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Environmental Monitoring of Tsetse Fly Spraying Impacts in the Okavango Delta – 2002

Final Report

Perkins, J.S. and L. Ramberg (Eds)

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Environmental Monitoring of Tsetse Fly Spraying Impacts in the Okavango Delta - 2002

Final Report

April 2004

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Executive Summary

After the successful spraying and control of tsetse fly in the northern portion of the Okavango Delta in 2001 the decision was made to continue with the aerial spraying using sequential aerosol doses of the insecticide deltamethrin in the remaining southernmost portion of the Delta in 2002.

Meteorological and spray deposition data showed considerable variation between sites. Wool strands used to investigate the extent of residual effects of an application of 0.3g/ha of deltamethrin during cycle 2, revealed that such effects were likely to have lasted for four or five days after the spray event.

An intensive monitoring programme of aquatic invertebrate families showed reduced total abundance of 25-46%. Due to changes in abundances, and the localized disappearance of several families after the spraying these differences became less apparent and a poorer, less diverse composition remained. Out of in total 65 taxa 23 were common, and of these, six taxa with several species in each, declined drastically during the spraying campaign and had disappeared by the fifth spray cycle. It is likely that at least the same proportion of the less common taxa was eliminated as well.

Terrestrial invertebrates were sampled from tree canopies before, during and after the five spray cycles. Abundances declined by up to 68%. The most affected group was beetles. The composition of species changed through the cycles. Around 30% of species were only sampled before the spray or in the first spray cycle, whilst a slightly lower proportion appeared for the first time in later cycles. An unsprayed control in Mopane woodland showed no change in abundance or composition, although there were some species changes over the monitoring period. Additional sampling techniques suggested there were limited effects on ground dwelling animals.

Bird species composition was recorded at several sites, but there was no detectable effect on birds.

A specific weevil is used to control infestations of the aquatic weed *Salvinia*. Mortalities of this weevil during the spray cycles was variable up to 46% per spray cycle.

Overall there was a significant and measurable effect of the spray on the abundance and community composition of non-target invertebrate organisms. There were indications that recovery from this effect was likely. A recovery study was strongly recommended.

Acknowledgements

Many individuals and institutions contributed towards making the 2002 monitoring programme a success. Apart from the key role played by the Ministry of Agriculture (Tsetse Fly Control Division), The Department of Wildlife and National Parks and the Department of Water Affairs, a great number of people contributed towards the exercise. They are too many to name but include the staff at the HOORC, the staff at the Sedia Hotel and Maun Lodge, and the reference group members and stakeholders, all of whom have consistently supported the project.

Thlamelo Mapila of Pangolin Enterprises, together with Uncle, Lamack, Gyps, Nchadi, Messiah and Goots, ran the catering and logistics at Nxaraxa, Xakanaxa and Khwai. Moss and Kupaza did an excellent job as field managers at Nxaraxa, while Thebe, Chris and Deeds provided the core of the aquatic invertebrate sampling team at Xakanaxa. Thuse kept the foggers going.

We are grateful to Okavango Wilderness Safaris for their continued support in allowing sampling visits to Pom Pom, while Peter and Andrew of Okavango Helicopters, provided expert logistical support.

The team dedicates this report to the late James Ngande.

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1. An Overview of Environmental effects caused by deltamethrin spraying of the Okavango Delta 2001 and 2002.

Lars Ramberg
Director, Harry Oppenheimer Okavango Research Centre,
Maun.

1.1. Background

After a ten year respite from aerial spraying to control tsetse flies in the Okavango Delta, and following the 1999 outbreak of trypansomiasis in cattle in the northeastern Delta and potential threats of people also becoming infected, the Department of Animal Health and Production (DAHP), Government of Botswana proposed a phased and integrated campaign of tsetse fly control measures in second half of year 2000.

The insecticide Deltamethrin was applied at 0.26 g/ha over five cycles from early June to late August 2001 in the 7,000-km2 northern spray block. The environmental monitoring could however not start until after the third spray event in the end of July, which meant that there were no pre-spray data. In order to compensate for this lack of pre-spray baseline data in the northern Delta spray block, a small trial block south of the main spray zone was sprayed at the start of cycle 5 in mid-August. This 3km x 3km area centred on PomPom, where the environmental monitoring team was in place to collect pre and post spray samples. This was a most important experiment and provided the basis for planning the impact study in 2002, and the subsequent recovery study on 2003.

Direct measurements of deltamethrin in the environment proved difficult. This reflects the fact that deltamethrin in its sunflower oil base is practically insoluble in water and therefore is either floating on the water surface or attach readily to any kind of surfaces or particles. Large variations between parallel samples were observed as well as irregular and inconsistent patterns over time, in particular in the aquatic environment. In addition there were fairly large variations between values from repeated analysis of the same sample. There were however enough data to confirm the generally very low levels of deltamethrin in the environment directly after the spraying.

There were no indications of any decline in abundance of fish. The only significant result was derived from the Pom Pom experiment where 3 species, all belonging to the family Cichlidae, were caught in significantly higher numbers after the spraying. The common denominator for these species is that they feed on sediments and what is living there. The high accumulation of deltamethrin that occurs on sediment surfaces may have resulted in these fishes getting a considerably higher dose than other species, which may have caused hyper activity and/or disturbed locomotion. Such effects are described in literature and are temporary.

There were no significant changes in the abundance of two species of reed frogs that due to their habitat and behaviour are the most likely amphibians to be exposed to the spray.

Aquatic invertebrates were sampled at PomPom in 2001, from lagoon, channel and shallow seasonal habitats. Samples collected immediately after the spray event had high proportions of

dead individuals. In 17 of the 47 different kinds of organisms collected, more than 50% of the individuals collected after the spray event, were dead. Of the more abundant of these organisms, the Notonectidae (backswimmers), Coleoptera (beetles) and Naucoridae (bugs) had deaths of 95-50%; the Hydrometridae (pond-skaters), Polymitarcydae (burrowing mayflies), Corixidae (water boatman), Baetidae (mayflies) and Caenidae (crawling mayflies) had deaths of 50-25%; and Chironomidae (midges) and Lestidae (damsel-flies) had deaths of 25-10%. Corduliidae (dragon flies) and Ostracoda (Crustacea) had low mortalities while the Mollusca (snails and mussels) had no mortalities. A species of burrowing mayfly emerged from the sediment after the spraying. Since deltamethrin is rapidly absorbed in the sediments this could be considered a stress response, as could the dramatic increase in plankton drift in the immediate post-spray period.

Experiments were carried out on *Cyrtobagous salviniae*; a weevil that has been introduced to control the problem water plant *Salvinia molesta.* There was a consistent and significant death rate of 40-60% after a single spraying. Extrapolated to 5 spray cycles this means that the natural population could be reduced to 3% by a full spraying campaign.

The relative abundance of ground active invertebrates caught in pitfall traps did not change as a result of the spray event at Pom Pom. Ant and Orthopteran morphospecies composition did not differ significantly before and after the spray event but there were differences in beetles and flies.

Rates of knockdown from tree crowns across all invertebrates were around 80 individuals m^{-2} and over half of these were mayflies and beetles. This took place within 48 hours after the spraying. More than 80% of all individuals in knockdown samples were from five groups; mayflies (Ephemeroptera, 26%), beetles (Coleoptera, 25%), bugs (Hemiptera, 11%), spiders (Aranae, 11%) and flies (Diptera, 9%). All dragonflies (Odonata) and booklice (Psocoptera) and more than 80% of mayflies (Ephemeroptera), flies (Diptera) and grasshoppers (Orthoptera) collected from knockdown traps died within 12 hours of being sampled. Out of in total 18 taxa with many species in each 16 had a death rate of 50% or more.

There was a significant decrease in grass living spiders in some of the experimental sites. This was most notable for Araneidae, Oxyopidae and Salticidae families. In the dry riparian zone all five studied spider families and a “rest” group showed a significant decrease after the spraying.

The results of the 2001 study guided the design of the 2002 environmental study:

- The methodological difficulties in the direct measurements of deltamethrin in the environment caused that study to be considerably reduced.

- Due to the absence of direct negative impacts on fish and amphibians these taxa were dropped from the 2002 design. The low impact of deltamethrin on vertebrates at large as evident from literature supported this exclusion.

- A bird study was however included due to the risk for secondary spray impacts caused by a possible reduction in food supply (mainly insects). The high conservation status of birds contributed to this inclusion.

- Consequently the core focus was on aquatic and terrestrial invertebrates, being the taxa most negatively affected by the aerial spraying.

- Within these susceptible taxa not all possible methods or habitats were used, but the emphasis was put on the habitats and methods that had shown the strongest and most significant negative results in terms of mortality causing reduction in number of individuals and/or reduction in species numbers.

- A special study was done on the *Salvinia* controlling weevil *Cyrtobagus salviniae.*

The 2002 monitoring programme was given the go ahead on 15th April 2002 contingent on an agreed budget, monitoring design and team which was finalised following the recommendations of an external reviewer on 30th April. The spray cycles commenced on the 16th May 2002.

1.2. Methods

The 2002 spray block was 8600 km^2 or 8722 km^2 when the northern buffer zone is included – that is the area of overlap with the 2001 spray block (Figure 1.1). The spraying was done with 3 – 4 turbo-prop planes flying at tree-top height at 220-250kph, 275m apart and completing each of the five spray cycles in 6 – 8 nights. As in 2001 the insecticide used was deltamethrin, carried in a sunflower oil base, and sprayed at 0.30 g a.i. (active ingredient)/ha for the first two cycles and 0.26g a.i./ha for cycles 3 – 5. This higher insecticide dosage for the first two cycles was based upon the resistance of pregnant tsetse flies to deltamethrin. Indeed, the difference between the 2001 and 2002 fly capture rates after cycles 1 and 2, bears out this fact, with flies caught several days after cycle 1 in 2001, but only a week after cycle 1 in 2002 (Allsopp, pers comm).

Spraying commenced just before dusk and finished in the early hours of the morning, with a very fine spray mist trapped by an inversion and drifting through the tree and shrub layers, with lethal consequences for all resident adult tsetse flies. However, as tsetse fly pupae of various ages are in the soil and are not affected by the insecticide, the application has to be repeated – with four consecutive applications and one safety application normally sufficient.

