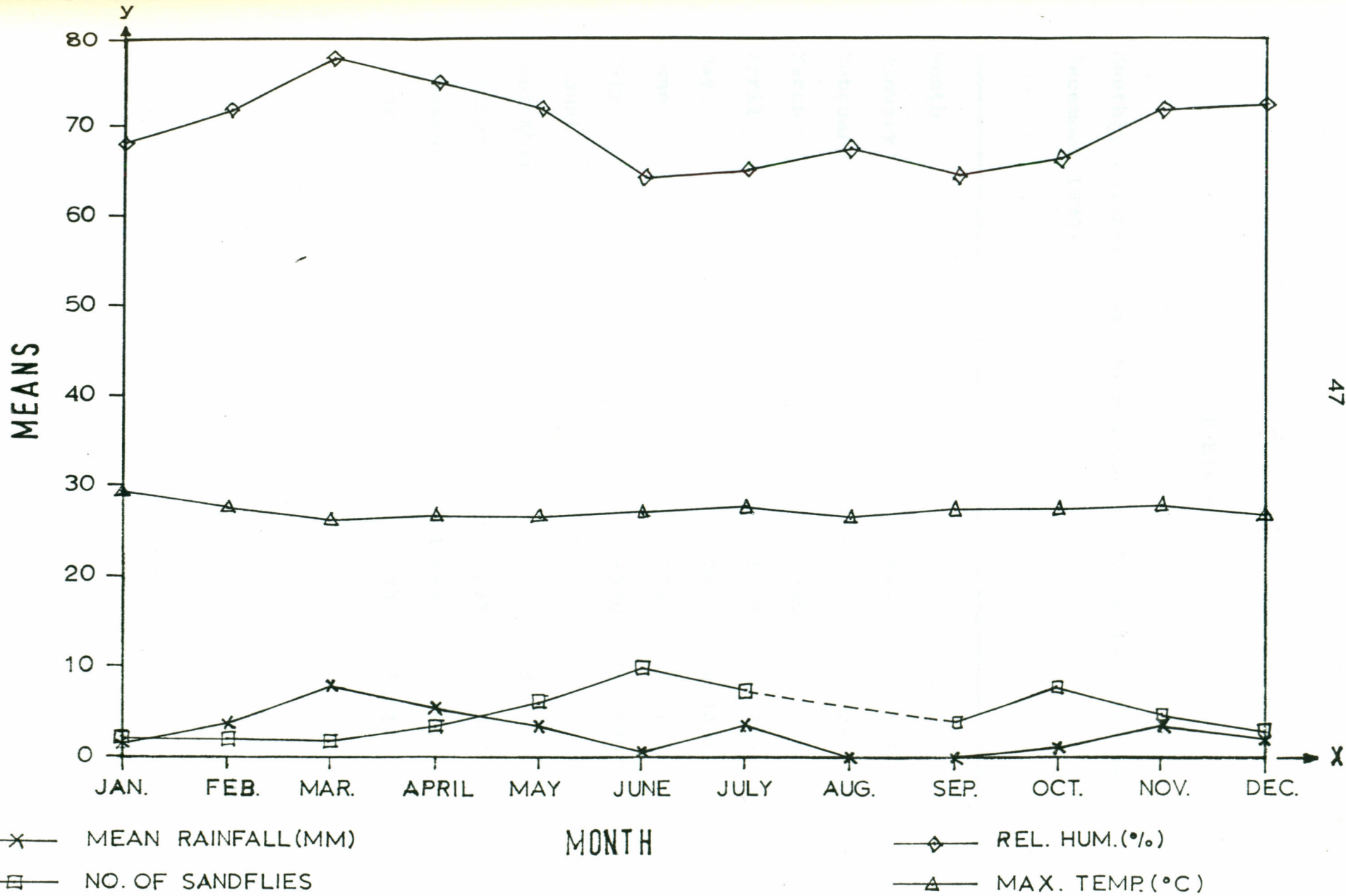


Table 4

Monthly rainfall (mm) during the study period (January -
December 1990)

Month	Amount (mm)	Mean	SD
January	49.4	1.594	5.1399
February	108.4	3.871	8.1267
March	218.1	7.0355	2.1309
April	167	5.5667	9.3069
May	124.2	4.0065	8.4404
June	16.6	0.5533	2.6171
July	120.9	3.9000	13.5953
August	0	0	0
September	0	0	0
October	23.8	0.7677	2.2907
November	119.5	3.983	9.3907
December	89.7	2.8935	5.5117

mm = millimetres

SD = Standard deviation

Table 5

Duncans' Multiple Range Test (Flies in the Sectors)

Means with the same letters are not significantly different

SECTOR	Mean	Duncan grouping
III	1.0614	A
IV	1.0572	A
V	1.0500	A
I	0.9802	B
II	0.9686	B

 $p < = 0.05$

Df = 1868

MSE = 0.017291

the various months, sectors, location and species while the sexes (male and female) showed no significant difference between them.

Further comparison of means using the Duncans' multiple range test revealed that there was no significant difference in the counts of the months of June, July, October and May (Table 3). The largest number of flies was caught during these months. Similarly the months of July, October, May, and November had no significant difference in terms of the count of the flies. They came second as far as the maximum mean number of flies caught was concerned. The months of May, November, September, and April were similar. The months of September, April, and December were similar too and there was no significant difference between the months of December, January, February and March. The least number of flies was caught during these later months.

The "peak season" for the flies was during the months of June, July and October, that is, just after the rainy season (February to May) and just before the onset of the short rains (November to December). The average number of flies was lowest during the months of January to March (Figures 4a and b). In April there was a significant negative relationship between fly abundance and rainfall ($r = -0.1968$, $P < 0.0177$, at 144 degrees of freedom).

There was correlation between relative humidity, wind-speed, hours of sunshine and the abundance of the flies (See Appendix 1). The correlation were on the whole negative in the case of relative humidity ($r = -0.1009$; $P < 0.0001$; $Df = 1897$) and wind-speed ($r = -0.0488$; $P < 0.0334$; $Df = 1897$), but were positive for hours of sunshine ($r = 0.0713$; $P < 0.0019$; $Df = 1897$).

In February there were significant relationships between fly count and minimum temperature and wind-speed ($r = 0.2962$, $P < 0.0296$, $Df = 53$ and $r = 0.3166$, $P < 0.0213$ $Df = 53$) respectively. Similar results were obtained in April between fly count and rainfall, wind-speed, wind direction, and hours of sunshine (Appendix 1).

When correlation analysis was done for all the months combined, relative humidity and fly count showed a significant relationship as mentioned earlier but when the individual months were considered there was no particular month in which fly count and relative humidity showed a significant relationship. On the other hand, when the overall analysis was done, some factors, for example, rainfall did not have any significant relationship with fly count (Appendix 1). Nevertheless there was significance in

some months; for example, minimum temperature in February; rainfall and wind direction in April (Appendix 1). During the other remaining months there was no significant relationship between the climatic factors (the ones showed in figures 5 a and b) and fly counts for the individual months. The summary of all the weather conditions is in Appendix 2.

There was a significant difference between the five sectors of the Island as far as the population of flies in them was concerned (Table 5).

In sectors 3, 4, and 5 (Figure 2), there was a larger number of flies caught compared to the other two on the southern side of the island (sectors 1 and 2). This was also reflected when correlation analysis was done for fly population and humidity in the different sectors. There was a significant negative relationship for the Southern sectors. (Appendix 1).

There was no significant relationship between count and humidity for sectors 4 and 5. The mean relative humidity in sectors 1 and 2 was higher than that of sectors 3, 4, and 5. In sector 4 there was a significant relationship between count and maximum temperature ($r = -0.1137$, $p < 0.0111$, $DF = 497$); minimum temperature ($r = -0.0974$, $p < 0.0298$, $Df = 497$); wind-speed ($r = 0.1206$, $p < 0.0045$, $Df = 497$). In

sector 3, there was a significant relationship between count and hours of sunshine ($r = 0.1206$, $p < 0.0071$ and $DF = 496$). There was a non-significant negative relationship between count and rainfall for all the sectors.

2.4.3. Seasonal Incidence of the Various Species of Sandflies

The occurrence of some of the sandflies was found to be "seasonal", in that some were caught throughout the year whilst others were not caught in certain months of the year. Five of the species, namely :- Sergentomyia antennatus, S. bedfordi, S. schwetzi, S. ingrami, and S. africanus were caught throughout the year (1990). These were the "perennial" species. Sergentomyia serratus was not caught during the month of February only. This was the month with the highest wind-speed and the least sunshine (Appendix II). Sergentomyia squamipleuris was absent during the months of June, January and May. The months of June and January had the longest mean hours of sunshine while May had relatively longer hours of sunshine compared to the remaining months. June and January were among the dry-seasons' months. Sergentomyia affinis was only caught during the months of June, October, November and December (1990) and it was absent during the rest of the year. Thus it was caught during the dry season (June and October) and

the period of the short rains (November and December).

Sergentomyia clydei was only caught in November; S. graingeri in November; S. inermis in July and Phlebotomus rodhaini in December only.

The months of November and December had the largest variation of the different species caught; whereas the months of January, March and September had the least variation of the species (only five species of the flies were caught then). During the months of February, March, June, September and December the species caught did not differ significantly in their counts.

Even though in the overall analysis, there was no significant difference between the male and female flies, in the months of January and September there was a significant difference between the counts of the sexes. The Anova results (Appendix IX) showed that in January the males were significantly more than the females (mean males = 0.9810 and females = 0.9444) whereas in September the females were more than the males (mean females = 1.0414 and males = 0.9749).

2.5. DISCUSSION

It is apparent that the rainfall pattern had an influence on the abundance of the sandflies in Rusinga. Thus the period just after the rainy season and just before the onset of the short rains had the highest abundance of the flies, whereas the periods when the rainfall amount was low had the lowest abundance of sandflies. This is in agreement with the findings of earlier workers like Smith (1959) who reported that the peak season of sandflies in Assam in India occurs soon after the monsoon rains and Mutinga (1981) has also reported very low populations of sandflies during the dry season. The influence of rainfall on both the incidence and prevalence was apparent and it appeared to influence the relative humidity which may influence the numbers of flies. This is in agreement with the findings of Minter (1964 b) that the effects of rainfall probably operate by changing the humidity of breeding places, increasing the length of life of adult flies and hence the reproductive turnover. The difference in the incidence of flies in the different sectors of the island affirms the fact that the relative humidity affects the incidence of the sandflies. There is an interplay of climatic factors involved in the seasonal incidence of the sandflies. This is so because humidity is affected by other

factors, for example, temperature (Anon. 1970). Correlation analysis (overall) showed that maximum and minimum temperatures, rainfall, duration of rain, wind direction and sunshine all have significant relationships with humidity. Maximum and minimum temperatures have a negative relationship with humidity, while the others mentioned above have a positive relationship with the same (Appendix III). The differences in the incidence of sandflies in the various sides of the island are thus explained; since the island has a leeward and windward side. The leeward side is on the southern side which is comparatively drier than the other side. It receives relatively less rain and its' humidity is also lower, so this befits the general impression given here that in Rusinga island as relative humidity increases the sandfly incidence decreases.

The sandfly species that were 'perennial' in Rusinga Island, for example, S. antennatus, S. bedfordi, and S. schwetzi were similarly found to be perennial in Kitui by Minter (1964 b). In Rusinga Island the studies indicate that other factors besides rainfall may have influenced the appearance and disappearance of some of the phlebotomine sandfly species. During the heavy rains such as in April, the adult population was low and it gradually increased as the rains subsided. This could imply that during the rainy season most

of the adult flies were destroyed by heavy rains; however the breeding sites were increased. Mutinga et al. (1989) also reported that in Kitui, Machakos and Baringo kala-azar foci, the sandflies adapted to many breeding sites during the rainy season. The adult stages then emerged as the rains decreased and the peak was reached when the rains were low.

