Strategy for monitoring and sustainable integrated control or eradication of *Glossina brevipalpis* and *G. austeni* (Diptera: Glossinidae) in South Africa

by

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SUMMARY

Glossina brevipalpis Newstead and *G. austeni* Newstead (Diptera: Glossinidae) are the vectors of trypanosomosis or nagana in cattle in N.E. KwaZulu-Natal, South Africa. Before intervention by means of target technology could be applied successfully to control these species, studies were still needed on the two species' attraction to natural host odours, trapping, their movement and dispersal, feeding responses towards hosts and their geographical distribution. Studies that were conducted with host odours to find an attractive odour for *G. austeni*, proved that CO_2 was seemingly the main attractive component of host odour for this species. The existing chemicals of the best SA odour, comprising of octenol released at *c.* 9,1 mg/h, 4-methylphenol released at *c.*15,5 mg/h and acetone released at *c.* 350 mg/h, still remained to be the main attractive components for *G. brevipalpis*. A sticky trap, namely a bicoloured electric blue/black XT, was refined to use in tsetse distribution surveys. A new trap, the H trap, was developed and proved to be effective in catching relatively high numbers of both species. This trap was used to capture live tsetse for mark-release-recapture studies to assess the population size and movement of a tsetse population. These studies revealed that target densities of about 4 targets/km^2 for *G. brevipalpis* and 7 targets/km^2 for *G. austeni* should be effective to control these species successfully with odour-baited insecticide-impregnated targets. *G. austeni* was confined to densely shaded areas but it still traversed short distances of up to 345 m of "unsuitable" habitat between pockets of vegetation. *G. brevipalpis* was considered a much more mobile fly and traversed wide areas of 1,345 m. Both species were readily attracted to cattle, but not to goats nor bushpig. They also fed more readily on cattle. Both species would also feed at night. It was recommended that insecticide-treated cattle could be used as mobile targets to control both *G. brevipalpis* and *G. austeni* in areas where cattle predominate.

Tsetse surveys through the northeastern parts of KwaZulu-Natal showed that there were two distinct bands of distribution for *G. brevipalpis*. The main sources of this species seemed to be the game reserves and other natural areas. *G. austeni* was more widespread with a continuous north to south distribution. A Geographic Information System was used to map tsetse distribution and their apparent densities. This was collated with trypanosomosis incidence and prevalence, diptank (cattle) distribution,

land tenure/designation, landcover and vegetation types, which were also mapped. Finally, a strategy was proposed for the monitoring and sustainable integrated control and eventual eradication of both *G. brevipalpis* and *G. austeni* throughout N.E. KwaZulu-Natal. This involves the subdivision of the area into five manageable zones with successive pre-suppression, suppression and eradication operations following in each of the zones. With this proposed strategy eradication of both species could be achieved within 8 - 12 years after initiation.

SAMEVATTING

Glossina brevipalpis Newstead en *G. austeni* Newstead (Diptera: Glossinidae) is vektore van trypasosomose (nagana) in beeste in noordoos KwaZulu-Natal, Suid-Afrika. Voordat ingryping deur middel van teiken-tegnologie suksesvol toegepas kan word om hierdie spesies te beheer, was dit nodig om die spesies se aanlokking na natuurlike gasheerreuke, vangmetodes, hulle beweging en verspreiding, voergedrag ten opsigte van gashere en hulle geografiese verspreiding, te bestudeer. Studies wat met gasheerreuke uitgevoer was om 'n aanloklike geur vir *G. austeni* te vind, het bewys dat CO_2 bleikbaar die hoof aanlokkingskomponent vir hierdie spesie is. Dit is bevestig dat die bestaande chemikalieë van die beste SA geur, wat bestaan uit oktenol vrygelaat teen *c.* 9,1 mg/h, 4-metielfenol vrygelaat teen *c.*15,5 mg/h en asetoon vrygelaat teen *c.* 350 mg/h, die hoof aanloklikkingskomponente vir *G. brevipalpis* is. 'n Gomval ("sticky trap"), naamlik 'n tweekleurige blou/swart XT, was aangepas om in tsetse verspreidingsopnames te gebruik. Die nuutontwikkelde H-val was bewys om doeltreffend te wees om relatiewe hoë getalle van beide tsetse spesies te kan versamel. Hierdie val was gebruik om lewende vlieë vir vang-merk-vrylaat studies te versamel om die populasie grootte en beweging van 'n tsetse populasie te bepaal. Laasgenoemde studies het getoon dat teikendigthede van omtrent 4 en 7 teikens/km^2 vir *G. brevipalpis* en *G. austeni* ondeskeidelik voldoende behoort te wees om hierdie spesies suksesvol met geurlokaas en insekmiddel-geïmpregneerde teikens te beheer. *G. austeni* is beperk tot digte skaduryke areas maar kan kort afstande, tot 345 m, van ongunstige habitat, tussen plate van digte plantegroei oorbrug. *G. brevipalpis* was beskou as 'n baie meer mobiele vlieg en het wye areas van 1,345 m oorbrug. Albei spesies word geredelik aangelok na beeste, maar nie na boerbokke of bosvarke nie. Hulle het ook meer geredelik op bees gevoed. Beide spesies kan ook in die nag voed. Dit word aanbeveel dat beeste wat met 'n insekmiddel behandel is as mobiele teikens gebruik word om beide *G. brevipalpis* en *G. austeni* te beheer, in areas waar beeste die oorheersende gasheer is.

Tsetse verspreidingopnames in die noordoostelike KwaZulu-Natal het gewys dat *G. brevipalpis* in twee hoofverspreidings-bande voorkom. Die belangrikste bron van hierdie spesie skyn natuur-reservate asook ander natuurlike gebiede te wees. *G. austeni* is meer wydverspreid met 'n aaneenlopende noord tot suid verspreiding. 'n

Geografiese Inligtingstelsel was gebruik om tsetse verspreiding en oënskynlike digthede te karteer. Trypanosomose-gevalle en -voorkoms, diptenkverspreiding, grondgebruik, gronbedekking en plantegroeitipes is ook gekarteer en met tsetse verspreiding en digthede vergelyk. Laastens, was 'n strategie vir die monitering en onderhoubare geïntegreerde beheer en uiteindelike totale uitwissing van beide *G. brevipalpis* en *G. austeni* in die hele N.O. KwaZulu-Natal voorgestel. Dit behels die onderverdeling van die gebied in vyf bestuurbare zones met opeenvolgende pre-suppressie, suppressie and uitroei operasies wat in elk van die zones volg. Met hierdie voorgestelde strategie kan beide tsetse spesies binne 8 - 12 jaar uitgeroei word.

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LIST OF ACRONYMS AND ABBREVIATIONS

3DT	3-Dimensional trap
Ag-ELISA	Antigen – Enzyme Linked Immuno-Sorbent Assay
ANOVA	Analysis of variance
AOP	Acetone, octenol and phenols mixture
ARC-ISCW	Agricultural Research Council-Institute for Soil, Climate and Water
ARC-ITSC	Agricultural Research Council-Institute for Tropical and Subtropical Crops
ARC-OVI	Agricultural Research Council-Onderstepoort Veterinary Institute
AVHRR	Advanced Very High Resolution Radiometer
BCT	Buffy Coat Technique
CCD	Cold Cloud Duration
CSIR	Centre/Council for Scientific and Industrial Research
DAVID	Disease and Vector Integrated Database
FAO	Food and Agriculture Organization
GIS	Geographic Information System
GPS	Global Positioning System
IAEA	International Atomic Energy Agency
IGR	Insect Growth Regulator
IPAR	Intercepted Photosynthetically Active Radiation
KZN	KwaZulu-Natal
KZNNCS	KwaZulu-Natal Nature Conservation Services
LIT	Lethal Insect Technique
LP	Legpanel
MP	Monopanel
NDVI	Normalized Difference Vegetation Index
NOAA	National Oceanic and Atmospheric Administration
NRI	Natural Resources Institute
PAAT	Programme Against African Trypanosomiasis
PATTEC	Pan African Tsetse and Trypanosomiasis Eradication Campaign
RT	Rectangular sticky screen
RTTCP	Regional Tsetse and Trypanosomiasis Control Programme
SAFCOL	South African Forestry Company Limited
SANDF	South African National Defense Force

SAT	Sequential Aerosol Technique
SC	Synthetic Cow
SIT	Sterile Insect Technique
XLP	Cross-shaped legpanel
XT	Cross-shaped target (sticky trap)

1. INTRODUCTION

1.1 LITERATURE REVIEW

1.1.1 Tsetse flies and their role in disease transmission

Tsetse flies belong to the small, but highly specialized genus *Glossina* (Diptera: Glossinidae) (Pont 1980). The genus *Glossina* is limited to Africa south of the Sahara, occurring in an area of an estimated 11 million square kilometers (Nash 1969; Jordan 1995), but have also been recorded in south-western Saudi Arabia (Moloo 1993). In Africa the distribution of tsetse flies extends from latitude 14° N to 29° S in 38 African countries, with either continuous or isolated areas of infestation (Ford 1970; Kuzoe 1991, Moloo 1993). There are 23 species and eight subspecies, belonging to three species groups. These are the fusca, morsitans and palpalis groups of species, which are distinguishable on the basis of the male and female genital armatures (Newstead *et al.* 1924; Moloo 1993; Jordan 1995). Tsetse flies are remarkable for their viviparity, females producing one fully-grown larva approximately every 9-11 days (FAO 1982). Both sexes feed solely on vertebrate blood. In the course of their feeding they transmit pathogenic flagellates of the genus *Trypanosoma* (Protozoa), which occur in the blood and organs of some of the African wild ungulates. Various species of *Trypanosoma* are the cause of trypanosomosis in humans and in domestic stock where they affect cattle, horses, sheep, goats and pigs (Connor 1994). They are harmless to game animals (except when animals are stressed) which, therefore, act as reservoirs of the disease. Tsetse act as the invertebrate hosts of the parasite and are, therefore, the principal biological vectors of trypanosomosis in Africa (Bruce 1895; Newstead *et al.* 1924; Aschcroft 1959). Other flies may play a lesser role as mechanical transmitters (Leak 1999).

1.1.2 Trypanosomosis – economic importance

Tsetse-transmitted trypanosomosis is recognized widely as a major animal and human disease (Jordan 1986). The wide distribution of tsetse and

trypanosomes, and their severe impact on animal production and agricultural production systems, makes animal trypanosomosis among the most important disease constraints of the agricultural sector, restraining agricultural advancement in over 46 % of the African continent (Buxton 1955; Glasgow 1963; Ford 1971; Ford & Katondo 1977; Rogers *et al.* 1994). Bovine trypanosomosis or "nagana" can cause anaemia, production losses, abortion and mortality in domestic herds and thus depresses all aspects of production: fertility is impaired, milk yields, growth and work output (draught power for ploughing) is reduced; and the mortality rate may reduce herd size (Connor 1994; Rogers *et al.* 1994). In its chronic form the disease results in poor health to ± 50 million people (in the form of human trypanosomosis or sleeping sickness) and enormous losses in livestock and, therefore, low industrial output (Kuzoe 1991). Losses of cattle are estimated at three million deaths, mainly young stock with up to 25 % mortality in pre-weaning calves. Mortality losses are combined by lower reproduction and less milk and weight gain. It is estimated that direct losses to agricultural production amount to about 4.5 billion USD in Africa annually (Budd 1999). Approximately 7 million km^2 of the tsetse-infested areas would probably be suitable for livestock and agricultural development if trypanosomosis were controlled (Finelle, 1974).

1.1.3 Disease and vector control aspects

There are different approaches to deal with the trypanosomosis problem (Jordan 1986). They vary from parasite control by treating infected cattle with therapeutic drugs, the use of trypanotolerant livestock, and finally control of the vector.

Parasite control

Since a broadly effective vaccine is unlikely to be developed (Williams & Williams 1992), the only effective treatment is the continuous dosage of trypanocidal drugs such as the therapeutic Diminazene (Berenil®) or the prophylactic Isometamidium (Samorin® and Trypamidium®). Drug therapy

has in the past been the main control activity in many countries. This has been partially effective in some circumstances where trypanosome resistance to drugs has developed due to drug mismanagement (Fox *et al.* 1993; Budd 1999). This is of particular concern as there are few drugs available and this situation is unlikely to change in the foreseeable future (Alsop 1993).

Trypanotolerant livestock

There is an increasing interest in using cattle breeds that are naturally resistant to trypanosomosis (trypanotolerant) (Murray *et al.* 1981). These breeds, however only comprise approximately five percent of the current cattle population in Africa (ILRAD 1989, cited in McMillan & Meltzer 1996). Furthermore, trypanotolerance does not offer complete protection and the breeds can still succumb to the disease under intense tsetse challenge.

Vector control

In much of Africa trypanosomosis control focused on the large-scale control of the vector with the ultimate aim being its eradication (Jordan 1985). Vector control aims to reduce or eliminate contact between tsetse and humans or livestock. Initially the resettling of people and, therefore, the movement of domestic stock away from tsetse infested areas was undertaken (Cockbill *et al.* 1963). This approach, however, shifted to the control and eradication of tsetse populations (Mulligan 1970; Jordan 1976, 1978, 1985; Dame & Jordan 1981).

ELIMINATION OF HOSTS AND CLEARING OF HABITAT

Early approaches to tsetse control included the extermination of the preferred host species of tsetse in infested areas (du Toit 1954; Cockbill 1960; Bursell 1970) as well as random clearance of woody vegetation (Nash 1940; du Toit 1954; Jordan 1974; Davies 1981; MacLennan 1981). These methods, however, are regarded as ecologically unacceptable and would often be unsuccessful as tsetse are capable of changing their breeding habitats and also their hosts in the

absence of their favourite species (Vale & Cumming 1976), while vegetation invariably grows back.

USE OF INSECTICIDES

Since the Second World War the control of *Glossina* has become increasingly dependent upon the use of insecticides. Ground and aerial application of DDT, HCH, dieldrin and endosulfan were widely used in Africa and have proved to be very effective for the management of crisis situations (du Toit 1954; Davies 1981; Turner 1984). These methods are, however, comparatively expensive and nowadays not favoured because of logistical requirements and for environmental reasons.

Currently, two vector control methods are preferred and are more selective and environmentally friendly. Improved understanding of visual and olfactory stimuli responsible for the host-seeking behaviour of tsetse flies, led to the development of artificial bait technology. In recent years reliance for the control of tsetse flies in parts of Africa has, therefore, increasingly been placed on attracting them to stationary targets, i.e. insecticide-impregnated odour-baited targets of the right colour (Vale *et al.* 1986; Vale *et al.* 1988a; Willemse 1991; Knols *et al.* 1993; Van Den Bossche 1997).

The application of insecticide to domestic animals, primarily cattle, can be regarded as a modification of the target method whereby, instead of stationary targets, the treated animals are used as attractive, mobile, living targets (referred to as "mobile targets"). This may be applied in the form of cattle dips or pour-ons and is technically feasible and a promising technique in tsetse-infested areas where cattle occur (Thomson 1987; Thomson *et al.* 1991; Fox *et al.* 1993; Okiria & Kalunda 1994; Bauer *et al.* 1995). This system could also be used together with targets to maintain tsetse fly barriers (Warnes *et al.* 1997; Warnes *et al.* 1999). Mobile targets are, therefore, more likely to be effective on commercial ranches and communal farms where the density of wild hosts is usually low (Williams & Williams 1992). They are, however, inappropriate when it comes to tsetse fly control in game reserves or other

tsetse-infested areas where the flies cannot, for obvious reasons, come into contact with treated cattle.

The Sequential Aerosol Technique (SAT) classically involves applying a sequence (usually 5 or 6 applications at 12-20 day intervals) of extremely low dosage, non-residual, insecticide (Endosulfan or synthetic pyrethroids) (Budd 1999) as a fine aerosol applied characteristically by fixed-wing aircraft. It has proven itself to be a good method of tsetse control and eradication over the last 20 years. Its main advantage is that large areas (2,500 - 20,000 km^2) could be treated very quickly (3 months) and reduce the number of flies to the point of eradication (Budd 1999) with a minimal reliance on ground support workers (Alsop 1993). With the use of deltamethrin the active ingredient is applied at a very low dose (0,25 g per spray per 10,000 m^2) so that non-target side effects are comparatively minimal and transient (Roussel Uclaf, Glossinex® information leaflet, undated).

ALTERNATIVE CONTROL OPTIONS

The biological basis for the Sterile Insect Technique (SIT) is that tsetse female flies only mate once. Operationally the technique involves the production and release of sterilized male flies into a community where they mate with the wild females, which are then unable to produce any offspring. As the population is not replenished with new young it gradually decreases eventually leading to eradication of the whole population. Inundated release of mass-reared, radiation sterilized tsetse males of several species has been used successfully to suppress populations of tsetse flies (Politzar *et al.* 1980, cited in Langley 1999; Williamson *et al.* 1983). This SIT has also been applied and was particularly successful to eradicate *G. austeni* Newstead from Unguja Island, Zanzibar (Saleh *et al.* 1999). It is also nowadays planned in other operational control programmes (FAO/IAEA 2000). This technology is, however, costly (Williams & Williams 1992) because huge colonies of tsetse have to be reared and facilities to sterilize these flies have to be established. Therefore, it is not sustainable for implementation at the local level. It is, however, justifiable if the objective is eradication. Its advantages of species-specificity and non-

contaminating nature are almost in all circumstances outweighed by the high costs and considerable sterile insect production and release logistics (Alsop 1993). However, although not yet proven on a very large scale, its reputation in other fields suggests that it could be another effective tool in the fight against trypanosomosis (Budd 1999).

The use of Insect Growth Regulators (IGRs) does provide an alternative to the use of insecticides control (Jordan *et al.* 1979) and works on the principle of sterilization rather than killing (Langley 1999). Sterilizing tsetse in the field by treating females with juvenile hormone, pyriproxyfen, through contaminating traps or targets has proven successful in the field and could be used with fair confidence in large-scale experimental control operations (Hargrove & Langley 1990, 1993). The chitin synthesis inhibitor triflumuron has also been proven to work as an alternative (Langley 1995; Bauer *et al.* 1999). However, it was suggested that IGRs as alternatives only be applied for control with targets if resistance to pyrethroids appears in tsetse populations (Langley 1999).

Biological control with the use of entomopathogenic Fungi *Beauveria bassiana* and *Metarhizium anisopliae* has also been demonstrated as a promising alternative (Kaaya *et al.* 1991). The value of this technique, now called the Lethal Insect Technique (LIT), has also been demonstrated in the field (Mahamat & Okech 1999).

1.1.4 Control/suppression and eradication prospects

The past trypanosomosis control policies of national control organizations, significantly supported by donor agencies, have placed emphasis on the eradication of the tsetse vector (FAO 1991, cited by Alsop 1993). Although often successful in achieving their objective of controlling trypanosomosis within the project area, these actions have usually not produced sustainable results because of the inability to consolidate and protect against reinvasion in the longer term. Financial continuity and commitment have rarely been adequate so that it was recommended that the problems experienced

necessitated a change of approach and re-defining of objectives (FAO 1991, cited by Alsop 1993) with a change in emphasis from vector eradication towards vector suppression.

Whereas eradication implies a once and for all cost and solution to the problem, suppression is an ongoing process with a recurrent budgetary commitment. Suppression can only be justified if it can be achieved and maintained at a reasonable cost, and is environmentally acceptable (Alsop 1993).

A major new initiative to eradicate tsetse flies from Africa was the recent establishment of PATTEC (Pan African Tsetse and Trypanosomiasis Eradication Campaign) which demonstrated the commitment of Heads of African States. It has only been possible to clear tsetse from 5 % of its range and the PATTEC objective is to drive towards larger and larger tsetse-free and disease-free zones in Africa (OAU/IBAR 2000; PAAT 2000).

1.1.5 Tsetse and trypanosomosis situation in South Africa

The historical tsetse and trypanosomosis situation in South Africa was reviewed by Kappmeier *et al.* (1998). Presently trypanosomosis or nagana occurs only in the north-eastern areas of KwaZulu-Natal Province where the two tsetse fly species, *G. brevipalpis* Newstead and *G. austeni* Newstead are its vectors. These flies are confined to evergreen forests and thickets, often associated with water-courses and other densely or semi-forested areas. They are responsible for the transmission of various trypanosome species to livestock. These are the severely pathogenic tsetse-transmitted *Trypanosoma* species *T. brucei* Plimmer & Bradford (in horses), *T. vivax* Ziemann (in cattle), *T. congolense* Broden (in cattle) and *T. simiae* Bruce *et al.* (in pigs) (Connor 1994).

Sleeping sickness has never occurred in South Africa (Kuzoe 1991) so that the focus is on nagana. The main nagana problem areas of KwaZulu-Natal Province are in the magisterial districts of Ingwavuma, Ubombo, Hlabisa and

Nongoma in the north-east (De Waal *et al.* 1998). The area is confined to some 16,000 km^2 and contains 426,000 humans, 130,000 small ruminants (De Waal *et al.* 1998), *c.* 350,000 cattle belonging to developing farmers in communal farming areas and *c.* 9,000 cattle on commercial farms. This number of cattle amounts to *c.* 10,8 % of those in the entire KwaZulu-Natal (A. Ilemobade, unpublished report 1997).

In 1990 an outbreak of nagana contributed to severe cattle mortalities in the communal areas of the magisterial districts mentioned above, involving *T. vivax* and *T. congolense* (De Waal *et al.* 1998). Between 1990 and 1994 surveys showed cattle served by 77 of 132 diptanks to be infected with nagana. Emergency control measures, which consisted of the treatment of cattle with homidium bromide (Ethidium®) and diminazene (Berenil®) as well as the weekly to fortnightly dipping of cattle in a pyrethroid, cyhalothrin (Grenade®) brought the disease under control (Kappmeier *et al.* 1998). This dipping was maintained for only two years so that ticks would not develop resistance as they have to the chlorinated hydrocarbons.

In 1992 odour-baited targets were used in a trial to control *G. brevipalpis* in the northern parts of the Hluhluwe-Umfolozi Game Reserve (Kappmeier *et al.* 1998). Use was made of the target technology then used in Zimbabwe for the control of *G. morsitans morsitans* Westwood and *G. pallidipes* Austen (Vale *et al.* 1988a). The target consisted of a 1,5 m wide x 1 m high black cloth baited with the synthetic odours 3-*n*-propylphenol, 1-octen-3-ol, 4-methylphenol and acetone. This trial was ineffective due to a number of possibilities (Kappmeier *et al.* 1998). Control efforts have, therefore, been put on hold until further research could provide the correct tools to use target technology for these species successfully. For this purpose, in 1992, the National Directorate of Veterinary Services contracted ARC-Onderstepoort Veterinary Institute (ARC-OVI) to develop a long-term strategy for the control of nagana and the present study formed part of this effort.

1.2 JUSTIFICATION

The use of drugs for the treatment and prevention of trypanosomosis is costly. Where a significant large-scale and long-term impact is needed, emphasis is placed on the control or eradication of the tsetse vector from the infested areas. In general the cheapest approach to tsetse control is by use of pyrethroid treated cattle. Where no cattle are present then targets are likely to be the best control tools. A practical and effective coloured target and attractive odour-bait system was developed which, it is considered, could prove effective for the control of both species when used in the field. The combination of the best South African (SA) odour developed for *G. brevipalpis*, namely 1-octen-3-ol (released at *c.* 9,1 mg/h), 4-methylphenol (released at *c.* 15,5 mg/h) and acetone (released at *c.* 350 mg/h) (Kappmeier & Nevill 1999a), together with a 1,75 m black/p.blue/black target (Kappmeier & Nevill 1999b), appeared to be an effective combination to employ as a control device for both species (Kappmeier & Nevill 1999c).

However, a number of studies were still needed in order to apply target technology successfully for long-term control and/or eradication of the tsetse flies in NE KwaZulu-Natal. These were the following:

1.2.1 Odour-bait for *G. austeni*

Odours have been used to attract tsetse flies to traps and targets (Snow 1980). However, none of the odour components tested in South Africa was attractive for *G. austeni* (Kappmeier & Nevill 1999a) except for carbon dioxide (Kappmeier, unpublished), which is impractical to use. The present lack of conventional odour attractants for *G. austeni* highlights the importance of searching for attractants specific for this species and efforts should thus focus on this. Although the recommended target was still effective for *G. austeni* in the absence of any odours (Kappmeier & Nevill 1999c), finding an appropriate odour for this species would mean that more of them could be attracted to the vicinity of a target or trap and they could be attracted from further away (Hargrove & Vale 1978; Torr 1990; Hargrove *et al.* 1995) so that faster control

should then be achieved. More efficient odour attractants would also allow fewer targets to be deployed per km^2 and would reduce costs (Leak 1999).

1.2.2 Trapping devices for *G. brevipalpis* and *G. austeni*

Population monitoring using traps provides essential information before and during tsetse control and eradication operations on the distribution of tsetse flies, the degree of control achieved at any given time and also on population composition, e.g. age structure. Sticky traps have been invaluable in determining presence or absence, sexes, age structure and whether marked with a fluorescence substance or not, as in the SIT eradication campaign on Unguja Island (Vreysen 1995, Vreysen *et al.* 1998). Following the early designs (Hall 1986) of sticky panels of various shapes and colours for *G. austeni*, these needed to be tested and improved for *G. brevipalpis* and *G. austeni* in South Africa. These sticky traps would, therefore, be useful tools for monitoring purposes.

To site insecticide treated targets successfully in optimal locations and densities in the field it is, however, also necessary to use a suitable trap to obtain base-line data on the behaviour, movement, population structure, density and ecology of the two species. Sticky traps kill the flies so that they cannot be used for mark-release-recapture studies and traps that capture live flies are therefore needed. For *G. austeni* no such trap exists as its behaviour is elusive and only low numbers are caught in existing tsetse fly traps elsewhere in Africa (Takken 1984, Hall 1986, Madubunyi 1990). For *G. brevipalpis* the only trap available for this purpose was the Siamese trap but it is only partially effective for this species in Kenya (Kyorku *et al.* 1993). It was, therefore, necessary to develop a trap for the purpose of catching live flies.

1.2.3 Population density and dispersal

For targets to be utilized effectively one needs to know the tsetse flies' population density and dispersal distances and rates. For the savanna species *G. m. morsitans, G. m. centralis* and *G. pallidipes* a target density close to four

targets per km^2 (with attractive synthetic odours) is necessary and sufficient to eradicate a tsetse population in nine months to a year (Hargrove 1993). What the required densities would be for *G. brevipalpis* and *G. austeni* has not yet been established. In order to control these species successfully with the newly developed targets, it was necessary that trials be conducted to establish more or less the number of targets needed per square kilometer.

Knowledge of target density is also needed if targets are to be deployed in barriers in control operations to prevent reinvasion from uncontrolled areas (Hargrove 1993). However, unsuitable habitat between pockets of forests and thickets can also act as "natural barriers". It is, therefore, also necessary to obtain knowledge on their dispersal and movement and thereby determine the distance of "unsuitable" habitat between pockets of forests and other suitable habitat, which could act as a natural barrier between controlled and infested areas.

1.2.4 The role of livestock in the control of tsetse and nagana

Demographic pressures and increases in livestock numbers are causing overgrazing, thereby forcing cattle owners to graze their cattle in tsetse habitat in their search for pasture. This is particularly the case in rural Zululand where it could be seen that cattle graze right up to the fences of the game reserves, which act as the main sources of tsetse flies. In these situations cattle can be used as mobile targets when treated with a suitable insecticide. What proportion of *G. brevipalpis* and *G. austeni* would feed on cattle is, however, not certain. There are also high numbers (*c.* 130 000) of small ruminants, i.e. goats in the rural areas (De Waal *et al.* 1998). If tsetse also feed on goats, these could accordingly be used as mobile targets. The role/importance of livestock, therefore, needs to be established in the Zululand situation for tsetse control management. Furthermore, since treatment with insecticides will not prevent infection, disease challenge could also be high in these high-risk areas and it may be necessary to protect cattle from tsetse fly attack. Improved knowledge on the times and situations when cattle are at most risk of tsetse challenge will aid in the development of strategies for minimizing contact.

1.2.5 Tsetse fly distribution surveys

The successful planning of a control/eradication campaign depends primarily on reliable knowledge of the tsetse distribution in an area and on what species occur in which areas. The last accurate information on the distribution of *G. brevipalpis* and *G. austeni* in South Africa was that obtained through tsetse surveys in the 1950s (du Toit 1954, 1956). Therefore, it was necessary that the relative distribution of both species in KwaZulu-Natal be re-surveyed.

Furthermore, reliable tsetse distribution mapping is necessary to define tsetse distributions. Geographical Information Systems (GIS) provide the ideal means for this. GIS, together with remote sensing techniques, also provide the means for overlaying layers of spatial and temporal information including land use patterns, vegetation, climate, topography and human, livestock and disease distributions, in effect the very parameters which characterize production systems (Lillesand & Kiefer 1994, cited in Erkelens *et al.* 2000). It will, therefore, be a useful tool for integrating tsetse distribution and abundance with disease distribution and prevalence, land use and tenure, livestock distribution and abundance to facilitate with the decision-making and assist with planning interventions of the nagana problem in KwaZulu-Natal.

1.3 PROBLEM AND HYPOTHESIS

Since no control measures against tsetse and trypanosomosis in South Africa are currently applied, it is necessary to strategically plan the control and/or eradication of tsetse flies throughout the infested areas of N.E. KwaZulu-Natal. This study should provide a sound basis for planning tsetse control/eradication operations in an area-wide integrated control/eradication strategy for both tsetse species in this region.

The north-eastern region of KwaZulu-Natal where nagana and tsetse are present consists of a number of land use categories. These consist of Game Reserves and nature conservation areas, communal farms, commercial farms, indigenous forests as well as commercial plantations.

The choice of control strategy appropriate to a particular land use category is determined by many factors. For example, aerial spraying may be considered inappropriate for a conservation area, while it could be used where tsetse flies occur outside the Game Reserves. Targets, which are considered most environmentally friendly (Nagel 1995), could be a good choice in conservation areas, while in communal areas, where cattle are present, pyrethroid application to livestock could be more effective and without the danger of target theft.

Thus, *Glossina brevipalpis* and *G. austeni* can be controlled and/or eradicated by the integration of various tsetse control options in various tsetse-infested areas. A strategy will be proposed for their sustainable control and/or eradication in N.E. KwaZulu-Natal.

1.4 OBJECTIVES

- To conduct studies to find an attractive odour(s) for *G. austeni* and to obtain a better understanding on the role of odours for both species. Simultaneously these studies will give results for *G. brevipalpis*.
- To develop a trap(s) that is suitable to survey the distribution of *G. brevipalpis* and *G. austeni* and to monitor future control programmes.
- To develop a trap to capture live flies of both species in relatively large numbers for population dynamics or other ecological studies.
- To determine the density and dispersal of *G. brevipalpis* and *G. austeni* in order to establish the required target density and what constitutes a natural barrier to dispersal.
- To determine the value of livestock for *G. brevipalpis* and *G. austeni* control and determine tsetse challenge to livestock in different sites at various times of day.
- To analyze, interpret and map data of a survey of the distribution of *G. brevipalpis* and *G. austeni* and to use a Geographical Information System (GIS) to integrate tsetse distribution and abundance, disease distribution

and prevalence, land use, land designation/tenure as well as cattle distribution so as to assist in the planning of sustainable tsetse control.

- To propose a strategy for the environmentally sound and sustainable control (or eradication) of *G. brevipalpis* and *G. austeni* in N.E. KwaZulu-Natal.

1.5 EXPECTED BENEFITS ARISING FROM THIS STUDY

The proposed research will contribute to an improved understanding on the ecology, behaviour, monitoring and control aspects of *G. brevipalpis* and *G. austeni*. This could also be used by other African countries, which host these species, since very little research is conducted elsewhere on these two difficult species. Accurate information on the distribution of tsetse flies in South Africa will become available which will benefit in the planning of control operations. Appropriate intervention methods in different land use areas will be proposed and the overall research will facilitate planning for the sustainable control of tsetse flies in N.E. KwaZulu-Natal. Ultimately, the benefits to farmers will depend on the extent that a reduction in trypanosomosis challenge translates into improved health and productivity of affected livestock populations, the extent that the opportunities presented for the development of more effective and profitable systems of livestock keeping are taken up by the rural community concerned (Alsop 1993) and the extent into how it could relieve other disease challenges, e.g. tick-borne diseases, which act as major constraints for the production of cattle in N.E. KwaZulu-Natal (De Waal *et al.* 1998).

2. GENERAL MATERIALS AND METHODS

2.1 STUDY AREA

The study was conducted at Hellsgate Military Base, situated on the Ndlozi peninsula north of Charter's Creek, Lake St. Lucia (28°02'40"S 32°25'50"E), which forms part of a nature conservation area. Both *G. brevipalpis* and *G. austeni* are present in habitat consisting of evergreen sand forest, bushland and thickets occurring in a ± 0,5 - 2,0 km wide stretch along the edge of the saltwater lake-system. This forms part of the coastal forest and thornveld (Acocks 1988). The remaining vegetation consists of thorn and palmveld (*Acacia* spp., *Syzygium cordatum, Phoenix reclinata, Hyphaene natalensis*) with patches of bushed grassland, dominantly on regic sands (MacVicar *et al.* 1986).

The area has an altitude between 0 - 50 m and forms part of the coastal belt, ± 12 km from the sea. The mean annual temperature is 21 - 22 °C (Schultze 1982). Climatic records from a Stevenson Screen at Hellsgate Tsetse Research Station (1994 - 1997), showed mean maximum temperatures for the hottest months to be *c.* 29,7 °C in February and March and the mean minimum for the coldest months (June and July) *c.* 11,6 °C. The relative humidity is high with an annual mean maximum of *c.* 96,5 % and an annual mean minimum of *c.* 62 %. Summer rainfall occurs with annual precipitation of *c.* 950 mm (minimum of 0 - 5 mm in June - July and maximum of *c.* 260 mm for each month between October - February [this may vary annually]). The prevailing winds are south-westerly and north-easterly.

The animal life in the area consists *i.a.* of hippopotamus (*Hippopotamus amphibius*), warthog (*Phacochoerus aethiopicus*), bushpig (*Potamochoerus porcus*), nyala (*Tragelaphus angasii*), bushbuck (*T. scriptus*), red duiker (*Cephalophus natalensis*), grey duiker (*Cep. monticola*), reedbuck (*Redunca arundinum*), vervet monkey (*Cercopithecus pygerythrus*), samango monkey (*Cer. albogularis*) and some nocturnal small mammals like the bushbaby (*Galago crassicaudatus*), porcupine (*Hystrix africaeaustralis*), genet (*Genetta*

sp.) and serval (*Felis serval*). Kudu (*T. strepsiceros*) were introduced to the area in September 1997. Crocodiles (*Crocodylis niloticus*) and water monitors (*Varanus niloticus*) are also common and there is abundant bird-life. There is no domestic stock in the area except for two experimental cattle introduced in 1997 (until 2000). Many of the mammals that occur at Hellsgate have been shown to be natural hosts of *G. brevipalpis* and *G. austeni* (Moloo 1993).

2.2 GENERAL TECHNIQUES AND EQUIPMENT

2.2.1 Tsetse fly evaluation techniques

The use of targets and electric grids

Visual targets incorporated into electric grids (Vale 1974a) are widely used as capturing devices and were used as capturing tools of the tsetse flies throughout some of the studies following. The grids consisted of various sizes of an aluminium frame with fine copper wires spaced close together electrifying both surfaces of either a coloured cloth target or an almost invisible black gauze screen, which was inserted in the frame, so incorporating the grid into a visual or non visual target (Fig. 2.1). A high voltage (20,000 – 30,000 V) was applied between the wires (alternate wires being charged and earthed) by means of a high-tension unit (energizer unit), which operated from a 12 volt car battery. The non-visual target will henceforth be referred to as an "electric net" and was at times used together with a visual target (as in Fig. 2.1) to intercept flies flying around the visual target, or could be used on its own, as will be described later in this work. Unless stated otherwise, the grids were placed at a right angle to the wind so that tsetse flies, which flew upwind, could see the visual target. A corrugated iron tray, painted with polybutene, was placed underneath the grid. Tsetse flies colliding with the grid were electrocuted and dropped down onto the sticky surface of the tray so that flies which were stunned could be retained, counted and sexed.

Fig. 2.1 Visual (1 x 1 m pthalogen blue) and non-visual (0,5 x 1 m net) electric grids incorporated to form a flanked target (i.e. p.blue/net).

Traps

Unless stated otherwise, all trap catches were made by either XT sticky traps or the newly developed H trap. Details of all traps and their designs will be described in Chapter 4.

2.2.2 Odour baits and dispensers

Synthetic tsetse fly attractants

Initially traps (or sometimes electrified grids) were baited with a blend of acetone (500 mg/h), 1-octen-3-ol (0,4 mg/h), 4-methylphenol (0,8 mg/h) and 3-*n*-propylphenol (0,1 mg/h) at rates known to increase the catch of tsetse flies in Zimbabwe (Vale *et al.* 1988b). Henceforth this blend of odours is termed the Zimbabwe mixture (**Zim-mix**). Alternatively, following experiments on the

South African species' attractiveness to the Zim-mix (Kappmeier & Nevill 1999a), the South African blend was used, which consisted of the same components as the Zim-mix, but without 3-*n*-propylphenol and with acetone released only at 350 mg/h. This blend is henceforth referred to as the **original SA blend**. Following further experiments (Kappmeier & Nevill 1999a) a more effective odour was used, namely acetone released at *c.* 350 mg/h, 1-octen-3-ol released at *c.* 9,1 mg/h and 4-methylphenol released at *c.* 15,5 mg/h, referred to as the **best SA blend** for *G. brevipalpis*. Therefore, traps (or sometimes electrified grids) were either baited with the Zim-mix, original SA blend or best SA blend.

In some experiments, studies were made using other synthetic odour components, e.g. carboxylic acids, butanone, phenol, 3-methylphenol, 3-and 4-ethylphenol, etc. Octenol, phenols and carboxylic acids were dispensed from heat-sealed sachets of low-density polyethylene tubing (wall thickness 100 microns). These have the advantage that the release rate remains reasonably constant with age. The surface area varied between *c.* 8-75 cm^2 for the various chemicals (or mixture of chemicals) to produce the different release rates desired.

Ketones (acetone and butanone) were mostly dispensed from a glass bottle. The release rate was controlled by a hole in the lid and could be varied by varying the apertures of the hole. When minute doses of the ketones were needed these were also dispensed by means of polythene sachets, which gave a slower release.

Carbon dioxide was released from 200 kg cylinders at the required rates controlled by means of flow regulators and appropriate flow meters.

Natural attractants

In some experiments, studies were made of the numbers of tsetse attracted to a source of natural host odour. Where natural odour needed to be tested without any interference from a visual effect (i.e. without the visibility of the animal

itself), odour was obtained by placing an animal in an underground, ventilated pit (chamber) (see Fig. 3.1). The details will be given in Chapter 3.

Otherwise, e.g. in feeding response studies (Chapter 6), the animals were tethered to a post at a particular site.

Experiments with animals

Approval was given by the ARC-OVI animal ethics committee regarding the use of all animals involved in this study.

2.3 EXPERIMENTAL DESIGN AND ANALYSIS

All field experiments were carried out during the hours between early morning until dark, when both *G. brevipalpis* and *G. austeni* were found active (Kappmeier 2000). Treatments were incorporated into a series of replicated Latin Squares described by Fisher (1935, cited in Perry *et al.* 1980) consisting of days x sites x treatments or randomized block designs were used. In the **Latin square designs** the number of sites and days equaled the number of treatments. Since each treatment occured once at each site and on each day the treatment means were independent of any differences due to sites and days. Only one treatment occupied a site on any one day, so treatment interaction was avoided (Perry *et al.* 1980). Sites were far enough apart to ensure that interactions between treatments could not occur, but near enough to experience similar weather on any given occasion. The design was randomized to prevent systematic errors. Where only one site was available, treatments were compared using a **randomized block design**; groups of adjacent days were regarded as different blocks and treatments were randomly allocated to days within these blocks.

Unless stated otherwise, the daily catches (n) were normalized using a $\log_{10}(n)$ or, where zero catches were obtained, a $\log_{10}(n+1)$ transformation and subjected to analysis of variance (ANOVA). Differences between more than two means were assessed by a least significant difference test. The general test level was P

= 0,05. Use was made of either a programme designed specifically for the analysis of series of Latin Squares or using GLIM4 (Francis *et al.* 1993, cited in Torr *et al.* 1996).

Male and female catches were usually analyzed separately. The detransformed means are generally reported as a catch index, which was estimated by expressing the detransformed mean catch with the test treatment as a proportion of the detransformed mean control (standard) catch. Thus catch indices significantly greater than the control indicated attraction/ superiority.

Further details on the methodology and analysis of the various types of studies will be explained in the following chapters.

3. STUDIES TO FIND AN ATTRACTIVE ODOUR BAIT

3.1 ABSTRACT

Studies were conducted on the attractiveness of carbon dioxide, synthetic and natural host odours to find an attractive odour for *Glossina austeni* and, less importantly, to improve attraction of *G. brevipalpis*. Carbon dioxide was the most attractive component for *G. austeni* at release rates between 2 - 20 l/min. Natural cow odour was also very attractive for both species. An attempt was made to evaluate the effect of natural odour components other than carbon dioxide on the attraction of especially *G. austeni*. For this species cow odour was found to be equally effective to CO_2 released at the same rate as that expired by the cow. Also there was no difference in attraction when adding supernormal doses of octenol, 4-methylphenol and acetone to cow odour or to carbon dioxide. This addition rather had a repellent effect, suggesting that the remaining components of cow odour were not attractive. For *G. brevipalpis* the presence of these high doses of octenol and 4-methylphenol played a major role in its attraction. In an attempt to simulate cow odour, chemical absorbent filters were used to absorb the main components of cow odour. When synthetically simulated cow and natural cow odours were compared there was no significant difference in their attractiveness for *G. austeni* but natural cow odour significantly increased catches of *G. brevipalpis* as compared to its synthetic concoct. For *G. austeni* it was assumed that the attractiveness of natural cow odour as well as other host odours could be attributed to the presence of carbon dioxide in their breath and that this is probably the single most important component in host odour. Vision was also found to play a very important role in the attraction of *G. austeni*. For *G. brevipalpis* there seemed to be additional unknown attractive components of cow odour. Compared to odour vision played a minor role in its attraction.

3.2 INTRODUCTION

Tsetse flies recognise potential hosts by olfactory and visual cues. They approach a stationary host or target baited with odour by upwind flights (Torr

1989; Gibson *et al.* 1991; Brady & Griffiths 1993; Willemse & Takken 1994; Groenendijk 1996) modulated by olfactory stimuli, with visual responses operating only at short range. Odours have, therefore, been used to enhance the effects of shape and colour in the attraction of tsetse flies to traps and targets (Snow 1980).

Studies in Zimbabwe (Vale 1974b, 1977a) demonstrated that ox-breath is an important odour bait for *G. morsitans morsitans* Westwood and *G. pallidipes* Austen. One of the effective components of ox-breath is carbon dioxide (Vale 1980; Owaga 1984), but it is too expensive and inconvenient to use. Acetone and a number of aldehydes and other ketones are attractive to tsetse (Vale 1980) and are cheaper and more convenient to use. The most attractive element in ox-breath was identified as 1-octen-3-ol (henceforth referred to as octenol) (Hall *et al.* 1984). This attractant enhances the effects of ketones and CO_2 (Vale & Hall 1985a). Butanone was identified as a substitute for acetone and can be used at a lower dosage rate (Vale & Hall 1985b). A further breakthrough occurred when Owaga (1985) demonstrated in Kenya that the urine of the African buffalo (*Syncerus caffer*) could be used for the attraction of *G. pallidipes*. Much of the efficacy of the urine is due to phenols (Hassanali *et al.* 1986; Bursell *et al.* 1988; Owaga *et al.* 1988; Vale *et al.* 1988b) comprising the parent phenol, 3- and 4-methylphenol (3- and 4-cresol), 3- and 4-ethylphenol as well as 3- and 4-*n*-propylphenol (Hassanali *et al.* 1986). Of these, 4-methylphenol and 3-*n*-propylphenol have been found to be the most compelling for tsetse flies (Saini 1990; Saini & Hassanali 1992) and act synergistically as the crucial components of the phenolic mix (Owaga *et al.* 1988; Vale *et al.* 1988b).

While workers in Kenya used acetone and cow urine (Okech & Hassanali 1990), the Zimbabwean workers used blends of synthetic octenol, 4-methylphenol and 3-*n*-propylphenol. Owaga (1992) tested African buffalo (*S. caffer)* and cow urine, acetone, 3-*n*-propylphenol, 4-methylphenol and carbon dioxide, in the form of dry-ice, for *G. austeni* in Kenya. Only dry ice attracted this species significantly. Kyorku (personal communication, 1994) also conducted studies on attractive odours (octenol and acetone) for *G. brevipalpis* in Kenya and found acetone and octenol to be the main attractive components.

In South Africa studies were conducted on the use of these conventional odour attractants for *G. brevipalpis* and *G. austeni* (Kappmeier & Nevill 1999a) and it was found that a very effective attractant combination with optimal release rates could be recommended for *G. brevipalpis*. This consisted of octenol released at *c.* 2,3 - 9,1 mg/h, 4-methylphenol released at *c.*15,5 mg/h and acetone released at *c.* 350 mg/h. This combination increased the catches of this species by 2,3 - 2,8 times when compared to the Zimbabwe mixture (Zim-mix) and by 10,1 - 12,3 times compared to 'no odour'. Unfortunately, none of the components or combinations attracted *G. austeni*. Further phenols, namely 4-*n*-propylphenol, 3-methylphenol, 3- and 4-ethylphenol as well as butanone were tested at low, medium and high release rates together with their synergistic effect to the Zim-mix. None of these components were, however, attractive to either species (Kappmeier 1999).

Considerably greater attraction of *G. pallidipes* and *G. m. morsitans* in Zimbabwe was obtained when Hargrove & Vale (1978) utilized live animals in pits and used the extracted odour as bait. It was suggested that other factors were also probably involved. Further detailed studies in Zimbabwe showed the attraction of these species to be greater to natural ox odour than to the synthetic ox odours (Torr *et al.* 1995; Hargrove *et al.* 1995) suggesting the presence of unidentified attractant(s) in ox odour.

The present lack of odour attractants for *G. austeni*, highlighted the importance of searching for attractants specific for this species and efforts during this study thus focused on this. It was suggested (G.A. Vale, personal communication, 1997) that since *G. austeni* did not react positively to any of the conventional odours, it might be that other more volatile components could be effective and that it could be the link to finding the unidentified odour components of ox odour. If present, the attractive odour component could be isolated and synthesized and so be used as part of an attractive odour bait.

In the present chapter, five types of study were undertaken to determine the significance of olfactory attraction for specifically *G. austeni*, as well as to find attractants in natural host odours. Firstly, studies were made on the effect of

carbon dioxide (CO_2) at various dosage rates on tsetse fly catches. Secondly, studies were made to determine the relative importance of vision vs. olfaction for their attraction as was done by Vale (1974b). Thirdly, studies were made to assess the responses of *G. brevipalpis* and *G. austeni* to natural cow odour. This included studies to determine if there is an animal odour besides CO_2, which is attractive for *G. austeni*. Comparisons were further made on the effect of adding large doses of known attractants to the natural cow odours and to CO_2, based on the concept that large doses of these components would eliminate the effect of the small quantities released by the cow. Following from this, comparisons were made of the numbers of tsetse attracted to natural cow odour and a synthetic cow odour simulate (henceforth referred to as synthetic cow) containing all the known attractants at their natural concentrations. Fourthly, studies were made to determine the attractiveness of other host odours in the search for an attractive odour for *G. austeni*. Lastly, studies were made on the effect of the presence of human odour for the attraction of both species.

3.3 MATERIALS AND METHODS

3.3.1 Evaluation methods

Tsetse flies orientate imprecisely to an odour source unless it is marked by a visual stimulus (Vale 1974b). To gauge the numbers of tsetse flies attracted to various odours, an electric grid consisting of a visual 1 x 1 m phthalogen blue part and an adjacent non-visual 0,5 x 1 m net (Fig. 2.1), was placed ± 30 - 50 cm downwind of the odour source to act as a focal point. This was at times replaced by a similar grid, but flanked with 2 x 1 m electric nets at each side of the visual target. The grids were operated from noon until dark, during which time both species could be attracted (Kappmeier 2000).

3.3.2 Odours

All odours (natural and synthetic), except CO_2 (released from cylinders), were extracted from a ventilated underground chamber (pit) (Fig. 3.1). The pit was constructed according to Vale (1974b). The pit dimensions are 3,5 x 2 x 2 m

with a roof of corrugated iron (at ground level) and an entrance door. Part of the roof contained a corrugated fibreglass sheet for light. A 5 m ramp led from ground level to the entrance of the chamber. The opposite side was fitted with a 3 m long (150 mm diam.) ventilation shaft of PVC piping, fitted in the top third of the pit wall. Air was exhausted from the pit at 2000 l/min via the ventilation shaft fitted with a 12 V (120 mm dia.) co-axial fan.

During these pit experiments, the electrified grid was placed 50 cm downwind of the extractor outlet (Fig. 3.2). Carbon dioxide cylinders were hidden away from the direct scene with CO_2 released through extended tubing placed at the extractor outlet at the release rates indicated in the experiments below.

Natural host odours

Natural host odours were obtained by placing the animals in the roofed pit and exhausting the air at 2000 l/min. The pit was cleaned daily to minimize the accumulation of phenolic materials present in animal excreta. The animals tested during the course of the study consisted of:

- a bull calf (*c.* 75 kg)
- a cow (*c.* 350 kg)
- three goats (total weight *c.* 68 kg)
- a bushpig (*c.* 100 kg)
- three men (total weight *c.* 270 kg)

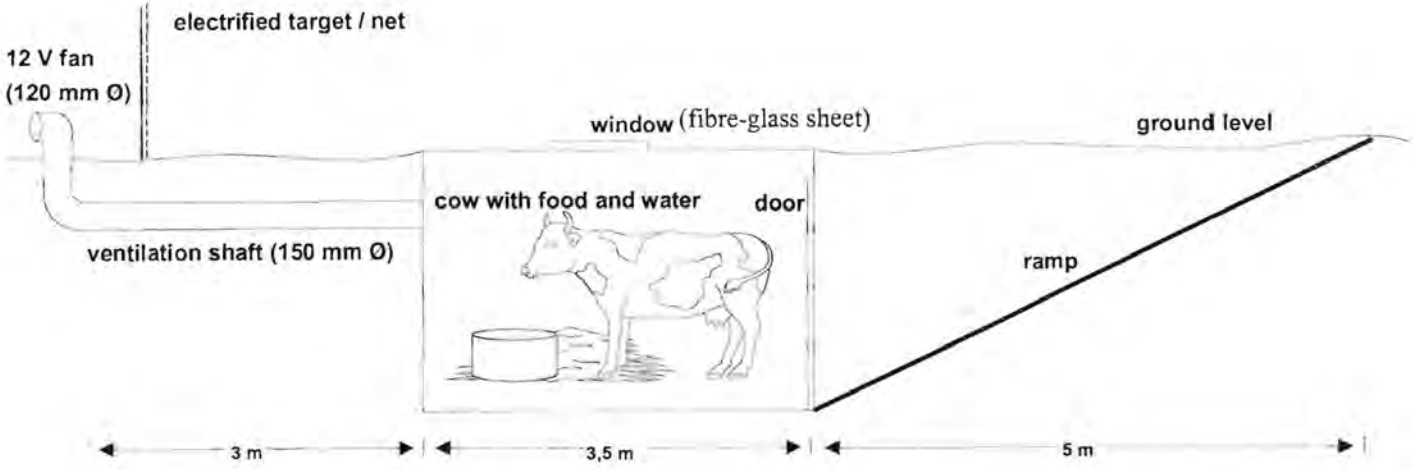


Fig. 3.1 Schematic representation of cow in underground ventilated pit (design after Vale 1974b)

Fig. 3.2 Extractor fan outlet placed *c.* 50 cm downwind of a flanked p/blue electric target

Synthetic odours

Blends of carbon dioxide and other synthetic odour components were dispensed at various rates as explained in Chapter 2.2.

3.3.3 Air sampling of attractants

Carbon dioxide

Measures of the natural CO_2 levels (ppm) released by the experimental cow were obtained. With this information, the release rate of the cow's CO_2 could be simulated at the correct rate for comparison purposes. Air was drawn at 300 ml/min via a tube inserted through a port in the pit ventilation shaft. The concentration of CO_2 was measured using an infrared gas analyzer (EGA, ADC Ltd., Hoddesdon, UK). The

output from the analyzer was recorded continuously by a data logger and the data were subsequently downloaded onto a personal computer for analysis. The logger recorded the mean levels of CO_2 at 1-minute intervals.

Natural cow

During October 1997 odour samples were taken of the 350 kg cow used in the experiments for the identification of its components and to obtain their release rates. This study was done in collaboration with researchers of the Natural Resources Institute[1] (NRI) where samples were analysed by the chemists, to be able to compare natural cow odour with synthesized cow odour.

During sampling at Hellsgate (initially by Dr. S.J. Torr (NRI)), the animal was housed in the underground pit (ventilated at *c.* 2000 l/min). Air was extracted via tubing with air pumps and filters were inserted through the sampling ports in the ventilation shaft of the pit (Fig. 3.3 a & b). Ketones and aldehydes (carbonyl compounds) were trapped with silica SepPak® (Waters Corporation, Milford, MA 01757, USA) cartridges containing DNPH (2,4-dinitrophenyl-hydrazine; 360 mg) at a rate of 0,5 l/min for approximately 2 h. Volatiles (i.e. phenols and octenol) were collected on Porapak® filters (200 mg; 50 – 80 mesh) at a rate of 2,0 l/min for 2 h. Collections of carboxylic acids were made by sampling at 2,0 l/min for 2 h on filters containing Chromasorb P AW impregnated with 2,5 % tetrabutyl-ammonium hydroxide (200 mg). Samples were taken of both the experimental cow as well as an empty pit (to be used as the background odour).

Since the samples taken in October 1997 showed contamination, which obscured the analyses of the ketones and octenol, these odours were resampled in December 1998 in order to get a proper analysis of the experimental cow odour for synthesis. Immediately after collection the filters were stored in a deep freeze and thereafter sent

[1] Natural Resources Institute/ University of Greenwich, Central Avenue, Chatham Maritime, Kent ME4 4TB, United Kingdom

by courier to the NRI for analyses where they were analysed within seven days of collection. Four samples (replicates) were taken of the cow and three of the empty pit with each of the three filter types during the first trial and five samples of each treatment during the second trial.

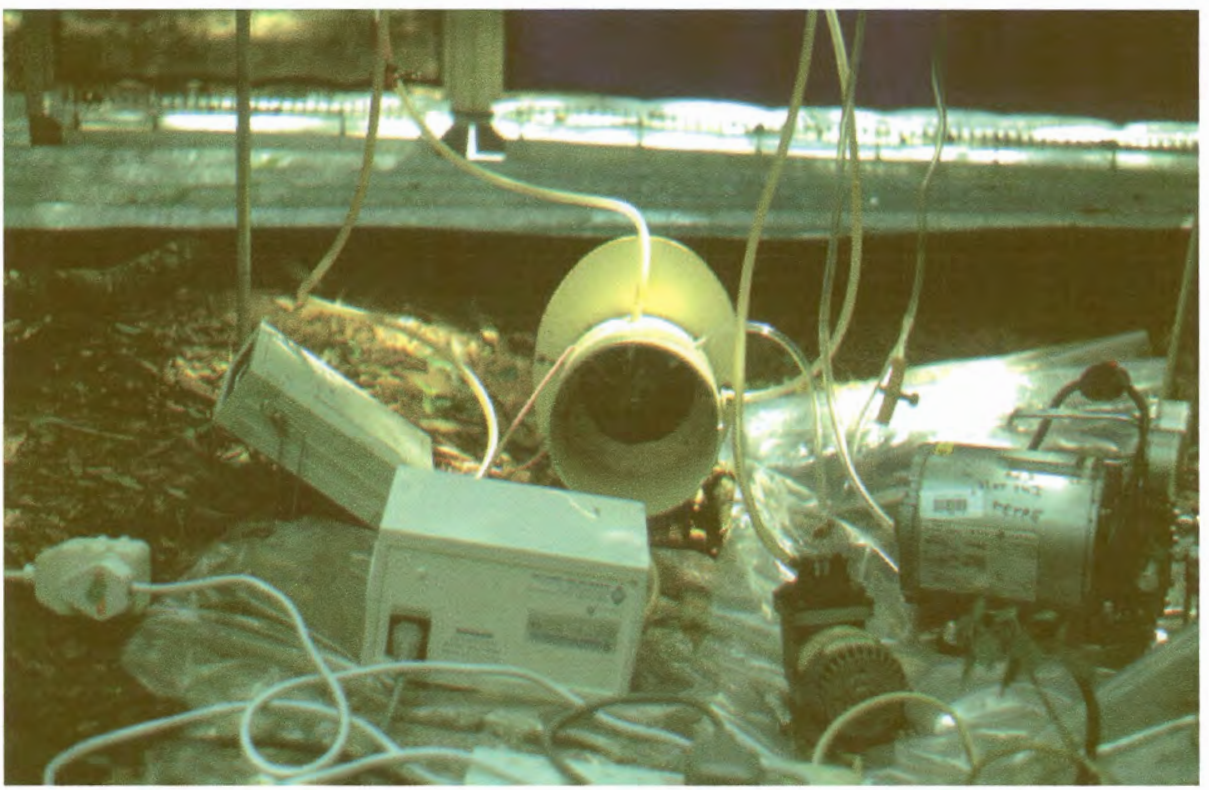


Fig. 3.3 a Odour extraction and sampling setup. Extracted air from the pit (housing an animal) was sampled via tubing with air pumps (shown right). Filters were inserted through the sampling ports in the ventilation shaft of the pit (centre). Carbon dioxide was measured similarly by means of an infrared gas analyzer (shown left).

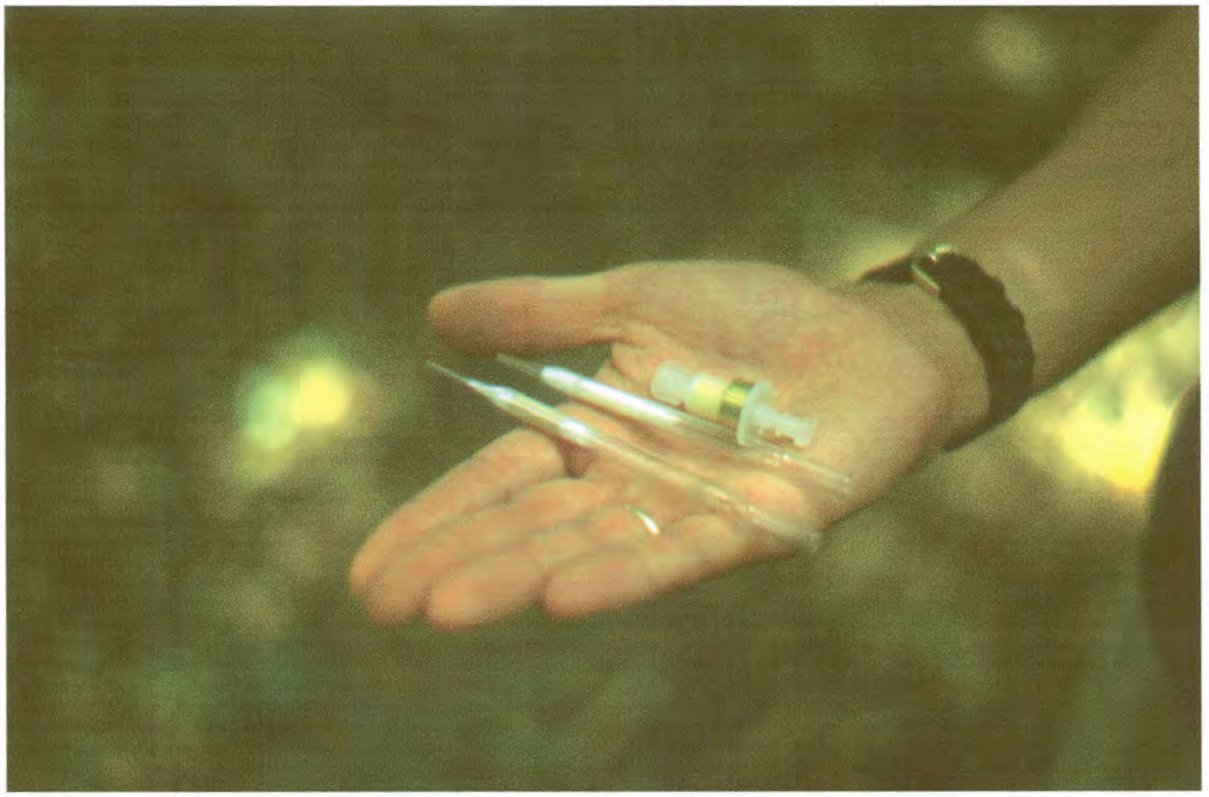


Fig. 3.3 b Collections of carboxylic acids were made by sampling through filters containing Chromasorb P AW filters (left), volatiles (i.e. phenols and octenol) were collected on Porapak® filters (centre) and ketones and aldehydes (carbonyl compounds) were trapped with silica SepPak® cartridges (right)

3.3.4. Details of chemical analyses by NRI

NRI chemists analyzed the filters following the procedures described below (D.R. Hall, unpublished report, 1998):

- *Carbonyl compounds:* At NRI, the trapped 2,4-dinitrophenylhydrazones (DNPHs) were eluted with 3 ml of HPLC grade acetonitrile and the eluate made up to 5 ml. Analyses used a Spherisorb5 ODS2 column (25 cm x 4,6 mm; HPLC Technologies) eluted with a 60:40 acetonitrile/water mixture at 1 ml/min. The eluate was monitored by an UV detector at 350 nm. DNPHs of acetone, butanone,

formaldehyde and acetaldehyde were synthesized at NRI and amounts in the test samples were quantified by external standard.

- Collections on Porapak: The Porapak was pre-purified by soxhlet extraction with dichloromethane and further washing with dichloromethane after making up the filters. At NRI trapped volatiles were eluted with dichloromethane (3 x 0,5 ml) and decyl acetate (2 μg) added as internal standard. The solution was analyzed by gas chromatography (GC) coupled directly to mass spectrometry (MS) using a Finnigan ITD 700 ion trap detector operated in electron impact mode. The GC column was fused silica (25 m x 0,25 mm i.d.) coated with polar CPWax52CB (Chrompack) with helium carrier gas (0,5 kg/cm^2) and oven temperature held at 50 °C for 2 min then programmed to 240 °C at 6 °C/min. Components were identified by their GC retention times and mass spectra and comparison with synthetic standards where possible. Components were quantified against the internal standard and rates of production calculated from the flow rates used.

- Collections of carboxylic acids: At NRI the tetrabutylammonium salts were eluted with acetone (3 x 0,5 ml) and benzyl bromide (2 μl) added to convert the salts to the benzyl esters. After standing for 2 hr, an internal standard, e.g. decyl acetate (2 μg), was added and the solution analyzed by GC-MS as above. Benzyl esters were detected by single ion scanning at m/z 91 and 108. Synthetic standard benzyl esters were prepared *via* the tetrabutylammonium salts on a preparative scale. The internal standard was calibrated against these and amounts of benzyl esters converted to amount of acid trapped.

3.3.5 Experimental design and analysis

In comparisons of different odour treatments, the various treatments were incorporated into a series of replicated Latin squares or, where only one site was available, treatments were compared using a randomized block design, as explained in Chapter 2. Unless stated otherwise, 'no odour' acted as the control treatment in all experiments. Details of each of the experiments will be given in the sections following. Detailed analyses of the results are described in section 2.3.

Where comparisons were made between the same treatments in more than one experiment and various indices (of detransformed mean catches) were obtained (e.g. comparisons with cow and 'no odour'), the overall mean index was calculated. Indices were transformed to log (n) and then the averages were calculated and detransformed. For more precise estimates the indices of the treatments were weighted by the reciprocal of the scale parameter (variance) for each experiment. This deals with problems of differences in the variance between different experiments when there are varying numbers of catches. The weighting procedure thus gives greater weight to the values with smaller variance.

3.4 EXPERIMENTS AND RESULTS

The experiments are described in detail in the following sections. The results will be summarized in Tables. Detransformed mean catches will be expressed as indices of increase relative to the control treatment. The detransformed mean catch of the control treatment (i.e. 'no odour') will be given in brackets. Indices followed by the same symbols (a, b or c) indicate no significant differences. Table summaries will incorporate the number of replicates (n) for each treatment, the degrees of freedom (df) for error, the transformed standard errors (s.e.) as well as the levels of probability (P) that the means are different at $P < 0,05$ (*), $P < 0,01$ (**), $P < 0,001$ (***), or not significantly different (n.s.).

3.4.1 Evaluation of carbon dioxide for the attraction of *G. brevipalpis* and *G. austeni*

Attraction with carbon dioxide at various release rates

Carbon dioxide was released from cylinders through appropriate flow regulators and evaluated for its attractiveness for the two species. Three treatments of CO_2 with release rates of 0,2 l/min, 2,0 l/min and 20,0 l/min were compared to 'no odour' as the control and to the best SA blend. Fifteen replicates were carried out during May - October 1997.

The results are given in Table 3.1 for both species. For *G. brevipalpis* males it was shown that only the best SA blend and none of the CO_2 treatments increased catches significantly compared to 'no odour'. For females CO_2 increased the catches significantly only at a rate of 2,0 l/min compared to 'no odour'. For *G. austeni* males no significant increase in the catches occurred when baited with any of the CO_2 treatments (or the best SA blend) and when released at 20,0 l/min only a 1,7 x increase in the catches was obtained when compared to 'no odour'. For *G. austeni* females, however, CO_2 increased the catches significantly at all three release rates by *c.* 2,3, 3,2 and 4,0 times, respectively, compared to those attracted to 'no odour'. The differences between the catches with these three CO_2 treatments were not significant, although catches increased as the release rates of CO_2 increased. CO_2 released at 20,0 l/min also increased the catches significantly (2,2 - 2,3 x) compared to the best SA blend, while the lower release rates were also better but not significant.

Table 3.1 Attractiveness of targets baited with various release rates of CO_2 and the best SA blend [Results are expressed as indices of increase relative to the control treatment (index = 1) with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*), $P < 0,01$ (**) levels of probability, or not significantly different (n.s.) are indicated]

	No odour	CO_2 (0,2 l/min)	CO_2 (2,0 l/min)	CO_2 (20 l/min)	Best SA blend	*n*	df	*P*	± s.e.
G.brevipalpis									
Males	1 (3,766)a	1,817a	2,020a	1,742a	4,506b	15	52	**	0,236
Females	1 (3,072)a	1,550ab	2,906bc	2,245abc	4,605c	15	52	*	0,231
G.austeni									
Males	1 (4,484)a	1,457a	1,361a	1,722a	1,048a	15	52	n.s.	0,331
Females	1 (4,711)a	2,252bc	3,172bc	3,971c	1,714ab	15	52	*	0,269

abc Treatments followed by the same symbol are not significantly different

Synergistic effect of carbon dioxide on components of the SA blend

The synergistic effect of CO_2 when added to other synthetic odours was determined in two experiments with treatments as set out in Tables 3.2 and 3.3. In these experiments CO_2 was always released at 2,0 l/min, since the increase in catches with CO_2 at a high dose of 20,0 l/min was not significant compared to the catches at 2,0 l/min and would not have warranted the expense. Fifteen replicates of each experiment were carried out from June - December 1997.

The results of the first experiment for both species are given in Table 3.2. For *G. brevipalpis* males and females it was shown that all treatments were significantly better than 'no odour'. The synergistic effect of CO_2 when added to the original SA blend is apparent but only significant for females.

For *G. austeni* males and females CO_2 also increased catches when added to the original SA blend, however, the original SA blend appears to have a repellent effect on the CO_2 catches.

The results for the second experiment are given in Table 3.3 for both species. For *G. brevipalpis* males and females all treatments were significantly better than 'no odour'. The addition of CO_2 to the original SA blend or the best SA blend increased the catches of both males and females (not significantly) compared to the respective blends. The best SA blend together with CO_2 increased the catches of males by 7,7 x and females by 6,9 x compared to 'no odour'.

For *G. austeni* males and females treatments were not significantly different to 'no odour' and the addition of CO_2 to the original and the best SA blends did not significantly increase the catches. It is possible that the components of the SA blends have a repellent effect on CO_2 (as was also indicated in the results from Table 3.2), since the blends were also less attractive than 'no odour'. Thus CO_2 is found attractive

Table 3.2 Indices of catches of targets baited with CO_2, phenols and acetone relative to the control treatment [Control index = 1 and detransformed mean catches of the control are given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) levels of probability are indicated]

	No odour	CO_2 (2,0 l/min)	Oct, 4mp + CO_2	Original SA blend[1]	Original SA blend + CO_2	*n*	df	*P*	± s.e.
G.brevipalpis									
Males	1 (2,647)a	3,297b	6,161bc	3,566bc	7,557c	15	52	**	0,277
Females	1 (2,428)a	3,785b	5,930bc	2,652b	8,787c	15	52	*	0,293
G.austeni									
Males	1 (4,059)ab	2,047b	1,741b	0,677a	1,679b	15	52	*	0,254
Females	1 (11,135)ab	1,450ab	1,508b	0,750a	1,232ab	15	52	*	0,225

abc Indices followed by the same symbol are not significantly different

[1] Original SA blend: octenol (0,4 mg/h); 4-methylphenol (0,8 mg/h); acetone (350 mg/h)

Table 3.3 Indices of catches of targets baited with CO_2, the SA blend and a combination of CO_2 added to the blends relative to the control treatment [Control index = 1 with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,01$ (**), $P < 0,001$ (***) levels of probability, or not significantly different (n.s.) are given]

	No odour	Original SA blend[1]	Original SA blend + CO_2	Best SA blend[2]	Best SA blend + CO_2	*n*	df	*P*	± s.e.
G.brevipalpis									
Males	1 (3,876)a	3,772b	4,064b	5,750b	7,714c	15	52	***	0,191
Females	1 (5,029)a	2,555b	3,622bc	3,900bc	6,858c	15	52	**	0,245
G.austeni									
Males	1 (3,475)a	0,786a	1,097a	0,987a	1,105a	15	52	n.s.	0,252
Females	1 (11,662)a	0,631a	1,288a	1,020a	1,319a	15	52	n.s.	0,290

abc Indices followed by the same symbols are not significantly different

[1] Original SA blend: octenol (0,4 mg/h); 4-methylphenol (0,8 mg/h); acetone (350 mg/h)

[2] Best SA blend: octenol (9,1 mg/h); 4-methylphenol (15,5 mg/h); acetone (350 mg/h)

for *G. austeni*, but it was suggested by the latter two experiments that the components of the SA blends might diminish its attractiveness.

3.4.2 Relation of visual vs. olfactory attraction (with natural odour)

In previous studies (Kappmeier & Nevill 1999a; 1999c) it was shown that vision could play a significant role for *G. austeni* since targets not baited with odour were still highly effective , while for *G. brevipalpis* odour clearly played a major role in its attraction. The importance of vision vs. olfaction for *G. austeni* and *G. brevipalpis* were evaluated before commencement to search for an odour, other than CO_2, for *G. austeni*. The numbers of tsetse attracted to a visual vs. non-visual target, each baited with and without natural cow odour (extracted from the ventilated pit), were compared. The treatments were, therefore, as follows:

- Non-visual target, not baited with odour (control)
- Visual target, not baited with odour
- Non-visual target, baited with cow odour
- Visual target, baited with cow odour

The non-visual target consisted of a 1,5 x 1 m electric net (Fig. 3.4) and the visual target incorporated a 1 x 1 m phthalogen blue target (flanked by 0,5 x 1 m net) as in Fig. 2.1. In one experiment (Exp. 1) a young bull calf of approximately 75 kg was used. A larger animal later replaced the small animal, producing more odour. The second experiment (Exp. 2) was, therefore, conducted with the full-grown cow weighing ± 350 kg. Six replicates were carried out for each treatment.

The results are summarized in Table 3.4. The control treatment was 'no odour' and no visual target (-/-).