The spray cycles occurred as follows:-

- Cycle 1 (16 – 23 May)
- Cycle 2 (3 – 9 June)
- Cycle 3 (27 June – 2nd July)
- Cycle 4 (21 – 26 July)
- Cycle 5 (11 – 16 Aug)

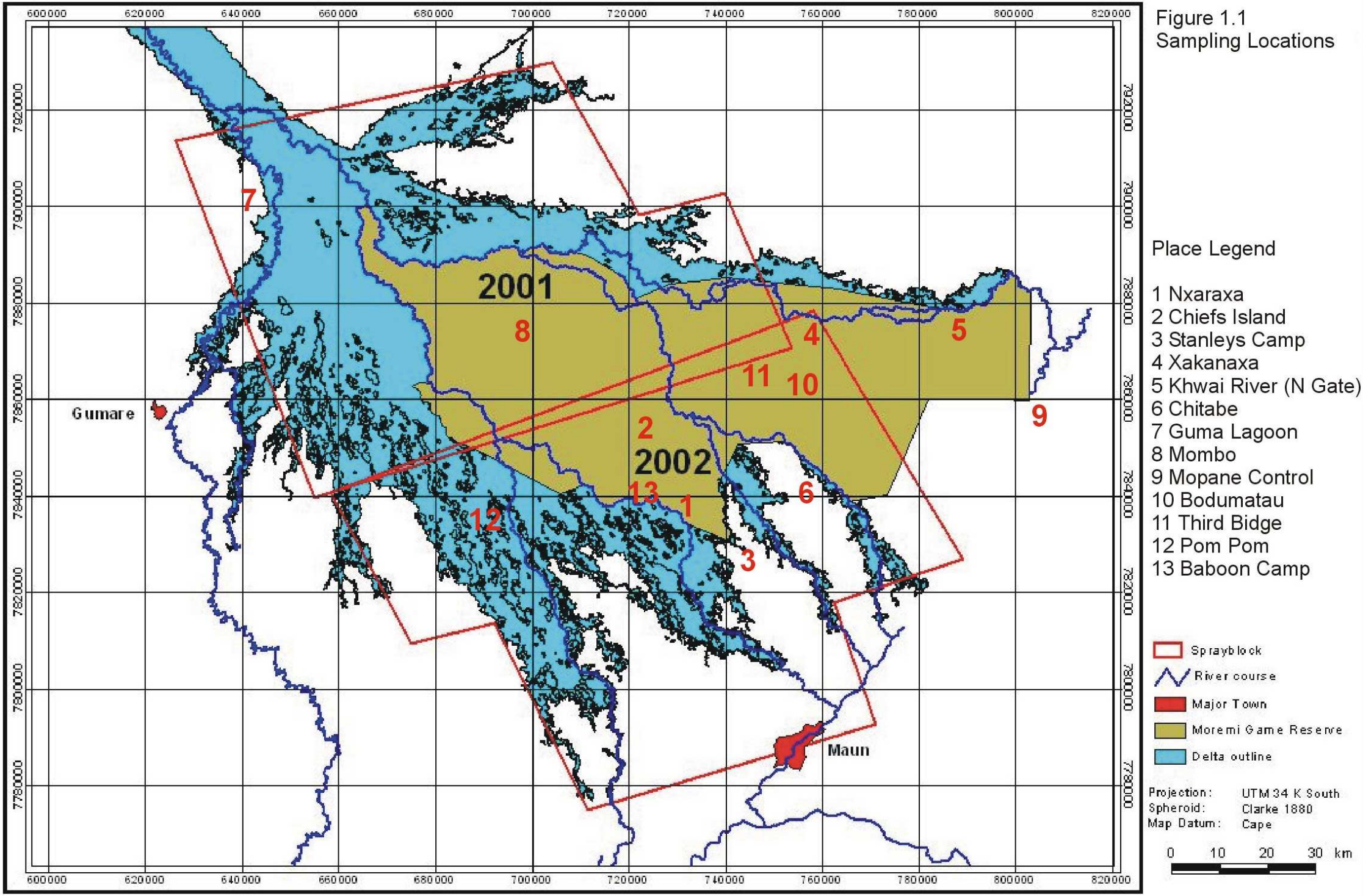
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Figure 1.1
Sampling Locations
Place Legend
1 Nxaraxa
2 Chiefs Island
3 Stanleys Camp
4 Xakanaxa
5 Khwai River (N Gate)
6 Chitabe
7 Guma Lagoon
8 Mombo
9 Mopane Control
10 Bodumatau
11 Third Bidge
12 Pom Pom
13 Baboon Camp
Sprayblock
River course
Major Town
Moremi Game Reserve
Delta outline
Projection: UTM 34 K South
Spheroid: Clarke 1880
Map Datum: Cape
0 10 20 30 km

The monitoring of terrestrial invertebrates was mainly done from the Harry Oppenheimer Okavango Research Centre's (HOORC) field station at Nxaraxa in the centre of the 2002 spray block, while the aquatic invertebrate and *Salvinia* studies focused upon Xakanaxa in the north eastern corner of the spray zone, with Khwai River (North Gate), used as an unsprayed control (Figure 1.1). Spray deposition and meteorological measurements were taken at the core terrestrial (Nxararxa) and aquatic (Xakanaxa) sites. Bird monitoring activities were undertaken at the same four sites in both 2001 and 2002. Chitabe and Nxaraxa located in the 2002 spray block, and Mombo and Guma in the northernmost zone – that had been sprayed in 2001.

Climate data and spray deposition monitoring were designed and undertaken by staff from the HOORC at the main study sites of Nxaraxa and Xakanaxa. Aquatic macroinverterbrate and zooplankton sampling was undertaken jointly by the Institute for Water Research, Grahamstown and Umgeni Water, South Africa with sampling centred on Xakanaxa and Khwai in Moremi Game Reserve. Terrestrial Invertebrates were sampled at Nxaraxa, Chief's Island and on the eastern border of Moremi Game Reserve following a design developed by BioTrack Australia (Pty) Ltd. Surveys of bird activity and abundance on four sites – Mombo and Guma in the northern spray block and Chitabe and Nxaraxa in the southern - were done both 2001 and 2002 by a research student from the University of California (Figure 1.1).

The biological sampling methods used are described briefly under Results for each study. They are all semi-quantitative and the quality of data depends to a large extent on the persons doing the sampling; that they do it in exactly the same way all the time otherwise comparisons will be jeopardized. An additional problem for both the aquatic and terrestrial studies has been the lack of benchmark (background) data and the lack of not sprayed reference areas. The lack of benchmark data was caused by the late involvement of the environmental monitoring team 2001 that started when the spraying was already well under way. Similarly the lack of reference areas could at least the first year have been avoided if the monitoring team had been given a chance to influence the design of the spraying. A division of spray blocks in a north-south direction would have been a great advantage in particular for the aquatic studies where the risk/possibility for down stream effects now has limited the possibilities to draw conclusions.

In this situation the analysis of data has been based on two methods:

1. Sampling just before a spraying event and just after. Significant changes in numbers of individuals in any taxa are likely to be caused by the spraying.

2. Analysis of trends in numbers of any taxa from cycle 1 to 5. Significant trends in particular if supported by method the first method are likely to be the effect of spraying.

Data management and statistical analysis for all biological monitoring components were designed and completed by BioTrack Australia (Pty) Ltd with summary results passed to the lead consultants for interpretation.

1.3. Results

1.3.1. Meteorological data and Spray deposition

Mean diurnal winds varied between 0.5 and 4 m/s during the spray campaign and the night winds were usually weaker than 0.5 m/s. Air temperatures varied between extremes of 5 – 33 degrees Centigrade, with mean air temperatures during the spray campaigns 1-5 being on average 20 (mid-May), 16 (beginning June), 15 (beginning July), 20 (end July) and 23 (mid-August). There

was usually a pronounced temperature inversion during night with cooler air close to the ground. This inversion, together with the low wind speed during night provides suitable conditions for effective aerial spraying.

Spray deposition varied considerably within and between sites – values between 23 and 867 drops cm^{-2} were recorded on rotating slides, with habitat variation and distance from the flight lines, the most likely explanation. Essentially the measurements confirmed however that all the study sites on all occasions received a considerable amount of spray.

To determine the residual effects of the deltamethrin spray in the environment a number of wool strands were exposed to the spraying during cycle 2 and collected and preserved on a daily basis. The concentrations declined exponentially and revealed that for tsetse flies lethal concentrations were likely to have lasted for four or five days after the spray event. This is in agreement with other studies.

1.3.2. Aquatic Invertebrates

Within the 2002 spray zone at Xakanaxa, aquatic macroinverterbrate sampling was undertaken at Paradise Pools on the Upper Khwai River, in the two typical biotopes: channels with flowing water and lagoons with still water (It is well known that flowing and still waters usually differ in composition of invertebrates). In addition, sampling was done at Bodumatau Bund (channel) and Third Bridge (lagoon and channel). Shallow seasonal habitats were sampled in August at all sites, including Pom Pom so as to allow comparisons with the 2001 study, and to include the seasonal habitat so widespread in the delta. The sampling followed methods developed for river quality monitoring in South Africa, using sweep net over a defined distance with a standardized number of sweeps. The Khwai River at North Gate served as an unsprayed 'control' site.

Crepuscular drift net sampling over a specific period, of both macroinvertebrates and zooplankton, was undertaken in channels at Xakanaxa and Khwai (North Gate). Trawl net sampling over a specific stretch of water for zooplankton was carried out at Paradise Pools.
A total of 695 macroinvertebrate samples and 200 zooplankton samples were collected, and 64 macroinvertebrate families were identified. Abundances were all adjusted to numbers per ten samples collected, and relative abundances were reported.

Xakanaxa channel and lagoon fauna.

At Xakanaxa, lagoon and channel aquatic macroinvertebrate fauna were distinctively different. There was considerable overlap of families and each habitat was characterised by variations in the abundance of common families, and the presence/absence of specific families. Changes in abundances, and in presence/absence data have been reported for channels and lagoons, and for the overall Xakanaxa condition. In channels abundances declined by 46% s after 5 spray cycles (927 individuals to 520) and in lagoons it declined by 25% (1230 individuals to 917).

A total of 47 taxa were found in channels and 49 in lagoons. The taxonomic composition differed between these two habitats and in total 65 taxa were identified of which 23 were common and occurred consistently in samples before the spraying. Out of these common taxa six showed distinct rapid declines after the first spray cycles and had disappeared completely after the fifth cycle. This corresponds to a loss of 26% of common taxa and is likely caused by the spraying. It is difficult to evaluate effects on the more rare taxa as they appear and disappear in the samples in an irregular way. The loss of rare taxa is of course at least as likely as the loss of common ones and a total loss of 20-30% of taxa is likely. As almost all taxa are made up of several species the total loss of species is considerably higher than six.

The North Gate site was unsprayed. It could not be termed a control as it was downstream of the spray zone, but it was not directly sprayed. There, abundances and number of taxa increased slightly from the benchmark to date of the final spray cycle 5 (789 individuals to 839, and 29 to 30 families). This confirms that the spraying in all likelihood caused the observed declines in these parameters in the spray zone.

In all cycles there was a significant increase the drift, and drifting is a well-recorded 'escape' phenomenon. The number of shrimps (Atyidae), and larvae of the Leptocerid cased caddis flies, caught in the drift nets after the spray events, represented an approximately 3000% and 660% increase, respectively, over the "normal" drift of these families within the sample area. The increased drift indicates a reduction in vitality and probably an increase in deaths, which is reflected in the results from the sweep net sampling.

Conclusions

A change in aquatic macroinvertebrate community structure related to the deltamethrin spray occurred at Xakanaxa in both lagoon and channel biotopes with 26% of families absent from the final state. In the initial state lagoon and channels each had a characteristic faunal composition. This difference was became smaller in the final state as more rare families, specific to each habitat disappeared, while the hardy ubiquitous ones survived. In addition, abundances decreased. The results from the other locations were in accordance with theses changes.

Table 1.1 The key aquatic invertebrate indicators of the spray effects 2002.

Taxa	**Nature of change**
Flathead mayflies (Heptageniidae) Stout crawler mayflies (Tricorythidae)	Restricted to channels and disappeared by Cycle 5.
Creeping water beetles (Noteridae)	Restricted to lagoons and reduced to single individuals.
Water Striders (Veliidae) Creeping water bugs (Naucoridae) Pygmy backswimmers (Pleidae)	Abundant in lagoons and channels. Reduced totally or to sampling of single individuals.
Shrimps (Atyidae) Damselflies (Coenagrionidae)	Dominant taxa that remained common, but were reduced in numbers in lagoons and channels.
Baetid mayflies (Baetidae)	Abundant and affected by spray but morphospecies identification required to discern pattern.

There is a general agreement in the results from 2002 and 2001 although the first year only describes results from the single spraying event at Pom Pom. In Table 1.2 spray effects on common families are compared. The data for 2001 is a combination of mortalities for the Shallow Water and Channel habitats, which showed consistent results. Only for two families the results are not consistent between the Pom Pom 2001 and the Xakanaxa 2002 results. The mayfly family Caenidae had large mortality and was completely eradicated in the channel habitat 2001, while

there were very small effects on it in the Xakanaxa habitats. There may have been different species, with different tolerances at the two sites.