As the wind-speed increased, the incidence decreased and this was seen in the month of February (Appendix II) when the speed had a significant effect on the count ($r = 0.31281$, $p < 0.0213$, $DF = 53$). The results indicated that still air was conducive for sandflies. It was apparent that winds and air currents had a deterrent effect on them and so they did not readily venture out into them; thus on windy days few flies were caught on the traps. As the sunshine increased the incidence also increased; this was well illustrated in the month of June when the incidence was highest and the hours of sunshine longest. It has also been reported that in China the peak period of sandflies is when temperatures of $25^{\circ} - 28^{\circ}\text{C}$ prevailed, and when the temperatures drop below 18°C no sandflies were seen. This could signify that the flies prefer relatively high temperatures to very low ones. When there are long hours of sunshine the temperatures remain constantly high as opposed to fewer hours of sunshine when the temperatures are low. It is known that sandflies prefer an optimum temperature of 28

degrees centigrade (Kirk and Lewis, 1951) and this was shown to be true for the sandflies of Rusinga Island.

COLLECTING SANDFLY SPECIES COMPOSITIONS AND IDENTIFICATION
RUSINGA ISLAND

1. INTRODUCTION

The adult sandfly is a blood-sucking insect with large wings and legs and a long proboscis. The female sandfly has a distinctive appearance and is easily identified. The species of sandfly found on Rusinga Island is *Phlebotomus* *sp.* The sandfly is a common pest of man and animals. It is a vector of several diseases. The sandfly is a blood-sucking insect with large wings and legs and a long proboscis. The female sandfly has a distinctive appearance and is easily identified. The species of sandfly found on Rusinga Island is *Phlebotomus* *sp.* The sandfly is a common pest of man and animals. It is a vector of several diseases.

The sandfly is a blood-sucking insect with large wings and legs and a long proboscis. The female sandfly has a distinctive appearance and is easily identified. The species of sandfly found on Rusinga Island is *Phlebotomus* *sp.* The sandfly is a common pest of man and animals. It is a vector of several diseases. The sandfly is a blood-sucking insect with large wings and legs and a long proboscis. The female sandfly has a distinctive appearance and is easily identified. The species of sandfly found on Rusinga Island is *Phlebotomus* *sp.*

CHAPTER III

PHLEBOTOMINE SANDFLY SPECIES COMPOSITION AND INCIDENCE IN
RUSINGA ISLAND

3.1 INTRODUCTION

The adult sandfly is a small insect with large eyes, long legs and a hairy body and wings. In the male the terminalia are generally conspicuous at the end of the abdomen. The species of sandflies in Kenya total thirty-eight in number. Their identification has mostly been based on detailed morphological studies but in some instances (Rogo et al. 1988), biochemical identification has been necessary where the sexes of some species, for example, females of Phlebotomus pedifer and Phlebotomus elgonensis are indistinguishable morphologically.

Some of the Kenyan species namely Phlebotomus rodhaini Parrot, Sergentomyia garnhami Heisch, Guggisberg and Teesdale, S. squamipleuris Newstead, S. kirki Parrot, S. ingrami Newstead, S. antennatus Newstead, S. graingeri Heisch, Guggisberg and Teesdale and S. clydei Sinton have been shown to carry various flagellates whose characters

have not been confirmed. Three species:- P. pedifer Lewis, Mutinga and Ashford, P. martini Parrot and P. duboscqi Neveu-Lemaire have been incriminated as vectors of Leishmania aethiopica, L. donovani and L. major respectively.

3.2 MATERIALS AND METHODS

3.2.1. Trapping, Collection and Mounting

These were done in the same way as described in Chapter two section 2.3.

3.2.2. Identification

The identification of the sandflies was done using keys developed by Lewis (1973), Abonnenc (1972), Kirk and Lewis (1951) and keys developed by Mutinga (unpublished). They were all based on detailed morphological studies.

The mounted sandflies were examined under a compound microscope. The head was used especially in the identification of the female flies. For the male flies, the terminalia were most useful although the head was

also used. Permanent mounts of the flies were made so that they could be kept for identification later on. A magnification of 100 or 400 was generally used but in cases where the fine details of the head, for example, were required, a magnification of 1000 was used. The identification was done to the species level. Those flies that were destroyed during their removal from the trap or the washing and mounting process were also counted although some could not be identified properly. In some of these cases, certain parts that are vital for identification for example the head, would be missing and only the sex of the fly would be known.

3.3.

RESULTS

Twelve sandfly species were found in Rusinga Island. Of these eleven belonged to the genus Sergentomyia and only one belonged to the genus Phlebotomus. These included:-

1. S. affinis Theodore
2. S. africanus magnus Newstead
3. S. antennatus Newstead
4. S. bedfordi Newstead
5. S. schwetzi Adler, Theodore and Parrot
6. S. ingrami Newstead
7. S. serratus Parrot and Malbrant

8. S. squamipleuris Newstead
9. S. clydei Sinton
10. S. inermis Theodore
11. S. graingeri Heisch, Guggisberg and Teesdale
12. P. rodhaini Parrot

The square-root transformation and the general linear models procedure were used to analyze the data. From Table 2 in Chapter two it was noted that in the overall analysis, there was a significant difference among the species of sandflies collected.

When the Duncans' Multiple Range Test (DMRT) was used to ascertain whether there were quantitative species differences it was found that S. antennatus had the highest mean number of flies and it was distinct from all the others. S. bedfordi came second but it was not significantly different from S. serratus and S. schwetzi. Sergentomyia serratus came third but it was not significantly different from S. schwetzi, S. ingrami, S. africanus, S. squamipleuris, S. affinis and the flies that were destroyed and were thus not identified (Table 6)

Table 6

Duncans' Multiple Range test (Species)

Means with the same letter are not significantly different

Species	Mean	Duncan Grouping
<u>S. antennatus</u>	1.0946	A
<u>S. bedfordi</u>	1.0210	B
<u>S. serratus</u>	0.9746	C B
<u>S. schwetzi</u>	0.9715	C B
<u>S. ingrami</u>	0.9519	C
<u>S. africanus</u>	0.9475	C
<u>S. squamipleuris</u>	0.9178	C
<u>S. affinis</u>	0.9164	C
Unknown	0.9148	C

P < 0.05 DF = 1868 MSE = 0.017291

TABLE 7

Duncans' Multiple Range Test (Flies in the Different Habitats)

Means with the same letters are not significantly different

HABITAT	Mean	Duncan grouping	
Tree-hole	1.0531	A	
Termite-hill	1.0419	B	A
Toilet	1.0313	B	A
House-Out	1.0274	B	A
House-In	1.0195	B	
Cow-shed	0.9537	C	
Lake-side	0.9237	D	

$p < 0.05$

DF = 1868

MSE = 0.017291

TABLE 8

Duncans' Multiple Range Test (Variation in the habitats in July)

Means with the same letter are not significantly different

HABITAT	Mean	Duncans' Grouping
Tree-hole	1.0841	A
Toilet	1.0723	A
Termite hill	1.0722	A
House-out	1.0547	A
House-in	1.0480	A
Lake	0.9512	B
Cow-shed	0.9410	B

$P < 0.05$

DF = 329 MSE = 0.02173

TABLE 9

Duncans' Multiple Range Test (Variation in the habitats in
October)

Means with the same letter are not significantly different

HABITAT	Mean	Duncans' Grouping
Termite hills	1.0795	A
Outside	1.0690	A
Tree-holes	1.0419	A
Inside	1.0338	A
Toilet	1.0252	A B
Cow-shed	0.9684	B
Lake-side	0.8950	B

$p < 0.05$

DF = 115

MSE = 0.0193559

3.3.1 Species Incidence in various Habitats

In the overall analysis using GLM (Table 2) there was a significant difference between the different habitats as far as the flies found within them were concerned. When DMRT (Table 7) was performed it was found that the tree-holes had the largest mean number of flies caught in them followed by the termite hills. But the abundance of flies in the tree-holes was not significantly different from those of the termite hills, toilets and outside the houses. The abundance of flies in the termite hills was also not significantly different from those in the toilets, outside houses and inside houses. The cow-sheds ranked sixth, and the least number of sandflies was found by the lake-side.

During some months of the year namely July and October, the mean number of flies in some habitats were significant. In July it was significant at 0.05 level of probability (Df = 6; $P > R = 0.0012$; $F = 3.78$; $N = 348$), just as it was in October (Df = 6; $P > R = 0.0033$; $F = 3.50$; $N = 135$). In July (Table 8) the tree-holes had the largest mean number of flies within them but this was not significantly different from the population within the toilets, termite hills, outside houses and inside houses. The lake-side ranked sixth and were not significantly different from the cow-shed which came last.

In October the termite hills (Table 9) took the lead but the mean number of flies in them was not significantly different from those outside the houses, in the tree-holes, inside the houses, toilets and cow-shed. The toilets ranked fifth but were not significantly different from the cow-shed and lake-side.

3.3.2 January Variation in the Species Composition in the the previous different Months

As had been mentioned earlier in Chapter Two, the sandflies have some perennial and 'seasonal' species. From Appendix VII it is noted that the perennial species that were caught throughout the year, also constituted the largest percentage of flies caught in any of the months. These perennial species consisted of species of S. antennatus, S. bedfordi and S. schwetzi, that is, the "Abs" group. Sergentomyia africanus was also amongst them. They made up relatively higher percentages of the total flies caught in the different months of the year.

During the months of January, April, May, July, October and November there were significant differences among the diverse habitats as far as the species caught in them was concerned (See Appendix X). Nevertheless, in all these months investigated S. antennatus was always the most abundant. In January S. antennatus had the highest mean number of flies caught but it was not significantly different from those of S. ingrami, S. bedfordi and S. schwetzi. Sergentomyia ingrami came second but its' mean was not significantly different from those of S. bedfordi, S. schwetzi, S. africanus and the destroyed flies. The results of the DMRT for

January and for the rest of the months referred to in the previous paragraph are in Appendix III. These results reveal that in all the months S. antennatus topped the list in terms of abundance and it was always followed by S. bedfordi and S. schwetzi except in the month of January. Although S. bedfordi had a high incidence, its' occurrence in various habitats was sporadic, and it was not as common as S. antennatus. When it was encountered, however the numbers would be high.

Only one male P. rodhaini was caught throughout the year, and it was caught in December (1990). Two male S. clydei were caught by the lake-side in November (1990). Two female S. graingeri were trapped by the lake-side in a termite hill in November; and one female S. inermis was caught outside a house in July (1990).

3.3.2.1. Sandfly Incidence in different Sectors during various Months

When analysis was done for the overall combined data, the various sectors sampled were found to be significantly different from each other, (Table 2). Sectors 3, 4 and 5 were significantly different from sectors 1 and 2 which were on the southern side of the

island. The numbers of sandflies caught during the months of April to December, except August, showed significant differences for the months within the various sectors when a GLM was done (Appendix V).

When the DMRT was performed for these months; it was shown that in April sector 4 had the largest mean number of sandflies, which did not significantly differ from sector 3 (Appendix V). Sector 3 came second but it was not significantly different from sector 1. Sector 2 had the least mean number of flies caught in April.

The results of the variation in the incidence of sandflies in the different sectors during the months of May, June and July are summarized in Appendix V. During all these months, (April, May, June and July) sectors 3, 4 and 5 had the highest incidence of sandflies and it was noted that these sectors were situated on the leeward side of the Island. The results for the variation in sandfly incidence in different sectors in October, November and December are also in Appendix V.

3.3.3. Species Variation within the different Sectors

When the individual sectors (Figure 2) were analyzed, in December sector 4 was found to have a significant result for the species (Appendix XI). In July too, the species in sector 4 had significant differences among them (Appendix XI) and when DMRT was carried out, S. antennatus had the highest mean. S. africanus ranked second but it was not significantly different from S. bedfordi and S. schwetzi (Table 10).

In sector 5 also, the species were significantly different from each other (DF = 6, N = 40, F = 4.89, P > F at 0.0018). S. antennatus had the highest mean number of flies but it was not significantly different from S. bedfordi which ranked second though it was not significantly different from the other species that were caught that month (Table 10).