Fig. 3.4 Front view of ramp of pit. Setup shows 1,5 x 1 m electric net at far side of pit where ventilation shaft exits. Note fibre-glass sheet in roof of pit allowing light into pit

Table 3. 4 Summary of the results to evaluate the importance of natural cow odour vs. visual stimuli [Results are expressed as the indices of increase relative to the control treatment (with (+) and without (-) odour, with (+) and without (-) visual) (index = 1) with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) levels of probability are given]

		Odour* / Visual**							
		- / -	- / +	+ / -	+ / +	*n*	df	*P*	± s.e
Exp.	*G.brevipalpis*								
1	Males	1 (3.036) a	3.945 b	6.291 bc	7.859 c	6	15	*	0,084
	Females	1 (2.772) a	3.260 b	7.534 c	10.370 c	6	15	**	0,073
Exp.	*G.brevipalpis*								
2	Males	1 (2.595) a	5.063 b	8.956 bc	11.625 c	6	15	*	0,109
	Females	1 (2,554) a	2.599 ab	5.501 bc	9.029 c	6	15	*	0,126
Exp.	*G.austeni*								
1	Males	1 (0.944) a	5.695 bc	2.082 ab	14.327 c	6	15	**	0.165
	Females	1 (1.836) a	7.142 b	2.708 ab	9.036 b	6	15	*	0.170
Exp.	*G.austeni*								
2	Males	1 (0.648) a	7.793 b	7.863 b	19.185 b	6	15	*	0,156
	Females	1 (2.573) a	10.382 b	4.010 c	12.082 b	6	15	*	0,105

+/- with/without (odour/visual)
* Odour : Exp. 1= 75 kg bull calf; Exp. 2 = 350 kg cow
** Visual target = 1x1 m p.blue plus 0,5x1 m net; Non-visual target = 1,5x1 m net
abc Means of treatments with the same symbols are not significantly different

For *G. brevipalpis* males it was shown that the presence of a visual stimulus with no odour (- / +) increased the catches significantly by *c.* 3,9 and 5,1 x in Exp. 1 and 2 respectively and *c.* 2,6 (not significant) to 3,3 x for females. The importance of odour for this species was confirmed when the presence of the 75 kg bull calf odour only (Exp. 1) and no visual target (+/ -) increased catches significantly by 6,3 x for males and 7,5 x for females and also significantly with the 350 kg cow (Exp. 2), i.e. *c.* 9,0 and 5,5 x for males and females, respectively. It also seems that odour plays by far a more significant role in the attraction of this species than does vision especially for females where the odour attraction (with the 75 kg calf) was even significantly greater than the visual attraction. For both sexes the addition of odour to the visual target (+/+) also increased the catches significantly compared to the visual target only (- /+), i.e. with the 75 kg calf an increase of 2,0 and 3,2 x (for males and females), and with the 350 kg cow an increase of 2,3 and 3,5 x for males and females, respectively.

For *G. austeni* the presence of a visual stimulus without any odour (- /+) increased catches significantly by 5,7 - 7,8 x for males and 7,1 - 10,4 x for females. The presence of the 75 kg calf odour (no visual), however, did not increase the catches of either sex significantly (2,1 x for males and 2,7 x for females) and vision played a more important role. However, with the 350 kg cow this addition of natural odour (no visual) (+/ -) increased the catches of both males and females significantly by 7,9 and 4,0 x respectively. Vision was, however, still significantly more important than odour for females. Also the addition of the natural odour to the visual target (+/+) did not increase the catches of both sexes significantly, although there was still a large increase, namely 2,5 x in males and 1,2 - 1,3 x in females in both experiments. It seems, therefore, that vision plays a greater role in the attraction of this species than does odour. There is, however, an indication, especially with the larger animal, that odour could play an important role in this species' attraction. Whether this odour attraction is due to the attractiveness of CO_2 given off by the animals will be determined in the experiments described below.

3.4.3 Studies to evaluate natural cow odour to find an attractive odour for *G. austeni*

Measurement of CO_2 levels of the experimental cow

The results of the CO_2 concentrations (ppm) as measured with the logger are given in Table 3.5 for various treatments together with their estimated actual rates in l/min. CO_2 measures (ppm) of the experimental cow were taken in the early morning (when metabolism is still low) and during the afternoon (when experiments were conducted). Measures from an empty pit and the background (natural levels in the air) were also taken.

Furthermore, the concentration of CO_2 was measured while releasing CO_2 from cylinders with flow-meters in the pit at various known (nominal) rates (0,2; 2,0; 4,0 and 6,0 l/min). With this information a regression (Fig. 3.5) was plotted of the known rates (l/min) against the known concentrations (ppm minus background of 356 ppm). The cow's morning and afternoon rates are also indicated on this graph. With the

Table 3.5 Summary of CO_2 measurements taken during October 1997

Nominal rate (l/min)	Concentration (ppm)	Estimated actual rate (l/min)
Background	356	0,0
Pit	371	0,1
Cow	993	2,9
0,2	427	0,3
2,0	654	1,4
4,0	1180	3,8
6,0	1703	6,2
3,4	954 – 969,75	2,8

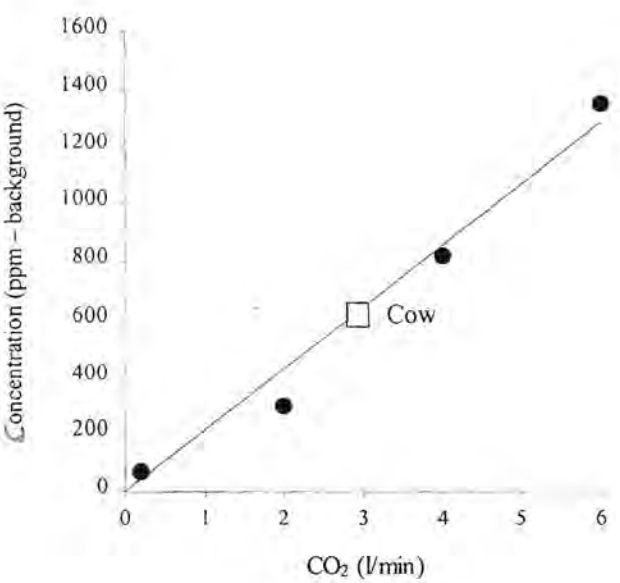


Fig. 3.5 CO_2 release rates of cow during the mornings and afternoons as determined by means of the regression between measured concentration (ppm minus background) against nominal CO_2 rates (l/min) obtained in Table 3.5

estimated actual rate of the experimental cow measured at *c.* 993 ppm (taken as an average over a number of afternoons' readings), it was on two occasions aimed to release CO_2 with the flow-meters at between 950 – 1000 ppm, in order to approximately simulate the CO_2 dose given off by the cow. The nominal rate of 3,4 l/min as indicated in Table 3.5 showed the obtained concentrations to be *c.* 954 – 970 ppm which could be estimated at an actual rate of *c.* 2,8 l/min. This rate was, therefore, used in future experiments to simulate the rate of CO_2 released by the cow.

Effect of CO_2 vs. natural cow odour

For *G. austeni* CO_2 was very attractive and is so far the only proven effective odour attractant (see section 3.4.1). To evaluate the importance of the natural cow odours, other than CO_2, the catches obtained with natural cow odour were compared with those obtained with CO_2 released at the dose of the natural cow, i.e. 2,8 l/min (as established in the previous section - see Table 3.5). The difference in attraction between the two treatments should indicate the attractiveness of the remaining odour components (other than CO_2). 'No odour' was included as the control treatment. Twelve replicates of each treatment were carried out. The results are summarized in Table 3.6.

Table 3.6 Mean catches of targets baited with natural cow odour and with CO_2 released at the same rate as produced by the cow [Expressed as the indices of increase relative to the control treatment (index = 1) with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) levels of probability are shown]

	No odour	Cow (350 kg)	CO_2 (2,8 l/min)	*n*	df	*P*	± s.e.
G.brevipalpis							
males	1 (14,15)a	4,298b	1,904c	12	22	*	0,086
females	1 (12,27)a	4,290b	2,580b	12	22	**	0,086
G.austeni							
males	1 (14,20)a	1,996b	2,137b	12	22	*	0,083
females	1 (29.29)a	2,140b	1,979b	12	22	*	0,074

abc Means of treatments followed by the same symbols are not significantly different at $P < 0,05$

The results showed that natural cow odour increased the catches of male and female *G. brevipalpis* significantly by *c.* 4,3 times compared to 'no odour'. CO_2 (released at 2,8 l/min) also increased catches significantly compared to 'no odour', namely a *c.*1,9 x increase for males and a *c.* 2,6 x increase for females. The comparison between CO_2 and cow odour showed significant differences between their attractiveness for *G. brevipalpis* males but not for females. This suggests that the remaining components of cow odour, other than CO_2, are very important, especially for males.

For *G. austeni* there was a significant increase in the catches of both males (*c.* 2,0 times) and females (*c.* 2,1 times) obtained by the cow odour compared to 'no odour'. There was also a significant increase with CO_2 compared to 'no odour', i.e. a *c.* 2,1 x increase for males and a *c.* 2,0 x increase for females. The comparison between CO_2 and cow odour showed no significant differences between their attractiveness. This suggests that CO_2 is the main attractive part of cow odour and no further important attractant is present in cow odour for *G. austeni*.

Effect of natural odour components other than carbon dioxide, acetone, octenol and 4-methylphenol

The following experiment was aimed to evaluate the importance of the natural aldehydes and ketones produced by the cow, other than octenol, 4-methylphenol, acetone as well as carbon dioxide. The experimental design included the synthetic odour chemicals, which are already in use to attract *G. brevipalpis*, namely 1-octen-3-ol, 4-methylphenol and acetone. This synthetic odour was included as the best SA blend. The best SA blend was added to CO_2 released at the equivalent rate produced by the cow, i.e. 2,8 l/min, and also to the cow. Note that the cow's natural levels of the components of the best SA blend are *c.* 0,1 mg/h (for 4-methylphenol), *c.* 5,0 – 9,0 mg/h (for acetone) and *c.* < 0,5 mg/h (for octenol). The additional large doses of the best SA blend, added to the natural cow odour, means that the small amount of phenols produced by the cow should not have any significant effect (i.e. the large doses of the SA blend will overwhelm the effect of these chemicals produced by the cow). A significant difference between the natural and synthetic odours will, therefore, indicate the presence of another attractive odour component(s).

In this experiment the visual focal point consisted once again of a 1 x 1 m phthalogen blue electric target, but instead of being flanked by a 0,5 x 1 m electric net at one side, it was flanked by two 1 x 1 m electric nets (non-visual) on each side of the visual part of the grid.

The results are summarized in Table 3.7. For *G. brevipalpis* the results indicated a significant increase in the catches with the Cow + best SA blend and the CO_2 + best SA blend for both sexes compared to 'no odour'. The comparison of catches obtained with the Cow + best SA blend and the CO_2 + best SA blend for males and females indicated no significance. This suggests that the increase in catches obtained by the remaining odour components will not make a significant difference and that CO_2, octenol, 4-methylphenol and acetone are the main attractive components of cow odour for this species.

Table 3.7 Summary of the results showing the importance of the remaining odour components other than octenol, 4-methylphenol, acetone and carbon dioxide [Expressed as indices relative to the control treatment (index = 1) with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) level of probability, or not significantly different (n.s.) are given]

	No odour	Cow (350kg) + best SA blend	CO_2 (2,8 l/min) + best SA blend	*n*	df	*P*	± s.e.
G.brevipalpis							
Males	1 (26,49)a	2,826b	1,824b	9	16	*	0,074
Females	1 (16,99)a	3,713b	2,216b	9	16	*	0,098
G.austeni							
Males	1 (20,48)a	1,195a	1,370a	9	16	n.s.	0,133
Females	1 (25,84)a	1,816a	1,595a	9	16	n.s.	0,108

ab Means of treatments followed by the same symbols are not significantly different

For *G. austeni* there was no significant difference between the treatments. Thus there is no significant indication that any of the remaining cow odour components would be attractive. It seems that the high doses of the best SA blend rather had a repellent effect and clearly suppressed the effect of CO_2. This repellent effect of the SA blend was also indicated in previous experiments.

Results of chemical analyses of experimental cow odour used in pit experiments

A summary of the results of the chemical analysis (by the NRI) of the odours absorbed from the 350 kg cow sampled at Hellsgate during 1997 and 1998 are given in Tables 3.8 a-d below (D.R. Hall, unpublished results, 1998). These analyses revealed the components and doses of the main carbonyl compounds (ketones and aldehydes), volatiles (octenol and phenols) and carboxylic acids present in the experimental cow. This will enable the simulation of the known components at the same doses as produced by the cow.

Table 3.8a Rates of production of acetone and butanone from cow and synthetic source (AOP) [AOP is a synthetic source of tsetse attractants consisting of a "mini-sachet" containing acetone and releasing at 6 mg/hr and a 5 cm x 5 cm x 150µm sachet containing a 100:3:1 mixture of 4-methylphenol + 3-*n*-propylphenol + octenol releasing at 1,0 mg/hr]

	RATE OF PRODUCTION[1] (mg/hr)			
	ACETONE	BUTANONE	MEAN ACETONE	MEAN BUTANONE
Cow	0,80	9,05	-2,79	7,39
Cow	-5,70	-1,72		
Cow	-7,25	9,42		
Cow	0,99	12,82		
AOP	1,01	12,36	-2,16	12,26
AOP	-9,57	5,91		
AOP	2,067	18,50		
Empty pit	-0,90	17,04	-0,99	12,86
Empty pit	-2,65	13,39		
Empty pit	0,58	8,15		

[1] corrected for blank

Table 3.8 b Analyses of volatiles collected on Porapak (ratio relative to 4-methylphenol = 100)

	RATE OF PRODUCTION (mg/hr)							
	OCTENOL	2-METHOXY PHENOL	PHENOL	4-METHYL PHENOL	3-METHYL PHENOL	4-ETHYL PHENOL	3-ETHYL PHENOL	3/4-PROPYL PHENOL
Empty pit	0,953	0,000	0,000	0,000	0,000	0,000	0,000	0,000
SE (n=3)	0,374	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Cow	0,500	0,000	0,008	0,107	0,000	0,001	0,001	0,001
SE (n=4)	0,102	0,000	0,008	0,107	0,000	0,001	0,001	0,001
Ratio	467,300	0,000	7,513	100,000	0,000	1,027	0,513	1,283
AOP	0,806	0,000	0,018	0,434	0,000	0,008	0,004	0,008
SE n=3	0,268	0,000	0,010	0,208	0,000	0,008	0,004	0,008
Ratio	185,956	0,000	4,143	100,000	0,000	1,779	0,889	1,779

AOP is a synthetic source of tsetse attractants consisting of a "mini-sachet" containing acetone and releasing at 6 mg/hr and a 5 cm x 5 cm x 150µ sachet containing a 100:3:1 mixture of 4-methylphenol + 3-*n*-propylphenol + octenol releasing at 1,0 mg/hr.

Table 3.8 c Rates of production of carboxylic acids in cattle odour

	RATE OF PRODUCTION[1] (mg/hr)													
SOURCE/REPS	FORM	ACET	i-BUTYR	PROPION	BUTYR	?	PENTAN	HEXAN	HEPTAN	LACTIC	OCTAN	4MP	NONAN	BENZ
Scan[2]	1115	1165	1235	1250	1340	1435	1454	1565	1670	1735	1776	1800	1881	2107
ECL[3]	10,10	10,52	11,10	11,23	11,98	12,77	12,93	13,87	14,86	15,48	15,87	16,11	16,95	19,28
Unused(3)	12,584	13,373	0,050	1,070	0,112	0,208	0,245	1,781	0,282	0,383	0,408	0,000	0,329	0,149
Empty pit (3)	13,475	13,849	0,069	1,030	0,114	0,211	0,262	1,781	0,191	0,561	0,444	0,064	0,358	0,176
Cow(4)	11,189	11,385	0,052	0,883	0,085	0,187	0,208	1,400	0,161	0,578	0,324	0,009	0,284	0,136

[1] Form = formic; acet = acetic; i-butyr = iso butyric; propion = propionic; butyr = butyric; pentan = pentanoic; hexan = hexanoic; heptan = heptanoic; octan = octanoic; nonan = nonanoic; 4MP = 4-methylphenol; benz = benzoic; ? = unknown.

[2] scan in GC-MS analysis (= seconds)

[3] ECL = GC retention time in equivalent chain length relative to retention times of straight-chain acetates.

Table 3.8 d Estimates of the mean rates of production of various tsetse attractants as obtained for the second run of chemical absorbtion (1998) and analyses [S.J. Torr & Sara Phythian, unpublished report, 1999. The mean rate for a cow is estimated from the difference between the blank filter and the cow+pit]

Odour	Treatment	Mean (mg/h)	s.e.	*n*
Octenol				
	Blank filter	0,027	0,02	5
	Empty pit	0,040	0,02	5
	Cow + pit	0,059	0,02	5
Mean rate (mg/cow/h)		0,03		
4-methylphenol				
	Blank filter	0	0,00	5
	Empty pit	0	0,00	5
	Cow + pit	0,06	0,06	5
Mean rate (mg/cow/h)		0,06		
Acetone				
	Blank filter	0,97	0	5
	Empty pit[1]	0,86	0,85	4
	Cow + pit	4,7	1,2	6
Mean rate (mg/cow/h)		3,7		
Butanone				
	Blank filter	0	0,00	5
	Empty pit	0,17	0,10	5
	Cow + pit	0,33	0,02	6
Mean rate (mg/cow/h)		0,33		

[1] Excludes the result for one (contaminated) filter which indicated a release rate of 25,4 mg/h compared to 0,4-1,1 mg/h for the remaining four filters

Analyses of carbonyl compounds showed samples were contaminated with UV-absorbing impurities and in some samples amounts of the acetone DNPH were lower than that in unused filters, such that no useful results could be obtained (Table 3.8 a). These collections and analyses were repeated in 1998 (Table 3.8 d). Presumably the impurities came from the pit set-up used, but the reason for the low acetone DNPH in some samples is less obvious. Possibly large amounts of moisture were a factor (D.R. Hall, unpublished report, 1998).

Rates of production of the volatiles by the cow were lower than from the synthetic source (AOP). Contamination of filters with octenol made it impossible to give a reliable figure for this component (Table 3.8 b) as rates of production are typically very low (D.R. Hall, personal communication, 1998). These measurements were repeated during 1998 with completely clean filters (Table 3.8 d).

Collections of carboxylic acids showed no significant amounts (< 1 mg/hr) of any of the carboxylic acids analyzed attributable to the cow.

Evaluation of natural vs. synthetic cow

The previous experiments showed that both natural cow odour and CO_2 increased the catches of *G. brevipalpis* and *G. austeni* males and females significantly compared to 'no odour'. The remaining cow odour components, other than carbon dioxide, were significant for the attraction of *G. brevipalpis*, especially for males, but not for *G. austeni*. It was also indicated that the very volatile components of natural cow odour might be important for the attraction of *G. brevipalpis* (although not significant), but not for *G. austeni*. From the previous results it could be concluded that no further attractant in cow odour, other than carbon dioxide, is present for *G. austeni*.

The final and ultimate test to prove this, or which could assist to find anything else that might attract *G. austeni*, will be to test the natural cow odour and compare it against synthetically simulated cow odour (henceforth synthetic cow (SC)). Any significant increase in the catches with the natural cow,

compared to SC, will suggest the presence of a very volatile and unidentified attractant.

SC consisted of a number of chemicals based on the analysis of chemists at the NRI (see previous section) as summarized in Table 3.9. SC was released more or less at the indicated dosages through the corresponding sachet sizes as set out in Table 3.9.

Table 3.9 List of synthetic cow (SC) components used to simulate the natural cow and the recommended release rates. The sachet sizes, which gave more or less the correct dosages, are indicated

Synthetic cow odour components	Release rate (mg/hr)	Sachet size (measurements in cm)
Octenol	0,03	
4-methylphenol	0,107	
4-ethylphenol	0,001 } 0,14	} 2x2
3-ethylphenol	0,001	
3-propylphenol	0,001	
2-methoxyphenol	zero	-
phenol	0,008	2,5 x 2
3-methylphenol	zero	-
acetone	3,7	2x2
butanone	0,33	2x2 in 3x3
acid mix*	0,002	2x2
carbon dioxide	2,8 l/min	released from cylinder

* Acids: Formic, acetic, iso butyric, propionic, butyric, pentanoic, hexanoic, heptanoic, octanoic, nonanoic, benzoic

Two weeks of experimentation was done so as to obtain the correct release rates for the components, by weighing chemicals released from different sized sachets. The acids were dispensed as a mixture of equal amounts of acids and combined with sunflower oil[2] to make a 30:1 solution (i.e. 1 part acids) and dispensed from the sachets to aim for a release rate of 0,002 mg/h. Carbon dioxide was released from a cylinder at 2,8 l/min as was measured to be the release rate of the cow during times of experimentation.

During the course of the experiment, sachets of the SC were weighed before and after each day's use in the pit, to keep record of the release rates.

Natural cow and SC odours were compared with each other and to 'no odour'. Eight replicates were carried out. A summary of the results is given in Table 3.10.

Table 3.10 Indices of mean catches of flies attracted to natural and synthetic cow (SC) odour relative to the control ['No odour' index = 1 with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) level of probability are indicated]

	No odour	Cow (± 350 kg)	Synthetic Cow (SC)	*n*	df	*P*	± s.e.
G. brevipalpis							
Males	1 (8,38)a	2,700b	1,143a	8	14	*	0,079
Females	1 (3,39)a	8,167b	2,997c	8	14	**	0,093
G. austeni							
Males	1 (10,05)a	4,293b	2,032ab	8	14	*	0,109
Females	1 (79,41)a	2,051b	1,528ab	8	14	*	0,073

abc Means of treatments followed by the same symbols are not significantly different at $P < 0,05$

[2] Acids are normally dissolved in dioctyl phthalate, however, this chemical was not available at the time and NRI chemists suggested the use of sunflower oil since this would be sufficiently polar and a good mimic for the former

The results showed SC to be significantly less attractive for both *G. brevipalpis* males and females, i.e. cow odour increased catches significantly by 2,4 and 2,7 times, respectively, for males and females compared to SC. For *G. austeni* males and females there were no significant differences between the SC and natural cow, although the cow still increased catches by 2,1 and 1,3 times, respectively, for males and females compared to SC.

3.4.4 Testing of other host odours

The attractiveness of other host odours was tested by extracting their odours from the ventilated pit, to see whether their odours could be more attractive and, therefore, hold something else than cow odour. The animals tested were a bushpig and goats, which were compared to cow and 'no odour'. The same target/net combination was used as shown in Fig. 3.4. Six replicates of each treatment were carried out. The results are given in Table 3.11.

Table 3.11 Indices of mean catches attracted to cow, bushpig and goat odours relative to 'no odour' ['No odour' index = 1 with detransformed mean catches of the control given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) and $P < 0,01$ (**) level of probability are given]

	No odour	Cow (± 350 kg)	Bushpig (± 100 kg)	Goats (±68 kg)	*n*	df	*P*	± s.e.
G. brevipalpis								
Males	1 (15,11)a	3,778b	1,870ab	1,535a	6	15	*	0,125
Females	1 (6,29)a	7,460c	4,298cb	2,183ab	6	15	*	0,142
G. austeni								
Males	1 (10,57)a	3,979b	4,470b	2,984b	6	15	**	0,114
Females	1 (29,81)a	3,360b	2,937b	2,133ab	6	15	*	0,122

abc Means of treatments followed by the same symbols are not significantly different at $P < 0,05$

The results indicated for *G. brevipalpis* males and females showed that cow odour was still the most attractive, increasing catches by *c.* 3,8 and 7,5 x respectively, compared to 'no odour'. For *G. brevipalpis* males, bushpig and goat odours did not increase the catches significantly compared to 'no odour'. For females, bushpig odour increased catches significantly by *c.* 4,3 x compared to 'no odour'. Goat odour was significantly inferior to cow odour for both sexes.

For *G. austeni* males, the cow, bushpig and goat odours were all significantly more attractive than 'no odour'. For females, cow and bushpig odours were significantly more attractive than 'no odour', but the goat odour was not significantly better. Bushpig odour was even more attractive than cow odour for males (although not significantly) but not for females. Considering the combined male and female catches, cow and bushpig odours were about equally effective with an increase of *c.* 3,6 with cow odour and *c.* 3,4 with bushpig odour, as compared to 'no odour'.

This suggests that no better attractants may be found in bushpigs and goats for either tsetse species.

3.4.5 Effect of human odour

Human smell was found to have a repellent effect when added to cow odour for *G. m. morsitans* and *G. pallidipes* in Zimbabwe (Vale 1979). The attractiveness of human odour for *G. brevipalpis* and *G. austeni* was determined together with its effect on the attractiveness of cow odour. Six replicates of the following treatments were compared in one experiment, the results of which are summarized in Table 3.12:

- No odour (control)
- Cow
- 3 Men
- Cow + 3 Men

Table 3.12 Indices of mean catches obtained with odours released from cow, man and a combination of cow and man relative to the control [Control/'no odour' index = 1 with detransformed mean catches of the control given in brackets. The number of replicates (n), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) or $P < 0,01$ (**) level of probability, or not significantly different (n.s.) are shown]

	No odour	Cow (± 350 kg)	3 Men (± 270 kg)	Cow + 3 Men	n	df	P	± s.e.
G. brevipalpis								
Males	1 (10,96) a	6,199 b	2,073 ac	3,461 bc	6	15	**	0,102
Females	1 (8,916) a	8,926 b	1,791 a	3,913 c	6	15	*	0,090
G. austeni								
Males	1 (6,583) a	3,609 a	2,289 a	2,091 a	6	15	n.s.	0,144
Females	1 (22,46) a	2,111 a	1,795 a	1,811 a	6	15	n.s.	0,100

abc Means of treatments with the same symbols indicate no significant difference at $p < 0,05$
The overall treatment F for *G. austeni* is not significant

For *G. brevipalpis* it was shown that the natural cow odour increased the catches of males and females significantly by 6,2 and 8,9 x respectively. Human odour, on the other hand, also increased the catches slightly, but not significantly (i.e. 2,1 and 1,8 x for males and females). The presence of human odour with cow reduced the attractiveness of the cow significantly for females but not for males, i.e. a 0,5 and 0,4 x reduction for females and males respectively.

For *G. austeni* the catches with the natural cow odour were also better than with no odour, i.e. 3,6 and 2,1 x for males and females respectively, but this was not significant. Further the same basic trend held for *G. austeni* as for *G. brevipalpis* in that human odour alone also increased the catches (not significantly). The presence of human odour with cow odour also seemed to have a repellent effect, however, this is not significant.

3.4.6 Overall mean index of cow odour

The indices of increase of natural cow odour relative to 'no odour' as obtained in the various experiments above are summarized in Table 3.13 for each

species and sex. To summarize these indices, the overall mean index of cow odour was obtained. Indices were transformed to log and then the averages were calculated and detransformed. The detransformed mean indices are calculated (Table 3.13) as 3,622 and 6,053 for *G. brevipalpis* males and females, respectively, and 3,133 and 2,051 for *G. austeni* males and females, respectively.

For more precise estimates these indices were weighted according to the reciprocal of the scale perimeter (variance) for each experiment as summarized in the Table. This deals with problems of differences in the variance between different experiments when there are different numbers of catches. The weighting procedure thus gives greater weight to the values with smaller variance. Weightings are indicated as 1 (low) - 5 (high). This procedure showed, for weightings of 5, indices of cow odour as 2,700 and 8,926 for *G. brevipalpis* males and females, respectively, and 3,979 and 2,051 for *G. austeni* males and females, respectively.

Table 3.13 Summary of indices of the attractiveness of natural cow odour vs. 'no odour' for five experiments (A-E) [The overall mean index is indicated in bold. Weights are allocated to the indices obtained in the various experiments]

	G. brevipalpis				*G. austeni*			
	Males		Females		Males		Females	
Exp.	Index	Weight[2]	Index	Weight	Index	Weight	Index	Weight
A	2,296	3	3,474	2	2,462	1	1,164	2
B	4,298	2	4,290	3	1,966	4	2,140	3
C	2,700	5	8,167	4	4,293	3	2,051	5
D	3,778	1	7,460	1	3,979	5	3,360	1
E	6,199	4	8,926	5	3,609	2	2,111	4
Mean[1]	**3,622**		**6,053**		**3,133**		**2,051**	

[1] Detransformed mean of log transformed indices
[2] Weighting index allocated to reciprocal of scaled parameters between 1 (low) – 5 (high)

3.5 DISCUSSION

CO_2 and other synthetic components

Carbon dioxide is an attractive component for *G. brevipalpis* and *G. austeni* in South Africa. For both these species the rate of CO_2 released from cylinders was comparatively suitable at 2-20 l/min and significantly increased the numbers of these flies attracted to a target. These rates are, however, regarded to be not worth the expense, since it is costly, impractical to use in the field and, furthermore, impossible to use on a large scale. Since studies started on testing conventional odour components for these species (Kappmeier & Nevill 1999a), CO_2 was the only single component found to attract significant numbers of *G. austeni*. It had also been found attractive, in the form of dry ice, for the Kenyan *G. austeni* population (Owaga 1992).

Carbon dioxide, acetone, octenol and 4-methylphenol all added synergistically to the attraction of *G. brevipalpis* males and females. Of these components it seemed that acetone and CO_2 act as the most important synergists, even at high doses of octenol and 4-methylphenol, in that the increase in catches were only significant when acetone and/or CO_2 were present. Torr (1990) also has found that acetone and CO_2 acted as valuable synergists. For *G. austeni* it seemed that the addition of octenol, 4-methylphenol and acetone had a repellent effect and diminished the attractiveness of CO_2. Kappmeier & Nevill (1999a) have noted a similar repellent effect of these components when targets baited with some combinations of these chemicals caught even less than those baited with no odour. Whether this repellency was the combined effect of all three components is not certain. Vale & Hall (1985a) have observed for *G. m. morsitans* and *G. pallidipes* that, even in the presence of CO_2, attraction can be depressed by high doses of octenol.

Natural cow odour

Natural cow odour also increased catches of *G. austeni* males and females with indices weighted at *c.* 4,0 and 2,0 times, respectively, compared to 'no

odour'. The emphasis of the study, therefore, focused on finding an attractive odour component(s) for *G. austeni*, other than CO_2, which may be present in natural cow odour and which could perhaps also further enhance the attraction of *G. brevipalpis*. The natural cow odours (other than CO_2) were confirmed to be valuable for the attraction of *G. brevipalpis*. However, the components of cow odour (other than CO_2) had no significant effect on the catches of *G. austeni*. These results were expected since some of the main synthetic components of ox odour were known to be very attractive for *G. brevipalpis* but not for *G. austeni* (Kappmeier & Nevill 1999a).

The components of natural cow odour excluding CO_2, acetone, 4-methylphenol and octenol looked promising for the attraction of *G. brevipalpis*, even when supernormal doses of the latter three components were added to cow odour, although the remaining components' contribution to the increase of catches were not significant. This was not the case for *G. austeni*, thus the results generally indicated that, other than carbon dioxide, no further attractant for *G. austeni* was present in cow odour. The implication that acetone, octenol and 4-methylphenol had a repellent effect for this species, was verified when the attractiveness of cow odour and CO_2 were both reduced so that their increases in catches compared to 'no odour' were unexpectedly not significant with the presence of supernormal doses of these components.

Natural vs. synthetic cow odour

When all identified attractants of the cow odour were dispensed synthetically at seemingly appropriate levels (this blend referred to as synthetic cow [SC]), no significant difference was found between catches of *G. austeni* obtained with the natural cow and its synthetic concoction. For *G. austeni* males, cow odour still doubled the catches compared to SC, although this was not significant. Therefore, despite the indication that there might be some attraction by a volatile component(s) present in the cow odour, this attraction is certainly not significant, so that most, and probably all of its attraction, especially for females, seems to be due to CO_2. For *G. brevipalpis* cow odour increased catches of males and females significantly by *c.* 2,4 and 2,7 times,

respectively, compared to SC. This supports the findings of Torr *et al.* (1995) and Hargrove *et al.* (1995) that there are possibly attractive components in cow odour, other than the acids, ketones and not so volatiles (octenol and phenols), which are probably relatively volatile and still remain to be identified, as was suggested in their respective studies on *G. pallidipes* and *G. m. morsitans* in Zimbabwe.

Other host odours

The subsequent testing of other host odours, which gave an idea of their relative attractiveness as compared to cow odour and 'no odour', showed that cow odour was still the most attractive for *G. brevipalpis* and bushpig odour to a lesser extent (not significant for males). Goat odour was relatively unattractive and did not increase the catches of this species significantly compared to 'no odour'. For *G. austeni* cow, bushpig and goat odours were all better than 'no odour', with cow and bushpig odours as the most attractive (significant) and goats to a much lesser extent (not significant). For *G. pallidipes* and *G. m. morsitans* it has been shown that increases in the body mass resulted in an increase in the catch of the tsetse flies (Hargrove *et al.* 1995). Therefore, it is possible that this is the reason that cow odour is still the best source of attraction for the two Zululand species. It could, therefore, be presumed that no better attractant might be found in either bushpigs or goats and that their effects on the increases in catches, specifically for *G. austeni*, were most probably due to CO_2. Thus the fact that the numbers of this species increased according to increased body mass can probably be attributed to increased carbon dioxide release.

Human odour

While human odour on its own was somewhat attractive (not significantly) for *G. brevipalpis* and *G. austeni*, its presence with cow odour clearly had a repellent effect on the attractiveness of cow odour for both species. This suggests that cow odour is attractive but the odour of man has a mixture of repellents and attractants. Similar results were obtained for *G. m. morsitans*

and *G. pallidipes* (Vale 1974b, 1977b, 1979) where it was shown that chemicals present in human odour reduce the numbers of tsetse attracted to a host and also the proportion of flies that feed. The identities of the repellents present in human odour remain unknown, however, work was underway to identify these repellents (Torr *et al.* 1996).

Vision vs. olfaction

In an experiment, separating the cues that might be responsible for attracting tsetse, the numbers of tsetse attracted to a visual and non-visual target with and without the smell of a cow were compared. Thus, when measuring the role of vision vs. olfaction the results for *G. brevipalpis* indicated that natural cow odour played a significantly more important role in its attraction than did vision. For *G. austeni*, on the contrary, vision seemed to play a major significant role in its attraction. However, there is a good indication that natural cow odour still played a role in its attraction, which is, as previously suggested, most likely exclusively due to CO_2.

This notable importance of vision and CO_2 is fascinating. Carbon dioxide normally occurs in large concentrations in nature, especially in woody vegetation where daytime concentrations of CO_2 exceed the concentration in the open (Gillies 1980). Carbon dioxide is naturally present at ≈ 300-400 ppm during the day (see Table 3.5), rising to as much as ≈ 1000 ppm at night (Gibson & Torr 1999). As *G. austeni* is most active during day-time (Kappmeier 2000), it would suggest that it would not easily detect host CO_2 which is released into a potentially competing background. However, the CO_2 released by the cow or via cylinders were measured at ≈ 993 ppm and ≈ 954-970 ppm respectively (see Table 3.5) (at the pit outlet). These CO_2 exhalations or releases could, therefore, be fairly dispersed and diluted before falling to background levels.

In a study on CO_2 odour plumes by G.E. Zollner (pers. comm., 2000), the background concentration of CO_2 at Hellsgate was very "noisy" in the middle

of the day in that the atmospheric concentrations of CO_2 fluctuated highly. Following release of natural or synthetic CO_2, it was difficult to detect CO_2 with gas analyzers at 16-32 m downwind from the source. Taking the foregoing into account, the background CO_2 will therefore probably not prevent detection of hosts by *G. austeni* at close range, but from further away host CO_2 should be lost in atmospheric "noise". It might, therefore, be difficult for tsetse in general to detect host-derived CO_2 from far away. This was also demonstrated in Zimbabwe, where it was shown that CO_2 stimulated close-range responses in tsetse, i.e. the alighting responses on targets (Vale 1983) as well as entering responses into traps (Vale & Hall 1985b).

G.E. Zollner (pers. comm., 2000) also showed during her study at Hellsgate that the catches of *G. austeni* decreased as they were measured from further away downwind of a CO_2 source. On the other hand, the catches improved as they were measured closer downwind of the CO_2 source (4-8 m). However, this range of attraction also probably coincides with the visual range of attraction of this species, which was clearly demonstrated earlier to be an important cue in their host-detecting behaviour. Their reliance on vision may on the other hand be linked to their smaller size and strength which may mean that they are unable to pursue odour trails for as far as *G. brevipalpis* and thus depend more on the visual detection of hosts (walking past its resting sites) than on the location of essentially stationary hosts using olfactory cues. However, Hargrove *et al.* (1995) indicated that one should be wary of any oversimplified explanations of differences based on differential flight ability.

The fact remains, however, that *G. austeni* is still attracted significantly more by a visual target baited with CO_2 than to an unbaited target. It may, therefore, be that this species (and other tsetse as well) probably have a highly sophisticated sense of smell and a higher sensitivity to CO_2. Zollner *et al.* (1998) suggested that tsetse may well be able to detect CO_2 from large distances (at least 64 m) downwind of an animal host in riverine habitats.

Implications

Consequently, it is concluded that *G. austeni* probably makes use of a combination of carbon dioxide and visual cues of a host. It could also be confidently deduced that the likely existence of other volatile components in cow odour would have no significant implications for this species. Due to its practical unsuitability, carbon dioxide can best be utilized with monitoring tools, such as electric grids, to confirm tsetse presence or absence. This can particularly be applied in such situations where *G. austeni* density is low, for example at the completion of control or eradication operations or at the limits of distribution so as to confirm their presence or absence. However, for practical application of odour-baits during monitoring and control operations, no odour bait is necessary to attract this species and reliance on the flies' visual capabilities should be sufficient to attract them to targets and traps.

For *G. brevipalpis*, on the other hand, the existence of an additional effective odour component is a possibility. It would, however, be worth doing an economic assessment to determine how much of an improvement such an odour would have over the present odours which have been found to be suitable for this species.

4. DEVELOPMENT OF SUITABLE TRAPS

4.1 ABSTRACT

Sticky traps of various shapes and colours were tested and improved for the purpose of surveying the distribution of *Glossina austeni* and *G. brevipalpis* in South Africa. For *G. austeni* and *G. brevipalpis* the 3-dimensional shapes of the XT and 3DT in light blue (l.blue) and white were better than the RT. An elelctric blue (e.blue)/l.blue odour-baited XT was effective to apply in surveys to monitor the distribution of the two species in N.E. KwaZulu-Natal. This sticky trap was later replaced by an e.blue/black XT proven to be more effective for *G. austeni* and similarly effective for *G. brevipalpis*. An increased size of the trap increased the numbers of *G. brevipalpis* females and both sexes of *G. austeni* significantly. For both species larger monopanels (95 x 80 cm and 120 x 100 cm) of which each side was painted half e.blue and black (vertical) were found equally attractive to the standard size (70 x 60 cm) XT. A new trap, named the "H trap", was developed for the simultaneous collection of live *G. brevipalpis* and *G. austeni*. Its design followed an evaluation of the responses of the two species towards traps that are used elsewhere in Africa for the collection of other tsetse fly species. These traps were found at Hellsgate to be unsuitable for capturing both *G. brevipalpis* and *G. austeni*. Some new trap designs and many modifications of these were tested, most of which were unsuccessful. The odour-baited blue and black H trap represents a different approach for trapping tsetse flies as it is fitted with lateral cones of white netting which induce the flies to take a more horizontal flight path once they have entered the trap, instead of the vertical flight paths they are forced to assume in existing tsetse fly traps. A number of modifications of the prototype H trap were devised (H1-H5), before the final design was established. Catches of up to 76 *G. brevipalpis* and 37 *G. austeni* were obtained per trap on a single day with the H3 modification. Further modifications improved on the trap's efficiency to capture *G. brevipalpis* and *G. austeni*. The final modification caught a record number of 180 *G. brevipalpis* and 57 *G. austeni* on a single day.

4.2 INTRODUCTION

Hargrove (1998) has defined a tsetse fly 'trap' as a device designed to induce tsetse to enter a space from which they cannot escape. Harris (1931) developed the first trap used for tsetse flies and employed it to capture large numbers of *G. pallidipes* Austen in South Africa. Since then, many traps have been designed for other species of tsetse in other parts of Africa (Morris & Morris 1949; Challier & Laveissière 1973, cited in Hargrove 1998; Moloo 1973; Hargrove 1977; Laveissière & Couret 1980; Vale 1982a; Flint 1985; Gouteux & Lancien 1986; Brightwell *et al.* 1987; Laveissière & Grébaut 1990; Brightwell *et al.* 1991; Gouteux 1991; FAO 1992; Kyorku *et al.* 1993; Mhindurwa 1994; Vreysen *et al.* 1996). Traps are, however, preferably used as monitoring tools but have shown to be very effective in control programmes.

4.2.1 Sticky traps

Initially sticky traps were developed in Zanzibar (Hall 1990; Schonefeld 1988, cited in Hall 1990) for the monitoring of *G. austeni*. These were light blue and white traps of the 3DT (3-dimensional trap), the XT (cross-shaped X target), and the RT (rectangular sticky screen) with 3-dimensional leg panels. The only traps earlier found to be effective for capturing *G. austeni* in KwaZulu-Natal have been these sticky panels of various shapes and colours. When baited with synthetic ox-odour, they also captured *G. brevipalpis* (Kappmeier, Venter & Nevill 1995). Since then more sticky traps have been developed, namely the Chuka trap by Madubunyi (1990) and the free rotating monopanel (MP) and legpanel (LP) by Vreysen *et al.* (1996).

Before the distribution of *G. austeni* and *G. brevipalpis* in northern KwaZulu-Natal could be surveyed, studies were needed to evaluate the sticky trap shapes and colours, so as to be able to select the best trap for these surveys. The three Zanzibar sticky traps available at that time (3DT, XT and RT) were, therefore, evaluated for their efficacy for the two tsetse species. Later, further studies were undertaken to improve on the design of the sticky traps used in initial surveys. The attractiveness of additional colours, colour combinations

and sizes were tested as well as simplifying the design for its manufacture and practical use in the field.

4.2.2 Cloth traps

Sticky traps proved to be useful tools for monitoring the relative distribution of both species in KwaZulu-Natal (Nevill *et al.* 1995; Nevill 1997), but, do not provide live flies suitable for mark-release-recapture studies. For this it is necessary to use a trap which catches live specimens in large enough numbers. No such trap exists for *G. austeni* as its behaviour is elusive and only low numbers are caught in existing tsetse fly traps elsewhere in Africa (Takken 1984; Hall 1986; Madubunyi 1990). The only trap available for this purpose for *G. brevipalpis* was the Siamese trap but it is only partially effective for this species in Kenya (Kyorku *et al.* 1993).

Preliminary studies in KwaZulu-Natal have indicated that, with the exception of sticky traps, most existing tsetse fly traps, which are effective for other species elsewhere in Africa, were not effective for the capture of *G. brevipalpis* and particularly not for *G. austeni* (Kappmeier, in press). Traps that have been tested in South Africa for capturing live *G. austeni* and *G. brevipalpis* include the Epsilon, Pyramidal, Biconical, Vavoua, Ngu (Ng2f) and Siamese (B) (Gouteux & Lancien 1986; Brightwell *et al.* 1987; Laveissière & Grébaut 1990; FAO 1992; Kyorku *et al.* 1993).

The best of these, namely the Ngu (Ng2f) and Siamese (B), caught mean daily numbers of 8,2 and 5,8 *G. brevipalpis* respectively (35 replicates) and 0,4 *G. austeni* (35 replicates) (Kappmeier, in press). In addition, the efficiencies of the Ngu and Siamese traps, as determined by comparing the results obtained with those when electrified nets were placed immediately adjacent to the traps, as suggested by Vale (1982a), were also found to be very low (Kappmeier, in press). The reason for the ineffectiveness of the traps for *G. brevipalpis* and *G. austeni* in KwaZulu-Natal was determined during further trap-orientated behavioural studies, as described by Vale (1982b), when, by the use of

electrified nets, it was shown that the upward flight responses of the flies were very low. Only 21-45 % of the *G. brevipalpis* that entered a Ngu and Siamese (B) trap, flew upwards towards the cone (Kappmeier, in press). The same basic trend also held true for *G. austeni*.

The poor vertical movement of these tsetse fly species led to the development of a prototype of a new trap using lateral or side-cones instead of vertical or top-cones so that the flies, once they had entered the trap, flew horizontally rather than upwards. In order to improve on the design, several modifications of this prototype trap, named the H trap, were assessed for trap-orientated responses of the flies as well as for efficiency.

Months of studies on numerous modifications of existing traps and on new designs preceded the development of the H trap. Because they were unsuccessful these efforts will only be referred to briefly and the main body of the chapter will concentrate on the evolution of the H trap.

4.3 Materials and Methods

4.3.1 Sticky traps

Trap designs and tests

Three types of sticky traps were made for testing at Hellsgate according to the description of Hall (1990). These were the 3 DT, XT and RT (Fig. 4.1). The traps were made from 3 mm tempered hardboard panels, painted light blue (l.blue), electric blue (e.blue), white or black with gloss enamel. All traps were hung from trees and were allowed to rotate with their lowest part 10-20 cm above ground level. They were painted with polybutene so that the flies that landed on the traps could be retained on the sticky surface. The polybutene was diluted with hexane to facilitate easier application. Once applied, the hexane evaporates and the surface remains sticky. To collect flies lost from the lower edges due to dripping, especially during the first day when the polybutene is still quite fluid, a plastic sheet was placed underneath each trap.

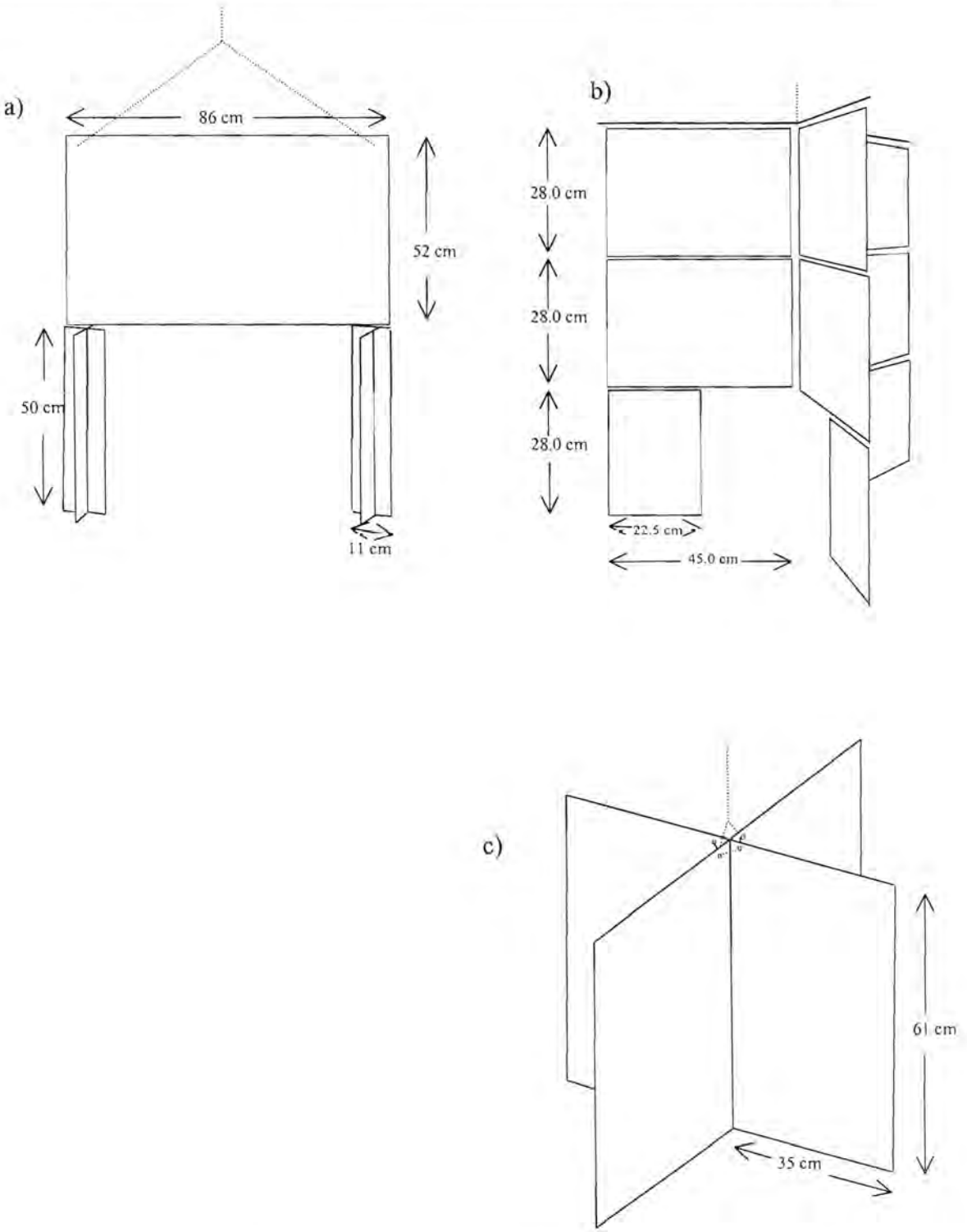


Fig. 4.1 Sticky traps for *G. austeni*: (a) Rectangular sticky screen (Hall 1986, cited in Hall 1990); (b) 3-DT (Schonefeld 1988, cited in Hall 1990); (c) cross-shaped target (XT) (Hall 1990)

This also reduced contamination of the trap with soil and leaves due to rain and wind. The polythene sheet was pinned down with 200 mm pegs made from heavy fencing wire.

A series of experiments were conducted between 1993 - 1996, firstly to evaluate the various sticky trap designs and compare black, white, l.blue and e.blue versions. Thereafter, the best design (XT) was improved and evaluated in single- and bicoloured combinations (i.e. one panel of the XT was painted a different colour to the second panel). It was then attempted to simplify the best colour combination of the XT in the form of monopanels (being a single panel of the XT) for more practical use in the field. These were also tested in various bicoloured combinations and sizes. The experiments will be described in more detail in the Experiments and Results section (4.4) below.

Sticky trap efficiencies

In order to determine the efficiency of traps, an electric net (1 x 1 m), was placed immediately adjacent to the trap. This electric net intercepted flies that were attracted to the trap, but which flew around it and which might never have landed on the trap. The number of flies captured by a trap (without an electric net) was expressed as a percentage of the total number of flies attracted to a trap (trap plus electric net), to give an estimate of trap efficiency (Vale 1982a; 1982b).

4.3.2 Cloth traps

Preceding trap tests and designs

Before the prototype of the H trap was designed, many modifications of existing traps were made and some other traps were originated at Hellsgate. The designs of all these traps took into account the flies' reluctance to fly upwards towards the cones. Some of these designs were also described in Kappmeier (in press).

The first designs consisted of modifications of the Ngu and Siamese traps where both were fitted with lowered or sunken cones so that the path towards the collecting devices was lower. Some of the latest tsetse traps were included in these tests, namely the M3 (Mhindurwa 1994) and the Nzi traps (Mihok 2001). The Nzi was also modified into what was referred to as the Nzi3 which consisted of three Nzi traps united back to back thus with three separate entrances. The Nzi was also further modified so that the rear netting part was incorporated into a horizontal and diagonally sloping cone plus collecting device, therefore doing away with the top/vertical cone. The Canopy trap used for Tabanidae (Catts 1970) was also tested and then modified, firstly by adding a pthalogen blue panel to the base (to enhance attraction), and later by providing openings in the blue pyramidal base, and simultaneously lowering the top cone part. Some new trap designs included what was referred to as the Monoscreen trap, which consisted of a blue and black cloth target with two thirds of the top part fixed with white mosquito netting which formed a "tent" over the target. A few modifications to the net part followed to encourage the horizontal movement of flies towards a collecting device. One of these modifications was further modified into what was called a 3-dimensional-screen trap (3DS), which, as seen from above, consisted of an X-shaped cloth target, also fixed with a tent-like cover of netting and collecting cages. The prototype H trap (with different modifications [H1-H5] as described below) was designed and developed together with a B trap (P.W. Trollip, personal communication, 1997) and its modifications B1-B5. The latter were similar to the H trap, but had only one horizontal cone.

Of all the above designs and modifications, other than the H trap modifications, only a few looked promising, namely the Nzi, Nzi3 and B1-B5 traps. Further experiments included the comparison of these traps with an e.blue/black XT sticky trap described in the previous section. These results will briefly be summarized in section 4.4.

The prototype H trap

The prototype design of the H trap (Fig. 4.2 a) consisted of a phthalogen blue cloth outer "box" (100 x 65 x 65 cm) with two opposite side entrances (40 x 45 cm), an inner black cloth X-target (which also acted as a baffle, attached to the centre of the roof), and then two "horizontal" cones of white mosquito netting extending laterally from the ends of the trap in opposite directions, therefore initially named the "Horizontal trap". Although the "cone"-device used here, was a hollow four-sided pyramid-shaped structure with a square base and straight (not curved) sides, it will here and henceforth be referred to as a "cone", which is an accepted term to use with tsetse fly traps (FAO 1992). The four corners of the trap body were fastened, with strings attached to the trap, to four poles pegged into the ground at the positions of the trap corners. The cones were held in position by attaching them each to a flexible rod which provided tension to keep them rigid (Fig. 4.2 a). The apex of each cone was fitted with the top third of a 750 ml polythene bottle on which a second bottle fitted as the collecting device (Fig. 4.2 e).

H trap modifications

Five modifications of the prototype trap (Fig. 4.2 a) were made, and referred to as the H1 – H5 traps/modifications. The following is a description of the modifications, also depicted in Figs. 4.2 b-d:

H1: The prototype H trap was modified by adding a black inner lining to the base of the cones to prevent flies from collecting at the corners at the cones' bases (Fig. 4.2 b).

H2: The H2 was made with an extension of the outer blue "body" over the cones of the prototype trap (Fig. 4.2 c) to attract the flies to the light and the trap cage (collecting device) at the apex of the cones.

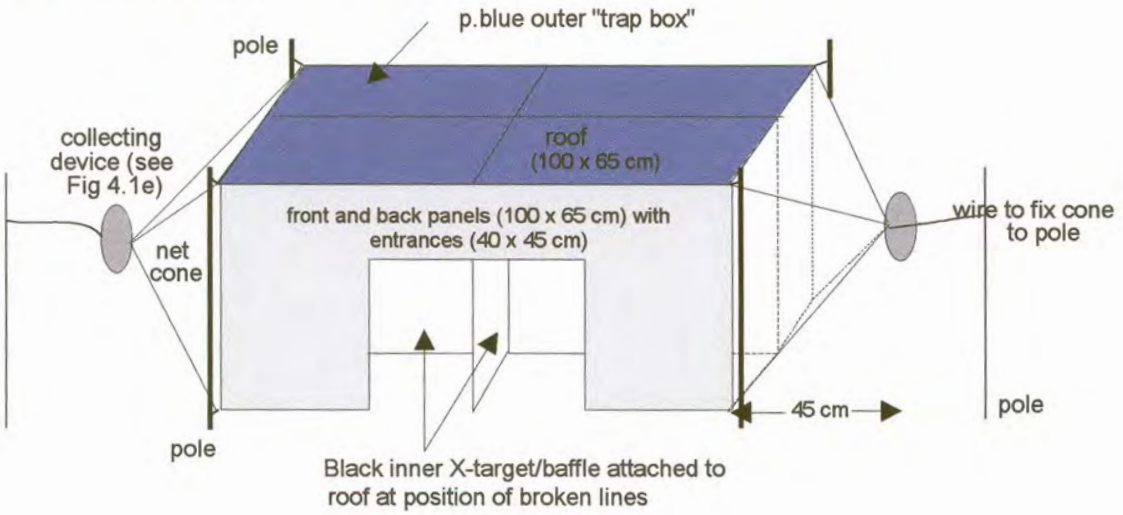
H3: A third modification, the H3, was designed with diagonal or upward-sloping cones to eliminate the problem of flies collecting at the corners of the bases (Fig. 4.2 d).

H4: The H4 modification was as the H3 but with bigger entrances (65 x 45 cm) and therefore a bigger blue body (125 x 65 x 65 cm).

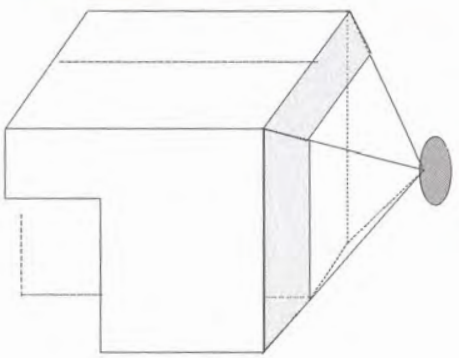
H5: The H5 modification was as the H4 but with bigger cones.

Final "H trap": See Fig. 4.3 and 4.4.

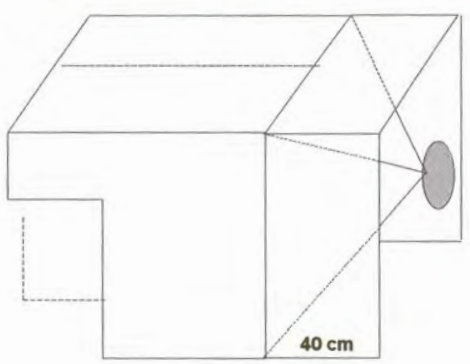
a) H trap (prototype)



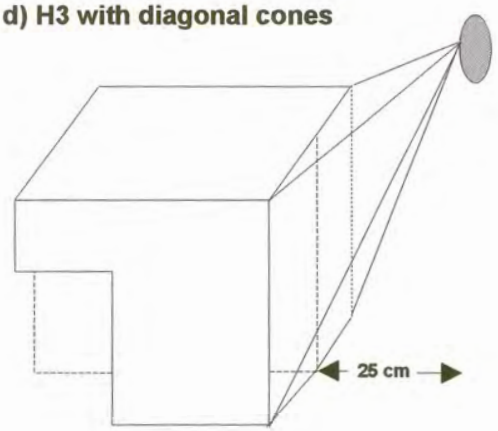
b) H1 with black lining added to inner bases of cones



c) H2 with blue outer box extended over cones



d) H3 with diagonal cones



e) Details of collecting device

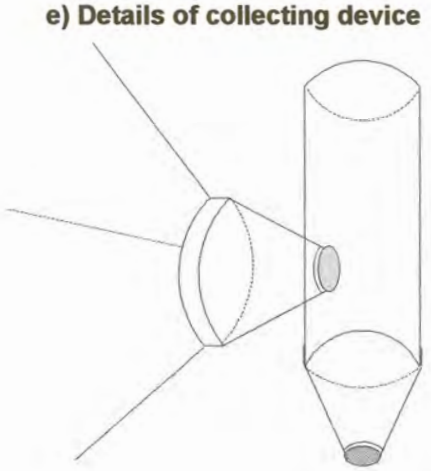


Fig. 4.2 Diagrammatic representations of the prototype H trap (a) with its H1, H2 and H3 modifications (b-d) and details of collecting device (e)

Trap efficiencies

The prototype H trap evolved from studying the behavioural responses of *G. brevipalpis* and *G. austeni* in and around the Siamese (B) and Ng2f traps (Kappmeier, in press). These behavioural studies were also conducted on later modifications of the H trap so as to be able to improve on its design. These trap-orientated responses and trap efficiencies of the H trap modification were evaluated by using electric nets (Vale 1974a) of various sizes and placements similar to those used by Vale (1982a; 1982b). All flies that were intercepted by the nets were electrocuted and retained on a tray painted sticky with polybutene so that they could be sexed and counted. In order to determine the efficiency of traps, an electric net (1 x 1 m), was placed immediately adjacent to the trap. This net intercepted flies that were attracted to the trap, but which flew around it, and which might never have been captured. The number of flies captured by the trap was expressed as a percentage of the total number of flies attracted to the trap, to give an estimate of trap efficiency. To determine the entering responses of flies, the trap's entrances were closed by means of smaller but similar electric nets, which were just large enough to fit into the trap entrances. All flies that attempted to enter the trap were therefore electrocuted and counted. The flies' horizontal flight responses were tested by placing small electric nets inside the traps, at the base of the cone, so that they intercepted all flies that flew horizontally towards the cone part of the trap. [These behavioural studies were also conducted with the Nzi and B3/B4 traps, the results of which are summarized in Kappmeier (in press)].

4.3.3 Odour baits

All treatments under comparison were baited either with the Zim-mix or the best SA blend as described in Chapter 2.2.2. The bait was placed about 20 cm away from the traps at ground level on the downwind side of sticky traps or in front of the downwind entrance of cloth traps.

4.3.4 Experimental design and analyses

All comparisons of traps as well as the efficiency and behavioural response tests with electric nets were tested by means of Latin squares. The comparisons of the traps and modifications were conducted over a 24-hour period, after which they were rotated between sites according to the Latin square design. The comparisons of trap efficiencies and trap-orientated behaviour of the flies, were determined from data collected from 10:00 until dark, the period of maximum activity of both species (Kappmeier 2000).

All data were analyzed, where numbers were adequate, by means of a statistical program for Latin squares, the details given in Chapter 2.3. Male and female catches were analyzed separately for *G. brevipalpis*, but numbers were usually too low for *G. austeni* to justify separate analyses according to the sex. Further details are given below in Experiments and Results.

4.4 Experiments and Results

4.4.1 Sticky traps

Tables 4.1 and 4.2 are summaries of the results of the experiments on the various colours and types of sticky traps tested for *G. brevipalpis* and *G. austeni* baited with the Zim-mix. Where catches were too low for separate analyses of the sexes, the pooled total catches are reported on. The overall trap catch of each treatment is given as an index of increase relative to the control treatment. The detransformed mean catch of the control treatment is given in brackets. Treatments followed by the same symbol are not significantly different from the control in the same experiment. Table summaries will incorporate the number of replicates (n) for each treatment, the transformed standard errors (s.e.) as well as the levels of probability (P) that the means are different at $P < 0,05$ (*), $P < 0,01$ (**), $P < 0,001$ (***), or not significantly different (n.s.).

In Experiment 1 (Table 4.1) the 3DT, XT and RT traps were each tested in l.blue, white and black. Nine replicates were carried out. For *G. brevipalpis* no significant difference was found between any of the treatments. However, very low numbers of this species were collected. For *G. austeni* the l.blue 3DT (control) was the best trap, which was significantly greater than all the black traps and all the RTs.

In Experiment 2 (Table 4.1) the best traps of Exp. 1 (XT and 3DT) were tested again in l.blue, e.blue and white. (Black was not included due to its lesser performance in the previous experiment, while e.blue was the colour most closely resembling phthalogen blue, the attractive blue part of a target for these species (Kappmeier & Nevill 1999b). Six replicates were carried out. For *G. brevipalpis* and *G. austeni* no significant differences were obtained between any treatments, suggesting the 3-dimensional shapes to be equally effective. White seemed very effective for *G. brevipalpis* but not for *G. austeni*.

Since no differences were obtained between the 3DT and XT and between the colours in experiment 2, the XT was chosen for upgrading due to its practicality for use in the field, consisting only of two panels as opposed to the nine panels of the 3DT. In Experiment 3 (Table 4.1) uni- and bicoloured XTs were tested in combinations of white, e.blue and l.blue, to try to improve on its design. Single coloured monopanels of the same size as one panel of the XT, were included for comparison. Ten replicates were carried out. For both species the e.blue/l.blue XT performed best and was then selected as the trap to use in tsetse distribution surveys, which started in December 1993.

In Experiment 4 the XTs were evaluated with different combinations of colour panels as set out in Table 4.1 to incorporate more uni- and bicoloured traps to try and find a better combination for use in surveys. This time black, which was previously left out, was once again included due to strong settling responses obtained for *G. brevipalpis* and *G. austeni* on black when added to a blue target (Kappmeier & Nevill 1999b). The e.blue/l.blue XT, selected for

Table 4.1 Comparisons of various shapes and colours of sticky traps in four experiments [Indices of increase are given relative to the control treatment (index = 1) in each experiment. Detransformed means of the controls are indicated in brackets. The number of replicates (*n*), the transformed standard errors (s.e.) and the probability that the means are different at the *P* < 0,05 (*), *P* < 0,001 (***) levels of probability or not significantly different (n.s.) are given]

	Trap	Colour / combination	*G. brevipalpis* totals			*G. austeni* totals			
			Index	*P*	s.e.	Index	*P*	s.e.	*n*
Exp. 1	3DT	l.blue	1 (1,113)a	n.s.	0,158	1 (12,466)d	*	0,168	9
		white	0,594a			0,684d			
		black	0,683a			0,049ab			
	XT	l.blue	0,422a			0,818d			
		white	0,734a			0,447cd			
		black	0,865a			0,112ab			
	RT	l.blue	0,324a			0,142abc			
		white	0,456a			0,144bc			
		black	0,117a			0,013a			
Exp. 2	3DT	l.blue	1 (0,414)a	n.s.	0,142	1 (11,462)a	n.s.	0,150	6
		e.blue	1,000a			1,124a			
		white	1,565a			0,698a			
	XT	l.blue	0,841a			1,141a			
		e.blue	0,295a			0,858a			
		white	1,973a			0,658a			
Exp. 3	XT	l.blue	1 (0,625)abc	***	0,141	1 (4,760)bc	***	0,202	10
		e.blue	2,274bc			0,739abc			
		white	0,512ab			0,187ab			
		e.blue/l.blue	2,918c			1,847c			
		l.blue/white	1,336abc			0,951bc			
		e.blue/white	2,770c			1,217bc			
	Mono	l.blue	0,115a			0,499abc			
		e.blue	0,314a			0,557abc			
		white	0,314a			0,046a			
Exp. 4	XT	l.blue	1,090c	***	0,170	0,790bcd	***	0,166	40
		e.blue	1,165c			1,008cde			
		white	0,608a			0,570ab			
		black	0,667ab			0,383a			
		e.blue/l.blue	1 (4,640)bc			1 (6,629)cde			
		e.blue/white	1,114c			1,160def			
		e.blue/black	1,080c			1,533f			
		l.blue/white	0,687ab			0,729bc			
		l.blue/black	1,076c			1,308ef			
		white/black	0,937bc			1,203ef			

abcdef Treatments followed by the same symbol are not significantly different

surveys, acted as the control trap. Forty replicates were conducted. For *G. brevipalpis* the e.blue, l.blue, e.blue/white, e.blue/black and l.blue/black XTs all caught better than the control, but this was not significant. For *G. austeni* the e.blue/black XT was significantly better than the control (e.blue/l.blue) XT and increased the catches by *c.* 1,5 times. The e.blue/black XT therefore replaced the e.blue/l.blue XT in surveys conducted from May 1995 onwards.

The next experiment (Table 4.2) attempted to improve on the recommended XT of the previous experiment (i.e. e.blue/black XT) by finding an optimal size, or to simplify it by using a bicoloured monopanel (single panel of the XT), making it more practical to use in the field. Three sizes of the XT and monopanels were tested, namely one panel measuring 70 x 60 cm (as original XT panel size); 95 x 80 cm and 120 x 100 cm. Monopanels consisted of a single XT panel, measuring the same sizes as given for the XT above. Two types of bicoloured monopanels were tested. In the first, referred to as Mono I, each side of the panel was painted both e.blue and black (split vertically in the centre). In the second, referred to as Mono II, one side of the panel was e.blue and the other side black.

For *G. brevipalpis* the larger monopanels (i.e. 95 x 80 cm and 120 x 100 cm) were all equally effective as the control XT (70 x 60 cm). It was also shown for both sexes that the larger the trap the better its performance. This was especially the case for females, where previously the small-sized XT was ineffective, the bigger size would capture 2,4 – 4,2 x more. For *G. austeni* males and females the bigger sized Mono I panels (95 x 80 cm and 120 x 100 cm) were mostly significantly better than the control XT (especially for females). Similarly as for *G. brevipalpis*, an increased size of the control XT meant better performance for both sexes (especially for females).

The results for both species, therefore, suggested that a single Mono I (or Mono II for *G. brevipalpis*) panel of a larger size, which would be more practical to handle in the field and cheaper to make, could replace the XT used in distribution surveys.

Table 4.2 Comparisons of e.blue/black 3-dimensional XTs with 2-dimensional Monopanels of various sizes [Indices of increase are given relative to the control treatment (index = 1) in each experiment. Detransformed means of the controls are indicated in brackets. The number of replicates (n), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,01$ (**) and $P < 0,001$ (***) levels of probability are given]

Species	Trap type	Size	Males			Females			
			Index	*P*	s.e.	Index	*P*	s.e.	*n*
G. brevipalpis	XT	70 x 60	1 (3,527)b	***	0,173	1 (0,655)abc	**	0,157	27
		95 x 80	1,435b			2,443cd			
		120 x 100	2,886c			4,246d			
	Mono I	70 x 60	0,347a			0,663ab			
		95 x 80	1,018b			1,641abc			
		120 x 100	1,178b			1,904bc			
	Mono II	70 x 60	0,430a			0,522a			
		95 x 80	1,232b			1,148abc			
		120 x 100	1,121b			1,321abc			
G. austeni	XT	70 x 60	1 (1,873)ab	***	0,105	1 (1,989)ab	***	0,165	27
		95 x 80	3,649d			5,884d			
		120 x 100	4,266d			10,595e			
	Mono I	70 x 60	0,820ab			1,338b			
		95 x 80	1,556bc			2,989c			
		120 x 100	1,954c			4,107cd			
	Mono II	70 x 60	0,442a			0,528a			
		95 x 80	1,009ab			2,708c			
		120 x 100	0,656a			1,165ab			

abcde Treatments followed by the same symbol are not significantly different

Estimates of trap efficiency

The efficiencies of the e.blue/l.blue and e.blue/black XTs were evaluated. Catches with and without a flanking net next to the trap for ten replicates were pooled. Trap efficiency is expressed as the proportion of flies of the pooled catch that were actually caught on the XTs (without flanking net) expressed as the percentage of flies that were caught by the traps with flanking nets (i.e. trap plus net). The overall trap efficiency of the e.blue/l.blue XT was 23 % for *G. brevipalpis* (33 % for males and 0 % for females) and 28 % for *G. austeni* (39 % for males and 24 % for females). The overall efficiency of the e.blue/black XT was 16 % for *G. brevipalpis* (22 % males, 2 % females) and 51 % for *G. austeni* (males 48 % and females 54 %). The low efficiencies of

the XTs for *G. brevipalpis* females explains the low catches obtained during preceding experiments.

4.4.2 Cloth traps

Results of the full series of Latin squares and comparisons with other designs and trap modifications are not given here. Apart from the H trap and its modifications, only a few traps and modifications, as described above, were worthwhile which included the Nzi, Nzi3 and B1-5 traps (Kappmeier, in press). Results, given below, are a summary of the work comparing only the H trap modifications and trap orientated behaviour around these modifications, which lead to the final design. Results of the final experiment comparing the H4 and H5 modifications with the Nzi, B4 and B5 traps are given.

Evaluation of initial H trap designs (H1 – H3)

It was observed with the prototype H trap that the flies tended to collect at the upper base corners of the cones (where they connect with the trap body). The prototype was then modified so that the H1, H2 and H3 modifications were developed as described earlier. The results of the H1-H3 modifications were originally compared with those of the Siamese trap, which acted as the control. All the results of the former were significantly ($p < 0,01$) better than those of the Siamese (i.e. 3,2 - 4,2 x for the total number of *G. brevipalpis* caught and at least 6,7 x for *G. austeni*). The H3 modification also consistently gave the best results when compared further with other promising traps, namely the XT, Nzi, Nzi3 and B3 traps (Kappmeier, in press) where it was found that the H3 caught twice as many *G. brevipalpis* as both the B3 and XT, and about three times more than the Nzi. The H3 caught significantly three times more *G. austeni* than the XT, while the remaining traps were ineffective for this species. The H3 caught mean daily catches of 12,0 *G. brevipalpis* (63 % females; 25 replicates), when baited with the Zimbabwe ox-odour blend, and was even more successful when baited with the best SA blend with mean daily numbers of 45,1 *G. brevipalpis* (64 % females; 12 replicates). The mean daily catches for *G. austeni* were 3,0 (82 % females, 25 replicates) when baited with

the Zimbabwe blend and 9,7 (64 % females; 12 replicates) when baited with the best SA blend. For *G. brevipalpis* the record catch in one day by an H3 trap was 76 flies and for *G. austeni* 37 flies.

Trap-orientated responses of tsetse in and around the H3 modification

In order to improve on the H3 design, the behavioural or trap-orientated responses of *G. brevipalpis* and *G. austeni* (Table 4.3 a & b) were determined by means of electric nets placed in and around the H3 trap, following the methods of Vale (1982a, 1982b). [Simultaneously this was done with the B3, B4 and Nzi traps, the results of which are given in Kappmeier (in press).] Only 16,8 % of the *G. brevipalpis* (total catches) that were initially attracted to the H3 trap actually attempted to enter them (Table 4.3 a). The lateral upward-sloping/diagonal cones were quite effective in inducing horizontally-directed flight responses, especially for *G. brevipalpis* for which it was found that all flies that found the entrances of the trap, thereafter flew in a horizontal direction and were captured. For *G. austeni* (Table 4.3 b) only 28,3 % of the flies that found the entrances flew towards the cones. Only four replicates (*n*) of this experiment were carried out. The statistical *P* and s.e. values are given in the Tables.