The second deviation is in the family Chironomidae. This is most probably caused by the fact that this family in 2001 was analyzed down to sub-family level and some of these were completely absent while others were not affected. In 2002 the analysis went only to family level and on that level no changes could be discerned. Chironomidae is a very large family and there is a significant difference in the ecology of the sub-families. There can in other words occur significant changes on Sub-Family, Genera and Species level that cannot be discovered by the used approach. Identification to family level is standard practice internationally for rapid bioassessments. The 2002 terrestrial study indicated the value of identification to a finer taxonomic level, and the more rapid identification to morphospecies allowed by the Biotrack system was undertaken for selected aquatic invertebrates for the 2001, 2002 and recovery samples and is reported in the final recovery report.

Otherwise there is a fairly clear picture in both channels and lagoons. The survivors here after spraying are molluscs that probably are more physiological resistant to spraying than insects. Together with the insect Families Chironomidae (non-biting midges), Ceratopogonidae (biting midges), Libellulidae (hairy dragon flies) and Caenidae (crawling mayflies) this forms a basic community of survivors. These entire insect families have in common that they live in the sediment, which may function as a protection against the spray, which is absorbed to organic sediment surfaces, is bound there, and is therefore less bio-available.

On the other hand, the high extermination rate recorded for the whole Order of Hemiptera (waterbugs) in particular, for most Ephemeroptera (mayflies) Families and some other Families from different Orders can be understood by their active behavior in the free water, on sediment and vegetation surfaces, and on the water surface. In particular the air breathing behavior that most Hemiptera have but also Coleoptera (beetles) will force them to come into contact with deltamethrin and the oil-based carrier that accumulate on water surfaces as a thin film. The Hemiptera that live both as nymphs and adults in the free water is therefore probably the most sensitive Order. Coleoptera are likely to be sensitive as well but most of the latter occur in low numbers and apart from Noteridae there are no significant negative effects. The free-living Ephemeroptera nymphs and Corduliidae (dragonflies) are obviously very susceptible to the spray and so are the adults in the terrestrial phase as indicated from the 2001 results. More than temporary and local exterminations are not unlikely for species from the groups mentioned above.

Table 1.2 .Tentative overview of common aquatic invertebrate taxa sensitivity to deltamethrin. For 2001: XXX= very high mortality or local extinction; XX= high mortality or reduction to low numbers; X= significant mortality or reduction of numbers; O= no mortality or no decrease in numbers. For 2002 the same symbols indicates changes in numbers only from before spraying in May to end of spraying in August.

Taxonomic group	2001	2002 channel	2002 lagoon
Ephmeroptera (Mayflies) (*Insecta)*			
Baetidae	XX	XX	
Heptageniidae		XXX	
Polymitarcyidae	XX		
Caenidae	XXX	O	O
Odonata (Dragonflies) (*Insecta)*			
Lestidae (Damselflies)	X		
Coenagrionidea (Damselflies)	X		X
Corduliidae (Large Dragon flies)	XX	XXX	
Libellulidae (Hairy Dragon flies)		O	O
Hemiptera (Waterbugs) (*Insecta)*			
Hydrometridae	XX		
Veliidae	XXX	XXX	
Corixidae	X		
Notonectidae	XXX		
Pleidae			XXX
Naucoridae			XXX
Coleoptera (Beetles) (*Insecta)*	XX		
Dytiscidae			O
Noteridae			XXX
Haliplidae	O		
Diptera (Flies) (*Insecta)*			
Simulidae	XXX		
Ceratopogonidae	O	O	O
Chironomidae	XXX-O	O	O
Trichoptera (Caddiesflies) (*Insecta)*			
Leptoceridae		XX	
Atyidae *(Crustacea)*		X	XX
Lymnaeidae *(Mollusca)*	O	O	O
Planorbinae *(Mollusca)*	O	O	O
Hirudinea (Leeches)			O

The main results and conclusions were:

1. There was a measurable impact on aquatic invertebrate biota as a result of 2002 deltamethrin spraying. The main impacts were: the numbers of some of the most abundant organisms were reduced; some common, but less abundant, taxa that were

specifically characteristic of either the channel or lagoon habitat “disappeared”; and the assemblage composition of the lagoon and channel habitats became more similar and were characterised by fewer, more resistant taxa.

2. Key sensitive taxa were: shrimps, pygmy backswimmers, damselflies, baetid mayflies, water striders, creeping water bugs and beetles, flathead mayflies, backswimmers and predaceous beetles.

3. The full impact of the 2002 deltamethrin spraying cannot be assessed without further monitoring, however it is likely that the aquatic invertebrate biota will recover. The mosaic of biotopes and occurrence of refugia, as well as the complex seasonal emergence and life cycles of the biota probably mean there is good recovery potential. Aerial life-history stages would assist with rapid recolonisation. A recovery study is recommended.

4. It is likely that identification to morphospecies would considerably enhance the information to be gained from the samples collected.

1.3.3. Terrestrial Invertebrates

It was shown in the 2001 study that most significant results were derived from invertebrates falling down from tree crowns as a result of spraying. Most sampling was therefore done in 2002 by collecting invertebrates that had been knocked down by the spraying, under tree crowns on plastic sheets with an area of just under 3 m^2. Complementary to this fogging with deltamethrin from the ground using handheld foggers were used. This made it possible to collect pre-spray data form the tree crowns and to see how much of potentially sensitive invertebrates remained in the trees after spraying. The knockdown was followed under three tree species (*Kigelia africana*, *Lonchocarpus capassa* and *Combretum imberbe*) in the riparian zone and under *Colophospermum mopane* in the drier inland. For the latter species an unsprayed control site along the south-eastern boundary of Moremi Game Reserve was also used. There were no un-sprayed riparian control sites left in the Delta. The number of flying insects was recorded to a limited extent by the use of one malaise trap and ground living invertebrates were captured in pitfall traps.

A total of 102,248 terrestrial invertebrates, covering 26 higher taxa, were captured from 746 samples of various types. Beetles (34%), flies (28%), ants (22%), Hemiptera (6%) and wasps (3%) were the most abundant taxa, collectively accounting for 93% of the total sampled and as with most samples of biodiversity the majority of the remaining taxa were infrequent.

Knockdown of invertebrates from riparian trees

Canopy fogging from hand-held foggers were more effective in knocking invertebrates from the canopy than aerosol applications of the same insecticide formulation from aircraft. Up to 70% more specimens were collected under fogged trees than sprayed ones. When a tree is fogged not all the invertebrates are killed, and invertebrates can be found in the tree canopies after five spray cycles. Fogging does not reduce subsequent catches from aerial applications, which may indicate high regeneration and mobility of the invertebrates in the canopy environment.

Canopy fogging with deltamethrin from the ground returned most canopy invertebrates with an average of 110 individuals per m^2 prior to the spray cycles. Trees fogged in this way after the five aerial application cycles produced on average 40 specimens per m^2 – a decline of 64%. This reduction – both in abundance and species richness - took primarily place after the first or second spray cycle. Most of this was caused by a dramatic reduction in the abundance of beetles by

84% (see Table 1.3). All other common taxa show significant reductions as well with the exception of Diptera (Flies). This is most likely correlated to the arrival of the flood in the area and increased temperatures. The Diptera Families Chironomidae and Ceratopogonidae that have their larval stages in water are common in Delta waters (see the aquatic chapter 4). They are known to produce mass swarming during spring and early summer. Most likely they are the main contributors to the increase in Diptera knockdown over time. Unfortunately the common Diptera were not analyzed to this taxonomic level in this study.

Table 1.3 Overview in knockdown from trees in the riparian zone 2002 in invertebrate abundance and species richness before and after spraying.

	Beetles	Ants	Flies	Hemipt.	Spiders	**Total**
ABUNDANCE CHANGES						
Nr. of specimens collected	30 000	17 603	649	774	1691	**50 717**
Pre-cycle nr. of spec. /m^2	72.0	23.4	2.1	6.3	3.1	**110**
Post-cycle nr. of spec. /m^2	11.5	12.6	10.8	2.8	1.0	**40**
Pre-cycle – post-cycle %	-84%	-46%	+517%	-56	-66	**-64**
SPECIES CHANGES						
Nr. of Families	22		32	12	4	
Identified morphospecies	133	35	117	59	23	**367**
Morphospecies only in pre-cycle or Cycle 1 or 2	45	4	48	27	7	**131**
Pre-cycle, Cycle1&2 – post-cycle %	-34	-11	-41	-46	-30	**-36**
Number of morphospecies in pre-cycle	74	23	40	27	12	**176**
Number of morphospecies in post-cycle	46	29	40	14	7	**136**

A total of 367 morphospecies were identified from selected families of beetles, spiders, flies, all Hemiptera and ants in samples taken from *Kigelia africana, Combretum imberbe* and *Lonchocarpus capassa* trees within the spray zone. Around 36% of these morphospecies were recorded in pre-cycle fogging and cycle 1 or 2 but not thereafter. Of these 131 morphospecies, only around 30 were sampled in any significant numbers prior to the spray cycles, the rest being recorded as singletons or just a handful of specimens. Proportionately beetles (34%), spiders (30%), flies (41%) and Hemiptera (46%) had the most morphospecies not sampled after cycle 1 and 2 and ants (11%) the least, raising questions as to the ability of these morphospecies to recover in the post-spray period.

There is a generally good agreement between the results from 2001 and 2002 (Table 1.4). With one exception the same seven higher taxa are making up 95-96% of the abundance for both years. The Order Ephemeroptera however, occurred in large numbers at Pom Pom 2001 but only in low numbers at Naxaraga 2002. This is likely to be a seasonal effect as the Pom Pom

experimental spraying took place on 21st August and post-spray sampling was done during the following 3-5 days and thus a few days later than the 2002 post-spray sampling. This taxon is known to have coordinated mass swarming in spring and early summer and in a few days dramatic changes in abundance can occur. It can however not be ruled out that the 2001 spraying which took place upstream of the Naxaraga site may have caused a high mortality on the aquatic living nymphs by aquatic drift of the spray. Such effects are not unlikely. For instance at Pom Pom 2001 located 35 km south of the spray zone significant levels of deltamethrin were found in the sediment before the experimental spraying.

Table 1.4. Comparison of the most common invertebrate taxa collected in the riparian zone in knockdown traps 2001 and 2002

Taxa	**Relative abundance % 2001**	**Mortality % 2001**	**Relative abundance % 2002**	**Decline Cycle1-5 % 2002**
Ephemeroptera	26	83	0.1	-
Coleoptera	25	66	7	84
Hemiptera	11	65	6	46
Aranae	11	67	2	67
Diptera	9	88	43	+517
Formicidae	8	68	33	46
Hymenoptera	4	79	4	20
Acarina	1	40	1	-
Sum % abundance	**95**		**96**	
Total number of taxa	**18**		**21**	
Total number of collected individuals	**5468 (1 cycle)**		**22860 (5 cycles)**	
Mean knockdown 1st cycle Individuals/m^2	**76**		**76**	

In 2001 the mortality was directly observed in experimental vials while during 2002 the decline in abundance from spray cycle 1 to 5 were recorded. High mortality correlate usually well with high reduction in abundance. As mentioned and discussed above the exception is the Diptera 2002 that show an increase in abundance over the five cycles. The total number of higher taxa is almost the same and the total knockdown from the first spraying is exactly the same for both years. There are thus good reasons to have confidence in the main results.

Invertebrate knockdown from dryland Mopane trees

Only for the *Colospermum mopane* knockdown sampling was it possible to locate a control site at the edge of Moremi Game Reserve 40 km from the spray zone, which was compared with a site on Chiefs Island that was sprayed in all five cycles. Only pre-spray and post-spray knockdown sampling was done.