Table 10

DMRT of the Species in Sector 4 and 5 in July

SECTOR 4

Means with the same letter are not significantly different

Species	Mean	Duncan Grouping
<u>S. antennatus</u>	1.2018	A
<u>S. africanus</u>	0.9889	B
<u>S. bedfordi</u>	0.9836	B
<u>S. schwetzi</u>	0.9661	B
Unknown	0.8950	B

$p < 0.05$ Df = 97 MSE = 0.02214

SECTOR 5

Species	Mean	Duncan Grouping
<u>S. antennatus</u>	1.255	A
<u>S. bedfordi</u>	1.130	B A
Unknown	1.050	B A
<u>S. africanus</u>	1.012	B
<u>S. schwetzi</u>	0.984	B
<u>S. ingrani</u>	0.942	B
<u>S. serratus</u>	0.895	B

$p < 0.05$ Df = 26 MSE = 0.016855

TABLE 11

DMRT for habitats in sector I and III in June

SECTOR I

Means with the same letter are not significantly different

HABITAT	Mean	Duncan Grouping	
Outside	1.1038	A	
Inside	1.0615	B	A
Termite Hill	1.0288	B	A
Cow-shed	0.9452	B	A
Lake-side	0.8950	B	

P < 0.05

DF = 14

MSE = 0.010954

SECTOR III

HABITATS	Mean	Duncan Grouping	
Termite Hill	1.1680	A	
Tree	1.0844	B	

P < 0.05

Df = 53

MSE = 0.02297

Table 12

Duncans' Multiple Range Test (Species in sector 3 In
June)

SPECIES	Mean	Duncan Grouping	
<u>S. antennatus</u>	1.253		A
<u>S. serratus</u>	1.235	B	A
<u>S. bedfordi</u>	1.193	B	A
<u>S. africanus</u>	1.032	B	A
<u>S. ingrami</u>	1.020	B	A
<u>S. schwetzi</u>	0.991	B	C
Unknown	0.926		C
<u>S. squamipleuris</u>	0.895		C

$P < 0.05$

DF = 53

MSE = 0.22971

TABLE 13

DMRT (Species in Sectors I, III, and IV in May)

SECTOR I				SECTOR III			
SPECIES	Mean	Duncan Grouping		SPECIES	Mean	Duncan Grouping	
<u>S. antennatus</u>	1.0806	A		<u>S. antennatus</u>	1.1833	A	
<u>S. africanus</u>	0.9630	B	A	<u>S. bedfordi</u>	1.1422	A	
Unknown	0.9571	B	A	<u>S. ingrami</u>	1.0555	B	A
<u>S. bedfordi</u>	0.9511	B	A	<u>S. serratus</u>	1.0470	B	A
<u>S. schwetzi</u>	0.9194	B		<u>S. schwetzi</u>	0.9283	B	A
<u>S. ingrami</u>	0.8950	B		Unknown	0.9283	B	
<u>S. serratus</u>	0.8950	B		<u>S. africanus</u>	0.9120	B	
P < 0.05 DF = 40 MSE = 0.01043				P < 0.05 DF = 61 MSE = 0.0191411			

SECTOR IV

SPECIES	Mean	Duncan Grouping
<u>S. antennatus</u>	1.2371	A
<u>S. schwetzi</u>	1.0426	B
<u>S. bedfordi</u>	1.0184	B
<u>S. africanus</u>	0.9302	B
Unknown	0.9106	B
<u>S. serratus</u>	0.8950	B
P < 0.05 DF = 53 MSE = 0.018686		

TABLE 14

GLM Results and DMRT for Species in Sector Four in September

GLM

SOURCE	DF	SS	MS	F	PR > F	
HABITAT	3	0.0392	0.0131	1.03	0.3950	NS
SEX	1	0.0655	0.0655	5.16	0.0310	*
SPECIES	3	0.4753	0.1584	12.4	0.0001	****
<u>HAB. * SPE.</u>	4	0.057	0.0144	1.1	0.3631	NS

HAB. * SPE. = HABITAT * SPECIES

DMRT

Means with the same letter are not significantly different

SPECIES	Mean	Duncan Grouping
<u>S. antennatus</u>	1.1302	A
<u>S. schwetzi</u>	0.9724	B
<u>S. bedfordi</u>	0.9392	B
<u>S. africanus</u>	0.9067	B

P < 0.05

DF = 28

MSE = 0.012711

TABLE 15

DMRT for Species in Sector One in October

Means with the same letter are not significantly different

SPECIES	Mean	Duncan Grouping	
<u>S. antennatus</u>	1.1218	A	
<u>S. schwetzi</u>	1.0034	B	A
<u>S. ingrami</u>	0.9724	B	A
<u>S. bedfordi</u>	0.9439	B	A
Unknown	0.9417	B	A
<u>S. serratus</u>	0.9262	B	
<u>S. africanus</u>	0.9184	B	
<u>S. affinis</u>	0.8950	B	
<u>S. squamipleuris</u>	0.8950	B	

P < 0.05

DF = 21

MSE = 0.008402

Table 16

DMRT for Species in Sector IV and V in November

SECTOR IV

SPECIES	Mean	Duncan Grouping
<u>S. antennatus</u>	1.0807	A
<u>S. africanus</u>	0.9137	B
<u>S. schwetzi</u>	0.8950	B
<u>S. affinis</u>	0.8950	B
<u>S. bedfordi</u>	0.8950	B

P < 0.05 DF = 46 MSE = 0.008088

SECTOR V

SPECIES	Mean	Duncan Grouping
<u>S. antennatus</u>	1.233	A
<u>S. bedfordi</u>	1.009	B A
<u>S. schwetzi</u>	1.007	B A
<u>S. africanus</u>	0.932	B A
<u>S. serratus</u>	0.895	B A

P < 0.05 DF = 46 MSE = 0.008088

In February none of the sectors had significant differences between the groups of species, location and sex. In January in sector 1 the species had a significant difference among them in numbers, as revealed by GLM, (DF = 5, N = 37, F = 3.55, P > F at 0.0140).

In June in sector 1 the habitat, species and sex all had significant differences among them (Appendix XII). When the DMRT was performed there were no significant differences for the means of the species and the sexes. But for the habitats, the traps set outside the houses had the highest mean number of sandflies, although they were not significantly different from those inside the houses which ranked second (Table 11).

In sector 3, the habitats, species and interaction between habitat and species were significant (Appendix XII). When DMRT was performed on the habitat data it was found that the termite hills had more sandflies than the trees (Table 11).

The DMRT done on the species revealed that S. antennatus had a higher mean (in sector 3) than the others but was not significantly different from S. serratus, S. bedfordi, S. africanus and S. ingrami (Table 12).

In March none of the sectors had any significant differences between them with respect to the sandflies caught in them.

In May the species had significant differences in sector 1; $DF = 6$, $F = 5.02$, $P > F$ at 0.0006 . When DMRT was performed, S. antennatus had the highest mean but it was not significantly different from S. africanus and S. bedfordi (Table 13).

In sector three in May the species and the interaction between location and species were significantly different from each other (Appendix XIII). When DMRT was performed S. antennatus was predominant but it was not significantly different from S. bedfordi, S. ingrami, S. serratus and S. schwetzi (Table 13).

In sector four again the numbers of various species and the interaction between habitat and species were significant (Appendix XIII). When DMRT was performed S. antennatus had a significantly higher mean than the other species (Table 13).

In September sectors one, two, three and five had no significant differences for the means of the groups within them. In sector four, the species were significant. For the species (DF = 3, F = 12.46, and P > F is 0.0001). When the DMRT was done S. antennatus had a significantly higher mean than the rest of the species (Table 14).

In October in sector one the species variation was significant. For the species (DF = 8, F = 6.49, P > F = 0.0003 . When DMRT was performed, S. antennatus was dominant although it was not significantly different from S. schwetzi, S. ingrami and S. bedfordi (Table 15). In sectors two, three and four during the month of October all the groups were insignificant.

In November sectors one and two were insignificant, but four and five were significant as far as the species were concerned. In sector four (DF = 4, MS = 0.1042, F = 12.89, Pr > F = 0.0001), whereas in sector five (DF = 4, MS = 0.1045, F = 3.87, Pr > F = 0.0205). The DMRT revealed that in sector four S. antennatus had a significantly higher mean than the other species which had no significant difference between their means (Table 16)

In sector five, S. antennatus had the highest mean number of sandflies again although it was not significantly different from S. bedfordi, S. schwetzi and S. africanus. S. serratus ranked sixth (Table 16).

3.3.4. Variation in the sexes of the flies

As mentioned earlier in Chapter two section 2.4, there was no significant difference between the male and female flies when the overall analysis (including all the factors involved in the experiment) was performed. But when the DMRT was done for all the flies of the different sexes, it was found that the female flies had a significantly higher mean than the male flies (Table 17). This could be due to the difference in some individual months when sex was significant; as is the case in January and September (Appendix IX). When the DMRT was performed the males had a higher mean than the females in January and vice versa in September (Table 18).

3.4 Altitudinal Variation

More sandflies were caught at the lower altitudes in comparison to the higher altitudes. As the altitudes increased there was less variation in the composition of flies. There was more variation of the flies species at medium altitude between (3500 and 3800 Ft) in comparison to those with the highest altitude (Figure 6).

TABLE 17

DMRT for the male and female flies

Means with the same letter are not significantly different

SEX	Mean	Duncan Grouping
FEMALE	1.03113	A
<u>MALE</u>	<u>1.01832</u>	<u>B</u>

P < 0.05 DF = 1868 MSE = 0.017291

TABLE 18

DMRT for Sex of the Flies in January and September

JANUARY

Means with the same letter are not significantly different

SEX	Mean	Duncan Grouping
MALE	0.9810	A
FEMALE	0.9444	B

$P < 0.05$ $DF = 77$ $MSE = 0.06856$

SEPTEMBER

SEX	Mean	Duncan Grouping
FEMALE	1.0414	A
MALE	0.9749	B

$P < 0.05$ $DF = 80$ $MSE = 0.014739$

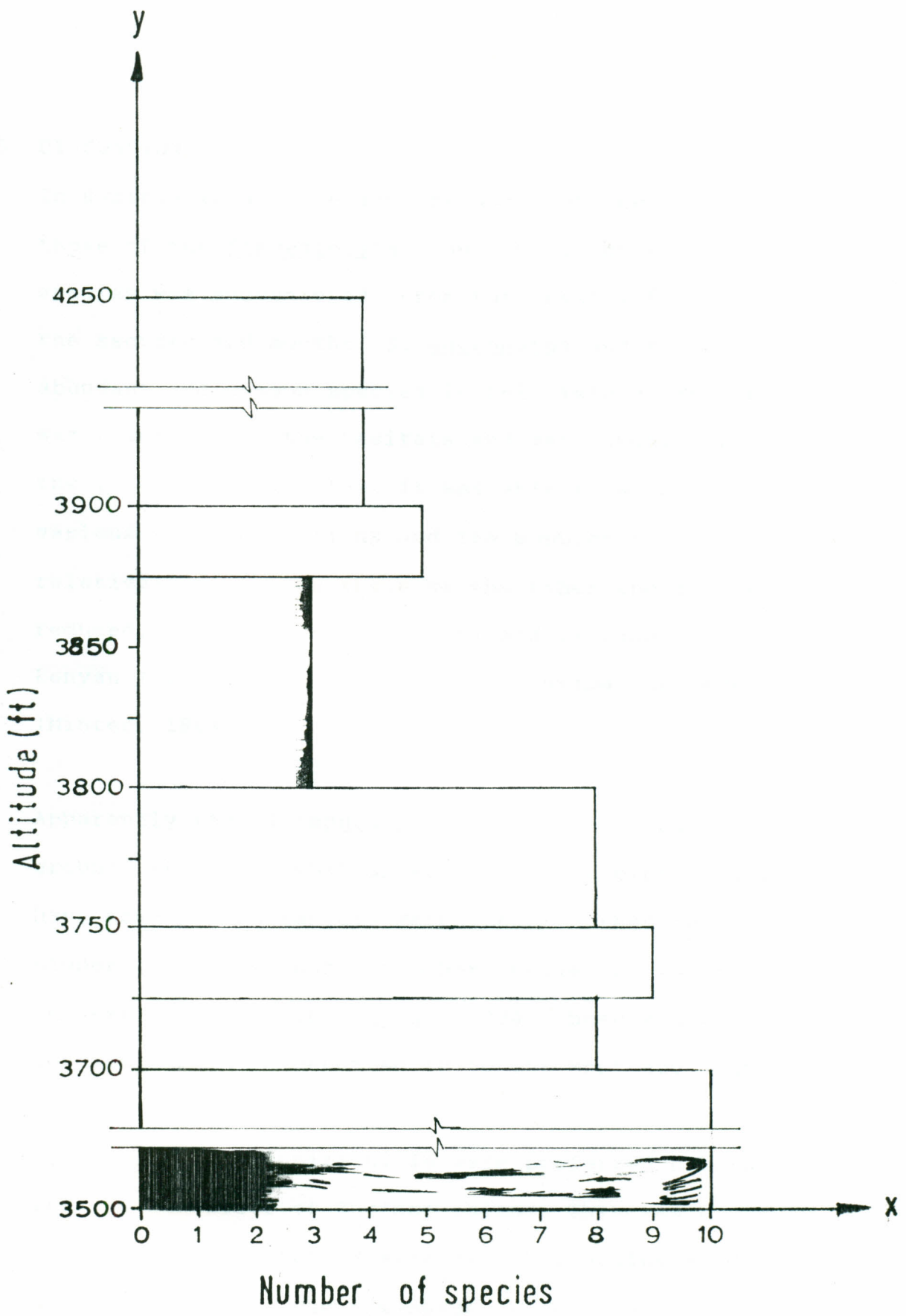


Fig.6. Altitudinal distribution of phlebotomine Sandflies in Rusinga Island.