Table 4.3 Behavioural responses of a) *G. brevipalpis* and b) *G. austeni* in and around the H3, H4 and H5 trap modifications as determined with electric nets [The results are expressed as a percentage relative to the mean daily number of the flies attracted to the traps (indicated as 100 %). The detransformed mean number of flies that were attracted are given in brackets for the control treatment. The number of replicates (n), the transformed standard errors (s.e.) and the probability (P) that the means are different or not significantly different (n.s.) are given.]

a) G. brevipalpis

Trap type and treatment	Males		Females		Totals	
H3						$P < 0{,}001$
Flies attracted	100 (26,308)	$P < 0{,}001$	100 (27,112)	$P < 0{,}01$	100 (54,422)	s.e.= 0,066
Entrance response	18,4	s.e.= 0,055	15,8	s.e.= 0,098	16,8	$n = 4$
Sideways flight response	22,2	$n = 4$	16,2	$n = 4$	19,2 (100 % of tsetse that entered)	
Eventually caught (efficiency)	34,9		42,4		38,2	
H4						
Flies attracted	100 (15,827)	$P < 0{,}001$	100 (12,856)	$P > 0{,}05$ n.s.	100 (29,360)	$P < 0{,}01$
Entrance response	60,3	s.e.= 0,529	61,3	s.e.= 0,071	62,6	s.e.= 0,057
Sideways flight response	44,7	$n = 14$	56,5	$n = 14$	50,0 (79,9 % of tsetse that entered)	$n = 14$
Eventually caught (efficiency)	37,7		59,7		47,9	
H5						
Flies attracted	100 (30,299)	$P < 0{,}001$	100 (21,264)	$P < 0{,}001$	100 (51,870)	$P < 0{,}001$
Entrance response	49,5	s.e.= 0,070	54,4	s.e.= 0,069	51,6	s.e.= 0,068
Sideways flight response	19,7	$n = 12$	24,0	$n = 12$	21,9 (42,4 % of tsetse that entered)	$n = 12$
Eventually caught (efficiency)	30,5		32,6		31,9	

Table 4.3 (Cont.)

b) G. austeni

Trap type and treatment	Males		Females		Totals	
H3						
Flies attracted	100 (8,836)	$P = 0,05$	100 (16,855)	$P < 0,01$	100 (26,964)	$P < 0,05$
Entrance response	36,5	s.e.= 0,140	20,3	s.e.= 0,1031	27,2	s.e.= 0,131
Sideways flight response	8,8	$n = 4$	5,9	$n = 4$	7,7 (28,3 % of tsetse that entered)	$n = 4$
Eventually caught (efficiency)	29,4		45,9		38,4	
H4						
Flies attracted	100 (6,920)	$P > 0,05$ n.s.	100 (14,983)	$P < 0,05$	100 (23,624)	$P > 0,05$ n.s.
Entrance response	57,8	s.e.= 0,148	43,6	s.e.= 0,110	44,4	s.e.= 0,122
Sideways flight response	58,8	$n = 8$	45,3	$n = 8$	44,3 (99,8 % of tsetse that entered)	$n = 8$
Eventually caught (efficiency)	31,6		26,9		29,0	
H5						
Flies attracted	100 (4,002)	$P > 0,05$ n.s.	100 (12,173)	$P < 0,01$	100 (16,819)	$P < 0,01$
Entrance response	60,1	s.e.= 0,105	66,6	s.e.= 0,077	69,4	s.e.= 0,0653
Sideways flight response	63,2	$n = 8$	33,8	$n = 8$	42,5 (61,2 % of tsetse that entered)	$n = 8$
Eventually caught (efficiency)	43,1		36,0		37,6	

Evaluation of H4 and H5 modifications

The H4 trap was a modification of the H3, and took into account its shortcomings as determined with electric nets. It, therefore, had bigger entrances (65 x 45 cm) and thus a slightly bigger body (125 x 65 x 65 cm) than the H3 to improve on the entrance responses of the flies. The H4 trap was further modified by providing it with somewhat larger cones to become the H5. The lower (bottom) side of each cone was at less of an acute angle (lower slope) to the body of the trap than the previous two modifications. This change was aimed at preventing flies from flying against the lower side and then bouncing off (especially in the case of the bigger *G. brevipalpis*), so that it was easier to progress to the trap collecting device.

The results for *G. brevipalpis* males, females and total catches and for *G. austeni* total catches as obtained with the H4 and H5 traps are compared in Table 4.4 with the B4 and B5 modifications (from Kappmeier, in press) and the Nzi. The results are given as indices of increase relative to the Nzi (with index = 1). The detransformed means of the catches obtained by the Nzi are given in brackets. Treatments' indices (for total catches) followed by the same symbols (a,b or c) are not significantly different.

The results showed the Nzi trap to be relatively effective for *G. brevipalpis* and although the H4 and H5 were better than the Nzi, this was not significant. The Nzi was poor for capturing *G. austeni* and the H4 and H5 increased catches significantly by *c.* 3,0 – 4,1 times respectively compared to the catches obtained with the Nzi. The larger cones of the H5 (compared to the H4) had no effect on the number of flies of either species captured. The mean daily catch for *G. brevipalpis* was 15,7 (69,5 % females) with the H4 trap and 16,9 (70,8 % females) with the H5 trap (28 replicates). For *G. austeni* the mean daily catch was 5,7 (99,0 % females) with the H4 trap and slightly better at 7,6 with the H5 trap (14 replicates).

Table 4.4 Final comparisons of the H4 and H5 modifications with the B4, B5 and Nzi traps [The results are expressed as the indices of increase relative to the Nzi trap (index = 1). The detransformed means of the Nzi are given in brackets. The number of replicates (*n*), the transformed standard errors (s.e.) and the probability (*P*) that the means are different are given]

Trap	*G. brevipalpis*						*G. austeni*	
type	Males		Females		Totals		Totals	
Nzi	1,000ab	(4,228)	1,000bc	(9,610)	1,000bc	(13,673)	1,000ab	(1,379)
B4	0,668a	$P < 0,05$	0,554a	$P < 0,05$	0,569a	$P < 0,05$	0,685a	$P < 0,001$
B5	0,919ab	s.e.=0,154	0,768ab	s.e.=0,146	0,804ab	s.e.=0,137	2,622bc	s.e.=0,177
H4	1,235b	$n = 28$	1,132bc	$n = 28$	1,145bc	$n = 28$	4,102c	$n = 14$
H5	1,170b		1,245c		1,236c		5,487c	

abc Treatments' indices followed by the same symbol are not significantly different from each other

Trap-orientated responses of tsetse in and around the H4 and H5 modifications

The behavioural or trap-orientated responses of tsetse flies in and around the H4 and H5 traps were tested in a final attempt to confirm whether the modifications of the H3 that were made were worthwhile, and also to make a final decision as to which of the modifications should be employed for future use. The results are given in Table 4.3 a and b for *G. brevipalpis* and *G. austeni* respectively. The number of replicates performed is indicated in the Tables. The various responses and trap efficiencies are given as a percentage relative to the mean daily number of flies that were attracted to the traps (detransformed means of the control trap are given in brackets). The statistical *P* and s.e. values and the numbers of replicates (*n*) are given in the Table.

For *G. brevipalpis* it was clear that the bigger entrances of the H4 and H5 modifications were an advantage in that more flies (51,6-62,6 %) attempted to enter these traps than the number entering the H3 (16,8 %). On the other hand, all flies that entered the H3 trap flew in a horizontal direction to the cones,

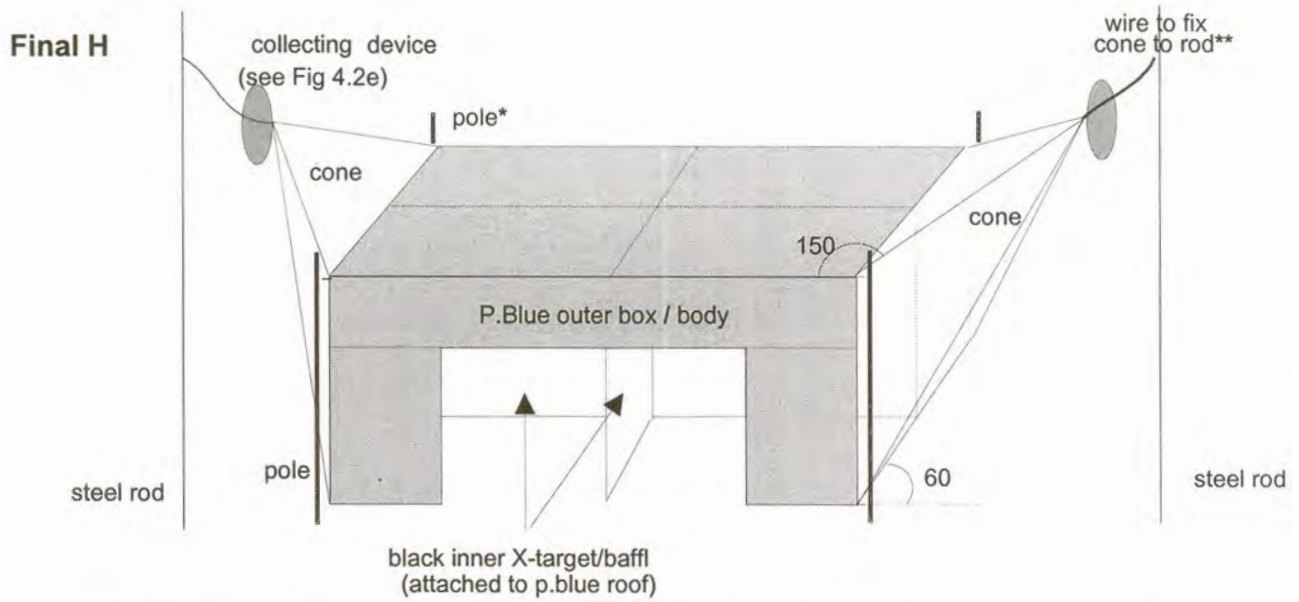
while only 42,4 - 79,9 % of the flies entering the H5 and H4 traps respectively, flew horizontally. It may, therefore, be suggested that because of the bigger entrances, more flies could fly directly out of the trap again, i.e. fewer of them advanced towards the cones. Nevertheless, the overall efficiency of the H4 trap was still better than the H3 (47,9 % versus 38,2 %). The efficiency of the H5 (with larger cones) was lower (31,9 %) than the previous modifications which might indicate that the flies get disorientated towards the apex of the cones and fewer of them enter the collecting device.

For *G. austeni* the efficiencies of the H4 and H5 traps were determined respectively at 29,0 % and 37,6 %. For this species the bigger entrances of the H4 and H5 traps also prompted more flies to enter the traps (44,4 - 69,4 %) compared to the number of those entering the H3 (27,2 %). Between 61 % and nearly 100 % of the flies that entered the H4 and H5 traps also flew horizontally towards the cones, indicating that, unlike *G. brevipalpis*, they do not often immediately fly out, but, as was observed, tend to "linger" once at the entrance to or inside a trap.

The final design

In accordance with the trap-orientated responses, the final H trap design incorporated entrances of the same size as those of the H4 and H5 traps but the cone sizes were in-between those of the H4 and H5 traps. Further comparisons between the H4 and H5 and the final design were not conducted. This final H trap design (Fig. 4.3) caught a record catch of 180 *G. brevipalpis* and 57 *G. austeni* in one day. A schematic representation of the final design is given in Fig. 4.3 with material measurements and construction procedures. The same method of erection, i.e. with the use of poles, is employed as was described previously and as indicated in the Figures.

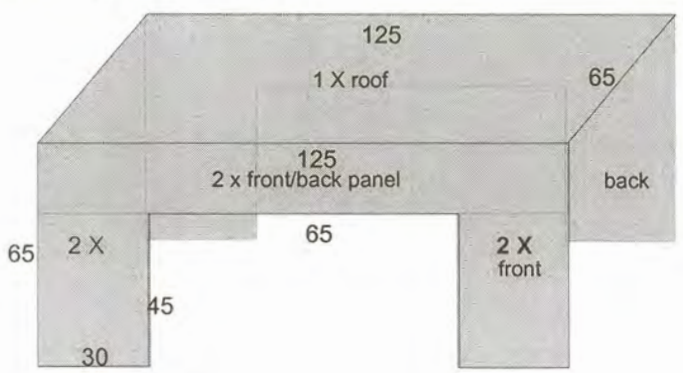
Fig. 4.3 Photograph of the final H trap design for the capture of *G. brevipalpis* and *G. austeni* (the trap is held upright by fastening the corners to four rigid metal poles (1,2 m long) and the cones are suspended from two flexible steel rods (1,4 m long)



* To erect the trap each corner of the trap is attached with string to a pole driven into the ground
**To keep the cones rigid they are attached with wire at the apex to tops of flexible steel rods

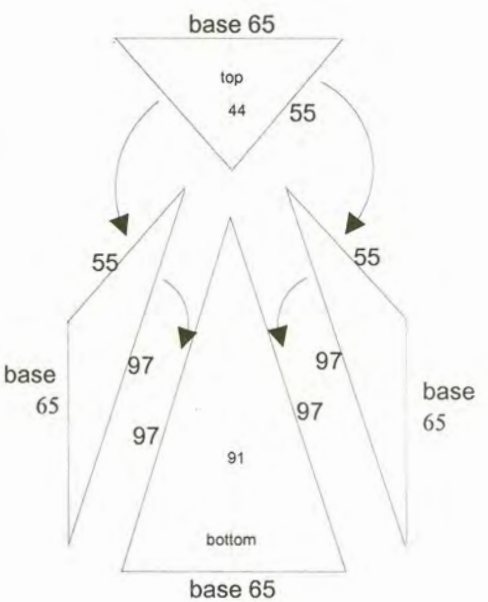
Material requirements (measurements in cm) and steps necessary for making:

P.blue outer box / body:



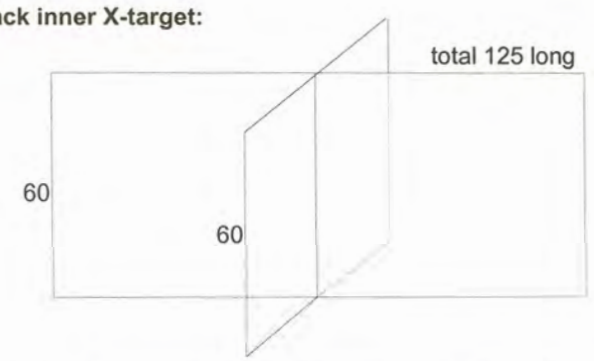
1. Sew the three blue pieces togethe (along the 125 cm sides) with roof panel between front and back panels

Net cone (x 2):



3. Sew pieces of netting togethe (as indicated) and then attac completed cone's top and side base to sides of blue outer box at the cones' positions

Black inner X-target:



2. Sew black target/baffle pieces together (as indicated) and attach target to centre of blue roof (indicated with broken lines in drawing of final H trap)

4. Cut apex of cone open to size of circumference of collecting system's base bottle (see Fig 4.2e) and attach reinforcing seam to prevent netting from tearing

Fig. 4.4 Diagrammatic representation of the final H trap with details of materials and measurements for trap construction

4.5 DISCUSSION

4.5.1 Sticky traps

Effect of colour and shape

For *G. brevipalpis* l.blue, white and black traps showed no significant difference in the catches during initial studies of the 3DT, XT and RT sticky traps. For *G. austeni*, l.blue and white were significantly better than black in initial studies with these traps. Later studies with the XT showed l.blue and e.blue to be superior over black and white traps for both species. Sticky traps tested concurrently by Vreysen *et. al.* (1996) for the monitoring of *G. austeni* in Zanzibar showed, however, that sky blue, baby blue and white monopanels (MP) showed no significant difference in the catch size.

The 3-dimensional shapes of the 3DT and XT were more or less equally effective for *G. austeni* and *G. brevipalpis* and significantly more attractive than the RT for *G. austeni*. The main advantage from the 3-dimensional traps over the RT seems to be that the trap is visible from all directions all of the time and this could account, together with the bigger surface area, for the higher catches. The XT was chosen for further upgrading, being more practical to make and use in the field. Initial experiments showed that bicoloured XTs of white were less effective than single coloured e.blue and l.blue traps for both species. For *G. brevipalpis* single e.blue trap was very effective, together with an e.blue/white and e.blue/l.blue, the latter which was most effective. These bicoloured traps were also most effective for *G. austeni* of which the e.blue/l.blue XT was also best for this species. Early studies of Vreysen *et al.* (1996) showed that the only occasion where a combination of colours significantly affected the catch rate of *G. austeni* in Zanzibar, was with legpanels (LP) coloured white on one panel side and sky blue on the other side. In this study, however, l.blue/white XTs were the most ineffective bicoloured traps for *G. austeni* and also for *G. brevipalpis*.

From December 1993 the e.blue/l.blue, due to its best performance for both species, used to survey the distribution of the two species in Zululand (Nevill *et al.* 1995). More tests with single and bicoloured XTs showed e.blue XTs to be as effective as the e.blue/black XT for both species while a black (and white) XTs was very ineffective for *G. austeni*. This corresponds with previous studies on cloth targets where phthalogen blue (e.blue is the closest resemblance of phthalogen blue) was also attractive for both species but black was very unattractive for *G. austeni* (Kappmeier & Nevill 1999b). Low trap catches of *G. austeni* were also obtained with single coloured black XTs on Unguja Island (Vreysen *et al.* 1998).

For *G. austeni* only the bicoloured e.blue/black XT was significantly better than the e.blue/l.blue XT and increased catches by 1,5 times, while e.blue/white, white/black and l.blue black bicoloured XTs were also relatively effective. For *G. brevipalpis* the e.blue/black XT was also, together with e.blue/white and l.blue/ black XTs, found to be more or less equally effective to the l.blue/e.blue XT for *G. brevipalpis*. For this reason the e.blue/l.blue XTs used in surveys were replaced by the e.blue/black XTs from May 1995 (Nevill 1997; Nevill *et al.* 1999). Phthalogen blue/black cloth target combinations were also most effective for the two species (Kappmeier & Nevill 1999b). Targets designed in blue/black combinations are also highly attractive for *G. morsitans* and *G. palpalis* species (Green 1993; Vale 1993; Merot & Filledier 1985; Laveissiere *et al.* 1987 cited in Vreysen *et al.* 1998). For *G. austeni* in Zanzibar poor catch results were obtained when black was combined with royal blue both as XT and XLP (cross-shaped legpanel) while royal blue/white XTs were more effective (Vreysen *et al.* 1998). Vreysen *et al.* (1998) ascribed the possible difference in the two *G. austeni* populations' behaviour to the genetic variations between the two populations or in differences of the spectral reflectance of the paint material used.

Trap efficiencies

The efficiencies of the e.blue/l.blue and e.blue/black were relatively high 33 - 39 % for *G. brevipalpis* males and very low for females (0 - 2 %). The low

efficiency for females could probably be ascribed to the relatively small size of the trap, in that catches of females increased with the bigger sized traps, as will be explained below. For *G. austeni* the traps were similarly effective for both sexes, i.e. 28 - 51 % for males and 24 - 54 % for females.

Effect of size of XT and monopanels

For *G. brevipalpis* the catches of both sexes increased as the size of the XTs and Monopanels increased although this was not always significant. Where previously the standard sized (70 x 60 cm) XT was ineffective for females, the bigger sizes increased catches of females 2,4 - 4,2 x. The larger monopanels (95 x 80 cm and 120 x 100 cm), i.e. Mono I (e.blue/black painted on both sides) and Mono II (e.blue painted one side and black on the other side) were equally effective as the standard XT. For *G. austeni* males and females an increase in the catches was related to an increase in the size of the trap. This was especially so with the XT where the bigger sized XTs increased the catches significantly by 3,6 - 4,3 x for males and 5,9 - 10,6 x for females. The bigger sizes of Mono I also improved the catches compared to the original sized XT by 1,6 - 1,9 x for males and 3,0 - 4,1 x for females, which was significant for females. Increased catches of these species were also obtained with an increase in the size of cloth targets (Kappmeier & Nevill 1999b). Vreysen *et al.* (1998) found an increase in the width of a blue XT (from 70 - 120 cm) doubled the catch of *G. austeni* in Zanzibar as compared to a standard sized blue XT.

Because larger sizes of e.blue/black monopanels of (Mono I) proved to be equally or even more effective than the XT for both species, it could therefore in future surveys be used instead of the XT. These would be cheaper in terms of construction material, paint, sticky material and kerosene for removal of sticky material. They are also lighter and easier to manipulate in the field, especially in densely forested areas.

4.5.2 Cloth traps

Following the improvements of the XT sticky trap, the H trap was developed and described for the monitoring and live collection of *G. brevipalpis* and *G. austeni*. It was designed after evaluating the behaviour of *G. brevipalpis* and *G. austeni* in and around Ngu (Ng2f) and Siamese (B) traps (Brightwell *et al.* 1987; Kyorku *et al.* 1993) in which it was shown that the two species were reluctant to fly upwards towards the cones (Kappmeier, in press). The H trap was, therefore, designed to do away with a top cone system, so that a totally different approach was employed, namely that of a trap fitted with two lateral devices (cones). This approach made use of the flies' preference to fly in a horizontal instead of a vertical flight path which is required in existing tsetse fly traps. The angled cones of the final trap incorporated an element of the ramp trap principle used extensively by mosquito ecologists (Service 1976).

This new H trap design proved to be effective, when baited with synthetic ox-odour, in catching *G. brevipalpis*, since it is known that this species is attracted by colour and odour (Kappmeier & Nevill 1999a, 1999b). It was, however, not as efficient in capturing *G. austeni*, probably because *G. austeni* is not attracted by the odours (Kappmeier & Nevill 1999a) although it responds strongly to colour (Kappmeier & Nevill 1999b). It may be that the odour does influence short-range trap entering behaviour (Vale & Hall 1985a) of *G. austeni*. The final version of the "horizontal" or H trap was developed after testing five modifications of the original prototype.

Some of the H trap modifications increased the sizes of the catches when compared to those of the XT sticky trap by up to 1,4 times (not significant) for *G. brevipalpis* and by up to 2,4 times (significantly) for *G. austeni* (Kappmeier, in press). The advantage of the H trap over the XT sticky trap, used in tsetse distribution surveys (Nevill 1997), is that flies are captured alive and can thus be used for studies on population dynamics. It can also be used for the automatic treatment of wild-caught flies with a variety of agents ranging from entomopathogenic fungi (Kaaya *et al.* 1991) to insect growth regulators (Hargrove & Langley 1990; Langley 1995, 1999).

Highest catches with the final H trap were 57 *G. austeni* and 180 *G. brevipalpis* in one day. Compared to the previous best live trap catches at Hellsgate with the Ng2f and Siamese traps, this new trap is a definite improvement. Although the Nzi also performs relatively well for capturing *G. brevipalpis*, the H trap is still better and it is significantly better for *G. austeni*. There is no doubt still room for improving the H trap, especially as far as *G. austeni* is concerned. The horizontally situated cones are, however, a major step forward for capturing both *G. brevipalpis* and *G. austeni* alive and facilitates studies, which require the use of live wild-caught *G. austeni* and *G. brevipalpis*. The H trap is certainly an advance for the trapping of these two previously "difficult" species of flies.

5. POPULATION DISPERSAL AND MOVEMENT

5.1 ABSTRACT

Mark-release-recapture studies were undertaken at Hellsgate Tsetse Research Station to determine the population density and dispersal rates of *Glossina brevipalpis* and *G. austeni* in order to estimate target densities suitable to control these species. The recommended target density, based on the assumption of killing 4 % of the female populations per day, was estimated first at nine and ten targets per square kilometer for *G. brevipalpis* and *G. austeni* females, respectively, but was then reconsidered and adjusted to four and seven targets/km^2 for the two species respectively. *Glossina brevipalpis* was by far the more mobile species while *G. austeni* appeared to be more static. The movement of flies over open areas of vlei and grassland was also investigated to determine their value as natural barriers in a strategy to protect controlled areas from reinvasion. From the results it is evident that both species do, to a certain extent, traverse open areas of "unsuitable" habitat.

5.2 INTRODUCTION

In 1990 a serious outbreak of nagana in N.E. KwaZulu-Natal Province, South Africa (Kappmeier *et al.* 1998), precipitated a need to develop a long-term control strategy for the two vector species *Glossina austeni* and *G. brevipalpis*. Studies on colour targets (Kappmeier & Nevill 1999b) and odours (Kappmeier & Nevill 1999a) have resulted in the development of an attractive odour-baited target (Kappmeier & Nevill 1999c) which, if treated with a suitable pyrethroid, could be used for the control of the two species in South Africa. No studies have, however, been conducted on the population dynamics of these species.

Rogers & Randolph (1985) pointed out the importance in understanding the population dynamics of tsetse flies in planning all types of tsetse control operations and also to assess tsetse control interventions. A precise knowledge of this subject is particularly important when the operation employs insecticide-treated targets. An optimal strategy in this type of operation would

involve the deployment of the smallest number of targets sufficient to eradicate the tsetse population in any desired time interval or rate of population reduction (Hargrove 1988).

A number of studies have been conducted on the movement or dispersal of tsetse flies as the distances they can travel will affect the success of tsetse control schemes (Leak 1999). For the successful target implementation to control or eradicate *G. brevipalpis* and *G. austeni*, base-line data on their movement and dispersal was, however, needed so that the targets could in future be sited successfully in optimal locations and densities in the field. Such studies would be critical for the planning of any future control operations in South Africa.

5.2.1 Estimates of dispersal rates and population size

The control of tsetse fly populations using traps or targets depends on the movement patterns of the flies, which determines how many flies find the targets, and on the efficiency of the targets, which determines the proportion of flies that are killed (Williams *et al.* 1992). It has been shown that traps or targets, used mainly for control rather than eradication, can reduce tsetse fly densities to acceptably low levels (Vale *et al.* 1988a; Dransfield *et al.* 1990). However, for such ongoing control strategies to be viable they must be cost-effective to livestock-producers in Africa and it is essential to make the most efficient use of targets. In order to reduce tsetse fly populations, targets must therefore kill the flies more rapidly than the flies can reproduce or invade the control area (Williams *et al.* 1992).

For the savannah species *G. m. morsitans*, *G. m. centralis* and *G. pallidipes* it appears that, with the attractive synthetic odours presently available (Vale 1993) a density of about four targets/km^2 is necessary and sufficient to eradicate a population in nine months to a year (Hargrove 1993). Because of their low natural birth rate, a population can be eradicated by superimposing and sustaining, on the natural death rate, a mortality of 4 % per day on any female tsetse population, for example through the use of targets or traps

(Hargrove 1981; Hargrove 1988). It seems likely that in most field conditions only an added 2–3 % is required (Hargrove 1988), which agrees with Williams *et al.* (1992) who stated that a population can be driven to extinction by imposing only an additional mortality rate greater than 2 % per day. This can easily be achieved with targets (Vale *et al.* 1988a).

Population studies often involve mark-release-recapture programmes, a technique that is potentially more promising by improved sampling devices. It raises the possibility that a high proportion of marked flies could be released into a small area to be recaptured (Hargrove & Vale 1979; Vale *et al.* 1984). Mark-release-recapture methods (Jolly 1965; Seber 1965) can be used to measure mortality for closed populations, but under more natural open conditions it is difficult to separate the effects of mortality and emigration, and the methods are generally complex and time consuming (Hargrove 1990, cited in Hargrove 1993). The recaptures obtained during mark-release-recapture operations, whilst providing an estimate of population size, also give some idea on the nature and extent of fly movement and dispersal (Rogers 1977; Hargrove & Lange 1989).

Many models concerning fly dispersal have been developed over the years by various authors. It was suggested that dispersal in tsetse flies could be viewed as a series of discrete daily steps each taken in a random direction (Bursell 1970). Although movement within a habitat appears to be random, Rogers (1977) assumed it to consist of fairly constant step lengths and that natural factors, e.g. humidity, availability of shade, host density and odour plumes, tend to limit movement to within the habitat and may reduce its randomness. He gave two methods for investigating the outcome of two-dimensional random movement appropriate to tsetse. The first model is a prediction of the mean distance d away from the starting point, assuming a constant step length s (the distance moved per unit time), and a variable number of steps x so that:

$$d \cong sx^{1/2}$$

Applying this to tsetse movement and defining a single step as the distance traversed in one day, Rogers (1977) proposed it is only necessary to know

accurately the mean population displacement over a period of time to calculate a value of s, the mean daily displacement. The second model is based on computer predictions of a series of random movements away from a release point. This involved the probability distributions for directions moved and distances covered per step. Hargrove (cited in Bursell & Taylor 1980) derived a more accurate definition of daily displacement d, based on modelled predictions of a series of random movements away from a release point, as:

$$d \cong 0{,}9sx^{½}$$

The predictions involved the probability distributions for the directions moved, and distances covered per step, but could be simplified by assuming that tsetse only fly for a few minutes per day and have a relatively constant step length. Hargrove (1981) further suggested the step length might vary and probably change with age and physiological stage of the fly. Hargrove & Lange (1989) suggested the 'rate' of dispersal to be simply defined as a diffusion coefficient rather than as a discrete step length. They therefore viewed tsetse dispersal as a diffusion process, with the position of a fly, relative to its origin, as a normally distributed random variable, i.e. the mean distance of a diffusing particle from the origin. Other models have estimated rates of advance of tsetse based on a root-mean-square displacement of 200 metres per day and a population growth rate of 1 % per day (Williams *et al.* 1992). Williams *et al.* (1992) implied that the dispersal of insects could be described by a Gaussian diffusion model with an exponential mortality term. The rate of diffusion (dispersal) was then defined by the root-mean-square displacement in one day (λ). If this is high, tsetse will disperse quickly into the vicinity of traps and there will be a rapid reduction of the population.

Bailey (1951) used simple recapture techniques to determine the maximum likelihood estimate of the population size P based on the number of flies marked and released M, the sample size recaptured N and the number of marked flies R in the sample N so that:

$$P = MN/R$$

However, he suggested that in certain ecological problems it may be more appropriate to use the reciprocal of the population size as the appropriate index, rather than the population size itself, so that:

$$1/P = R/MN$$

To improve the precision of mark-release-recapture technique it would be necessary to increase the expected number of recaptures, by increasing the number of marked flies released or increasing the expected recapture percentage (Vale *et al.* 1984).

In order to control tsetse flies successfully with targets and to ascertain the density of targets needed for the control of the two tsetse species in Zululand, it was necessary to initiate trials to evaluate the movement and dispersal rate of the two tsetse species. A good trap was, therefore, necessary to capture live *G. brevipalpis* and *G. austeni* in large enough numbers. The H trap described in the previous chapter was specifically developed for this purpose. Questions that needed to be answered were: At what rate does each of the species disperse? What is the population density at the research site? At what density should targets be placed in order to kill *c.* 3 - 4 % of the population per day?

The present study involved mark-release-recapture experiments to estimate the population size by determining the probability of recapture and then using the inverse of the population size as a population estimate as suggested by (Bailey 1951). Because the results were based on the degree of trapping efficiency of the H trap and the required levels of population control were based on this, it was essential to relate the results to the required effect to obtain control with targets. The relative performance of the H trap used in this study was, therefore, compared to the recommended control target. The target density required for certain levels of tsetse control based on the results obtained with the H trap could then be assessed.

5.2.2 Estimates of composition of natural barriers

As tsetse flies are relatively mobile, there is a constant reinvasion pressure against areas from which the flies have not been removed or controlled unless these measures are taken up to natural boundaries, or an effective barrier is maintained (Leak 1999).

Several methods of preventing reinvasion have been attempted over the years, often with little long-term success. In early days barriers of bush clearings to prevent reinvasion were used. The distance a fly could travel was, therefore, critical in determining the width for effective clearings. In Zambia, a bush clearing of one kilometer wide was standard for a 'holding line' (Wooff 1968, cited in Leak 1999) while Jackson (1954b, cited in Leak 1999) referred to the use of a 3,2 km wide clearing, which was necessary to stop the passage of flies. In Uganda, much wider clearings, up to 8 km wide, were used for tsetse (Wooff 1968, cited in Leak 1999).

Target barriers to prevent reinvasion or emigration into a controlled area could be used in control campaigns (Williams *et al.* 1992; Hargrove 1993). These barriers normally consist of stationary targets only but could also consist of a combination of stationary and mobile targets (i.e. cattle treated with deltamethrin) (Warnes *et al.* 1999). Use could also be made of natural barriers, e.g. large water masses, which tsetse could not traverse.

Tsetse flies do not normally venture far from trees during their daily activities as they seem to need to rest frequently and they also need cover to prevent exposure during flight. Therefore, it is unlikely that a tsetse fly would set off into a large open area such as an extensive body of water when there was no suitable object in sight to provide the next stopping point or, more important, shade. Barriers that have therefore been identified are higher ground and unsuitable temperature, natural and man-made bodies of water including large rivers, desert sands, natural treeless areas including grassland, flood plain and seasonal or permanent swamps, arid areas, mountains and expanding areas of

human settlement. The critical factor is the width of the particular body of water or natural treeless areas and lack of shade if it is to serve as a barrier to tsetse movement (Lovemore 1996).

In South Africa *G. brevipalpis* and *G. austeni* are confined to riverine, coastal, and low-lying forests and thickets of the N.E. KwaZulu-Natal area. The distribution of the two species is sometimes patchy, especially where forests are patchy and isolated. In this study it was proposed to establish the distance of apparently "unsuitable" habitat between pockets of forests and other suitable habitat. This could indicate whether such situations could in future act as a natural barrier between populations, or between controlled and infested areas. For *G. brevipalpis* it had already been recorded that they could roam out of these forested areas, especially during their times of main activity at dawn and dusk, and at night (Kappmeier 2000). However, it is not known what distance they will cross over these more open areas between forest pockets. For *G. austeni* the experience was that they are restricted to the pockets of suitable habitat, but whether they could cross small sections of unsuitable habitat, perhaps at night, was not known. This study was, therefore, designed to establish the distance that the two species may or may not cross between forest pockets over open areas of vlei and grassland at the Hellsgate research area.

5.3 MATERIALS AND METHODS

5.3.1 Relative efficiency of target vs. H trap

The relative performance (catch) of the H trap, compared to the recommended target, was established at Hellsgate Tsetse Research Station. This was needed because many experiments are done only with targets or only with traps and the question always arises as to how much better the target performs as they are generally more effective than traps. A comparative test was therefore necessary to establish the relative increase of catches obtained by the recommended SA target (Kappmeier & Nevill 1999c) vs. the H trap.

This comparison was also necessary for the present studies on population densities, with mark-release-recapture techniques. When the fly density in an area, and recapture rate of marked flies, are for example determined by traps (which they mostly are), it can be determined what number of traps will be needed per square kilometre to control the population at a certain rate (e.g. between 3 - 4 % female reduction per day). However, when targets are substituted for traps to conduct a control trial, it is necessary to adjust the calculations to determine what target density is required to have the same effect.

The performance of the H trap was compared with the recommended target to be used for control purposes, namely a 1,75 m black/blue/black target (Kappmeier & Nevill 1999b). Eighteen replicates were tested by means of a Latin Square design. The targets were tested by the use of electric grids, as described in Chapter 2. Targets and traps were baited with the best SA blend (see Chapter 2) placed ± 30 cm downwind of the target or trap. Treatments were operated daily from about 09:00 until dark, and electric grids were supplied with fully charged replacement batteries, halfway during the daily trial to remain effective throughout.

Flies were sexed and recorded, and then analysed statistically. The catches (n) were normalized using a $\log_{10}(n+1)$ transformation and subjected to analysis of variance (Anova), using GLIM4.

5.3.2 Mark-release-recapture trials

Estimates of dispersal rates and population size

Based on the trials that were conducted on *G. pallidipes* and *G. m. morsitans* in Zimbabwe (G.A. Vale, pers. comm., 1998) a mark-release-recapture trial was conducted at Hellsgate. H traps, designed specifically for the purpose of capturing live flies for mark-release-recapture studies, were used. Traps were placed in 'concentric' squares around a central release point, following an example used in Zimbabwe where 'concentric' squares radiated out at 500 m

intervals from the centre and traps were sited 500 m apart to form a grid (G.A. Vale, pers. comm., 1998). However, since nothing was yet known of the dispersal of *G. brevipalpis* and *G. austeni*, a smaller grid lay-out was designed for the present study.

The initial design of the grid was established in a stretch of sand-forest with pockets of dense thickets during August - December 1998. Since the type of vegetation is not optimal for visibility and flight as in a savanna situation, a series of squares was cut through the bush at 'linear' distances of 200, 400 and 600 m from the point of marking and release, which thus became the centre of three 'concentric squares'. The three squares were each marked out with white sisal twine, which made it easier to follow and not loose track in the forest. Traps, on each concentric line, were initially placed at intervals of 400 m, but afterwards changed to 200 m (indicated in Fig. 5.1 Block A) to be sure of results, especially for *G. austeni* (since the feeling was that this species might not disperse very fast).

H traps were set up before commencement of the trial, which started on 13 January 1999. The approximate trap positions are indicated with dots (Fig. 5.1, Block A). One of the two openings of the traps faced the downwind side of the prevailing wind direction.

Marking of flies at the centre was carried out for a total of 24 consecutive days, starting at about 07:00 until 17:30. For this, a set of 10 traps, which were located separately (*c.* 3 km away) to provide freshly-caught flies, were emptied approximately 2-hourly. Flies were kept cool and dark during transportation to the release site. This was done to ensure that all flies that were released were as fresh and viable as possible with the greatest chance of survival. Each day's mark was coded differently with yellow spots of artist's oil paint, on various positions on the thorax (see Fig. 5.2 a & b), so that recaptures could be tracked back to the day they were released, and so determine the period from release to recapture and distance traveled during that period. Score was kept on the number of males and females of each species that were released each day.

The recapture traps in the grid were numbered 1 - 46 (according to distance from centre) to keep track of the position where flies were recaptured. The 'centre trap' (no. 1) was not placed directly at the marking site but 30 m from the site so that it would not directly interfere with the flies' release. The trap catches were collected daily (early morning during the same time) by a party of two catchers during the 24 days that flies were marked and released and for a further 28 consecutive days until no more marked flies were recaptured (last eight days for *G. brevipalpis* and last four days for *G. austeni*). Thus over the period of the experiment there was more than one daily opportunity to recapture flies marked at a certain day previously. Daily records were kept of the species, sex, total number of unmarked flies for each trap, and the number and code of marked flies for each trap position for that day. This enabled the calculation of dispersal rates and population density for each species and sex.

Dispersal over open areas

In order to determine various distances of open (supposedly unsuitable) habitat, mark-release-recapture trials were conducted. Initially three separate release blocks were used at Hellsgate between September and December 1998, named Block B, C and D where flies were marked on position 10 of the thorax (see Fig. 5.2 b) with blue, red and green artist's oil paint for the three release areas respectively. They were recaptured in H traps placed in certain positions surrounding the release sites and separated by various distances of open areas between the release and recapture sites. Ten independent traps at a separate location provided a daily (early mornings) supply of freshly-caught flies which were transported to one of the release points where they were marked and released.

Fig. 5.1 shows the layout of the positions of the release and recapture sites of Block B (in blue), Block C (in red) and Block D (in green). Each of the release points in the different blocks is indicated with an X and marked B, C and D respectively. The recapture sites of each block in the surrounding areas are marked in the respective block colours, i.e. recapture sites B1-9 in Block B (blue), sites C1-11 in Block C (red) and sites D1-8 in Block D (green). Flies at

Block B were released every second day for 17 days (between 3 Sep. - 2 Nov. 1998) and at block C for 5 days (between 4 Nov. - 16 Nov. 1998). On every alternative second day, flies were released at block D for 21 days (between 9 Sep. - 17 Nov. 1998). The recapture traps were checked every second and alternative second day for each of the blocks, from the time that flies were marked until 17 Dec. 1998. Record was kept of the number of flies released at each block and the number of marked and unmarked flies recaptured at the surrounding traps.

For Blocks B - D results could only be obtained for *G. brevipalpis*, probably due to open areas being too extensive for *G. austeni* to cross. Therefore, a new trial (Block E – indicated in Fig. 5.1 in orange/yellow) was planned, with the release site (indicated with xE) being an isolated small pocket of bush in grassland. This bush was also much closer to other patches of bush, so that the recapture traps were mainly placed within these surrounding patches of bush. The distances between the release site and the ten recapture sites were, therefore, much shorter than in previous trials (B, C and D). The trial started on 16 March 1999. Flies were marked and released until 27 March 1999. The recapture traps were checked during this period on a daily basis, and continued until 9 April 1999.

5.3.3 Odours

All traps and electrified targets in this study were baited with the best SA blend, as described in Chapter 2.

5.3.4 Marking techniques

Marking was done with artist's oil paint (Fig. 5.2 a) on different positions of the thorax (Fig. 5.2 b). For each trial (release points) different colours and coding were used. In the dispersal study (Trial E) positions of the markings were varied so that all flies released on a specific day were differently coded and records could be kept of the time it took a fly to reach a specific trap from the release point.

Fig. 5.1 Copy of airphoto of Ndlozi peninsula, Lake St. Lucia, showing the vegetation of the Hellsgate study area. The positions of various Blocks (A-E) used in mark-release-recapture trials are shown. [Release sites are indicated with x and trapping sites for the recapture of flies are numbered in different colours for each block as described in the text. The straight line distances are indicated between release and recapture sites that were crossed over open grassland and vlei areas, i.e. for *G. brevipalpis* males (green), *G. brevipalpis* females (blue), *G. austeni* males (black) and G. austeni females (red). Distances covered in block C are indicated as broken lines. (In Block E only *G. austeni* recaptures are indicated).]

Fig. 5.2 a Yellow artists' oilpaint was used to colour-code flies on positions of thorax as also indicated in Fig 5.2 b.

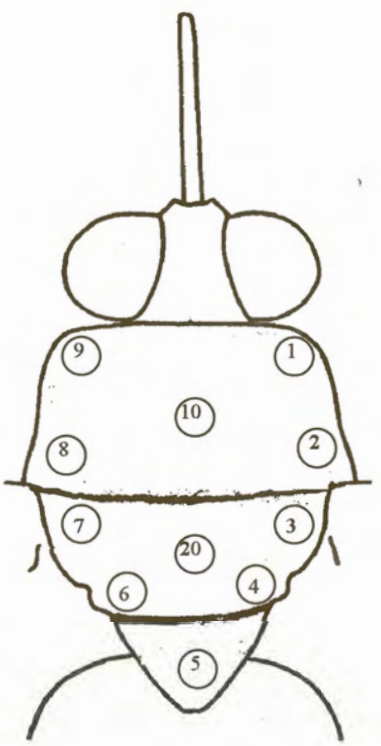


Fig 5.2 b Positions on thorax used for marking (e.g. for position 18, positions 10 + 8 are marked)

5.4 RESULTS

5.4.1 Relative efficiency of target vs. H trap

The results for the experiment, which compared the catches obtained by an H trap to a target, are given in Table 5.1. The results are expressed as an index of increase of the detransformed mean catches of the H trap (index = 1) relative to the mean target catches. Geometric means for the trap catches are given in brackets. The levels of probability (*P*) that the means are different at $P < 0,01$ (**) or $P < 0,001$ (***) are indicated in the Table as well as the number of replicates (*n*) for each treatment, the degrees of freedom (df) for error and the transformed standard errors (s.e.).

As expected, the recommended target increased catches significantly compared to the H trap for both *G. brevipalpis* and *G. austeni* males and females. For *G. brevipalpis* the target increased male and female catches, respectively, by *c.* 2,8 and 2,2 times compared to the H trap. For *G. austeni* the target increased catches of males and females by *c.* 33,4 and 6,8 times, respectively. These results can now be applied to determine target densities when making use of the results of a mark-release-recapture trial where H traps instead of targets were used to determine population density (see Table 5.3).

Table 5.1 Indices of increase of the recommended target[1] relative to the H trap [Geometric means of the H trap are given in brackets]

		Indices of increase					
		H trap	Target[1]	*n*	df	*P*	± s.e.
G. brevipalpis	Males	1 (23,140)	2,811	18	24	***	0,073
	Females	1 (29,690)	2,181	18	24	**	0,064
G. austeni	Males	1 (0,565)	33,445	18	24	***	0,066
	Females	1 (7,196)	6,815	18	24	***	0,053

[1] 1,75 m black/blue/black (50/75/50 cm) target (best SA target)

5.4.2 Mark-release-recapture trials

Estimates of dispersal rates

Table 5.2 is a summary of the total number of each species and sex released at the release site over the 24 mark-and-release days and the total number of flies captured and recaptured at traps number 1 - 46 over the 52 recapture days. The approximate linear distances of each trap (1 - 46) from the centre are also indicated in the Table. Recaptured flies are given as the total number of unmarked and marked flies captured over the period at each trap site. During the period of study a total of 2,683 male and 3,563 female *G. brevipalpis* and 1,518 male and 6,977 female *G. austeni* were marked and released at the centre. A total of 8,627 male and 13,697 female *G. brevipalpis* and 1,984 male and 9,436 female *G. austeni* were caught in the recapture traps (no. 1 - 46). Of these 159 male (1,8 %) and 112 female (0,8 %) *G. brevipalpis* and 21 male (1,1 %) and 291 female (3,1 %) *G. austeni* were marked. Most of the recaptures, 66 male and 29 female *G. brevipalpis* and 25 female *G. austeni*, were made at the 'centre trap' no.1. The direction of fly movement from the release point appeared to be random.

To investigate the movement of tsetse within the block, the recaptures made in the block were separated according to various distances from the centre of the block, at various times after release. Figs. 5.3a-d summarizes, for *G. brevipalpis* and *G. austeni* males and females, respectively, the concentration of flies expressed as the total number of recaptured flies, which dispersed over the various distances from the centre over the various number of days in time. This gives a clear 3-dimensional picture of dispersal rates of the flies.

Table 5.2 Summary of details on the number of flies released and recaptured at the various trap sites – 13 January to 5 March 1999

		G.b males		*G.b* females		*G.a* males		*G.a* females	
Total released		2683		3563		1518		6977	
Total (re)captured		Un-marked	Marked	Un-marked	Marked	Un-marked	Marked	Un-marked	Marked
Trap no.	Distance from rel. site								
1	30	241	66	377	29	42	0	254	25
2	200	128	3	213	6	84	2	395	21
3	200	126	7	164	3	50	1	253	6
4	200	111	3	225	2	53	3	276	17
5	200	245	10	277	3	74	0	289	5
6	283	99	6	172	3	73	1	245	10
7	283	136	0	229	8	23	0	120	2
8	283	154	3	237	2	55	1	194	6
9	283	171	4	282	2	45	1	215	11
10	400	160	0	172	0	28	0	180	9
11	400	128	1	241	3	35	0	200	6
12	400	144	0	296	3	43	1	221	4
13	400	184	2	283	2	29	0	120	5
14	447	123	1	248	1	59	0	373	9
15	447	31	1	122	1	18	0	86	4
16	447	69	0	115	1	40	0	127	2
17	447	127	1	222	2	46	0	223	2
18	447	112	3	226	1	26	0	185	8
19	447	353	5	455	1	40	3	234	12
20	447	504	3	788	3	61	2	234	9
21	447	241	1	350	0	25	0	209	4
22	566	78	1	149	0	56	0	376	12
23	566	89	1	153	0	24	0	73	0
24	566	229	3	386	0	49	1	154	6
25	566	711	5	1 088	2	78	0	243	9
26	600	130	2	202	1	59	0	327	6
27	600	55	2	86	0	9	0	31	1
28	600	155	0	279	1	34	0	137	4
29	600	744	1	1 112	4	83	0	364	3
30	632	291	2	563	3	60	0	388	12
31	632	177	4	324	3	15	0	39	2
32	632	79	2	161	1	28	0	88	4
33	632	203	2	295	3	46	0	209	3
34	632	160	1	311	0	54	2	419	15
35	632	219	0	243	0	28	1	135	3
36	632	173	1	267	3	48	0	163	1
37	721	130	2	282	1	59	0	298	3
38	721	72	1	83	1	16	0	35	0
39	721	64	2	112	3	29	0	54	2
40	721	89	2	116	1	42	0	111	2
41	721	169	0	232	3	38	1	260	3
42	721	221	0	357	3	39	1	196	4
43	721	364	2	513	0	42	0	149	10
44	849	94	0	214	2	23	0	48	4
45	849	64	2	172	1	25	0	70	4
46	849	121	1	191	0	30	0	145	1
TOT		8 468	159	13 585	112	1 963	21	9 145	291

The proportions of *G. brevipalpis* males and females recaptured at the traps most distant from the release point, that is recaptured at 600 - 849 m from the centre, increased soon after release. From this it seems that they reached the outer limits (600 - 849 m) of the recapture block within a short time (*c.* 1 - 7 days for males and *c.* 1 - 9 days for females), after which only a very few individual flies were recaptured within the block as time passed. One hypothesis to explain the recapture of *G. brevipalpis* later in the recapture period, is that many of the released flies rapidly diffused out of the area and some of these probably diffused back again later, so appearing in the traps. This is supported by the recapture of marked flies on occasions at the collection traps, placed at linear distances approximately 2,585 m from the centre release point of the block as well as further at two sites, i.e. 3,138 m and 3,310 m away.

For *G. austeni* not many males were recaptured, but the proportions of both males and females recaptured at the most distant traps from the release point, seemed to increase more or less with time. Although some specimens reached the outer limits of the block after only one or a few days, for most flies the distance dispersed depended on time. Some flies also remained close to the release point. It appears that this species dispersed much slower than *G. brevipalpis*. No significant data could be obtained for *G. austeni* males, since the H trap is biased for females and therefore very few males are captured in the first place (note the small number of males marked and released compared to females).

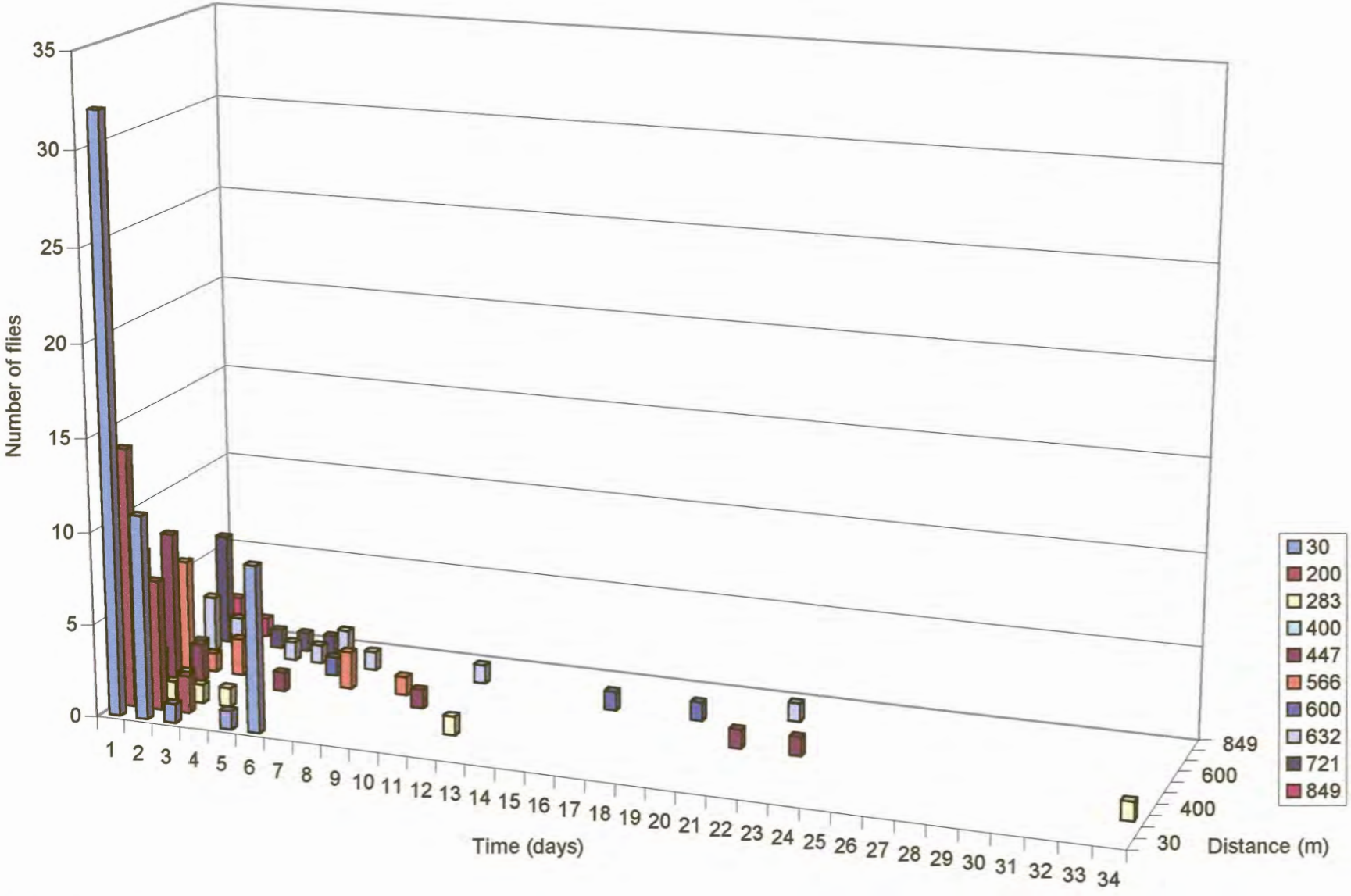


Fig. 5.3 a Summary of the dispersal rates for *G. brevipalpis* males

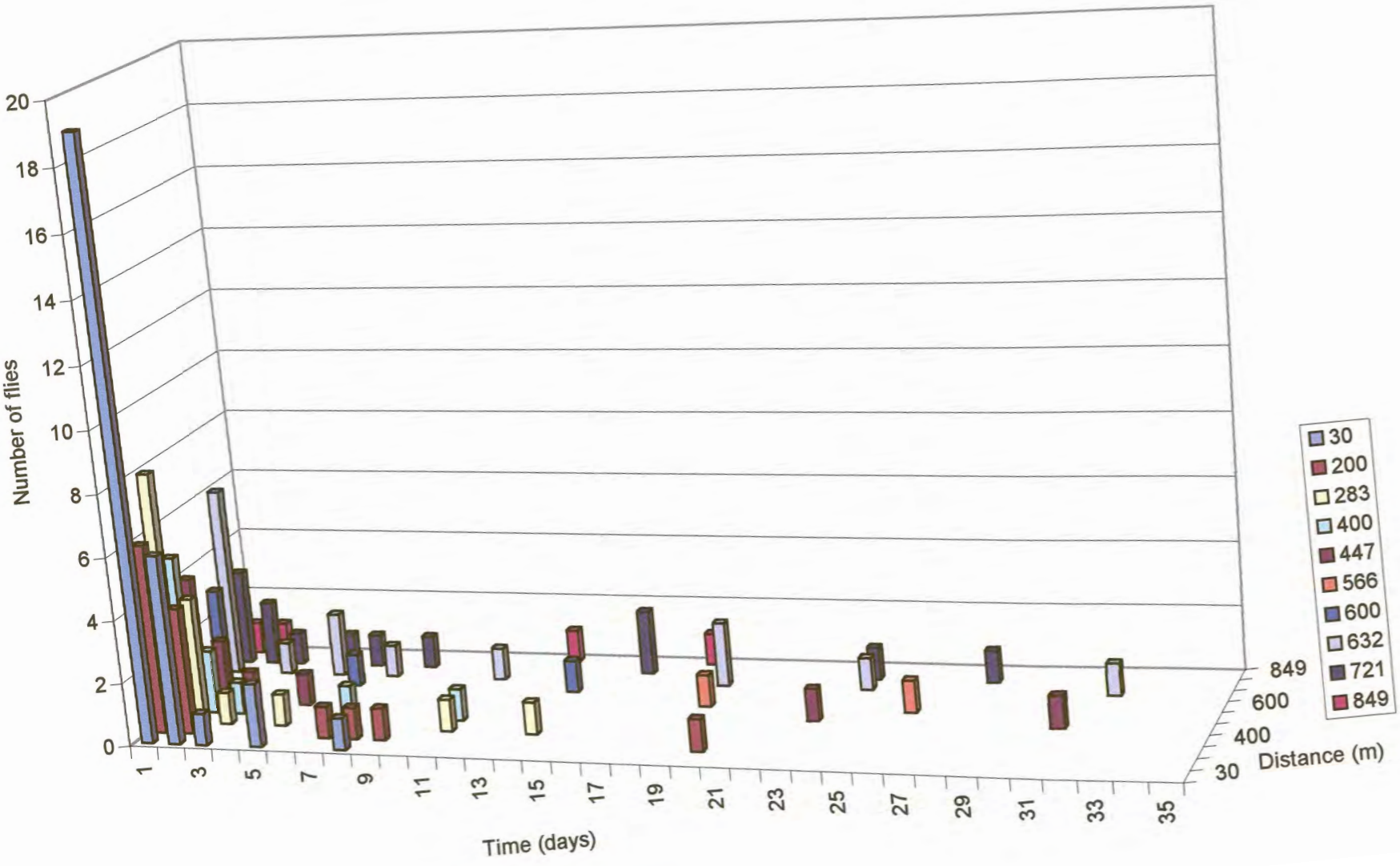


Fig. 5.3 b Summary of the dispersal rates for *G. brevipalpis* females

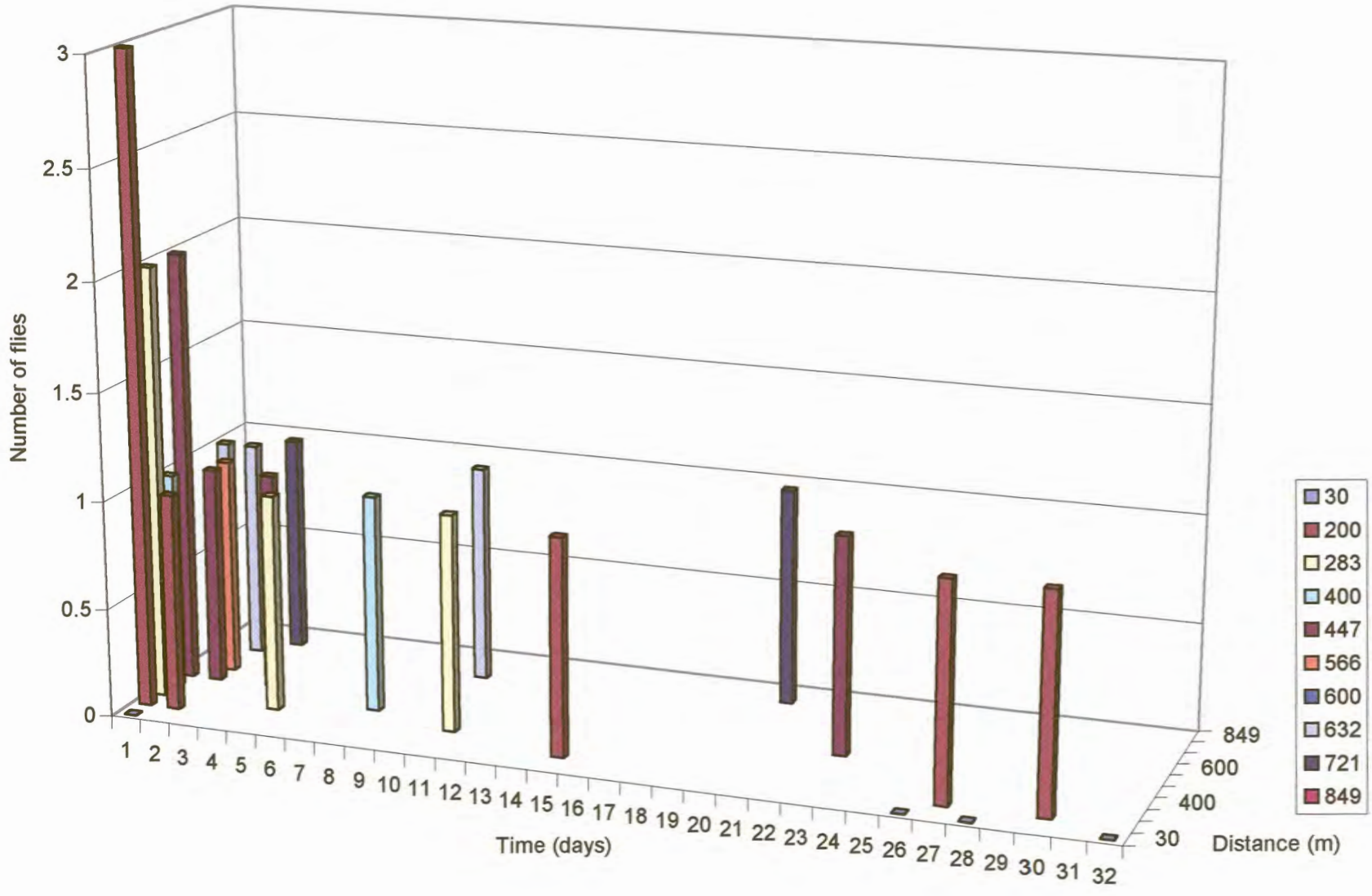


Fig. 5.3 c Summary of dispersal rates for *G. austeni* males

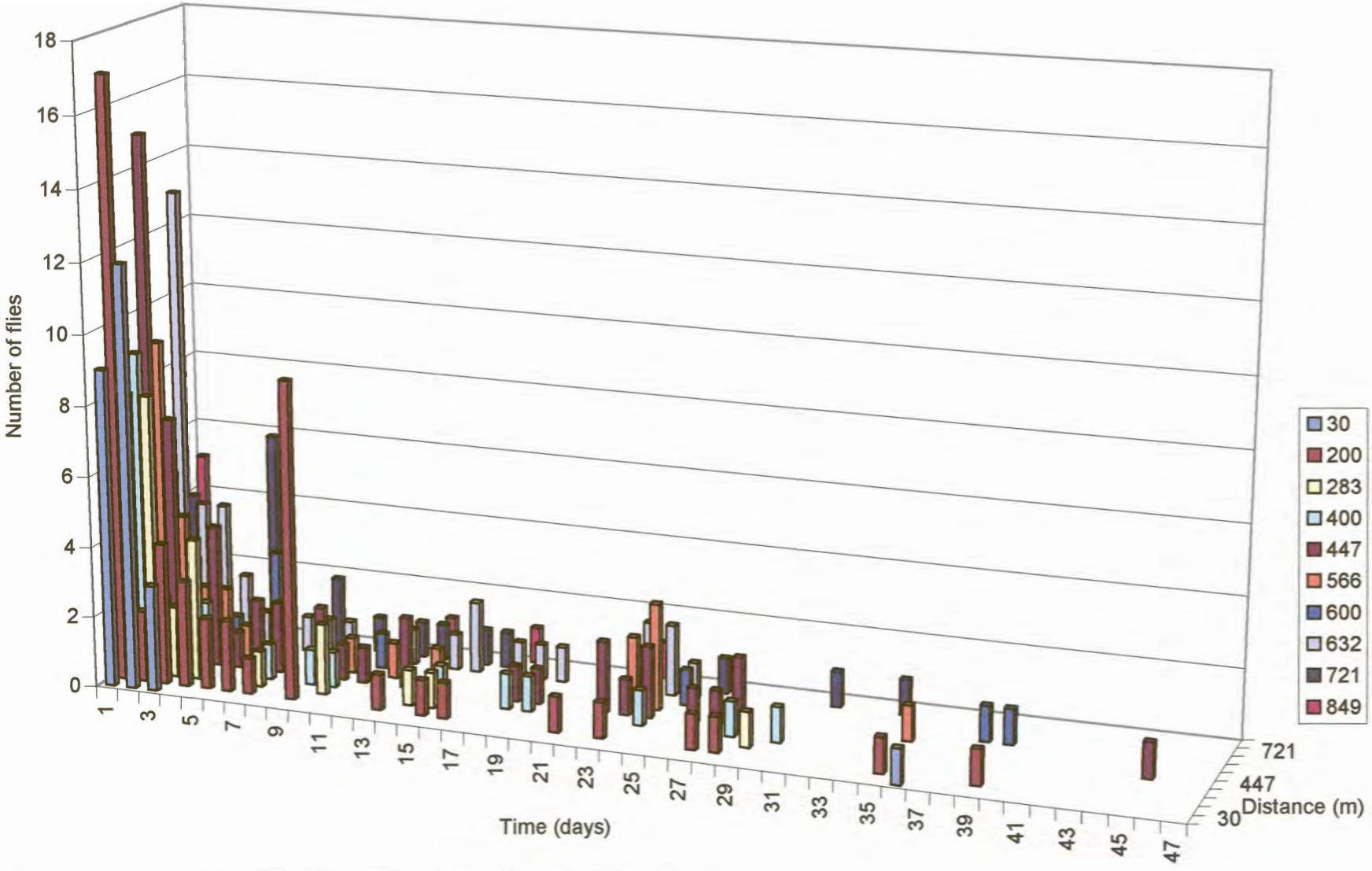


Fig. 5.3 d Summary of the dispersal rates for *G. austeni* females

For both species it is, therefore, notable that the concentration of recaptures decreased with distance from the release point. Both the distribution of recaptures in the traps and the decrease in concentration with distance suggests that the movement of *G. brevipalpis* and *G. austeni* in this habitat is a simple diffusion from the point of release.

The data for *G. brevipalpis* and *G. austeni* is inappropriate to determine the exact rate of movement, since some flies moved fairly rapidly to the outermost concentric square traps and, presumably, beyond, so that the mean distance moved away from a starting point and the daily step length could not accurately be estimated in this study.

Estimates of population size

Further analyses, done to determine the population size, were based on estimates that were basically made on the way in which the capture probability (in all traps taken jointly) changed with time-since-release (see Fig. 5.4 for *G. brevipalpis* and Fig. 5.5 for *G. austeni*). Steps involved in the procedure are as follows (J.W. Hargrove, pers. comm., 1999):

1. Totals were obtained for marked releases, and unmarked and marked recaptures for each day of the trial.
2. A matrix of recaptures was then formed (using the daily totals as in point 1 above) by columnizing the daily total catches of unmarked flies and the sum of the daily marked and unmarked captures, together with the numbers of flies released on each day. The matrix further summarized the number of recaptures caught during each capture day after release (i.e. time after release).
3. In order to calculate the probability of recapture on each day after release, it was needed to know how many marked flies were released and were available for recapture. In doing this provision was made for the marked flies which were being removed from the population by means of trapping. Another matrix was designed to do this.

4. The population can be estimated by (Bailey 1951):

$$Population = MN / R$$

where M is the number of marked flies released and N is a random sample of flies taken some short time later, and R will be the sample of marked flies recaptured:

The probability of capture (p) is just the inverse of this and can be estimated by:

$$p = R / MN$$

One can then get estimates, and variances, of the probability as it changes with time after release. If n experiments were conducted and on the ith day there were M_i marked flies from that experiment in the population, a sample can then be taken on day i and in this sample there could be N_i flies, of which R_i were marked. Then as before:

$$p_i = R_i / M_i N_i$$

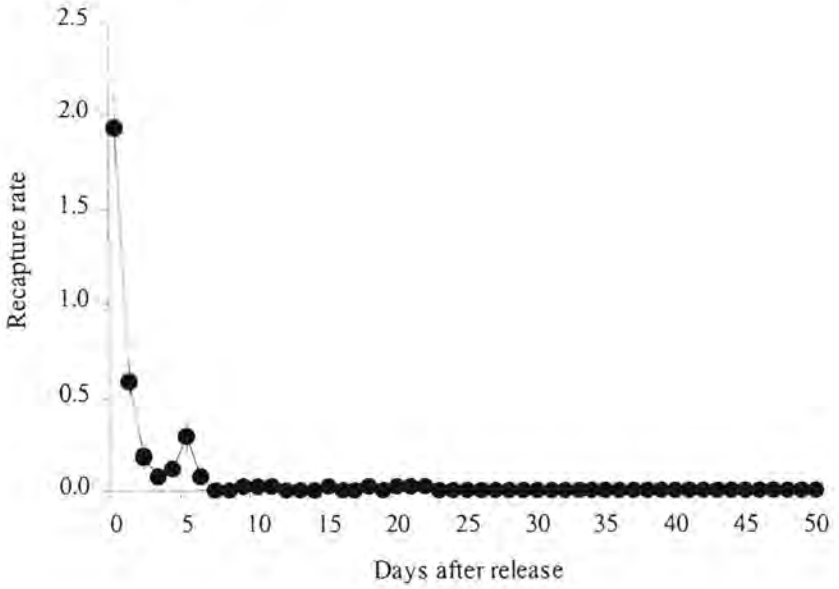
It can be shown that, if the data from all n experiments are used, the maximum likelihood estimate of p_i is given by:

$$p_i = \sum_{i=1}^{i=n} R_i / M_i N_i \qquad \text{Equation 1.}$$

Equation 1 has been used to calculate the capture probabilities.

The probabilities of recapture are given in Fig 5.4 at various days after release for male and female *G. brevipalpis* and in Fig. 5.5 for *G. austeni*.

a) *G. brevipalpis* males



b) *G. brevipalpis* females

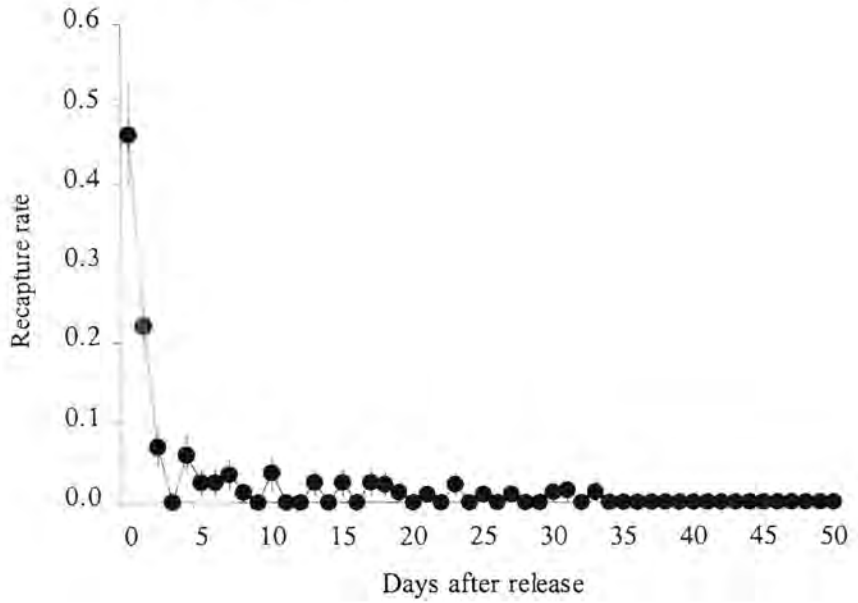
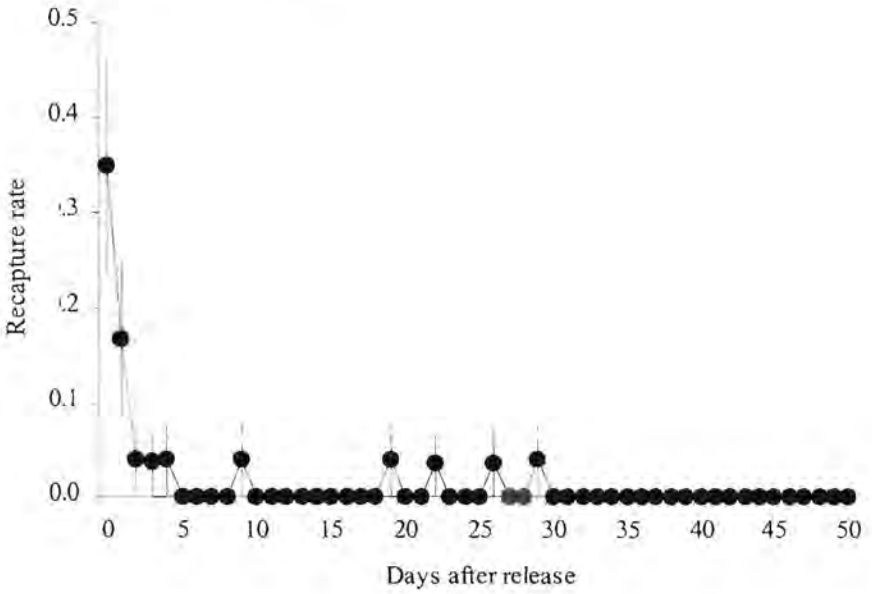


Fig. 5.4 Daily recapture rate at various days after release for *G. brevipalpis* a) males and b) females

a) *G. austeni* males



b) *G. austeni* females

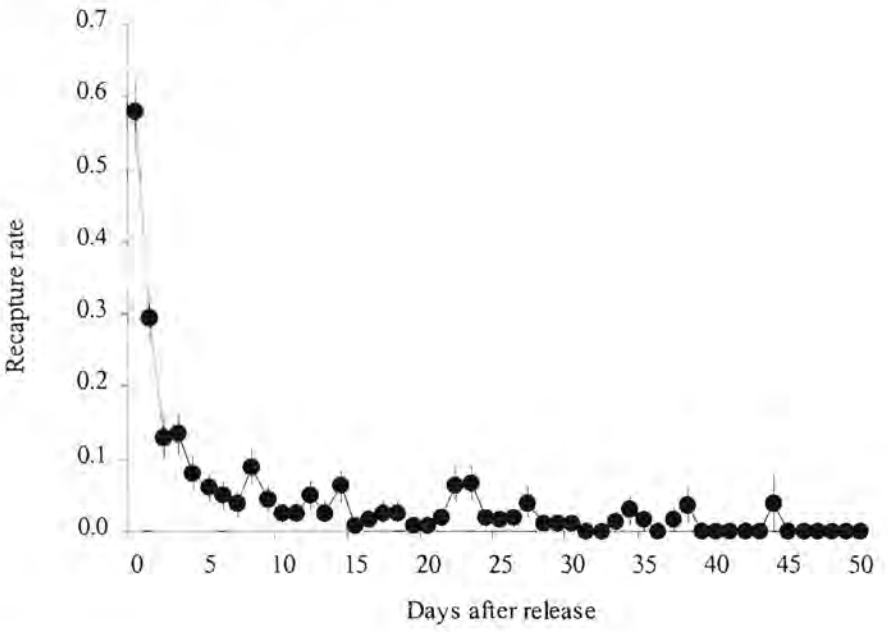


Fig. 5.5 Daily recapture rate at various days after release for *G. austeni* a) males and b) females

Once the probability of recapture had been calculated, the population in the mark-recapture area could be estimated from above indicated formula, using the day 1 recapture rates and probability of recapture (i.e. obtaining the reciprocal of the recapture rate of *G. austeni* of e.g. 0,5779 which then gives 1,7305 x 10 000 – see Table 5.3).