When fogged, *Colophospermum mopane* trees returned far more individuals; as a mean 220 per m^2 for Moremi and 240 per m^2 for Chiefs Island in pre-spray samples to compare with 110 specimens per $m^{2\ for}$ the riverine trees. Catches were dominated by beetles, particularly two morphospecies, and between the pre-cycle and post-cycle sampling they more than doubled on the unsprayed site compared to a very significant decline on the sprayed site. At the post spray sampling the total abundance for all taxa increased by 35% on the unsprayed site, while it declined significantly with 60% at the sprayed site on Chiefs Island.

There was a significant difference in taxa compositions in that the sprayed Moremi site had an increase in Diptera. This was probably due to an increase in species with aquatic nymphs as discussed above. The Moremi site was in this respect not completely comparable as it was located far away from watercourses. There was a small not significant increase in morphospecies composition at the unsprayed site, while there was a significant decrease in species numbers with 30% on the sprayed site.

The trend in the unsprayed site with an increase in both abundance and in species numbers are in agreement with what can be expected with the higher temperatures in August and it is very likely that the same pattern would have been observed also in the riverine trees if they had not been sprayed. The declines observed there are therefore very likely to be caused by the spraying itself and the overall effects as summarized in Table 1.3 and 1.4 are probably under estimates.

The total number of lost species is however impossible to estimate. The spraying may not cause a complete extermination of all species that were not found after cycle 1or 2. Some may have been reduced to very low numbers and if so they have a much smaller chance to appear in the samples. Some may disappear due to natural causes such as end of the life cycle, and many species here are small and have short life spans. The results from the mortality experiments in 2001, from the Mopane knockdown and from the three riverine tree species are however in good agreement and it is reasonable to state that 30-40% of invertebrate species in trees became extinct by the deltamethrin spraying.

Recovery by invertebrates from spraying

Trays impregnated with insecticide (to prevent recovery from spray cycle knockdown) captured on average 20% more taxa than adjacent untreated trays. Mantodea, Neuroptera, Orthoptera and Pseudoscorpionida were only sampled from the treated trays as well as 11 fly morphospecies and three of these were not sampled in any other method.

Flying insects

7,500 individuals, mostly flies, were sampled. Within each cycle catches were lower in the days after the spray event but then increased between the spray cycles to be much greater in subsequent pre-cycle catches. Composition of samples changed over the cycles mostly due to an increase in the abundance of flies, most likely due to increasing temperatures and the arrival of the annual flood as discussed above.

Ground living invertebrates

5,700 individuals from 18 higher taxa were sampled in the pitfall traps. There were no significant trends in abundance or richness through the cycles and inconsistent changes in the pre-cycle and post-cycle samples. This is in agreement with the results from 2001.

1.3.4. Birds

Deltamethrin is highly unlikely to kill birds directly due to its low toxicity to vertebrates. Effects on bird populations may however be caused by reduced food supply (Insects) and be detected as population declines caused either by increased emigration out of the spray block or in reduction in breeding success. The later effect would however not be detectable by population census methods the year of the spraying.

Bird populations change naturally from season to season and from year to year. Climatic factors are important and the Okavango Delta received above average rainfall 2001 and below average 2002 causing the food supply to probably be better the first year. In addition the seasonal flood distribution has large variations from year to year and fires are frequent. Also these factors have impact on food supply. Two of the study sites (Chitabe and Nxaraxa) burned in fact after the last pre-spray survey 2001. With all these factors as part of the equation it is unlikely that smaller effects of the spraying can be identified and isolated from other factors. Large consistent and catastrophic declines in species or in guilds (group of species with similar ecology) can however readily be discovered by the survey methods used here.

Methods

Four study sites were followed in both 2001 and 2002. Mombo and Guma in the northern spray block were sprayed 2001 and Chitabe and Nxaraxa in the southern block were sprayed 2002. No suitable not-sprayed reference sites could be found.

Because of the variety of habitat types a number of monitoring techniques were used. Circular point counts and calling stations were used for monitoring forest birds, transects were used for acacia thornveld species, and boat surveys for water dependent species. Transects gave in most cases too few data for statistical analysis and were then excluded from further analysis, while some water survey sites dried up during the latter part of the spraying cycles 2002 and became useless.

During 2001 the monitoring started 10th April and went on until 10th June with two sampling occasions at Mombo and Nxaraxa and three at Guma and Chitabe. The following year 2002 the sampling started 25th March and ended 25th July with three sampling occasions in Mombo and Guma, four in Chitabe and five in Nxaraxa. During these whole periods, fieldwork was done by a two-person team, and ongoing practically all the time.

Results

In the forest habitat a total of 21,045 birds were detected from 162 species at 605 point count surveys at two sites in the 2001 spray block (Mombo and Guma) and the two sites in the 2002 spray zone (Nxaraxa and Chitabe). All sites were surveyed in both 2001 and 2002 enabling spatial and temporal comparisons of the observed bird populations to be made. Only one sampling 2001 was done after spraying (in Guma June) all other were “pre spray”. These data are therefore only used as benchmarks for the spraying 2002 and can to some extent answer whether there was a longterm effect on bird numbers or species richness.

The result of the diet guild analysis of these two sites, Mombo and Guma 2001 and 2002, showed that the non-insectvorous species in Guma had no significant difference in abundance between the years, while they declined at Mombo 2002. The same comparison for insectivorous birds showed an increase at both sites during 2002 – in other words opposite to what would be expected if spraying had a negative effect.

Chitabe showed declines in insectivorous birds when comparing data from 2001 to 2002 before the spraying, and this trend continued during the whole spraying campaign. Chitabe non-insect-

dependent birds showed no changes. Nxaraxa however had no significant differences in abundance from 2001 to 2002 and both guilds of birds increased during the sampling period. Again the results indicate that other natural factors rather than the spraying are at play.

The resident insectivorous bird, the Greybacked bleating warbler, showed no significant change in numbers at Nxaraxa, Guma or Mombo between years, with a decline evident at Chitabe when pre spray 2001 and 2002 data, was compared with post spray 2002.

Comparisons between 2001 and 2002 bird populations in Acacia thornveld at Mombo showed a decline in non-insectivorous birds, but no decline in insectivorous birds. The majority of species were detected in both years and twice as many species were only sighted in 2002 than species only sighted in 2001. This was probably due to a larger number of observations in 2002.

The results were similar for water birds. There was no significant decrease in numbers of insectivorous or non-insect-dependent species. More insectivorous bird species were sighted in 2001 only, than in 2002, but the magnitude of loss was small and not a cause for concern.

In summary there were some local changes in bird populations during the spraying, but these changes cannot be attached with any significance to the spraying exercise. For that several bird species or guilds would have to show declines, which could be correlated with the spraying. No such observations were made in spite of extensive fieldwork.

1.3.5. Salvinia weevils

Experiments were carried out with *Salvinia* weevils that were either kept in basins on the riverbank, or in perforated basins in shallow water. They were exposed to the routine spraying and the experiments were carried out for all five spray cycles. Controls were run 30 km outside of the spray block. In addition *Salvinia* samples were collected in various watercourses after each spray cycle and the number of weevils extracted and counted. These studies were conducted at Xakanaxa.

There were no differences in mortality between the experiments kept on the riverbank and those kept floating on the water surface. The mortality was significant for all five cycles with as a mean 28% after 48 hours and with a variation between cycles of 17% to 40%. The variation in mortality is clearly correlated to the amount of deposition of deltamethrin that was collected on aluminum foils at the time of spraying by the experimental site.

The number of weevils in the *Salvinia* mats collected on water surfaces declined from cycle to cycle in an exponential way with an about 50% reduction from cycle to cycle.

Theses results are in agreement with those from 2001 where experiments were done very similar to the ones 2002. That first year the mortality was as a mean 50%. The results after five spray cycles gives a survival of 3% of the original population, while the lower mortality of 28% gives a survival of 20% at the end of the spraying campaign. Whichever figure is more true is not of concern. The important result is that there is a consistent survival of weevils after each spray cycle observed for both years both in experiments and in nature. This consistent survival is in all likelihood enough to secure a quick recovery of the weevil population.

1.4. Conclusions

The studies of environmental effects of deltamethrin spraying on other species than the target tsetse fly have not found any decline in abundance or loss of species for fish, amphibians or birds. Although the studies in particular of birds have been fairly comprehensive it is as almost always with this kind of studies not possible to exclude that negative effects might have occurred. The documented low toxicity of deltamethrin on vertebrates makes it however likely that such effects were small if they occurred at all.

There was a significant reduction in abundance of the *Salvinia* controlling weevil *Cyrtobagus salvinae.* After five spray cycles the weevil population had in all likelihood been pressed down to very low numbers and did for some time probably not control the growth of *Salvinia.* In all experiments and direct observations in the field there were however always some surviving weevils left after spraying. The weevil has a fast reproduction rate and re-distribute easily. This is well known from the numerous translocations of the weevil done in many *Salvinia* infested waters all over the sub-tropical and tropical regions. The negative effects of the spraying on the *Salvinia* controlling weevil are therefore likely to be very temporary.

There were clear negative effects on both terrestrial and aquatic invertebrates. The results from both the aquatic and terrestrial studies are in agreement between each other, between the two years, and between the various sites and various methods used. Observed differences have logical explanations and do not affect any important conclusions. Deltamethrin spraying in the Okavango Delta 2001 and 2002 is likely to have reduced the abundance of aquatic invertebrates with 30-50% and the terrestrial with 60-70%. It is likely that the overall abundance – but perhaps by other species – will move back to the natural level within a short while after the spraying.

The loss of higher invertebrate taxa in the aquatic environment is about 50% and the loss of species in the tree canopies is 30-40%. It remains to see how much of this will come back in the coming years. The terrestrial study based on morphospecies has a reasonable chance to answer this as the taxonomic precision is high and in particular the knockdown sampling brings in very large samples. The aquatic study that is planning to use the morphospecies approach as well has the drewbacks that sampling is cumbersome, is less reproducible, and gives comparatively small samples. This in turn reduces the possibilities to draw significant conclusions.

If a permanent loss of a number of taxa occur it remains to evaluate the actual effect of this. It is likely to be difficult or impossible. Ecological processes such as pollination, decomposition, control of populations by parasites or predators, could be affected but there are in the Okavango Delta no measurements of these processes. The ecological functions of most species are hardly known either and many species are probably not described. The relation between biodiversity and ecological function is an area of cutting-edge research and it is in retrospect regrettable that such functional studies were not done during and after these spraying campaigns. They were actually gigantic ecological experiments from which much more could have been learnt.

2. Meteorology and Spray Deposition

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2.1 Methods

Weather conditions during the tsetse fly spraying campaign were monitored using automatic weather stations installed at three sites: Nxaraxa, Xakanaxa wetland and Xakanaxa dryland. Each station was recording a suite of environmental measurements, out of which only those relevant to the assessment of spraying conditions are reported here, namely: wind speed, wind direction and air temperature. Measurements were done at different heights – 1 and 6 m at Nxaraxa, 2 and 10 m at Xakanaxa wet and 10 m at Xakanaxa dry. At each station data were recorded at 1-minute intervals, averaged into 30-minute averages and stored in a logger. The 30 minute averages were retrieved from loggers and further processed in MS Excel.

2.2 Results

2.2.1 Environmental measurements

2.2.1.1 Wind

In the period of spraying campaign wind conditions were in general relatively stable, and no significant temporal trends were observed. Mean diurnal winds varied between 0.5 and 4 m/s. At Nxaraxa winds were stronger that at Xakanaxa (Figure 2.2). Diurnal variation was strongly dominated by differences between night and daytime winds (Figure 2.3). Night winds were usually weaker than 0.5 m/s. An increase in wind speed was observed between 8 am and 10 a.m., and between 10 and 11 a.m. the wind speed was reaching its diurnal maximum (usually more than 3.5 m/s). Subsequently, a gradual decrease in wind speed was observed, with wind speed dropping to 0.5 m/s around 5 p.m.
Conditions on 21 July 2002, shown in Figure 2.3, were slightly different – i.e. night wind is stronger, reaching 1-3 m/s. Such conditions were, however, rather exceptional and during spraying were recorded only on 22 May, 3 June, 2 July and the mentioned 21 July.