3.5 DISCUSSION

In Rusinga island the most predominant species are those of the Sergentomyia genus. Only one Phlebotomus species was encountered. From the results found in all the sectors and months, S. antennatus was the most abundant and common species in this island. The species was found in all the habitats and was caught throughout the year. It seemed that it was able to withstand various tough conditions and its population remained relatively high when those of the other species were reduced. It is a robust species and is found in most Kenyan lowland areas for example, Marigat and Kauriro (Minter, 1964 b).

Apparently the (Antennatus, bedfordi and schwetzi group), that is, "Abs" group of phlebotomine sandflies have some common factors which enabled them to have a higher incidence than the other species of sandflies. In West Pokot (Mutinga et al. 1984) these sandfly species were also reported to be the most abundant.

Tree-holes and termite hills were preferred habitats for the sandflies of Rusinga island. This was probably because they did not receive direct sunshine as do the other places like the cow-sheds, rock-crevices or open vegetation by the lake-side. According to Noirot

(1970), termite hills have a high moisture content and they also provide thermal isolation. Luscher (1961) reported that some African termite nests are designed such that temperature and humidity within the nest is always maintained. He found that relative humidity was always above 96 % in nests of 5 species of termites in Ivory Coast. Findings by Mutinga et al. (1989) show that breeding sites of termite hills are associated with moisture shade and organic matter. The results indicate from Rusinga that darkness, high moisture content and thus high relative humidity are some of the optimal physical factors affecting sandfly abundance. This phenomenon has previously been observed by Kirk and Lewis (1951); Adler (1964) and Minter (1964 a). Termite hills have been found to be breeding and resting sites for many of the Eastern Africa sandfly species, for example, Phlebotomus martini (Mutinga et al. 1989, and Mutinga and Kamau, 1986).

The sectors on the windward side of the island had generally low incidence of sandfly population. Usually in the late afternoons and evenings there was a strong wind blowing from the northern side of the island. Sandflies are fragile and can not resist strong winds, so this could have been the cause of the lower incidence of flies in sectors 1 and 2. On the other hand, the winds could have blown the flies to the

southern side. It has been previously observed that sandflies tend to arrive in waves of wind, especially in the evenings (Ashford, 1974).

POTENTIAL OF THE BOSTON SANDFLY AS A VECTOR OF LEISHMANIASIS

1. INTRODUCTION

The sandfly, *Lutzomyia longipalpis*, is a vector of leishmaniasis in the tropics and subtropics. It is a blood-sucking insect that feeds on humans and animals. The sandfly is a common pest in many areas, and its bites can be very annoying. In some areas, sandfly bites can cause serious health problems, such as leishmaniasis. Leishmaniasis is a disease caused by a parasite that is transmitted by sandflies. There are several different types of leishmaniasis, and they can affect different parts of the body. Some types of leishmaniasis can be fatal. The sandfly is a very important vector of leishmaniasis, and it is important to understand its biology and behavior in order to control the disease. This paper will discuss the potential of the Boston sandfly as a vector of leishmaniasis. It will describe the sandfly's life cycle, its feeding habits, and its role in the transmission of leishmaniasis. It will also discuss the importance of sandfly control in the prevention of leishmaniasis.

The sandfly, *Lutzomyia longipalpis*, is a vector of leishmaniasis in the tropics and subtropics. It is a blood-sucking insect that feeds on humans and animals. The sandfly is a common pest in many areas, and its bites can be very annoying. In some areas, sandfly bites can cause serious health problems, such as leishmaniasis. Leishmaniasis is a disease caused by a parasite that is transmitted by sandflies. There are several different types of leishmaniasis, and they can affect different parts of the body. Some types of leishmaniasis can be fatal. The sandfly is a very important vector of leishmaniasis, and it is important to understand its biology and behavior in order to control the disease. This paper will discuss the potential of the Boston sandfly as a vector of leishmaniasis. It will describe the sandfly's life cycle, its feeding habits, and its role in the transmission of leishmaniasis. It will also discuss the importance of sandfly control in the prevention of leishmaniasis.

CHAPTER FOUR

4.0 POTENTIAL OF THE RUSINGA SANDFLIES AS VECTORS OF LEISHMANIASIS

4.1. INTRODUCTION

Sandflies are of great economic importance since they carry parasites and viruses that cause diseases in the tropical and sub-tropical regions of the world. It is because of this that it is crucial to know which species of sandflies could be the potential vectors in any given place. Among the tools used for this are blood-meal analyses of fed sandflies and the isolation and characterization of parasites accompanied by dissecting naturally infected wild-caught sandflies for disease-causing Leishmania parasites in nature.

Sandflies are attracted to specific animals for their blood-meals and this has been the basis for performing blood-meal analyses to determine upon which hosts a particular sandfly species feeds (Leeuwenburg and Lawyer, 1987). Host blood-meal analysis in sandflies is useful in determining the possible reservoir hosts of the leishmanial parasite species and whether the flies are anthropophilic or not. Blood-meal analysis can also

be utilized to find out natural host preference and can be a useful tool for incriminating both the vectors and reservoirs of leishmaniasis (Mutinga et al. 1990).

4.2. MATERIALS AND METHODS

4.2.1. DISSECTION

4.2.1.1. Trapping and Collection of Sandflies

This was done in the same way as described in Chapter two sections 2.3.1.2, 2.3.1.3. and 2.3.1.4.

4.2.1.2. Dissection of female flies

After the flies had been removed from the traps they were washed in a dilute solution of "Teepol" detergent for a short time and then transferred to a solution of normal saline (Sinton, 1932). The dissection of female flies was carried out as described by Mutinga and Odhiambo (1986) whereby the head was severed using entomological pins and the musculature between the last two abdominal segments was torn and the gut pulled backwards with one pin while the other pin is placed on

the thorax to hold the rest of the body onto the slide. Each fly was dissected in a drop of saline solution and the gut was transferred to a clean drop of saline with a coverslip over it for the entire gut to be examined under the microscope. The head was however mounted separately in a drop of gum chloral solution, and it aided in taxonomical identification up to species level of the dissected fly.

4.2.2. Parasite Culturing

Where a sandfly gut was suspected to have promastigotes, it was triturated into a drop of saline and the mixture aspirated into a syringe and innoculated into Novy, Mac'Neal and Nicolle's (NNN) medium (Taylor and Baker, 1986). The culture media were kept for five days at room temperature and then smears were made to check for any Leishmania parasites. The slides were stained with Giemsa stain, and then observed under the microscope at a magnification of 1000 using oil immersion.

4.2.3.

BLOODMEAL ANALYSIS

4.2.3.1 Trapping and Collection of Flies

This was the same as described in section 4.2.1.1. but the light trap (section 2.3.1.3.) was mostly used in this case to collect live flies that would have fresh blood-meals.

4.2.3.2. Analysis of the Meals

The fed female flies were separated from the rest of the unfed flies. Their engorged posterior midguts were individually transferred to Whatman filter paper Number 2, sealed in polythene bags and sent to Dr. C. Staak at the Robert Von Ostertag Institut in Berlin, (a World Health Organization (WHO) collaborative centre on blood-meal analysis using seroprecipitation) Germany. Twenty seven samples were analysed. The head and the rest of the body of the fed female sandflies were used for the identification of the sandfly specimen sent for blood-meal analysis.

4.3.

RESULTS

4.3.1. Dissection Results

A total of one thousand five hundred female flies were dissected and they were all negative for Leishmania parasites. In twenty flies flagellates were observed moving about in the guts. However, when these were cultured in the NNN medium, the flagellates did not grow.

4.3.2 Blood-meal Analysis

The blood-meal analysis results (Appendix VII) revealed that the sandflies in Rusinga Island mostly fed on monitor lizards and chicken. The sandflies caught and analyzed did not reveal any anthropophilic tendencies as far as the blood-meal analysis was concerned. 33.33 % of the flies fed on chicken while 66.67 % fed on monitor lizards. The flies that were engorged and whose blood-meals were assessed belonged to four species namely S. schwetzi, S. antennatus, S. squamipleuris and S. africanus (Figure 7). The blood fed S. squamipleuris was trapped from a tree-hole and had fed on a monitor lizard. S. africanus were found in a toilet and inside a

house having fed on a monitor lizard. 71.43 % of the S. antennatus analyzed fed on monitor lizards while the rest of the S. antennatus fed on chicken. Likewise 50 % of the S. schwetzi fed on monitor lizards and the other half fed on chicken (Tables 19 and 20). The S. schwetzi were caught around households. 81.82 % were found inside the houses while the remaining percentage was encountered outside the households.

44.44 % of the S. antennatus were found inside the houses while 27.78 % were caught outside the houses and in the toilets (Table 20).

The chi-squared independence test was performed, to find out whether there was any relationship between the fly species and its' host or any relationship between the habitat of the fed sandflies and the hosts they fed upon. Yates' Correction Factor was applied to avoid increasing Type I error, that is, the tendency of rejecting a perfectly true null hypothesis.