Table 5.3 is a summary of the probabilities of recapture (for day 1) and estimated population densities for each species and sex. The mean catch per trap as determined in Table 5.1 and the expected catch per target, are also indicated together with estimated target densities and options of killing percentages e.g. 1-10 % per day.

Table 5.3 Summary of estimates on population density and expected target densities needed for various options of killing percentages

	G. brevipalpis		*G. austeni*	
	Males	Females	Males	Females
Probability of recapture on day 1	1,9335	0,4618	0,3485	0,5779
Population in mark-release-recapture area	5172	21653	28691	17305
Area (sq.km)	1,44	1,44	1,44	1,44
Density per sq.km	3591,7	15037	19924	12017
Mean catch per H trap*	23	30	0,6	7
Increase per target*	2,8	2,2	33,4	6,8
Catch per target	64,4	66	20,04	47,6
Killing percentage:	Required target densities:			
1	0,6	2,3	9,9	2,5
2	1,1	4,6	19,9	5,0
3	1,7	6,8	29,8	7,6
4	**2,2**	**9,1**	**39,8**	**10,1**
5	2,8	11,4	49,7	12,6
6	3,3	13,7	59,7	15,1
7	3,9	15,9	69,6	17,7
9	5,0	20,5	89,5	22,7
10	5,6	22,8	99,4	25,2

* The mean catch per H trap and increase per targets indicated were obtained from the results of comparisons the effectiveness of the H trap vs. target (5.3.1)

Looking at females only, the above Table would then suggest the use of nine targets for the females of *G. brevipalpis* and 10 targets for *G. austeni*, at a 4 % killing rate (indicated in bold in Table 5.3).

It was, therefore, initially proposed that 9 targets/km^2 would work well for both species. However, it was decided that the population estimates as indicated in Table 5.3 were certainly far too high in that they are about an order of magnitude higher than the estimates for the tsetse population density in the Rifa Triangle (G.A. Vale & J.W. Hargrove, pers. comm. 1999). The population estimates are also a first approximation and the reasons for expecting that they are too high (J.W. Hargrove, pers. comm. 1999) are that:

a) Only the day 1 recapture rates were used to estimate the population. Using the day 0 level will probably give a higher capture probability and hence a lower population estimate. A crude estimate indicates that the population may be 35 % lower than the figure in Table 5.3. For *G. austeni* one could therefore use 7 targets /km^2 where 10 were originally suggested and for *G. brevipalpis* 4 targets /km^2 instead of 9.

b) It is assumed that there was no movement out of the study (mark-recapture) area. If there was such movement (of marked flies), which there definitely was, this will further inflate the population estimate. (If the marked flies leave the area one under-estimates the recapture probability and hence over-estimates the population.)

Dispersal over open areas of unsuitable habitat

Table 5.4 is a summary of the number of flies marked and released at the four different release sites (Blocks B, C, D and E). The total number of marked and unmarked flies captured at each recapture site is also given in the table. Approximate straight distances between each release site and the block's corresponding recapture sites, are also indicated (also refer to Fig. 5.1).

Table 5.4 Summary of mark-release-recapture results for Blocks B, C, D and E to investigate the use of open areas as natural barriers to the movement of *G. brevipalpis* and *G. austeni* – 3 September to 17 December 1998

Flies released		Gb males		Gb females		Ga males		Ga females	
Block	B	543		662		65		190	
	C	103		175		36		89	
	D	509		582		109		314	
	E	1806		1856		1399		2979	
Flies recaptured		Un-	Marked	Un-	Marked	Un-	Marked	Un-	Marked
(m) from release pt	Trap No.	marked		marked		marked		marked	
775	B1	39	0	73	0	0	0	0	0
520	B2	14	1	18	1	0	0	0	0
1120	B3	21	0	19	0	1	0	0	0
1085	B4	12	1	18	0	0	0	0	0
1190	B5	33	0	31	0	0	0	0	0
1105	B6	52	0	82	0	2	0	6	0
1155	B7	25	0	28	1	0	0	1	0
1225	B8	30	0	30	0	2	0	7	0
1345	B9	27	1	25	0	0	0	1	0
275	C1	40	0	88	0	0	0	0	0
85	C2	19	0	29	0	0	0	0	0
465	C3	87	2	121	0	0	0	0	0
500	C4	77	0	195	0	5	0	45	0
725	C5	42	1	51	0	0	0	0	0
605	C6	41	2	57	0	0	0	1	0
655	C7	23	0	28	0	0	0	0	0
725	C8	3	0	6	0	0	0	0	0
860	C9	50	1	63	0	0	0	0	1
690	C10	33	0	51	1	0	0	0	0
485	C11	123	0	237	0	11	0	68	0
725	D1	35	0	31	0	0	0	0	0
550	D2	105	2	157	0	1	0	4	0
550	D3	84	2	131	0	0	0	1	0
450	D4	91	0	132	0	2	0	11	0
515	D5	200	2	264	0	2	0	9	0
725	D6	119	1	246	2	9	0	35	0
400	D7	35	2	48	1	0	0	2	0
415	D8	59	0	91	2	0	0	4	0
345	E1	49	7	912	1	45	2	189	10
275	E2	479	5	760	3	35	0	158	7
310	E3	380	6	656	4	36	8	241	6
240	E4	328	5	506	4	26	2	198	20
240	E5	516	9	886	1	22	1	211	4
295	E6]	153	2	220	0	0	0	0	0
310	E7]**	147	2	168	0	0	0	1	1
205	E8]	45	1	64	0	0	0	1	1
85	E9*	205	33	301	23	11	27	16	34
140	E10	166	14	198	7	2	2	3	3

* Trap E9 was positioned inside the same patch where flies were marked and released.

** Traps E6, 7 and 8 were all small clusters of bush where ants removed many of the catches. Site 6 consisted of very dense thickets, so that the trap was hidden, therefore the low numbers. Note that trap E10 was located about 140 m from the release site under a copse of two *Syzygium* trees (note the high number of unmarked catches of *G. brevipalpis* and even the presence of *G. austeni* that were found at this unusual site.

The distances that were crossed over the unsuitable grass and vlei areas are also depicted in Fig. 5.1. Different colours are used for each species and sex, i.e. *G. brevipalpis* males (green), *G. brevipalpis* females (blue), *G. austeni* males (black) and *G. austeni* females (red). Although these flight distances are indicated as straight lines and as the shortest linear distances between the release and each recapture site, it is unlikely that it is a true representation of the actual path that was traversed. A fly may, for example, have crossed at a particular point (closer or further than the straight distance to the recapture sites), and then have followed the bush before it was attracted and captured by a particular trap. The flies released at point A of Block B, may have followed the bush to the recapture sites, since the release point of Block B is in actual fact indirectly connected to the bush where its recapture traps were set. However, the release points of Blocks C, D and E are totally separated from their respective recapture sites, and so give a truer reflection on what is happening.

From the recapture results obtained from Blocks C and D it is clear that *G. brevipalpis* males and females readily cross all distances of vlei and grassland to reach patches of bushed areas. It is probable that the *G. brevipalpis* males and females recaptured in Block B crossed the open section of grassland and did not follow the bush all the way round.

For *G. austeni* only one recaptured female was obtained in Block C. However, in this case it is more likely that it did not actually cross this distance, but followed the bush all the way round, since no flies were recaptured in Blocks B and D.

The final results for Block E showed that *G. brevipalpis* males and females crossed all distances of open areas, as was expected from the previous results. This time *G. austeni* was also found to cross various distances of open areas (up to 345 m). It seems that, due to a lower percentage of recaptured marked flies, *G. brevipalpis* moved out quicker from the isolated pockets (while unmarked flies move into the patches). *G. austeni*, on the other hand, showed a

greater percentage of recovered marked flies, which may suggest that this species is more static. This is especially supported by trap E9's results.

5.5 DISCUSSION

Attempts to control tsetse flies in much of Africa rely increasingly on the use of odour baited targets (Vale *et al.* 1986; Vale *et al.* 1988a; Willemse 1991; Knols *et al.* 1993; Van den Bossche 1997). In order to implement sustainable control of *G. brevipalpis* and *G. austeni* in South Africa by means of targets at the right density, the probability of recapture and population density of each species and sex was estimated. The re-adapted results suggested an estimated use of four targets per square kilometer to control *G. brevipalpis* females and seven targets per square kilometer for *G. austeni* females.

The recommended target density was based on the assumption of killing 4 % of the female population per day. Although ground and aerial spraying techniques produce much higher mortalities than this (Leak 1999), they may often not be sustained for sufficiently long enough periods to achieve eradication. When odour-baited targets are used the increased death rate is much smaller, but it can be sustained as required (Hargrove 1988). A number of four targets per square kilometer was suggested for *G. brevipalpis*. It compares with the defined absolute lower limit for target densities for the savanna species *G. m. morsitans* and *G. pallidipes* (Hargrove 1993), for which one cannot be sure of eradicating these tsetse populations with a target density lower than 4/km^2. For *G. austeni* the recommended seven targets per square kilometer is also significantly less and much more economical than the 70 blue targets per square kilometer that were used to suppress *G. austeni* numbers in the Jozani forest on the Unguja Island of Zanzibar prior to applying SIT (Tanzania Government/FAO/IAEA 1994).

Fly movement is important, at least in the short-term regulation of fly numbers, especially for particularly mobile species (Leak 1999). One of the factors responsible in the lack of success in sustaining control of tsetse is their high mobility resulting in continual invasion pressure into cleared areas. *G.*

brevipalpis proved to be very mobile in the forested areas, since it appeared to move out of the 850 m range (of this study) in a short period of time (1 - 7 days) (see Fig. 5.3 a-b). It is clear that this species should, therefore, be regarded and treated the same way as the mobile savanna species. *G. austeni,* however, shows a much slower rate of dispersal as seen in Fig. 5.3 c-d. Data for movement of marked *G. m. morsitans* and *G. pallidipes* suggested that the minimal daily rates of movement were about 700 m for *G. m. morsitans* males and 800 m for *G. m. morsitans* females and *G. pallidipes* (Vale *et al.* 1984). Their displacement averages up to 1 km/day in random steps (Laveissiere *et al.* 1990, cited in Leak 1999).

The pattern of movement of *G. brevipalpis* and *G. austeni* appears also to be random, suggested by the low recapture rates in Figs. 5.4 and 5.5. The decrease in recapture rates with time demonstrates how quickly the marked population is lost from the sampling area. Most of this loss is probably attributable to emigration rather than mortality. The decrease in the concentration of marked flies with distance from the point of marking and release is also similar to the diffusion patterns of other invertebrates (Southwood 1966).

As tsetse flies are relatively mobile, there is a constant reinvasion pressure into areas from which the fly has been removed or controlled (Leak 1999). The utilization of natural barriers to protect areas cleared of tsetse flies from reinvasion is a great advantage (Lovemore 1996) and was investigated for use against *G. brevipalpis* and *G. austeni.* At the Hellsgate study area *G. brevipalpis* readily crossed various distances of vlei and grassland between patches of bushed areas (up to 1,345 m or more). *G. austeni* were also found to cross distances of open areas (up to 345 m). In some situations the open grassveld areas had single standing shrubs or small trees (sometimes in very small clusters) which might have still given sufficient shade for protection. However, keeping in mind *G. brevipalpis*' times of peak activity, i.e. early morning and late afternoon until dark (Kappmeier 2000), the crossing of open areas of this species will most probably occur at these times, when the sun and heat factor is less, and when these distances of open areas, could easily be

bridged. It could also be at night, during which time *G. brevipalpis* was often found entering moving vehicles in open areas (Kappmeier 2000). Taking into account *G. austeni*'s activity times being mainly during the middle morning to late afternoon (Kappmeier 2000), it is questionable whether it traverses even the 345 m, shown during this study, in this warmest part of the day and probably rather does so at night. *G. swynnertoni* was able to cross a 800 m clearing, although flies crossing were mainly hungry and presumably in search of a blood-meal (Lloyd 1935, cited in Leak 1999).

Although the type of "unsuitable" habitat at Hellsgate, i.e. an open grassland situation with patches of small bushes situated between their preferred habitat of forests, would have no value as a natural barrier for *G. brevipalpis*, it may be more suitable for *G. austeni*. It is apparent that natural barriers could be effective, especially for the less mobile *G. austeni*, and should be adopted in the preparation of the comprehensive strategic plan. Many barriers could be identified in the N.E. KwaZulu-Natal region as suitable for this purpose, e.g. numerous lakes, a mountain range, reed and sedge swamps, and open grassland areas, and should be used to advantage. However, there is a need to conduct special studies of the various types of barrier identified to understand their mode of operation more fully and to confirm their effectiveness in limiting tsetse movement. It is also essential to identify any possible weaknesses in these natural barriers so that the necessary precautionary measures can be instituted from the outset. Passive movement of tsetse flies by human traffic, especially for *G. brevipalpis*, which enters vehicles easily, would have to be controlled and eliminated where possible.

Because these studies have revealed and proven that both species of tsetse do cross certain distances of these "unsuitable" open areas adjacent and between forests, and that they (especially *G. brevipalpis*) readily roam out of "suitable" habitat of dense bush, it is important not to ignore these "unsuitable" or open areas when setting traps/targets in a control campaign. This was also concluded in a separate study in which traps were placed along a 12 km transect through different vegetation types. Both *G. brevipalpis* and *G. austeni* were captured in open areas of shrubveld and grassland, although their

numbers were comparatively (but not significantly) lower than in forested areas (J.R. Esterhuizen, pers. comm., 2000). In a target control trial for *G. brevipalpis* in 1992 (Kappmeier *et al.* 1998), targets were only concentrated inside the forests, and not in adjacent open areas, and this could have been one of the reasons for the failure of this trial. It is, therefore, clear that control devices such as targets should also be placed strategically in open areas adjacent to dense bush. Whether the concentration of targets needs to be lower in open situations, should still be investigated.

Where natural barriers are unavailable the use of target barriers will have to be implemented. Efficiency of barriers constructed from lines of traps/targets depends on the width of the barrier, the mobility of the flies and the mortality rate within the barrier (Williams *et al.* 1992). Hargrove (1993) made estimates of the width of target barriers required to prevent reinvasion, and attempted to establish the relationship between barrier width, target density and economic costs (the widest barrier is cheapest and uses smallest number of targets). He suggested that targets should be deployed in barriers exactly as they are in normal control operations, when that density is chosen to provide local eradication in 9 - 12 months, while the width of such a barrier should be *c.* 8 times the daily step length of the tsetse species concerned. For the two Zululand species they should, therefore, consist of four targets per square kilometer and for *G. brevipalpis* and eight targets per square kilometer for *G. austeni*. Since the daily step length was not calculated in this study, the width of the barriers for each species could not be estimated. The presence of a target barrier has a marked depressing effect on tsetse populations outside its boundaries, and barriers will be most effective if they are positioned before the treatment of the areas they are meant to protect (Hargrove 1993).

In conclusion an estimate of four and seven targets/km^2 for *G. brevipalpis* and *G. austeni*, respectively, should be sufficient to control *c.* 4 % of the female populations per day. Eight instead of seven targets/km^2 would, however, be advisable for *G. austeni*, as this would make the lay-out of targets easier. It is, however, essential that a small-scale control trial be conducted first before implementing these results on a large-scale to make sure that the target density

estimates are correct and to refine the recommendations. Such a trial is currently underway in the Hellsgate area. This trial will simultaneously be used to evaluate the width of targets in a barrier for both *G. brevipalpis* and *G. austeni*.

Adjacent areas of open grassland next to forested tsetse infested areas should not be ignored when setting targets and traps in a control trial, although the target density would probably decrease in such areas. The distances between main pockets of tsetse distribution (suitable tsetse habitat), which will act as natural barriers between populations, should be reconsidered, especially for *G. brevipalpis*.

6. FEEDING RESPONSES

6.1 ABSTRACT

Living hosts, namely a cow, goats and bushpig, were placed within an incomplete ring of electric nets, within forest vegetation. Observations were made of the numbers of *Glossina brevipalpis* and *G. austeni* attracted to the hosts as well as the feeding responses of tsetse. The cow attracted significantly more *G. brevipalpis* and *G. austeni* than did goats or bushpig. Significantly more flies also fed on the cow with less than *c.* 10 % feeding on either goats or bushpig. Comparisons were also made on the attraction and feeding responses of tsetse during different periods of day as well as at night at two sites, the first being inside their preferred habitat, namely forest vegetation, and the second at about 750 m away from the forest. Both *G. brevipalpis* and *G. austeni* were attracted to the cow inside the forest at all times of day, even at night, while the largest percentage of flies feeding occurred after dark. *G. brevipalpis* was also attracted to the cow outside the forest area during the daytime until dark and significantly fewer after dark, while very few flies fed during all occasions. Only a few individual *G. austeni* were occasionally attracted to the cow at the latter site during the day, while virtually no flies fed.

6.2 INTRODUCTION

The use of pour-ons to control tsetse flies works on the principle that the flies, which come to feed on cattle or other treated domestic livestock, will be killed by picking up lethal deposits of insecticide (Leak 1999). The treated livestock are then equivalent to moving insecticide-treated targets, complete with built-in odour attractants, and have been referred to as "mobile targets". Cattle have often been used with success as mobile targets, where they are treated with a pyrethroid pour-on or dip (Thomson 1987; Thomson *et al.* 1991; Horeth-Bontgen 1992, cited in Vreysen 1995; Bauer *et al.* 1995; Warnes *et al.* 1997; 1999). The control of tsetse flies by this method, however, depends upon a relatively large proportion of feeds being taken from domestic rather than wild animals (Leak 1999). The proportion of tsetse flies that feed on hosts also has

a major bearing on the epidemiology of tsetse-borne diseases (Vale 1977b). To assess the importance of this problem it is necessary to know the proportion of flies that feed after visiting a natural host. Furthermore, since treatment with insecticides will not prevent infection, disease challenge could also be high in these areas and it may be necessary to protect cattle from tsetse fly attack. Improved knowledge of the times and situations when cattle are at most risk of tsetse challenge could be beneficial to develop strategies for minimizing contact.

In N.E. KwaZulu-Natal there is a network of plunge dips mainly for the control of ticks on cattle and thereby tick-borne diseases. There is also a high number (*c.* 130 000) of small ruminants, i.e. goats in the rural areas (De Waal *et al.* 1998), however, they are never dipped. In many communal farming areas virtually no wild animals remain on which tsetse flies can feed so their main blood sources have to be cattle or goats. For the mobile target approach to have a chance of success most tsetse must then attempt to feed on treated cattle. What proportion of *G. brevipalpis* and *G. austeni* would feed on cattle, is however, not certain. If tsetse also feed on goats, these could accordingly be used as mobile targets. The role/importance of livestock, therefore, needs to be established in the Zululand situation for tsetse control management.

This work attempted to evaluate the potential of cattle and goats as mobile targets. It consisted of studies where the attraction and feeding responses of tsetse to cattle, goats and bushpig (*Potamochoerus porcus*) were measured. The bushpig was included since bushpigs are an important host for *G. austeni* (Moloo 1993). The responses of flies to these hosts were compared in ideal tsetse habitat, i.e. in sand forest situation. Furthermore, because *G. brevipalpis* and *G. austeni* have different times of activity and since the former has also been found active at night (Kappmeier 2000), attraction and feeding responses of tsetse to cattle were also determined at various times of day including dark. Since it was indicated in the previous chapter, that both species traverse open areas to an extent, this study was also conducted in open grassland areas adjacent to forest vegetation. This could also indicate whether tsetse challenge will be lower in open grassland areas adjacent to forested areas.

6.3 MATERIALS AND METHODS

6.3.1 Attraction and feeding

Responses of tsetse to various hosts

Studies were made of the responses of *G. brevipalpis* and *G. austeni* to various host animals, namely an adult cow (*c.* 350 kg), three adult goats (*c.* 68 kg) and a juvenile bushpig (female, 6 months, *c.* 50 kg), within sand forest where high densities of both tsetse species occur. The animals were placed in the centre of an incomplete ring (8 m diam.) of six electric nets following the methods of Vale (1977b) and Torr (1994). The six nets covered 35 % of the circumference of the ring. The animals were tethered to a pole(s) at the centre of the ring to prevent them from touching the nets. They were allowed to move freely within a 2 m radius of the pole (Fig. 6.1). Fresh water and food were supplied throughout the experimentation.

The electric nets (1,5 x 1 m) were mounted on corrugated iron sheets as explained in Chapter 2.2. Tsetse flies that were attracted to the cow were either electrocuted on approach or passed unscathed between the electric nets. Once in the circle, they either escaped directly, or were electrocuted on the insides of the nets or fed on the animal(s). Thereafter fed flies either escaped or were electrocuted on the inside of the nets. A mathematical formula exists which allows these results to be analysed and interpreted (Vale 1977b). It was, therefore, necessary that electrocuted tsetse flies were separated according to the side of the net on which they were caught and categorized as fed or unfed by the presence or absence of red blood visible through the abdominal wall. Flies caught on the outside or inside of the ring were presumed to be approaching or leaving the vicinity of the animals, respectively (Vale 1977b).

Fig. 6.1 Cow in the centre of an incomplete ring of six electric nets (8 m diam.) covering 35 % of the circumference of the ring

Experiments of the comparison of various host treatments were carried out from noon until dark, a period when both tsetse species are active (Kappmeier 2000). Wind direction was noted by means of observations with a wind pane after the start of each daily experiment.

Comparison of tsetse responses inside and outside forest vegetation

Attraction and feeding responses of flies to a cow were determined at various times of day, with a similar incomplete ring of nets as described above. These incorporated the two species' main activity times (Kappmeier 2000), i.e. from noon - 16:00 (for *G. austeni*), from 16:00 - dark (for *G. brevipalpis*) and at night from dark -23:00. These respective treatments will be referred to as Times I, II and III, respectively. The experiment was repeated in an open area about 750 m away from the main forest. Wind direction was recorded.

6.3.2 Experimental design and analysis

In any one experiment only one site was used so that the various treatments being compared were incorporated in a randomized block design. To normalize data, catches (n) and proportions (p) were transformed to $\log_{10}(n+1)$ and $\arcsin(\sqrt{p})$, respectively, and then subjected to analysis of variance using GLIM4.

6.4 Experiments and Results

6.4.1 Ring of nets

The ring was operated for a total of 53 days within sand forest in three separate experiments. During these experiments the mean inside catch of the ring comprised 46,9 % (± 0,079 s.e.) and 53,8 % (± 0,024 s.e.) of total *G. brevipalpis* male and female catches, respectively, and 28,7 % (± 0,192 s.e.) and 48,1 % (± 0,0213 s.e.) of total *G. austeni* males and females, respectively. If the ring was operated in the way described by Vale (1977b), who assumed a random approach and departure of flies to and from a host, then the inside catch should make up 39 % of the total (inside + outside) catch instead (Torr 1994).

For both the inside and outside catches for the three experiments combined (53 replicates), some of the nets caught significantly more than others ($p < 0,001$) for both *G. brevipalpis* and *G. austeni* (males and females combined). This suggested that arrivals and departures from the ring were not random (as suggested by Vale's (1977b) assumption). Outside catches of individual nets, expressed as a percentage of the total outside catch, ranged from 0 - 90 % for *G. brevipalpis* and 0 - 57,7 % for *G. austeni*. Furthermore, nets with significantly larger outside catches correlated with wind direction. The downwind nets had outside catches of 24,6 % (± 0,624 s.e.) for *G. brevipalpis* and 20,1 % (± 0,0711 s.e.) for *G. austeni* as opposed to the opposite upwind catches of 3,1 % and 2,1 % for the two species, respectively. The downwind

catches were significantly higher ($P < 0,05$) than all the remaining nets for *G. austeni* and also for *G. brevipalpis* ($P < 0,001$) and specifically 9,6 times and 7,9 times higher than the opposite upwind net catch for the two species, respectively.

6.4.2 Attraction and feeding

Since the presumption of random approaches was not supported by the data, it was not possible to obtain absolute estimates of the numbers of tsetse attracted to a host. Therefore, attraction was determined as an index of the total catches obtained from the inside plus outside ring of nets as was also suggested by Torr (1994). The feeding proportion consisted of the percentage of fed flies on the inside ring. Indices of the detransformed means are reported.

Responses of tsetse to various hosts

Studies of tsetse responding to a live cow, goats and a bushpig were carried out in two separate experiments (Exp. 1 & 2). Attraction of the two tsetse species to these animals is summarized in Table 6.1. The results are expressed as an index of attraction relative to the attraction to the cow with detransformed mean catches obtained by the latter given in brackets. The proportion of flies caught on the inside of the ring of nets that had fed are given in Table 6.2. These results are expressed as detransformed percentages. The number of replicates (n) for each treatment, the degrees of freedom (df) for error, the transformed standard errors (s.e.) as well as the levels of probability (P) that the means are different at $P < 0,05$ (*), $P < 0,01$ (**), $P < 0,001$ (***), or not significantly different (n.s.) are given in the Tables.

The results showed that *G. brevipalpis* males and females were significantly more attracted to cow (*c.* 4,0 - 6,5 x and *c.* 2,7 - 2,8 x) than to either bushpig or goats, respectively. The percentage of males and females feeding on cow (*c.* 19,2 - 49,7 %) were also significantly greater than those feeding on bushpig (*c.* 0,01 - 9,60 %) or goats (*c.* 0,3 - 0,4 %).

Table 6.1 Relative attraction of *G. brevipalpis* and *G. austeni* males and females to cow, bushpig and goats (in two experiments within sand forest) [Results are expressed as indices of increase relative to the cow (index = 1) with detransformed mean catches of the cow given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*), $P < 0,01$ (**), $P < 0,001$ (***) levels of probability or not significantly different (n.s.) are indicated]

	Exp. 1						Exp. 2					
	Indices of increase						Indices of increase					
Species/sex	Cow	Bushpig	*n*	df	*P*	± s.e.	Cow	Goats	*n*	df	*P*	± s.e.
G. brevipalpis												
Males	1(40,534)	0,153	7	13	***	0,089	1(41,870)	0,377	9	17	***	0,040
Females	1(43,040)	0,253	7	13	*	0,109	1(33,390)	0,354	9	17	***	0,038
G. austeni												
Males	1(11,667)	0,331	7	13	n.s.	0,136	1(8,267)	0,904	9	17	n.s.	0,061
Females	1(42,720)	0,470	7	13	*	0,088	1(65,49)	0,400	9	17	**	0,070

Table 6.2 Feeding percentages of *G. brevipalpis* and *G. austeni* males and females on cow, bushpig and goats (in two experiments within sand forest) [The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*), $P < 0,01$ (**) and $P < 0,001$ (***) levels of probability are indicated]

	Exp. 1						Exp. 2					
	Feeding %						Feeding %					
Species/sex	Cow	Bushpig	*n*	df	*P*	± s.e.	Cow	Goats	*n*	df	*P*	± s.e.
G. brevipalpis												
Males	43,9	9,60	7	13	**	0,206	19,2	0,30	9	17	**	0,161
Females	42,0	0,01	7	13	***	0,132	49,7	0,40	9	17	**	0,176
G. austeni												
Males	24,5	9,70	7	13	*	0,212	8,9	0,04	9	17	*	0,272
Females	45,0	5,00	7	13	**	0,164	80,0	11,10	9	17	*	0,117

For *G. austeni* males the attraction towards the cow was not significantly different from attraction to bushpig and especially to goats, while the percentage of males feeding on cow (*c.* 8,9 - 24,5 %) was still significantly more than those feeding on either bushpig (*c.* 9,7 %) or goats (*c.* 0,04 %). For females attraction to cow was significantly greater (c. 2,1 - 2,5 %) than for bushpig and goats. The percentage of females feeding on cow (*c.* 45 - 80 %) was also significantly higher than those feeding on bushpig (*c.* 5 %) and goats (*c.* 11 %).

Comparison of tsetse responses inside and outside forest vegetation

Comparisons of the responses of *G. brevipalpis* and *G. austeni* to a live cow were compared at two sites i.e. inside and outside forest vegetation (referred to as Site 1 and 2, respectively) during various times (I - III) of tsetse activity as set out in Materials and Methods above. The results for attraction of tsetse to the cow for the two sites and various times are summarized in Table 6.3. These results are given as the indices of increase relative to a time period when each species was most active (Kappmeier 2000), i.e. for *G. austeni* it was Time I (noon - 16:00) and for *G. brevipalpis* this is Time II (16:00 - dark). The proportion of fed flies, caught on the inside of the ring of nets, is given in Table 6.4. These results are once again expressed as detransformed percentages. Again, the number of replicates (n) for each treatment, the degrees of freedom (df) for error, the transformed standard errors (s.e.) as well as the levels of probability (P) that the means are different at $P < 0,05$ (*), $P < 0,01$ (**), $P < 0,001$ (***), or not significantly different (n.s.) are given in the Tables.

For *G. brevipalpis* there were no significant differences between the number of both males and females attracted to a cow inside the forest for the various times of experimentation. A high percentage of these males and females also fed on the cow, especially after dark (*c.* 58,5 - 70,0 %) when the percentage of fed females were significantly higher than those fed during the preceding test times until dark. Outside the forest, more males and females were attracted to the cow during daytime hours (noon until dark) than after dark. However,

virtually no flies (*c.* 0,02 – 4,6 %) fed on the cow outside during any of the times experimented.

For *G. austeni* males and females there were no significant differences in attraction to cow inside the forest between any of the times and they were also attracted to the cow at night. However, the greatest percentage of feeds took place between 16:00 until dark, although some feeding took also place at night (*c.* 4,4 - 15,7 %) Outside the forest, basically no males or females (mean number of flies attracted *c.* 1,2 - 1,3) were attracted during daytime periods until dark. Also no flies were attracted to the cow at night, thus the feeding percentage of the cow at all times outside the forest was essentially zero (0 - 0,02 %).

Table 6.3 Relative attraction of *G. brevipalpis* and *G. austeni* males and females at various times of day inside sand forest (Site 1) and in the adjacent open grassland area (Site 2) [Results are expressed as indices of increase relative to Time I for *G. austeni* and Time II for *G. brevipalpis* (index = 1). Detransformed mean catches of the control Time are given in brackets. The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) level of probability or not significantly different (n.s.) are indicated]

	Site 1 (Forest)							Site 2 (Grassland)						
	Indices of increase							Indices of increase						
Species/sex	Time I	Time II	Time III	*n*	Df	*P*	± s.e.	Time I	Time II	Time III	*N*	df	*P*	± s.e.
G. brevipalpis														
Males	0,712	1(49,38)	0,896	7	20	n.s.	0,085	1,160	1(19,877)	0,431	6	17	n.s.	0,152
Females	1,009	1(51,33)	0,957	7	20	n.s.	0,082	0,754ab	1(32,788)a	0,290b	6	17	*	0,123
G. austeni														
Males	1(7,958)	1,004	1,122	7	20	n.s.	0,131	1(1,245)a	0,085a	0,000b	6	17	*	0,098
Females	1(24,71)	0,534	0,513	7	20	n.s.	0,111	1(1,289)a	1,059a	0,000b	6	17	*	0,108

ab treatments followed by the same symbols are not significantly different

Table 6.4 Feeding percentages of *G. brevipalpis* and *G. austeni* males and females at various times of day inside sand forest (Site 1) and in the adjacent open grassland area (Site 2) [The number of replicates (*n*), the degrees of freedom for error (df), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,05$ (*) levels of probability or not significantly different (n.s.) are indicated]

	Site 1 (Forest)							Site 2 (Grassland)						
	Feeding percentage							Feeding percentage						
Species/sex	Time I	Time II	Time III	*n*	df	*P*	± s.e.	Time I	Time II	Time III	*n*	df	*P*	± s.e.
G. brevipalpis														
Males	13,8	9,5	58,5	7	20	n.s.	0,196	0,45	0,48	0,70	6	17	n.s.	0,294
Females	39,6a	38,0a	70,0b	7	20	*	0,044	0,02	0,02	4,60	6	17	n.s.	0,260
G. austeni														
Males	6,7	23,5	4,4	7	20	n.s.	0,313	0,00	0,02	0,00	6	17	n.s.	0,150
Females	22,0	68,7	15,7	7	20	n.s.	0,200	0,02	0,02	0,00	6	17	n.s.	0,223

ab Treatments followed by the same symbol are not significantly different

6.5 DISCUSSION

6.5.1 Ring of nets

Except for *G. austeni* males, the proportion of flies caught on the inside ring of nets was about 9 % (for *G. austeni* females) and 8 – 15 % (for *G. brevipalpis*) greater than the 39 % expected if a random approach or departure to hosts occurs. Torr (1994) found exactly the same proportion of greater than expected inside catches for tsetse in Zimbabwe as here obtained for *G. brevipalpis*. Vale (1977b) implied that flies approaching a host were flying higher than those leaving after feeding, therefore the higher inside catch. According to Torr's (1994) explanation the estimated 75 % efficiency of electric nets would increase the inside catch of nets. However, he indicated that, as a result of lower efficiency, the inside catch would actually comprise 46 % of the total catch, which is more or less identical to the proportion observed for *G. brevipalpis* males, but still a little less than for female *G. brevipalpis* and *G. austeni*.

Torr (1994) furthermore suggested another reason why flight to and from a host is unlikely to be random. Some nets are in the flight paths to and from the host, in that tsetse attracted to the odour of the host will approach from downwind (Vale 1974b), while also departing downwind after feeding (Vale 1977a). This study showed that some nets caught significantly more than others both at the inside and outside of the ring, thus also suggesting that arrivals and departures were not random. It was furthermore shown for both species that the downwind nets had significantly more outside catches than any of the other nets, which suggests an upwind flight response towards the host. This is expected for *G. brevipalpis* which reacts strongly to odours, but is a very interesting observation for *G. austeni*, for which olfaction played a less important role compared to visual attraction, as indicated in Chapter 3.

6.5.2 Attraction and feeding

The present study showed that male and female *G. brevipalpis* and female *G. austeni* were significantly more attracted to cow than to either bushpig or goats, with a significantly higher percentage (19,2 - 49,7 % *G. brevipalpis* and 45 - 80 % *G. austeni*) feeding on cow. Even though a low number of *G. austeni* males were attracted to cow (as well as to goats and bushpig) there was still a significant proportion of them feeding on cow as compared to goats and bushpig. Vale (1977a) found similar results for tsetse in Zimbabwe where, amongst other hosts, goats and bushpig were less attractive than cow and the proportion of flies feeding was also very low (8 - 15 %).

During the various times of experimentation, i.e. between noon until well after dark, *G. brevipalpis* and *G. austeni* males and females were equally attracted to a cow inside the forest vegetation. A high percentage of the *G. brevipalpis* fed on the cow, especially after dark when 58,5 % of the males and 70,0 % of the females that were attracted, fed. However, the greatest percentage of feeds for *G. austeni* took place from 16:00 until dusk, although some feeding also took place at night. The results of the "cow in forest" attraction trials differed in many ways from the results of "target trials" conducted during the same season (autumn) (Kappmeier 2000). For example, only individual *G. brevipalpis* were attracted to targets between noon and 16:00 and were mainly active during the two hours preceding dark. *G. austeni* was attracted to targets from noon until darkness fell. Both species were, however, "unavailable" to targets as soon as it was dark. The reason for this was thought that, although odour was present to attract flies to the vicinity of the target, the flies could not visually perceive the target. The cow, however, attracted both species well after dark, with many feeding. This indicates that non-visual host-finding mechanisms continued to act in the dark. Apart from host-odour, which was also present during the day, radiating body heat would appear to be the most likely source of close-range attraction in the absence of visual stimuli. This heat factor might also be the important factor for *G. brevipalpis* during the day when flies were largely active around a live host but not around an odour-baited target.

At the site in an adjacent grassland area about 750 m outside the forest, more *G. brevipalpis* were attracted to a cow during daytime hours including dusk than after dark. Since this experiment was conducted during winter, when daytime temperatures are much lower, it is not surprising that the flies left the cover of shade since they were also found "available" to targets during daytime in winter (Kappmeier 2000). It also supports the findings in the previous chapter, which showed that this species has the tendency to roam out of the forest especially at lower temperatures. However, virtually no *G. brevipalpis* fed on the cow during any of these times with at most only 4,6 % of the females feeding after dark. Only individual *G. austeni* were attracted during daytime periods until dusk and no flies were attracted at night. The feeding percentage was thus basically naught. In the previous chapter it was also shown that this species was able to traverse open distances, but essentially not as far as 750 m.

6.5.3 Implications

The control of a tsetse population by means of pour-ons used on domestic animals depends upon a relatively large proportion of flies feeding on the treated animals. The present investigation suggested that cattle could be used as viable mobile targets for the control of *G. brevipalpis* and *G. austeni* in communal farming areas where cattle are present. Since domestic goats were relatively unattractive to, and infrequently fed upon by tsetse, there would be little point in treating them for use as mobile targets. Also untreated goats are unlikely to provide a major alternative blood-source to the treated cattle.

Regarding the protection of cattle from tsetse fly attack, it is clear that disease challenge by these two forest-dwelling species will be lower if cattle are kept from direct contact with forest situations. This may not always be possible, since cattle are nowadays forced to make closer contact with tsetse habitat because of overgrazing. However, since tsetse feeding outside the forest areas seems to take place mostly at night and also takes place less outside forest vegetation, it might be of benefit to at least shelter animals at night at a

distance from forested areas. For *G. austeni* this distance is obviously less than for *G. brevipalpis*.

7. TSETSE DISTRIBUTION AND ABUNDANCE

7.1 ABSTRACT

Tsetse surveys were conducted from 1993 - 1999 in the northeastern parts of KwaZulu-Natal. A large proportion of the nagana-infected area of N.E. KwaZulu-Natal has now been surveyed. A successful surveying system was developed and has been refined at each successive survey. Trap sites were mapped as positive or negative for tsetse fly presence. Maps of the apparent densities of each species in terms of flies/trap/day are also presented. For *Glossina brevipalpis* there appeared to be two distinct bands of distribution, i.e. one in the southern one-third of the area and the other just south of the Mozambique border, but it seems to be inexplicably absent from Tembe Elephant Park, the central Makhathini/Mkuze area and most of the Coastal Reserve between the Eastern Shores of Lake St. Lucia and Kosi Bay. *G. austeni* appeared to be more widespread from north to south but is absent from Hluhluwe/Umfolozi Game Reserve and from the Eastern Shores of Lake St. Lucia northwards along the Coastal Reserve to Kosi Bay. Results were incorporated into a Geographic Information System (GIS). Reference was made to the historical distribution of the two species and current trends in cattle distribution, trypanosomosis prevalence, landcover and vegetation types, which were also mapped.

7.2 INTRODUCTION

Before a tsetse control campaign is implemented it is necessary to be able to answer certain questions, for example: Where exactly do tsetse flies occur? Which species occur where? Are they restricted to certain types of vegetation? For some activities, such as planning a control campaign where the same solution is to be applied throughout the affected area, only accurate information on distribution is required. For others, such as determining the amount of control/intervention, abundance and prevalence information is also required (Rogers & Randolph 1986).

Early work on tsetse flies concentrated on their distribution and habitats, with the objective of determining priority areas for the control of the flies and areas where people or domestic livestock are at risk. The general distribution of tsetse flies, determined principally by climate and influenced by altitude, vegetation and the presence of suitable host animals, has been known for a long time. However, more precise limits of distribution, particularly in areas of low population density, were not well defined. Since the 1960s more precise limits of tsetse distribution have been obtained from surveys using various sampling techniques (Leak 1999).

A long-term solution to the nagana problem in KwaZulu-Natal depends on the control and/or eradication of the tsetse flies. It is, therefore, essential to know the distribution of the two tsetse species and to relate this to outbreaks of the disease so that possible control strategies can be developed. Ford and Katondo's (1977) distribution maps are not very accurate regarding the present distribution of *G. brevipalpis* and *G. austeni* in South Africa. The historical distribution of these two species (Fig. 7.1) in N.E. KwaZulu-Natal was given more accurately by Du Toit in 1954 and in a small area surrounding Lake St. Lucia in 1956. Aerial smoking with DDT and HCH also eradicated *G. pallidipes* from the Zululand region (Du Toit 1954) and possibly also *G. brevipalpis* and *G. austeni* where their distributions coincided with that of *G. pallidipes*. However, since 1954 various changes in land use and development have occurred in the region (Kappmeier *et al.* 1998). In certain areas the human and stock population increase resulted in bush removal, making those areas less favourable for the two shade requiring tsetse species. On the other hand, the planting of pine and eucalypt trees between 1953 - 1960, which was mostly on grassland and shrubland in the Hlabisa District, may have created artificial but suitable habitat for shelter and even reproduction of the two species. This also resulted in the expansion of thickets in certain areas, due to the excessive use of water by the plantations and concomitant lowering of the water table, as well as their protection from fire and clearing for cropping (Jacobs, Schafer & Robertson 1989).

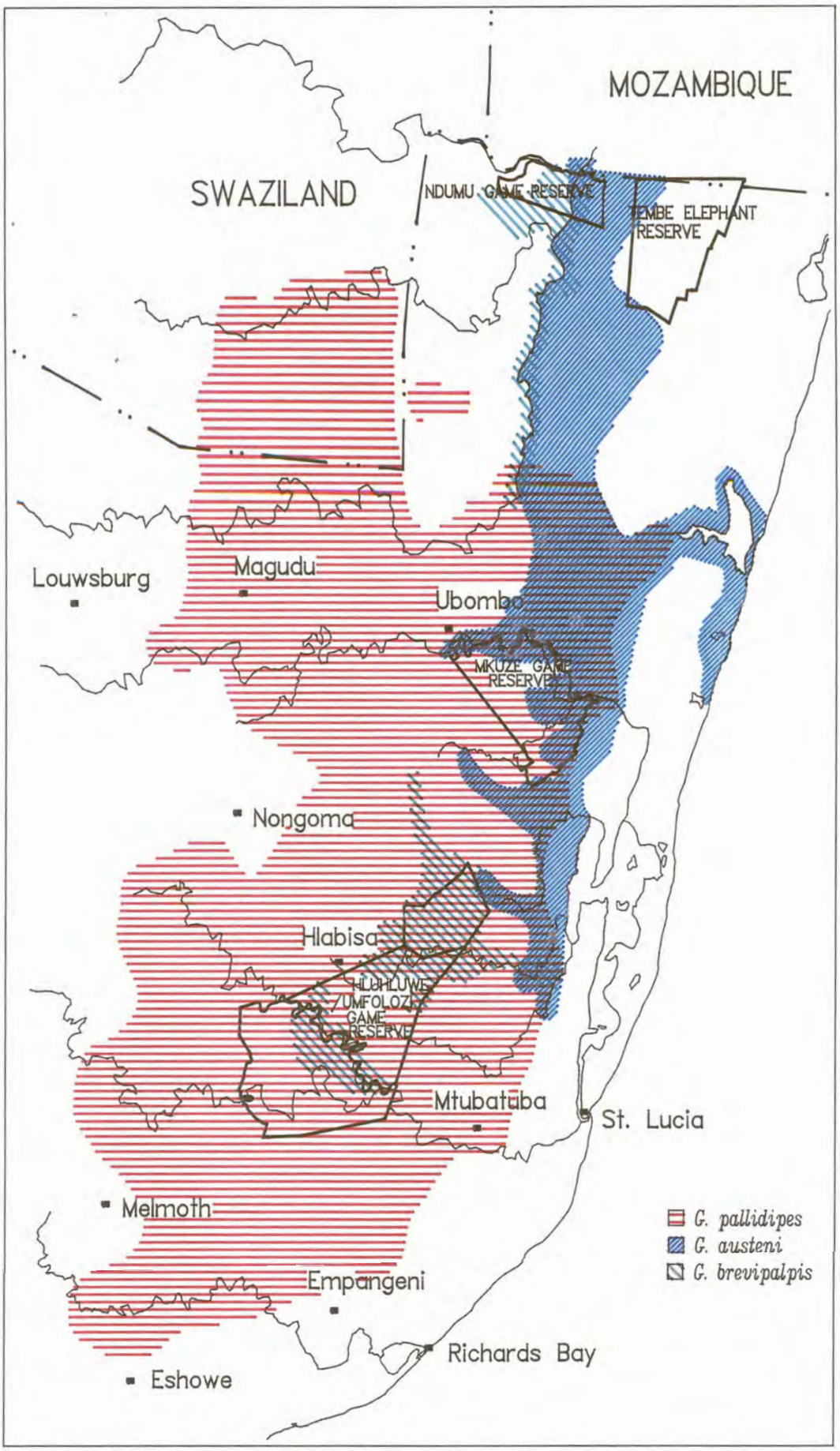


Fig. 7.1 Historical distribution of the tsetse flies *Glossina pallidipes*, *G. austeni* and *G. brevipalpis* (after Du Toit 1954)

Meaningful changes could, therefore, have occurred regarding the distribution of both tsetse species given by Du Toit in (1954) so that accurate information on the present distribution of tsetse and/or trypanosomosis in South Africa is not available. Moreover, the information that is available and obtained through tsetse surveys in the late 1940s is dated. Also, within that wide geographical range, the distribution of *G. brevipalpis* and *G. austeni* seems to be rather patchy and their abundance will vary dramatically from one location to another. Greater knowledge of the detailed distribution of these species is essential for surveillance and control measures. In order to develop a strategy to plan tsetse fly eradication or control in the region successfully it was necessary to re-survey the area to establish the present relative distribution of the two species. Such a survey was possible using technology, which has been developed elsewhere in Africa (Schonefeld 1988 cited in Hall 1990) and modified for our species (see Chapter 4).

Recently remote sensing techniques and GIS (Geographic Information System) have been utilized as means of supplementing and evaluating survey information to obtain more precise limits of distribution. GIS is an invaluable tool for integrating and manipulating available data sources into useful studies. GIS contain spatial data sets that are accessible to display, manipulation and analysis (Clarke *et al.* 1996; Ndegwa & Dwinger 1998) and has been applied to tsetse and trypanosomosis (Rogers & Williams 1993; Rogers *et al.* 1996; Robinson *et al.* 1997a, 1997b; Allsopp 1998; Robinson 1998a, 1998b; Rogers 1998; Erkelens *et al.* 2000; Hendrickx *et al.* 2000). Most of the studies referred to above used GIS to combine survey results with climate and remote sensing data to predict the distributions of the disease vectors. The critical role of GIS is to collect, rationalize and merge information in a way that facilitates data analysis to help with decision-making and to assist with planning interventions and can be used to model epidemiological problems in time and space (Erkelens *et al.* 2000).

The effective control of tsetse and trypanosomosis must be carried out in relation to the threat to cattle and land use. It is clear that a GIS would be a great help in mapping the distribution flies from survey data. Accurate tsetse distribution maps are needed if tsetse distribution data are to be integrated with

data on trypanosomosis prevalence and/or incidence, livestock distribution and current land use, in order to identify areas where trypanosomosis is a constraint to rural development and to determine high priority intervention areas. The principal function of the GIS for this study will ultimately be to manage tsetse survey data and to collate it with other relevant information. This will be used to assist with the planning and intervention of trypanosomosis and allocating resources to tsetse control, which will be dealt with in Chapter 8. The data needed to do this will be, e.g. livestock (cattle) distribution and/or abundance, trypanosomosis incidence and/or prevalence as well as land tenure/designation, landcover and vegetation types.

7.3 MATERIALS AND METHODS

7.3.1 Sampling area

The sampling/survey area extended from about 28°31' S in the south to 26°50' S in the north (up to the Mozambique border) and from about 31°40' E in the west up to the eastern coastline of N.E. KwaZulu-Natal. The area of interest has a total surface of about 12,000 km^2. It covers the magisterial districts of Ingwavuma, Ubombo, Hlabisa and parts of Nongoma. The region mainly consists of a number of Game Reserves and conservation areas, communal and commercial farming areas, State Forests and commercial plantations as well as a number of large lakes and a network of rivers. The Ubombo Mountain range lies from north to south on the western border of Swaziland and ranges southward to the southwest of the Mkuze Game Reserve (see Fig. 7.2).

Tsetse surveys were done systematically from December 1993 based on collections with XT sticky traps (see Chapter 4), which were at that time found to be the most appropriate survey tool. The surveys covered in the first phase (1993-1995) the major part of game reserves, nature conservation areas and commercial farming areas since accessibility to these areas was easy to arrange. In a following phase (1996-1999) surveys were mainly concentrated in the communal farming and diptank areas, where permission from Zulu chiefs was needed prior to surveys and where a close liaison between Animal Health Technicians and the communal populations was essential.

7.3.2 Sampling method

Survey sites were selected using 1:50,000 topographical maps. Initially the survey method that was developed in Zambia was adopted (T. Robinson, pers. comm., 1993). The 1:50,000 maps were divided in 3 x 3 minutes of latitude and longitude (*c.* 5 x 5 km 'cells'). The first consideration was to have an even distribution of traps throughout the area to be surveyed. The traps were placed systematically along roads and tracks to cover most of the 'cells'. It was attempted to place an optimum of about 4 - 5 traps/cell. However, it became clear that this system was not appropriate for detailed surveys of these particular species of tsetse fly. Because of the cryptic habitats of *G. austeni* and because of the preference of both species for dense bush, traps needed to be set in what was considered to be suitable habitat. Thereafter the most suitable tsetse habitats per survey site were sampled at about 2 km intervals (in suitable areas) or greater in apparently unsuitable areas. Traps were sited either along the edge of inaccessible bush or thickets, where possible along roads that wound through the bush, or were hung 5 - 50 metres into the bush in what was considered to be the most suitable situation for both fly species.

For practical reasons each survey lasted approximately 12 days and involved setting up to 75 traps. The traps operated over a period of 5 - 7 days. Due to the physical or ownership differences between the various areas surveyed during this period the different areas were designated as sampling or survey units. These were, for example, different farms, plantations, game reserves, diptank areas, etc. Trap sites were selected inside and adjacent to thickets and wooded areas and also in plantations where colonization of *G. brevipalpis* and *G. austeni* has taken place. The exact location of each trap site was recorded using a handheld Pyxis®, Magellan® or Garmin II Plus® Global Positioning System (GPS). In order to relocate traps quickly on returning after about five days, numbered markers (chevron tape) were tied to a tree near the trap or at the roadside near the spot where traps were hung.

Initially e.blue/l.blue XTs (as described in Chapter 4) were used in the surveys. During 1995 these were replaced by e.blue/black XTs which proved at that time to be significantly more effective for *G. austeni* (see Chapter 4).

Traps were painted with polybutene, which was first diluted with hexane for easy application. Because *G. austeni* is known to be a low-flier, traps were hung about 10 - 15 cm above the ground from tree branches. A plastic sheet was pegged below the sticky traps to collect flies that dripped off the trap over the sampling period. Traps were baited with the Zim-mix, as described in Chapter 2.2.2, to attract *G. brevipalpis*.

During the first phase of surveys a total of 11 surveys and 51 survey units were sampled. The survey units and the number of traps placed per unit for this phase are summarized in Table 7.1 in the Results. GIS coordinates for each trap placed in the various survey units are given in Appendix 1. As the first phase of the survey only included areas of easy access i.e. Game Reserves, conservation areas, game farms, commercial cattle/sugar-cane/pineapple farms, State Forests and commercial pine and eucalypt plantations, these will be referred to as "natural and commercial areas". The second phase of surveys, which covered diptank and communal farming areas, was divided into State Veterinary Areas. A total of 14 surveys consisting of 38 survey units were sampled in the Hluhluwe State Veterinary Area (i.e. Hlabisa and Nongoma Magisterial Districts) and 10 surveys consisting of 56 survey units in the Jozini State Veterinary Area (i.e. Ubombo and Ingwavuma Districts). The survey units and the number of traps placed per unit for the second phase are summarized in Table 7.2 in the Results. GIS coordinates of each trap site are also given in Appendix 1.

On the day of setting the trap the following data were recorded: date and time trap set; coordinates; map code; survey unit/locality; general vegetation type and soil type. Upon removal of the trap the two tsetse species, if present, were identified, sexed and counted. The date and time that the trap was removed was recorded. The approximate numbers of other biting flies were also recorded and then placed in 80 % alcohol and identified back in the lab.

7.3.3 Sampling data

From field data books the tsetse distribution data were entered onto tsetse data input sheets. From these the data were entered into a Disease and Vector

Integrated Database (DAVID), which was developed at Oxford University and installed at ARC-OVI for use and collaboration in its de-bugging and development to ensure that it met with operational requirements. DAVID is a geographical information management system for tsetse, trypanosomosis and livestock field data (Robinson *et al.* 1997c; Robinson 1998b). It greatly facilitates entry, display and analysis of field data, and their integration with other data within GISs. The input fields consisted of: Trap number/sample site; Longitude; Latitude; Sample method; Start date; Start time; End date; End time; Species; Flies caught (males and females). For the present work the database was used for the trap coordinates and to calculate the trapping period as well as the apparent densities expressed as numbers of flies/trap/day to be incorporated into a GIS and to facilitate mapping in ArcView GIS.

7.3.4 Base maps

Results of tsetse distribution surveys, diptank localities, landcover and vegetation types were mapped using ArcView GIS version 3.2. All other maps were produced with ArcInfo. Separate coverages of some base maps of the region, consisting of national and magisterial district boundaries, major towns, lakes, major rivers, game reserves, other major conservation areas and forestry areas, were used in the generation of the maps produced in the Results below. A background map (Fig. 7.2) shows the locality of the study area and should be referred to for locations of the magisterial districts, major game reserves and conservation areas, lakes, major rivers and the Lebombo Mountain range. Also refer to this map for the scale.

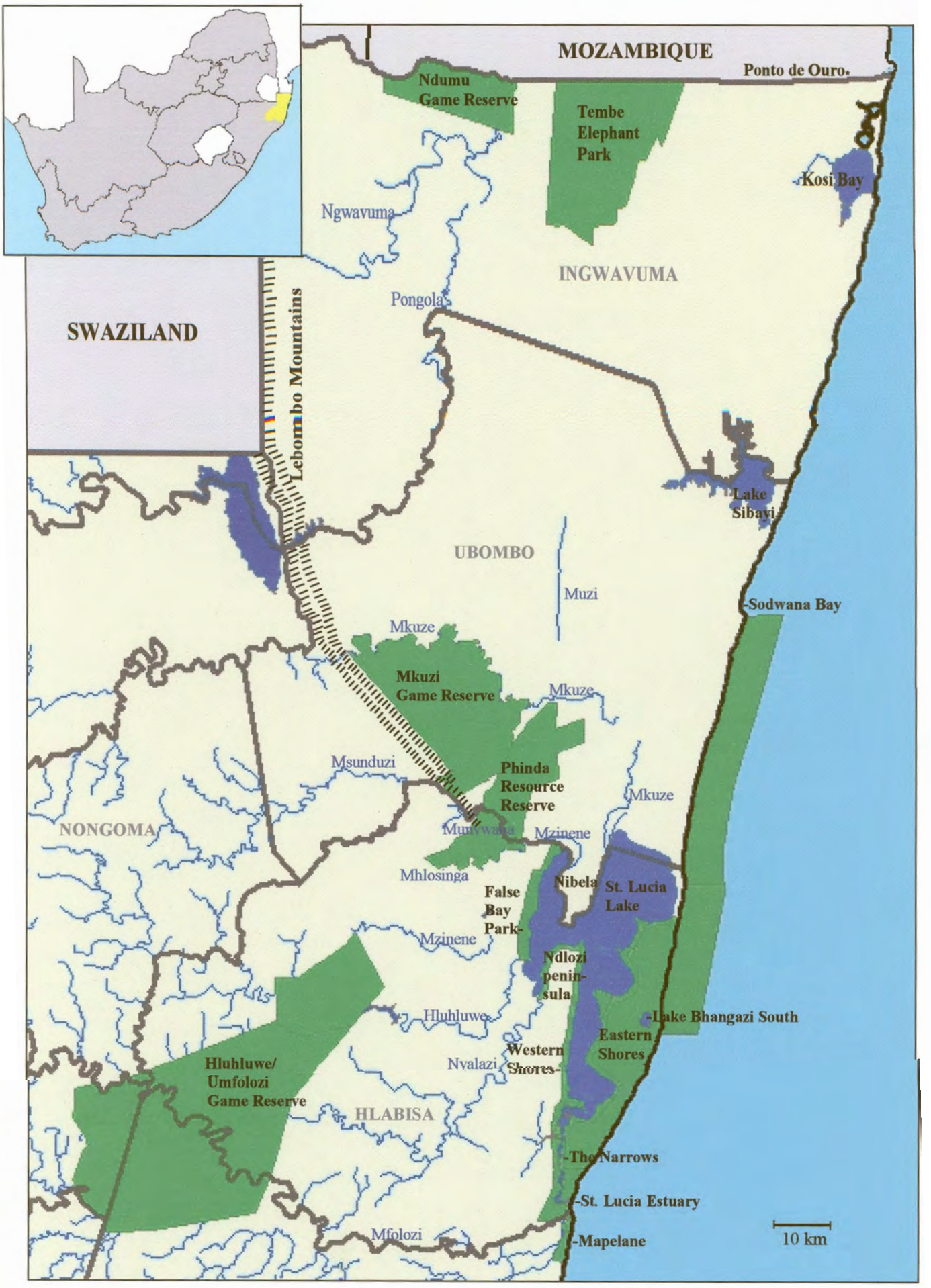


Fig. 7.2 Reference map to indicate localities of magisterial districts, major game reserves and conservation areas, lakes and major rivers

7.4 RESULTS

7.4.1 Tsetse distribution and apparent densities

Other biting flies

The sticky traps also caught a variety of flies other than tsetse flies. Some of these were specifically attracted to these traps (as evidenced by their numbers) and were therefore, identified to family or genus level and recorded. Many of these flies are also known to feed on the blood exudates and lachrymal secretions of livestock and wild animals (Lancaster & Meisch 1986). Their identification, especially the biting flies, is important for two main reasons. Firstly, because of their possible role in the mechanical transmission of trypanosomosis (Jordan 1986). Secondly, because they, especially blood-dependent *Stomoxys* spp., are an indicator that the flies had access to host animals nearby. This confirms that the absence of tsetse flies on such traps cannot be attributed to a lack of host animals. The biting flies identified were Tabanidae (*Tabanus* spp., *Haematopota* spp., *Philoliche* spp. and other Tabanidae) as well as the Stomoxyinae (*Stomoxys nigra*, other *Stomoxys* spp., *Parastomoxys* spp., *Prostomoxys* spp., *Haematobia* spp. and *Haematobosca* spp.). So-called non-biting flies of the subfamily Muscinae (*Musca* spp., *Morellia* spp. and others), although fewer in numbers than the biting flies, were identified to genus. The present study will concentrate only on analysis of the tsetse fly catches.

Tsetse positive/negative trapping sites

The e.blue/l.blue and e.blue/black XTs, developed in Chapter 4, were successful in surveying the distribution of both *G. austeni* and *G. brevipalpis*. A summary of the surveys with details of survey units, month and year of survey, the number of traps operated and the number of positive traps for each species is given in Table 7.1 for natural and commercial areas and in Table 7.2 for communal farming areas. The distribution of the two species was mapped (Fig. 7.3) according to catches of flies in terms of fly presence (one or both species) and where zero catches were obtained. Details of the survey results

are discussed separately for the natural and commercial areas and for the communal areas. The results will be discussed under three main geographical areas, i.e. south (Hlabisa and Nongoma Districts), central (Ubombo District) and north (Ingwavuma District).

- **Natural and commercial areas**

A total of 514 traps were placed in the 51 survey units sampled in the natural and commercial areas. Refer to Fig. 7.2 for locations of game reserves, lakes and rivers and to Table 7.1 for the details of the survey units and the survey number. (The number of positive traps for each species was also given in the Table. Detailed summaries of results at each trap site are given in Appendix 1.)

SOUTH

Around Lake St. Lucia (surveys no. 1, 7 & 10), *G. brevipalpis* was found as far north as northern False Bay Park, as far south as St. Lucia Estuary and along the western and eastern shores of the lake. *G. austeni* was found accordingly except on the Eastern Shores and to the west of The Narrows of Lake St. Lucia (namely Dukuduku/ Futululu Forest area) where only *G. brevipalpis* was caught.

The survey (no. 4), which filled the gap between the rivers in the central area and the Hluhluwe River in the south, included the Mhlosinga, Mzinene, Ngweni (north of Mzinene – not indicated in Fig. 7.2) and Ncemane (north of Hluhluwe River – not indicated in Fig. 7.2) rivers.

Table 7.1 Summary of survey units surveyed in natural and commercial areas* (Details of trap localities and catches in each survey unit is given in Appendix 1)

Survey	Survey unit (locality)	No. of traps set	No. of positive traps	
			G.brevipalpis	*G. austeni*
1) Dec 1993	Nyalazi plantation (Mondi Forest, SAFCOL)	26	20	15
	Dukuduku plantation (SAFCOL)	6	1	-
	Fernwood plantation (Mondi Forest)	7	2	-
	Shire plantation (Mondi Forest)	8	-	-
	Futululu Research Station (CSIR-Forestek)	7	1	-
	Boomerang (cattle farm)	8	-	-
	Total	**62**	**24**	**15**
2) Feb 1994	Mkuzi Game Reserve (KZNNCS)	43	-	8
	Sodwana State Forest	13	-	4
	Phinda Resource Reserve	20	-	16
	Total	**76**	**0**	**28**
3) May 1994	Sungulwane Game Lodge (game and cattle)	3	-	-
	Mduna Estates (game and cattle farm)	4	-	-
	Sipofu (cattle farm)	3	-	-
	Kube Yini (game farm)	6	-	2
	Sutton (game farm)	5	-	3
	Panata (game farm)	5	-	5
	Mziki (game farm)	2	-	2
	Zulu Nyala (game farm)	5	-	5
	Total	**33**	**0**	**17**
4) Jun 1994	False Bay Park (KZNNCS)	13	8	12
	Kuleni (ARC-ITSC pineapple research farm)	5	-	5
	Somerset (cattle farm)	6	-	4
	Doringkuil (cattle farm)	5	-	1
	Ezulwini (game farm)	4	-	4
	Bonamanzi (game farm)	5	1	5
	HH Ranch (cattle farm)	4	-	-
	Ubizane Game Reserve (game farm)	3	1	2
	Total	**45**	**10**	**33**
5) Sept 1994	Ndumu Game Reserve (KZNNCS)	34	25	8
	Tembe Elephant Park (KZNNCS)	29	-	3
	Total	**63**	**25**	**11**
6) Jan 1995	Hluhluwe-Umfolozi Game Reserve (KZNNCS)	71	58	-
	Total	**71**	**58**	**0**
7) Jan 1995	Eastern Shores (KZNNCS)	15	14	-
	Mapelane (KZNNCS)	8	1	1
	St. Lucia	3	3	-
	Boomerang (cattle farm)	5	-	-
	Southern limit farms	6	-	-
	Teza plantation (Mondi Forest)	4	-	-
	Shire plantation (Mondi Forest)	3	-	-
	Fernleas plantation (Mondi Forest)	5	-	-
	Nyalazi plantation (SAFCOL)	5	2	2
	Dukuduku plantation (SAFCOL)	11	5	-
	Futululu plantation (Sappi Forest)	6	-	-
	Futululu Research Station (CSIR-Forestek)	4	-	-
	Total	**75**	**25**	**3**

Survey	Survey unit (locality)	No. of traps set	No. of positive traps	
			G.brevipalpis	*G. austeni*
8) Jun 1995	Sodwana Bay Park (KZNNCS)	14	-	-
	Lake Bhangazi North	9	-	-
	KwaMbila	7	-	-
	Lake Sibaya (KwaZulu-Natal Coastal Reserve)	14	-	-
	Manzengwenya (")	3	-	-
	Mabaso plantation (KwaZulu-Natal Forestry)	4	-	-
	Mbazwane plantation (")	12	-	-
	Manzengwenya plantation (")	8	-	-
	Total	**71**	**0**	**0**
9) Oct 1996	Ndumo Game Reserve (KZNNCS)	10	4	5
	Total	**10**	**4**	**5**
10) Nov 1996	Hellsgate Military Base (SANDF)	8	8	6
	Total	**8**	**8**	**6**
11) Apr 1998	Lake Sibaya (KZN Coastal Reserve)	10	0	0
	Total	**10**	**0**	**0**
	Grand Total	**524**	**154**	**118**

*Natural and commercial areas: included areas of Game Reserves, conservation areas, game farms, commercial farms, State Forests and commercial pine and eucalypt plantations.

These flow in various directions but all eventually enter the Mzinene and flow through a treeless flood plain into the northern part of False Bay Park (Lake St. Lucia). Both species of tsetse were captured. *G. austeni* was present in False Bay Park and on all the farms except HH Ranch (28°04'45"S 32°12'58"E), which may, however, not have been adequately surveyed (farms are not indicated on maps but listed in Table 7.1). *G. brevipalpis* was restricted to False Bay Park, Bonamanzi (which adjoins it) and Ubizane Game Reserve (along the Mzinene River).

Only *G. brevipalpis* was collected in the Hluhluwe-Umfolozi Game Reserve (survey no. 6). Despite traps being set in suitable *G. austeni* habitat in these areas no *G. austeni* were collected. Only half the traps were positive in the Umfolozi area, which were concentrated mainly around the rivers, in *Spirostachys* sp. thickets and along drainage systems. The remaining area has a more savanna type vegetation, especially in the southwest where all traps were negative. The southern part of Umfolozi Game Reserve is a declared Wilderness Area and no roads exist in this part of the reserve. As it was not possible to set traps in this area, the

southernmost distribution of *G. brevipalpis* in the reserve could not be established.

To determine the southern limits of tsetse distribution, a survey (no. 7) included areas south and north of the Mfolozi River as well as forestry areas north of the river. Single specimens of each of *G. brevipalpis* and *G. austeni* were caught at Mapelane. They were the only tsetse caught south of the Mfolozi River and were collected in coastal dune forest. On the northern side of Mfolozi single specimens of *G. brevipalpis* were caught but no *G. austeni*. In the Safcol Dukuduku plantations, to the west of St. Lucia Estuary, only *G. brevipalpis* was trapped. No tsetse were collected in the plantations of the southwesterly areas.

CENTRAL

G. austeni was also found to the east of the Lebombo Mountains (see locality on Fig. 7.2) throughout parts of the Mkuze Game Reserve, the north-western part of Sodwana State forest (see locality west of Sodwana Bay in Fig. 7.7) and Phinda Resource Reserve (survey no. 2). The Sodwana State Forest area is used for cattle grazing. Most of this area is sandy with copses of dense bush. The traps that were positive in the Mkuze Game Reserve were mostly placed in the sandveld area where vegetation is dense and in the north-east in a well wooded area near the Mkuzi river (E.M. Nevill & J. Greger, unpublished report, 1994; also see Fig.7.9). Phinda Resource Reserve consists, in the north, mostly of dense sand bush and sand forest while in the south it contains mostly mixed bush ranging from *Acacia*, *Combretum*, *Schotia* to *Spirostachys* (also see Fig. 7.9). The southern part has three rivers (Munywana, Mzinene and Mhlosinga) and traps were often set near these rivers or in bushed gullies leading to these rivers. Of much significance was the collection of *G. austeni* along the Mhlosinga River at a point where it breaks through the last hills of the Lebombo Mountains. No *G. brevipalpis* were found in these areas to the east of the Lebombo even though traps were set along rivers (Mkuzi, Msunduzi, Munywana, Mzinene and Mhlosinga) where

they could be expected to occur (nor west of the Lebombo Mountains) (E.M. Nevill & J. Greger, unpublished report, 1994).

A survey (no. 3) west of the southern extent of the Lebombo Mountains, an area which included both game farms and/or cattle ranches, revealed that 50 % traps placed in this area were positive for *G. austeni*. No tsetse were caught along the Msunduzi River although *G. austeni* was caught on an adjoining game farm in a wooded gully leading to the river. The absence of tsetse along the Msunduzi is thought to be due to dipping of cattle with pyrethroids at that time in the diptank area (diptank no. 766 – see Fig. 7.6) along the river. The traps placed along the Mhlosinga and Munywana rivers west of the Lebombo Mountains were positive for *G. austeni* but not for *G. brevipalpis*.

The survey (no. 8) in the Lake Sibayi/Sodwana Bay area (June 1995) included coastal reserves (dune forests) as well as other natural and forestry areas between Lake Sibayi to Lake Bhangazi North (situated about halfway between Lake St. Lucia and Lake Sibayi – not indicated on the map). The plantations concerned were Mabaso, Mbazwana and Manzengwenya to the south and north of Lake Sibayi (see localities in Figs. 7.7 and 7.8). No tsetse flies were trapped during this survey. The narrow dune forest strip between Lake Sibayi and the Indian Ocean, which seemed to be suitable tsetse habitat, was re-surveyed in April 1998. Once again no flies were caught. Also, no sign of wild animals or cattle was seen in that strip.

NORTH

G. austeni was present at both Ndumu Game Reserve and Tembe Elephant Park (surveys no. 5 & 9). However, only 11 of the 63 traps set were positive and numbers low. This is probably a reflection of reduced habitat due to many leafless trees at the time surveyed (Sept. 1994). *G. brevipalpis* was only present in Ndumu.

- **Communal areas**

Surveys of communal areas followed the completion of surveys in most of the conserved and commercial areas. A total of 24 surveys was carried out in which 644 traps were placed in the communal areas surrounding 79 diptank localities. Of these, 311 traps were set in the Hluhluwe State Veterinary Area (34 diptank localities) and the remaining traps were set in the Jozini S.V. Area (also see Table 7.2). Details of trap sites and catches are given in Appendix 1.

SOUTH

In the Hluhluwe S.V. Area (Hlabisa & Nongoma Districts - surveys no. 1 - 14) only one diptank was found positive for *G. austeni*, namely Qakweni (diptank no. 692 – see Fig. 7.6), where traps were actually set across the Nyalazi River in a conserved area. *G. brevipalpis* was widespread with 21 of the 34 diptank localities being positive (positive diptanks are those numbered in Fig. 7.6). The average number of *G. brevipalpis* collected on each of the 71 positive traps (out of 300) was 4,34.

CENTRAL

In the Jozini S.V. Area the Ubombo magisterial district was covered by surveys 15 - 21 (also see Table 7.2). The Makhathini Flats cover a great deal of the area. The vegetation in the area consists of thicket and bushland as well as forest and woodland (see Fig. 7.9). *G. austeni* was collected at 8 of 21 diptank localities. No *G. brevipalpis* were collected.