As far as wind direction is concerned two different wind regimes could be distinguished (Figure 2.4). South-easterly winds dominated during the first part of spray cycle 1 (16-21 May 2002) and during spray cycles 2 and 3. North to north-easterly winds dominated during the second part of spray cycle 1 (22-23 May 2002) and cycles 4 and 5.

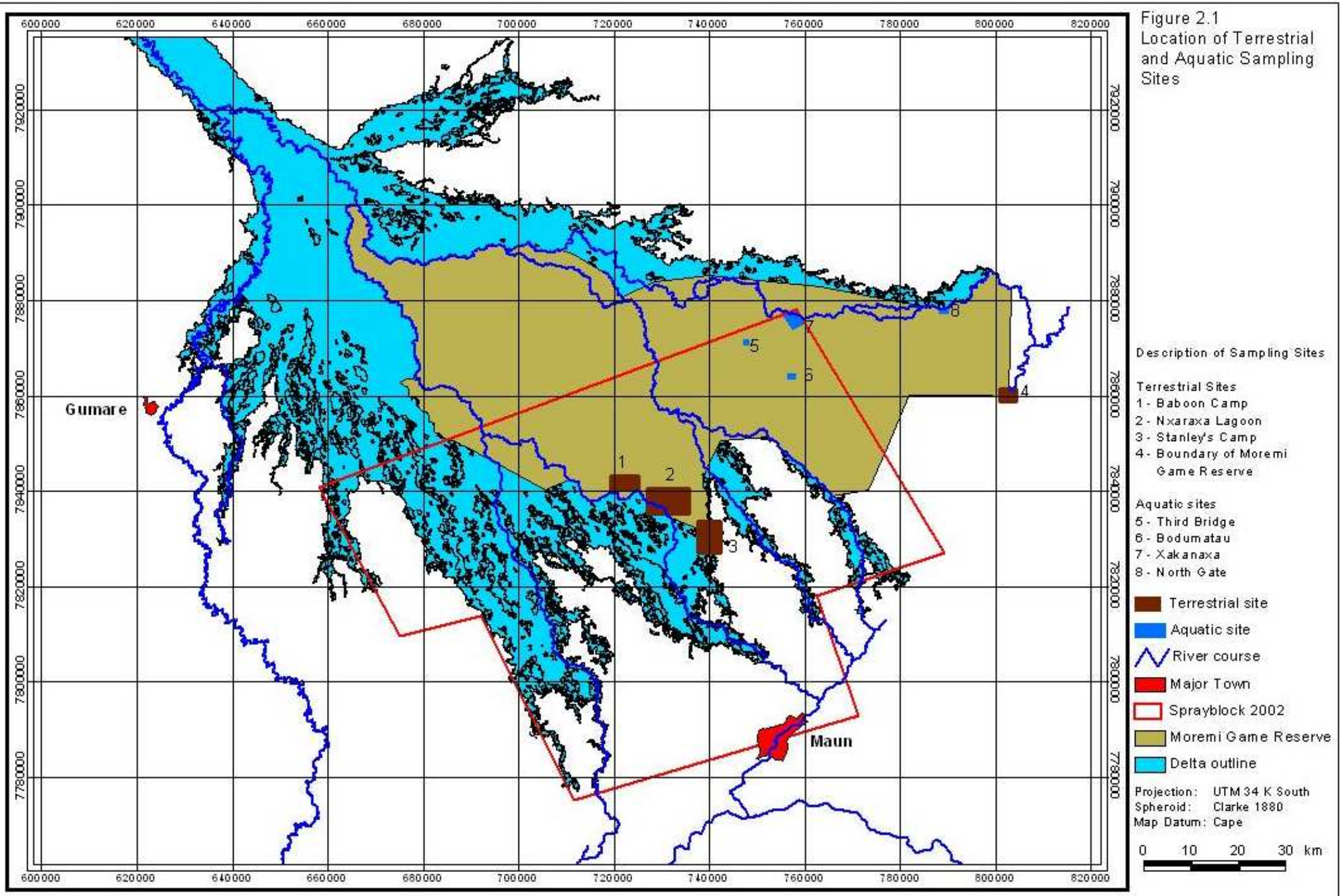

Figure 2.1
Location of Terrestrial and Aquatic Sampling Sites

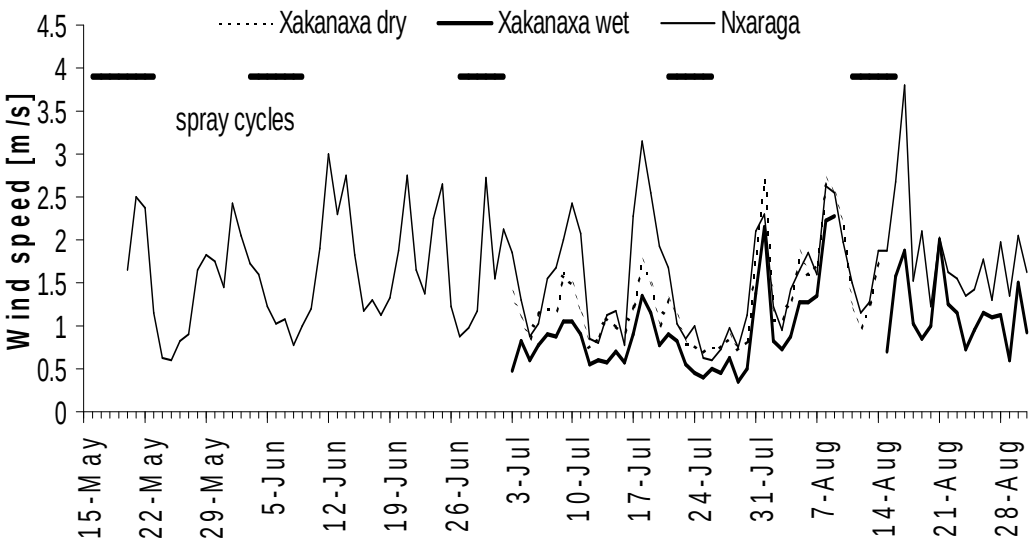

Figure 2.2 Variation of mean diurnal wind speed throughout the period of spraying campaign

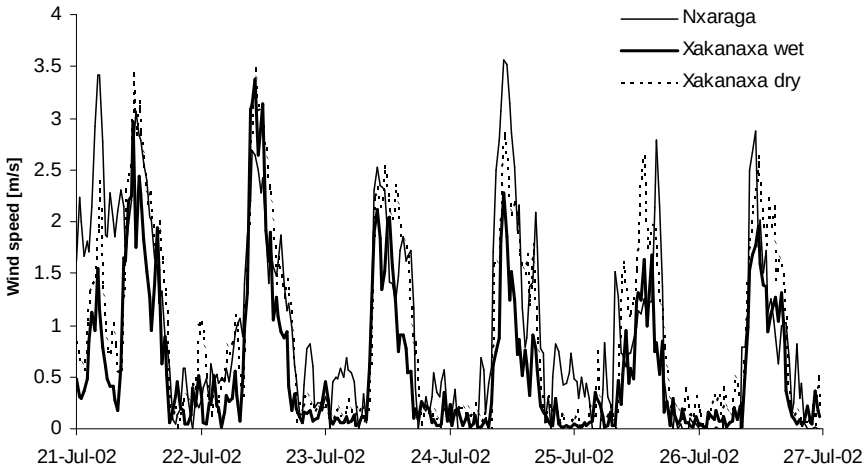

Figure 2.3 Diurnal variation of wind speed, spray cycle 4

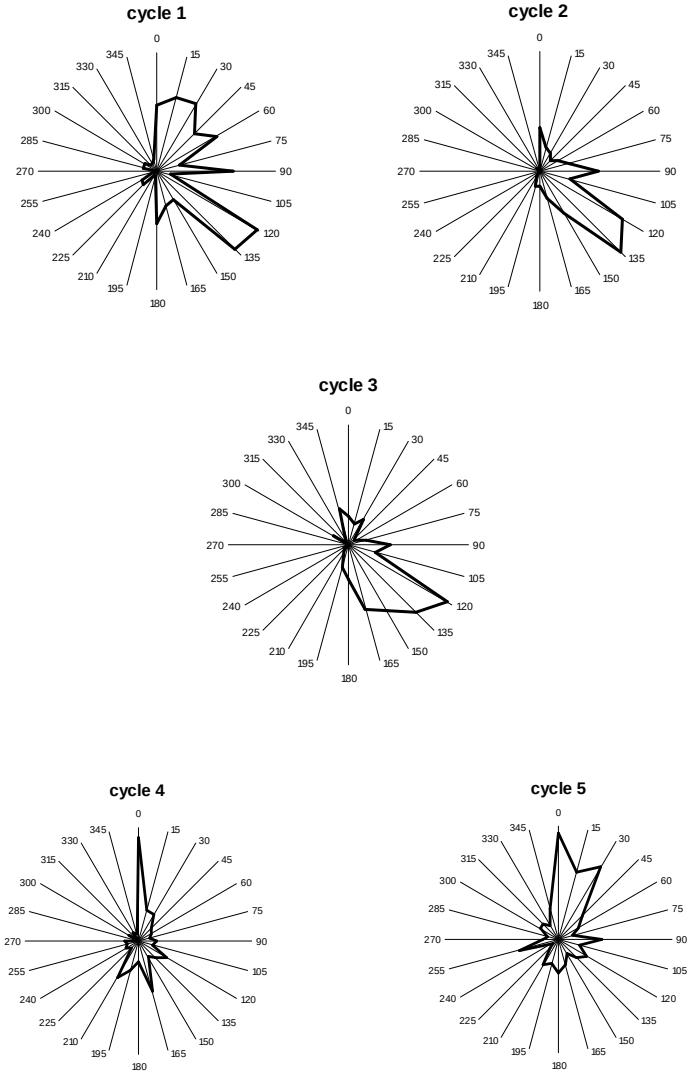

Figure 2.4 Wind roses for each of the spray cycles (6-8 days period), Nxaraxa site

2.2.1.2 Temperature

Air temperatures varied between 5 and 33 deg C (Figure 2.5). First part of the spraying campaign (until 31 of May) was somewhat warmer, with average diurnal temperature in order of 20 deg C and minimum diurnal temperature around 15 deg C. June and July (cycles 2 and 3) were cooler, with minimum temperatures dropping to 5 deg C, and average diurnal temperature varying between 15 and 20 deg C. In end of July temperature started rising, and during cycle 5, average diurnal temperature was around 20 deg C, and minimum diurnal temperature was around 15 deg C.