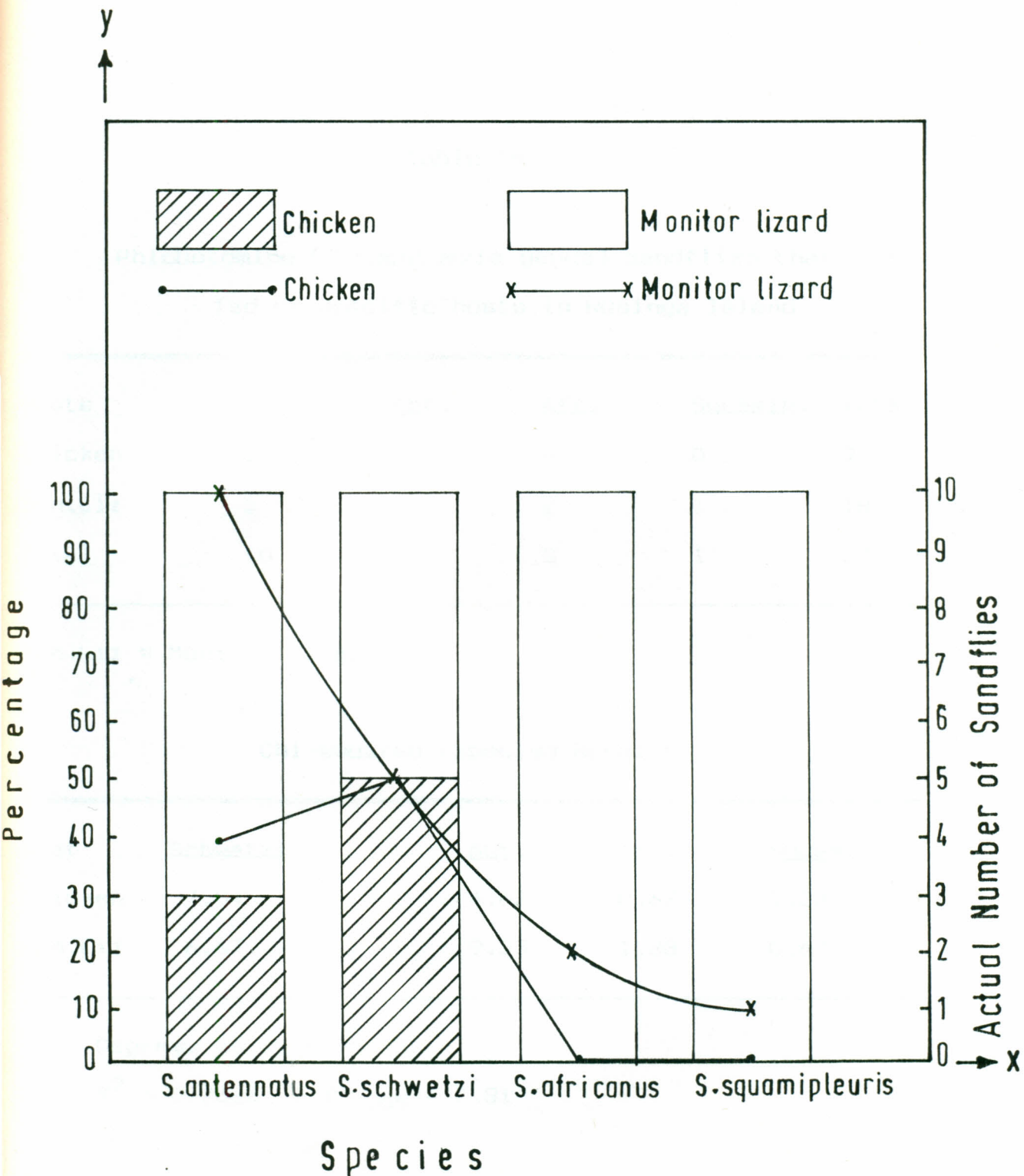


Fig.7. Percent host preference of phlebotomine species (Rusinga island)

Table 19

Phlebotomine (*Sergentomyia* genus) sandflies that had fed on specific hosts in Rusinga Island

Hosts	<u>Sch.</u>	<u>Ant.</u>	<u>Afr.</u>	<u>Squamip.</u>	Total
Chicken	5	4	0	0	9
Mon.Liz	5	10	2	1	18
Total	10	14	2	1	27

Mon.Liz = Monitor Lizard

Chi-squared Expected Results

Host	<u>Schwetzi</u>	<u>Ant.</u>	<u>Afr.</u>	<u>Squam.</u>
Chicken	7.04	4.67	0.67	0.33
Mon.Liz	6.67	9.33	1.33	0.67

Degrees of freedom =3

$$X^2 = 0.7366 \quad X^2_{0.05} = 7.81$$

$$X^2_{0.0001} = 13.82$$

Probability is greater than 0.05 so the null hypotheses of non significance is accepted.

Table 20

Phlebotomine sandflies (fed) collected from various habitats

Host	Inside	Outside	Tree	Total
	Houses	Houses	Holes Toilets	
Chicken	6	2	0 1	9
Mon.Liz	8	4	1 5	18
Total	14	6	1 6	27

H_0 = The habitats of the flies have no effect on the hosts they feed on.

Chi-squared Expected Results

	Inside	Outside	Toilet	Tree-hole
Chicken	4.67	2	2	0.33
Mon.Liz	9.33	4	4	0.67

$$\chi^2 = 1.723 \quad \text{Degrees of freedom} = 3$$

$$\chi^2_{0.05} = 7.81 \quad \chi^2_{0.0001} = 13.82$$

Probability is greater than 0.05 so the null hypothesis of non significance is accepted.

4.4. DISCUSSION

Presently, the phlebotomine sandflies in Rusinga Island are apparently, as far as the presence of Leishmania parasites are concerned, not carriers of leishmaniasis of man. The species caught belonged to the Sergentomyia genus except Phlebotomus rodhaini. This genus has only very few species suspected elsewhere as vectors of leishmaniasis, although some members of the Sergentomyia group of phlebotomine sandflies have previously been shown to harbor parasites resembling those that cause disease in man (Mutinga and Odhiambo 1982, and Mutinga et al. 1986b). None of the known suspected vectors were encountered in Rusinga Island. Of the Sergentomyia group S. bedfordi, S. graingeri, S. clydei, and S. schwetzi have been reported to bite man (Wijers and Minter, 1962; Mutinga and Ngoka, 1978; and Mutinga and Odhiambo, 1982). Experimental infections in the laboratory have shown that although S. antennatus picks up the human parasites (Kaddu et al. 1986), the parasites, however, do not develop in the gut in the known usual manner (Kaddu and Mutinga, 1984).

Furthermore it was established from the blood-meal analysis results that the engorged fed sandflies did not feed on humans even though some of them were

encountered inside the houses. The results show that the flies that were caught inside the houses had fed on chicken and monitor lizards. The chi-square results show that there was some relationship between the fed sandflies and the habitats in which they were caught. The inhabitants of Rusinga Island keep chicken in the houses at night and this explains the finding that some of the flies caught inside the houses fed on them. Monitor lizards are also common in the island especially in the bushy places, and at times they can even enter the houses during their hunting 'sprees' in search of chicken. Thus these sandflies fed on hosts that were around their resting sites. Sandflies have a weak flying ability and they usually hop about in their resting sites (Kirk and Lewis, 1951; Mutinga et al. 1986c) thus making it easy for them to feed on hosts that are within their surroundings. Furthermore it has been reported (Foster et al. 1972) that sandflies remain in their resting places to digest their meals.

From the relatively higher percentages of flies that fed on the monitor lizards it was evident that sandflies preferred to feed more on the monitor lizards than the chicken. Sandflies have previously been known to feed on lizards in nature (Mutinga et al. 1986c and 1990) and some of the lizards even act as reservoir hosts of human Leishmania parasites (Okot-kot'ber et

al. 1989). The current results indicate that S. antennatus preferred to feed on lizards and this has also been shown by Mutinga et al. (1990). Sergentomyia schwetzi showed equal preference for birds and reptiles (monitor lizards).

The chi-squared results indicate that there is a relationship between the fed sandflies and their hosts and habitats of the sandflies. This suggests that these specific sandflies were attracted more to some hosts than to others. Similar results have been reported by Mutinga et al. (1986c), found that the Sergentomyia species generally fed mainly on reptilian hosts.

CHAPTER FIVE

5.0 GENERAL DISCUSSION AND CONCLUSIONS

In Kenya studies have formerly been concentrated on known endemic foci of the disease but now new areas which pose the problem of being potential foci are being investigated, for example, Trans-Mara and Laikipia, (Lawyer et al. 1991). These areas need to be studied and their potential for disease outbreaks known. In the Mbita division of South Nyanza District, phlebotomine sandflies had been seen by the Medical Vectors Research Programme of ICIPE in 1986 but no detailed work had been carried out (Anon. 1988). This prompted current detailed study to determine the species of sandflies in this area and any potential harm they could cause.

The procedures followed in this study were those of a descriptive research (Best, 1981) which involves the description, recording, analysis and interpretation of conditions that exist. It comprises of some type of comparison and contrast and attempts to discover relationships between existing non-manipulated variables. The results revealed that Rusinga island has

32 % of the total number of sandfly species known in Kenya to date. Of the twelve species, eleven belong to the genus Sergentomyia and one (rodhaini) belongs to the Phlebotomus genus. It was apparent that the rainfall pattern had an influence on both the prevalence and incidence of the phlebotomine sandflies in Rusinga island. Relative humidity also influenced the abundance of sandflies. Some species of the sandflies especially the "Abs" group were perennial. These flies have also been previously found to be perennial in Kitui by Minter (1964b) and in Marigat by Basimike (1988). It seems that other factors apart from rainfall could have affected the occurrence of some of the sandfly species. The adults were suppressed by heavy rains, such as in April, and their population gradually increased as the rains subsided. But the breeding sites have earlier on been reported by Mutinga et al. (1989) to increase during the rainy season. This means that as the rains recede the adult population emerges until it finally reaches the peak.

Wind-speed and temperature also influenced the incidence of the sandflies. Still air appears to be conducive to the flies as opposed to strong winds and currents which have a deterrent effect on them. In the overall analysis the windward side of the island did not have a high incidence of sandflies. Since sandflies are fragile and tend to arrive in

waves of wind (Ashford, 1974), they could have been blown to the leeward side. The phlebotomine sandflies of Rusinga Island preferred mean temperatures of 27.5° centigrade. This phenomenon concurs with what Kirk and Lewis (1951) reported on the optimal temperature for sandflies. None of the known suspected vectors of leishmaniasis were encountered in Rusinga island. But of the Sergentomyia species caught, S. bedfordi and S. schwetzi have been reported biting man (Heisch et al. 1956; and Mutinga and Ngoka, 1978).

The tree-holes and termite hill habitats were preferred by the sandflies to other habitats. These habitats offered a suitable environment for resting especially during the daytime when it is usually very hot in Rusinga island. The relatively high moisture content in them is a suitable physical factor for sandflies as advanced by Kirk and Lewis (1951), Adler (1964) and Minter (1964a). The most predominant sandfly species in the island were those of the "Abs" group. This is in agreement with Minters findings (1964a) that both S. antennatus and S. bedfordi are common and widespread African species in Kenya. Sergentomyia schwetzi was previously reported (Heisch et al. 1956) to have a high incidence in Kitui and was caught throughout the year. Similar results were reported by Mutinga et al. (1982) in West Pokot for the "Abs" members.

The blood-meal analysis revealed that phlebotomine sandflies in Rusinga island fed on monitor lizards and chicken, with more of them feeding upon the former. There are other animals in Rusinga such as rats, snakes, monkeys (although these are confined to a small area which was not sampled), crocodiles, and hippopotamuses, but none of the fed flies that were caught had fed on these animals. It would appear that the species tested did have preferred hosts for feeding, that is, reptiles and chicken.

Kenyan sandflies have been shown to feed on lizards (Mutinga *et al.* 1984) and it has been reported (Mutinga *et al.* 1988) that lizards also harbor the Leishmania parasites including those which affect man in nature and malarial parasites as well (Dipeolu and Mutinga, 1989; Mutinga and Dipeolu, 1989). Similarly the parasites can even be maintained within the lizard host and more research performed on the Leishmania parasite as has been done by Forawi (1986) when L. major was isolated from a lizard in West Pokot.

It was concluded from these studies that phlebotomine sandflies in Rusinga island do not harbor Leishmania parasites, although this does not rule out future introduction of both vectors and parasites. The island has a causeway with a lot of movements of people and vehicles which could easily transport and introduce new vectors of

the disease. Some of the fly species caught, for example, S. ingrami has been indicated to be an alternate zoonotic vector in Baringo District (Mutinga et al. 1986a) although the parasites that cause the disease are absent in Rusinga at the moment. Experimental evidence on the vectorial capability of most of the various species of phlebotomine sandflies in Kenya is an important aspect of the epidemiology of leishmaniases (Kaddu, 1986). This would enhance the knowledge and facilitate the forecasting of potential disease (leishmaniasis) outbreak in any new area, where potential vectors are found. People would then not have to be taken "unawares" as it happened in the mid-fifties (Mutinga and Kamau, 1986) when the study of sandflies "received a sudden impetus after a serious epidemic of kala-azar in Kitui District" (Minter, 1964a) or recent outbreaks of the disease among soldiers stationed in Saudi Arabia.

5.1. Recommendations

In view of the fact that time and manpower were limiting factors in this study, no experiments were carried out in the Mbita mainland although phlebotomine sandflies had originally been sited there. It would be interesting to study the sandfly fauna in the mainland and compare them with those of Rusinga island. Since

the island is generally detached from the mainland (except for the narrow cause-way connecting them); there is a possibility of there being differences in their sandfly populations.

To further determine the possibility that the Rusinga Island sandflies can bite humans (The inhabitants of Rusinga claimed that they were usually bitten in the evenings when going fishing); human-baiting experiments should be done using volunteers and more host blood-meals analyses should be carried out.

Screening of leishmanial parasites from domestic and wild animals could also be carried out in this area in view of widespread wild reservoirs of leishmaniasis in Kenya and particularly in small ruminants (goats and sheep) which have been found to harbour human leishmaniasis and which travel far in form of trade.