NORTH

In the northern parts of Jozini S.V. Area (Ingwavuma district), surveys 22 - 24 were covered (also see Table 7.2). *G. austeni* was present in six and *G. brevipalpis* in seven of 24 diptank localities. The vegetation and topography ranged from coastal dune forest, coastal lakes and marsh areas

Table 7.2 Summary of survey units (diptank localities) surveyed in communal farming areas. (Details of trap localities and catches in each survey unit is given in Appendix 1)

Survey	Survey unit (diptank locality)	No. of traps set	No. of positive traps	
			G.brevipalpis	*G. austeni*
	HLUHLUWE STATE VETERINARY AREA:			
1) Oct 1995	Mahiya 517	2	-	-
	Qakweni 692	3	-	-
	Ngodini 944	6	3	-
	Total	**11**	**3**	**0**
2) Jan 1996	Ngodini 944	10	1	-
	Total	**10**	**1**	**0**
3) Feb 1996	Mzinene 526	7	2	-
	Qakwini 692	10	8	-
	Mahiya 517	7	3	-
	Gunjaneni 523	9	4	-
	Mvutshini 945	10	9	-
	Hlazane 519	10	1	-
	Machibini 746	3	3	-
	Total	**56**	**30**	**0**
4) Mar 1996	Nhlwathi 525	9	-	-
	Mquthungu 726	9	4	-
	Hlambanyathi 754	6	2	-
	Total	**24**	**6**	**0**
5) Apr 1996	Mpenbeni 528	8	4	-
	Gwegwede 524	10	-	-
	Sangoyana 946	10	2	-
	Total	**28**	**6**	**0**
6) Nov 1996	Ngwenyambili 778	7	4	-
	Hluhluwe 518	5	-	-
	Total	**12**	**4**	**0**
7) Oct 1996	Makhatini communal areas	6	-	-
	Total	**6**	**0**	**0**
8) Mar 1997	Mahlabinyathi 963	9	7	-
	Sakwini 842	10	-	-
	Bukhipha 962	10	-	-
	Total	**29**	**7**	**0**
9) Jun 1997	Matshamhlophe 326	10	1	-
	Mfanelo 327	7	-	-
	Qunwane 964	6	-	-
	Mgangado 694	6	-	-
	Total	**29**	**1**	**0**
10) Jan 1998	Dabedabe 527	5	-	-
	Total	**5**	**0**	**0**
11) Mar 1998	Nkolokotho 744	10	3	-
	Hoho 522	9	-	-
	Nyalazi 520	10	-	-
	Dukuduku 967	10	1	-
	Gwedla 669	10	2	-
	Total	**49**	**6**	**0**
12) Apr 1998	Nsane 521	9	-	-
	Nomathiya 966	10	-	-
	Total	**19**	**0**	**0**
13) May 1998	Kwamsane 323	10	1	-
	Ekuphudisweni 328	7	4	-
	Total	**17**	**5**	**0**
14) Jun 1998	Mpanzakazi 325	6	2	-
	Total	**6**	**2**	**0**

Survey	Survey unit (diptank locality)	No. of traps set	No. of positive traps	
			G.brevipalpis	*G. austeni*
	JOZINI STATE VETERINARY AREA:			
15) Oct 1996	Zidlele 701	3	-	-
	Zineshe 743	3	-	-
	Biva 936	3	-	-
	Total	**9**	**0**	**0**
16) May 1997	Zidlele 701	10	-	-
	Zineshe 743	10	-	3
	Biva 936	10	-	-
	Total	**30**	**0**	**3**
17) Mar 1998	Siphondweni 819	2	-	-
	Hlazane 937	7	-	-
	Mozane 938	11	-	-
	Total	**20**	**0**	**0**
18) Apr 1998	Munyu 820	11	-	1
	Mkhumbikazana 514	10	-	-
	Mseleni 512	10	-	8
	Total	**31**	**0**	**9**
19) May 1998	Manaba 500	20	-	13
	Mbazwana 513	4	-	3
	Ntenga 678	1	-	-
	Nibela 510	5	-	-
	Masakeni 679	4	-	2
	Total	**34**	**0**	**18**
20) Oct 1998	Zineshe 743	5	-	2
	Nibela 510	10	-	-
	Masakeni 679	9	-	2
	Mpempe 302	10	-	-
	Nkomo 698	7	-	-
	Total	**41**	**0**	**4**
21) Nov 1998	Biva 936	3	-	-
	Siphondweni 819	5	-	1
	Hlazane 006	4	-	1
	Munyu 820	6	-	-
	Mbazwana 530	5	-	1
	Mpini 849	1	-	-
	Manzibomvu 813	10	-	-
	Total	**34**	**0**	**3**
22) Apr 1999	Shemula 494	4	-	-
	Madlakude 803	5	-	2
	Namaneni 814	14	1	-
	Mengu 802	5	2	2
	Nhlathu 906	1	-	1
	Mpophomeni 682	1	-	-
	Phelandaba 503	3	-	-
	Kosibay 722	2	1	-
	Thengane 908	1	-	-
	Ndumu 130	2	-	-
	Nhlanjwana 320	6	2	-
	Ntabayengwe 827	8	-	-
	Nkawini 896	1	-	-
	Ezulwini 311	3	-	-
	Total	**56**	**6**	**5**
23) May 1999	Mahhashi 895	5	-	-
	Malangeni 864	11	2	-
	Thengane 908	3	-	-
	Mloli 683	14	4	1
	Phelandaba 503	3	1	1
	Manzibomvu 907	4	0	1
	Kosibay 722	8	3	0
	Total	**48**	**10**	**3**

Survey	Survey unit (diptank locality)	No. of traps set	No. of positive traps	
			G.brevipalpis	*G. austeni*
24) Nov 1999	Mlambongwenya 506	4	-	-
	Lubambo 789	5	-	-
	Singeni 787	5	-	-
	Mpeshane 898	2	-	-
	Mzinyeni 497	6	-	-
	Manqwashu 732	7	-	-
	Total	**29**	**0**	**0**
	Grand Total	**633**	**87**	**45**

in the east, to Lala palm grasslands in the central area (see Fig. 7.9) and sand forest near Tembe (see Fig. 7.10).

The Muzi depression (wetland - see Fig. 7.9) extends fingerlike south from the Mozambique border to south of Phelandaba (diptank no. 503 – see Fig. 7.6). Forest intrusions extended southwards from Mozambique into the more northerly areas (see Fig. 7.9). More to the west lie a number of pans, which form part of the Pongola River system. The results suggested an absence of tsetse along the Ingwavuma River and a north to south distribution of *G. brevipalpis* just south of Ndumu Game Reserve on the Pongola River and eastwards to the southwestern corner of Tembe Elephant Park. *G. brevipalpis* was also present in many parts of the area between Tembe and Ponto de Ouro border post (see locality in Fig. 7.2) in association with the forest intrusions and Muzi depression. The highest numbers of this species were recorded in Kosi Bay Reserve. *G. austeni* was found east from Pongola River to the southern boundary of Tembe. It was also collected in sandy bush areas roughly between Phelandaba and the Mozambique border, not very far from Tembe Elephant Park. No *G. austeni* were collected in the Kosi Bay coastal lake strip. No *G. brevipalpis* nor *G. austeni* were found in a survey concentrated west of the Pongola River, south of the Ingwavuma River and east of the Lebombo Mountains, mainly due to a lack of suitable tsetse habitat.

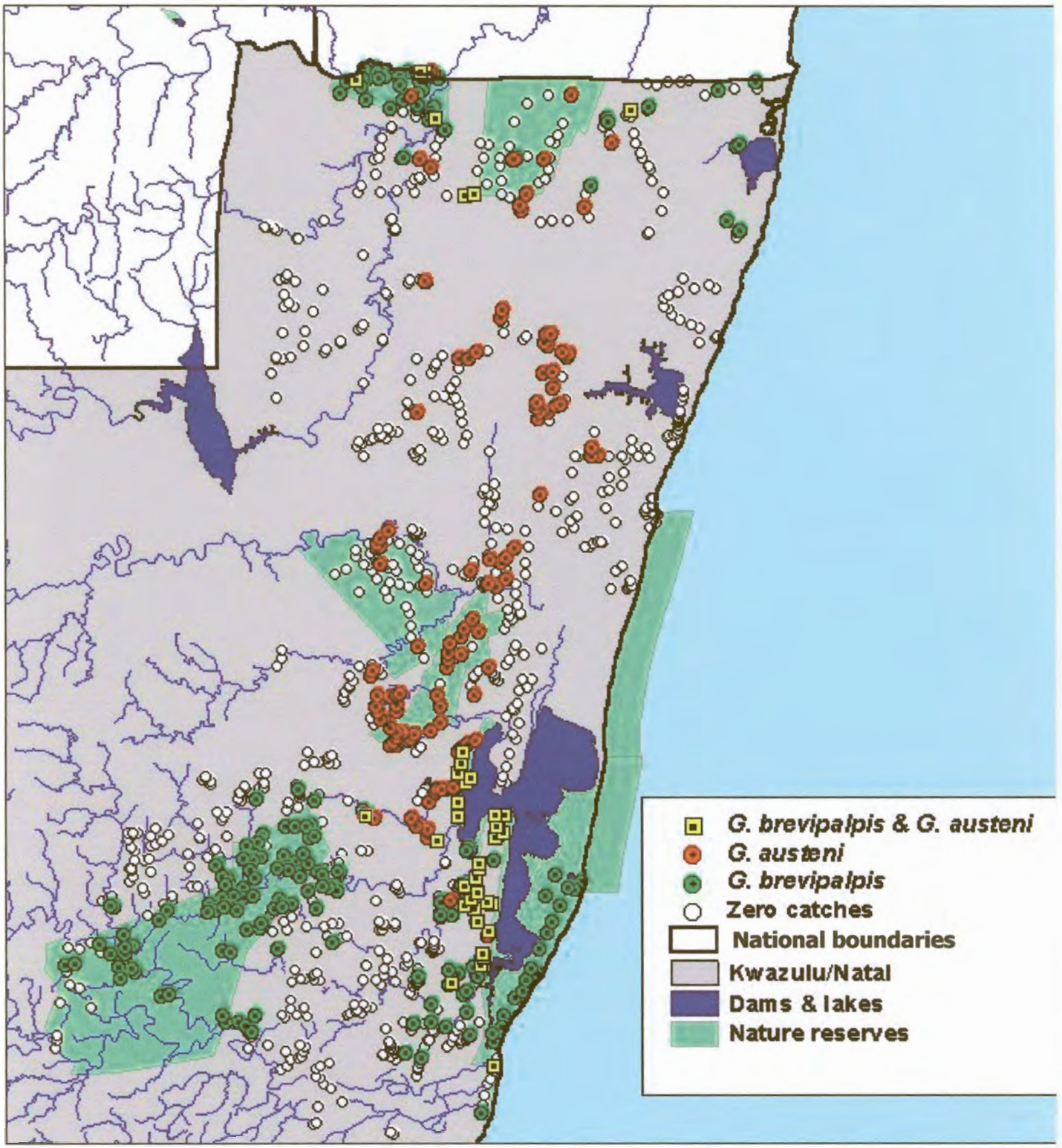


Fig. 7.3 Distribution of *Glossina brevipalpis* and *G. austeni* expressed as positive and negative trap catches

Apparent densities

To compare the apparent tsetse densities in the areas surveyed, catches were transformed to the number of flies/trap/day (also see Appendix 1). Maps showing the apparent densities of *G. brevipalpis* (Fig. 7.4) and *G. austeni* (Fig. 7.5) were generated using five density classes: for *G. brevipalpis* 0; 0,09 - 1,89; 1,89 - 4,87; 4,87 - 9,87 and 9,87 - 24,01 and for *G. austeni* 0; 0,11 - 2; 2 - 5; 5 - 10 and 10 - 15.

The maps might not be a true reflection of apparent densities for the two species, since surveys were conducted over several years and during various months of the year, although surveys were specifically not carried out during the cool dry season when tsetse numbers are at their lowest. Notwithstanding these short-comings it is still clear that both *G. brevipalpis* (Fig. 7.4) and *G. austeni* (Fig. 7.5) were both more plentiful in game reserves and other natural areas than in surrounding communal farming areas. Most *G. brevipalpis* (Fig. 7.4) were captured in an old established pine plantation in Eastern Shores of Lake St. Lucia (i.e. 24,01 and 21,47 flies/trap/day). *G. austeni* (Fig. 7.5) was most abundant in False Bay Park (12,76 flies/trap/day) and in a natural area adjacent to Lake St. Lucia (on the Nyalazi River – 11,14 flies/trap/day). It was noted during the survey that high tsetse populations usually accompany the presence of large numbers of wild animals, such as in game reserves.

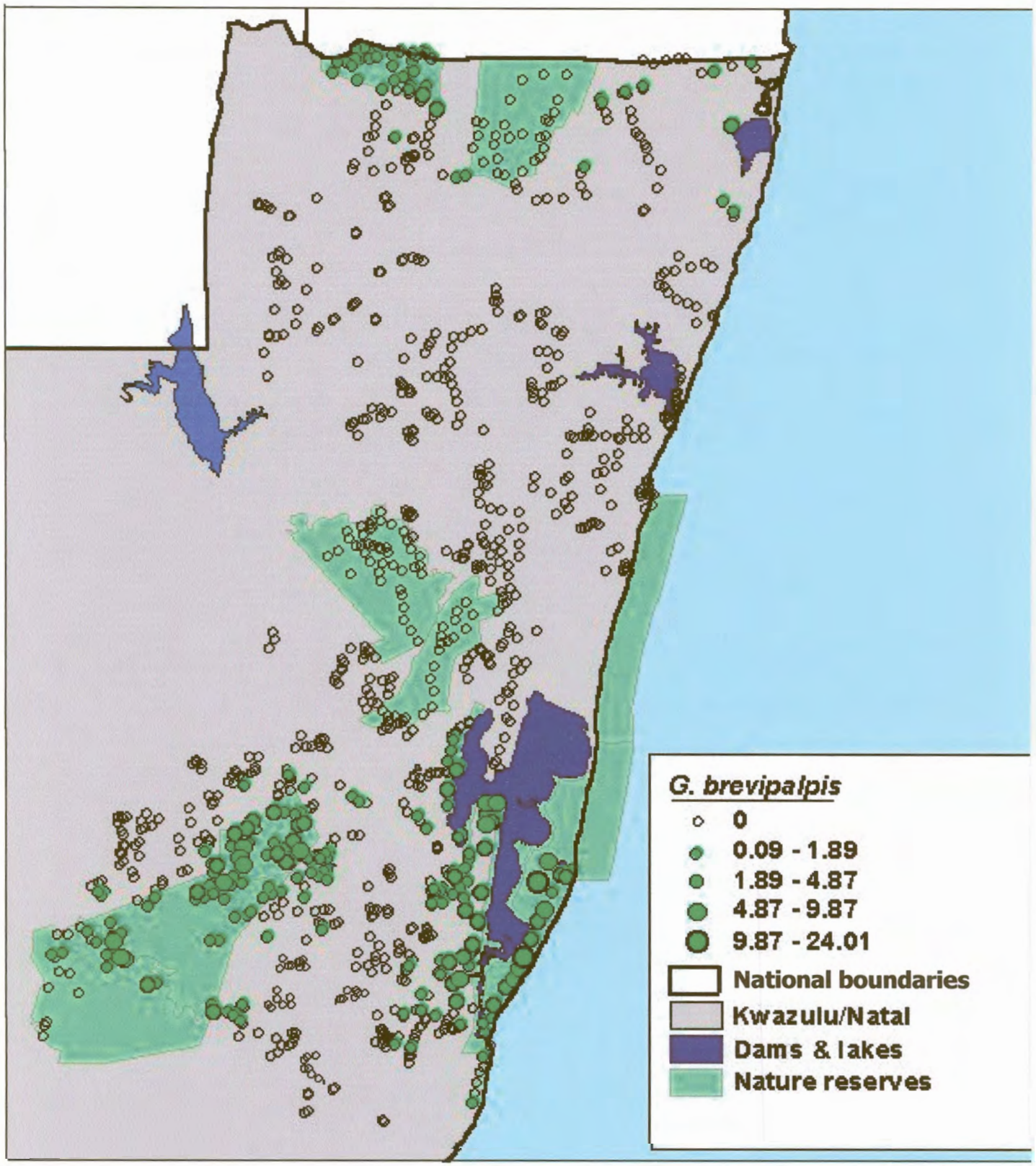


Fig. 7.4 Apparent density of *Glossina brevipalpis* expressed as the number of flies/trap/day

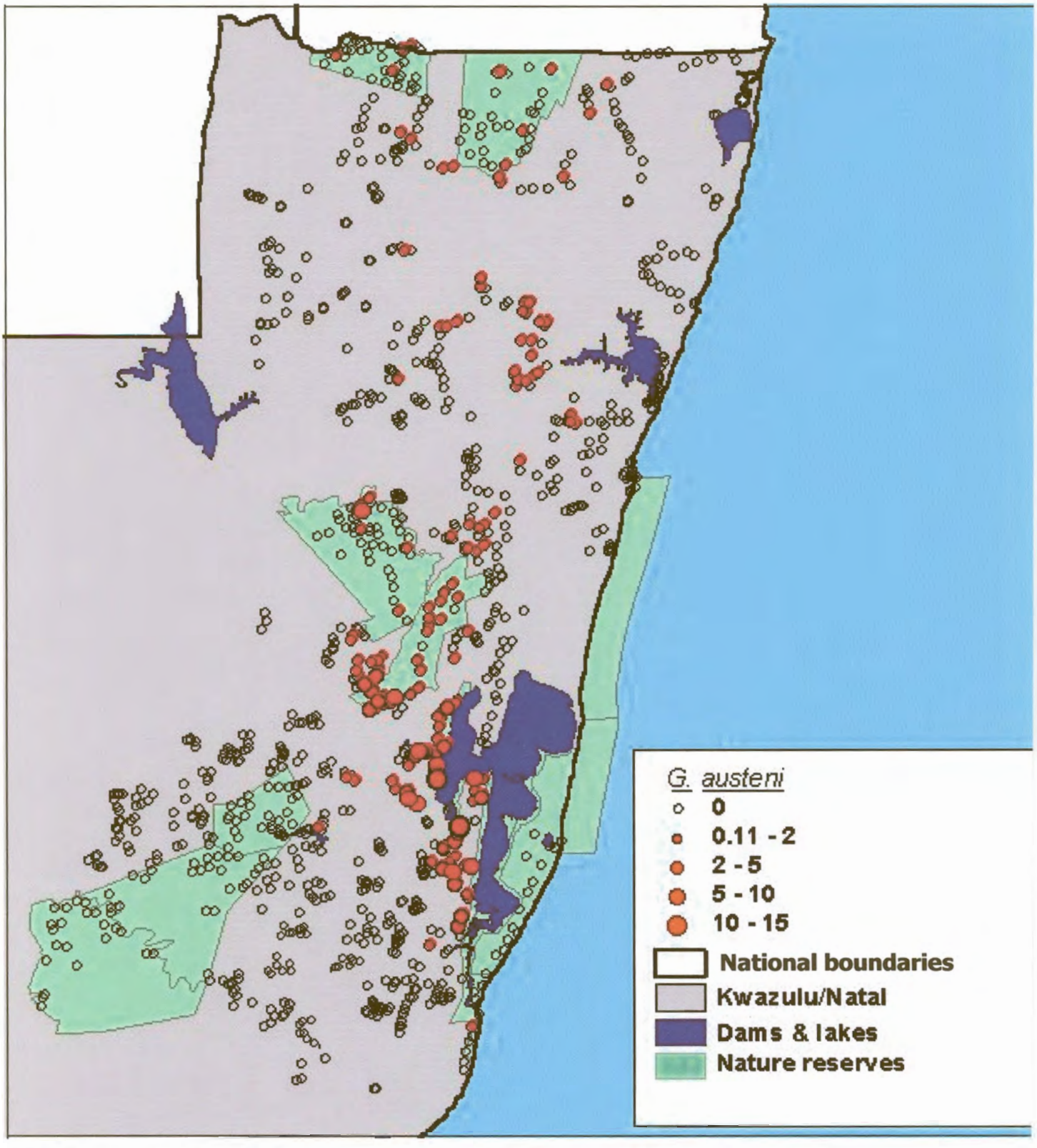


Fig. 7.5 Apparent density of *Glossina austeni* expressed as the number of flies/trap/day

7.4.2 Livestock distribution

Limited livestock census data are available for the area of concern so that accurate cattle densities could not be established and mapped (R. Williams, pers comm., 2001). Although some data are available on the number of cattle served by each diptank, these data are not accurate and will not be presented here. However, there is a network of plunge dips for the control of ticks on cattle in the region and cattle are allocated to diptanks according to the nearest walking distance. Topography and rivers/waterbodies also play a role in deciding diptank allocation (R.J. Bagnall, pers. comm., 2001). Coordinates of the diptanks in the area of interest (Hlabisa, Ingwavuma, Mhlabatini, Nongoma and Ubombo) were obtained (G.C. Bishop, Allerton Veterinary Laboratory, KZN Veterinary Services) and entered into DAVID. The diptank positions are given in Fig. 7.6.

The map of the distribution of diptanks can give a good estimate of the distribution of the *c.* 350,000 cattle belonging to communal farmers in the tsetse infested area, since they graze in permitted areas surrounding the game reserves and other nature conservation and forest areas.

It is assumed that where cattle, visiting a diptank, were diagnosed positive for trypanosomosis then all cattle in that diptank area were at risk of contracting nagana. Diptank areas that were found positive for tsetse are those numbered in Fig. 7.6 (number of traps positive are given in Table 7.2). They were the following: in Ingwavuma - 802, 803, 814, 906, 503, 907, 683, 684, 323, 722 and 937; in Ubombo - 819, 500, 512, 820, 937, 936, 960, 513, 813, 743, 679 and 766; in Hlabisa - 726, 528, 946, 325, 523, 328, 326, 944, 526, 517, 945, 519, 746, 744, 323, 669, 967, 963, 962 and 788; and in Nongoma - 754.

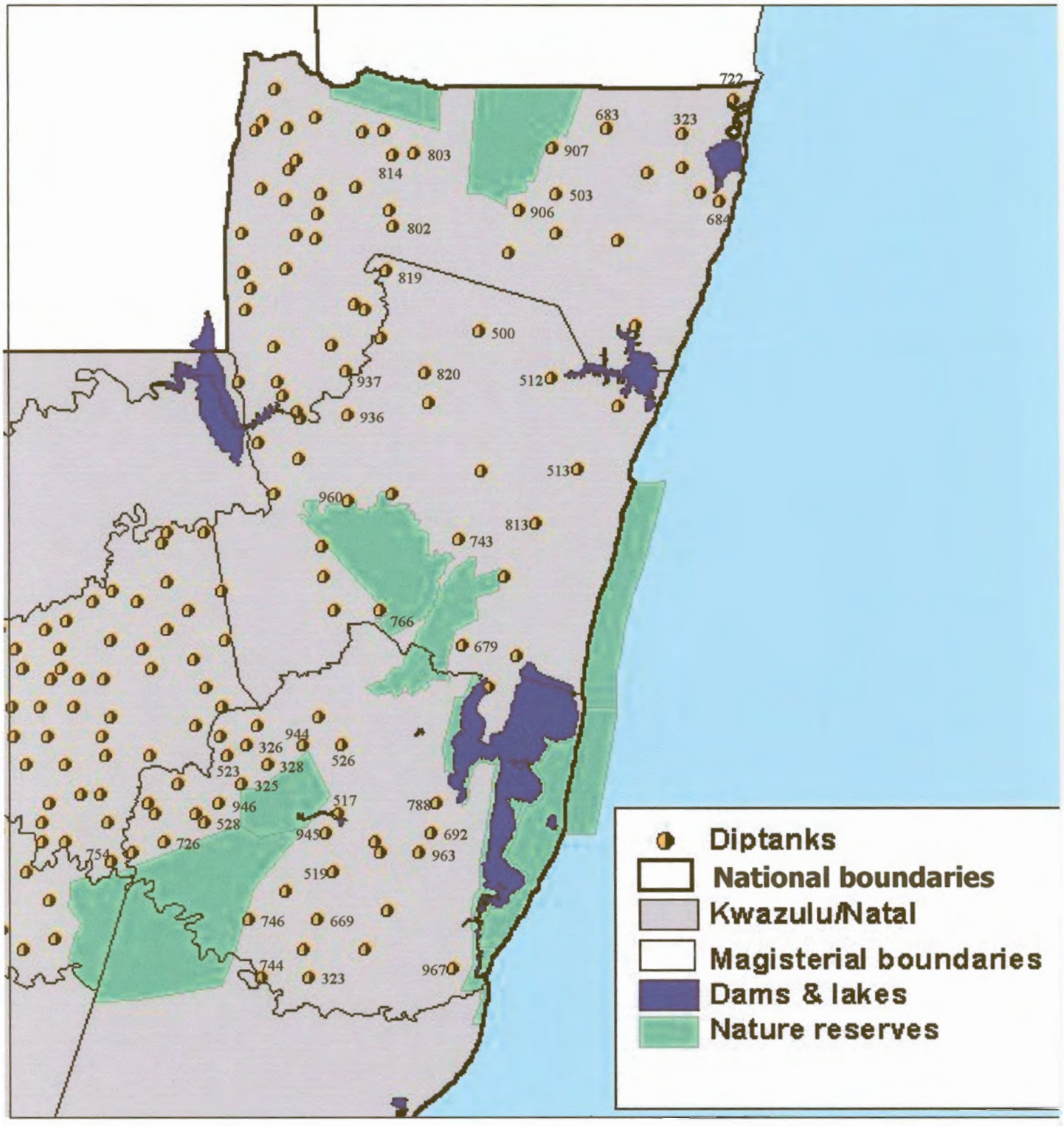


Fig. 7.6 Diptank positions in magisterial districts of Ingwavuma, Ubombo, Hlabisa, Nongoma and Mhlabathini indicating the distribution of cattle of communal farmers (diptank areas positive for tsetse during surveys are numbered)

7.3.5 Trypanosomosis distribution and prevalence

In 1990 trypanosomosis was diagnosed in cattle served by diptanks close to Hluhluwe-Umfolozi Game Reserve (Bagnall 1993). These were infected with both *Trypanosoma congolense* and *T. vivax*. A survey of infection in cattle was started in May 1990, making use of thick and thin blood smears from cattle showing signs of chronic trypanosomosis, to determine the extent of the outbreak. By the end of 1992, infection was found at 61 out of 132 diptank areas between the Mfolozi River and the Mozambique border (Carter 1993; 1994; Bagnall 1993) and 16 additional diptank areas of infection were confirmed by 1994 (R.F. Carter, unpublished information, 1998). Fig. 7.7 is a summary of trypanosome infected areas based on Carter's (1993, 1994) results of positive and negative diptank areas. The areas endemic for trypanosomosis extended from Mozambique in the north to the Mfolozi River in the south, including the low lying areas in Ingwavuma, Ubombo and Hlabisa districts as well as those areas adjacent to the Hluhluwe-Umfolozi Game Reserve in the Mahlabathini and Nongoma districts. This map also highlights the fact, as mentioned under 7.4.2, that cattle are uniformly distributed around diptanks except in game reserves and forest areas (where grazing is prohibited).

Fifty-nine diptank areas were again surveyed in 1994 (Bagnall 1994). The geographical prevalence of *Trypanosoma* spp. as determined by Ag-ELISA and BCT (Buffy Coat Technique) is summarized in Fig. 7.8 (from De Waal *et al.* 1998). According to BCT results the highest trypanosomosis prevalence (10 - 15 % and > 15 %) was in the Ubombo district. Medium prevalence (4 - 10 %) was indicated in the Ingwavuma District south of the Ndumu Game Reserve, east of the Pongola River and in the southern parts of Ubombo. Low (0 - 2 % and 2 - 4 %) to zero prevalence occurred in areas surrounding the Hluhluwe-Umfolozi Game Reserve (Hlabisa and Nongoma districts) and also west of the Pongola River (Ingwavuma district). The estimated prevalence of trypanosomosis as determined by Ag-ELISA (Fig. 7.8) indicated generally higher prevalence in all areas, with Ingwavuma and Ubombo Districts being the highest.

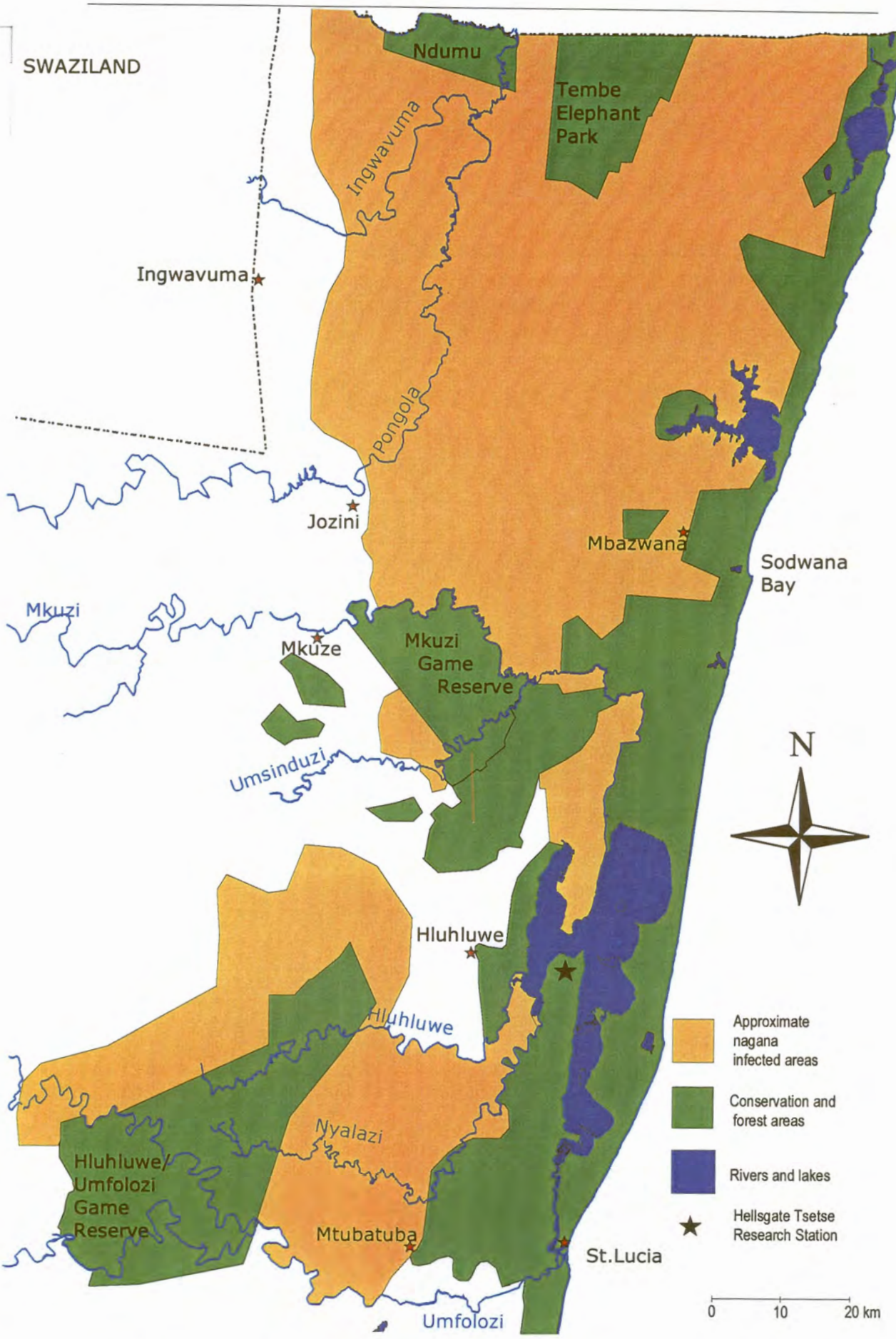


Fig. 7.7 Approximate distribution of cattle affected by trypanosomosis during 1990 - 1992 in N.E. KwaZulu-Natal (drawn from results from Carter 1993, 1994)

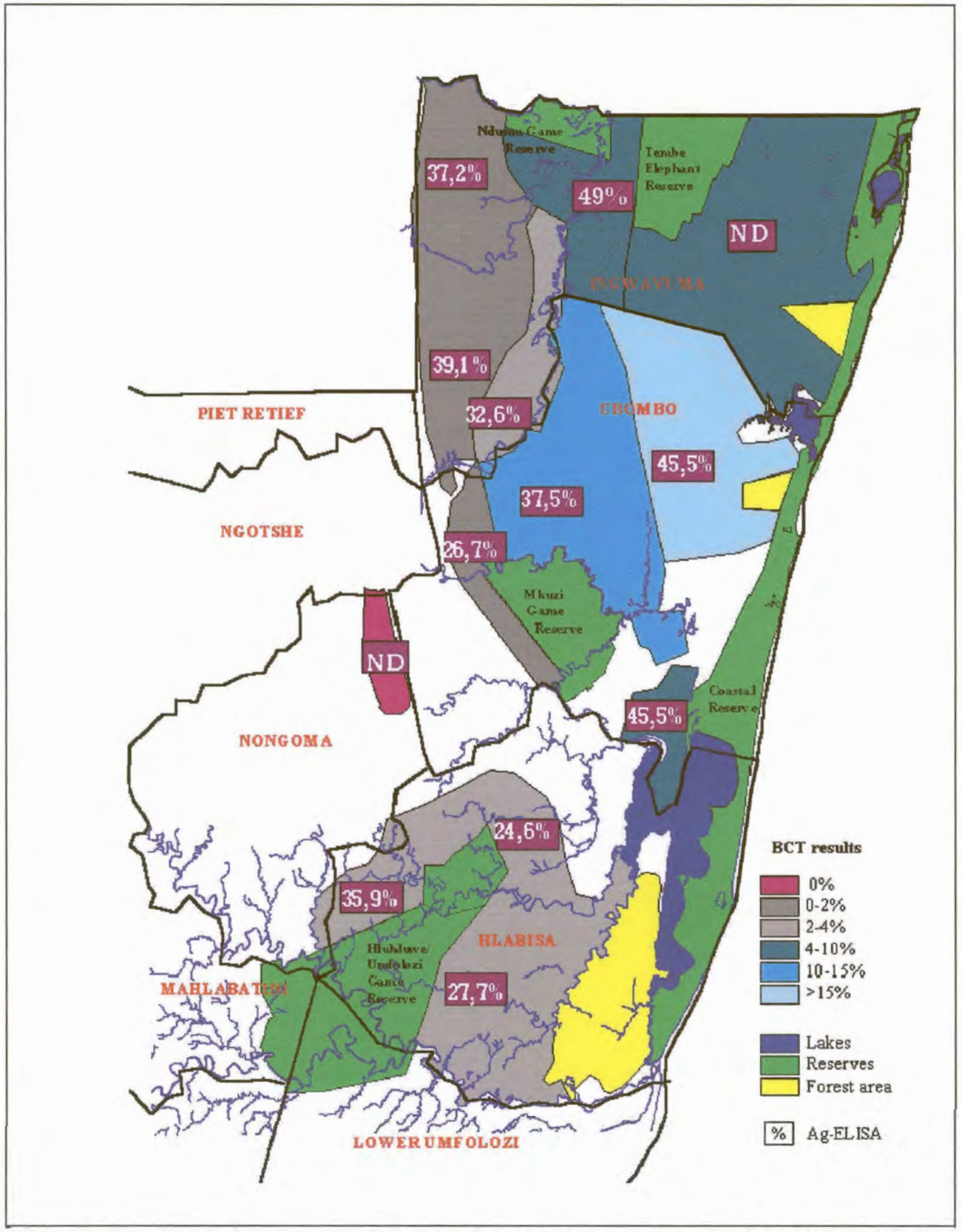


Fig. 7.8 Prevalence of trypanosomosis in N.E. KwaZulu-Natal as determined by BCT and Ag-ELISA (from De Waal *et al.* 1998)

However, according to De Waal *et al.* (1998), the estimated prevalence obtained with Ag-ELISA appeared to be much higher than expected and did not reflect the clinical position on the ground.

7.3.6 Landcover and vegetation type

Land use covers a range of types of data, many of which are relevant to tsetse-transmitted trypanosomosis. Furthermore, a classification of the area into different vegetation types is of importance particularly in determining the habitat suitability for tsetse. Datasets on landcover (Fairbanks & Thompson 1996; Fairbanks *et al.* 2000) and vegetation types (Low & Rebelo 1996) were obtained from ARC-ISCW (Agricultural Research Council – Institute for Soil, Climate and Water). Fig. 7.9 shows the landcover data for the region. Vegetation types are indicated in Fig. 7.10.

According to the landcover map (Fig. 7.9) the tsetse infested area consists mainly of thicket and bushland, and forest and woodland with patches of forest, sandforest, degraded thicket and bushland, and degraded forest and woodland. There are also great open areas of degraded grassland and wetlands along the eastern parts, which have not yet been surveyed due to unsuitability of the habitat for tsetse flies.

The map of vegetation types (Fig. 7.10) shows the tsetse infested area to contain mainly coastal bushveld/grassland with patches of sand forest, bordered by subhumid lowveld bushveld and Natal lowveld bushveld (Low & Rebelo 1996).

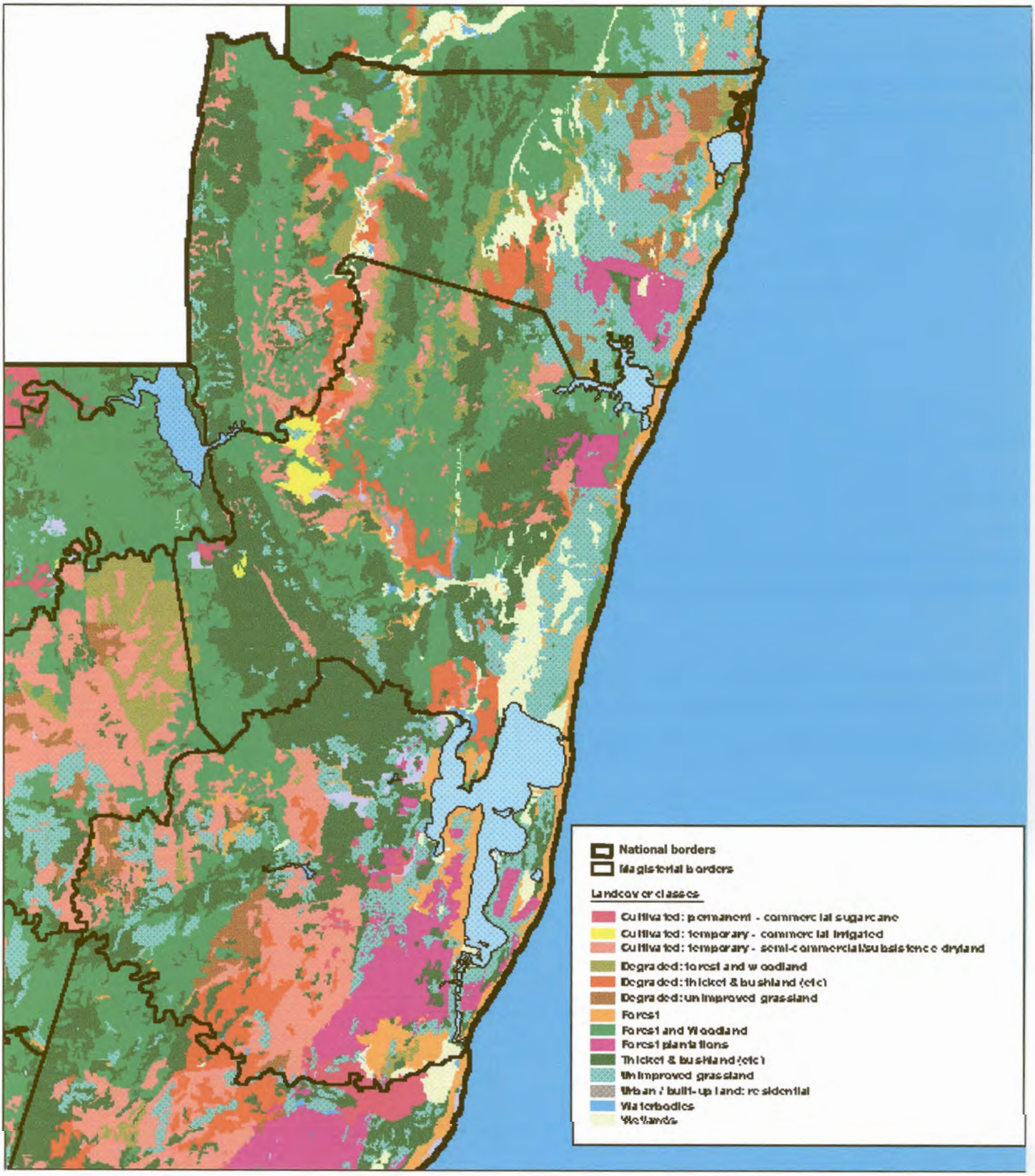


Fig. 7.9 Landcover map (Fairbanks & Thompson 1996; Fairbanks *et al.* 2000 – ArcView data from ARC-ISCW)

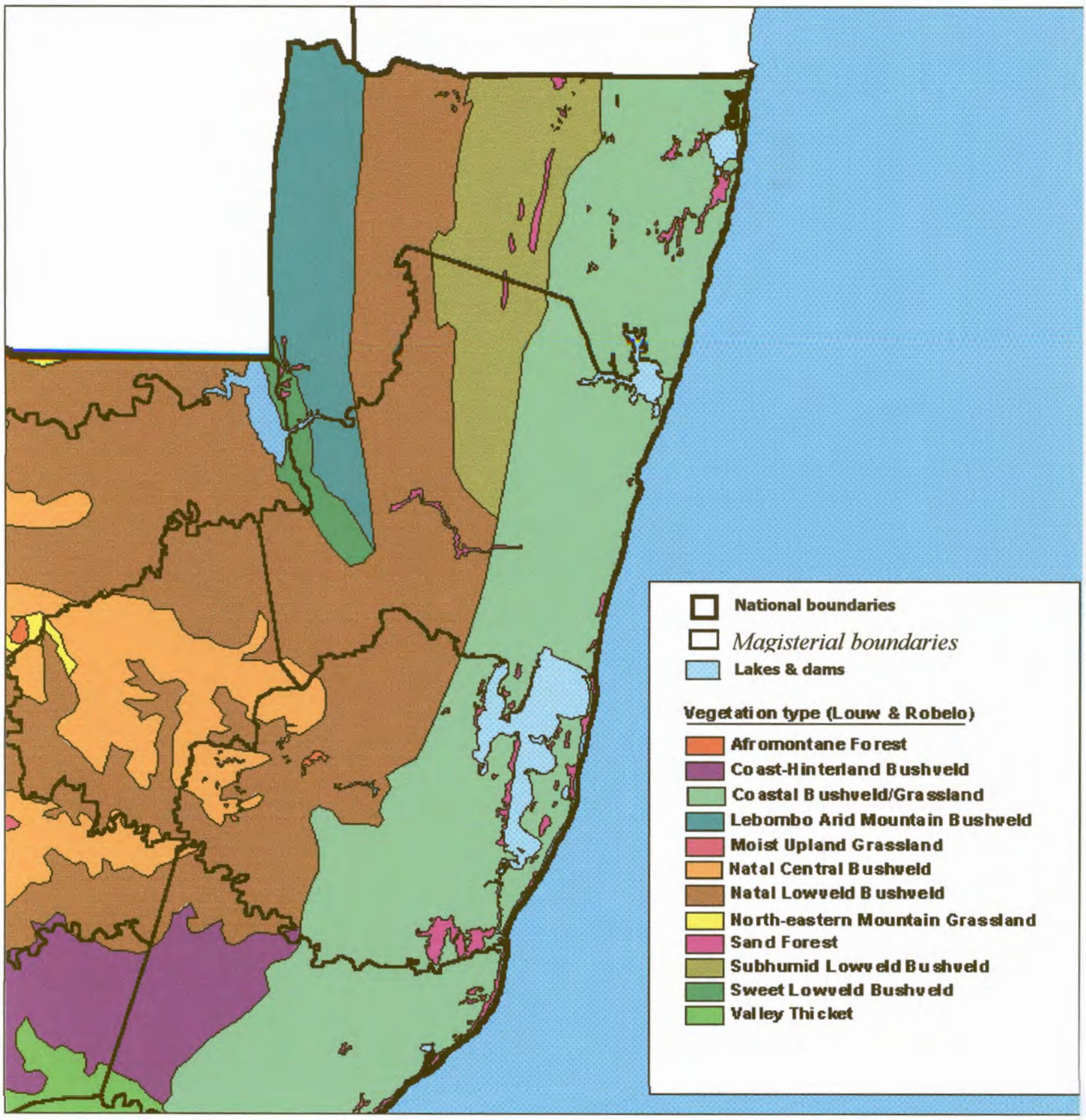


Fig. 7.10 Vegetation type map (Low & Rebelo 1996) (data from ARC-ISCW)

7.3.7 Land designation and tenure

In managing the intervention of tsetse-transmitted trypanosomosis it is essential to have accurate and updated information on land designation and tenure. This will distinguish for example areas classified as Game Reserves and other game or conservation management areas, state forests, commercial plantations, commercial farming areas and communal farming areas. Fig. 7.7 indicates areas designated as game and nature conservation areas as well as forest plantations in the region of concern. Forest areas and/or commercial plantations are also indicated in Fig. 7.9. Communal farming areas occur in all areas surrounding the diptanks shown in Fig. 7.6. It has not been possible to obtain precise data covering commercial cattle farm boundaries and these are therefore not indicated in any of the Figures. They are, however, mostly situated in areas between the west of False Bay Park (i.e. around Hluhluwe village) and surrounding the southern parts of Phinda Resource Reserve (see areas around Hluhluwe village, which form gaps (indicated white) between the nagana infected communal areas shown in Fig. 7.7)

7.5 DISCUSSION

7.5.1 Comparison of past and present distributions of *G. brevipalpis* and *G. austeni*

The historical survey by Du Toit (1954) (Fig. 7.1) showed the presence of *G. brevipalpis* to be mainly in and around the Ndumu Game Reserve and along the Pongola River in the north. In the south it occurred in the Hluhluwe-Umfolozi Game Reserve and its surroundings with a finger-like extension to the north and following the Hluhluwe River to the east. Du Toit (1956) also indicated *G. brevipalpis* in areas around Lake St. Lucia in a later survey. *G. austeni* was more widely distributed and was present in areas surrounding Lake Sibayi on the coast and in a low-lying belt *c.* 145 km long reaching from the Mozambique border in the north to about the Nyalazi River in the south, with a width ranging from *c.* 15 - 54 km inland from the coast.

The present survey results (Fig. 7.3) showed that there are two distinct bands of occurrence for *G. brevipalpis* and that *G. austeni* is more widely distributed, as was also the case in Du Toit's survey (Fig. 7.1). Certain changes in land use and development have occurred during the past fifty years. This is clearly illustrated by the differences of historical and present distribution maps. Possible reasons for the changes in distribution are discussed below.

North

Starting from the north, the present survey results indicated that *G. austeni* was present at both Ndumu Game Reserve and Tembe Elephant Park (in apparent densities < 5 flies/trap/day (Fig. 7.5)) while *G. brevipalpis* was only present in Ndumu (in apparent densities < 5 flies/trap/day (Fig. 7.4))[1]. For the past 19 years *G. brevipalpis* has not been seen in Tembe (E.M. Nevill & G.J. Venter, unpublished report 1994). Nevill and Venter theorized the reason for this as follows: Ndumu is bounded in the north by the Usuthu River. The Pongola River passes through the eastern part of Ndumu. Planned flooding, through releases from the Jozini Dam leads to inundation of the flood plain and filling of pans, which abound with animal life such as nyala and impala. In the large pans hippos are also abundant. A human-inhabited corridor, approximately 5 km wide, separates Tembe from Ndumu. Tembe is predominantly sandy except for the Muzi swamp in the east. The swamp is covered with reeds and has only the occasional open area of water. This is the only part of Tembe that has permanent natural surface water. There had been a marked difference in the types and numbers of animals seen at Tembe during the survey as opposed to those seen at Ndumu. Red and grey duiker and suni were very common in Tembe, while very few Nyala were seen. The impression they obtained is that at Tembe *G. austeni* would flourish since it prefers to feed on small antelope (Moloo 1993). However, *G. brevipalpis*, a stronger flier, would have to search far and wide at Tembe in order to find the same large animals on which it prefers to feed (Moloo 1993) and probably feeds on at Ndumu, such as nyala, hippo, etc. In Du Toit's 1946 survey (Du

[1] The low apparent densities of both species could give a wrong impression in that those surveys were conducted at the beginning of the warm season (Oct. 1994) before any rains had yet fallen when apparent densities are still relatively low. Densities could in reality be expected to be as high as in other game reserves.

Toit 1954) *G. austeni* was also shown to be present in the Tembe area, although only in the northwestern corner, and in the eastern parts of Ndumu. *G. brevipalpis* was only found in and around Ndumu and along the Pongola valley. In this area no profound changes have occurred in their distribution over the last fifty years, except that *G. austeni* is nowadays more widely distributed in Tembe, which had not yet been established at the time of Du Toit's survey. The factors that govern tsetse distribution must therefore be very basic and constant.

Surveys in the communal and diptank areas of the Ingwavuma district in the north indicated no tsetse to be present along the Ingwavuma River, except for one positive site between the Ingwavuma and Pongola Rivers just south of Ndumu Game Reserve. This supports du Toit's (1954) negative survey results of 1954 along this river. The area between the Ingwavuma River and Ndumu Game Reserve, which was positive for *G. brevipalpis* in 1946, was not surveyed. However, Fig. 7.9 indicates patches of semi-commercial/subsistence dryland, which would be unsuitable for tsetse. No *G. brevipalpis* were found along the Pongola River outside the Ndumu Game Reserve. However, in 1946 *G. brevipalpis* occurred in a narrow band along the river (Du Toit 1954). The area had at some stage in the past been deforested for cultivation and grazing (see Fig. 7.9 – degraded forest and woodland). The area immediately bordering the Pongola River is at present being cultivated (see Fig. 7.9 – semi-commercial/subsistence dryland). It would seem that the permanent breeding of this species could no longer take place along the river but it is possible that there is a southerly movement from Ndumu Game Reserve, at least for a short distance which extends eastwards to the southwestern corner of Tembe where this species was found. No *G. brevipalpis* were captured further to the west of the Pongola River, mainly due to lack of suitable habitat (degraded forest and woodland) as is presented in Fig. 7.9. *G. brevipalpis* was present in the area between Tembe and Ponto de Ouro border post and Kosi Bay with highest numbers at Kosi Bay (apparent density between 5 - 10 – Fig. 7.4). No tsetse were indicated on du Toit's (1954) map in this area (Fig. 7.1), but it may be that this area was not surveyed by him.

G. austeni was also found along the Pongola River just south of Ndumu Game Reserve but not further southward as was indicated by Du Toit (1954). The present survey suggests that the southern section of the Pongola River is no longer suitable for either tsetse species possibly due to population growth accompanied by deforestation and cultivation (see Fig. 7.9). During the 1946 survey by Du Toit (1954), the Pongola River formed the western boundary for *G. austeni* and for some reason it did not (and still does not) occur west of the river. *G. austeni* was also found west and east of Tembe at very low densities (< 2 flies/trap/day – Fig. 7.5), whereas no *G. austeni* were indicated east of Tembe on du Toit's (1954) map (Fig. 7.1). It is possible that Du Toit's surveys did not extend as far east.

Central

Du Toit (1954) indicated that *G. brevipalpis* was absent from Lake Sibayi and its surrounds but that *G. austeni* was present in a narrow strip around the lake and in a 5 km-wide coastal strip from Lake Sibayi southwards as far as Lake Bhangazi North (indicated in Fig. 7.1). It is not known, however, if Du Toit's survey extended any further. However, the area further south currently consists of unimproved grassland and wetland (see Fig. 7.9), which was probably also the case at that time. The present results of the survey in the Lake Sibayi/Sodwana Bay area concluded that no tsetse flies were present in this area. Moloo (1993) summarized all known results of tsetse blood-meal identifications made to date. The apparent favoured hosts of *G. brevipalpis* are bushpig (42,5 %), hippo (30,0 %), buffalo (8,4 %), bushbuck (6,3 %) and elephant (2,1 %) and of *G. austeni*, bushpig (46,6 %), cattle (14,5 %), duiker (7,1 %) and humans (4,9 %). With these facts as a guide one can theorize that the presence or absence of these tsetse species could largely be affected by the presence and abundance of their apparent hosts. It is accepted by many of the people consulted that there has been heavy poaching resulting in no wild animals nor signs of animals seen in the plantations nor in the indigenous bush around Lake Sibayi. Except for a narrow strip along the eastern shore of the lake where no cattle were seen, cattle were, however, common in the plantations, especially grazing along fire-breaks, so would be a possible blood-meal source. This is supported by the high numbers of *Stomoxys* spp. present

on the sticky traps. The apparent absence of tsetse from this specific area, which had been surveyed twice (June 1995 and again in April 1998) could therefore be due to lack of sufficient hosts or the failure of the survey system to reveal low tsetse numbers.

The survey in the communal farming and diptank areas of the Makhathini Flats, which lie west of Lake Sibayi indicated that *G. austeni* are widespread although in low densities (Fig. 7.5) and eight of 43 diptank areas were found positive. No *G. brevipalpis* were found here as was also reflected by Du Toit (1954) (Fig. 7.1).

The present distribution of *G. austeni* in the central area (Ubombo District) agrees to a great degree with du Toit's (1954) distribution. No *G. brevipalpis* were found in these areas even though traps were set along rivers where it could be expected to occur (nor west of the Lebombo). Du Toit (Fig. 7.1) also reflected the absence of *G. brevipalpis* in these areas.

South

Around Lake St. Lucia *G. brevipalpis* was found as far north as northern False Bay Park, as far south as St. Lucia as well as on the Eastern Shores of the lake. Highest apparent densities were found here (with highest density of 24 flies/trap/day in an old established pine plantation (indicated in Fig. 7.9)). *G. austeni* was also found around Lake St. Lucia in relatively high numbers (highest apparent densities at False Bay Park and Nyalazi River – Fig. 7.5). However, although vegetation and presence of hosts were suitable, this species was not collected on the Eastern Shores. They were also absent west of St. Lucia where cattle had been dipped in pyrethroids. Du Toit's (1954) survey only indicated *G. austeni* in the False Bay area (Fig. 7.1). However, *G. brevipalpis* was also found during a later survey (Du Toit 1956) in False Bay and also other areas surrounding Lake St. Lucia (i.e. on Eastern Shores, Nibela Peninsula, Ndlozi Peninsula and Western Shores). Without proper knowledge of landcover for those years, it is not possible to make comparisons for the whole of the area. The reason for the absence of *G. austeni* from Eastern Shores should ideally be determined as this could throw light on its dispersal

and reinvasion behaviour. Conversely the reasons for the large population of *G. brevipalpis* on Eastern Shores (Fig. 7.4) could provide clues as to why this species occurs in some areas of N.E. KwaZulu-Natal and not in others.

On one particular farm (Boomerang – 28°14'00"S 32°18'15"E) located east of Charter's Creek (Lake St. Lucia), which was surveyed on two occasions (Dec. 1993 and Apr. 1995), no tsetse and very few biting flies were captured even though the traps were set in what appeared to be "ideal" indigenous bush and tsetse had been captured immediately south and north of this farm. The reason for the absence of tsetse and biting flies appeared to be the regular treatment of 300 cattle on this farm with a 1 % deltamethrin pour-on. The cattle are allowed to graze all over the farm so would serve to attract and kill any tsetse flies which might be present. However, for two successive years (both during March) the farmer had outbreaks of nagana on his farm (five of 20 cattle bled were positive for trypanosomes). In May 2000 ten H traps were deployed for 14 days. The traps captured both *G. brevipalpis* (3 females and 2 males) and *G. austeni* (4 females) for the first time (J.R. Esterhuizen, pers. comm., 2000). Many of the other negative catches obtained during surveys with the XT could, therefore, have been false negatives. It may also be that the H trap, due to its better performance (as was discussed in Chapter 4), would have been a better survey tool than the XT. However, its development was only completed in 1998 and it was decided to use the same sampling method throughout the surveys for suitable comparison of apparent fly densities.

Only *G. brevipalpis* were collected in the Hluhluwe-Umfolozi Game Reserve. Despite traps being set in suitable *G. austeni* habitat in these areas no *G. austeni* were collected (Figs. 7.3 & 7.5). The southernmost distribution of *G. brevipalpis* in the reserve could not be established due to lack of roads. Du Toit (1954) also recorded only *G. brevipalpis* in this area (Fig. 7.1). Aerial smoking with DDT and HCH, which eradicated *G. pallidipes* from Zululand, also eradicated *G. brevipalpis* from Hluhluwe-Umfolozi Game Reserve. However, according to Hargrove (2000) a treated area of 10,000 km^2, which is subjected to reinvasion from all sides, could be invaded and tsetse populations can recover within two years. Reinvasion of *G. brevipalpis* must, therefore, have occurred from outside the reserve and the large numbers of hosts present

as well as suitable breeding grounds encouraged the current stable population in this reserve.

The surveys in the communal farming and diptank areas of the Hluhluwe State Veterinary Area, surrounding the game reserve, revealed that *G. brevipalpis* was widespread with 21 of the 34 diptank areas being positive. Although the apparent density of this species was relatively low in certain areas (Fig. 7.4), it could still be suggested that local breeding and/or invasion from nearby game reserves constantly takes place.

7.5.2 Southernmost limit

Neither species was collected south of the Mfolozi River (except at Mapelane). The southern-limit survey has shown that the present surveying system, designed to determine the broad distribution limits of each tsetse species, is too coarse to determine actual limits (even if these were static). Using more traps placed for much longer and also using the better H trap with more artificial odour would obtain finer results, as was discussed earlier, but this may still not be sufficiently intensive. The limit of transmission in these marginal areas will have to be determined by sentinel cattle. Until finer data are obtained the southernmost limit of tsetse distribution in South Africa, and therefore Africa, could roughly be regarded as the southernmost extent of the Mfolozi River.

7.5.3 Distribution in association with cattle distribution

According to the survey results, tsetse are more plentiful in game reserves and seem to be positively associated with the presence of game. However, they were also present in surrounding communal farming areas, where 42 diptank localities (Fig. 7.6) were found positive with tsetse during the surveys. Although communal areas are often deforested, there are usually trees along the rivers and drainage lines. These won't be indicated on vegetation or landcover maps (Figs. 7.9 & 7.10) but *G. brevipalpis* and *G. austeni* were caught in such situations. Furthermore, shortage of sufficient grazing in communal areas places pressure on cattle so that they are forced into forested

areas in their search for grazing and they also graze right up to the fences of the game reserves. In total 350,000 cattle are at risk of contracting nagana.

In the past (prior to 1992) the cattle-dipping regime in Zululand consisted of 2-weekly dipping of cattle in Amitraz [Triatix® - Hoechst Roussel Vet. (Pty) Ltd.], a tick-detaching agent, which does not kill flies. This programme was interrupted for two years (1992-1993) by dipping cattle in a pyrethroid cyhalothrin [Grenade® - Hoechst Roussel Vet. (Pty) Ltd.] during an attempt to control tsetse flies during an outbreak of nagana (Kappmeier *et al.* 1998). Since then diptanks reverted to Amitraz so as to prevent the development of tick resistance to pyrethroids. Currently, the state provides Amitraz for dipping once a month (R.J. Bagnall & M. Nel, pers. comm., 2001), which, however, will not be of any use for the control of tsetse. Novartis SA (Pty) Ltd. provides Ektoban®, a pyrethroid (cypermethrin) and cymiazol combination dip, for sale to the community to use as a handspray (M. Nel, pers. comm., 2001).

The surveys in the communal farming areas of Jozini State Veterinary Area (magisterial districts of Ubombo and Ingwavuma) revealed that *G. austeni* was more widespread than *G. brevipalpis*. Since *G. austeni* doesn't fly far, is restricted to dense bush and appears, in these areas, to feed mainly on cattle, control of *G. austeni* by treating cattle with pyrethroids should have a profound effect on nagana transmission and could lead to eradication of the fly. However, the success of control by means of mobile targets (insecticide-treated livestock) depends upon a sufficient proportion of the livestock population treated, and a sufficiently low level of reinfestation (Leak 1999). Chances of reinvasion from untreated areas are high in the Zululand situation, since the adjacent Game Reserves have high populations of flies (Fig. 7.4 & 7.5).

7.5.4 Distribution in association with trypanosomosis prevalence

By comparing the distribution of the two tsetse species (Fig. 7.3) to the distribution of nagana (Figs. 7.7 & 7.8) it is clear that the cause of nagana in the Mkuzi area (Ubombo District) is *G. austeni* and not *G. brevipalpis*, as the latter species does not occur in this area while the areas surrounding

Hluhluwe-Umfolozi Game Reserve are infested with *G. brevipalpis*. The high prevalence of trypanosomosis (10 - 15 % and >15 % - as determined by BCT) (Fig. 7.8) in the Ubombo district is associated with *G. austeni* while very low prevalence (2 - 4 %) occurs in the *G. brevipalpis* areas around Hluhluwe-Umfolozi Game Reserve. The prevalence of *Trypanosoma* spp. as determined by Ag-ELISA (Fig. 7.8) was higher in areas where *G. austeni* occurs but is also quite high in the surrounding areas of Hluhluwe-Umfolozi where *G. brevipalpis* was found. De Waal *et al.* (1998) estimated the prevalence obtained with Ag-ELISA to be much higher than expected and that it did not reflect the clinical position on the ground. They discussed shortcomings of the Ag-ELISA test.

7.5.5 Distribution in association with land designation, landcover and vegetation

The presence/absence of flies in relation to land designation (i.e. game reserves, plantations, communal farming areas, etc.), landcover and vegetation types has already been mentioned. From the surveys it was clear that the presence of tsetse appeared to be mostly associated with forests, forests and woodland, forest plantations, thickets and bushland, while fly absence in this survey could be associated with unimproved grassveld, degraded forests and woodland, subsistence dryland and wetland areas (Low & Rebelo 1996).

A large area of the southern part of the affected area of N.E. KwaZulu-Natal is under pine and eucalypt plantations. Both tsetse species have been trapped in both types of plantation and, in fact, the highest number of *G. brevipalpis* (144) ever collected on one trap was in an isolated 35 year-old pine plantation on Eastern Shores of Lake St. Lucia. Many of these flies were also teneral. There is, therefore, a strong indication that at least *G. brevipalpis* can breed in such an established plantation where there are suitable hosts. This was not previously certain.

7.5.6 Other flies

Much effort has been spent estimating the number of other flies attracted to and captured on the sticky traps used in the surveys. They were all identified to family, subfamily or genus level, but are not reported on in this study. Analysing total biting fly catches has the following value: There is an ongoing debate as to whether other biting flies play a role in the mechanical transmission of nagana (Jordan 1986; Leak 1999). The survey shows that if this is so then the most likely mechanical transmitters would be the Stomoxyinae and Tabanidae and that these flies are found in the bush where cattle feed. Stomoxyine biting flies are extremely common in plantations and some bush situations. Both sexes of *Stomoxys* and *Glossina* spp. survive solely on blood (Lancaster & Meisch 1986) and females must feed even more often to produce their eggs. Therefore, the presence of large numbers of *Stomoxys* spp. is an indicator that sufficient blood is available to support the survival of tsetse flies and that if tsetse flies are not caught it is not due to a shortage of hosts. *Stomoxys* and *Glossina* spp., however, differ in other respects. For example *Stomoxys* may breed in rotting vegetation, sometimes cow dung, and does not like densely shaded situations. The two tsetse species, however, require densely shaded areas in which to breed and are very selective as to the sites where they will deposit their larvae. Stable flies are, therefore, prime candidates for the mechanical transmission of trypanosomes. They should not be overlooked in a study of the epidemiology of trypanosomosis. The experience in Zanzibar, however, indicated that despite the presence of a widespread *Stomoxys* fly population on the island at high densities (up to 1000 flies/trap/day in certain areas), these stable flies failed to sustain a trypanosome prevalence in domestic livestock in the absence of tsetse flies (Saleh *et al.* 2001).

7.5.7 Shortcomings

There are certain variables and shortcomings that make it impossible to draw absolute conclusions on the distribution results.

- The surveys were conducted over several years and over several months each year so that direct comparison regarding the apparent density results is not possible.
- Trap catches that were negative do not necessarily reflect total fly absence (and could be false negatives) as the trapping system might have been too coarse. Better results may have been obtained if a trap like the H trap was available at the time. This trap could also have been baited with a better odour (best SA odour – see Chapter 2.2.2), which was also not available at the time.
- The same persons did not carry out the surveys each time, so that the selection of optimal trap sites would have been inconstant.
- The period over which the traps were set could have been insufficient, especially where populations were very low.
- The lack of roads in many of the areas left large areas unsurveyed.

7.5.8 Implications/Future use of GIS

The present results of the surveys, i.e. positive/negative sites (Fig. 7.3), are incomplete descriptions of fly distribution in terms of fly presence vs. zero catches. For example flies may be present in some areas but have not yet been caught (i.e. false negative sites) or the region may contain areas suitable for, but are presently uninhabited by flies. Therefore, the present results may be inaccurate especially where zero catches are indicated. Furthermore some areas that have not yet been surveyed include the eastern coastal area between Lake Bhangazi North (situated about halfway between Lakes Sibayi and St. Lucia) and Lake Bhangazi South; from Kosi Bay to Lake Sibayi; and commercial farming areas to the west of the Lebombo Mountains between Mkuzi River and approximately the Ncemane River, especially the river valleys. However, these are relatively small areas. The use of GIS and remote sensing to define tsetse distributions and update distribution maps in the Zululand area will be of enormous value. Remote sensing can also be used to predict the levels of abundance of the two species.

In the last few years several attempts have been made to improve tsetse distribution and abundance maps using relationships between historical map

data and recent satellite imagery (Rogers & Randolph 1993; Rogers *et al.* 1996; Robinson *et al.* 1997a, 1997b) as well as present satellite and fly data (Hendrickx *et al.* 1993; Rogers *et al.* 1994; Hendrickx 1999, cited in Hendrickx *et al.* 2000). Various studies and application of GIS technology defining the epidemiological and related aspects of trypanosomosis have been published. For example, the use of remote sensing technology to better understand the natural habitat and epidemiology of tsetse and to explain or predict tsetse and disease distribution in East, West and Southern Africa was described (Rogers & Randolph 1985, 1991; Rogers 1991; Rogers & Williams 1993; Rogers *et al.* 1994; Rogers *et al.* 1996; Robinson *et al.* 1997a, 1997b; Rogers 1998). For West Africa the use of GIS and remote sensing was used as a decision and management tool for trypanosomosis control (Hendrickx 1998, cited in Erkelens *et al.* 2000). Focusing on smaller scale areas, GIS and remote sensing was used to study the effects of tsetse fly presence and control methods on land-use, environmental change and biodiversity, (Reid *et al.* 1997; Reid *et al.* 1999; Bourn *et al.* 2001) and also to select priority areas for tsetse control (Robinson 1998a; Erkelens *et al.* 2000).

In order to improve the present maps on the distribution and abundance of *G. brevipalpis* and *G. austeni*, we need to increase our understanding of why the two species are distributed in the way they are, and to produce empirical models that enable prediction on the likely distribution of these two species. When working with *G. brevipalpis* and *G. austeni* the over-riding question is: "Why do the two species sometimes occur in the same area and sometimes not?" Correlation with climate and other predictor variables can in future assist in answering this question and in predicting distribution and abundance of the two species in N.E. KwaZulu-Natal.

The principal factors that influence tsetse population development are climate, vegetation cover (which provides shade for tsetse) and host availability (Robinson *et al.* 1997a). Tsetse flies are particularly sensitive to temperature, rainfall and saturation deficit (Rogers & Randolph 1991). Other predictor variables may include atmospheric moisture, heavy rainfall and interactions with hosts (Rogers *et al.* 1994). Information on each of these factors may be gathered together with GIS to facilitate further analysis. Computerized maps

of climate variables such as rainfall, temperature and saturation deficit can be generated by ground-based measurements (Hutchinson 1989, cited in Robinson *et al.* 1997a). Modern satellites produce data that can be analyzed to reveal the ecological condition of the lands they pass over (Brady 1991). Especially useful is the Normalized Difference Vegetation Index (NDVI), which, in effect, indicates the level of photosynthetic activity in plants (Tucker & Sellers 1986, cited in Rogers *et al.* 1994), and thus the vegetation cover. Channel 1 and 2 data of the Advanced Very High Resolution Radiometer (AVHRR) of the National Oceanographic and Atmospheric Administration (NOAA) meteorological satellites, which circle the polar orbit, are used to produce NDVIs related to the intercepted photosynthetically active radiation (IPAR) and vegetation type on continental scales (Rogers *et al* .1994). Satellite data have also been shown to act as surrogates for a number of meteorological variables. From NDVIs can be inferred functions of moisture, such as soil moisture (Narasimha Rao *et al.* 1993, cited in Baylis *et al.* 1999), saturation deficit (Rogers & Randolph 1991; Rogers *et al.* 1994) and recent levels of rainfall (Rogers & Randolph 1991; Schultz & Halpert 1993, cited in Baylis *et al.* 1999). Rainfall has also been correlated with cloud-top temperatures and cold-cloud duration (CCD) from METEOSAT data. It was shown that the abundance of clouds with cloud-top temperatures of between -30 °C and -60 °C or less is correlated with rainfall at ground level (Rogers *et al.* 1994).

The ground-collected tsetse distribution data of N.E. KwaZulu-Natal can now be complemented with information derived from remote sensing satellites (e.g. high-resolution satellite data) to make increasingly accurate predictions in areas where ground data are sparse or absent. Once data have been incorporated into a GIS, extensive multivariate analyses will have to be carried out in order to produce reliable predictions. Such analysis can distinguish between sites that are suitable and unsuitable for these species. It will also provide challenging insights into the relationships between vectors, hosts, habitats and disease agents and a prediction of the importance of tsetse-transmitted diseases in the KwaZulu-Natal area.

7.5.9 Conclusion

In conclusion, the map of the distribution of the two tsetse species indicates that there are two distinct bands of distribution for *G. brevipalpis*. These are well separated and could be treated as independent distributions when planning for intervention. The surveys also indicated that the main sources of *G. brevipalpis* are the game reserves and natural areas whereas this is not necessarily the case for *G. austeni*. The latter species is more widely distributed and may be able to be satisfactorily controlled by reversion to pyrethroid dipping when the incidence of trypanosomosis warrants it. The results can be fruitfully used to indicate where resources for disease control should be directed. With such information it should be possible to plan cost effective control measures, which may improve the productivity of the livestock sector in Zululand. The completed surveys will help decide if control or eradication should be undertaken, i.e. where, for what species, in what order and with what techniques.

4. DEVELOPMENT OF SUITABLE TRAPS

4.1 ABSTRACT

Sticky traps of various shapes and colours were tested and improved for the purpose of surveying the distribution of *Glossina austeni* and *G. brevipalpis* in South Africa. For *G. austeni* and *G. brevipalpis* the 3-dimensional shapes of the XT and 3DT in light blue (l.blue) and white were better than the RT. An elelctric blue (e.blue)/l.blue odour-baited XT was effective to apply in surveys to monitor the distribution of the two species in N.E. KwaZulu-Natal. This sticky trap was later replaced by an e.blue/black XT proven to be more effective for *G. austeni* and similarly effective for *G. brevipalpis*. An increased size of the trap increased the numbers of *G. brevipalpis* females and both sexes of *G. austeni* significantly. For both species larger monopanels (95 x 80 cm and 120 x 100 cm) of which each side was painted half e.blue and black (vertical) were found equally attractive to the standard size (70 x 60 cm) XT. A new trap, named the "H trap", was developed for the simultaneous collection of live *G. brevipalpis* and *G. austeni*. Its design followed an evaluation of the responses of the two species towards traps that are used elsewhere in Africa for the collection of other tsetse fly species. These traps were found at Hellsgate to be unsuitable for capturing both *G. brevipalpis* and *G. austeni*. Some new trap designs and many modifications of these were tested, most of which were unsuccessful. The odour-baited blue and black H trap represents a different approach for trapping tsetse flies as it is fitted with lateral cones of white netting which induce the flies to take a more horizontal flight path once they have entered the trap, instead of the vertical flight paths they are forced to assume in existing tsetse fly traps. A number of modifications of the prototype H trap were devised (H1-H5), before the final design was established. Catches of up to 76 *G. brevipalpis* and 37 *G. austeni* were obtained per trap on a single day with the H3 modification. Further modifications improved on the trap's efficiency to capture *G. brevipalpis* and *G. austeni*. The final modification caught a record number of 180 *G. brevipalpis* and 57 *G. austeni* on a single day.

4.2 INTRODUCTION

Hargrove (1998) has defined a tsetse fly 'trap' as a device designed to induce tsetse to enter a space from which they cannot escape. Harris (1931) developed the first trap used for tsetse flies and employed it to capture large numbers of *G. pallidipes* Austen in South Africa. Since then, many traps have been designed for other species of tsetse in other parts of Africa (Morris & Morris 1949; Challier & Laveissière 1973, cited in Hargrove 1998; Moloo 1973; Hargrove 1977; Laveissière & Couret 1980; Vale 1982a; Flint 1985; Gouteux & Lancien 1986; Brightwell *et al.* 1987; Laveissière & Grébaut 1990; Brightwell *et al.* 1991; Gouteux 1991; FAO 1992; Kyorku *et al.* 1993; Mhindurwa 1994; Vreysen *et al.* 1996). Traps are, however, preferably used as monitoring tools but have shown to be very effective in control programmes.

4.2.1 Sticky traps

Initially sticky traps were developed in Zanzibar (Hall 1990; Schonefeld 1988, cited in Hall 1990) for the monitoring of *G. austeni*. These were light blue and white traps of the 3DT (3-dimensional trap), the XT (cross-shaped X target), and the RT (rectangular sticky screen) with 3-dimensional leg panels. The only traps earlier found to be effective for capturing *G. austeni* in KwaZulu-Natal have been these sticky panels of various shapes and colours. When baited with synthetic ox-odour, they also captured *G. brevipalpis* (Kappmeier, Venter & Nevill 1995). Since then more sticky traps have been developed, namely the Chuka trap by Madubunyi (1990) and the free rotating monopanel (MP) and legpanel (LP) by Vreysen *et al.* (1996).