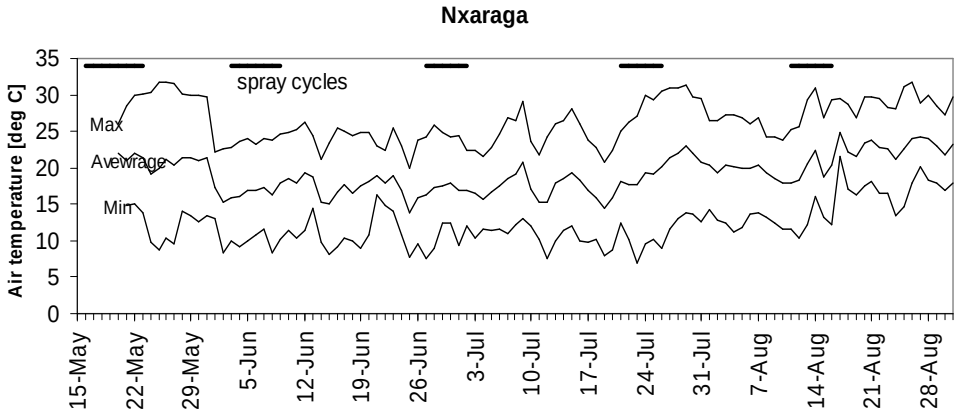

Figure 2.5 Air temperature during the period of spraying campaign, Nxaraxa site

There is some variation in temperature conditions between various sites. Figure 2.6 shows that the wet site at Xakanaxa was in general warmer that the other two sites. Figure 2.7 reveals that the differences between sites are accentuated in the diurnal cycle. Daytime temperatures are generally higher at Xakanaxa (both sites) that those at Nxaraxa. Nighttime temperatures at Xakanaxa dry site are significanly lower than those at the two other sites. The difference in daytime temperature seem to result from regional differences in air temperature - that is why both sites at Xakanaxa, which are close to each other are similar, but different from the distant Nxaraxa. Night temperatures seem to be affected more by local conditions. Wet site at Xakanaxa and Nxaraxa site represent “wet” conditions, where water stores daytime heat and releases it during night. At dry site of Xakanaxa such heat release does not occur and thus the site is characterized by lower night temperatures. The fact that there is night time heat release from water does not, however, affect night temperature inversion, which is necessary for effective spraying. Figure 2.8 shows that strong temperature inversion (reaching 5 degrees over 9 m height difference) was developed during spray cycle 4 at Xakanaxa wet site. Similar conditions occured at Nxaraxa, and during other spray cycles.

It is important to note, that the arrival of annual flood at Nxaraxa that took place on July, 6, did not have any significant influence on temperature.

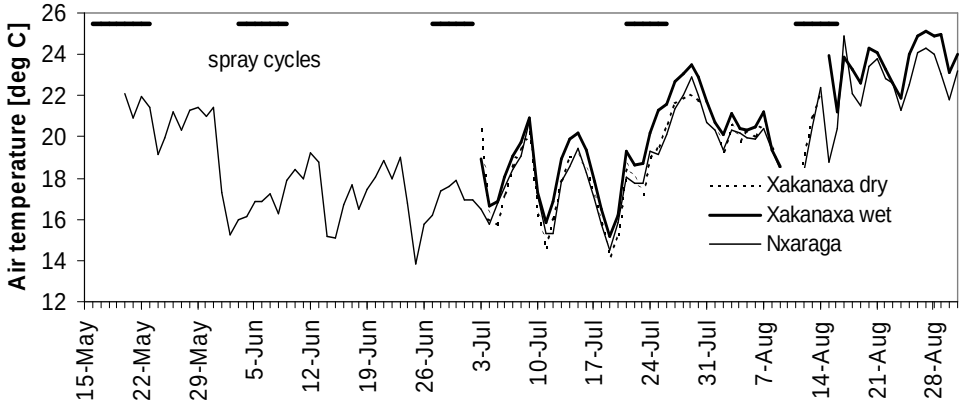

Figure 2.6 Mean diurnal air temperature during the period of spraying campaign, comparison of sites

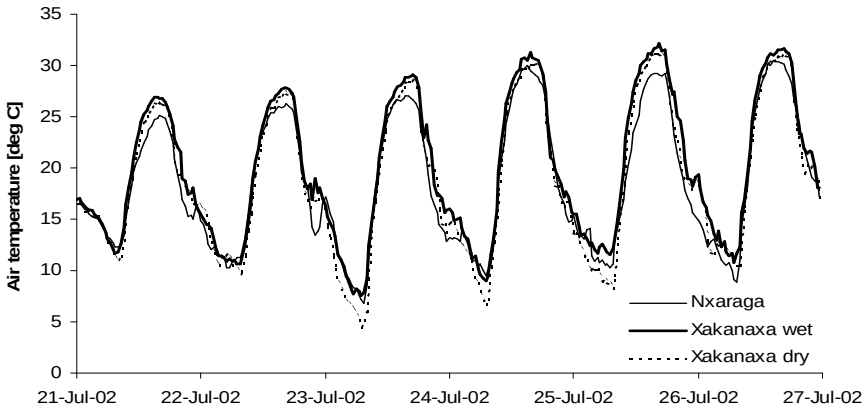

Figure 2.7 Air temperature during the 4th spray cycle, comparison of sites

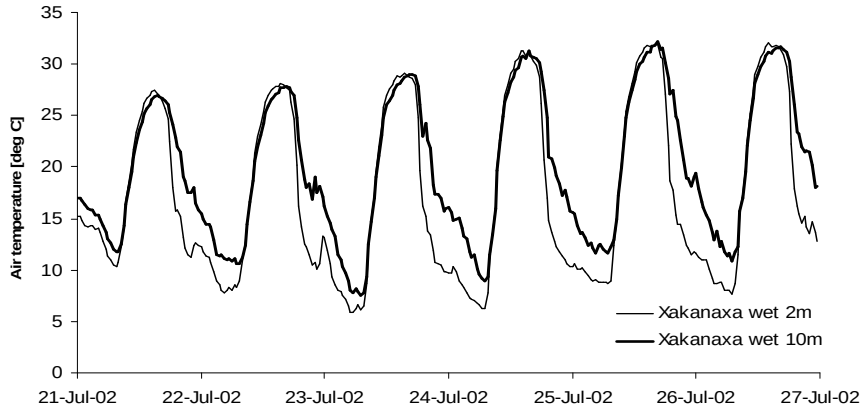

Figure 2.8 Air temperature at various hights, Xakanaxa wet site

2.2.2 Spray deposition

The objectives of the spray deposition research were to determine:-

- presence/absence of spray at the sites where sampling and monitoring of terrestrial and aquatic insects were carried out,
- characteristics of the spray that could affect spray effectiveness (droplets diameter, amount of droplets present).

Due to logistical reasons it was not possible to set spray sampling devices in all the sites used for sampling of insects. In order to obtain information on spray presence and characteristics in the non-sampled sites it was decided to establish a qualitative relationship between spray deposition, distance from flight line and land cover. In practice the following approach was undertaken:

- various spray samplers were set at key insect sampling sites,
- for each spray sampling site GPS position and land cover type was recorded,
- for each of the spray sampling sites the distance of the airplane flight line was calculated using a GIS system,
- relationship between the variables were analysed both qualitatively (visually) and quantitatively (correlation) to check whether relation between land cover and flight line distance and spray deposition exists.

2.2.2.1 Materials and Methods

For the determination of presence of spray and its physical characteristics a physical method of droplet sampling using magnesium oxide-coated slides was applied. The method has an advantage of being easy to use and cheap. The slides were set in three various ways:

- rotating slides
- stationary slides positioned horizontally
- stationary slides positioned vertically

Additionally, on one occasion stretches of wool were hang on trees, which after spraying were collected and send for quantitative analysis for the pesticide.

The rotating slides were 5mm by 25mm in size, while the stationary slides were 24 mm by 40 mm in size. All slides were set up in evenings before spraying and collected either in the following mornings, or during nights, approximately 4 to 6 hours after spraying took place.

Slides were subsequently inspected under a microscope in the following manner:

- for the rotating slides three transects, each 10 mm long, and 1.6 mm wide (the view field of the microscope) were inspected.
- For the stationary slides, three transects of 30 mm in length and 1.6 mm in width were inspected.

All the craters in the magnesium oxide coating noticed in each of the transects were counted and classified according to size classes. Size class was assessed using Porton graticule fitted in the eyepiece of the microscope. Crater sizes were subsequently converted to droplet size classes. Volume Median Diameter (VMD) and Number Median Diameter (NMD) were calculated. To assess droplets density two measures were used: number/cm^2 and volume/cm^2.

2.2.2.2 Results

Spray sampling was conducted at two locations – at Nxaraxa and at Xakanaxa. Logistical difficulties and deficiencies in information flow between spray operator and the monitoring team caused that not all the spray cycles were sampled at both locations. Table below presents schedule of sampling.

Table 2.1 Schedule of spray sampling

Site	Cyle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Nxaraxa	R,H	R,H		R,H	R,H
Xakanaxa	R,H	R,H	R,V	R,H,W	R,H

R – rotating slides
V – vertical slides
H – horizontal slides
W - wool

Comparison of various types of samplers
In general, the rotating samplers were collecting much more droplets than the horizontal stationary ones. Surprising is the fact that the vertical stationary samplers, which were used only on one occasion, collected virtually no droplets. This is probably attributed to the primary vertical movement of spray during windless nights.

Stationary slides in many cases were affected by dew. Although it was possible to detect some craters on the dew-covered slides, droplet counting done on them was by no means comparable to that done on the dry slides. Thus, in practice, dew cover rendered slides uninterpretable. The rotating slides were affected by dew in much lower extent.

Comparison of results from rotating slides and from horizontal stationary slides (Figure 2.9) set at the same sites show that there is very little relationship between them. Thus, those results cannot be interpreted simultaneously. In view of that, only the results from rotating slides are interpreted below.

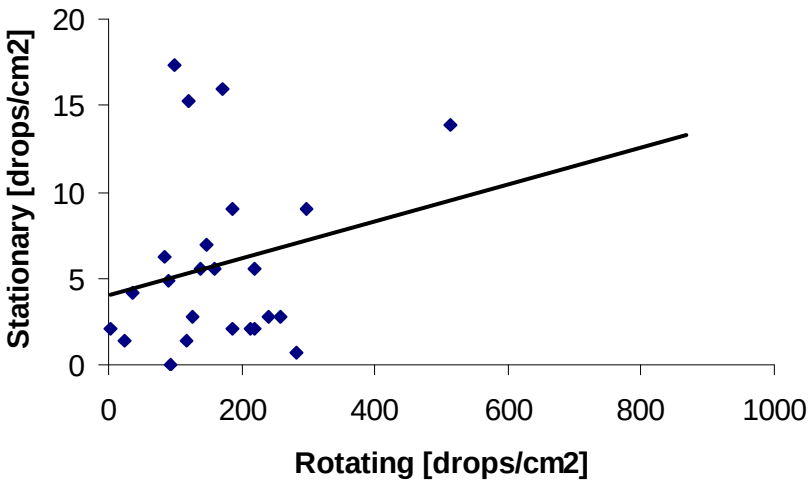

Figure 2.9 Comparison of drop counts on rotating and stationary slides.