REFERENCES

- ABDALLA, R.E. (1982). Parasites in Sudanese cutaneous and mucosal leishmaniasis. Ann. Trop. Med. Parasit.; 76 (3): 229-307.
- ABONNENC, E. (1972). Les Phlebotomines de la region Ethiopienne (Diptera: Psychodidae) Memoires ORSTOM, No. 55 Off. Rech. Sci.techn. Outre-Mer. Paris.
- ADLER, S. (1964). Leishmania, in Advances in Parasitology. Vol, II (Ed. Ben Daves) pp 35-96. Academic Press, London.
- ANDERSON, T.F. AND AYALA, S.C. (1968). Trypanosome transmitted by Phlebotomus. First report from the Americas. Science. 161: 1023-1025.
- ANON. (1988). ICIPE Sixteenth Annual Report, 1988.
- ANON. (1984). Report of a WHO Expert Committee on the Leishmaniases, WHO Technical report Series, 701. Geneva.

- ANON. (1980) Studies on Leishmaniasis vectors/reservoirs and their control in the Old World. Part IV. World Health Organization. Division of vector biology and control, 81.25. Geneva.
- ANON. (1970). Sectional map-west in National atlas of Kenya. Survey of Kenya. p 9.
- ASHFORD, R.W. (1974). Sandflies (Diptera: Phlebotominae) from Ethiopia: Taxonomic and biological notes. J. Med. Entomol. II: 605-616.
- ASHFORD, R.W. AND BETTINI, S. (1987). Ecology and Epidemiology. New World in Peters and Killick-Kendrick (eds), The Leishmaniasis in biology and Medicine, Vol. I, pp 365-424, Academic Press, New York.
- AYALA, S.C. (1971). Gregarine Infections in the Californian sandfly Lutzomyia vexatrix occidentalis. J. Invertebrate Pathology 17: 440-441.
- BASIMIKE, M. (1988). Studies on Factors affecting the Distribution and Abundance of Phlebotomine Sandflies in a leishmaniasis Endemic Focus in Marigat, District, Kenya. Ph.D Thesis, Riverstate University of Science and Technology, Nigeria.

- BELSHAW, R. (1980). Homabay, in Kenya Map and Photo Studies., p. 19 Survey of Kenya.
- BEST. J.W. (1981). Research in Education. Prentice-Hall, Inc., N.J., USA, 1981.
- BETTINI, S. AND GRADONI, L. (1986). Canine leishmaniasis in the Mediterranean area and its' implications for human leishmaniasis Insect Sci. Applic., 7 (20): 241-245.
- CHANIOTIS, B.N., CORREA, M.A., TESH, R.B., AND JOHNSON, K.M. (1988). Daily and Seasonal Man-biting activity of phlebotomine sandflies in Panama. J. Med. Entomol., 8 (4): 415-420
- CHENG, T.C. (1986). General Parasitology. Academic Press, Orlando, 1986.
- DIPEOLU, O.O. AND MUTINGA, M.J. (1989). Saurian malaria in Kenya: Description of ten new species from West Pokot district. Insect Sci. Applic., 10 (3): 413-436
- FORAWI, W.S. (1986). Studies on leishmania of lizards. Ph.D Thesis. University of Khartoum.
- FOSTER, W.A., BOREHAM, P.F.L. AND TEMPELIS, C.H. (1972). Studies on leishmaniasis in Ethiopia IV Feeding behaviour of P. longipes. Annals of Trop. and Med. Parasit. 66:433.

- HEISCH, R.B. (1954). Studies in leishmaniasis in East Africa. I. The epidemiology of an outbreak of kala-azar in Kenya. Trans. R. Soc. Trop. Med. Hyg. 48:449.
- HEISCH, R.B., GUGGISBERG, C.A.W. AND TEESDALE, C. (1956). Studies on leishmaniasis in East Africa. The Sandflies of the Kitui Kala-azar azar in Kenya, with descriptions of six new Species. Trans. R. Soc. Trop. Med. Hyg. 50 (3): 209-226.
- HERRER, A. AND CHRISTENSEN, H.A. (1975). Implications of phlebotomus sandflies as vectors of bartonellosis and leishmaniasis as early as 1764. Science., 190: 154-15.
- HOOGSTAL. H. AND HEYNEMAN, D. (1969). Leishmaniasis in the Sudan Republic: 30. Final Epidemiology report. Am. J. Trop. Med. Hyg. 18:1087-1112.
- JOETSOLD, R. AND R. AND SCHMIDT, H. (Ed) (1982). Ministry of Agriculture, Nairobi. Vol. II.
- KADDU, J.B. (1986). Leishmania in Kenyan phlebotomine sandflies - III. Advances in the vector-parasite relationships of various species of sandflies in Kenya. Insect Sci. Applic., 7 (2): 207-212.

KADDU, J.B. AND MUTINGA, M.J. (1984). Leishmaniasis in Kenyan Phlebotomine Sandflies - II. Natural infection in the malphigian tubules of Sergentomyia garnhami and S. antennatus. Insect sci. Applic., 5: 239-243.

KADDU, J.B., MUTINGA, M.J., AND NYAMORI, M.P. (1986). Leishmania in Kenyan Phlebotomine Sandflies - IV . Artificial feeding and attempts to infect six species of laboratory-reared sandflies with Leishmania donovani. Insect Sci. Applic., 7 (6): 731-735.

KADDU, J.B., MUTINGA, M.J., CHIMTAWI, M., OKOT-KOT'BER, B.M., NYAMORI, M.P. AND MUSYOKI, R. (1988). Leishmaniasis in Kenyan Phlebotomine Sandflies - V. Leishmania aethiopica in the oesophagus of Phlebotomus pedifer.

KATZELLENBOGEN, I. (1947). Cutaneous leishmaniasis in Palestine with a report of a new endemic focus in the Negev (Revivim) Harefuah 33:98-100.

KILLICK-KENDRICK, R. (1979). Biology of Leishmania in phlebotomine sandflies in Biology of the Kinetoplastida Vol. 2 (Eds. Lumsden, W.H.R. and Evans, D.A.) Academic Press London.

KIRK, R. AND LEWIS, D.J. (1955). Studies in leishmaniasis in the Anglo-Egyptian Sudan. Phlebotomus in relation to leishmaniasis in the Sudan. Trans. R. Soc. Trop. Med. Hyg., 49 (3): 229-240.

KIRK, R. AND LEWIS, D.J. (1951). The Phlebotominae of the Ethiopian region. Trans. Roy. Ent. Soc. Lond., 102 (8): 388-393.

KIRK, R. AND LEWIS, D.J. (1946). Taxonomy of the Ethiopian sandflies (Phlebotomus) I. Classification and synonymy. Ann. trop. Med. Parasit. 40: 34-51.

LAINSON, R. AND SHAW, J.J. (1978). Epidemiology and Ecology of Leishmaniasis in Latin-America. Nature 273: 595-600.

LAWYER, P.G., MEBRAHTU, Y.B., NGUMBI, P.M., MWANYUMBA, P., MBUGUA, J., KIILU, G., KIPKORECH, D., NZOVU, J. AND ANJILI, C.O. (1991). Phlebotomus guggisbergi (Diptera: Psychodidae), A vector of Leishmania tropica in Kenya. Am. J. Trop. Med. Hyg., 44(3), pp. 290-298.

LEEUWENBURG, J. AND LAWYER, P.G. (1987). Diagnostic Methods and criteria pertaining to epidemiological aspects of the leishmaniasis In Research on Control Strategies for the leishmaniasis - Proceedings of an International Workshop IDRC. CRDI. CILD. 184e: 241-242.

- LEWIS, D.J. (1982). A taxonomic review of the genus Phlebotomus (Diptera, Psychodidae) Bulletin of the British Museum (Natural History) Entomology Series, 45 (2): 122-209.
- LEWIS, D.J. (1973). Phlebotomidae and Psychodidae In Insects and other Arthropods of Medical importance (Smith, K.G.V. ed) pp 155. Bri. Mus. Nat. Hist. London.
- LEWIS, D.J. AND WARD, R.D. (1987). Transmission and Vectors In: The Leishmaniases in Biology and Medicine. Peters and Kellick-Kendrick (eds) Vol. I, 235-262. Academy Press.
- LUSCHER, M. (1961). Air-conditoined termite nests. Sci. Am. 205:138-145.
- MARQUARDT, W.C. AND DEMAREE, R.S. (1985). "How are parasitic diseases controlled?" In Parasitology p. 9-11 MacMillan, U.S.A.
- MINTER, D.M. (1964 a). The Distribution of Sandflies (Diptera, Psychodidae) in Kenya. Bull. Ent. Res., 55:205-217.
- MINTER, D.M. (1964 b). Seasonal changes in Populations of Phlebotomine sandflies (Diptera: Psychodidae) in Kenya. Bull. Ent. Res., 55 (3): 421-435.

- MOLYNEUX, D.H. AND ASHFORD, R.W. (1983). Introduction to the leishmaniases. In: The biology of Trypanosoma and Leishmania: parasites of man and domestic animals; pp 185-223. Taylor and Francis, London.
- MUTINGA, M.J. (1987). Leishmaniasis in Kenya. In: Research on control strategies for the leishmaniases-proceedings of an international workshop IDRC.CRDI.CILD. 184e: 24-33 Ottawa, Canada.
- MUTINGA, M.J. (1986). Epidemiology of leishmaniases in Kenya. Advances in research on vectors and animal reservoirs, and possible control measures. Insect Sci. Applic., 7 (2):199-206.
- MUTINGA, M.J. (1981). An efficient trap for sandflies (Diptera: Phlebotomidae). Insect Sci. Applic., 1: 203-206.
- MUTINGA, M.J. (1975). Phlebotomus fauna in the cutaneous leishmaniasis focus of Mt. Kenya. E. Afr. Med. J. 52:340-347.
- MUTINGA, M.J. (1971). Phlebotomus longipes, a vector of cutaneous leishmaniasis in Kenya. Trans. R. Soc. Trop. Hyg., 65:106.

- MUTINGA, M.J. AND DIPEOLU, O.O. (1989). Saurian malaria in Kenya: Description of new species of haemoproteid and haemogregarine parasites, anaplasma-like and pirhemocytion-like organisms in the blood of lizards in West-Pokot District. Insect Sci. Applic., 10 (3):401-413.
- MUTINGA, M.J. AND KAMAU. C.C. (1986). Investigations on the epidemiology of leishmaniasis in Kenya II: The breeding sites of phlebotomine sandflies in Marigat, Baringo District, Kenya. Insect Sci. Applic., 7:37.
- MUTINGA, M.J. AND NGOKA, J.M. (1978). Incrimination of vectors of visceral leishmaniasis in Kenya. E. Afr. med. J. 55: 332-336.
- MUTINGA, M.J. AND ODHIAMBO, T.R. (1986). Cutaneous leishmaniasis in Kenya - II. Studies on vector potential of Phlebotomus pedifer (Dipteran: Phlebotomidae) in Kenya. Insect Sci. Applic., 7 (2): 171-174.
- MUTINGA, M.J. AND ODHIAMBO, T.R. (1982). Studies on Infection rates of human-baited anthropophilic sandflies in Machakos District, Kenya. Insect Sci. Applic., 3: 211-214

MUTINGA, M.J., KAMAU, C.C. AND KYAI, F.M. (1986 a).

Investigations on the epidemiology of leishmaniasis in Kenya- IV. Breeding habitat of Phlebotomus duboscqi (Diptera, Psychodidae) a vector of Leishmania major in Marigat, Baringo District, Kenya. Insect Sci. Applic., 7 (2): 727-729.

MUTINGA, M.J., KYAI, F.K., AND OMOGO, D.M. (1986 b).

Investigations on the epidemiology of leishmaniasis in Kenya- I. studies on vectors of Leishmania major in Marigat, Baringo District, Kenya. Insect Sci. Applic., 7 (2) :181-186.

MUTINGA, M.J., NGOKA, J.M. AND ODHIAMBO, R.O. (1984).

Epidemiological investigations of visceral leishmaniasis in the West Pokot District, Kenya. Insect Sci. Applic., 6:521-525.