Before the distribution of *G. austeni* and *G. brevipalpis* in northern KwaZulu-Natal could be surveyed, studies were needed to evaluate the sticky trap shapes and colours, so as to be able to select the best trap for these surveys. The three Zanzibar sticky traps available at that time (3DT, XT and RT) were, therefore, evaluated for their efficacy for the two tsetse species. Later, further studies were undertaken to improve on the design of the sticky traps used in initial surveys. The attractiveness of additional colours, colour combinations

and sizes were tested as well as simplifying the design for its manufacture and practical use in the field.

4.2.2 Cloth traps

Sticky traps proved to be useful tools for monitoring the relative distribution of both species in KwaZulu-Natal (Nevill *et al.* 1995; Nevill 1997), but, do not provide live flies suitable for mark-release-recapture studies. For this it is necessary to use a trap which catches live specimens in large enough numbers. No such trap exists for *G. austeni* as its behaviour is elusive and only low numbers are caught in existing tsetse fly traps elsewhere in Africa (Takken 1984; Hall 1986; Madubunyi 1990). The only trap available for this purpose for *G. brevipalpis* was the Siamese trap but it is only partially effective for this species in Kenya (Kyorku *et al.* 1993).

Preliminary studies in KwaZulu-Natal have indicated that, with the exception of sticky traps, most existing tsetse fly traps, which are effective for other species elsewhere in Africa, were not effective for the capture of *G. brevipalpis* and particularly not for *G. austeni* (Kappmeier, in press). Traps that have been tested in South Africa for capturing live *G. austeni* and *G. brevipalpis* include the Epsilon, Pyramidal, Biconical, Vavoua, Ngu (Ng2f) and Siamese (B) (Gouteux & Lancien 1986; Brightwell *et al.* 1987; Laveissière & Grébaut 1990; FAO 1992; Kyorku *et al.* 1993).

The best of these, namely the Ngu (Ng2f) and Siamese (B), caught mean daily numbers of 8,2 and 5,8 *G. brevipalpis* respectively (35 replicates) and 0,4 *G. austeni* (35 replicates) (Kappmeier, in press). In addition, the efficiencies of the Ngu and Siamese traps, as determined by comparing the results obtained with those when electrified nets were placed immediately adjacent to the traps, as suggested by Vale (1982a), were also found to be very low (Kappmeier, in press). The reason for the ineffectiveness of the traps for *G. brevipalpis* and *G. austeni* in KwaZulu-Natal was determined during further trap-orientated behavioural studies, as described by Vale (1982b), when, by the use of

electrified nets, it was shown that the upward flight responses of the flies were very low. Only 21-45 % of the *G. brevipalpis* that entered a Ngu and Siamese (B) trap, flew upwards towards the cone (Kappmeier, in press). The same basic trend also held true for *G. austeni*.

The poor vertical movement of these tsetse fly species led to the development of a prototype of a new trap using lateral or side-cones instead of vertical or top-cones so that the flies, once they had entered the trap, flew horizontally rather than upwards. In order to improve on the design, several modifications of this prototype trap, named the H trap, were assessed for trap-orientated responses of the flies as well as for efficiency.

Months of studies on numerous modifications of existing traps and on new designs preceded the development of the H trap. Because they were unsuccessful these efforts will only be referred to briefly and the main body of the chapter will concentrate on the evolution of the H trap.

4.3 Materials and Methods

4.3.1 Sticky traps

Trap designs and tests

Three types of sticky traps were made for testing at Hellsgate according to the description of Hall (1990). These were the 3 DT, XT and RT (Fig. 4.1). The traps were made from 3 mm tempered hardboard panels, painted light blue (l.blue), electric blue (e.blue), white or black with gloss enamel. All traps were hung from trees and were allowed to rotate with their lowest part 10-20 cm above ground level. They were painted with polybutene so that the flies that landed on the traps could be retained on the sticky surface. The polybutene was diluted with hexane to facilitate easier application. Once applied, the hexane evaporates and the surface remains sticky. To collect flies lost from the lower edges due to dripping, especially during the first day when the polybutene is still quite fluid, a plastic sheet was placed underneath each trap.

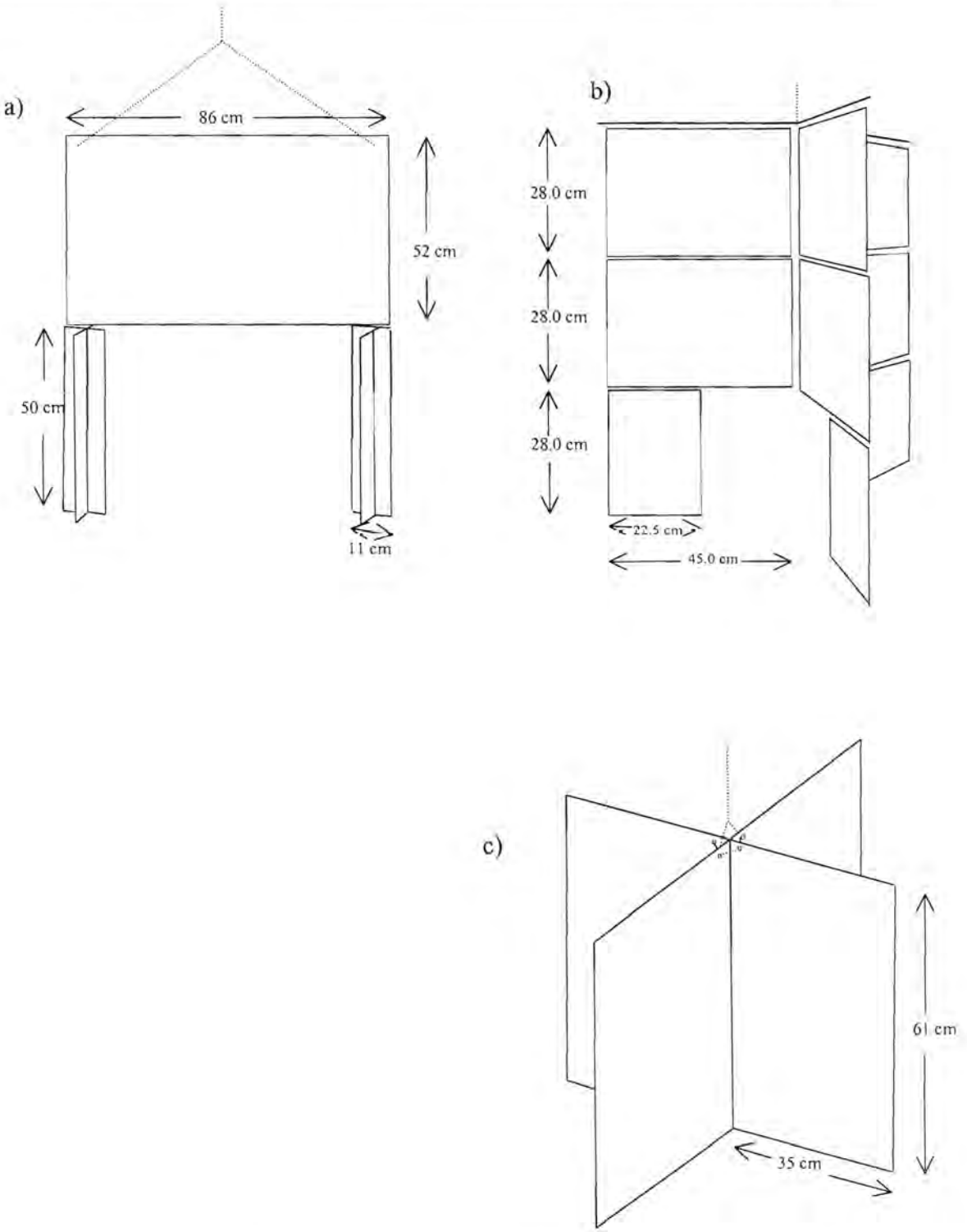


Fig. 4.1 Sticky traps for *G. austeni*: (a) Rectangular sticky screen (Hall 1986, cited in Hall 1990); (b) 3-DT (Schonefeld 1988, cited in Hall 1990); (c) cross-shaped target (XT) (Hall 1990)

This also reduced contamination of the trap with soil and leaves due to rain and wind. The polythene sheet was pinned down with 200 mm pegs made from heavy fencing wire.

A series of experiments were conducted between 1993 - 1996, firstly to evaluate the various sticky trap designs and compare black, white, l.blue and e.blue versions. Thereafter, the best design (XT) was improved and evaluated in single- and bicoloured combinations (i.e. one panel of the XT was painted a different colour to the second panel). It was then attempted to simplify the best colour combination of the XT in the form of monopanels (being a single panel of the XT) for more practical use in the field. These were also tested in various bicoloured combinations and sizes. The experiments will be described in more detail in the Experiments and Results section (4.4) below.

Sticky trap efficiencies

In order to determine the efficiency of traps, an electric net (1 x 1 m), was placed immediately adjacent to the trap. This electric net intercepted flies that were attracted to the trap, but which flew around it and which might never have landed on the trap. The number of flies captured by a trap (without an electric net) was expressed as a percentage of the total number of flies attracted to a trap (trap plus electric net), to give an estimate of trap efficiency (Vale 1982a; 1982b).

4.3.2 Cloth traps

Preceding trap tests and designs

Before the prototype of the H trap was designed, many modifications of existing traps were made and some other traps were originated at Hellsgate. The designs of all these traps took into account the flies' reluctance to fly upwards towards the cones. Some of these designs were also described in Kappmeier (in press).

The first designs consisted of modifications of the Ngu and Siamese traps where both were fitted with lowered or sunken cones so that the path towards the collecting devices was lower. Some of the latest tsetse traps were included in these tests, namely the M3 (Mhindurwa 1994) and the Nzi traps (Mihok 2001). The Nzi was also modified into what was referred to as the Nzi3 which consisted of three Nzi traps united back to back thus with three separate entrances. The Nzi was also further modified so that the rear netting part was incorporated into a horizontal and diagonally sloping cone plus collecting device, therefore doing away with the top/vertical cone. The Canopy trap used for Tabanidae (Catts 1970) was also tested and then modified, firstly by adding a pthalogen blue panel to the base (to enhance attraction), and later by providing openings in the blue pyramidal base, and simultaneously lowering the top cone part. Some new trap designs included what was referred to as the Monoscreen trap, which consisted of a blue and black cloth target with two thirds of the top part fixed with white mosquito netting which formed a "tent" over the target. A few modifications to the net part followed to encourage the horizontal movement of flies towards a collecting device. One of these modifications was further modified into what was called a 3-dimensional-screen trap (3DS), which, as seen from above, consisted of an X-shaped cloth target, also fixed with a tent-like cover of netting and collecting cages. The prototype H trap (with different modifications [H1-H5] as described below) was designed and developed together with a B trap (P.W. Trollip, personal communication, 1997) and its modifications B1-B5. The latter were similar to the H trap, but had only one horizontal cone.

Of all the above designs and modifications, other than the H trap modifications, only a few looked promising, namely the Nzi, Nzi3 and B1-B5 traps. Further experiments included the comparison of these traps with an e.blue/black XT sticky trap described in the previous section. These results will briefly be summarized in section 4.4.

The prototype H trap

The prototype design of the H trap (Fig. 4.2 a) consisted of a phthalogen blue cloth outer "box" (100 x 65 x 65 cm) with two opposite side entrances (40 x 45 cm), an inner black cloth X-target (which also acted as a baffle, attached to the centre of the roof), and then two "horizontal" cones of white mosquito netting extending laterally from the ends of the trap in opposite directions, therefore initially named the "Horizontal trap". Although the "cone"-device used here, was a hollow four-sided pyramid-shaped structure with a square base and straight (not curved) sides, it will here and henceforth be referred to as a "cone", which is an accepted term to use with tsetse fly traps (FAO 1992). The four corners of the trap body were fastened, with strings attached to the trap, to four poles pegged into the ground at the positions of the trap corners. The cones were held in position by attaching them each to a flexible rod which provided tension to keep them rigid (Fig. 4.2 a). The apex of each cone was fitted with the top third of a 750 ml polythene bottle on which a second bottle fitted as the collecting device (Fig. 4.2 e).

H trap modifications

Five modifications of the prototype trap (Fig. 4.2 a) were made, and referred to as the H1 – H5 traps/modifications. The following is a description of the modifications, also depicted in Figs. 4.2 b-d:

H1: The prototype H trap was modified by adding a black inner lining to the base of the cones to prevent flies from collecting at the corners at the cones' bases (Fig. 4.2 b).

H2: The H2 was made with an extension of the outer blue "body" over the cones of the prototype trap (Fig. 4.2 c) to attract the flies to the light and the trap cage (collecting device) at the apex of the cones.

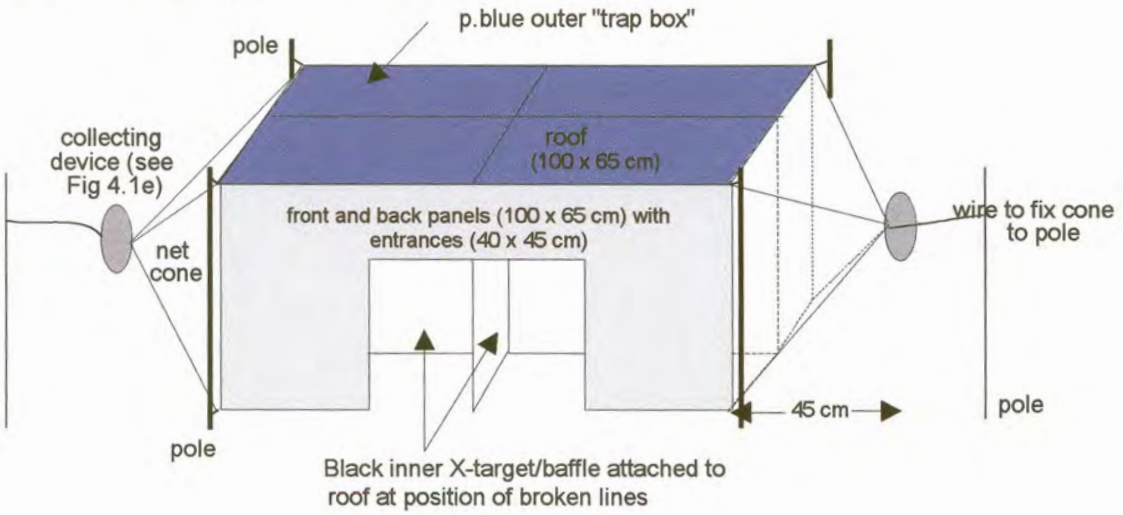
H3: A third modification, the H3, was designed with diagonal or upward-sloping cones to eliminate the problem of flies collecting at the corners of the bases (Fig. 4.2 d).

H4: The H4 modification was as the H3 but with bigger entrances (65 x 45 cm) and therefore a bigger blue body (125 x 65 x 65 cm).

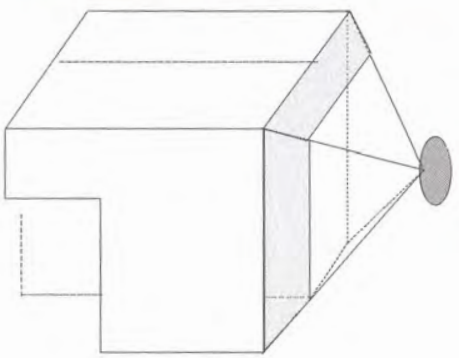
H5: The H5 modification was as the H4 but with bigger cones.

Final "H trap": See Fig. 4.3 and 4.4.

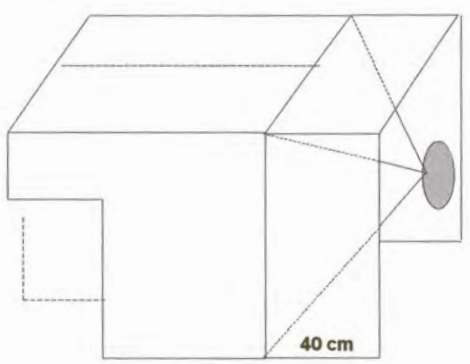
a) H trap (prototype)



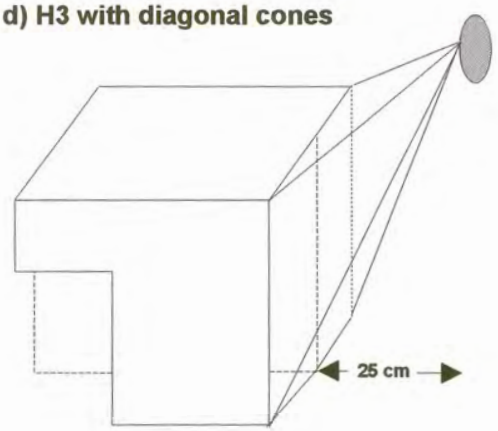
b) H1 with black lining added to inner bases of cones



c) H2 with blue outer box extended over cones



d) H3 with diagonal cones



e) Details of collecting device

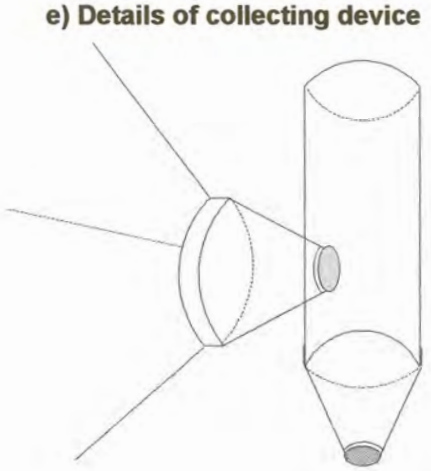


Fig. 4.2 Diagrammatic representations of the prototype H trap (a) with its H1, H2 and H3 modifications (b-d) and details of collecting device (e)

Trap efficiencies

The prototype H trap evolved from studying the behavioural responses of *G. brevipalpis* and *G. austeni* in and around the Siamese (B) and Ng2f traps (Kappmeier, in press). These behavioural studies were also conducted on later modifications of the H trap so as to be able to improve on its design. These trap-orientated responses and trap efficiencies of the H trap modification were evaluated by using electric nets (Vale 1974a) of various sizes and placements similar to those used by Vale (1982a; 1982b). All flies that were intercepted by the nets were electrocuted and retained on a tray painted sticky with polybutene so that they could be sexed and counted. In order to determine the efficiency of traps, an electric net (1 x 1 m), was placed immediately adjacent to the trap. This net intercepted flies that were attracted to the trap, but which flew around it, and which might never have been captured. The number of flies captured by the trap was expressed as a percentage of the total number of flies attracted to the trap, to give an estimate of trap efficiency. To determine the entering responses of flies, the trap's entrances were closed by means of smaller but similar electric nets, which were just large enough to fit into the trap entrances. All flies that attempted to enter the trap were therefore electrocuted and counted. The flies' horizontal flight responses were tested by placing small electric nets inside the traps, at the base of the cone, so that they intercepted all flies that flew horizontally towards the cone part of the trap. [These behavioural studies were also conducted with the Nzi and B3/B4 traps, the results of which are summarized in Kappmeier (in press)].

4.3.3 Odour baits

All treatments under comparison were baited either with the Zim-mix or the best SA blend as described in Chapter 2.2.2. The bait was placed about 20 cm away from the traps at ground level on the downwind side of sticky traps or in front of the downwind entrance of cloth traps.

4.3.4 Experimental design and analyses

All comparisons of traps as well as the efficiency and behavioural response tests with electric nets were tested by means of Latin squares. The comparisons of the traps and modifications were conducted over a 24-hour period, after which they were rotated between sites according to the Latin square design. The comparisons of trap efficiencies and trap-orientated behaviour of the flies, were determined from data collected from 10:00 until dark, the period of maximum activity of both species (Kappmeier 2000).

All data were analyzed, where numbers were adequate, by means of a statistical program for Latin squares, the details given in Chapter 2.3. Male and female catches were analyzed separately for *G. brevipalpis*, but numbers were usually too low for *G. austeni* to justify separate analyses according to the sex. Further details are given below in Experiments and Results.

4.4 Experiments and Results

4.4.1 Sticky traps

Tables 4.1 and 4.2 are summaries of the results of the experiments on the various colours and types of sticky traps tested for *G. brevipalpis* and *G. austeni* baited with the Zim-mix. Where catches were too low for separate analyses of the sexes, the pooled total catches are reported on. The overall trap catch of each treatment is given as an index of increase relative to the control treatment. The detransformed mean catch of the control treatment is given in brackets. Treatments followed by the same symbol are not significantly different from the control in the same experiment. Table summaries will incorporate the number of replicates (n) for each treatment, the transformed standard errors (s.e.) as well as the levels of probability (P) that the means are different at $P < 0,05$ (*), $P < 0,01$ (**), $P < 0,001$ (***), or not significantly different (n.s.).

In Experiment 1 (Table 4.1) the 3DT, XT and RT traps were each tested in l.blue, white and black. Nine replicates were carried out. For *G. brevipalpis* no significant difference was found between any of the treatments. However, very low numbers of this species were collected. For *G. austeni* the l.blue 3DT (control) was the best trap, which was significantly greater than all the black traps and all the RTs.

In Experiment 2 (Table 4.1) the best traps of Exp. 1 (XT and 3DT) were tested again in l.blue, e.blue and white. (Black was not included due to its lesser performance in the previous experiment, while e.blue was the colour most closely resembling phthalogen blue, the attractive blue part of a target for these species (Kappmeier & Nevill 1999b). Six replicates were carried out. For *G. brevipalpis* and *G. austeni* no significant differences were obtained between any treatments, suggesting the 3-dimensional shapes to be equally effective. White seemed very effective for *G. brevipalpis* but not for *G. austeni*.

Since no differences were obtained between the 3DT and XT and between the colours in experiment 2, the XT was chosen for upgrading due to its practicality for use in the field, consisting only of two panels as opposed to the nine panels of the 3DT. In Experiment 3 (Table 4.1) uni- and bicoloured XTs were tested in combinations of white, e.blue and l.blue, to try to improve on its design. Single coloured monopanels of the same size as one panel of the XT, were included for comparison. Ten replicates were carried out. For both species the e.blue/l.blue XT performed best and was then selected as the trap to use in tsetse distribution surveys, which started in December 1993.

In Experiment 4 the XTs were evaluated with different combinations of colour panels as set out in Table 4.1 to incorporate more uni- and bicoloured traps to try and find a better combination for use in surveys. This time black, which was previously left out, was once again included due to strong settling responses obtained for *G. brevipalpis* and *G. austeni* on black when added to a blue target (Kappmeier & Nevill 1999b). The e.blue/l.blue XT, selected for

Table 4.1 Comparisons of various shapes and colours of sticky traps in four experiments [Indices of increase are given relative to the control treatment (index = 1) in each experiment. Detransformed means of the controls are indicated in brackets. The number of replicates (*n*), the transformed standard errors (s.e.) and the probability that the means are different at the *P* < 0,05 (*), *P* < 0,001 (***) levels of probability or not significantly different (n.s.) are given]

	Trap	Colour / combination	*G. brevipalpis* totals			*G. austeni* totals			
			Index	*P*	s.e.	Index	*P*	s.e.	*n*
Exp. 1	3DT	l.blue	1 (1,113)a	n.s.	0,158	1 (12,466)d	*	0,168	9
		white	0,594a			0,684d			
		black	0,683a			0,049ab			
	XT	l.blue	0,422a			0,818d			
		white	0,734a			0,447cd			
		black	0,865a			0,112ab			
	RT	l.blue	0,324a			0,142abc			
		white	0,456a			0,144bc			
		black	0,117a			0,013a			
Exp. 2	3DT	l.blue	1 (0,414)a	n.s.	0,142	1 (11,462)a	n.s.	0,150	6
		e.blue	1,000a			1,124a			
		white	1,565a			0,698a			
	XT	l.blue	0,841a			1,141a			
		e.blue	0,295a			0,858a			
		white	1,973a			0,658a			
Exp. 3	XT	l.blue	1 (0,625)abc	***	0,141	1 (4,760)bc	***	0,202	10
		e.blue	2,274bc			0,739abc			
		white	0,512ab			0,187ab			
		e.blue/l.blue	2,918c			1,847c			
		l.blue/white	1,336abc			0,951bc			
		e.blue/white	2,770c			1,217bc			
	Mono	l.blue	0,115a			0,499abc			
		e.blue	0,314a			0,557abc			
		white	0,314a			0,046a			
Exp. 4	XT	l.blue	1,090c	***	0,170	0,790bcd	***	0,166	40
		e.blue	1,165c			1,008cde			
		white	0,608a			0,570ab			
		black	0,667ab			0,383a			
		e.blue/l.blue	1 (4,640)bc			1 (6,629)cde			
		e.blue/white	1,114c			1,160def			
		e.blue/black	1,080c			1,533f			
		l.blue/white	0,687ab			0,729bc			
		l.blue/black	1,076c			1,308ef			
		white/black	0,937bc			1,203ef			

abcdef Treatments followed by the same symbol are not significantly different

surveys, acted as the control trap. Forty replicates were conducted. For *G. brevipalpis* the e.blue, l.blue, e.blue/white, e.blue/black and l.blue/black XTs all caught better than the control, but this was not significant. For *G. austeni* the e.blue/black XT was significantly better than the control (e.blue/l.blue) XT and increased the catches by *c.* 1,5 times. The e.blue/black XT therefore replaced the e.blue/l.blue XT in surveys conducted from May 1995 onwards.

The next experiment (Table 4.2) attempted to improve on the recommended XT of the previous experiment (i.e. e.blue/black XT) by finding an optimal size, or to simplify it by using a bicoloured monopanel (single panel of the XT), making it more practical to use in the field. Three sizes of the XT and monopanels were tested, namely one panel measuring 70 x 60 cm (as original XT panel size); 95 x 80 cm and 120 x 100 cm. Monopanels consisted of a single XT panel, measuring the same sizes as given for the XT above. Two types of bicoloured monopanels were tested. In the first, referred to as Mono I, each side of the panel was painted both e.blue and black (split vertically in the centre). In the second, referred to as Mono II, one side of the panel was e.blue and the other side black.

For *G. brevipalpis* the larger monopanels (i.e. 95 x 80 cm and 120 x 100 cm) were all equally effective as the control XT (70 x 60 cm). It was also shown for both sexes that the larger the trap the better its performance. This was especially the case for females, where previously the small-sized XT was ineffective, the bigger size would capture 2,4 – 4,2 x more. For *G. austeni* males and females the bigger sized Mono I panels (95 x 80 cm and 120 x 100 cm) were mostly significantly better than the control XT (especially for females). Similarly as for *G. brevipalpis*, an increased size of the control XT meant better performance for both sexes (especially for females).

The results for both species, therefore, suggested that a single Mono I (or Mono II for *G. brevipalpis*) panel of a larger size, which would be more practical to handle in the field and cheaper to make, could replace the XT used in distribution surveys.

Table 4.2 Comparisons of e.blue/black 3-dimensional XTs with 2-dimensional Monopanels of various sizes [Indices of increase are given relative to the control treatment (index = 1) in each experiment. Detransformed means of the controls are indicated in brackets. The number of replicates (n), the transformed standard errors (s.e.) and the probability that the means are different at the $P < 0,01$ (**) and $P < 0,001$ (***) levels of probability are given]

Species	Trap type	Size	Males			Females			
			Index	*P*	s.e.	Index	*P*	s.e.	*n*
G. brevipalpis	XT	70 x 60	1 (3,527)b	***	0,173	1 (0,655)abc	**	0,157	27
		95 x 80	1,435b			2,443cd			
		120 x 100	2,886c			4,246d			
	Mono I	70 x 60	0,347a			0,663ab			
		95 x 80	1,018b			1,641abc			
		120 x 100	1,178b			1,904bc			
	Mono II	70 x 60	0,430a			0,522a			
		95 x 80	1,232b			1,148abc			
		120 x 100	1,121b			1,321abc			
G. austeni	XT	70 x 60	1 (1,873)ab	***	0,105	1 (1,989)ab	***	0,165	27
		95 x 80	3,649d			5,884d			
		120 x 100	4,266d			10,595e			
	Mono I	70 x 60	0,820ab			1,338b			
		95 x 80	1,556bc			2,989c			
		120 x 100	1,954c			4,107cd			
	Mono II	70 x 60	0,442a			0,528a			
		95 x 80	1,009ab			2,708c			
		120 x 100	0,656a			1,165ab			

abcde Treatments followed by the same symbol are not significantly different

Estimates of trap efficiency

The efficiencies of the e.blue/l.blue and e.blue/black XTs were evaluated. Catches with and without a flanking net next to the trap for ten replicates were pooled. Trap efficiency is expressed as the proportion of flies of the pooled catch that were actually caught on the XTs (without flanking net) expressed as the percentage of flies that were caught by the traps with flanking nets (i.e. trap plus net). The overall trap efficiency of the e.blue/l.blue XT was 23 % for *G. brevipalpis* (33 % for males and 0 % for females) and 28 % for *G. austeni* (39 % for males and 24 % for females). The overall efficiency of the e.blue/black XT was 16 % for *G. brevipalpis* (22 % males, 2 % females) and 51 % for *G. austeni* (males 48 % and females 54 %). The low efficiencies of

the XTs for *G. brevipalpis* females explains the low catches obtained during preceding experiments.

4.4.2 Cloth traps

Results of the full series of Latin squares and comparisons with other designs and trap modifications are not given here. Apart from the H trap and its modifications, only a few traps and modifications, as described above, were worthwhile which included the Nzi, Nzi3 and B1-5 traps (Kappmeier, in press). Results, given below, are a summary of the work comparing only the H trap modifications and trap orientated behaviour around these modifications, which lead to the final design. Results of the final experiment comparing the H4 and H5 modifications with the Nzi, B4 and B5 traps are given.

Evaluation of initial H trap designs (H1 – H3)

It was observed with the prototype H trap that the flies tended to collect at the upper base corners of the cones (where they connect with the trap body). The prototype was then modified so that the H1, H2 and H3 modifications were developed as described earlier. The results of the H1-H3 modifications were originally compared with those of the Siamese trap, which acted as the control. All the results of the former were significantly ($p < 0,01$) better than those of the Siamese (i.e. 3,2 - 4,2 x for the total number of *G. brevipalpis* caught and at least 6,7 x for *G. austeni*). The H3 modification also consistently gave the best results when compared further with other promising traps, namely the XT, Nzi, Nzi3 and B3 traps (Kappmeier, in press) where it was found that the H3 caught twice as many *G. brevipalpis* as both the B3 and XT, and about three times more than the Nzi. The H3 caught significantly three times more *G. austeni* than the XT, while the remaining traps were ineffective for this species. The H3 caught mean daily catches of 12,0 *G. brevipalpis* (63 % females; 25 replicates), when baited with the Zimbabwe ox-odour blend, and was even more successful when baited with the best SA blend with mean daily numbers of 45,1 *G. brevipalpis* (64 % females; 12 replicates). The mean daily catches for *G. austeni* were 3,0 (82 % females, 25 replicates) when baited with

the Zimbabwe blend and 9,7 (64 % females; 12 replicates) when baited with the best SA blend. For *G. brevipalpis* the record catch in one day by an H3 trap was 76 flies and for *G. austeni* 37 flies.

Trap-orientated responses of tsetse in and around the H3 modification

In order to improve on the H3 design, the behavioural or trap-orientated responses of *G. brevipalpis* and *G. austeni* (Table 4.3 a & b) were determined by means of electric nets placed in and around the H3 trap, following the methods of Vale (1982a, 1982b). [Simultaneously this was done with the B3, B4 and Nzi traps, the results of which are given in Kappmeier (in press).] Only 16,8 % of the *G. brevipalpis* (total catches) that were initially attracted to the H3 trap actually attempted to enter them (Table 4.3 a). The lateral upward-sloping/diagonal cones were quite effective in inducing horizontally-directed flight responses, especially for *G. brevipalpis* for which it was found that all flies that found the entrances of the trap, thereafter flew in a horizontal direction and were captured. For *G. austeni* (Table 4.3 b) only 28,3 % of the flies that found the entrances flew towards the cones. Only four replicates (*n*) of this experiment were carried out. The statistical *P* and s.e. values are given in the Tables.

Table 4.3 Behavioural responses of a) *G. brevipalpis* and b) *G. austeni* in and around the H3, H4 and H5 trap modifications as determined with electric nets [The results are expressed as a percentage relative to the mean daily number of the flies attracted to the traps (indicated as 100 %). The detransformed mean number of flies that were attracted are given in brackets for the control treatment. The number of replicates (n), the transformed standard errors (s.e.) and the probability (P) that the means are different or not significantly different (n.s.) are given.]

a) G. brevipalpis

Trap type and treatment	Males		Females		Totals	
H3						$P < 0{,}001$
Flies attracted	100 (26,308)	$P < 0{,}001$	100 (27,112)	$P < 0{,}01$	100 (54,422)	s.e.= 0,066
Entrance response	18,4	s.e.= 0,055	15,8	s.e.= 0,098	16,8	$n = 4$
Sideways flight response	22,2	$n = 4$	16,2	$n = 4$	19,2 (100 % of tsetse that entered)	
Eventually caught (efficiency)	34,9		42,4		38,2	
H4						
Flies attracted	100 (15,827)	$P < 0{,}001$	100 (12,856)	$P > 0{,}05$ n.s.	100 (29,360)	$P < 0{,}01$
Entrance response	60,3	s.e.= 0,529	61,3	s.e.= 0,071	62,6	s.e.= 0,057
Sideways flight response	44,7	$n = 14$	56,5	$n = 14$	50,0 (79,9 % of tsetse that entered)	$n = 14$
Eventually caught (efficiency)	37,7		59,7		47,9	
H5						
Flies attracted	100 (30,299)	$P < 0{,}001$	100 (21,264)	$P < 0{,}001$	100 (51,870)	$P < 0{,}001$
Entrance response	49,5	s.e.= 0,070	54,4	s.e.= 0,069	51,6	s.e.= 0,068
Sideways flight response	19,7	$n = 12$	24,0	$n = 12$	21,9 (42,4 % of tsetse that entered)	$n = 12$
Eventually caught (efficiency)	30,5		32,6		31,9	

Table 4.3 (Cont.)

b) G. austeni

Trap type and treatment	Males		Females		Totals	
H3						
Flies attracted	100 (8,836)	$P = 0,05$	100 (16,855)	$P < 0,01$	100 (26,964)	$P < 0,05$
Entrance response	36,5	s.e.= 0,140	20,3	s.e.= 0,1031	27,2	s.e.= 0,131
Sideways flight response	8,8	$n = 4$	5,9	$n = 4$	7,7 (28,3 % of tsetse that entered)	$n = 4$
Eventually caught (efficiency)	29,4		45,9		38,4	
H4						
Flies attracted	100 (6,920)	$P > 0,05$ n.s.	100 (14,983)	$P < 0,05$	100 (23,624)	$P > 0,05$ n.s.
Entrance response	57,8	s.e.= 0,148	43,6	s.e.= 0,110	44,4	s.e.= 0,122
Sideways flight response	58,8	$n = 8$	45,3	$n = 8$	44,3 (99,8 % of tsetse that entered)	$n = 8$
Eventually caught (efficiency)	31,6		26,9		29,0	
H5						
Flies attracted	100 (4,002)	$P > 0,05$ n.s.	100 (12,173)	$P < 0,01$	100 (16,819)	$P < 0,01$
Entrance response	60,1	s.e.= 0,105	66,6	s.e.= 0,077	69,4	s.e.= 0,0653
Sideways flight response	63,2	$n = 8$	33,8	$n = 8$	42,5 (61,2 % of tsetse that entered)	$n = 8$
Eventually caught (efficiency)	43,1		36,0		37,6	

Evaluation of H4 and H5 modifications

The H4 trap was a modification of the H3, and took into account its shortcomings as determined with electric nets. It, therefore, had bigger entrances (65 x 45 cm) and thus a slightly bigger body (125 x 65 x 65 cm) than the H3 to improve on the entrance responses of the flies. The H4 trap was further modified by providing it with somewhat larger cones to become the H5. The lower (bottom) side of each cone was at less of an acute angle (lower slope) to the body of the trap than the previous two modifications. This change was aimed at preventing flies from flying against the lower side and then bouncing off (especially in the case of the bigger *G. brevipalpis*), so that it was easier to progress to the trap collecting device.

The results for *G. brevipalpis* males, females and total catches and for *G. austeni* total catches as obtained with the H4 and H5 traps are compared in Table 4.4 with the B4 and B5 modifications (from Kappmeier, in press) and the Nzi. The results are given as indices of increase relative to the Nzi (with index = 1). The detransformed means of the catches obtained by the Nzi are given in brackets. Treatments' indices (for total catches) followed by the same symbols (a,b or c) are not significantly different.

The results showed the Nzi trap to be relatively effective for *G. brevipalpis* and although the H4 and H5 were better than the Nzi, this was not significant. The Nzi was poor for capturing *G. austeni* and the H4 and H5 increased catches significantly by *c.* 3,0 – 4,1 times respectively compared to the catches obtained with the Nzi. The larger cones of the H5 (compared to the H4) had no effect on the number of flies of either species captured. The mean daily catch for *G. brevipalpis* was 15,7 (69,5 % females) with the H4 trap and 16,9 (70,8 % females) with the H5 trap (28 replicates). For *G. austeni* the mean daily catch was 5,7 (99,0 % females) with the H4 trap and slightly better at 7,6 with the H5 trap (14 replicates).

Table 4.4 Final comparisons of the H4 and H5 modifications with the B4, B5 and Nzi traps [The results are expressed as the indices of increase relative to the Nzi trap (index = 1). The detransformed means of the Nzi are given in brackets. The number of replicates (*n*), the transformed standard errors (s.e.) and the probability (*P*) that the means are different are given]

Trap	*G. brevipalpis*						*G. austeni*	
type	Males		Females		Totals		Totals	
Nzi	1,000ab	(4,228)	1,000bc	(9,610)	1,000bc	(13,673)	1,000ab	(1,379)
B4	0,668a	$P < 0,05$	0,554a	$P < 0,05$	0,569a	$P < 0,05$	0,685a	$P < 0,001$
B5	0,919ab	s.e.=0,154	0,768ab	s.e.=0,146	0,804ab	s.e.=0,137	2,622bc	s.e.=0,177
H4	1,235b	$n = 28$	1,132bc	$n = 28$	1,145bc	$n = 28$	4,102c	$n = 14$
H5	1,170b		1,245c		1,236c		5,487c	

abc Treatments' indices followed by the same symbol are not significantly different from each other

Trap-orientated responses of tsetse in and around the H4 and H5 modifications

The behavioural or trap-orientated responses of tsetse flies in and around the H4 and H5 traps were tested in a final attempt to confirm whether the modifications of the H3 that were made were worthwhile, and also to make a final decision as to which of the modifications should be employed for future use. The results are given in Table 4.3 a and b for *G. brevipalpis* and *G. austeni* respectively. The number of replicates performed is indicated in the Tables. The various responses and trap efficiencies are given as a percentage relative to the mean daily number of flies that were attracted to the traps (detransformed means of the control trap are given in brackets). The statistical *P* and s.e. values and the numbers of replicates (*n*) are given in the Table.

For *G. brevipalpis* it was clear that the bigger entrances of the H4 and H5 modifications were an advantage in that more flies (51,6-62,6 %) attempted to enter these traps than the number entering the H3 (16,8 %). On the other hand, all flies that entered the H3 trap flew in a horizontal direction to the cones,

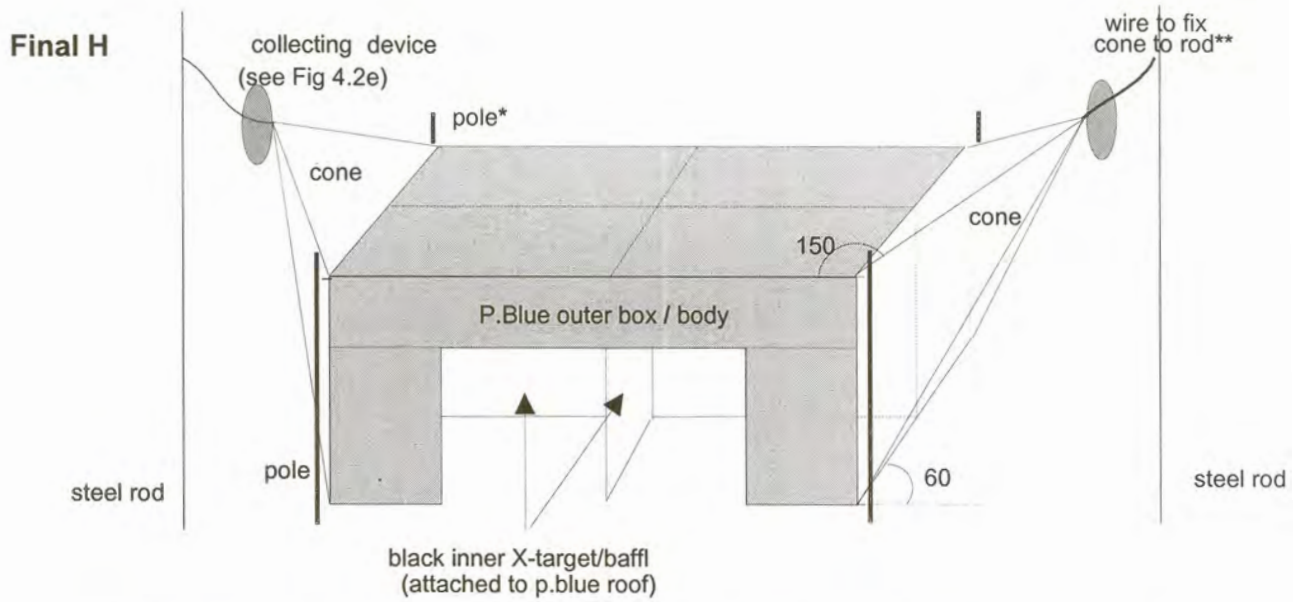
while only 42,4 - 79,9 % of the flies entering the H5 and H4 traps respectively, flew horizontally. It may, therefore, be suggested that because of the bigger entrances, more flies could fly directly out of the trap again, i.e. fewer of them advanced towards the cones. Nevertheless, the overall efficiency of the H4 trap was still better than the H3 (47,9 % versus 38,2 %). The efficiency of the H5 (with larger cones) was lower (31,9 %) than the previous modifications which might indicate that the flies get disorientated towards the apex of the cones and fewer of them enter the collecting device.

For *G. austeni* the efficiencies of the H4 and H5 traps were determined respectively at 29,0 % and 37,6 %. For this species the bigger entrances of the H4 and H5 traps also prompted more flies to enter the traps (44,4 - 69,4 %) compared to the number of those entering the H3 (27,2 %). Between 61 % and nearly 100 % of the flies that entered the H4 and H5 traps also flew horizontally towards the cones, indicating that, unlike *G. brevipalpis*, they do not often immediately fly out, but, as was observed, tend to "linger" once at the entrance to or inside a trap.

The final design

In accordance with the trap-orientated responses, the final H trap design incorporated entrances of the same size as those of the H4 and H5 traps but the cone sizes were in-between those of the H4 and H5 traps. Further comparisons between the H4 and H5 and the final design were not conducted. This final H trap design (Fig. 4.3) caught a record catch of 180 *G. brevipalpis* and 57 *G. austeni* in one day. A schematic representation of the final design is given in Fig. 4.3 with material measurements and construction procedures. The same method of erection, i.e. with the use of poles, is employed as was described previously and as indicated in the Figures.

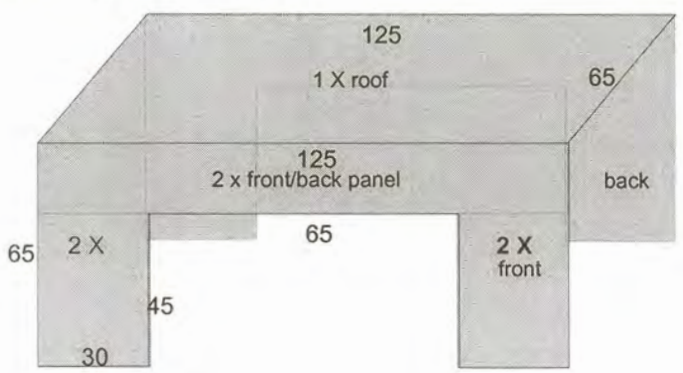
Fig. 4.3 Photograph of the final H trap design for the capture of *G. brevipalpis* and *G. austeni* (the trap is held upright by fastening the corners to four rigid metal poles (1,2 m long) and the cones are suspended from two flexible steel rods (1,4 m long)



* To erect the trap each corner of the trap is attached with string to a pole driven into the ground
**To keep the cones rigid they are attached with wire at the apex to tops of flexible steel rods

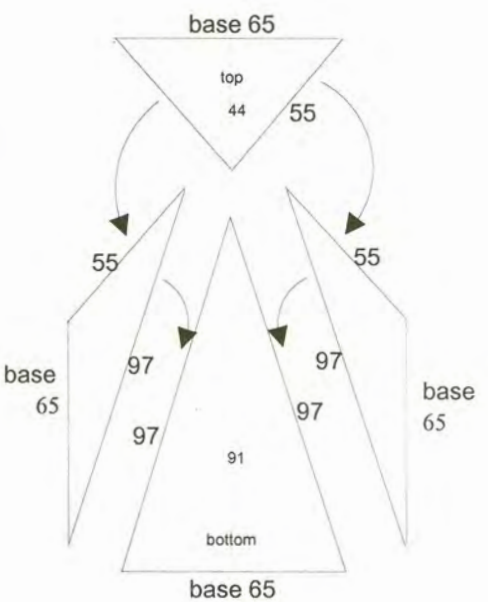
Material requirements (measurements in cm) and steps necessary for making:

P.blue outer box / body:



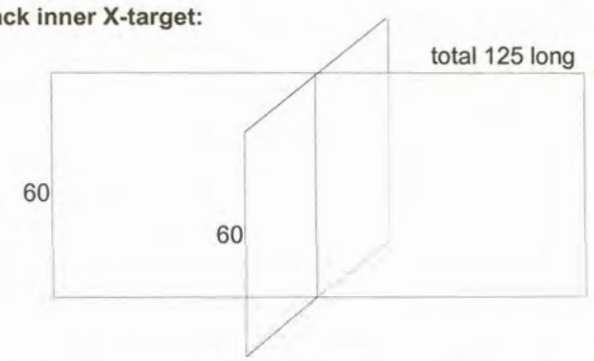
1. Sew the three blue pieces togethe (along the 125 cm sides) with roof panel between front and back panels

Net cone (x 2):



3. Sew pieces of netting togethe (as indicated) and then attac completed cone's top and side base to sides of blue outer box at the cones' positions

Black inner X-target:



2. Sew black target/baffle pieces together (as indicated) and attach target to centre of blue roof (indicated with broken lines in drawing of final H trap)

4. Cut apex of cone open to size of circumference of collecting system's base bottle (see Fig 4.2e) and attach reinforcing seam to prevent netting from tearing

Fig. 4.4 Diagrammatic representation of the final H trap with details of materials and measurements for trap construction

4.5 DISCUSSION

4.5.1 Sticky traps

Effect of colour and shape

For *G. brevipalpis* l.blue, white and black traps showed no significant difference in the catches during initial studies of the 3DT, XT and RT sticky traps. For *G. austeni*, l.blue and white were significantly better than black in initial studies with these traps. Later studies with the XT showed l.blue and e.blue to be superior over black and white traps for both species. Sticky traps tested concurrently by Vreysen *et. al.* (1996) for the monitoring of *G. austeni* in Zanzibar showed, however, that sky blue, baby blue and white monopanels (MP) showed no significant difference in the catch size.

The 3-dimensional shapes of the 3DT and XT were more or less equally effective for *G. austeni* and *G. brevipalpis* and significantly more attractive than the RT for *G. austeni*. The main advantage from the 3-dimensional traps over the RT seems to be that the trap is visible from all directions all of the time and this could account, together with the bigger surface area, for the higher catches. The XT was chosen for further upgrading, being more practical to make and use in the field. Initial experiments showed that bicoloured XTs of white were less effective than single coloured e.blue and l.blue traps for both species. For *G. brevipalpis* single e.blue trap was very effective, together with an e.blue/white and e.blue/l.blue, the latter which was most effective. These bicoloured traps were also most effective for *G. austeni* of which the e.blue/l.blue XT was also best for this species. Early studies of Vreysen *et al.* (1996) showed that the only occasion where a combination of colours significantly affected the catch rate of *G. austeni* in Zanzibar, was with legpanels (LP) coloured white on one panel side and sky blue on the other side. In this study, however, l.blue/white XTs were the most ineffective bicoloured traps for *G. austeni* and also for *G. brevipalpis*.

From December 1993 the e.blue/l.blue, due to its best performance for both species, used to survey the distribution of the two species in Zululand (Nevill *et al.* 1995). More tests with single and bicoloured XTs showed e.blue XTs to be as effective as the e.blue/black XT for both species while a black (and white) XTs was very ineffective for *G. austeni*. This corresponds with previous studies on cloth targets where phthalogen blue (e.blue is the closest resemblance of phthalogen blue) was also attractive for both species but black was very unattractive for *G. austeni* (Kappmeier & Nevill 1999b). Low trap catches of *G. austeni* were also obtained with single coloured black XTs on Unguja Island (Vreysen *et al.* 1998).

For *G. austeni* only the bicoloured e.blue/black XT was significantly better than the e.blue/l.blue XT and increased catches by 1,5 times, while e.blue/white, white/black and l.blue black bicoloured XTs were also relatively effective. For *G. brevipalpis* the e.blue/black XT was also, together with e.blue/white and l.blue/ black XTs, found to be more or less equally effective to the l.blue/e.blue XT for *G. brevipalpis*. For this reason the e.blue/l.blue XTs used in surveys were replaced by the e.blue/black XTs from May 1995 (Nevill 1997; Nevill *et al.* 1999). Phthalogen blue/black cloth target combinations were also most effective for the two species (Kappmeier & Nevill 1999b). Targets designed in blue/black combinations are also highly attractive for *G. morsitans* and *G. palpalis* species (Green 1993; Vale 1993; Merot & Filledier 1985; Laveissiere *et al.* 1987 cited in Vreysen *et al.* 1998). For *G. austeni* in Zanzibar poor catch results were obtained when black was combined with royal blue both as XT and XLP (cross-shaped legpanel) while royal blue/white XTs were more effective (Vreysen *et al.* 1998). Vreysen *et al.* (1998) ascribed the possible difference in the two *G. austeni* populations' behaviour to the genetic variations between the two populations or in differences of the spectral reflectance of the paint material used.

Trap efficiencies

The efficiencies of the e.blue/l.blue and e.blue/black were relatively high 33 - 39 % for *G. brevipalpis* males and very low for females (0 - 2 %). The low

efficiency for females could probably be ascribed to the relatively small size of the trap, in that catches of females increased with the bigger sized traps, as will be explained below. For *G. austeni* the traps were similarly effective for both sexes, i.e. 28 - 51 % for males and 24 - 54 % for females.

Effect of size of XT and monopanels

For *G. brevipalpis* the catches of both sexes increased as the size of the XTs and Monopanels increased although this was not always significant. Where previously the standard sized (70 x 60 cm) XT was ineffective for females, the bigger sizes increased catches of females 2,4 - 4,2 x. The larger monopanels (95 x 80 cm and 120 x 100 cm), i.e. Mono I (e.blue/black painted on both sides) and Mono II (e.blue painted one side and black on the other side) were equally effective as the standard XT. For *G. austeni* males and females an increase in the catches was related to an increase in the size of the trap. This was especially so with the XT where the bigger sized XTs increased the catches significantly by 3,6 - 4,3 x for males and 5,9 - 10,6 x for females. The bigger sizes of Mono I also improved the catches compared to the original sized XT by 1,6 - 1,9 x for males and 3,0 - 4,1 x for females, which was significant for females. Increased catches of these species were also obtained with an increase in the size of cloth targets (Kappmeier & Nevill 1999b). Vreysen *et al.* (1998) found an increase in the width of a blue XT (from 70 - 120 cm) doubled the catch of *G. austeni* in Zanzibar as compared to a standard sized blue XT.

Because larger sizes of e.blue/black monopanels of (Mono I) proved to be equally or even more effective than the XT for both species, it could therefore in future surveys be used instead of the XT. These would be cheaper in terms of construction material, paint, sticky material and kerosene for removal of sticky material. They are also lighter and easier to manipulate in the field, especially in densely forested areas.

4.5.2 Cloth traps

Following the improvements of the XT sticky trap, the H trap was developed and described for the monitoring and live collection of *G. brevipalpis* and *G. austeni*. It was designed after evaluating the behaviour of *G. brevipalpis* and *G. austeni* in and around Ngu (Ng2f) and Siamese (B) traps (Brightwell *et al.* 1987; Kyorku *et al.* 1993) in which it was shown that the two species were reluctant to fly upwards towards the cones (Kappmeier, in press). The H trap was, therefore, designed to do away with a top cone system, so that a totally different approach was employed, namely that of a trap fitted with two lateral devices (cones). This approach made use of the flies' preference to fly in a horizontal instead of a vertical flight path which is required in existing tsetse fly traps. The angled cones of the final trap incorporated an element of the ramp trap principle used extensively by mosquito ecologists (Service 1976).

This new H trap design proved to be effective, when baited with synthetic ox-odour, in catching *G. brevipalpis*, since it is known that this species is attracted by colour and odour (Kappmeier & Nevill 1999a, 1999b). It was, however, not as efficient in capturing *G. austeni*, probably because *G. austeni* is not attracted by the odours (Kappmeier & Nevill 1999a) although it responds strongly to colour (Kappmeier & Nevill 1999b). It may be that the odour does influence short-range trap entering behaviour (Vale & Hall 1985a) of *G. austeni*. The final version of the "horizontal" or H trap was developed after testing five modifications of the original prototype.

Some of the H trap modifications increased the sizes of the catches when compared to those of the XT sticky trap by up to 1,4 times (not significant) for *G. brevipalpis* and by up to 2,4 times (significantly) for *G. austeni* (Kappmeier, in press). The advantage of the H trap over the XT sticky trap, used in tsetse distribution surveys (Nevill 1997), is that flies are captured alive and can thus be used for studies on population dynamics. It can also be used for the automatic treatment of wild-caught flies with a variety of agents ranging from entomopathogenic fungi (Kaaya *et al.* 1991) to insect growth regulators (Hargrove & Langley 1990; Langley 1995, 1999).

Highest catches with the final H trap were 57 *G. austeni* and 180 *G. brevipalpis* in one day. Compared to the previous best live trap catches at Hellsgate with the Ng2f and Siamese traps, this new trap is a definite improvement. Although the Nzi also performs relatively well for capturing *G. brevipalpis*, the H trap is still better and it is significantly better for *G. austeni*. There is no doubt still room for improving the H trap, especially as far as *G. austeni* is concerned. The horizontally situated cones are, however, a major step forward for capturing both *G. brevipalpis* and *G. austeni* alive and facilitates studies, which require the use of live wild-caught *G. austeni* and *G. brevipalpis*. The H trap is certainly an advance for the trapping of these two previously "difficult" species of flies.

5. POPULATION DISPERSAL AND MOVEMENT

5.1 ABSTRACT

Mark-release-recapture studies were undertaken at Hellsgate Tsetse Research Station to determine the population density and dispersal rates of *Glossina brevipalpis* and *G. austeni* in order to estimate target densities suitable to control these species. The recommended target density, based on the assumption of killing 4 % of the female populations per day, was estimated first at nine and ten targets per square kilometer for *G. brevipalpis* and *G. austeni* females, respectively, but was then reconsidered and adjusted to four and seven targets/km^2 for the two species respectively. *Glossina brevipalpis* was by far the more mobile species while *G. austeni* appeared to be more static. The movement of flies over open areas of vlei and grassland was also investigated to determine their value as natural barriers in a strategy to protect controlled areas from reinvasion. From the results it is evident that both species do, to a certain extent, traverse open areas of "unsuitable" habitat.

5.2 INTRODUCTION

In 1990 a serious outbreak of nagana in N.E. KwaZulu-Natal Province, South Africa (Kappmeier *et al.* 1998), precipitated a need to develop a long-term control strategy for the two vector species *Glossina austeni* and *G. brevipalpis*. Studies on colour targets (Kappmeier & Nevill 1999b) and odours (Kappmeier & Nevill 1999a) have resulted in the development of an attractive odour-baited target (Kappmeier & Nevill 1999c) which, if treated with a suitable pyrethroid, could be used for the control of the two species in South Africa. No studies have, however, been conducted on the population dynamics of these species.

Rogers & Randolph (1985) pointed out the importance in understanding the population dynamics of tsetse flies in planning all types of tsetse control operations and also to assess tsetse control interventions. A precise knowledge of this subject is particularly important when the operation employs insecticide-treated targets. An optimal strategy in this type of operation would

involve the deployment of the smallest number of targets sufficient to eradicate the tsetse population in any desired time interval or rate of population reduction (Hargrove 1988).

A number of studies have been conducted on the movement or dispersal of tsetse flies as the distances they can travel will affect the success of tsetse control schemes (Leak 1999). For the successful target implementation to control or eradicate *G. brevipalpis* and *G. austeni*, base-line data on their movement and dispersal was, however, needed so that the targets could in future be sited successfully in optimal locations and densities in the field. Such studies would be critical for the planning of any future control operations in South Africa.

5.2.1 Estimates of dispersal rates and population size

The control of tsetse fly populations using traps or targets depends on the movement patterns of the flies, which determines how many flies find the targets, and on the efficiency of the targets, which determines the proportion of flies that are killed (Williams *et al.* 1992). It has been shown that traps or targets, used mainly for control rather than eradication, can reduce tsetse fly densities to acceptably low levels (Vale *et al.* 1988a; Dransfield *et al.* 1990). However, for such ongoing control strategies to be viable they must be cost-effective to livestock-producers in Africa and it is essential to make the most efficient use of targets. In order to reduce tsetse fly populations, targets must therefore kill the flies more rapidly than the flies can reproduce or invade the control area (Williams *et al.* 1992).

For the savannah species *G. m. morsitans*, *G. m. centralis* and *G. pallidipes* it appears that, with the attractive synthetic odours presently available (Vale 1993) a density of about four targets/km^2 is necessary and sufficient to eradicate a population in nine months to a year (Hargrove 1993). Because of their low natural birth rate, a population can be eradicated by superimposing and sustaining, on the natural death rate, a mortality of 4 % per day on any female tsetse population, for example through the use of targets or traps

(Hargrove 1981; Hargrove 1988). It seems likely that in most field conditions only an added 2–3 % is required (Hargrove 1988), which agrees with Williams *et al.* (1992) who stated that a population can be driven to extinction by imposing only an additional mortality rate greater than 2 % per day. This can easily be achieved with targets (Vale *et al.* 1988a).

Population studies often involve mark-release-recapture programmes, a technique that is potentially more promising by improved sampling devices. It raises the possibility that a high proportion of marked flies could be released into a small area to be recaptured (Hargrove & Vale 1979; Vale *et al.* 1984). Mark-release-recapture methods (Jolly 1965; Seber 1965) can be used to measure mortality for closed populations, but under more natural open conditions it is difficult to separate the effects of mortality and emigration, and the methods are generally complex and time consuming (Hargrove 1990, cited in Hargrove 1993). The recaptures obtained during mark-release-recapture operations, whilst providing an estimate of population size, also give some idea on the nature and extent of fly movement and dispersal (Rogers 1977; Hargrove & Lange 1989).

Many models concerning fly dispersal have been developed over the years by various authors. It was suggested that dispersal in tsetse flies could be viewed as a series of discrete daily steps each taken in a random direction (Bursell 1970). Although movement within a habitat appears to be random, Rogers (1977) assumed it to consist of fairly constant step lengths and that natural factors, e.g. humidity, availability of shade, host density and odour plumes, tend to limit movement to within the habitat and may reduce its randomness. He gave two methods for investigating the outcome of two-dimensional random movement appropriate to tsetse. The first model is a prediction of the mean distance d away from the starting point, assuming a constant step length s (the distance moved per unit time), and a variable number of steps x so that:

$$d \cong sx^{1/2}$$

Applying this to tsetse movement and defining a single step as the distance traversed in one day, Rogers (1977) proposed it is only necessary to know

accurately the mean population displacement over a period of time to calculate a value of s, the mean daily displacement. The second model is based on computer predictions of a series of random movements away from a release point. This involved the probability distributions for directions moved and distances covered per step. Hargrove (cited in Bursell & Taylor 1980) derived a more accurate definition of daily displacement d, based on modelled predictions of a series of random movements away from a release point, as:

$$d \cong 0{,}9sx^{½}$$

The predictions involved the probability distributions for the directions moved, and distances covered per step, but could be simplified by assuming that tsetse only fly for a few minutes per day and have a relatively constant step length. Hargrove (1981) further suggested the step length might vary and probably change with age and physiological stage of the fly. Hargrove & Lange (1989) suggested the 'rate' of dispersal to be simply defined as a diffusion coefficient rather than as a discrete step length. They therefore viewed tsetse dispersal as a diffusion process, with the position of a fly, relative to its origin, as a normally distributed random variable, i.e. the mean distance of a diffusing particle from the origin. Other models have estimated rates of advance of tsetse based on a root-mean-square displacement of 200 metres per day and a population growth rate of 1 % per day (Williams *et al.* 1992). Williams *et al.* (1992) implied that the dispersal of insects could be described by a Gaussian diffusion model with an exponential mortality term. The rate of diffusion (dispersal) was then defined by the root-mean-square displacement in one day (λ). If this is high, tsetse will disperse quickly into the vicinity of traps and there will be a rapid reduction of the population.

Bailey (1951) used simple recapture techniques to determine the maximum likelihood estimate of the population size P based on the number of flies marked and released M, the sample size recaptured N and the number of marked flies R in the sample N so that:

$$P = MN/R$$

However, he suggested that in certain ecological problems it may be more appropriate to use the reciprocal of the population size as the appropriate index, rather than the population size itself, so that:

$$1/P = R/MN$$

To improve the precision of mark-release-recapture technique it would be necessary to increase the expected number of recaptures, by increasing the number of marked flies released or increasing the expected recapture percentage (Vale *et al.* 1984).

In order to control tsetse flies successfully with targets and to ascertain the density of targets needed for the control of the two tsetse species in Zululand, it was necessary to initiate trials to evaluate the movement and dispersal rate of the two tsetse species. A good trap was, therefore, necessary to capture live *G. brevipalpis* and *G. austeni* in large enough numbers. The H trap described in the previous chapter was specifically developed for this purpose. Questions that needed to be answered were: At what rate does each of the species disperse? What is the population density at the research site? At what density should targets be placed in order to kill *c.* 3 - 4 % of the population per day?

The present study involved mark-release-recapture experiments to estimate the population size by determining the probability of recapture and then using the inverse of the population size as a population estimate as suggested by (Bailey 1951). Because the results were based on the degree of trapping efficiency of the H trap and the required levels of population control were based on this, it was essential to relate the results to the required effect to obtain control with targets. The relative performance of the H trap used in this study was, therefore, compared to the recommended control target. The target density required for certain levels of tsetse control based on the results obtained with the H trap could then be assessed.

5.2.2 Estimates of composition of natural barriers

As tsetse flies are relatively mobile, there is a constant reinvasion pressure against areas from which the flies have not been removed or controlled unless these measures are taken up to natural boundaries, or an effective barrier is maintained (Leak 1999).

Several methods of preventing reinvasion have been attempted over the years, often with little long-term success. In early days barriers of bush clearings to prevent reinvasion were used. The distance a fly could travel was, therefore, critical in determining the width for effective clearings. In Zambia, a bush clearing of one kilometer wide was standard for a 'holding line' (Wooff 1968, cited in Leak 1999) while Jackson (1954b, cited in Leak 1999) referred to the use of a 3,2 km wide clearing, which was necessary to stop the passage of flies. In Uganda, much wider clearings, up to 8 km wide, were used for tsetse (Wooff 1968, cited in Leak 1999).

Target barriers to prevent reinvasion or emigration into a controlled area could be used in control campaigns (Williams *et al.* 1992; Hargrove 1993). These barriers normally consist of stationary targets only but could also consist of a combination of stationary and mobile targets (i.e. cattle treated with deltamethrin) (Warnes *et al.* 1999). Use could also be made of natural barriers, e.g. large water masses, which tsetse could not traverse.

Tsetse flies do not normally venture far from trees during their daily activities as they seem to need to rest frequently and they also need cover to prevent exposure during flight. Therefore, it is unlikely that a tsetse fly would set off into a large open area such as an extensive body of water when there was no suitable object in sight to provide the next stopping point or, more important, shade. Barriers that have therefore been identified are higher ground and unsuitable temperature, natural and man-made bodies of water including large rivers, desert sands, natural treeless areas including grassland, flood plain and seasonal or permanent swamps, arid areas, mountains and expanding areas of

human settlement. The critical factor is the width of the particular body of water or natural treeless areas and lack of shade if it is to serve as a barrier to tsetse movement (Lovemore 1996).

In South Africa *G. brevipalpis* and *G. austeni* are confined to riverine, coastal, and low-lying forests and thickets of the N.E. KwaZulu-Natal area. The distribution of the two species is sometimes patchy, especially where forests are patchy and isolated. In this study it was proposed to establish the distance of apparently "unsuitable" habitat between pockets of forests and other suitable habitat. This could indicate whether such situations could in future act as a natural barrier between populations, or between controlled and infested areas. For *G. brevipalpis* it had already been recorded that they could roam out of these forested areas, especially during their times of main activity at dawn and dusk, and at night (Kappmeier 2000). However, it is not known what distance they will cross over these more open areas between forest pockets. For *G. austeni* the experience was that they are restricted to the pockets of suitable habitat, but whether they could cross small sections of unsuitable habitat, perhaps at night, was not known. This study was, therefore, designed to establish the distance that the two species may or may not cross between forest pockets over open areas of vlei and grassland at the Hellsgate research area.

5.3 MATERIALS AND METHODS

5.3.1 Relative efficiency of target vs. H trap

The relative performance (catch) of the H trap, compared to the recommended target, was established at Hellsgate Tsetse Research Station. This was needed because many experiments are done only with targets or only with traps and the question always arises as to how much better the target performs as they are generally more effective than traps. A comparative test was therefore necessary to establish the relative increase of catches obtained by the recommended SA target (Kappmeier & Nevill 1999c) vs. the H trap.

This comparison was also necessary for the present studies on population densities, with mark-release-recapture techniques. When the fly density in an area, and recapture rate of marked flies, are for example determined by traps (which they mostly are), it can be determined what number of traps will be needed per square kilometre to control the population at a certain rate (e.g. between 3 - 4 % female reduction per day). However, when targets are substituted for traps to conduct a control trial, it is necessary to adjust the calculations to determine what target density is required to have the same effect.

The performance of the H trap was compared with the recommended target to be used for control purposes, namely a 1,75 m black/blue/black target (Kappmeier & Nevill 1999b). Eighteen replicates were tested by means of a Latin Square design. The targets were tested by the use of electric grids, as described in Chapter 2. Targets and traps were baited with the best SA blend (see Chapter 2) placed ± 30 cm downwind of the target or trap. Treatments were operated daily from about 09:00 until dark, and electric grids were supplied with fully charged replacement batteries, halfway during the daily trial to remain effective throughout.

Flies were sexed and recorded, and then analysed statistically. The catches (n) were normalized using a $\log_{10}(n+1)$ transformation and subjected to analysis of variance (Anova), using GLIM4.

5.3.2 Mark-release-recapture trials

Estimates of dispersal rates and population size

Based on the trials that were conducted on *G. pallidipes* and *G. m. morsitans* in Zimbabwe (G.A. Vale, pers. comm., 1998) a mark-release-recapture trial was conducted at Hellsgate. H traps, designed specifically for the purpose of capturing live flies for mark-release-recapture studies, were used. Traps were placed in 'concentric' squares around a central release point, following an example used in Zimbabwe where 'concentric' squares radiated out at 500 m

intervals from the centre and traps were sited 500 m apart to form a grid (G.A. Vale, pers. comm., 1998). However, since nothing was yet known of the dispersal of *G. brevipalpis* and *G. austeni*, a smaller grid lay-out was designed for the present study.

The initial design of the grid was established in a stretch of sand-forest with pockets of dense thickets during August - December 1998. Since the type of vegetation is not optimal for visibility and flight as in a savanna situation, a series of squares was cut through the bush at 'linear' distances of 200, 400 and 600 m from the point of marking and release, which thus became the centre of three 'concentric squares'. The three squares were each marked out with white sisal twine, which made it easier to follow and not loose track in the forest. Traps, on each concentric line, were initially placed at intervals of 400 m, but afterwards changed to 200 m (indicated in Fig. 5.1 Block A) to be sure of results, especially for *G. austeni* (since the feeling was that this species might not disperse very fast).

H traps were set up before commencement of the trial, which started on 13 January 1999. The approximate trap positions are indicated with dots (Fig. 5.1, Block A). One of the two openings of the traps faced the downwind side of the prevailing wind direction.

Marking of flies at the centre was carried out for a total of 24 consecutive days, starting at about 07:00 until 17:30. For this, a set of 10 traps, which were located separately (*c.* 3 km away) to provide freshly-caught flies, were emptied approximately 2-hourly. Flies were kept cool and dark during transportation to the release site. This was done to ensure that all flies that were released were as fresh and viable as possible with the greatest chance of survival. Each day's mark was coded differently with yellow spots of artist's oil paint, on various positions on the thorax (see Fig. 5.2 a & b), so that recaptures could be tracked back to the day they were released, and so determine the period from release to recapture and distance traveled during that period. Score was kept on the number of males and females of each species that were released each day.

The recapture traps in the grid were numbered 1 - 46 (according to distance from centre) to keep track of the position where flies were recaptured. The 'centre trap' (no. 1) was not placed directly at the marking site but 30 m from the site so that it would not directly interfere with the flies' release. The trap catches were collected daily (early morning during the same time) by a party of two catchers during the 24 days that flies were marked and released and for a further 28 consecutive days until no more marked flies were recaptured (last eight days for *G. brevipalpis* and last four days for *G. austeni*). Thus over the period of the experiment there was more than one daily opportunity to recapture flies marked at a certain day previously. Daily records were kept of the species, sex, total number of unmarked flies for each trap, and the number and code of marked flies for each trap position for that day. This enabled the calculation of dispersal rates and population density for each species and sex.

Dispersal over open areas

In order to determine various distances of open (supposedly unsuitable) habitat, mark-release-recapture trials were conducted. Initially three separate release blocks were used at Hellsgate between September and December 1998, named Block B, C and D where flies were marked on position 10 of the thorax (see Fig. 5.2 b) with blue, red and green artist's oil paint for the three release areas respectively. They were recaptured in H traps placed in certain positions surrounding the release sites and separated by various distances of open areas between the release and recapture sites. Ten independent traps at a separate location provided a daily (early mornings) supply of freshly-caught flies which were transported to one of the release points where they were marked and released.

Fig. 5.1 shows the layout of the positions of the release and recapture sites of Block B (in blue), Block C (in red) and Block D (in green). Each of the release points in the different blocks is indicated with an X and marked B, C and D respectively. The recapture sites of each block in the surrounding areas are marked in the respective block colours, i.e. recapture sites B1-9 in Block B (blue), sites C1-11 in Block C (red) and sites D1-8 in Block D (green). Flies at

Block B were released every second day for 17 days (between 3 Sep. - 2 Nov. 1998) and at block C for 5 days (between 4 Nov. - 16 Nov. 1998). On every alternative second day, flies were released at block D for 21 days (between 9 Sep. - 17 Nov. 1998). The recapture traps were checked every second and alternative second day for each of the blocks, from the time that flies were marked until 17 Dec. 1998. Record was kept of the number of flies released at each block and the number of marked and unmarked flies recaptured at the surrounding traps.

For Blocks B - D results could only be obtained for *G. brevipalpis*, probably due to open areas being too extensive for *G. austeni* to cross. Therefore, a new trial (Block E – indicated in Fig. 5.1 in orange/yellow) was planned, with the release site (indicated with xE) being an isolated small pocket of bush in grassland. This bush was also much closer to other patches of bush, so that the recapture traps were mainly placed within these surrounding patches of bush. The distances between the release site and the ten recapture sites were, therefore, much shorter than in previous trials (B, C and D). The trial started on 16 March 1999. Flies were marked and released until 27 March 1999. The recapture traps were checked during this period on a daily basis, and continued until 9 April 1999.

5.3.3 Odours

All traps and electrified targets in this study were baited with the best SA blend, as described in Chapter 2.

5.3.4 Marking techniques

Marking was done with artist's oil paint (Fig. 5.2 a) on different positions of the thorax (Fig. 5.2 b). For each trial (release points) different colours and coding were used. In the dispersal study (Trial E) positions of the markings were varied so that all flies released on a specific day were differently coded and records could be kept of the time it took a fly to reach a specific trap from the release point.