Table 2.2 Summary of results from rotating slides

cycle	Location	site	Land cover	Count -	VMD microns	NMD microns	Deposition drops/cm2	Deposition mm3/cm2
1	Nxaraxa	c15	floodplain	50.0	89	52	104	0.028
1	Nxaraxa	c16	riparian	17.0	65	50	35	0.006
1	Nxaraxa	c17	riparian	77.0	55	41	160	0.015
1	Nxaraxa	c18	dry plain	70.0	61	43	146	0.016
1	Nxaraxa	c19	riparian	66.0	54	44	138	0.014
1	Xakanaxa	x0	riparian/floodplain	58.0	64	51	121	0.018
1	Xakanaxa	x11	pool	102.0	61	50	213	0.029
1	Xakanaxa	x3	riparian	48.0	69	47	100	0.015
1	Xakanaxa	x5	dry grassland	48.0	60	49	100	0.013
2	Nxaraxa	c15	floodplain/water edge	11.0	57	48	23	0.003
2	Nxaraxa	c20	riparian	1.0	n.a.	n.a.	2	0.000
2	Nxaraxa	c1	floodplain	105.0	64	44	219	0.027
2	Nxaraxa	c3	floodplain	60.0	51	44	125	0.011
2	Nxaraxa	c4	riparian	43.0	46	38	90	0.006
2	Xakanaxa	x1	dry grassland	246.0	46	35	513	0.027
2	Xakanaxa	x4	pool	136.0	52	37	283	0.021
2	Xakanaxa	x8	riparian/floodplain	102.0	48	38	213	0.014
2	Xakanaxa	x12	floodplain	135.0	51	37	281	0.022
2	Xakanaxa	X11	pool	82.8	50	46	173	0.012
3	Xakanaxa	x5	dry grassland	87.0	49	36	181	0.011
3	Xakanaxa	x3	riparian	113.0	59	38	235	0.022
3	Xakanaxa	x0	riparian/water edge	27.0	48	41	56	0.004
3	Xakanaxa	x12	floodplain	44.0	61	44	92	0.011
3	Xakanaxa	x11	pool	416.0	50	36	867	0.059
4	Nxaraxa	c15	floodplain/water edge	90	54	36	188	0.014
4	Nxaraxa	c1	floodplain	53.0	71	49	110	0.019
4	Nxaraxa	c3	floodplain	135.0	76	43	281	0.049
4	Nxaraxa	c20	riparian	143.0	53	28	298	0.014
4	Xakanaxa	WOOL	short mopane	105.0	69	40	219	0.026
4	Xakanaxa	x4	floodplain	24.0	64	48	50	0.006
4	Xakanaxa	X11	pool	93.0	74	47	194	0.034
4	Xakanaxa	X12	floodplain	50.0	69	48	104	0.017
5	Nxaraxa	c111	dry grassland	70.0	47	36	146	0.009
5	Nxaraxa	c112	dry grassland	56.0	59	39	117	0.011
5	Nxaraxa	c113	dry grassland	89.0	49	39	185	0.014
5	Nxaraxa	c114	dry grassland	115.0	59	44	240	0.026
5	Nxaraxa	c115	dry grassland	45.0	65	45	94	0.012
5	Xakanaxa	x5	dry grassland	124.0	53	32	258	0.015
5	Xakanaxa	x3	riparian	58.0	49	39	121	0.009
5	Xakanaxa	x0	riparian/water edge	40.0	52	45	83	0.008
5	Xakanaxa	x12	floodplain	308.0	41	35	642	0.031
5	Xakanaxa	x11	pool	296.0	45	34	617	0.032

The results above (Table 2.2) indicate that essentially all the sampled sites received a considerable amount of spray. One exception is a riparian site in Nxaraxa (c20, cycle 2), where only 1 droplet (or 2 droplets per cm^2) was recorded on the rotating slides. Also, the deposition recorded at the nearby site (C15, cycle 2) was somewhat lower. However, as the deposition values recorded at that site during other campaigns were comparable to those recorded elsewhere, it seems that the low values recorded there during cycle 2 were exceptional and probably resulted from a combination of reasons. Namely, that site c20 is covered with dense bushy vegetation, localised thermal air movements occurred (very localised differences in air temperature were frequently felt in the field) and the fact that the overall distance from the flight line was relatively large, probably resulted in the small

deposition recorded. Such low recorded values confirm that it is possible that the spray does not reach some points within the spray block, although it's one-time occurrence also indicates that such a situation is exceptional.

Deposition varied considerably between sites - values between 23 and 867 drops/cm^2 were recorded. There is no obvious relationship between land cover and the recorded deposition, apart from the fact that open areas, be it dry or wet, seem to receive more spray than the wooded or bushy areas. However, in some of the cases, particularly at Xakanaxa, it is possible that the floodplain and pool sites were sprayed twice.

Even in the case of open areas there is a considerable variation in spray deposition. During cycle 5, for example, samplers at Nxaraxa were set in a transect perpendicular to the flight line in an area of homogenous dry grassland. Despite such habitat uniformity the observed deposition varied between 94 and 240 drops per cm^2, variation that can only be attributed to differences in the distance of a sampling point from the flightline.

Some variation in values of VMD and NMD was observed, but in general those values did not depart considerably from the values for which spraying nozzles were set (R. Allsop, pers.comm.). This confirms that the monitoring sites were sprayed with the spray of suitable characteristics.

2.2.3 Insecticide Residue Analysis

2.2.3.1 Materials and Methods

To determine the extent of residual effects of an application of 0.3g/ha during cycle 2, an experiment was set up using strands of wool. Lengths of acrylic wool, each approximately 75cm long were exposed to the spray. Over a five day period, the ten strands were collected, noting the time of collection, and stored in hexane for analysis of Deltamethrin by NRI in the United Kingdom. Mike Andrews did similar work in Botswana using angora wool (ISCTRC, 1981 in Allsopp, 2002) – only acrylic wool is available in Botswana. Using his method for converting the residue on the wool, the potential dosages to tsetse fly in ng/fly can be calculated. This then gives and indication of the residue of the spray remaining in the habitat at intervals after the spray event (Table 2.3).

2.2.3.2 Results

The results of Gas Chromotography of the wool strands exposed to deltamethrin over time are shown below.

Table 2.3. Residues measured by GC of wool exposed to 0.3g/ha of Deltamethrin

NRI residue analysis results

Analytical code	Field code	Residue (i)	(ii)	Mean residue,	length of wool
Sample 02 AB1:	Day 1, 9.00	0.27	0.33	0.3	78cm
Sample 02 AB2:	Day 1, 21.00	0.14	0.17	0.16	78cm
Sample 02 AB3:	Day 2, 9.00	0.13	0.15	0.14	78cm
Sample 02 AB4:	Day 2, 21.00	0.1	0.08	0.09	77cm
Sample 02 AB5:	Day 3, 9.00	0.11	0.12	0.12	75.5cm
Sample 02 AB6:	Day 3, 21.00	0.1	0.12	0.11	74.5cm
Sample 02 AB7:	Day 4, 9.00	0.06	0.09	0.08	78cm
Sample 02 AB8:	Day 4, 21.00	0.07	0.08	0.08	78cm
Sample 02 AB9:	Day 5, 9.00	0.05	0.05	0.05	79cm

Table 2.4 Residue analysis converted to dosage in ng/fly

	Recalculated according to Andrews 1981		
	Andrews correction		
Hours Post-spray	residue per m	equivalent to ng/fly*	exceeds LD90 by x
9	0.385	3.846	13.7
21	0.205	2.051	7.3
33	0.179	1.795	6.4
45	0.117	1.169	4.2
57	0.159	1.589	5.7
69	0.148	1.477	5.3
81	0.103	1.026	3.7
93	0.103	1.026	3.7
105	0.063	0.633	2.3

Allsopp (2002) quotes results from the 1970s for wild *Glossina morsitans centralis* that show the LD50 and LD90 for deltamethrin to be 0.092 and 0.282, respectively. Consequently, even allowing for the poor collecting efficiency of the wool there is a strong likelihood that residual effects were experienced four or five days after the spraying of 0.3g/ha during cycle 2 (Table 2.4). As Allsopp (2002) points out this could help to explain the inordinately low numbers of tsetse fly emerging between applications.

2.3 References

Allsopp, R (2002) Report on the 2002 Aerial Spraying Operation to Control Tsetse Flies in Northern Botswana. For Tsetse Control Division, Department of Animal Health and Production, Maun. Botswana.

3. Data Management and Analyses

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3.1 Background

A significant problem with projects involving many specialists is to keep some consistency and standardisation in the analysis and presentation of data. Not all specialists use the same statistical approaches to data analyses or have equivalent experience in statistics, particularly in complex multivariate analyses. This often results in reports that are somewhat inconsistent in their data treatments. Similarly data collected in different sub-projects is rarely collated into one central data source for reliable archiving and access.

The tsetse monitoring project was able to overcome some of these constraints by engaging BioTrack Australia to capture, archive and analyse all data collected during the 2002 Environmental Monitoring of Tsetse fly eradication.

3.2 Data Management

All data from the bird census, freshwater macroinvertebrate, zooplankton and terrestrial invertebrate sampling were entered into the relational database Biota® (Colwell 1996). Biota® was designed specifically for specimen based biodiversity assessments and can be customized to take into account different survey designs. In the current study all sampling sites were designated as locations and given a location code. Any samples taken were then tagged to their appropriate locality.

The samples were given a unique code and a record that included information on the type of sample, when it was collected and by whom. Similarly any specimens identified from each collection were given unique specimen codes that were, in turn, tagged to the collection record. This means that the details for any particular specimen that has been identified can be easily retrieved.

All the terrestrial invertebrate material was also managed with a barcode system. The unique collection and specimen codes were attached to the vials and collection bottles as barcodes. Each collection, for example a pitfall trap, was given a unique barcode (collection code) attached to the trap with a duplicate on a field datasheet. At each stage of the identification process a new barcode was applied and linked in the database to the original collection code. In the case of aquatic invertebrates and birds, where species or Family identification was immediate, only codes for each collection event were required.

All data generated in the 2002 monitoring program, from aquatic invertebrate, terrestrial invertebrate and bird census studies were exported from Biota into standardised Microsoft Excel spreadsheet formats. Matrices of taxa by sample were generated with abundance values in the cells of the matrix. Matrices were provided categorised by taxa, site and method as well as a master matrix of all the data for each taxon.

All these data matrices and the Biota database files were archived to CD—ROM and delivered to the project coordinator on 30th November 2002.

3.3 Data Analyses

The focus of this study was to determine the impact of aerial insecticide application on non-target taxa. Empirical evidence for significant changes in species is difficult to achieve when nothing is known about their natural variation in abundance or distribution. For example, failure to sample a species after a spray event could be the result of behavioural changes that affect catch rates but have no long-term harm to the species.

In addition to this focus on individual taxa statistical hypotheses to test difference in composition between samples were completed. The objective was to assess if samples taken before and after the spray event showed any greater change in their make-up and the relative abundance of taxa than would be expected by chance. This shifts the initial focus onto overall changes in assemblages, which integrate the effects on individual taxa into a measure more relevant at the ecosystem level.

The way to achieve this for samples that contain many different types of organisms (where each type is a variable) is to first simplify the multivariate data using ordination procedures. Clarke & Warwick (1994) describe an ordination as "*a map of the samples, usually in two or three dimensions, in which the placement of the samples reflect the similarity of their biological communities*". So, the distance between two points on an ordination diagram reflects the dissimilarity in assemblage composition. If points are far apart they either share few taxa in common or the same taxa but at very different levels of abundance.

There are many different types of ordination procedure. Here, multi-dimensional scaling (MDS) was used for its simplicity, best use of sample information and general applicability (Clarke & Warwick 1994). MDS ordinations focus on a similarity matrix that is relevant to the question and were computed for both ordinal level data and for morphospecies. First the matrices of taxa by samples were exported from Biota® according to each sampling method and using abundance data. These matrices were imported to PRIMER v5 (Clarke & Gorley, 2001) where the raw data were transformed; similarity matrices computed using Bray-Curtis similarity and non-metric MDS plots produced. These plots display the relative positions of each sample in two dimensions which reflect biological difference.

Another advantage of ordination is that statistical comparisons of *a priori* sample groupings can be made using permutation procedures. These procedures, that use *Monte Carlo* approaches to generate significance levels, test questions about biological distance between samples of a given category. For example, is the grouping of samples taken before a spray event significantly different to those taken after the spray; do they have a different biological composition? The specific procedure used was Analysis of Similarities (ANOSIM), which is a rough multivariate analogue of one-way analysis of variance. The null hypothesis tested is that there are no assemblage differences between groups of samples specified in advance. The interpretation of a significant ANOSIM result is that differences in composition between samples within a group compared to those between groups is less than would be expected by chance. ANOSIM is a non-parametric permutation procedure that makes minimal assumptions about the normality of the data and uses the assemblage relationships between samples as summarised in the ranks of the biotic similarity matrix (Clarke & Gorley, 2001). This makes the test robust to the types of comparisons attempted in this study where the lack of benchmarks made estimates of relative difference the key measure.

If a significant ANOSIM result is obtained, further procedures can identify which taxa primarily account for the observed assemblage difference. The percentage contribution each taxa makes to the average dissimilarity between two groups can be established and listed in decreasing order of importance. This is achieved in the SIMPER routine in PRIMER v5 (Clarke & Gorley, 2001). This makes it possible to identify the taxa that discriminate between two groups of samples.