MUTINGA, M.J., BASIMIKE, M., KAMAU, C.C., MUTERO, C.M.

(1990). Epidemiology of leishmaniasis in Kenya. A natural Host preference of wild caught phlebotomine sandflies in Baringo District, Kenya. East Afr. Med. J. Vol. 67 No. 5: 319-327.

- MUTINGA, M.J., KYAI, F., KAMAU, C. AND OMOGO, D.M. (1986 c).
Epidemiology of leishmaniasis in Kenya III. Host Preference studies using various types of animal baits at animal burrows in Marigat, Baringo District. Insect Sci. Applic., 7 (2): 191-197.
- MUTINGA, M.J., KAMAU, C.C., KYAI, F.M. AND OMOGO, D.M. (1989). epidemiology of leishmaniasis in Kenya - V Wider search for breeding habitats of phlebotomine sandflies in three kala-azar endemic foci E. Afr. Med. J., 63:173-182.
- MUTINGA, M.J., MUTERO, C., NGINDU, A.. AND AMIMO, F. (1988). The isolation of leishmanial parasites from domestic and wild goats as reservoirs of leishmaniasis. Insect Sci. Applic., 9 (3): 347-349.
- NEAVE, S. (1904). Leishmania donovani in the Sudan. Brit. Med. J., I : 1252.
- NOIROT, C.H. (1970). The Nests of Termites In: Biology of termites Vol. II. (Eds. Krishna, K. and Weesner, F.M.) Translated by Passont, M. and Weesner, F.M. Academic Press, London.

- OKOT-KOT'BER, B.M. AND NDURURU, R. (1984) Biochemical Characterization of leishmanial isolates from humans and wild animals. ICIPE Twelfth Annual Report. pp. 35-37.
- PRATT, D.J., GREENWAY, P.J. AND GWYNNE, M.D. (1966). A Classification of East African Rangeland, (With an Appendix on Terminology) J. Appl. Ecology Vol. 3 and 4, 369-382
- PUNYUA, D.K. (1988). Rusinga Island Survey: Common ticks on livestock. In: Progress in Acarology VOL.I Eds' (G.P Channabasavanna and C.A. Vivaktamath) Oxford and IBH Publishing Co. Pvt. Ltd.
- ROGO, L.M., KHAMALA, C.P.M., AND MUTINGA, M.J. (1988). Biochemical identification of Phlebotomus (Larrousius) pedifer and Phlebotomus (Larrousius) elgonensis. Biochemical Systematics and Ecology, Vol. 16, No. 7/8. Pp 655-659.
- SCHLEIN, Y., WARBURG, A., SCHNUR, L.F., Le BLANQ, S.M., AND GUNDERS, A.E. (1984). Leishmaniasis in Israel: Reservoir hosts, sandfly vectors and leishmanial strains in Israel: The Negev, Central Arava and along the Dead sea. Trans. of the Roy. Soc. of Trop. Med. Hyg., 78: 480-484.

- SCHNUR, L.F. (1986). Identity and taxonomy within the genus Leishmania. Insect Sci. Applic., 2: 213-223
- SERVICE, M.W. (1986 a). Phlebotomine sandflies. In: Blood-sucking insects: vectors of disease Edward Arnold (Publishers) Ltd. London pp 34-35.
- SERVICE, M.W. (1986 b). Phlebotomine sandflies. In: Lecture notes on medical entomology. Blackwell Scientific Publications. London p. 106.
- SERVICE, M.W. (1976). Mosquito Ecology. Field sampling methods. Applied Science Publishers Ltd. London. 583 pp.
- SINTON, J.A. (1932). Some further records of Phlebotomus from Africa. Indian J. Med. Res. 20:565-576.
- SMITH, R.O.A. (1959). Bionomics of Phlebotomus argentipes. Bull. Calcutta Sch. trop. Med. & Hyg., 7:17-21.
- STERNFELD, G. (1944). Leishmaniasis of the skin in Haifa and the North Palestine. Harefuah, 26:43-45.

TAYLOR, A. E.R. AND BAKER, J. R. (1986). The Cultivation of Parasites In Vitro. Blackwell, Oxford.

WIJERS, D.J.B. AND MINTER, D.M. (1962). Studies on the vector of kala-azar in Kenya I. Entomological Evidence. Ann. trop. Med. Parasit., 56:462-472.

WIJEYARATNE, P.M. (1987). The leishmaniasis and development. In: Research on control strategies for the leishmaniasis- proceedings of an International workshop IDRC.CRDI, 184e:24-33. Ottawa, Canada.

ZAR, J.H. (1974). Biostatistical Analysis. P. 185. Prentice-Hall Inc. N.J. USA.

ZUCKERMANN, A. AND LAINSON, R. (1977). "Leishmania". In: Parasitic Protozoa, Vol. I Taxonomy, Kinetoplastids and Flagellates of Fish. Ed. (Julius P. Kreier). Academic Press Inc. New York.

APPENDIX I

PEARSON'S CORRELATION COEFFICIENTS (RELATIONSHIPS BETWEEN CLIMATIC FACTORS AND SANDFLY CATCH IN DIFFERENT SECTORS AND MONTHS)

	TOTAL	APRIL	FEB	I	II	III	IV
C/RH	-0.1009 0.0001***	-0.1212 0.1466	-0.1837 0.1836	-0.1738 0.0002***	-0.1298 0.0224*	-0.1304 0.0036**	-0.0607 0.1764
C/MAX	0.0067 0.7703	0.0337 0.6874	0.2015 0.1440	-0.0589 0.2040	-0.0177 0.7567	0.0775 0.0844	-0.1137 0.0111**
C/MIN	-0.0296 0.1970	0.0709 0.3969	0.2962 0.0296	0.0080 0.8627	0.1055 0.0641	-0.0227 0.6133	-0.0974 0.0298*
C/RN	-0.0313 0.1726	-0.1968 0.0177*	0.0681 0.6245	-0.0857 0.0644	-0.0674 0.2372	-0.0103 0.8197	-0.0326 0.4681
C/WSP	-0.0488 0.0336*	-0.1699 0.0410*	0.3128 0.0213*	-0.0132 0.7759	0.0694 0.2241	-0.0459 0.3066	-0.1270 0.0045**
C/WD	0.0131 0.5681	0.1725 0.0381*	.	-0.0061 0.8953	-0.0268 0.6387	0.0143 0.7512	0.0655 0.1443
C/SS	0.0713 0.0019**	0.1788 0.0314*	0.1366 0.3247	0.0679 0.1433	0.0960 0.0921	0.1206 0.0071**	-0.0130 0.7720

* = Significance level of 0.05

N = 1898 C = CATCH

** = Significance level of 0.01

RH = RELATIVE HUMIDITY

*** = Significance level of 0.001

MAX = MAXIMUM TEMPERATURE

MIN = MINIMUM TEMPERATURE

RN = RAINFALL

WD = WIND DIRECTION

SS = SUNSHINE FEB = FEBRUARY (N = 54) APRIL (N = 145)

TOTAL = OVERALL ANALYSIS INCLUDING ALL FACTORS, MONTHS, SPECIES, ETC;

I = SECTOR I

II = SECTOR II

III = SECTOR III

IV = SECTOR IV

APPENDIX II

SUMMARY OF THE WEATHER CONDITIONS (MEANS) DURING THE STUDY PERIOD (JANUARY
- DECEMBER)

MONTH	RH(%)	MAXT	MINT	RAIN	WINDSP	SUN
JAN	67.86	29.55	19.13	1.594	215.09	9.68
FEB	71.58	27.73	19.32	3.871	228.81	6.17
MAR	77.62	26.26	15.90	7.0355	181.11	7.48
APR	74.69	26.74	14.88	5.5667	175.94	7.76
MAY	71.75	26.75	15.11	4.0065	177.86	8.19
JUNE	64.15	27.27	15.64	0.5533	184.16	9.88
JULY	64.98	27.85	18.71	3.9	189.19	9.01
AUG	67.19	26.53	19.64	0.0	198.03	7.51
SEPT	64.50	27.38	20.89	0.0	191.42	8.36
OCT	66.31	27.66	20.66	0.7677	196.02	7.34
NOV	71.94	28.09	19.90	3.983	203.85	8.17
DEC	72.43	27.07	19.45	2.8935	195.51	7.74

RH : RELATIVE HUMIDITY

MAXT : MAXIMUM TEMPERATURE ($^{\circ}$ C)MINT : MINIMUM TEMPERATURE ($^{\circ}$ C)

RAIN : RAINFALL (MM)

WINDSP : WINDSPEED (KM/HR)

SUN : SUNSHINE (HOURS)

APPENDIX III

RELATIONSHIPS BETWEEN THE CLIMATIC FACTORS (CORRELATION COEFFICIENTS)

	RH	MAXT	MINT	RAIN	DUR	WDSP	WDDR	SNSN
RH	1.0000 0.0***	-0.3839 0.0001***	-0.2129 0.0001***	-0.3093 0.0001***	0.2889 0.0001***	-0.0288 0.2096	-0.631 0.0060**	-0.5111 0.0001***
MAXT	-0.3839 0.0001***	1.0000 0.0***	0.2211 0.0001***	0.2714 0.0001***	0.1459 0.0001***	0.5303 0.0001***	0.0724 0.0016**	0.4106 0.0001***
MINT	-0.2129 0.0001***	0.2211 0.0001***	1.0000 0.0***	-0.234 0.0001***	-0.1925 0.0001***	0.4268 0.0001***	-0.0507 0.0271*	-0.0394 0.0863
RAIN	0.3093 0.0001***	0.2714 0.0001***	-0.234 0.0001***	1.0000 0.0***	0.7904 0.0***	0.1418 0.0001***	0.0842 0.0002***	-0.3509 0.0001***
DUR	0.2889 0.0001***	0.1459 0.0001***	-0.1925 0.0001***	0.7904 0.0***	1.000 0.0***	0.0729 0.0015**	-0.1083 0.0001***	-0.4085 0.0001***
WDSP	-0.0288 0.2096	0.5303 0.0001***	0.4268 0.0001***	0.1418 0.0001***	0.0729 0.0015**	1.000 0.0***	-0.1813 0.0001***	-0.0302 0.1890
WDDR	0.0631 0.0060**	0.0724 0.0016**	-0.0507 0.0271*	0.0842 0.0002***	-0.1083 0.0001***	-0.1813 0.0001***	1.000 0.0***	0.0955 0.0001***
SNSN	-0.5111 0.0001***	0.4106 0.0001***	-0.0394 0.0863	-0.3509 0.000***	-0.4085 0.0001***	-0.0302 0.1890	0.0955 0.0001***	1.000 0.0***

RH = Relative Humidity MAXT = Maximum Temperature MINT = Minimum Temperature

RAIN = Rainfall DUR = Duration of Rainfall WDSP = Windspeed

WDDR = Wind-direction SNSN = Sunshine

APPENDIX IV

DMRT for the months when species numbers varied significantly

JANUARY				APRIL			
SPECIES	Mean	Duncan Grouping		SPECIES	Mean	Duncan grouping	
S. antennatus	1.0010		A	S. antennatus	1.0732		A
S. ingrami	0.9729	B	A	S. bedfordi	1.0038	B	A
S. bedfordi	0.9590	B	A	S. squamipleuris	0.9950	B	A
S. schwetzi	0.9546	B	A	S. schwetzi	0.9931	B	A
S. africanus	0.9205	B		S. serratus	0.9773	B	A
Unknown	0.9054	B		S. africanus	0.9493	B	A
				Unknown	0.9028	B	
				S. ingrami	0.8950	B	
p < 0.05 df = 77 MSE = 0.006856				p < 0.05 df = 127 MSE = 0.012586			
MAY				JULY			
SPECIES	Mean	Duncan Grouping		SPECIES	Mean	Duncan Grouping	
S. antennatus	1.1396		A	S. antennatus	1.1582		A
S. bedfordi	1.0766	B	A	S. bedfordi	1.0349	B	A
S. ingrami	1.0097	B	C	S. africanus	0.9735	B	A
S. schwetzi	0.9944	B	C	S. schwetzi	0.9525	B	
S. serratus	0.9829	B	C	S. serratus	0.9364	B	
S. africanus	0.9324		C	S. ingrami	0.9262	B	
Unknown	0.9235		C	S. squamipleuris	0.8950	B	
p < 0.05 df = 224 MSE = 0.0191				p < 0.05 df = 329 MSE = 0.021729			

continued

....cont'd.