Fig. 5.1 Copy of airphoto of Ndlozi peninsula, Lake St. Lucia, showing the vegetation of the Hellsgate study area. The positions of various Blocks (A-E) used in mark-release-recapture trials are shown. [Release sites are indicated with x and trapping sites for the recapture of flies are numbered in different colours for each block as described in the text. The straight line distances are indicated between release and recapture sites that were crossed over open grassland and vlei areas, i.e. for *G. brevipalpis* males (green), *G. brevipalpis* females (blue), *G. austeni* males (black) and G. austeni females (red). Distances covered in block C are indicated as broken lines. (In Block E only *G. austeni* recaptures are indicated).]

Fig. 5.2 a Yellow artists' oilpaint was used to colour-code flies on positions of thorax as also indicated in Fig 5.2 b.

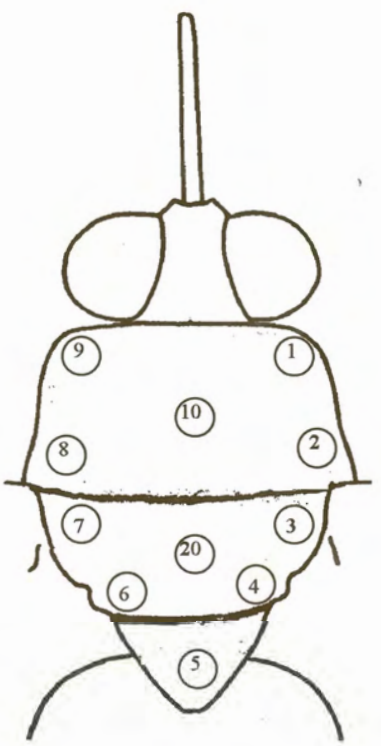


Fig 5.2 b Positions on thorax used for marking (e.g. for position 18, positions 10 + 8 are marked)

5.4 RESULTS

5.4.1 Relative efficiency of target vs. H trap

The results for the experiment, which compared the catches obtained by an H trap to a target, are given in Table 5.1. The results are expressed as an index of increase of the detransformed mean catches of the H trap (index = 1) relative to the mean target catches. Geometric means for the trap catches are given in brackets. The levels of probability (*P*) that the means are different at $P < 0,01$ (**) or $P < 0,001$ (***) are indicated in the Table as well as the number of replicates (*n*) for each treatment, the degrees of freedom (df) for error and the transformed standard errors (s.e.).

As expected, the recommended target increased catches significantly compared to the H trap for both *G. brevipalpis* and *G. austeni* males and females. For *G. brevipalpis* the target increased male and female catches, respectively, by *c.* 2,8 and 2,2 times compared to the H trap. For *G. austeni* the target increased catches of males and females by *c.* 33,4 and 6,8 times, respectively. These results can now be applied to determine target densities when making use of the results of a mark-release-recapture trial where H traps instead of targets were used to determine population density (see Table 5.3).

Table 5.1 Indices of increase of the recommended target[1] relative to the H trap [Geometric means of the H trap are given in brackets]

		Indices of increase					
		H trap	Target[1]	*n*	df	*P*	± s.e.
G. brevipalpis	Males	1 (23,140)	2,811	18	24	***	0,073
	Females	1 (29,690)	2,181	18	24	**	0,064
G. austeni	Males	1 (0,565)	33,445	18	24	***	0,066
	Females	1 (7,196)	6,815	18	24	***	0,053

[1] 1,75 m black/blue/black (50/75/50 cm) target (best SA target)

5.4.2 Mark-release-recapture trials

Estimates of dispersal rates

Table 5.2 is a summary of the total number of each species and sex released at the release site over the 24 mark-and-release days and the total number of flies captured and recaptured at traps number 1 - 46 over the 52 recapture days. The approximate linear distances of each trap (1 - 46) from the centre are also indicated in the Table. Recaptured flies are given as the total number of unmarked and marked flies captured over the period at each trap site. During the period of study a total of 2,683 male and 3,563 female *G. brevipalpis* and 1,518 male and 6,977 female *G. austeni* were marked and released at the centre. A total of 8,627 male and 13,697 female *G. brevipalpis* and 1,984 male and 9,436 female *G. austeni* were caught in the recapture traps (no. 1 - 46). Of these 159 male (1,8 %) and 112 female (0,8 %) *G. brevipalpis* and 21 male (1,1 %) and 291 female (3,1 %) *G. austeni* were marked. Most of the recaptures, 66 male and 29 female *G. brevipalpis* and 25 female *G. austeni*, were made at the 'centre trap' no.1. The direction of fly movement from the release point appeared to be random.

To investigate the movement of tsetse within the block, the recaptures made in the block were separated according to various distances from the centre of the block, at various times after release. Figs. 5.3a-d summarizes, for *G. brevipalpis* and *G. austeni* males and females, respectively, the concentration of flies expressed as the total number of recaptured flies, which dispersed over the various distances from the centre over the various number of days in time. This gives a clear 3-dimensional picture of dispersal rates of the flies.

Table 5.2 Summary of details on the number of flies released and recaptured at the various trap sites – 13 January to 5 March 1999

		G.b males		*G.b* females		*G.a* males		*G.a* females	
Total released		2683		3563		1518		6977	
Total (re)captured		Un-marked	Marked	Un-marked	Marked	Un-marked	Marked	Un-marked	Marked
Trap no.	Distance from rel. site								
1	30	241	66	377	29	42	0	254	25
2	200	128	3	213	6	84	2	395	21
3	200	126	7	164	3	50	1	253	6
4	200	111	3	225	2	53	3	276	17
5	200	245	10	277	3	74	0	289	5
6	283	99	6	172	3	73	1	245	10
7	283	136	0	229	8	23	0	120	2
8	283	154	3	237	2	55	1	194	6
9	283	171	4	282	2	45	1	215	11
10	400	160	0	172	0	28	0	180	9
11	400	128	1	241	3	35	0	200	6
12	400	144	0	296	3	43	1	221	4
13	400	184	2	283	2	29	0	120	5
14	447	123	1	248	1	59	0	373	9
15	447	31	1	122	1	18	0	86	4
16	447	69	0	115	1	40	0	127	2
17	447	127	1	222	2	46	0	223	2
18	447	112	3	226	1	26	0	185	8
19	447	353	5	455	1	40	3	234	12
20	447	504	3	788	3	61	2	234	9
21	447	241	1	350	0	25	0	209	4
22	566	78	1	149	0	56	0	376	12
23	566	89	1	153	0	24	0	73	0
24	566	229	3	386	0	49	1	154	6
25	566	711	5	1 088	2	78	0	243	9
26	600	130	2	202	1	59	0	327	6
27	600	55	2	86	0	9	0	31	1
28	600	155	0	279	1	34	0	137	4
29	600	744	1	1 112	4	83	0	364	3
30	632	291	2	563	3	60	0	388	12
31	632	177	4	324	3	15	0	39	2
32	632	79	2	161	1	28	0	88	4
33	632	203	2	295	3	46	0	209	3
34	632	160	1	311	0	54	2	419	15
35	632	219	0	243	0	28	1	135	3
36	632	173	1	267	3	48	0	163	1
37	721	130	2	282	1	59	0	298	3
38	721	72	1	83	1	16	0	35	0
39	721	64	2	112	3	29	0	54	2
40	721	89	2	116	1	42	0	111	2
41	721	169	0	232	3	38	1	260	3
42	721	221	0	357	3	39	1	196	4
43	721	364	2	513	0	42	0	149	10
44	849	94	0	214	2	23	0	48	4
45	849	64	2	172	1	25	0	70	4
46	849	121	1	191	0	30	0	145	1
TOT		8 468	159	13 585	112	1 963	21	9 145	291

The proportions of *G. brevipalpis* males and females recaptured at the traps most distant from the release point, that is recaptured at 600 - 849 m from the centre, increased soon after release. From this it seems that they reached the outer limits (600 - 849 m) of the recapture block within a short time (*c.* 1 - 7 days for males and *c.* 1 - 9 days for females), after which only a very few individual flies were recaptured within the block as time passed. One hypothesis to explain the recapture of *G. brevipalpis* later in the recapture period, is that many of the released flies rapidly diffused out of the area and some of these probably diffused back again later, so appearing in the traps. This is supported by the recapture of marked flies on occasions at the collection traps, placed at linear distances approximately 2,585 m from the centre release point of the block as well as further at two sites, i.e. 3,138 m and 3,310 m away.

For *G. austeni* not many males were recaptured, but the proportions of both males and females recaptured at the most distant traps from the release point, seemed to increase more or less with time. Although some specimens reached the outer limits of the block after only one or a few days, for most flies the distance dispersed depended on time. Some flies also remained close to the release point. It appears that this species dispersed much slower than *G. brevipalpis*. No significant data could be obtained for *G. austeni* males, since the H trap is biased for females and therefore very few males are captured in the first place (note the small number of males marked and released compared to females).

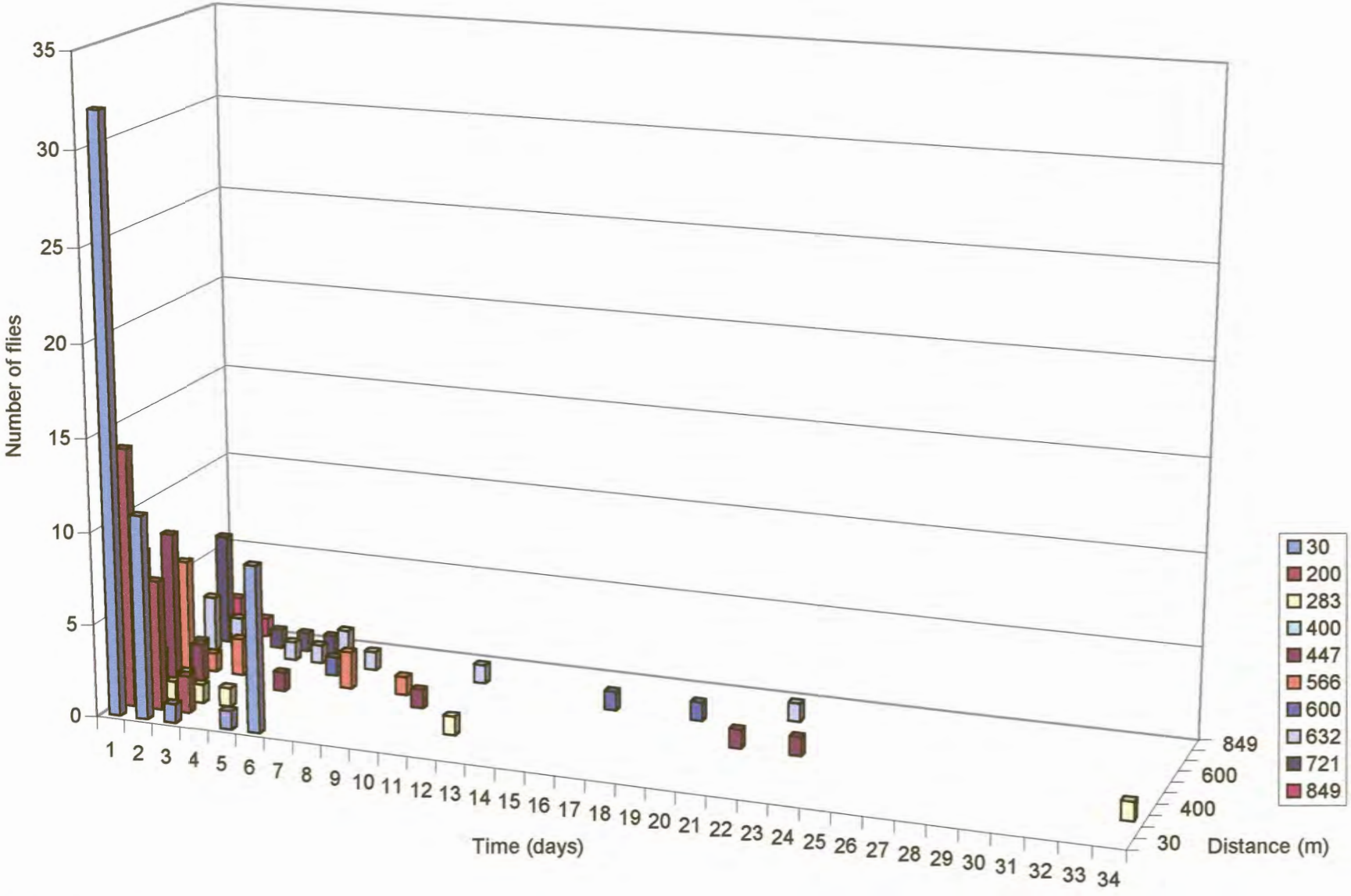


Fig. 5.3 a Summary of the dispersal rates for *G. brevipalpis* males

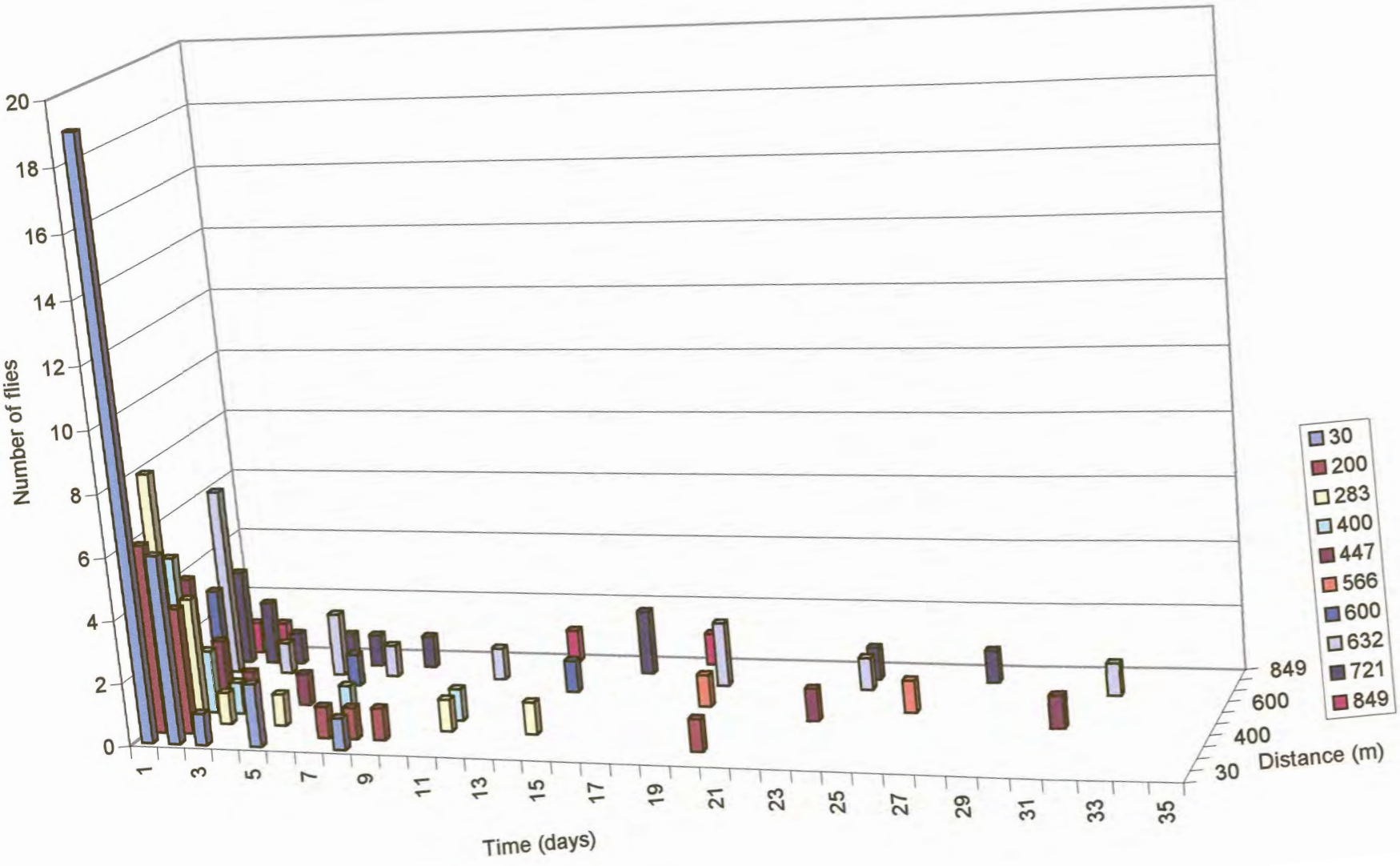


Fig. 5.3 b Summary of the dispersal rates for *G. brevipalpis* females

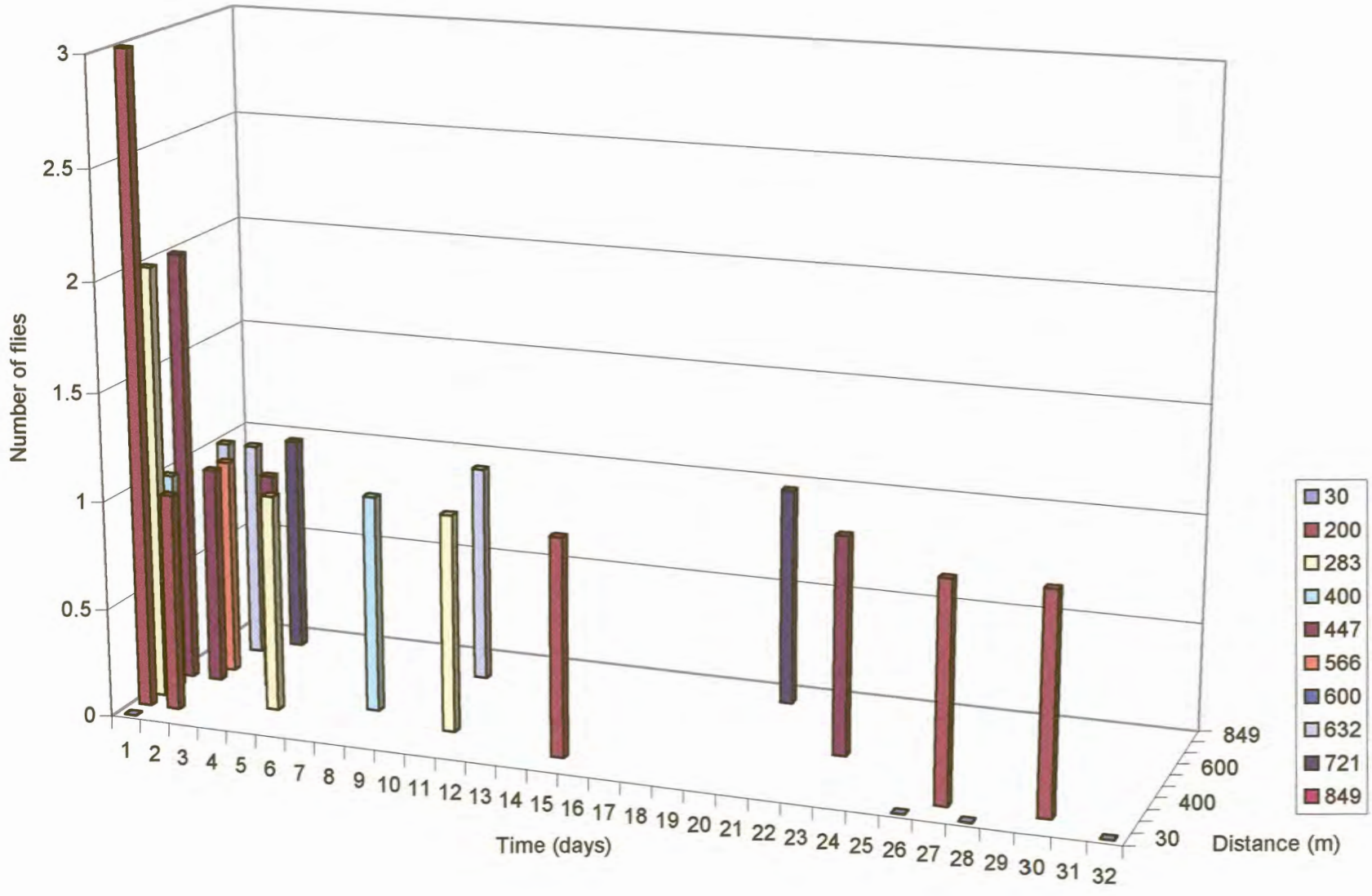


Fig. 5.3 c Summary of dispersal rates for *G. austeni* males

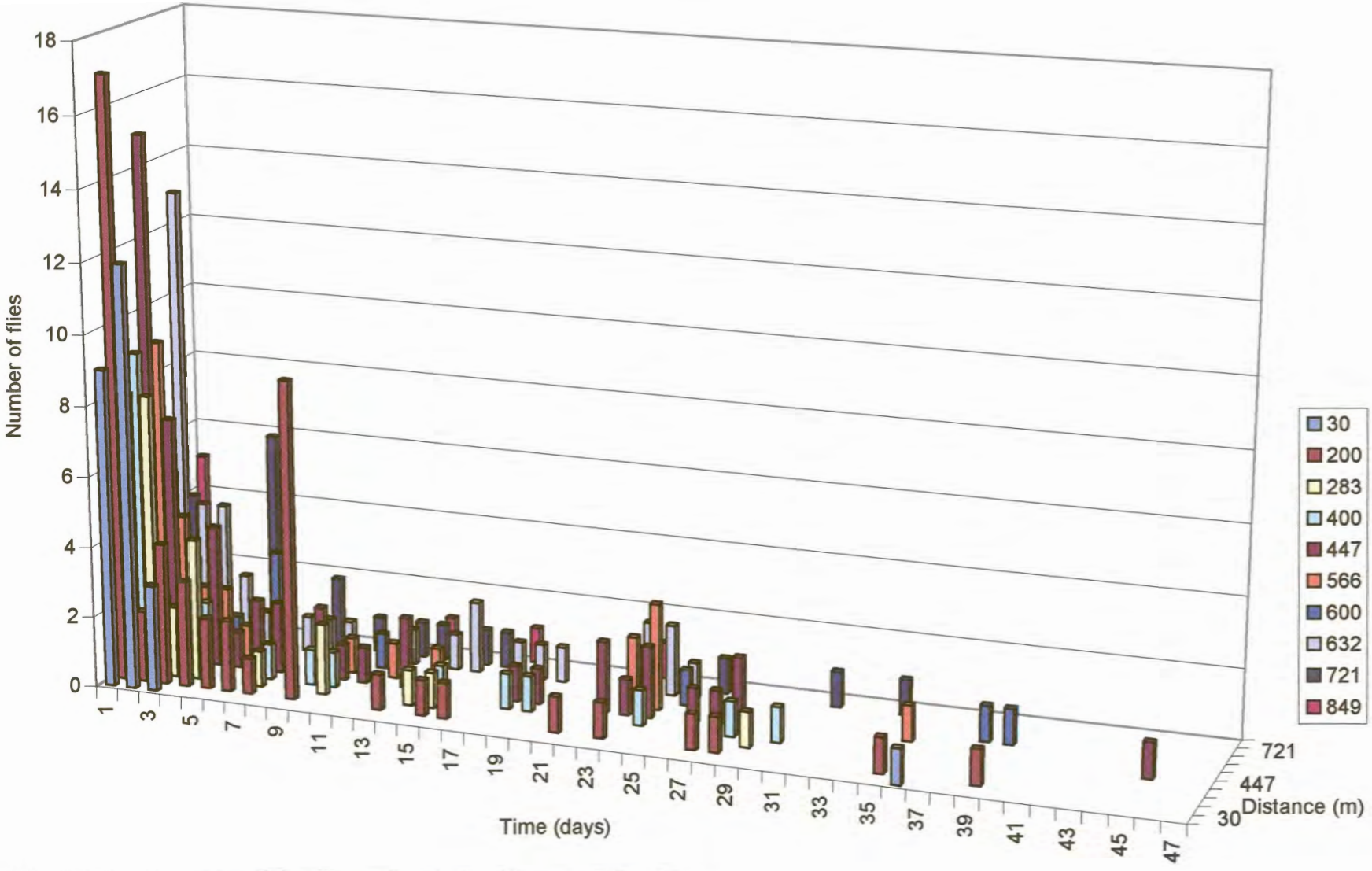


Fig. 5.3 d Summary of the dispersal rates for *G. austeni* females

For both species it is, therefore, notable that the concentration of recaptures decreased with distance from the release point. Both the distribution of recaptures in the traps and the decrease in concentration with distance suggests that the movement of *G. brevipalpis* and *G. austeni* in this habitat is a simple diffusion from the point of release.

The data for *G. brevipalpis* and *G. austeni* is inappropriate to determine the exact rate of movement, since some flies moved fairly rapidly to the outermost concentric square traps and, presumably, beyond, so that the mean distance moved away from a starting point and the daily step length could not accurately be estimated in this study.

Estimates of population size

Further analyses, done to determine the population size, were based on estimates that were basically made on the way in which the capture probability (in all traps taken jointly) changed with time-since-release (see Fig. 5.4 for *G. brevipalpis* and Fig. 5.5 for *G. austeni*). Steps involved in the procedure are as follows (J.W. Hargrove, pers. comm., 1999):

1. Totals were obtained for marked releases, and unmarked and marked recaptures for each day of the trial.
2. A matrix of recaptures was then formed (using the daily totals as in point 1 above) by columnizing the daily total catches of unmarked flies and the sum of the daily marked and unmarked captures, together with the numbers of flies released on each day. The matrix further summarized the number of recaptures caught during each capture day after release (i.e. time after release).
3. In order to calculate the probability of recapture on each day after release, it was needed to know how many marked flies were released and were available for recapture. In doing this provision was made for the marked flies which were being removed from the population by means of trapping. Another matrix was designed to do this.

4. The population can be estimated by (Bailey 1951):

$$Population = MN / R$$

where M is the number of marked flies released and N is a random sample of flies taken some short time later, and R will be the sample of marked flies recaptured:

The probability of capture (p) is just the inverse of this and can be estimated by:

$$p = R / MN$$

One can then get estimates, and variances, of the probability as it changes with time after release. If n experiments were conducted and on the ith day there were M_i marked flies from that experiment in the population, a sample can then be taken on day i and in this sample there could be N_i flies, of which R_i were marked. Then as before:

$$p_i = R_i / M_i N_i$$

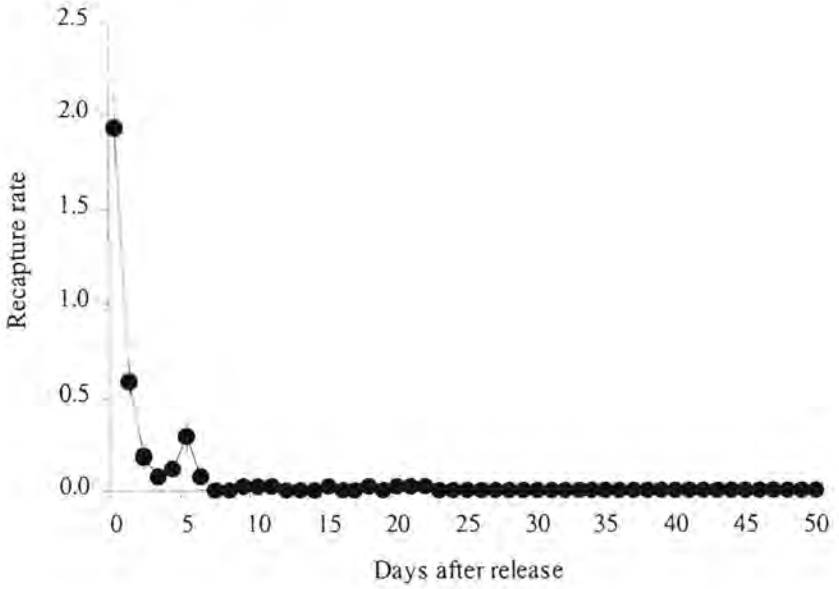
It can be shown that, if the data from all n experiments are used, the maximum likelihood estimate of p_i is given by:

$$p_i = \sum_{i=1}^{i=n} R_i / M_i N_i \qquad \text{Equation 1.}$$

Equation 1 has been used to calculate the capture probabilities.

The probabilities of recapture are given in Fig 5.4 at various days after release for male and female *G. brevipalpis* and in Fig. 5.5 for *G. austeni*.

a) *G. brevipalpis* males



b) *G. brevipalpis* females

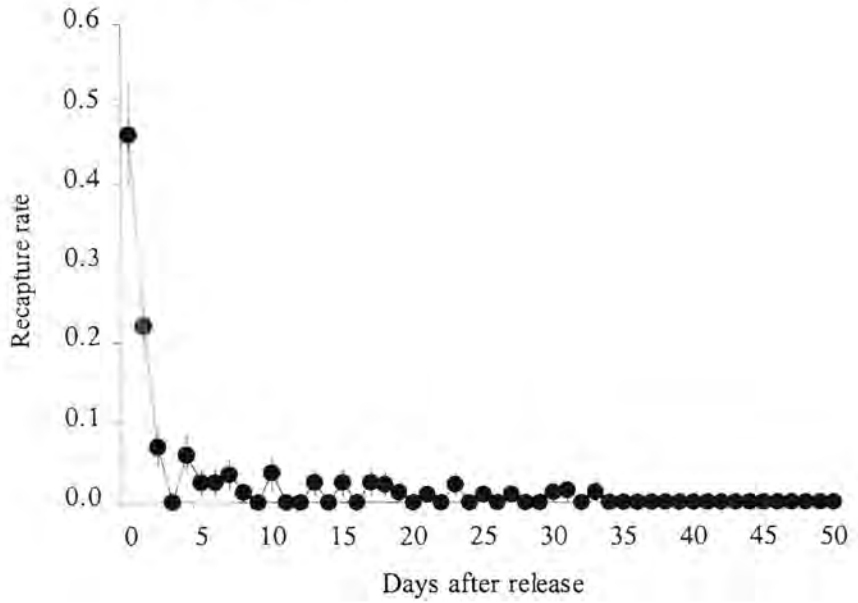
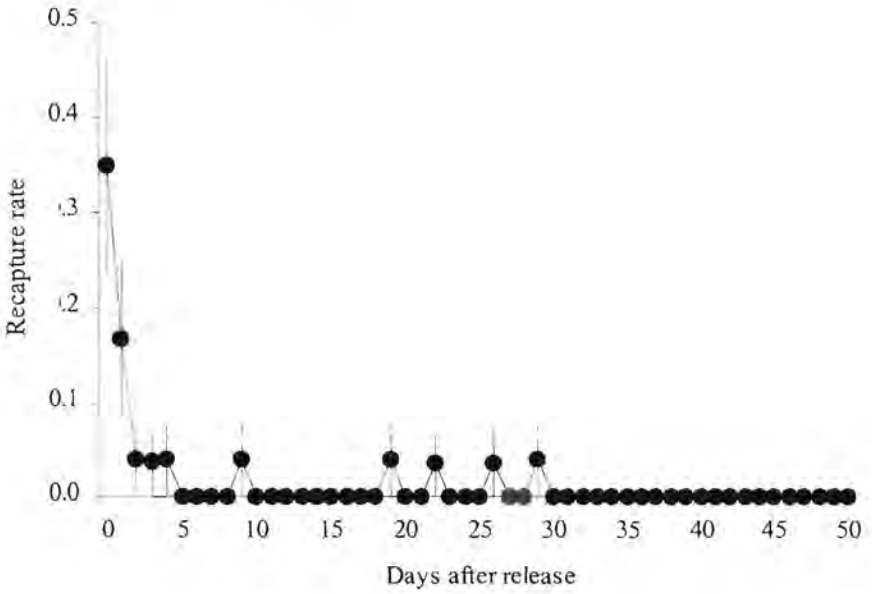


Fig. 5.4 Daily recapture rate at various days after release for *G. brevipalpis* a) males and b) females

a) *G. austeni* males



b) *G. austeni* females

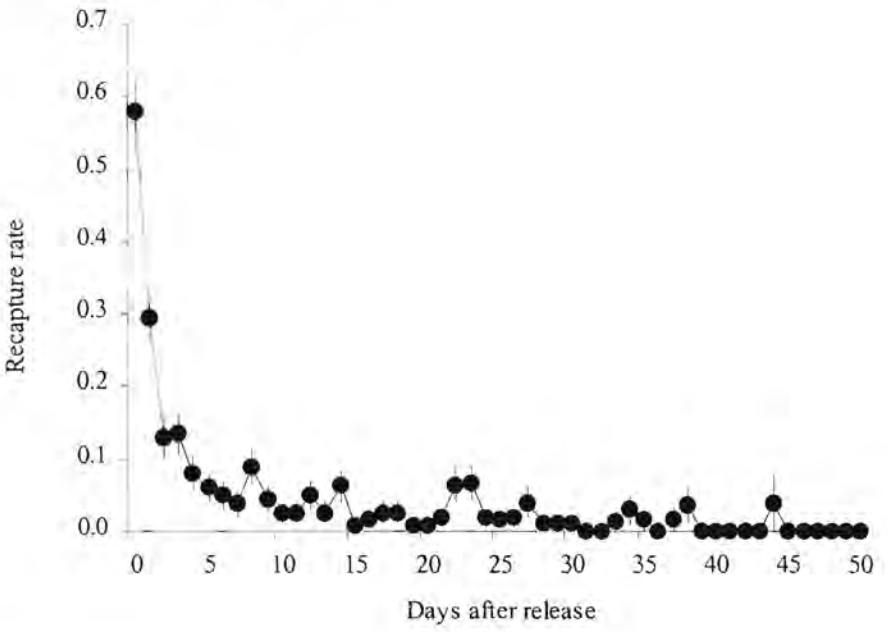


Fig. 5.5 Daily recapture rate at various days after release for *G. austeni* a) males and b) females

Once the probability of recapture had been calculated, the population in the mark-recapture area could be estimated from above indicated formula, using the day 1 recapture rates and probability of recapture (i.e. obtaining the reciprocal of the recapture rate of *G. austeni* of e.g. 0,5779 which then gives 1,7305 x 10 000 – see Table 5.3).

Table 5.3 is a summary of the probabilities of recapture (for day 1) and estimated population densities for each species and sex. The mean catch per trap as determined in Table 5.1 and the expected catch per target, are also indicated together with estimated target densities and options of killing percentages e.g. 1-10 % per day.

Table 5.3 Summary of estimates on population density and expected target densities needed for various options of killing percentages

	G. brevipalpis		*G. austeni*	
	Males	Females	Males	Females
Probability of recapture on day 1	1,9335	0,4618	0,3485	0,5779
Population in mark-release-recapture area	5172	21653	28691	17305
Area (sq.km)	1,44	1,44	1,44	1,44
Density per sq.km	3591,7	15037	19924	12017
Mean catch per H trap*	23	30	0,6	7
Increase per target*	2,8	2,2	33,4	6,8
Catch per target	64,4	66	20,04	47,6
Killing percentage:	Required target densities:			
1	0,6	2,3	9,9	2,5
2	1,1	4,6	19,9	5,0
3	1,7	6,8	29,8	7,6
4	**2,2**	**9,1**	**39,8**	**10,1**
5	2,8	11,4	49,7	12,6
6	3,3	13,7	59,7	15,1
7	3,9	15,9	69,6	17,7
9	5,0	20,5	89,5	22,7
10	5,6	22,8	99,4	25,2

* The mean catch per H trap and increase per targets indicated were obtained from the results of comparisons the effectiveness of the H trap vs. target (5.3.1)

Looking at females only, the above Table would then suggest the use of nine targets for the females of *G. brevipalpis* and 10 targets for *G. austeni*, at a 4 % killing rate (indicated in bold in Table 5.3).

It was, therefore, initially proposed that 9 targets/km^2 would work well for both species. However, it was decided that the population estimates as indicated in Table 5.3 were certainly far too high in that they are about an order of magnitude higher than the estimates for the tsetse population density in the Rifa Triangle (G.A. Vale & J.W. Hargrove, pers. comm. 1999). The population estimates are also a first approximation and the reasons for expecting that they are too high (J.W. Hargrove, pers. comm. 1999) are that:

a) Only the day 1 recapture rates were used to estimate the population. Using the day 0 level will probably give a higher capture probability and hence a lower population estimate. A crude estimate indicates that the population may be 35 % lower than the figure in Table 5.3. For *G. austeni* one could therefore use 7 targets /km^2 where 10 were originally suggested and for *G. brevipalpis* 4 targets /km^2 instead of 9.

b) It is assumed that there was no movement out of the study (mark-recapture) area. If there was such movement (of marked flies), which there definitely was, this will further inflate the population estimate. (If the marked flies leave the area one under-estimates the recapture probability and hence over-estimates the population.)

Dispersal over open areas of unsuitable habitat

Table 5.4 is a summary of the number of flies marked and released at the four different release sites (Blocks B, C, D and E). The total number of marked and unmarked flies captured at each recapture site is also given in the table. Approximate straight distances between each release site and the block's corresponding recapture sites, are also indicated (also refer to Fig. 5.1).

Table 5.4 Summary of mark-release-recapture results for Blocks B, C, D and E to investigate the use of open areas as natural barriers to the movement of *G. brevipalpis* and *G. austeni* – 3 September to 17 December 1998

Flies released		Gb males		Gb females		Ga males		Ga females	
Block	B	543		662		65		190	
	C	103		175		36		89	
	D	509		582		109		314	
	E	1806		1856		1399		2979	
Flies recaptured		Un-	Marked	Un-	Marked	Un-	Marked	Un-	Marked
(m) from release pt	Trap No.	marked		marked		marked		marked	
775	B1	39	0	73	0	0	0	0	0
520	B2	14	1	18	1	0	0	0	0
1120	B3	21	0	19	0	1	0	0	0
1085	B4	12	1	18	0	0	0	0	0
1190	B5	33	0	31	0	0	0	0	0
1105	B6	52	0	82	0	2	0	6	0
1155	B7	25	0	28	1	0	0	1	0
1225	B8	30	0	30	0	2	0	7	0
1345	B9	27	1	25	0	0	0	1	0
275	C1	40	0	88	0	0	0	0	0
85	C2	19	0	29	0	0	0	0	0
465	C3	87	2	121	0	0	0	0	0
500	C4	77	0	195	0	5	0	45	0
725	C5	42	1	51	0	0	0	0	0
605	C6	41	2	57	0	0	0	1	0
655	C7	23	0	28	0	0	0	0	0
725	C8	3	0	6	0	0	0	0	0
860	C9	50	1	63	0	0	0	0	1
690	C10	33	0	51	1	0	0	0	0
485	C11	123	0	237	0	11	0	68	0
725	D1	35	0	31	0	0	0	0	0
550	D2	105	2	157	0	1	0	4	0
550	D3	84	2	131	0	0	0	1	0
450	D4	91	0	132	0	2	0	11	0
515	D5	200	2	264	0	2	0	9	0
725	D6	119	1	246	2	9	0	35	0
400	D7	35	2	48	1	0	0	2	0
415	D8	59	0	91	2	0	0	4	0
345	E1	49	7	912	1	45	2	189	10
275	E2	479	5	760	3	35	0	158	7
310	E3	380	6	656	4	36	8	241	6
240	E4	328	5	506	4	26	2	198	20
240	E5	516	9	886	1	22	1	211	4
295	E6]	153	2	220	0	0	0	0	0
310	E7]**	147	2	168	0	0	0	1	1
205	E8]	45	1	64	0	0	0	1	1
85	E9*	205	33	301	23	11	27	16	34
140	E10	166	14	198	7	2	2	3	3

* Trap E9 was positioned inside the same patch where flies were marked and released.

** Traps E6, 7 and 8 were all small clusters of bush where ants removed many of the catches. Site 6 consisted of very dense thickets, so that the trap was hidden, therefore the low numbers. Note that trap E10 was located about 140 m from the release site under a copse of two *Syzygium* trees (note the high number of unmarked catches of *G. brevipalpis* and even the presence of *G. austeni* that were found at this unusual site.

The distances that were crossed over the unsuitable grass and vlei areas are also depicted in Fig. 5.1. Different colours are used for each species and sex, i.e. *G. brevipalpis* males (green), *G. brevipalpis* females (blue), *G. austeni* males (black) and *G. austeni* females (red). Although these flight distances are indicated as straight lines and as the shortest linear distances between the release and each recapture site, it is unlikely that it is a true representation of the actual path that was traversed. A fly may, for example, have crossed at a particular point (closer or further than the straight distance to the recapture sites), and then have followed the bush before it was attracted and captured by a particular trap. The flies released at point A of Block B, may have followed the bush to the recapture sites, since the release point of Block B is in actual fact indirectly connected to the bush where its recapture traps were set. However, the release points of Blocks C, D and E are totally separated from their respective recapture sites, and so give a truer reflection on what is happening.

From the recapture results obtained from Blocks C and D it is clear that *G. brevipalpis* males and females readily cross all distances of vlei and grassland to reach patches of bushed areas. It is probable that the *G. brevipalpis* males and females recaptured in Block B crossed the open section of grassland and did not follow the bush all the way round.

For *G. austeni* only one recaptured female was obtained in Block C. However, in this case it is more likely that it did not actually cross this distance, but followed the bush all the way round, since no flies were recaptured in Blocks B and D.

The final results for Block E showed that *G. brevipalpis* males and females crossed all distances of open areas, as was expected from the previous results. This time *G. austeni* was also found to cross various distances of open areas (up to 345 m). It seems that, due to a lower percentage of recaptured marked flies, *G. brevipalpis* moved out quicker from the isolated pockets (while unmarked flies move into the patches). *G. austeni*, on the other hand, showed a

greater percentage of recovered marked flies, which may suggest that this species is more static. This is especially supported by trap E9's results.

5.5 DISCUSSION

Attempts to control tsetse flies in much of Africa rely increasingly on the use of odour baited targets (Vale *et al.* 1986; Vale *et al.* 1988a; Willemse 1991; Knols *et al.* 1993; Van den Bossche 1997). In order to implement sustainable control of *G. brevipalpis* and *G. austeni* in South Africa by means of targets at the right density, the probability of recapture and population density of each species and sex was estimated. The re-adapted results suggested an estimated use of four targets per square kilometer to control *G. brevipalpis* females and seven targets per square kilometer for *G. austeni* females.

The recommended target density was based on the assumption of killing 4 % of the female population per day. Although ground and aerial spraying techniques produce much higher mortalities than this (Leak 1999), they may often not be sustained for sufficiently long enough periods to achieve eradication. When odour-baited targets are used the increased death rate is much smaller, but it can be sustained as required (Hargrove 1988). A number of four targets per square kilometer was suggested for *G. brevipalpis*. It compares with the defined absolute lower limit for target densities for the savanna species *G. m. morsitans* and *G. pallidipes* (Hargrove 1993), for which one cannot be sure of eradicating these tsetse populations with a target density lower than 4/km^2. For *G. austeni* the recommended seven targets per square kilometer is also significantly less and much more economical than the 70 blue targets per square kilometer that were used to suppress *G. austeni* numbers in the Jozani forest on the Unguja Island of Zanzibar prior to applying SIT (Tanzania Government/FAO/IAEA 1994).

Fly movement is important, at least in the short-term regulation of fly numbers, especially for particularly mobile species (Leak 1999). One of the factors responsible in the lack of success in sustaining control of tsetse is their high mobility resulting in continual invasion pressure into cleared areas. *G.*

brevipalpis proved to be very mobile in the forested areas, since it appeared to move out of the 850 m range (of this study) in a short period of time (1 - 7 days) (see Fig. 5.3 a-b). It is clear that this species should, therefore, be regarded and treated the same way as the mobile savanna species. *G. austeni,* however, shows a much slower rate of dispersal as seen in Fig. 5.3 c-d. Data for movement of marked *G. m. morsitans* and *G. pallidipes* suggested that the minimal daily rates of movement were about 700 m for *G. m. morsitans* males and 800 m for *G. m. morsitans* females and *G. pallidipes* (Vale *et al.* 1984). Their displacement averages up to 1 km/day in random steps (Laveissiere *et al.* 1990, cited in Leak 1999).

The pattern of movement of *G. brevipalpis* and *G. austeni* appears also to be random, suggested by the low recapture rates in Figs. 5.4 and 5.5. The decrease in recapture rates with time demonstrates how quickly the marked population is lost from the sampling area. Most of this loss is probably attributable to emigration rather than mortality. The decrease in the concentration of marked flies with distance from the point of marking and release is also similar to the diffusion patterns of other invertebrates (Southwood 1966).

As tsetse flies are relatively mobile, there is a constant reinvasion pressure into areas from which the fly has been removed or controlled (Leak 1999). The utilization of natural barriers to protect areas cleared of tsetse flies from reinvasion is a great advantage (Lovemore 1996) and was investigated for use against *G. brevipalpis* and *G. austeni.* At the Hellsgate study area *G. brevipalpis* readily crossed various distances of vlei and grassland between patches of bushed areas (up to 1,345 m or more). *G. austeni* were also found to cross distances of open areas (up to 345 m). In some situations the open grassveld areas had single standing shrubs or small trees (sometimes in very small clusters) which might have still given sufficient shade for protection. However, keeping in mind *G. brevipalpis*' times of peak activity, i.e. early morning and late afternoon until dark (Kappmeier 2000), the crossing of open areas of this species will most probably occur at these times, when the sun and heat factor is less, and when these distances of open areas, could easily be

bridged. It could also be at night, during which time *G. brevipalpis* was often found entering moving vehicles in open areas (Kappmeier 2000). Taking into account *G. austeni*'s activity times being mainly during the middle morning to late afternoon (Kappmeier 2000), it is questionable whether it traverses even the 345 m, shown during this study, in this warmest part of the day and probably rather does so at night. *G. swynnertoni* was able to cross a 800 m clearing, although flies crossing were mainly hungry and presumably in search of a blood-meal (Lloyd 1935, cited in Leak 1999).

Although the type of "unsuitable" habitat at Hellsgate, i.e. an open grassland situation with patches of small bushes situated between their preferred habitat of forests, would have no value as a natural barrier for *G. brevipalpis*, it may be more suitable for *G. austeni*. It is apparent that natural barriers could be effective, especially for the less mobile *G. austeni*, and should be adopted in the preparation of the comprehensive strategic plan. Many barriers could be identified in the N.E. KwaZulu-Natal region as suitable for this purpose, e.g. numerous lakes, a mountain range, reed and sedge swamps, and open grassland areas, and should be used to advantage. However, there is a need to conduct special studies of the various types of barrier identified to understand their mode of operation more fully and to confirm their effectiveness in limiting tsetse movement. It is also essential to identify any possible weaknesses in these natural barriers so that the necessary precautionary measures can be instituted from the outset. Passive movement of tsetse flies by human traffic, especially for *G. brevipalpis*, which enters vehicles easily, would have to be controlled and eliminated where possible.

Because these studies have revealed and proven that both species of tsetse do cross certain distances of these "unsuitable" open areas adjacent and between forests, and that they (especially *G. brevipalpis*) readily roam out of "suitable" habitat of dense bush, it is important not to ignore these "unsuitable" or open areas when setting traps/targets in a control campaign. This was also concluded in a separate study in which traps were placed along a 12 km transect through different vegetation types. Both *G. brevipalpis* and *G. austeni* were captured in open areas of shrubveld and grassland, although their

numbers were comparatively (but not significantly) lower than in forested areas (J.R. Esterhuizen, pers. comm., 2000). In a target control trial for *G. brevipalpis* in 1992 (Kappmeier *et al.* 1998), targets were only concentrated inside the forests, and not in adjacent open areas, and this could have been one of the reasons for the failure of this trial. It is, therefore, clear that control devices such as targets should also be placed strategically in open areas adjacent to dense bush. Whether the concentration of targets needs to be lower in open situations, should still be investigated.

Where natural barriers are unavailable the use of target barriers will have to be implemented. Efficiency of barriers constructed from lines of traps/targets depends on the width of the barrier, the mobility of the flies and the mortality rate within the barrier (Williams *et al.* 1992). Hargrove (1993) made estimates of the width of target barriers required to prevent reinvasion, and attempted to establish the relationship between barrier width, target density and economic costs (the widest barrier is cheapest and uses smallest number of targets). He suggested that targets should be deployed in barriers exactly as they are in normal control operations, when that density is chosen to provide local eradication in 9 - 12 months, while the width of such a barrier should be *c.* 8 times the daily step length of the tsetse species concerned. For the two Zululand species they should, therefore, consist of four targets per square kilometer and for *G. brevipalpis* and eight targets per square kilometer for *G. austeni*. Since the daily step length was not calculated in this study, the width of the barriers for each species could not be estimated. The presence of a target barrier has a marked depressing effect on tsetse populations outside its boundaries, and barriers will be most effective if they are positioned before the treatment of the areas they are meant to protect (Hargrove 1993).

In conclusion an estimate of four and seven targets/km^2 for *G. brevipalpis* and *G. austeni*, respectively, should be sufficient to control *c.* 4 % of the female populations per day. Eight instead of seven targets/km^2 would, however, be advisable for *G. austeni*, as this would make the lay-out of targets easier. It is, however, essential that a small-scale control trial be conducted first before implementing these results on a large-scale to make sure that the target density

estimates are correct and to refine the recommendations. Such a trial is currently underway in the Hellsgate area. This trial will simultaneously be used to evaluate the width of targets in a barrier for both *G. brevipalpis* and *G. austeni*.

Adjacent areas of open grassland next to forested tsetse infested areas should not be ignored when setting targets and traps in a control trial, although the target density would probably decrease in such areas. The distances between main pockets of tsetse distribution (suitable tsetse habitat), which will act as natural barriers between populations, should be reconsidered, especially for *G. brevipalpis*.

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APPENDIX 1

Table A.1 Details of survey sampling site coordinates and trap catches

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
	Natural and commercial farming areas									
1	Nyalazi plantation	28 17 29 S	32 20 40 E	2	0	2	0.33	0.00	0.33	Gb
		28 18 24 S	32 22 00 E	16	3	19	2.67	0.50	3.17	Gb
		28 12 37 S	32 24 25 E	1	4	5	0.14	0.57	0.71	Ga
		28 12 37 S	32 24 25 E	14	3	17	2.00	0.43	2.43	Gb
		28 12 52 S	32 24 27 E	0	1	1	0.00	0.14	0.14	Ga
		28 12 52 S	32 24 27 E	1	0	1	0.14	0.00	0.14	Gb
		28 11 30 S	32 23 10 E	0	0	0	0.00	0.00	0.00	
		28 06 09 S	32 23 31 E	26	52	78	3.71	7.43	11.14	Ga
		28 06 09 S	32 23 31 E	3	1	4	0.43	0.14	0.57	Gb
		28 06 39 S	32 23 31 E	10	24	34	1.43	3.43	4.86	Ga
		28 06 39 S	32 23 31 E	0	1	1	0.00	0.14	0.14	Gb
		28 07 29 S	32 23 10 E	11	14	25	1.57	2.00	3.57	Ga
		28 07 29 S	32 23 10 E	3	0	3	0.43	0.00	0.43	Gb
		28 08 14 S	32 22 08 E	2	2	4	0.29	0.29	0.57	Ga
		28 08 14 S	32 22 08 E	1	2	3	0.14	0.29	0.43	Gb
		28 08 58 S	32 23 15 E	3	2	5	0.43	0.29	0.71	Ga
		28 08 58 S	32 23 15 E	2	0	2	0.29	0.00	0.29	Gb
		28 09 49 S	32 24 16 E	3	11	14	0.43	1.57	2.00	Ga
		28 09 49 S	32 24 16 E	2	0	2	0.29	0.00	0.29	Gb
		28 10 05 S	32 20 28 E	0	0	0	0.00	0.00	0.00	
		28 09 57 S	32 24 39 E	26	31	57	3.71	4.43	8.14	Ga
		28 09 57 S	32 24 39 E	22	2	24	3.14	0.29	3.43	Gb
		28 09 32 S	32 20 51 E	0	2	2	0.00	0.29	0.29	Ga
		28 16 46 S	32 23 31 E	1	1	2	0.14	0.14	0.29	Gb
		28 18 05 S	32 22 25 E	21	5	26	7.00	1.67	8.67	Gb
		28 10 45 S	32 19 59 E	0	0	0	0.00	0.00	0.00	
		28 10 30 S	32 19 59 E	0	0	0	0.00	0.00	0.00	
		28 10 04 S	32 22 04 E	9	8	17	1.80	1.60	3.40	Ga
		28 10 04 S	32 22 04 E	2	0	2	0.40	0.00	0.40	Gb
		28 09 27 S	32 22 39 E	2	3	5	0.40	0.60	1.00	Ga
		28 09 27 S	32 22 39 E	3	2	5	0.60	0.40	1.00	Gb
		28 10 28 S	32 22 56 E	20	15	35	4.00	3.00	7.00	Ga
		28 10 28 S	32 22 56 E	8	0	8	1.60	0.00	1.60	Gb
		28 11 35 S	32 23 14 E	8	8	16	1.60	1.60	3.20	Ga
		28 11 35 S	32 23 14 E	15	0	15	3.00	0.00	3.00	Gb
		28 15 04 S	32 23 53 E	0	2	2	0.00	0.40	0.40	Ga
		28 15 04 S	32 23 53 E	11	0	11	2.20	0.00	2.20	Gb
		28 15 50 S	32 22 58 E	14	1	15	2.80	0.20	3.00	Gb
		28 16 30 S	32 21 39 E	16	1	17	3.20	0.20	3.40	Gb
		28 16 12 S	32 20 45 E	2	0	2	0.40	0.00	0.40	Gb
	Dukuduku plantation	28 21 13 S	32 17 15 E	0	0	0	0.00	0.00	0.00	
		28 21 58 S	32 19 22 E	0	0	0	0.00	0.00	0.00	
		28 21 25 S	32 21 48 E	0	0	0	0.00	0.00	0.00	
		28 20 10 S	32 22 23 E	12	2	14	2.00	0.33	2.33	Gb
		28 19 46 S	32 19 32 E	0	0	0	0.00	0.00	0.00	
		28 20 59 S	32 23 40 E	0	0	0	0.00	0.00	0.00	
	Fernwood plantation	28 17 03 S	32 17 22 E	0	0	0	0.00	0.00	0.00	
		28 17 06 S	32 17 42 E	0	1	1	0.00	0.25	0.25	Gb
		28 17 15 S	32 17 38 E	0	0	0	0.00	0.00	0.00	
		28 17 29 S	32 17 32 E	0	0	0	0.00	0.00	0.00	
		28 17 37 S	32 17 19 E	0	0	0	0.00	0.00	0.00	
		28 16 26 S	32 17 57 E	0	0	0	0.00	0.00	0.00	
		28 16 45 S	32 17 44 E	1	0	1	0.25	0.00	0.25	Gb
	Shire plantation	28 23 14 S	32 15 06 E	0	0	0	0.00	0.00	0.00	
		28 23 26 S	32 15 12 E	0	0	0	0.00	0.00	0.00	
		28 24 03 S	32 15 39 E	0	0	0	0.00	0.00	0.00	
		28 23 49 S	32 16 02 E	0	0	0	0.00	0.00	0.00	
		28 23 37 S	32 15 38 E	0	0	0	0.00	0.00	0.00	
		28 23 37 S	32 15 27 E	0	0	0	0.00	0.00	0.00	
		28 22 58 S	32 14 58 E	0	0	0	0.00	0.00	0.00	
		28 21 37 S	32 14 37 E	0	0	0	0.00	0.00	0.00	
	Futululu (CSIR - Forestek)	28 22 46 S	32 18 38 E	0	0	0	0.00	0.00	0.00	
		28 23 21 S	32 18 19 E	0	0	0	0.00	0.00	0.00	
		28 23 48 S	32 18 12 E	0	0	0	0.00	0.00	0.00	
		28 24 23 S	32 18 01 E	1	1	2	0.14	0.14	0.29	Gb
		28 24 57 S	32 17 51 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
1	Futululu (CSIR - Forestek)	28 25 25 S	32 17 44 E	0	0	0	0.00	0.00	0.00	
		28 26 09 S	32 17 36 E	0	0	0	0.00	0.00	0.00	
	Boomerang	28 14 14 S	32 18 58 E	0	0	0	0.00	0.00	0.00	
		28 14 10 S	32 19 10 E	0	0	0	0.00	0.00	0.00	
		28 14 04 S	32 19 45 E	0	0	0	0.00	0.00	0.00	
		28 13 57 S	32 19 19 E	0	0	0	0.00	0.00	0.00	
		28 13 39 S	32 19 21 E	0	0	0	0.00	0.00	0.00	
		28 13 25 S	32 19 22 E	0	0	0	0.00	0.00	0.00	
		28 13 29 S	32 17 58 E	0	0	0	0.00	0.00	0.00	
		28 13 29 S	32 17 58 E	0	0	0	0.00	0.00	0.00	
2	Mkuze Game Reserve	27 35 28 S	32 13 55 E	1	6	7	0.12	0.69	0.81	Ga
		27 35 04 S	32 14 09 E	2	2	4	0.23	0.23	0.46	Ga
		27 34 20 S	32 14 59 E	2	6	8	0.23	0.70	0.93	Ga
		27 35 07 S	32 15 02 E	0	0	0	0.00	0.00	0.00	
		27 35 27 S	32 16 01 E	0	0	0	0.00	0.00	0.00	
		27 36 05 S	32 17 12 E	0	0	0	0.00	0.00	0.00	
		27 36 34 S	32 17 56 E	0	0	0	0.00	0.00	0.00	
		27 37 00 S	32 17 23 E	0	0	0	0.00	0.00	0.00	
		27 37 45 S	32 17 51 E	0	0	0	0.00	0.00	0.00	
		27 38 25 S	32 18 41 E	0	0	0	0.00	0.00	0.00	
		27 39 19 S	32 18 35 E	1	0	1	0.21	0.00	0.21	Ga
		27 40 05 S	32 18 44 E	0	0	0	0.00	0.00	0.00	
		27 39 26 S	32 18 08 E	0	0	0	0.00	0.00	0.00	
		27 39 43 S	32 16 58 E	0	0	0	0.00	0.00	0.00	
		27 39 17 S	32 16 07 E	0	0	0	0.00	0.00	0.00	
		27 38 10 S	32 15 43 E	0	0	0	0.00	0.00	0.00	
		27 37 32 S	32 14 51 E	0	0	0	0.00	0.00	0.00	
		27 37 17 S	32 13 51 E	0	0	0	0.00	0.00	0.00	
		27 36 26 S	32 13 18 E	0	0	0	0.00	0.00	0.00	
		27 38 23 S	32 09 54 E	0	0	0	0.00	0.00	0.00	
		27 37 59 S	32 11 02 E	0	0	0	0.00	0.00	0.00	
		27 37 26 S	32 13 12 E	0	0	0	0.00	0.00	0.00	
		27 39 22 S	32 12 39 E	0	0	0	0.00	0.00	0.00	
		27 40 08 S	32 12 10 E	0	0	0	0.00	0.00	0.00	
		27 46 26 S	32 18 24 E	0	0	0	0.00	0.00	0.00	
		27 45 24 S	32 17 48 E	0	1	1	0.00	0.21	0.21	Ga
		27 44 29 S	32 17 11 E	0	0	0	0.00	0.00	0.00	
		27 43 16 S	32 17 12 E	0	0	0	0.00	0.00	0.00	
		27 35 32 S	32 13 06 E	0	0	0	0.00	0.00	0.00	
		27 36 07 S	32 11 51 E	0	0	0	0.00	0.00	0.00	
		27 36 45 S	32 11 57 E	0	0	0	0.00	0.00	0.00	
		27 38 03 S	32 13 27 E	0	0	0	0.00	0.00	0.00	
		27 38 56 S	32 13 59 E	0	0	0	0.00	0.00	0.00	
		27 42 11 S	32 17 02 E	0	0	0	0.00	0.00	0.00	
		27 41 33 S	32 16 45 E	0	0	0	0.00	0.00	0.00	
		27 40 48 S	32 14 56 E	0	0	0	0.00	0.00	0.00	
		27 39 55 S	32 14 45 E	0	0	0	0.00	0.00	0.00	
		27 39 07 S	32 15 09 E	0	0	0	0.00	0.00	0.00	
		27 37 48 S	32 14 55 E	0	0	0	0.00	0.00	0.00	
		27 37 30 S	32 14 14 E	3	3	6	0.73	0.73	1.47	Ga
		27 35 57 S	32 13 58 E	1	4	5	0.33	1.31	1.64	Ga
		27 35 45 S	32 14 09 E	6	10	16	1.97	3.28	5.25	Ga
		27 37 32 S	32 15 30 E	0	0	0	0.00	0.00	0.00	
	Sodwana State Forest	27 38 15 S	32 25 16 E	0	0	0	0.00	0.00	0.00	
		27 37 14 S	32 24 54 E	0	0	0	0.00	0.00	0.00	
		27 36 35 S	32 24 34 E	0	0	0	0.00	0.00	0.00	
		27 35 30 S	32 24 48 E	0	0	0	0.00	0.00	0.00	
		27 37 23 S	32 25 44 E	3	4	7	0.62	0.83	1.45	Ga
		27 36 57 S	32 26 13 E	0	1	1	0.00	0.21	0.21	Ga
		27 37 55 S	32 26 18 E	0	0	0	0.00	0.00	0.00	
		27 38 53 S	32 26 10 E	0	3	3	0.00	0.63	0.63	Ga
		27 39 17 S	32 27 05 E	0	0	0	0.00	0.00	0.00	
		27 38 29 S	32 28 02 E	0	0	0	0.00	0.00	0.00	
		27 37 20 S	32 28 00 E	0	0	0	0.00	0.00	0.00	
		27 35 51 S	32 28 21 E	0	0	0	0.00	0.00	0.00	
		27 35 56 S	32 27 04 E	1	2	3	0.21	0.43	0.64	Ga
	Phinda Resource Reserve	27 45 04 S	32 20 46 E	0	4	4	0.00	0.82	0.82	Ga
		27 45 59 S	32 20 44 E	1	2	3	0.21	0.41	0.62	Ga
		27 46 36 S	32 20 41 E	1	0	1	0.21	0.00	0.21	Ga
		27 46 27 S	32 21 24 E	0	0	0	0.00	0.00	0.00	
		27 46 09 S	32 21 53 E	1	1	2	0.21	0.21	0.42	Ga
		27 44 25 S	32 21 57 E	0	3	3	0.00	0.63	0.63	Ga
		27 43 36 S	32 22 13 E	1	1	2	0.21	0.21	0.42	Ga

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
2	Phinda Resource Reserve	27 42 46 S	32 22 59 E	0	2	2	0.00	0.42	0.42	Ga
		27 44 02 S	32 23 35 E	1	1	2	0.21	0.21	0.42	Ga
		27 45 19 S	32 22 58 E	0	0	0	0.00	0.00	0.00	
		27 53 34 S	32 16 21 E	0	2	2	0.00	0.41	0.41	Ga
		27 53 28 S	32 15 41 E	1	0	1	0.20	0.00	0.20	Ga
		27 53 03 S	32 15 12 E	0	0	0	0.00	0.00	0.00	
		27 53 03 S	32 19 12 E	4	8	12	0.84	1.68	2.52	Ga
		27 53 18 S	32 19 00 E	0	1	1	0.00	0.21	0.21	Ga
		27 52 39 S	32 19 51 E	0	1	1	0.00	0.21	0.21	Ga
		27 51 05 S	32 19 54 E	2	6	8	0.43	1.28	1.70	Ga
		27 50 00 S	32 19 40 E	1	7	8	0.21	1.50	1.71	Ga
		27 48 53 S	32 19 59 E	0	0	0	0.00	0.00	0.00	
		27 47 17 S	32 20 31 E	0	1	1	0.00	0.22	0.22	Ga
3	Sungulwane Game Lodge	27 50 30 S	32 10 53 E	0	0	0	0.00	0.00	0.00	
		27 50 13 S	32 10 37 E	0	0	0	0.00	0.00	0.00	
		27 49 37 S	32 10 49 E	0	0	0	0.00	0.00	0.00	
	Mduna Estates	27 49 13 S	32 11 21 E	0	0	0	0.00	0.00	0.00	
		27 49 04 S	32 11 16 E	0	0	0	0.00	0.00	0.00	
		27 48 33 S	32 11 32 E	0	0	0	0.00	0.00	0.00	
		27 48 07 S	32 11 21 E	0	0	0	0.00	0.00	0.00	
		27 47 11 S	32 12 09 E	0	0	0	0.00	0.00	0.00	
	Sipofu	27 46 23 S	32 04 54 E	0	0	0	0.00	0.00	0.00	
		27 47 13 S	32 04 24 E	0	0	0	0.00	0.00	0.00	
		27 45 40 S	32 04 49 E	0	0	0	0.00	0.00	0.00	
	Kube Yini	27 48 09 S	32 13 17 E	0	1	1	0.00	0.25	0.25	Ga
		27 47 37 S	32 13 25 E	1	0	1	0.25	0.00	0.25	Ga
		27 47 03 S	32 13 31 E	0	0	0	0.00	0.00	0.00	
		27 47 15 S	32 13 49 E	0	0	0	0.00	0.00	0.00	
		27 47 33 S	32 14 01 E	0	0	0	0.00	0.00	0.00	
		27 47 57 S	32 14 26 E	0	0	0	0.00	0.00	0.00	
	Sutton	27 52 20 S	32 14 34 E	12	4	16	3.04	1.01	4.06	Ga
		27 51 27 S	32 13 53 E	4	1	5	1.01	0.25	1.27	Ga
		27 52 14 S	32 14 10 E	2	1	3	0.51	0.26	0.77	Ga
		27 52 06 S	32 13 18 E	0	0	0	0.00	0.00	0.00	
		27 52 47 S	32 13 35 E	0	0	0	0.00	0.00	0.00	
	Panata	27 50 00 S	32 15 48 E	1	1	2	0.26	0.26	0.52	Ga
		27 49 46 S	32 16 08 E	0	1	1	0.00	0.26	0.26	Ga
		27 50 11 S	32 15 13 E	2	3	5	0.52	0.78	1.30	Ga
		27 50 02 S	32 13 57 E	0	1	1	0.00	0.26	0.26	Ga
		27 50 59 S	32 13 42 E	3	4	7	0.79	1.05	1.83	Ga
	Mziki	27 51 33 S	32 15 42 E	4	5	9	1.04	1.30	2.34	Ga
		27 50 53 S	32 15 47 E	1	1	2	0.26	0.26	0.52	Ga
	Zulu Nyala	27 54 02 S	32 15 42 E	3	3	6	0.76	0.76	1.52	Ga
		27 54 02 S	32 15 47 E	8	5	13	2.04	1.28	3.32	Ga
		27 54 47 S	32 15 07 E	8	5	13	2.03	1.27	3.29	Ga
		27 54 28 S	32 15 28 E	3	2	5	0.77	0.51	1.28	Ga
		27 54 41 S	32 15 45 E	0	1	1	0.00	0.25	0.25	Ga
4	False Bay Park	27 58 41 S	32 21 12 E	2	5	7	0.40	1.01	1.41	Ga
		27 57 34 S	32 21 31 E	1	2	3	0.20	0.40	0.61	Ga
		27 57 34 S	32 21 31 E	1	0	1	0.20	0.00	0.20	Gb
		27 56 30 S	32 21 47 E	3	6	9	0.61	1.21	1.82	Ga
		27 56 30 S	32 21 47 E	1	0	1	0.20	0.00	0.20	Gb
		27 55 33 S	32 21 58 E	1	3	4	0.20	0.61	0.81	Ga
		27 55 33 S	32 21 58 E	1	0	1	0.20	0.00	0.20	Gb
		27 54 33 S	32 22 33 E	8	16	24	1.63	3.25	4.88	Ga
		27 54 03 S	32 23 18 E	0	0	0	0.00	0.00	0.00	
		27 54 13 S	32 23 19 E	3	6	9	0.62	1.23	1.85	Ga
		27 58 01 S	32 22 38 E	1	0	1	0.20	0.00	0.20	Ga
		27 58 01 S	32 22 38 E	1	0	1	0.20	0.00	0.20	Gb
		27 58 23 S	32 22 15 E	5	8	13	1.02	1.64	2.66	Ga
		27 58 23 S	32 22 15 E	9	3	12	1.84	0.61	2.46	Gb
		28 01 40 S	32 21 26 E	23	40	63	4.66	8.10	12.76	Ga
		28 01 40 S	32 21 26 E	4	2	6	0.81	0.41	1.22	Gb
		28 00 28 S	32 21 27 E	6	8	14	1.23	1.64	2.87	Ga
		28 00 28 S	32 21 27 E	4	1	5	0.82	0.21	1.03	Gb
		28 00 14 S	32 21 28 E	9	17	26	1.86	3.52	5.38	Ga
		28 00 14 S	32 21 28 E	11	2	13	2.28	0.41	2.69	Gb
		27 58 52 S	32 21 20 E	4	6	10	0.82	1.24	2.06	Ga
	Kuleni	27 55 05 S	32 21 57 E	1	2	3	0.21	0.41	0.62	Ga
		27 55 02 S	32 22 02 E	2	4	6	0.40	0.79	1.19	Ga
		27 54 57 S	32 22 11 E	1	4	5	0.20	0.79	0.99	Ga
		27 55 38 S	32 21 50 E	0	0	0	0.00	0.00	0.00	
		27 55 22 S	32 21 38 E	2	4	6	0.40	0.80	1.20	Ga

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
4	Somerset	27 54 04 S	32 17 09 E	6	4	10	1.19	0.79	1.98	Ga
		27 53 42 S	32 17 23 E	25	19	44	4.97	3.77	8.74	Ga
		27 54 12 S	32 16 48 E	2	2	4	0.40	0.40	0.79	Ga
		27 54 15 S	32 16 25 E	0	1	1	0.00	0.20	0.20	Ga
		27 55 24 S	32 17 32 E	0	0	0	0.00	0.00	0.00	
		27 54 50 S	32 18 02 E	0	0	0	0.00	0.00	0.00	
	Doringkuil	27 59 15 S	32 18 13 E	0	0	0	0.00	0.00	0.00	
		27 59 19 S	32 18 38 E	0	0	0	0.00	0.00	0.00	
		28 00 07 S	32 18 47 E	0	1	1	0.00	0.20	0.20	Ga
		27 59 56 S	32 17 43 E	0	0	0	0.00	0.00	0.00	
		28 00 02 S	32 18 26 E	0	0	0	0.00	0.00	0.00	
	Ezulwini	27 58 55 S	32 20 26 E	6	9	15	1.20	1.80	3.01	Ga
		27 58 51 S	32 20 08 E	9	14	23	1.81	2.82	4.63	Ga
		27 58 54 S	32 19 34 E	15	25	40	3.02	5.03	8.05	Ga
		27 59 08 S	32 19 28 E	4	6	10	0.81	1.21	2.02	Ga
	Bonamanzi	28 03 56 S	32 19 27 E	10	15	25	2.01	3.02	5.03	Ga
		28 03 56 S	32 19 27 E	1	0	1	0.20	0.00	0.20	Gb
		28 03 27 S	32 18 38 E	15	23	38	3.03	4.64	7.66	Ga
		28 02 44 S	32 18 40 E	4	7	11	0.81	1.41	2.22	Ga
		28 02 44 S	32 18 40 E	1	1	2	0.20	0.20	0.40	Gb
		28 01 38 S	32 17 11 E	2	3	5	0.41	0.61	1.01	Ga
		28 02 29 S	32 17 33 E	1	0	1	0.20	0.00	0.20	Ga
	HH Ranch	28 04 41 S	32 12 57 E	0	0	0	0.00	0.00	0.00	
		28 04 45 S	32 12 58 E	0	0	0	0.00	0.00	0.00	
		28 04 41 S	32 12 18 E	0	0	0	0.00	0.00	0.00	
		28 04 41 S	32 12 18 E	0	0	0	0.00	0.00	0.00	
	Ubizane Game Reserve	28 01 23 S	32 12 45 E	4	2	6	0.81	0.40	1.21	Ga
		28 01 22 S	32 13 04 E	0	1	1	0.00	0.20	0.20	Gb
		28 01 38 S	32 13 34 E	1	1	2	0.20	0.20	0.40	Ga
5	Ndumu Game Reserve	26 51 59 S	32 18 06 E	1	1	2	0.20	0.20	0.40	Gb
		26 51 32 S	32 18 25 E	2	1	3	0.40	0.20	0.60	Ga
		26 51 32 S	32 18 25 E	5	2	7	1.00	0.40	1.40	Gb
		26 51 48 S	32 19 58 E	7	1	8	1.40	0.20	1.60	Gb
		26 51 21 S	32 19 31 E	8	0	8	1.60	0.00	1.60	Gb
		26 51 10 S	32 19 04E	4	3	7	0.80	0.60	1.40	Ga
		26 51 10 S	32 19 04E	1	1	2	0.20	0.20	0.40	Gb
		26 51 23 S	32 18 36 E	1	2	3	0.20	0.40	0.60	Ga
		26 51 23 S	32 18 36 E	1	0	1	0.20	0.00	0.20	Gb
		26 51 22 S	32 18 06 E	2	5	7	0.40	1.00	1.40	Ga
		26 51 22 S	32 18 06 E	8	0	8	1.60	0.00	1.60	Gb
		26 51 07 S	32 16 58 E	2	0	2	0.40	0.00	0.40	Gb
		26 51 28 S	32 16 15 E	0	1	1	0.00	0.20	0.20	Gb
		26 51 40 S	32 14 52 E	3	1	4	0.60	0.20	0.80	Gb
		26 50 49 S	32 13 41 E	2	0	2	0.40	0.00	0.40	Gb
		26 50 50 S	32 12 49 E	2	0	2	0.40	0.00	0.40	Gb
		26 51 47 S	32 13 13 E	0	1	1	0.00	0.20	0.20	Gb
		26 54 21 S	32 19 25 E	1	1	2	0.20	0.20	0.40	Gb
		26 55 04 S	32 19 12 E	15	2	17	3.01	0.40	3.41	Gb
		26 55 20 S	32 19 24 E	3	2	5	0.61	0.40	1.01	Gb
		26 55 37 S	32 18 58 E	2	0	2	0.47	0.00	0.47	Gb
		26 54 37 S	32 17 53 E	1	1	2	0.20	0.20	0.40	Gb
		26 55 15 S	32 17 43 E	0	0	0	0.00	0.00	0.00	
		26 54 54 S	32 16 33 E	0	0	0	0.00	0.00	0.00	
		26 54 33 S	32 15 17 E	2	0	2	0.41	0.00	0.41	Gb
		26 51 11 S	32 14 03 E	1	0	1	0.20	0.00	0.20	Gb
		26 53 51 S	32 12 55 E	2	1	3	0.41	0.20	0.61	Gb
		26 53 09 S	32 10 35 E	0	1	1	0.00	0.20	0.20	Gb
		26 52 08 S	32 11 11 E	8	0	8	1.64	0.00	1.64	Gb
		26 52 10 S	32 11 55 E	1	0	1	0.21	0.00	0.21	Ga
		26 52 10 S	32 11 55 E	4	0	4	0.83	0.00	0.83	Gb
		26 51 50 S	32 12 45 E	1	1	2	0.21	0.21	0.42	Gb
		26 52 29 S	32 12 31 E	3	1	4	0.62	0.21	0.83	Gb
		26 51 51 S	32 14 11 E	0	1	1	0.00	0.21	0.21	Gb
		26 52 23 S	32 16 16 E	7	2	9	1.46	0.42	1.88	Gb
		26 52 55 S	32 18 19 E	4	0	4	0.95	0.00	0.95	Gb
		26 53 10 S	32 17 53 E	1	1	2	0.24	0.24	0.48	Gb
		26 53 32 S	32 17 15 E	0	1	1	0.00	0.24	0.24	Ga
		26 53 32 S	32 17 15 E	1	1	2	0.24	0.24	0.48	Gb
		26 53 45 S	32 16 33 E	5	0	5	1.22	0.00	1.22	Gb
		26 54 15 S	32 17 59 E	1	0	1	0.26	0.00	0.26	Gb
	Tembe Elephant Park	26 58 09 S	32 26 45 E	0	0	0	0.00	0.00	0.00	
		26 59 08 S	32 27 14 E	6	2	8	1.23	0.41	1.64	Gb
		26 58 58 S	32 28 21 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
5	Tembe Elephant Park	26 57 35 S	32 30 33 E	0	0	0	0.00	0.00	0.00	
		26 59 21 S	32 29 59 E	1	0	1	0.17	0.00	0.17	Ga
		27 02 36 S	32 25 56 E	0	0	0	0.00	0.00	0.00	
		27 01 20 S	32 25 56 E	0	0	0	0.00	0.00	0.00	
		26 59 59 S	32 25 43 E	0	0	0	0.00	0.00	0.00	
		26 59 08 S	32 25 48 E	0	0	0	0.00	0.00	0.00	
		26 58 01 S	32 24 45 E	0	0	0	0.00	0.00	0.00	
		26 57 46 S	32 23 58 E	0	0	0	0.00	0.00	0.00	
		26 59 14 S	32 24 13 E	0	0	0	0.00	0.00	0.00	
		27 00 23 S	32 23 38 E	0	0	0	0.00	0.00	0.00	
		27 01 42 S	32 24 36 E	0	0	0	0.00	0.00	0.00	
		27 02 50 S	32 26 44 E	0	0	0	0.00	0.00	0.00	
		27 03 40 S	32 27 37 E	2	0	2	0.51	0.00	0.51	Ga
		27 02 31 S	32 28 16 E	2	1	3	0.51	0.25	0.76	Ga
		27 01 31 S	32 29 30 E	0	0	0	0.00	0.00	0.00	
		27 01 13 S	32 30 01 E	0	0	0	0.00	0.00	0.00	
		27 00 18 S	32 30 26 E	0	0	0	0.00	0.00	0.00	
		26 59 12 S	32 30 34 E	0	0	0	0.00	0.00	0.00	
		26 57 51 S	32 30 55 E	0	0	0	0.00	0.00	0.00	
		26 56 57 S	32 31 18 E	0	0	0	0.00	0.00	0.00	
		26 55 53 S	32 31 44 E	0	0	0	0.00	0.00	0.00	
		26 53 24 S	32 32 36 E	1	0	1	0.25	0.00	0.25	Ga
		26 53 23 S	32 30 26 E	0	0	0	0.00	0.00	0.00	
		26 54 02 S	32 28 18 E	0	0	0	0.00	0.00	0.00	
		26 55 47 S	32 27 10 E	0	0	0	0.00	0.00	0.00	
6	Hluhluwe-Umfolozi Game Reserve	28 02 17 S	32 07 40 E	1	0	1	0.20	0.00	0.20	Gb
		28 03 15 S	32 03 03 E	10	1	11	1.95	0.20	2.15	Gb
		28 03 55 S	32 02 32 E	11	2	13	2.15	0.39	2.55	Gb
		28 05 17 S	32 02 50 E	2	1	3	0.40	0.20	0.59	Gb
		28 04 15 S	32 03 18 E	0	0	0	0.00	0.00	0.00	
		28 05 15 S	32 05 02 E	1	0	1	0.20	0.00	0.20	Gb
		28 05 46 S	32 06 08 E	7	1	8	1.42	0.20	1.62	Gb
		28 06 46 S	32 06 18 E	9	1	10	1.84	0.20	2.04	Gb
		28 07 03 S	32 06 18 E	8	4	12	1.67	0.83	2.50	Gb
		28 06 46 S	32 06 18 E	1	1	2	0.20	0.20	0.41	Gb
		28 07 12 S	32 06 33 E	8	0	8	1.68	0.00	1.68	Gb
		28 06 02 S	32 07 22 E	24	6	30	5.10	1.28	6.38	Gb
		28 04 58 S	32 06 52 E	8	3	11	1.62	0.61	2.23	Gb
		28 03 33 S	32 07 25 E	11	2	13	2.22	0.40	2.63	Gb
		28 02 27 S	32 05 09 E	12	0	12	3.03	0.00	3.03	Gb
		28 02 26 S	32 06 06 E	4	0	4	1.01	0.00	1.01	Gb
		28 02 29 S	32 07 04 E	3	1	4	0.76	0.25	1.01	Gb
		28 03 18 S	32 07 54 E	11	2	13	2.80	0.51	3.31	Gb
		28 06 16 S	32 05 21 E	14	0	14	3.36	0.00	3.36	Gb
		28 06 36 S	32 05 23 E	0	2	2	0.00	0.48	0.48	Gb
		28 06 25 S	32 04 24 E	7	0	7	1.71	0.00	1.71	Gb
		28 08 20 S	32 01 12 E	2	0	2	0.40	0.00	0.40	Gb
		28 09 14 S	32 01 06 E	3	2	5	0.60	0.40	1.00	Gb
		28 09 07 S	32 00 12 E	41	3	44	8.22	0.60	8.82	Gb
		28 09 15 S	31 59 09 E	3	2	5	0.60	0.40	1.00	Gb
		28 09 53 S	31 57 56 E	15	0	15	3.01	0.00	3.01	Gb
		28 10 27 S	31 58 34 E	3	0	3	0.60	0.00	0.60	Gb
		28 10 07 S	31 59 21 E	4	1	5	0.80	0.20	1.01	Gb
		28 10 30 S	32 00 28 E	9	0	9	1.81	0.00	1.81	Gb
		28 04 19 S	32 01 27 E	12	4	16	2.90	0.97	3.87	Gb
		28 05 42 S	32 02 02 E	24	0	24	5.82	0.00	5.82	Gb
		28 05 17 S	32 01 20 E	1	0	1	0.25	0.00	0.25	Gb
		28 05 34 S	32 01 08 E	1	2	3	0.25	0.50	0.74	Gb
		28 06 38 S	32 01 45 E	24	1	25	5.78	0.24	6.02	Gb
		28 07 25 S	32 02 09 E	25	6	31	6.14	1.47	7.61	Gb
		28 09 05 S	32 02 05 E	1	1	2	0.25	0.25	0.49	Gb
		28 09 22 S	32 04 33 E	3	1	4	0.72	0.24	0.95	Gb
		28 10 19 S	32 04 07 E	5	0	5	1.20	0.00	1.20	Gb
		28 11 42 S	32 02 56 E	4	0	4	0.96	0.00	0.96	Gb
		28 12 06 S	32 02 37 E	3	0	3	0.63	0.00	0.63	Gb
		28 10 35 S	31 57 41 E	5	1	6	1.24	0.25	1.48	Gb
		28 14 20 S	31 59 06 E	7	2	9	1.47	0.42	1.89	Gb
		28 14 28 S	31 59 51 E	2	0	2	0.42	0.00	0.42	Gb
		28 12 17 S	32 01 24 E	4	0	4	0.85	0.00	0.85	Gb
		28 16 20 S	31 50 54 E	22	5	27	5.49	1.25	6.74	Gb
		28 16 01 S	31 50 28 E	26	5	31	6.53	1.26	7.79	Gb
		28 15 46 S	31 49 19 E	1	1	2	0.25	0.25	0.50	Gb
		28 14 36 S	31 49 51 E	18	3	21	4.55	0.76	5.31	Gb

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
6	Hluhluwe-Umfolozi Game Reserve	28 13 49 S	31 50 40 E	3	0	3	0.76	0.00	0.76	Gb
		28 14 02 S	31 49 57 E	1	0	1	0.25	0.00	0.25	Gb
		28 14 32 S	31 48 27 E	4	0	4	1.01	0.00	1.01	Gb
		28 14 30 S	31 47 38 E	0	0	0	0.00	0.00	0.00	
		28 13 29 S	31 46 42 E	0	0	0	0.00	0.00	0.00	
		28 13 37 S	31 47 28 E	2	1	3	0.51	0.25	0.76	Gb
		28 14 04 S	31 45 36 E	0	0	0	0.00	0.00	0.00	
		28 14 02 S	31 44 29 E	0	0	0	0.00	0.00	0.00	
		28 15 41 S	31 44 08 E	2	0	2	0.51	0.00	0.51	Gb
		28 17 48 S	31 45 37 E	0	0	0	0.00	0.00	0.00	
		28 17 35 S	31 44 43 E	0	0	0	0.00	0.00	0.00	
		28 16 15 S	31 44 20 E	0	0	0	0.00	0.00	0.00	
		28 15 35 S	31 45 12 E	1	0	1	0.31	0.00	0.31	Gb
		28 23 34 S	31 43 14 E	0	0	0	0.00	0.00	0.00	
		28 22 41 S	31 43 25 E	0	0	0	0.00	0.00	0.00	
		28 22 20 S	31 43 30 E	0	0	0	0.00	0.00	0.00	
		28 19 10 S	31 43 41 E	0	0	0	0.00	0.00	0.00	
		28 19 37 S	31 46 36 E	0	0	0	0.00	0.00	0.00	
		28 16 58 S	31 49 23 E	1	0	1	0.34	0.00	0.34	Gb
		28 18 32 S	31 53 17 E	6	2	8	1.60	0.53	2.13	Gb
		28 18 28 S	31 54 01 E	1	0	1	0.27	0.00	0.27	Gb
7	Eastern Shores	28 20 22 S	32 25 54 E	12	1	13	1.99	0.17	2.16	Gb
		28 18 42 S	32 27 01 E	20	4	24	3.33	0.67	4.00	Gb
		28 18 06 S	32 27 44 E	34	2	36	5.68	0.33	6.01	Gb
		28 17 05 S	32 28 07 E	53	6	59	8.87	1.00	9.87	Gb
		28 16 28 S	32 29 05 E	18	1	19	3.02	0.17	3.19	Gb
		28 16 02 S	32 28 35 E	106	22	128	17.78	3.69	21.47	Gb
		28 14 10 S	32 29 19 E	19	0	19	3.18	0.00	3.18	Gb
		28 12 33 S	32 30 02 E	18	4	22	3.01	0.67	3.68	Gb
		28 11 32 S	32 30 30 E	29	12	41	4.85	2.01	6.86	Gb
		28 09 55 S	32 31 18E	1	1	2	0.17	0.17	0.33	Gb
		28 07 39 S	32 33 16 E	1	1	2	0.17	0.17	0.33	Gb
		28 08 21 S	32 32 42 E	14	1	15	2.33	0.17	2.50	Gb
		28 08 15 S	32 32 09 E	12	1	13	2.00	0.17	2.16	Gb
		28 07 04 S	32 30 47 E	35	6	41	5.83	1.00	6.83	Gb
		28 09 07 S	32 29 52 E	118	26	144	19.68	4.34	24.01	Gb
	Mapelane	28 24 25 S	32 25 19 E	0	0	0	0.00	0.00	0.00	
		28 25 17 S	32 24 53 E	0	1	1	0.00	0.15	0.15	Ga
		28 25 17 S	32 24 53 E	1	0	1	0.15	0.00	0.15	Gb
		28 25 40 S	32 25 00 E	0	0	0	0.00	0.00	0.00	
		28 26 28 S	32 24 45 E	0	0	0	0.00	0.00	0.00	
		28 26 53 S	32 24 25 E	0	0	0	0.00	0.00	0.00	
		28 27 58 S	32 24 01 E	0	0	0	0.00	0.00	0.00	
		28 28 53 S	32 23 52 E	0	0	0	0.00	0.00	0.00	
		28 29 40 S	32 23 47 E	1	0	1	0.15	0.00	0.15	Gb
	St. Lucia	28 22 57 S	32 25 00 E	1	1	2	0.14	0.14	0.29	Gb
		28 21 35 S	32 25 43 E	6	0	6	0.86	0.00	0.86	Gb
		28 22 11 S	32 24 48 E	4	0	4	0.58	0.00	0.58	Gb
	Boomerang	28 13 27 S	32 18 01 E	0	0	0	0.00	0.00	0.00	
		28 13 30 S	32 17 51 E	0	0	0	0.00	0.00	0.00	
		28 14 23 S	32 19 05 E	0	0	0	0.00	0.00	0.00	
		28 14 05 S	32 19 17 E	0	0	0	0.00	0.00	0.00	
		28 14 17 S	32 19 25 E	0	0	0	0.00	0.00	0.00	
	Southern limit farms	28 28 34 S	32 10 29 E	0	0	0	0.00	0.00	0.00	
		28 28 32 S	32 10 32 E	0	0	0	0.00	0.00	0.00	
	Teza plantation	28 29 02 S	32 08 10 E	0	0	0	0.00	0.00	0.00	
		28 29 05 S	32 08 09 E	0	0	0	0.00	0.00	0.00	
		28 30 36 S	32 08 08 E	0	0	0	0.00	0.00	0.00	
		28 30 46 S	32 07 44 E	0	0	0	0.00	0.00	0.00	
		28 31 36 S	32 15 23 E	0	0	0	0.00	0.00	0.00	
		28 31 30 S	32 15 13 E	0	0	0	0.00	0.00	0.00	
	Shire plantation	28 22 59 S	32 15 06 E	0	0	0	0.00	0.00	0.00	
		28 23 46 S	32 15 55 E	0	0	0	0.00	0.00	0.00	
		28 23 35 S	32 15 27 E	0	0	0	0.00	0.00	0.00	
	Fernleas plantation	28 18 18 S	32 17 00 E	0	0	0	0.00	0.00	0.00	
		28 18 29 S	32 17 17 E	0	0	0	0.00	0.00	0.00	
		28 17 17 S	32 17 37 E	0	0	0	0.00	0.00	0.00	
		28 17 03 S	32 17 26 E	0	0	0	0.00	0.00	0.00	
		28 15 58 S	32 17 42 E	0	0	0	0.00	0.00	0.00	
	Nyalazi plantation	28 14 57 S	32 16 41 E	0	0	0	0.00	0.00	0.00	
		28 15 25 S	32 16 50 E	0	0	0	0.00	0.00	0.00	
		28 15 41 S	32 16 35 E	0	0	0	0.00	0.00	0.00	
		28 15 45 S	32 23 32 E	1	13	14	0.21	2.68	2.89	Ga

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
7	Nyalazi plantation	28 15 45 S	32 23 32 E	4	0	4	0.83	0.00	0.83	Gb
		28 14 45 S	32 23 47 E	4	15	19	0.83	3.10	3.92	Ga
		28 14 45 S	32 23 47 E	13	3	16	2.68	0.62	3.30	Gb
	Dukuduku plantation	28 21 48 S	32 17 05 E	0	0	0	0.00	0.00	0.00	
		28 21 14 S	32 17 14 E	1	0	1	0.17	0.00	0.17	Gb
		28 21 08 S	32 18 18 E	0	0	0	0.00	0.00	0.00	
		28 20 59 S	32 19 02 E	1	0	1	0.17	0.00	0.17	Gb
		28 21 27 S	32 20 21 E	4	1	5	0.67	0.17	0.84	Gb
		28 21 52 S	32 19 12 E	0	0	0	0.00	0.00	0.00	
		28 22 41 S	32 18 53 E	0	0	0	0.00	0.00	0.00	
		28 21 02 S	32 16 18 E	0	0	0	0.00	0.00	0.00	
		28 19 24 S	32 19 02 E	2	0	2	0.41	0.00	0.41	Gb
		28 17 32 S	32 20 46 E	0	1	1	0.00	0.21	0.21	Ga
		28 17 32 S	32 20 46 E	1	4	5	0.21	0.82	1.03	Gb
	Fututulu plantation	28 24 33 S	32 15 32 E	0	0	0	0.00	0.00	0.00	
		28 24 19 S	32 15 06 E	0	0	0	0.00	0.00	0.00	
		28 24 07 S	32 14 29 E	0	0	0	0.00	0.00	0.00	
		28 24 05 S	32 14 26 E	0	0	0	0.00	0.00	0.00	
		28 24 02 S	32 16 31 E	1	1	2	0.21	0.21	0.42	Gb
		28 23 41 S	32 16 36 E	0	0	0	0.00	0.00	0.00	
	Fututulu (CSIR - Forestek)	28 25 30 S	32 17 43 E	0	0	0	0.00	0.00	0.00	
		28 24 21 S	32 18 02 E	0	0	0	0.00	0.00	0.00	
		28 23 50 S	32 18 13 E	0	0	0	0.00	0.00	0.00	
		28 25 26 S	32 17 45 E	0	0	0	0.00	0.00	0.00	
8	Sodwana Bay Park	27 32 09 S	32 39 35 E	0	0	0	0.00	0.00	0.00	
		27 32 24 S	32 39 27 E	0	0	0	0.00	0.00	0.00	
		27 32 45 S	32 39 37 E	0	0	0	0.00	0.00	0.00	
		27 32 34 S	32 40 01 E	0	0	0	0.00	0.00	0.00	
		27 33 32 S	32 40 00 E	0	0	0	0.00	0.00	0.00	
		27 33 58 S	32 39 57 E	0	0	0	0.00	0.00	0.00	
		27 34 00 S	32 39 58 E	0	0	0	0.00	0.00	0.00	
		27 32 40 S	32 40 41 E	0	0	0	0.00	0.00	0.00	
		27 32 38 S	32 40 13 E	0	0	0	0.00	0.00	0.00	
		27 32 35 S	32 40 19 E	0	0	0	0.00	0.00	0.00	
		27 31 59 S	32 40 13 E	0	0	0	0.00	0.00	0.00	
		27 31 49 S	32 40 15 E	0	0	0	0.00	0.00	0.00	
		27 31 11 S	32 40 10 E	0	0	0	0.00	0.00	0.00	
		27 31 12 S	32 40 02 E	0	0	0	0.00	0.00	0.00	
	Lake Bhangazi North	27 39 03 S	32 38 04 E	0	0	0	0.00	0.00	0.00	
		27 39 26 S	32 38 10 E	0	0	0	0.00	0.00	0.00	
		27 39 29 S	32 38 01 E	0	0	0	0.00	0.00	0.00	
		27 39 38 S	32 37 55 E	0	0	0	0.00	0.00	0.00	
		27 39 39 S	32 37 52 E	0	0	0	0.00	0.00	0.00	
		27 39 44 S	32 37 50 E	0	0	0	0.00	0.00	0.00	
		27 38 49 S	32 36 10 E	0	0	0	0.00	0.00	0.00	
		27 38 02 S	32 36 45 E	0	0	0	0.00	0.00	0.00	
		27 37 37 S	32 37 21 E	0	0	0	0.00	0.00	0.00	
	KwaMbila	27 35 51 S	32 33 48 E	0	0	0	0.00	0.00	0.00	
		27 35 48 S	32 33 48 E	0	0	0	0.00	0.00	0.00	
		27 35 49 S	32 34 15 E	0	0	0	0.00	0.00	0.00	
		27 35 33 S	32 34 30 E	0	0	0	0.00	0.00	0.00	
		27 35 21 S	32 34 56 E	0	0	0	0.00	0.00	0.00	
		27 35 14 S	32 35 17 E	0	0	0	0.00	0.00	0.00	
		27 35 22 S	32 35 29 E	0	0	0	0.00	0.00	0.00	
	Lake Sibayi	27 25 11 S	32 41 46 E	0	0	0	0.00	0.00	0.00	
		27 25 14 S	32 42 01 E	0	0	0	0.00	0.00	0.00	
		27 25 55 S	32 42 05 E	0	0	0	0.00	0.00	0.00	
		27 26 06 S	32 42 09 E	0	0	0	0.00	0.00	0.00	
		27 25 04 S	32 42 31 E	0	0	0	0.00	0.00	0.00	
		27 24 58 S	32 42 33 E	0	0	0	0.00	0.00	0.00	
		27 23 58 S	32 42 44 E	0	0	0	0.00	0.00	0.00	
		27 23 49 S	32 42 41 E	0	0	0	0.00	0.00	0.00	
		27 22 59 S	32 42 49 E	0	0	0	0.00	0.00	0.00	
		27 21 55 S	32 43 02 E	0	0	0	0.00	0.00	0.00	
		27 20 57 S	32 43 12 E	0	0	0	0.00	0.00	0.00	
		27 20 56 S	32 43 13 E	0	0	0	0.00	0.00	0.00	
		27 21 30 S	32 43 13 E	0	0	0	0.00	0.00	0.00	
		27 25 21 S	32 41 40 E	0	0	0	0.00	0.00	0.00	
	Manzengwenya Coastal Reserve	27 15 51 S	32 46 22 E	0	0	0	0.00	0.00	0.00	
		27 15 51 S	32 46 19 E	0	0	0	0.00	0.00	0.00	
		27 15 53 S	32 46 17 E	0	0	0	0.00	0.00	0.00	
	Mabaso plantation	27 27 14 S	32 33 08 E	0	0	0	0.00	0.00	0.00	
		27 27 07 S	32 32 55 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
8	Mabaso plantation	27 27 08 S	32 34 03 E	0	0	0	0.00	0.00	0.00	
		27 26 53 S	32 34 29 E	0	0	0	0.00	0.00	0.00	
	Mbazwane plantation	27 27 09 S	32 36 09 E	0	0	0	0.00	0.00	0.00	
		27 27 11 S	32 37 18 E	0	0	0	0.00	0.00	0.00	
		27 27 10 S	32 38 48 E	0	0	0	0.00	0.00	0.00	
		27 27 16 S	32 39 53 E	0	0	0	0.00	0.00	0.00	
		27 25 35 S	32 38 13 E	0	0	0	0.00	0.00	0.00	
		27 26 37 S	32 38 12 E	0	0	0	0.00	0.00	0.00	
		27 27 30 S	32 37 33 E	0	0	0	0.00	0.00	0.00	
		27 28 46 S	32 37 27 E	0	0	0	0.00	0.00	0.00	
		27 29 55 S	32 37 16 E	0	0	0	0.00	0.00	0.00	
		27 30 21 S	32 36 04 E	0	0	0	0.00	0.00	0.00	
		27 29 13 S	32 36 05 E	0	0	0	0.00	0.00	0.00	
		27 26 22 S	32 39 39 E	0	0	0	0.00	0.00	0.00	
	Manzengwenya plantation	27 12 12 S	32 41 21 E	0	0	0	0.00	0.00	0.00	
		27 13 05 S	32 41 15 E	0	0	0	0.00	0.00	0.00	
		27 13 13 S	32 41 45 E	0	0	0	0.00	0.00	0.00	
		27 13 44 S	32 42 06 E	0	0	0	0.00	0.00	0.00	
		27 14 18 S	32 42 55 E	0	0	0	0.00	0.00	0.00	
		27 14 44 S	32 43 57 E	0	0	0	0.00	0.00	0.00	
		27 15 00 S	32 44 44 E	0	0	0	0.00	0.00	0.00	
		27 16 29 S	32 44 46 E	0	0	0	0.00	0.00	0.00	
9	Ndumu Game Reserve	26 51 17S	32 19 32 E	0	0	0	0.00	0.00	0.00	
		26 51 13S	32 19 11 E	1	1	2	0.37	0.37	0.74	Ga
		26 51 39S	32 18 28 E	1	0	1	0.34	0.00	0.34	Gb
		27 51 39S	33 18 28 E	1	2	3	0.34	0.69	1.03	Ga
		26 51 41S	32 18 28 E	2	2	4	0.69	0.69	1.38	Ga
		26 51 25S	32 18 10 E	0	0	0	0.00	0.00	0.00	
		26 51 25S	32 18 11 E	2	6	8	0.69	2.06	2.75	Ga
		26 51 21S	32 17 54 E	3	0	3	1.03	0.00	1.03	Gb
		26 52 35S	32 18 22 E	0	0	0	0.00	0.00	0.00	
		26 55 41S	32 19 23 E	4	1	5	1.37	0.34	1.71	Gb
		26 55 41S	32 19 23 E	0	1	1	0.00	0.34	0.34	Ga
		26 55 16S	32 19 20 E	10	2	12	3.48	0.70	4.18	Gb
10	Hellsgate Military Base	28 01 33 S	32 25 59 E	0	2	2	0.00	0.41	0.41	Ga
		29 01 33 S	33 25 59 E	29	10	39	5.95	2.05	8.00	Gb
		28 02 36 S	32 25 14 E	0	1	1	0.00	0.20	0.20	Ga
		29 02 36 S	33 25 14 E	9	9	18	1.84	1.84	3.69	Gb
		28 02 33 S	32 25 11 E	0	1	1	0.00	0.20	0.20	Ga
		29 02 33 S	33 25 11 E	12	2	14	2.46	0.41	2.87	Gb
		28 01 30 S	32 24 56 E	11	14	25	2.25	2.86	5.11	Ga
		29 01 30 S	33 24 56 E	26	3	29	5.31	0.61	5.92	Gb
		28 03 37 S	32 25 01 E	0	1	1	0.00	0.20	0.20	Ga
		29 03 37 S	33 25 01 E	32	7	39	6.51	1.42	7.93	Gb
		28 05 40 S	32 25 00 E	10	4	14	2.03	0.81	2.85	Gb
		28 03 13 S	32 25 39 E	19	5	24	3.86	1.02	4.87	Gb
		28 03 13 S	32 25 48 E	3	7	10	0.61	1.42	2.03	Ga
		29 03 13 S	33 25 48 E	12	2	14	2.44	0.41	2.85	Gb
11	Lake Sibayi	27 24 59 S	32 42 33 E	0	0	0	0.00	0.00	0.00	
		27 24 59 S	32 42 33 E	0	0	0	0.00	0.00	0.00	
		27 24 39 S	32 42 43 E	0	0	0	0.00	0.00	0.00	
		27 24 14 S	32 42 43 E	0	0	0	0.00	0.00	0.00	
		27 23 43 S	32 42 42 E	0	0	0	0.00	0.00	0.00	
		27 23 26 S	32 42 42 E	0	0	0	0.00	0.00	0.00	
		27 23 08 S	32 42 48 E	0	0	0	0.00	0.00	0.00	
		27 22 49 S	32 42 51 E	0	0	0	0.00	0.00	0.00	
		27 21 33 S	32 43 13 E	0	0	0	0.00	0.00	0.00	
		27 25 11 S	32 42 14 E	0	0	0	0.00	0.00	0.00	
	Communal farming areas									
1	Mahiya 517	28 07 05 S	32 11 07 E	0	0	0	0.00	0.00	0.00	
		28 07 08 S	32 11 07 E	0	0	0	0.00	0.00	0.00	
	Qakweni 692	28 08 58 S	32 19 47 E	0	0	0	0.00	0.00	0.00	
		28 09 19 S	32 19 37 E	0	0	0	0.00	0.00	0.00	
		28 08 41 S	32 20 13 E	0	0	0	0.00	0.00	0.00	
	Ngodini 944	27 58 37 S	32 06 09 E	0	0	0	0.00	0.00	0.00	
		27 58 46 S	32 06 24 E	1	0	1	0.45	0.00	0.45	Gb
		27 59 42 S	32 06 30 E	2	0	2	0.92	0.00	0.92	Gb
		28 00 08 S	32 05 27 E	0	0	0	0.00	0.00	0.00	
		28 00 54 S	32 05 20 E	0	0	0	0.00	0.00	0.00	
		27 59 57 S	32 07 06 E	1	0	1	0.47	0.00	0.47	Gb
2		27 58 59 S	32 06 30 E	0	0	0	0.00	0.00	0.00	
		27 59 13 S	32 06 30 E	0	0	0	0.00	0.00	0.00	
		27 59 24 S	32 06 30 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
2	Ngodini 944	28 00 57 S	32 05 19 E	0	0	0	0.00	0.00	0.00	
		28 00 54 S	32 05 19 E	0	0	0	0.00	0.00	0.00	
		28 00 06 S	32 05 30 E	0	0	0	0.00	0.00	0.00	
		28 00 34 S	32 06 26 E	0	0	0	0.00	0.00	0.00	
		27 59 41 S	32 06 27 E	0	0	0	0.00	0.00	0.00	
		27 58 30 S	32 06 10 E	0	0	0	0.00	0.00	0.00	
		27 58 53 S	32 06 24 E	1	0	1	0.15	0.00	0.15	Gb
3	Mzinene 526	27 59 59 S	32 07 52 E	0	1	1	0.00	0.14	0.14	Gb
		27 59 22 S	32 08 24 E	0	0	0	0.00	0.00	0.00	
		28 01 57 S	32 08 39 E	0	0	0	0.00	0.00	0.00	
		28 02 20 S	32 07 53 E	0	0	0	0.00	0.00	0.00	
		28 02 09 S	32 08 22 E	0	0	0	0.00	0.00	0.00	
		28 00 02 S	32 11 11 E	0	0	0	0.00	0.00	0.00	
		28 00 56 S	32 12 27 E	0	1	1	0.00	0.14	0.14	Gb
	Qakwini 692	28 10 50 S	32 20 58 E	1	0	1	0.14	0.00	0.14	Gb
		28 08 18 S	32 22 04 E	9	2	11	1.28	0.29	1.57	Gb
		28 08 10 S	32 22 02 E	1	0	1	0.14	0.00	0.14	Gb
		28 08 13 S	32 21 58 E	1	0	1	0.14	0.00	0.14	Gb
		28 07 59 S	32 22 01 E	4	1	5	0.57	0.14	0.71	Gb
		28 08 13 S	32 22 04 E	4	0	4	0.57	0.00	0.57	Gb
		28 08 25 S	32 22 05 E	0	2	2	0.00	0.28	0.28	Ga
		28 08 25 S	32 22 05 E	12	5	17	1.70	0.71	2.42	Gb
		28 08 39 S	32 22 08 E	3	2	5	0.43	0.28	0.71	Gb
		28 08 59 S	32 19 43 E	0	0	0	0.00	0.00	0.00	
		28 08 01 S	32 21 09 E	0	0	0	0.00	0.00	0.00	
	Mahiya 517	28 06 46 S	32 10 08 E	1	1	2	0.17	0.17	0.34	Gb
		28 06 11 S	32 10 21 E	1	0	1	0.16	0.00	0.16	Gb
		28 05 39 S	32 10 20 E	0	0	0	0.00	0.00	0.00	
		28 05 01 S	32 10 33 E	0	0	0	0.00	0.00	0.00	
		28 06 29 S	32 08 49 E	0	0	0	0.00	0.00	0.00	
		28 06 29 S	32 09 05 E	0	0	0	0.00	0.00	0.00	
		28 06 12 S	32 09 56 E	1	2	3	0.17	0.34	0.52	Ga
		28 06 12 S	32 09 56 E	2	0	2	0.34	0.00	0.34	Gb
	Gunjaneni 523	28 09 52 S	32 05 44 E	4	0	4	0.58	0.00	0.58	Gb
		28 10 03 S	32 04 54 E	5	0	5	0.72	0.00	0.72	Gb
		28 11 05 S	32 05 49 E	0	0	0	0.00	0.00	0.00	
		28 12 13 S	32 05 19 E	0	0	0	0.00	0.00	0.00	
		28 11 30 S	32 04 57 E	0	0	0	0.00	0.00	0.00	
		28 10 48 S	32 03 48 E	0	0	0	0.00	0.00	0.00	
		28 11 42 S	32 02 53 E	1	1	2	0.16	0.16	0.31	Gb
		28 12 24 S	32 03 54 E	0	0	0	0.00	0.00	0.00	
		28 14 09 S	32 04 25 E	1	0	1	0.15	0.00	0.15	Gb
	Mvutshini 945	28 07 58 S	32 09 52 E	1	0	1	0.14	0.00	0.14	Gb
		28 08 39 S	32 10 04 E	2	0	2	0.28	0.00	0.28	Gb
		28 08 20 S	32 09 16 E	1	0	1	0.14	0.00	0.14	Gb
		28 06 44 S	32 09 31 E	10	1	11	1.44	0.14	1.58	Gb
		28 06 48 S	32 08 13 E	4	0	4	0.58	0.00	0.58	Gb
		28 07 03 S	32 08 37 E	2	0	2	0.29	0.00	0.29	Gb
		28 08 07 S	32 08 37 E	3	0	3	0.44	0.00	0.44	Gb
		28 08 34 S	32 07 18 E	3	0	3	0.44	0.00	0.44	Gb
		28 09 33 S	32 07 48 E	0	0	0	0.00	0.00	0.00	
		28 10 20 S	32 07 59 E	2	0	2	0.29	0.00	0.29	Gb
	Hlazane 519	28 11 39 S	32 09 38 E	0	0	0	0.00	0.00	0.00	
		28 11 44 S	32 10 09 E	0	0	0	0.00	0.00	0.00	
		28 12 52 S	32 10 59 E	0	0	0	0.00	0.00	0.00	
		28 13 07 S	32 10 33 E	0	0	0	0.00	0.00	0.00	
		28 13 28 S	32 09 38 E	0	1	1	0.00	0.14	0.14	Gb
		28 13 08 S	32 09 23 E	0	0	0	0.00	0.00	0.00	
		28 12 50 S	32 09 05 E	0	0	0	0.00	0.00	0.00	
		28 11 58 S	32 08 49 E	0	0	0	0.00	0.00	0.00	
		28 11 58 S	32 07 05 E	0	0	0	0.00	0.00	0.00	
		28 14 30 S	32 07 59 E	0	0	0	0.00	0.00	0.00	
	Machibini 746	28 21 02 S	32 00 45 E	1	0	1	0.16	0.00	0.16	Gb
		28 21 02 S	31 59 25 E	22	2	24	3.54	0.32	3.86	Gb
		28 20 30 S	31 59 10 E	8	0	8	1.29	0.00	1.29	Gb
4	Nhlwathi 525	28 01 51 S	31 59 08 E	0	0	0	0.00	0.00	0.00	
		28 01 52 S	31 59 09 E	0	0	0	0.00	0.00	0.00	
		28 01 33 S	31 59 13 E	0	0	0	0.00	0.00	0.00	
		28 09 28 S	31 54 19 E	0	0	0	0.00	0.00	0.00	
		28 00 59 S	31 59 32 E	0	0	0	0.00	0.00	0.00	
		28 00 58 S	31 58 42 E	0	0	0	0.00	0.00	0.00	
		28 02 11 S	31 56 52 E	0	0	0	0.00	0.00	0.00	
		28 03 34 S	31 57 04 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
4	Nhlwathi 525	28 03 33 S	31 57 01 E	0	0	0	0.00	0.00	0.00	
	Mquthungu 726	28 08 59 S	31 54 22 E	0	0	0	0.00	0.00	0.00	
		28 09 50 S	31 53 39 E	0	0	0	0.00	0.00	0.00	
		28 10 26 S	31 53 54 E	2	0	2	0.18	0.00	0.18	Gb
		28 11 19 S	31 53 10 E	1	0	1	0.09	0.00	0.09	Gb
		28 10 57 S	31 52 03 E	0	0	0	0.00	0.00	0.00	
		28 12 45 S	31 49 44 E	13	1	14	1.17	0.09	1.26	Gb
		28 12 41 S	31 49 47 E	4	0	4	0.36	0.00	0.36	Gb
		28 08 13 S	31 51 36 E	0	0	0	0.00	0.00	0.00	
		28 06 46 S	31 52 18 E	0	0	0	0.00	0.00	0.00	
	Hlambanyathi 754	28 10 08 S	31 49 01 E	0	0	0	0.00	0.00	0.00	
		28 10 10 S	31 48 53 E	0	1	1	0.00	0.12	0.12	Gb
		28 09 03 S	31 48 38 E	0	0	0	0.00	0.00	0.00	
		28 09 09 S	31 48 22 E	0	0	0	0.00	0.00	0.00	
		28 09 33 S	31 47 46 E	0	0	0	0.00	0.00	0.00	
		28 09 58 S	31 48 34 E	0	1	1	0.00	0.13	0.13	Gb
5	Mpenbeni 528	28 07 21 S	31 59 43 E	12	3	15	1.97	0.49	2.46	Gb
		28 07 22 S	31 59 39 E	3	1	4	0.49	0.16	0.66	Gb
		28 07 14 S	31 59 11 E	3	1	4	0.50	0.17	0.66	Gb
		28 08 01 S	31 59 23 E	8	0	8	1.34	0.00	1.34	Gb
		28 04 58 S	31 55 58 E	0	0	0	0.00	0.00	0.00	
		28 06 13 S	31 55 59 E	0	0	0	0.00	0.00	0.00	
		28 08 09 S	31 56 16 E	0	0	0	0.00	0.00	0.00	
		28 08 04 S	31 56 46 E	0	0	0	0.00	0.00	0.00	
	Gwegwede 524	28 05 42 S	31 50 48 E	0	0	0	0.00	0.00	0.00	
		28 05 39 S	31 50 46 E	0	0	0	0.00	0.00	0.00	
		28 06 22 S	31 50 35 E	0	0	0	0.00	0.00	0.00	
		28 06 36 S	31 50 30 E	0	0	0	0.00	0.00	0.00	
		28 06 25 S	31 50 24 E	0	0	0	0.00	0.00	0.00	
		28 07 16 S	31 50 40 E	0	0	0	0.00	0.00	0.00	
		28 07 38 S	31 50 22 E	0	0	0	0.00	0.00	0.00	
		28 08 44 S	31 51 43 E	0	0	0	0.00	0.00	0.00	
		28 05 43 S	31 51 49 E	0	0	0	0.00	0.00	0.00	
		28 05 27 S	31 52 10 E	0	0	0	0.00	0.00	0.00	
	Sangoyana 946	28 03 22 S	31 59 34 E	0	0	0	0.00	0.00	0.00	
		28 05 13 S	31 58 47 E	0	0	0	0.00	0.00	0.00	
		28 05 11 S	31 58 50 E	0	0	0	0.00	0.00	0.00	
		28 05 45 S	31 58 40 E	0	0	0	0.00	0.00	0.00	
		28 06 05 S	31 58 37 E	1	0	1	0.17	0.00	0.17	Gb
		28 06 06 S	31 58 34 E	1	0	1	0.17	0.00	0.17	Gb
		28 06 05 S	31 58 34 E	0	0	0	0.00	0.00	0.00	
		28 05 24 S	31 57 43 E	0	0	0	0.00	0.00	0.00	
		28 05 18 S	31 57 42 E	0	0	0	0.00	0.00	0.00	
		28 04 38 S	31 58 28 E	0	0	0	0.00	0.00	0.00	
6	Ngwenyambili 778	28 05 45 S	32 20 19 E	0	0	0	0.00	0.00	0.00	
		28 05 59 S	32 20 24 E	0	0	0	0.00	0.00	0.00	
		28 05 52 S	32 20 21 E	0	0	0	0.00	0.00	0.00	
		28 04 28 S	32 22 31 E	0	1	1	0.00	0.20	0.20	Gb
		28 04 45 S	32 22 26 E	1	0	1	0.20	0.00	0.20	Gb
		28 08 12 S	32 21 41 E	3	2	5	0.60	0.40	1.01	Gb
		28 07 57 S	32 21 59 E	3	3	6	0.61	0.61	1.21	Gb
	Hluhluwe 518	28 08 43 S	32 16 03 E	0	0	0	0.00	0.00	0.00	
		28 08 24 S	32 15 53 E	0	0	0	0.00	0.00	0.00	
		28 08 17 S	32 15 38 E	0	0	0	0.00	0.00	0.00	
		28 07 54 S	32 15 45 E	0	0	0	0.00	0.00	0.00	
		28 09 35 S	32 13 44 E	0	0	0	0.00	0.00	0.00	
7	Makhatini communal areas	27 34 45 S	32 17 33 E	0	0	0	0.00	0.00	0.00	
		27 34 45 S	32 17 41 E	0	0	0	0.00	0.00	0.00	
		27 34 38 S	32 18 03 E	0	0	0	0.00	0.00	0.00	
		27 37 10 S	32 25 49 E	0	0	0	0.00	0.00	0.00	
		27 25 07 S	32 14 20 E	0	0	0	0.00	0.00	0.00	
		27 25 21 S	32 14 10 E	0	0	0	0.00	0.00	0.00	
8	Mahlabinyathi 963	28 09 43 S	32 20 45 E	0	1	1	0.00	0.15	0.15	Gb
		28 09 42 S	32 20 21 E	2	2	4	0.29	0.29	0.59	Gb
		28 09 35 S	32 20 05 E	2	0	2	0.29	0.00	0.29	Gb
		28 09 36 S	32 20 08 E	7	1	8	1.03	0.15	1.18	Gb
		28 09 41 S	32 19 56 E	0	1	1	0.00	0.15	0.15	Gb
		28 10 57 S	32 20 03 E	0	0	0	0.00	0.00	0.00	
		28 11 00 S	32 19 56 E	1	0	1	0.15	0.00	0.15	Gb
		28 10 58 S	32 19 57 E	0	1	1	0.00	0.15	0.15	Gb
		28 10 32 S	32 19 44 E	0	0	0	0.00	0.00	0.00	
	Sakwini 842	28 11 39 S	32 14 20 E	0	0	0	0.00	0.00	0.00	
		28 11 31 S	32 14 00 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
8	Sakwini 842	28 11 48 S	32 14 21 E	0	0	0	0.00	0.00	0.00	
		28 11 39 S	32 14 35 E	0	0	0	0.00	0.00	0.00	
		28 11 46 S	32 14 37 E	0	0	0	0.00	0.00	0.00	
		28 12 21 S	32 14 08 E	0	0	0	0.00	0.00	0.00	
		28 12 16 S	32 14 20 E	0	0	0	0.00	0.00	0.00	
		28 12 15 S	32 14 20 E	0	0	0	0.00	0.00	0.00	
		28 11 41 S	32 15 39 E	0	0	0	0.00	0.00	0.00	
		28 12 02 S	32 15 35 E	0	0	0	0.00	0.00	0.00	
	Bukhipha 962	27 56 27 S	32 09 59 E	0	0	0	0.00	0.00	0.00	
		27 55 38 S	32 09 40 E	0	0	0	0.00	0.00	0.00	
		27 55 38 S	32 09 36 E	0	0	0	0.00	0.00	0.00	
		27 55 44 S	32 09 15 E	0	0	0	0.00	0.00	0.00	
		27 55 58 S	32 09 09 E	0	0	0	0.00	0.00	0.00	
		27 56 17 S	32 07 58 E	0	0	0	0.00	0.00	0.00	
		27 56 36 S	32 06 58 E	0	0	0	0.00	0.00	0.00	
		27 55 24 S	32 07 22 E	0	0	0	0.00	0.00	0.00	
		27 56 23 S	32 09 15 E	0	0	0	0.00	0.00	0.00	
		27 56 20 S	32 08 23 E	0	0	0	0.00	0.00	0.00	
9	Matshamhlophe 326	27 59 54 S	32 01 01 E	0	0	0	0.00	0.00	0.00	
		27 59 44 S	32 00 39 E	0	0	0	0.00	0.00	0.00	
		27 59 11 S	32 00 54 E	0	0	0	0.00	0.00	0.00	
		27 58 38 S	32 01 14 E	0	0	0	0.00	0.00	0.00	
		27 59 15 S	32 01 33 E	0	0	0	0.00	0.00	0.00	
		27 59 09 S	32 01 57 E	0	0	0	0.00	0.00	0.00	
		27 59 22 S	32 01 57 E	0	0	0	0.00	0.00	0.00	
		27 59 53 S	32 02 20 E	1	0	1	0.25	0.00	0.25	Gb
		27 59 59 S	32 02 10 E	0	0	0	0.00	0.00	0.00	
		27 58 48 S	32 01 27 E	0	0	0	0.00	0.00	0.00	
	Mfanelo 327	27 58 29 S	31 58 05 E	0	0	0	0.00	0.00	0.00	
		27 58 06 S	31 58 02 E	0	0	0	0.00	0.00	0.00	
		27 57 52 S	31 57 32 E	0	0	0	0.00	0.00	0.00	
		27 57 57 S	31 57 15 E	0	0	0	0.00	0.00	0.00	
		27 57 42 S	31 57 31 E	0	0	0	0.00	0.00	0.00	
		28 03 04 S	31 51 05 E	0	0	0	0.00	0.00	0.00	
		28 03 05 S	31 51 04 E	0	0	0	0.00	0.00	0.00	
	Qunwane 964	28 04 43 S	31 50 45 E	0	0	0	0.00	0.00	0.00	
		28 04 22 S	31 50 30 E	0	0	0	0.00	0.00	0.00	
		28 03 36 S	31 50 17 E	0	0	0	0.00	0.00	0.00	
		28 03 03 S	31 50 15 E	0	0	0	0.00	0.00	0.00	
		28 03 08 S	31 50 41 E	0	0	0	0.00	0.00	0.00	
		28 03 03 S	31 50 36 E	0	0	0	0.00	0.00	0.00	
	Mgangado 694	28 04 17 S	31 52 22 E	0	0	0	0.00	0.00	0.00	
		28 03 49 S	31 53 28 E	0	0	0	0.00	0.00	0.00	
		28 03 12 S	31 54 01 E	0	0	0	0.00	0.00	0.00	
		28 04 06 S	31 52 47 E	0	0	0	0.00	0.00	0.00	
		28 03 24 S	31 52 25 E	0	0	0	0.00	0.00	0.00	
		28 02 39 S	31 52 50 E	0	0	0	0.00	0.00	0.00	
10	Dabedabe 527	27 58 48 S	32 02 45 E	0	0	0	0.00	0.00	0.00	
		27 58 18 S	32 03 05 E	0	0	0	0.00	0.00	0.00	
		27 57 20 S	32 03 22 E	0	0	0	0.00	0.00	0.00	
		27 57 25 S	32 02 40 E	0	0	0	0.00	0.00	0.00	
		27 58 40 S	32 03 05 E	0	0	0	0.00	0.00	0.00	
11	Nkolokotho 744	28 21 26 S	32 01 26 E	0	0	0	0.00	0.00	0.00	
		28 21 22 S	32 01 14 E	1	0	1	0.16	0.00	0.16	Gb
		28 21 10 S	32 01 27 E	0	0	0	0.00	0.00	0.00	
		28 21 57 S	32 01 51 E	1	0	1	0.17	0.00	0.17	Gb
		28 22 29 S	32 01 56 E	0	0	0	0.00	0.00	0.00	
		28 22 09 S	32 01 37 E	0	0	0	0.00	0.00	0.00	
		28 20 55 S	32 02 07 E	0	0	0	0.00	0.00	0.00	
		28 20 29 S	32 02 09 E	0	1	1	0.00	0.17	0.17	Gb
		28 23 32 S	32 01 38 E	0	0	0	0.00	0.00	0.00	
		28 23 15 S	32 03 23 E	0	0	0	0.00	0.00	0.00	
	Hoho 522	28 23 49 S	32 06 51 E	0	0	0	0.00	0.00	0.00	
		28 24 19 S	32 06 38 E	0	0	0	0.00	0.00	0.00	
		28 24 31 S	32 05 39 E	0	0	0	0.00	0.00	0.00	
		28 23 51 S	32 05 11 E	0	0	0	0.00	0.00	0.00	
		28 23 18 S	32 07 01 E	0	0	0	0.00	0.00	0.00	
		28 25 30 S	32 04 40 E	0	0	0	0.00	0.00	0.00	
		28 23 13 S	32 06 32 E	0	0	0	0.00	0.00	0.00	
		28 24 27 S	32 06 19 E	0	0	0	0.00	0.00	0.00	
		28 25 11 S	32 05 15 E	0	0	0	0.00	0.00	0.00	
	Nyalazi 520	28 15 42 S	32 12 51 E	0	0	0	0.00	0.00	0.00	
		28 14 14 S	32 14 00 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
11	Nyalazi 520	28 15 03 S	32 14 48 E	0	0	0	0.00	0.00	0.00	
		28 17 06 S	32 12 36 E	0	0	0	0.00	0.00	0.00	
		28 16 02 S	32 14 29 E	0	0	0	0.00	0.00	0.00	
		28 16 35 S	32 14 32 E	0	0	0	0.00	0.00	0.00	
		28 16 17 S	32 15 15 E	0	0	0	0.00	0.00	0.00	
		28 17 11 S	32 15 32 E	0	0	0	0.00	0.00	0.00	
		28 17 33 S	32 14 11 E	0	0	0	0.00	0.00	0.00	
		28 16 59 S	32 13 45 E	0	0	0	0.00	0.00	0.00	
	Dukuduku 967	28 22 49 S	32 21 37 E	0	0	0	0.00	0.00	0.00	
		28 22 43 S	32 21 53 E	0	0	0	0.00	0.00	0.00	
		28 22 54 S	32 21 01 E	0	0	0	0.00	0.00	0.00	
		28 22 22 S	32 21 12 E	0	0	0	0.00	0.00	0.00	
		28 21 51 S	32 21 24 E	0	0	0	0.00	0.00	0.00	
		28 22 12 S	32 22 37 E	6	0	6	1.54	0.00	1.54	Gb
		28 22 43 S	32 22 20 E	0	0	0	0.00	0.00	0.00	
		28 22 39 S	32 22 36 E	0	0	0	0.00	0.00	0.00	
		28 22 58 S	32 21 49 E	0	0	0	0.00	0.00	0.00	
		28 23 20 S	32 20 33 E	0	0	0	0.00	0.00	0.00	
	Gwedla 669	28 17 29 S	32 07 42 E	0	0	0	0.00	0.00	0.00	
		28 16 46 S	32 07 24 E	0	0	0	0.00	0.00	0.00	
		28 16 33 S	32 07 27 E	0	1	1	0.00	0.06	0.06	Gb
		28 17 02 S	32 06 56 E	0	0	0	0.00	0.00	0.00	
		28 17 03 S	32 05 23 E	0	0	0	0.00	0.00	0.00	
		28 17 10 S	32 05 28 E	0	0	0	0.00	0.00	0.00	
		28 16 32 S	32 06 27 E	0	0	0	0.00	0.00	0.00	
		28 16 42 S	32 06 34 E	1	1	2	0.06	0.06	0.12	Gb
		28 17 34 S	32 07 22 E	0	0	0	0.00	0.00	0.00	
		28 17 37 S	32 08 33 E	0	0	0	0.00	0.00	0.00	
12	Nsane 521	28 19 45 S	32 13 13 E	0	0	0	0.00	0.00	0.00	
		28 19 38 S	32 13 17 E	0	0	0	0.00	0.00	0.00	
		28 19 30 S	32 13 25 E	0	0	0	0.00	0.00	0.00	
		28 20 05 S	32 13 16 E	0	0	0	0.00	0.00	0.00	
		28 20 15 S	32 12 52 E	0	0	0	0.00	0.00	0.00	
		28 20 14 S	32 12 32 E	0	0	0	0.00	0.00	0.00	
		28 19 48 S	32 12 05 E	0	0	0	0.00	0.00	0.00	
		28 19 31 S	32 11 45 E	0	0	0	0.00	0.00	0.00	
		28 19 22 S	32 11 38 E	0	0	0	0.00	0.00	0.00	
	Nomathiya	28 20 58 S	32 06 31 E	0	0	0	0.00	0.00	0.00	
		28 21 00 S	32 06 21 E	0	0	0	0.00	0.00	0.00	
		28 21 10 S	32 06 30 E	0	0	0	0.00	0.00	0.00	
		28 20 40 S	32 06 28 E	0	0	0	0.00	0.00	0.00	
		28 20 36 S	32 06 19 E	0	0	0	0.00	0.00	0.00	
		28 20 30 S	32 05 19 E	0	0	0	0.00	0.00	0.00	
		28 20 39 S	32 04 23 E	0	0	0	0.00	0.00	0.00	
		28 21 15 S	32 04 25 E	0	0	0	0.00	0.00	0.00	
		28 19 31 S	32 05 10 E	0	0	0	0.00	0.00	0.00	
		28 21 09 S	32 04 05 E	0	0	0	0.00	0.00	0.00	
13	KwaMsane 323	28 25 16 S	32 08 06 E	0	0	0	0.00	0.00	0.00	
		28 25 35 S	32 08 15 E	0	0	0	0.00	0.00	0.00	
		28 25 09 S	32 08 20 E	0	0	0	0.00	0.00	0.00	
		28 25 04 S	32 08 31 E	0	0	0	0.00	0.00	0.00	
		28 24 44 S	32 08 19 E	1	0	1	0.13	0.00	0.13	Gb
		28 26 45 S	32 09 23 E	0	0	0	0.00	0.00	0.00	
		28 26 07 S	32 07 49 E	0	0	0	0.00	0.00	0.00	
		28 26 37 S	32 07 57 E	0	0	0	0.00	0.00	0.00	
		28 27 24 S	32 08 24 E	0	0	0	0.00	0.00	0.00	
		28 27 17 S	32 08 24 E	0	0	0	0.00	0.00	0.00	
	Ekuphindiseni 328	28 01 30 S	32 03 36 E	1	1	2	0.20	0.20	0.40	Gb
		28 01 42 S	32 02 42 E	1	0	1	0.20	0.00	0.20	Gb
		28 03 17 S	32 03 02 E	26	24	50	5.20	4.80	10.00	Gb
		28 03 15 S	32 02 57 E	11	13	24	2.20	2.60	4.80	Gb
		28 02 34 S	32 02 49 E	0	0	0	0.00	0.00	0.00	
		28 02 41 S	32 02 49 E	0	0	0	0.00	0.00	0.00	
		28 00 59 S	32 03 34 E	0	0	0	0.00	0.00	0.00	
14	Mpamzakazi 325	28 03 11 S	31 59 55 E	0	0	0	0.00	0.00	0.00	
		28 02 58 S	31 59 58 E	1	0	1	0.32	0.00	0.32	Gb
		28 03 52 S	31 59 25 E	0	0	0	0.00	0.00	0.00	
		28 02 34 S	32 01 43 E	0	0	0	0.00	0.00	0.00	
		28 01 54 S	32 01 35 E	0	0	0	0.00	0.00	0.00	
		28 03 24 S	32 01 07 E	1	0	1	0.34	0.00	0.34	Gb
15	Zidlele 701	27 34 45 S	32 17 33 E	0	0	0	0.00	0.00	0.00	
		27 34 45 S	32 17 41 E	0	0	0	0.00	0.00	0.00	
		27 34 38 S	32 18 03 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
15	Zineshe 743	27 38 33 S	32 22 28 E	0	0	0	0.00	0.00	0.00	
		27 37 10 S	32 25 49 E	0	0	0	0.00	0.00	0.00	
	Biva	27 25 07 S	32 14 20 E	0	0	0	0.00	0.00	0.00	
		27 25 21 S	32 14 10 E	0	0	0	0.00	0.00	0.00	
16	Zidlele 701	27 34 33 S	32 17 33 E	0	0	0	0.00	0.00	0.00	
		27 34 33 S	32 17 33 E	0	0	0	0.00	0.00	0.00	
		27 34 40 S	32 17 29 E	0	0	0	0.00	0.00	0.00	
		27 34 46 S	32 17 44 E	0	0	0	0.00	0.00	0.00	
		27 34 29 S	32 17 43 E	0	0	0	0.00	0.00	0.00	
		27 34 13 S	32 17 43 E	0	0	0	0.00	0.00	0.00	
		27 34 18 S	32 17 50 E	0	0	0	0.00	0.00	0.00	
		27 34 38 S	32 18 00 E	0	0	0	0.00	0.00	0.00	
		27 34 37 S	32 18 00 E	0	0	0	0.00	0.00	0.00	
		27 34 11 S	32 17 21 E	0	0	0	0.00	0.00	0.00	
	Zineshe 743	27 38 33 S	32 22 36 E	0	0	0	0.00	0.00	0.00	
		27 38 37 S	32 22 51 E	0	0	0	0.00	0.00	0.00	
		27 39 23 S	32 24 24 E	1	3	4	0.20	0.60	0.80	Ga
		27 39 19 S	32 24 31 E	2	3	5	0.40	0.61	1.01	Ga
		27 39 35 S	32 24 01 E	0	0	0	0.00	0.00	0.00	
		27 39 13 S	32 23 59 E	0	0	0	0.00	0.00	0.00	
		27 38 08 S	32 22 52 E	0	1	1	0.00	0.20	0.20	Ga
		27 37 14 S	32 23 06 E	0	0	0	0.00	0.00	0.00	
		27 37 55 S	32 21 08 E	0	0	0	0.00	0.00	0.00	
		27 38 24 S	32 22 14 E	0	0	0	0.00	0.00	0.00	
	Biva 936	27 25 52 S	32 12 15 E	0	0	0	0.00	0.00	0.00	
		27 25 53 S	32 12 27 E	0	0	0	0.00	0.00	0.00	
		27 26 14 S	32 12 04 E	0	0	0	0.00	0.00	0.00	
		27 26 39 S	32 24 43 E	0	0	0	0.00	0.00	0.00	
		27 27 09 S	32 12 45 E	0	0	0	0.00	0.00	0.00	
		27 25 35 S	32 13 19 E	0	0	0	0.00	0.00	0.00	
		27 25 30 S	32 13 38 E	0	0	0	0.00	0.00	0.00	
		27 24 53 S	32 15 14 E	0	0	0	0.00	0.00	0.00	
		27 24 38 S	32 14 08 E	0	0	0	0.00	0.00	0.00	
		27 24 33 S	32 14 07 E	0	0	0	0.00	0.00	0.00	
17	Siphondweni 819	27 11 50 S	32 14 51 E	0	0	0	0.00	0.00	0.00	
		27 11 54 S	32 14 59 E	0	0	0	0.00	0.00	0.00	
	Hlazane 937	27 16 08 S	32 14 32 E	0	0	0	0.00	0.00	0.00	
		27 16 08 S	32 14 32 E	0	0	0	0.00	0.00	0.00	
		27 16 18 S	32 14 31 E	0	0	0	0.00	0.00	0.00	
		27 22 18 S	32 17 02 E	0	0	0	0.00	0.00	0.00	
		27 22 21 S	32 17 10 E	0	0	0	0.00	0.00	0.00	
		27 22 44 S	32 17 35 E	0	0	0	0.00	0.00	0.00	
		27 23 09 S	32 17 40 E	0	0	0	0.00	0.00	0.00	
	Mozane 938	27 24 52 S	32 19 18 E	0	0	0	0.00	0.00	0.00	
		27 25 06 S	32 19 57 E	0	0	0	0.00	0.00	0.00	
		27 25 17 S	32 19 35 E	0	0	0	0.00	0.00	0.00	
		27 25 39 S	32 20 18 E	0	0	0	0.00	0.00	0.00	
		27 27 10 S	32 17 38 E	0	0	0	0.00	0.00	0.00	
		27 26 49 S	32 17 28 E	0	0	0	0.00	0.00	0.00	
		27 27 31 S	32 18 03 E	0	0	0	0.00	0.00	0.00	
		27 26 01 S	32 22 14 E	0	0	0	0.00	0.00	0.00	
		27 25 14 S	32 22 32 E	0	0	0	0.00	0.00	0.00	
		27 26 41 S	32 17 59 E	0	0	0	0.00	0.00	0.00	
		27 26 32 S	32 18 02 E	0	0	0	0.00	0.00	0.00	
18	Munyu 820	27 23 58 S	32 22 08 E	0	0	0	0.00	0.00	0.00	
		27 23 03 S	32 21 59 E	0	0	0	0.00	0.00	0.00	
		27 22 15 S	32 21 43 E	0	0	0	0.00	0.00	0.00	
		27 21 44 S	32 20 59 E	0	0	0	0.00	0.00	0.00	
		27 21 32 S	32 20 21 E	0	0	0	0.00	0.00	0.00	
		27 21 30 S	32 19 23 E	0	0	0	0.00	0.00	0.00	
		27 21 37 S	32 21 29 E	0	0	0	0.00	0.00	0.00	
		27 20 47 S	32 21 02 E	0	0	0	0.00	0.00	0.00	
		27 20 09 S	32 21 31 E	0	0	0	0.00	0.00	0.00	
		27 18 55 S	32 21 43 E	0	0	0	0.00	0.00	0.00	
		27 17 57 S	32 21 50 E	1	0	1	0.17	0.00	0.17	Ga
	Mkhumbikazana 514	27 30 58 S	32 25 09 E	0	0	0	0.00	0.00	0.00	
		27 30 53 S	32 25 05 E	0	0	0	0.00	0.00	0.00	
		27 29 48 S	32 25 09 E	0	0	0	0.00	0.00	0.00	
		27 30 44 S	32 24 28 E	0	0	0	0.00	0.00	0.00	
		27 31 38 S	32 24 40 E	0	0	0	0.00	0.00	0.00	
		27 31 50 S	32 25 03 E	0	0	0	0.00	0.00	0.00	
		27 33 27 S	32 24 16 E	0	0	0	0.00	0.00	0.00	
		27 32 27 S	32 24 12 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
18	Mkhumbikazana 514	27 30 46 S	32 24 07 E	0	0	0	0.00	0.00	0.00	
		27 30 20 S	32 24 17 E	0	0	0	0.00	0.00	0.00	
	Mseleni 512	27 21 52 S	32 31 57 E	0	0	0	0.00	0.00	0.00	
		27 22 19 S	32 31 25 E	0	2	2	0.00	0.39	0.39	Ga
		27 22 28 S	32 31 04 E	0	1	1	0.00	0.20	0.20	Ga
		27 23 06 S	32 30 16 E	1	1	2	0.19	0.19	0.39	Ga
		27 23 51 S	32 30 21 E	0	0	0	0.00	0.00	0.00	
		27 23 38 S	32 29 16 E	0	1	1	0.00	0.20	0.20	Ga
		27 23 02 S	32 29 24 E	0	1	1	0.00	0.20	0.20	Ga
		27 22 32 S	32 29 08 E	0	1	1	0.00	0.20	0.20	Ga
		27 22 08 S	32 29 09 E	0	1	1	0.00	0.20	0.20	Ga
		27 20 41 S	32 30 45 E	0	1	1	0.00	0.20	0.20	Ga
19	Manaba 500	27 19 38 S	32 31 37 E	0	0	0	0.00	0.00	0.00	
		27 19 13 S	32 30 39 E	1	2	3	0.14	0.29	0.43	Ga
		27 19 16 S	32 29 48 E	0	2	2	0.00	0.29	0.29	Ga
		27 17 43 S	32 32 05 E	2	1	3	0.29	0.14	0.43	Ga
		27 17 23 S	32 31 32 E	3	6	9	0.43	0.86	1.30	Ga
		27 16 40 S	32 30 12 E	1	0	1	0.14	0.00	0.14	Ga
		27 16 28 S	32 29 57 E	4	1	5	0.58	0.14	0.72	Ga
		27 15 40 S	32 30 04 E	0	1	1	0.00	0.14	0.14	Ga
		27 15 37 S	32 30 32 E	4	8	12	0.58	1.15	1.73	Ga
		27 17 25 S	32 32 12 E	5	3	8	0.72	0.43	1.15	Ga
		27 15 51 S	32 28 14 E	0	0	0	0.00	0.00	0.00	
		27 16 04 S	32 27 56 E	0	0	0	0.00	0.00	0.00	
		27 15 33 S	32 26 05 E	0	0	0	0.00	0.00	0.00	
		27 14 28 S	32 25 44 E	0	0	0	0.00	0.00	0.00	
		27 14 30 S	32 26 05 E	0	0	0	0.00	0.00	0.00	
		27 14 06 S	32 25 49 E	0	1	1	0.00	0.13	0.13	Ga
		27 13 24 S	32 25 52 E	1	0	1	0.13	0.00	0.13	Ga
		27 17 14 S	32 24 32 E	0	0	0	0.00	0.00	0.00	
		27 17 22 S	32 23 29 E	0	2	2	0.00	0.25	0.25	Ga
		27 17 53 S	32 22 37 E	0	3	3	0.00	0.38	0.38	Ga
	Mbazwana 513	27 27 07 S	32 34 24 E	3	2	5	0.38	0.25	0.63	Ga
		27 27 08 S	32 35 01 E	0	1	1	0.00	0.13	0.13	Ga
		27 26 26 S	32 34 27 E	2	0	2	0.25	0.00	0.25	Ga
		27 28 42 S	32 32 36 E	0	0	0	0.00	0.00	0.00	
	Ntenga 678	27 20 11 S	32 12 45 E	0	0	0	0.00	0.00	0.00	
	Nibela 510	27 50 43 S	32 27 22 E	0	0	0	0.00	0.00	0.00	
		27 51 25 S	32 27 00 E	0	0	0	0.00	0.00	0.00	
		27 52 33 S	32 27 26 E	0	0	0	0.00	0.00	0.00	
		27 51 58 S	32 26 11 E	0	0	0	0.00	0.00	0.00	
		27 51 08 S	32 26 47 E	0	0	0	0.00	0.00	0.00	
	Masakeni 679	27 47 53 S	32 25 12 E	0	0	0	0.00	0.00	0.00	
		27 47 16 S	32 24 18 E	0	0	0	0.00	0.00	0.00	
		27 48 06 S	32 23 11 E	0	0	0	0.00	0.00	0.00	
		27 49 03 S	32 22 49 E	0	0	0	0.00	0.00	0.00	
20	Zineshe 743	27 39 35 S	32 25 19 E	2	2	4	0.34	0.34	0.68	Ga
		27 38 16 S	32 25 18 E	0	0	0	0.00	0.00	0.00	
		27 37 03 S	32 24 37 E	2	3	5	0.34	0.51	0.85	Ga
		27 37 28 S	32 23 08 E	0	0	0	0.00	0.00	0.00	
	Nibela 510	27 48 24 S	32 27 45 E	0	0	0	0.00	0.00	0.00	
		27 49 09 S	32 28 11 E	0	0	0	0.00	0.00	0.00	
		27 49 33 S	32 27 30 E	0	0	0	0.00	0.00	0.00	
		27 53 36 S	32 26 54 E	0	0	0	0.00	0.00	0.00	
		27 54 05 S	32 27 12 E	0	0	0	0.00	0.00	0.00	
		27 55 32 S	32 26 36 E	0	0	0	0.00	0.00	0.00	
		27 56 41 S	32 26 10 E	0	0	0	0.00	0.00	0.00	
		27 57 37 S	32 25 54 E	0	0	0	0.00	0.00	0.00	
		27 58 03 S	32 25 42 E	0	0	0	0.00	0.00	0.00	
		27 58 19 S	32 26 13 E	0	0	0	0.00	0.00	0.00	
	Masakeni 679	27 48 38 S	32 25 24 E	0	0	0	0.00	0.00	0.00	
		27 48 29 S	32 25 08 E	0	0	0	0.00	0.00	0.00	
		27 48 04 S	32 25 03 E	0	0	0	0.00	0.00	0.00	
		27 47 45 S	32 24 47 E	0	0	0	0.00	0.00	0.00	
		27 47 16 S	32 24 34 E	0	1	1	0.00	0.14	0.14	Ga
		27 47 13 S	32 24 20 E	0	0	0	0.00	0.00	0.00	
		27 47 37 S	32 23 37 E	0	0	0	0.00	0.00	0.00	
		27 48 19 S	32 23 09 E	0	0	0	0.00	0.00	0.00	
		27 49 56 S	32 23 09 E	0	1	1	0.00	0.14	0.14	Ga
	Mpempe 302	27 41 52 S	32 26 21 E	0	0	0	0.00	0.00	0.00	
		27 42 21 S	32 26 28 E	0	0	0	0.00	0.00	0.00	
		27 43 01 S	32 26 27 E	0	0	0	0.00	0.00	0.00	
		27 43 42 S	32 26 04 E	0	0	0	0.00	0.00	0.00	

Survey	Locality	Latitiude	Longitude	Males	Females	Totals	Males/ trap/day	Females/ trap/day	Totals/ trap/day	Species
23	Kosibay 722	26 57 58 S	32 48 28 E	2	2	4	0.26	0.26	0.51	Gb
		26 52 40 S	32 48 06 E	0	0	0	0.00	0.00	0.00	
		26 52 53 S	32 46 35 E	1	0	1	0.16	0.00	0.16	Gb
		26 53 21 S	32 45 19 E	0	0	0	0.00	0.00	0.00	
		26 52 01 S	32 43 48 E	0	0	0	0.00	0.00	0.00	
		26 52 02 S	32 43 02 E	0	0	0	0.00	0.00	0.00	
		26 52 09 S	32 41 50 E	0	0	0	0.00	0.00	0.00	
24	Mlambongwenya 506	27 10 36 S	32 04 42 E	0	0	0	0.00	0.00	0.00	
		27 10 20 S	32 05 16 E	0	0	0	0.00	0.00	0.00	
		27 10 06 S	32 05 27 E	0	0	0	0.00	0.00	0.00	
		27 10 46 S	32 06 09 E	0	0	0	0.00	0.00	0.00	
	Lubambo 789	27 11 54 S	32 05 04 E	0	0	0	0.00	0.00	0.00	
		27 13 03 S	32 05 12 E	0	0	0	0.00	0.00	0.00	
		27 15 27 S	32 04 56 E	0	0	0	0.00	0.00	0.00	
		27 12 41 S	32 05 33 E	0	0	0	0.00	0.00	0.00	
		27 12 59 S	32 06 00 E	0	0	0	0.00	0.00	0.00	
	Singeni 787	27 17 43 S	32 04 26 E	0	0	0	0.00	0.00	0.00	
		27 17 58 S	32 04 26 E	0	0	0	0.00	0.00	0.00	
		27 19 23 S	32 03 56 E	0	0	0	0.00	0.00	0.00	
		27 17 56 S	32 05 03 E	0	0	0	0.00	0.00	0.00	
		27 17 47 S	32 05 43 E	0	0	0	0.00	0.00	0.00	
		27 16 43 S	32 06 55 E	0	0	0	0.00	0.00	0.00	
	Mpeshane 898	27 21 35 S	32 04 19 E	0	0	0	0.00	0.00	0.00	
		27 21 35 S	32 04 19 E	0	0	0	0.00	0.00	0.00	
	Mzinyeni 497	27 15 06 S	32 12 17 E	0	0	0	0.00	0.00	0.00	
		27 14 57 S	32 12 30 E	0	0	0	0.00	0.00	0.00	
		27 14 47 S	32 12 38 E	0	0	0	0.00	0.00	0.00	
		27 08 18 S	32 12 40 E	0	0	0	0.00	0.00	0.00	
		27 08 13 S	32 12 42 E	0	0	0	0.00	0.00	0.00	
	Manqwashu 732	27 15 21 S	32 07 06 E	0	0	0	0.00	0.00	0.00	
		27 17 05 S	32 09 05 E	0	0	0	0.00	0.00	0.00	
		27 17 23 S	32 08 58 E	0	0	0	0.00	0.00	0.00	
		27 16 21 S	32 10 30 E	0	0	0	0.00	0.00	0.00	
		27 16 16 S	32 10 32 E	0	0	0	0.00	0.00	0.00	
		27 13 28 S	32 07 52 E	0	0	0	0.00	0.00	0.00	
		27 11 28 S	32 08 53 E	0	0	0	0.00	0.00	0.00	