APPENDIX IV

DMRT for the months when species numbers varied significantly

OCTOBER			NOVEMBER		
SPECIES	Mean	Duncan Grouping	SPECIES	Mean	Duncan Grouping
<u>S. antennatus</u>	1.1715	A	<u>S. antennatus</u>	1.0929	A
<u>S. bedfordi</u>	0.9963	B A	<u>S. bedfordi</u>	1.0115	B A
<u>S. schwetzi</u>	0.9779	B	<u>S. schwetzi</u>	0.9630	B A
<u>S. ingrami</u>	0.9724	B	<u>S. affinis</u>	0.9447	B A
<u>S. serratus</u>	0.9417	B	<u>S. africanus</u>	0.9411	B A
<u>S. squamipleuris</u>	0.9417	B	<u>S. ingrami</u>	0.8950	B
<u>S. africanus</u>	0.9192	B	Unknown	0.8950	B
Unknown	0.9184	B	<u>S. serratus</u>	0.8950	B
<u>S. affinis</u>	0.9106	B	<u>S. squamipleuris</u>	0.8950	B

p < 0.05 df = 115 MSE = 0.019359

p < 0.05 df = 245 MSE = 0.0156

APPENDIX V

MONTHS WHEN THE SECTORS WERE SIGNIFICANT
(GLM)

SOURCE	DF	TYPE I SS	MS	F VALUE	Pr > F
APRIL					
SECTOR	3	0.2902	0.0967	7.69	0.0001****
MAY					
SECTOR	3	0.6638	0.2213	11.62	0.0001****
JUNE					
SECTOR	3	0.5337	0.1779	7.44	0.0001****
JULY					
SECTOR	4	0.4491	0.11226	5.17	0.0005***
OCTOBER					
SECTOR	4	0.3750	0.0937	4.84	0.0012***
NOVEMBER					
SECTOR	4	0.6423	0.1606	10.29	0.0001****
DECEMBER					
SECTOR	4	0.5077	0.1269	12.52	0.0001****

DF = Degrees of freedom

SS = Sum of Squares

MS = Mean Sum of Squares

*** = Significance level of 0.001

APPENDIX VI

DMRT for the months during which the fly numbers in the sectors varied significantly

APRIL			MAY				
SECTOR	Mean	Duncan Grouping		SECTOR	Mean	Duncan Grouping	
IV	1.0557		A	IV	1.0920		A
III	1.0190	B	A	III	1.0723		A
I	0.9831	B		I	0.9862		B
II	0.9254		C	I	0.9584		B

JUNE			JULY				
SECTOR	Mean	Duncan Grouping		SECTOR	Mean	Duncan Grouping	
III	1.1331		A	V	1.1092		A
IV	1.0727	B	A	III	1.0766	B	A
I	1.0229	B	C	IV	1.0715	B	A
II	1.0998		C	I	1.0234	B	C
				II	0.9949		C

OCTOBER			NOVEMBER				
SECTOR	Mean	Duncan Grouping		SECTOR	Mean	Duncan Grouping	
IV	1.1138		A	III	1.0908		A
III	1.0839	B	A	V	1.0668	B	A
V	1.0594	B	A	IV	1.0275	B	
I	1.0118		C	II	1.0176	B	
II	0.919		C	I	0.9552		C

DECEMBER			DECEMBER				
SECTOR	Mean	Duncan Grouping		SECTOR	Mean	Duncan grouping	
III	1.0511		A	I	0.9425	D	C
IV	1.1118	B	A	II	0.9243	D	
V	0.9830	B	C				

APPENDIX VII

Percentage Composition of Monthly Catches in Rusinga Island
from January to December 1990

MONTH

SPECIES	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE	JULY	SEPTEMBER	OCTOBER	NOVEMBER	DECEMBER
A	54.78	43.52	41.61	57.03	61.16	77.61	77.53	72.92	85.35	75.76	67.78
B	6.92	6.48	24.82	17.15	20.16	10.21	11.82	16.89	5.27	9.74	13.53
C	17.55	27.78	16.06	13.38	9.98	2.99	2.61	4.29	4.79	8.19	11.09
D	7.98	3.70	1.46	0.62	1.42	0.64	0.12	.	0.38	0.31	0.30
E	7.45	12.96	8.76	5.99	3.84	4.27	6.75	5.36	2.11	4.29	4.56
F	.	0.93	2.92	1.03	.	.	0.04	.	0.28	0.16	0.76
G	.	.	.	1.86	1.69	4.03	0.39	0.27	0.57	0.31	0.15
H	0.05	.	.	0.67	0.62	0.61
I	0.04
J	0.16	.
K	0.08	0.15
L	0.15	.
Unknown	5.32	4.63	4.38	2.68	1.75	0.19	0.70	0.27	0.48	0.39	0.91
TOTAL	188	110	142	497	1483	2037	2563	373	1044	1283	659

GRAND TOTAL = 10379

A = *S. antennatus*B = *S. bedfordi*C = *S. schwetzi*D = *S. ingrami*E = *S. africanus*F = *S. squamipleuris*G = *S. serratus*H = *S. affinis*I = *S. inermis*J = *S. clydei*K = *S. graingeri*L = *P. rodhaini*

APPENDIX VIII

BLOODMEAL ANALYSIS RESULTS

SPECIES	HABITAT	HOST
1. <u>S. schwetzi</u>	Inside House	Monitor Lizard
2. <u>S. schwetzi</u>	Inside House	Chicken
3. <u>S. schwetzi</u>	Inside House	Chicken
4. <u>S. schwetzi</u>	Inside House	Chicken
5. <u>S. schwetzi</u>	Inside House	Chicken
6. <u>S. schwetzi</u>	Inside House	Chicken
7. <u>S. schwetzi</u>	Inside House	Monitor Lizard
8. <u>S. schwetzi</u>	Inside House	Monitor Lizard
9. <u>S. schwetzi</u>	Inside House	Monitor Lizard
10. Unknown	Toilet	Chicken
11. <u>S. antennatus</u>	Outside House	Chicken
12. <u>S. antennatus</u>	Toilet	Monitor Lizard
13. Unknown	Toilet	Monitor Lizard
14. <u>S. antennatus</u>	Toilet	Chicken
15. <u>S. antennatus</u>	Toilet	Monitor Lizard
16. <u>S. antennatus</u>	Toilet	Monitor Lizard
17. Unknown	Inside House	Monitor Lizard
18. <u>S. schwetzi</u>	Outside House	Monitor Lizard
19. <u>S. antennatus</u>	Outside House	Monitor Lizard
20. <u>S. antennatus</u>	Inside House	Monitor Lizard
21. <u>S. antennatus</u>	Outside House	Monitor Lizard
22. <u>S. antennatus</u>	Inside House	Monitor Lizard
23. <u>S. squamipleuris</u>	Tree-hole	Monitor Lizard
24. <u>S. schwetzi</u>	Inside House	Chicken
25. <u>S. antennatus</u>	Inside House	Chicken
26. <u>S. africanus</u>	Toilet	Monitor Lizard
27. <u>S. antennatus</u>	Outside House	Monitor Lizard

APPENDIX IX

ANOVA RESULTS FOR VARIOUS FACTORS IN JANUARY AND SEPTEMBER

JANUARY

SOURCE	DF	SS	MS	F	Pr > F
SECTOR	4	0.0102	0.0034	0.50	0.6862
HABITAT	3	0.0244	0.0081	1.19	0.3202
SPECIES	5	0.1468	0.0294	4.28	0.0017**
SEX	1	0.0501	0.0501	7.30	0.0085**
ERROR	77	0.5279	0.0068		

SEPTEMBER

SOURCE	DF	SS	MS	F	Pr > F
SECTOR	4	0.0881	0.0220	1.49	0.2118
HABITAT	6	0.1343	0.0224	1.52	0.1827
SPECIES	5	0.3413	0.0683	4.63	0.0009***
SEX	1	0.1425	0.1425	9.67	0.00026***
ERROR	80	1.1792	0.0147		

APPENDIX X

ANOVA RESULTS FOR MONTHS WHEN SPECIES AND HABITATS SHOWED
SIGNIFICANT RELATIONSHIPS

SOURCE	DF	SS	MS	F	Pr > F
JANUARY					
HABITAT	3	0.0244	0.0081	1.19	0.3202
SPECIES	5	0.1468	0.0294	4.28	0.0017**
APRIL					
HABITAT	6	0.0356	0.0059	0.47	0.8282
SPECIES	7	0.4804	0.0686	5.45	0.0001****
MAY					
HABITAT	6	0.1429	0.0238	1.25	0.2813
SPECIES	6	1.8436	0.3073	16.13	0.0001****
JULY					
HABITAT	6	0.2168	0.0361	1.66	0.1294
SPECIES	7	3.1374	0.4482	20.63	0.0001****
OCTOBER					
HABITAT	6	0.0536	0.0295	1.89	0.0834
SPECIES	8	1.5010	0.1876	12.03	0.0001****

APPENDIX XI

GLM RESULTS SHOWING RELATIONSHIPS BETWEEN SPECIES, HABITATS AND SEX
IN SECTOR IV IN JULY AND DECEMBER

JULY

SOURCE	DF	SS	MS	F	Pr > F
HABITAT	3	0.0601	0.0200	0.9	0.4421
SEX	1	0.0286	0.0286	1.29	0.2589
SPECIES	4	1.6058	0.4015	18.13	0.0001***
HABITAT * SPECIES	9	0.1599	0.0178	0.8	0.6647
ERROR	28	0.3559	0.0127		

DECEMBER

SOURCE	DF	SS	MS	F	Pr > F
HABITAT	3	0.0277	0.0092	0.73	0.5372
SEX	1	0.0291	0.0291	2.31	0.1356
SPECIES	5	0.3301	0.0660	5.25	0.0007***
HABITAT * SPECIES	6	0.338	0.0056	0.45	0.8423
ERROR	97	2.1479	0.0221		

APPENDIX XII

GLM TABLE SHOWING INTERACTION BETWEEN HABITAT, SEX AND SPECIES FOR
SECTOR III IN JUNE

SOURCE	DF	SS	MS	F	Pr > F
MODEL	13	1.8329	0.1410	6.14	0.0001
ERROR	53	1.2174	0.0229		
CORRECTED TOTAL	66	3.0503			
	R-SQUARE	C.V		ROOT MSE	
	0.6009	13.37620		0.1515	

SOURCE	DF	SS	MS	F	Pr > F
HABITAT	1	0.1138	0.1138	4.95	0.0303*
SEX	1	0.0007	0.0007	0.03	0.8565
SPECIES	7	0.9951	0.1422	6.19	0.0001****
HABITAT * SPECIES	4	0.7233	0.1808	7.87	0.0001****

APPENDIX XIII

GLM FOR SECTORS I, III, AND IV IN MAY

SECTOR I

SOURCE	DF	SS	MS	F	Pr > F
HABITAT	3	0.0335	0.0112	1.07	0.3719
SEX	1	0.0014	0.0014	0.14	0.7126
SPECIES	6	0.3141	0.0524	5.02	0.0006****
HABITAT * SPECIES	11	0.0485	0.0044	.042	0.9369
ERROR	40	0.4172	0.0104		

SECTOR III

SOURCE	DF	SS	MS	F	Pr > F
HABITAT	1	0.0125	0.0125	0.65	0.4249
SEX	1	0.0326	0.0326	1.68	0.1999
SPECIES	6	0.6890	0.1148	5.92	0.0001****
HABITAT * SPECIES	6	0.3024	0.0504	2.60	0.0263*
ERROR	61	1.1841	0.0194		

SECTOR IV

SOURCE	DF	SS	MS	F	Pr > F
HABITAT	3	0.1859	0.0619	3.32	0.0267*
SEX	1	0.0323	0.0323	1.73	0.1943
SPECIES	5	1.3838	0.2767	14.81	0.0001****
HABITAT * SPECIES	8	0.3574	0.0447	2.39	0.0279*
ERROR	53	0.9904	0.0187		