Data were summarised using standard methods to include analyses of changes in relative abundance and composition of taxa. The focus was in establishing difference in abundance between samples before and after spray events and over the cycles in both higher taxa and morphospecies. These summaries also included species or morphospecies specific comparisons to identify and describe patterns for affected or sensitive taxa.

Data were also analysed using comprehensive multivariate methods, including ordination and permutation test procedures described above. These analyses identified changes in composition that are the result of spray cycles.

The standard sequence of analyses completed for the aquatic macroinvertebrate, zooplankton and terrestrial invertebrate data was:

1. Patterns in abundance of individual taxa using average numbers of speciemens per sample though each cycle
2. Rank-abundance patterns for multi-taxa samples for each cycle
3. Comparisons of taxon richness and diversity between the cycles.
4. Compositional change measurements using non-metric multidimensional scaling and significances tested with analysis of similarities
5. Indicator taxa analyses using the SIMPER procedure

3.4 Data receipt and data summary returns

Raw data for aquatic macroinvertebrate surveys were received from Umgeni Water on 18th October 2002. Worksheets were tidied and analyses completed by 18th November 2002. Results were sent as MS Word and windows metafiles. Copies of all the output from these analyses are available on the data CD-ROM.

Raw data for zooplankton surveys were also received from Umgeni Water on 18th October with additional material supplied on 25th October 2002. Analyses for the zooplankton were returned on 18th November 2002.

Raw data from the bird survey were delayed by several weeks and only received at BioTrack in early November 2002. Data were analysed and returned to the specialist by 15th November 2002.

All MS Excel raw data files, average abundance histograms, average richness histograms, diversity index histograms, rank-abundance plots, non-metric multi-dimensional scaling plots, summaries of ANOSIM and summaries of SIMPER analyses are provided on the accompanying CD-ROM for all the aquatic macroinvertebrate, zooplankton, terrestrial invertebrate and bird survey data.

3.5 References

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Clarke, K.R. and Warwick, R.M. (1994) *Changes in marine communities: an approach to statistical analysis and interpretation.* Hitchings and Mason, Plymouth.

4. Monitoring of Aquatic Invertebrates

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4.1 Introduction

Aerial spraying to control tsetse flies in the Okavango Delta effectively eliminated associated diseases from Botswana between the early 1980s and 1999 (Allsop 2001). However, after a 1999 outbreak of trypanosomiasis in cattle in the north-eastern Delta and potential threats to people, the Botswana Department of Animal Health and Production (DAHP) proposed a phased and integrated campaign of tsetse fly control measures. A programme of aerial spraying with Deltamethrin commenced in 2001 in the northern half of the Delta (Perkins and Ramberg 2002). Deltamethrin was selected as specifically targeting tsetse fly and as having lower levels of general environmental impact (Scott Wilson Consultants 2001). The 2002 spray programme covered the remainder of the Delta (the southern half) and because of the greater lead-time was monitored more intensively. This report documents the results of that monitoring.

Aquatic habitats are exposed during aerial spraying with insecticide, and the diverse aquatic fauna is consequently at risk. The monitoring of aquatic invertebrates was based on the same approach as that followed for the terrestrial invertebrates (Chapter 5). Pre-spray "benchmark" samples were collected, and biological assemblage composition was used to describe the initial state of the study area. Samples were then collected at the same sites before and after spraying in each of the 5 spray cycles. Samples collected after the 5th spray cycle were used to describe the final biological composition state. The effect of the spray is reported as a change from the initial state, taking into account natural changes that would occur over the spray cycle period. Information about the natural seasonal change through the spray cycle was collected at an unsprayed site 30 km downstream of the spray zone. Aquatic habitats in the delta form a complex mosaic in time and space. Therefore the benchmark, initial state, and the effects of the spray were described for a range of sites and habitats.

The aims of the study were therefore to:

1 sample a range of aquatic habitats and sites before the 2002 spray cycle and describe the benchmark initial state of the aquatic biota;
2 sample the same habitats and site through the spray cycles and describe the impact of spray in terms of changes in biotic assemblage structure; and
3 provide an information base from which recovery could be monitored and assessed.

The 2001 pilot study at the Pom-Pom site (Palmer 2002) was used to select methods and approaches, and to provide information on the kind and magnitude of response to Deltamethrin spray that could be expected. The monitoring of the 2001 spray event was less intensive and the results more speculative. Important results from that survey were:

1 there were measurable and observable post-spray effects on aquatic biota as a result of a single Deltamethrin spray event;
2 there was an increase in the number of dead aquatic invertebrates collected;
3 there were measurable changes in community structure in permanent water habitats;

4 burrowing organisms emerged and some were dead when collected;
5 there was a dramatic increase in planktonic drift immediately post-spray; but
6 there was no measurable change in community structure in the shallow seasonal sedge habitat.

Based on these results, lagoon and channel habitats were the focus of attention, drift was intensively sampled, and the numbers of burrowing mayflies were noted. The core sampling procedure of repeated sweep netting was followed as in 2001.

The selection of aquatic sampling sites for the 2002 monitoring was difficult and Prof. Ian Grant, from the Natural Resources Institute in the UK, was the primary advisor. The Xakanaxa site was selected as it has permanent water and may have been expected to have a high natural diversity. Lagoon and channel margin habitats were sampled. There was also the opportunity of sampling a downstream, unsprayed site. However it was noted that the unsprayed site was vulnerable to pesticide contamination from the upstream spray zone. The other disadvantages of the Xakanaxa site were that it is on the periphery of the spray zone (Figure 5.1) and that the shallow seasonal habitat of seasonally inundated sedges and grasses, so characteristic of much of the delta, was minimally represented.

In order to counteract these disadvantages two sites deeper in the spray zone (Bodumatau Bund and Third Bridge) were added to the Xakanaxa sampling regime, and shallow seasonal habitats were sampled widely after the 5th spray cycle.

In order to meet the aim of providing a good basis for recovery monitoring, and in order to maximise on the value of the investment made in the 2001 experimental spray monitoring, a more widespread final state sampling was undertaken. This included sites on the Khwai system that had not been sampled during each spray cycle (termed the “Northern Zone“ site), a repeated sampling of the 2001 Pom-Pom sites, and samples from aquatic habitats adjacent to the terrestrial site at Nxaraxa. These samples will be most useful in reporting on longer-term post-spray changes.

In this study 625 macroinvertebrate sweep net samples were collected from Xakanaxa, Third Bridge, and Bodumatau Bund; 70 additional samples were collected from Pom-Pom, Nxaraxa, and the Northern Zone; 200 drift and trawl samples were collected and a total of 64 macroinvertebrate taxa (mainly families) were identified. The identification of aquatic invertebrate taxa to family, rather than to morphospecies, is the greatest difference between the aquatic and terrestrial approaches. Identification to morphospecies undoubtedly provides a greater degree of understanding of the effects of deltamethrin spraying. In this report the coarse level of identification limits the level of data interpretation.

4.2 Study sites

The locations of the full range of sampling sites is shown in Figure 4.1 with the detailed location of the sample sites within the 2002 spray zone (Xakanaxa, Third Bridge and Bodumatau Bund) shown in Figure 4.2.

4.2.1 Xakanaxa

Khwai Main Channel: The KB 1 site (Khwai main channel immediately outside Xakanaxa camp - GR’s S 19°11’ 20.2”, E 23°25’ 40.3’’) was the d rift net sampling site.

Khwai South Branch: The KSB 1 site (Khwai south branch at Paradise Pools - GR’s S 19°11’ 49.8”, E 23°27’ 41.5”) was surveyed daily. Lagoon and channel habitats were sweep-net sampled, and the pool was trawled for zooplankton.

The channel habitat was approximately 2m wide and 1-2m deep, lined by densely packed grass (principally *Miscanthus junceus*) and some sedge stems, forming an almost impenetrable wall of vegetation defining the channel. Samples were collected randomly over

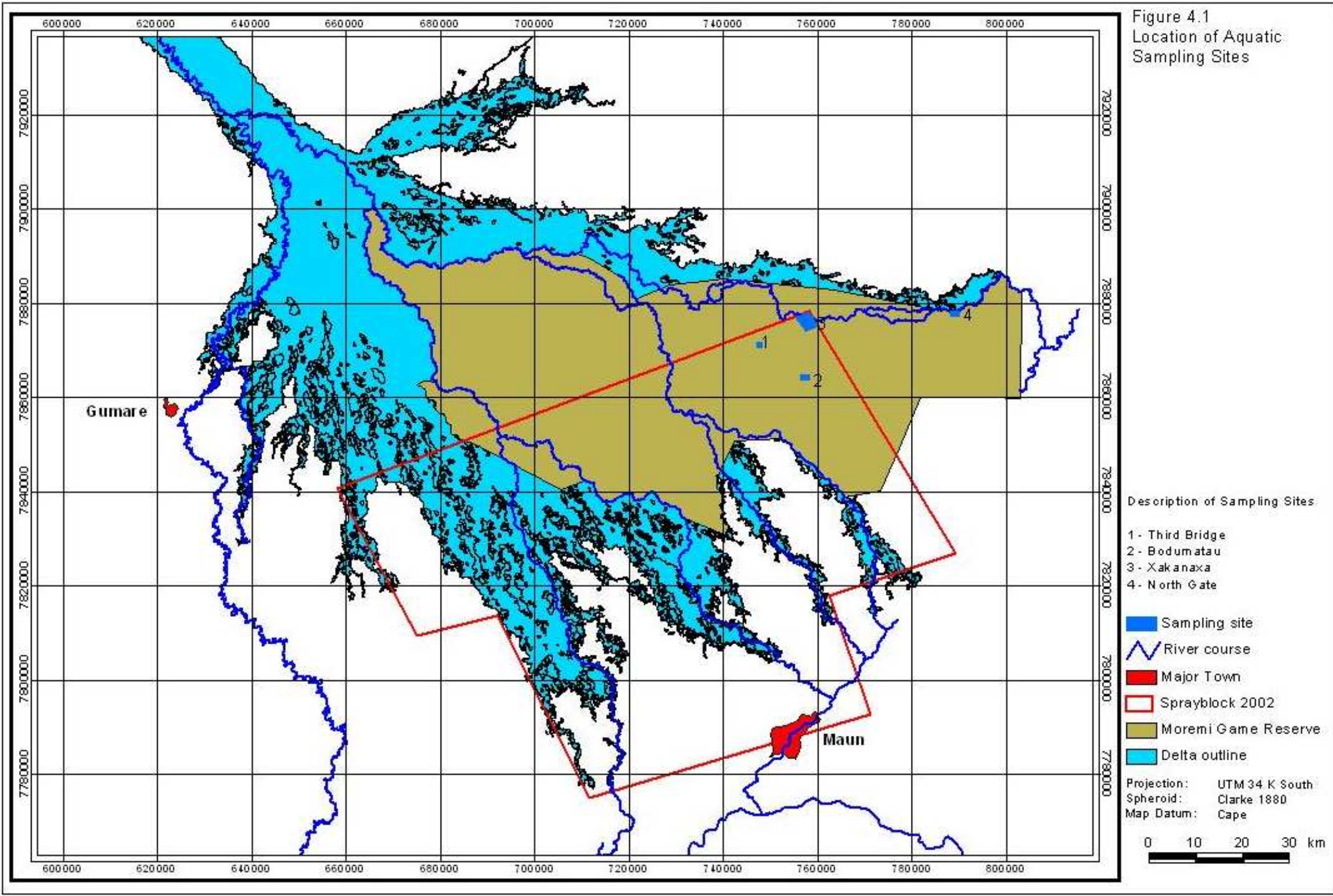


Figure 4.1
Location of Aquatic Sampling Sites

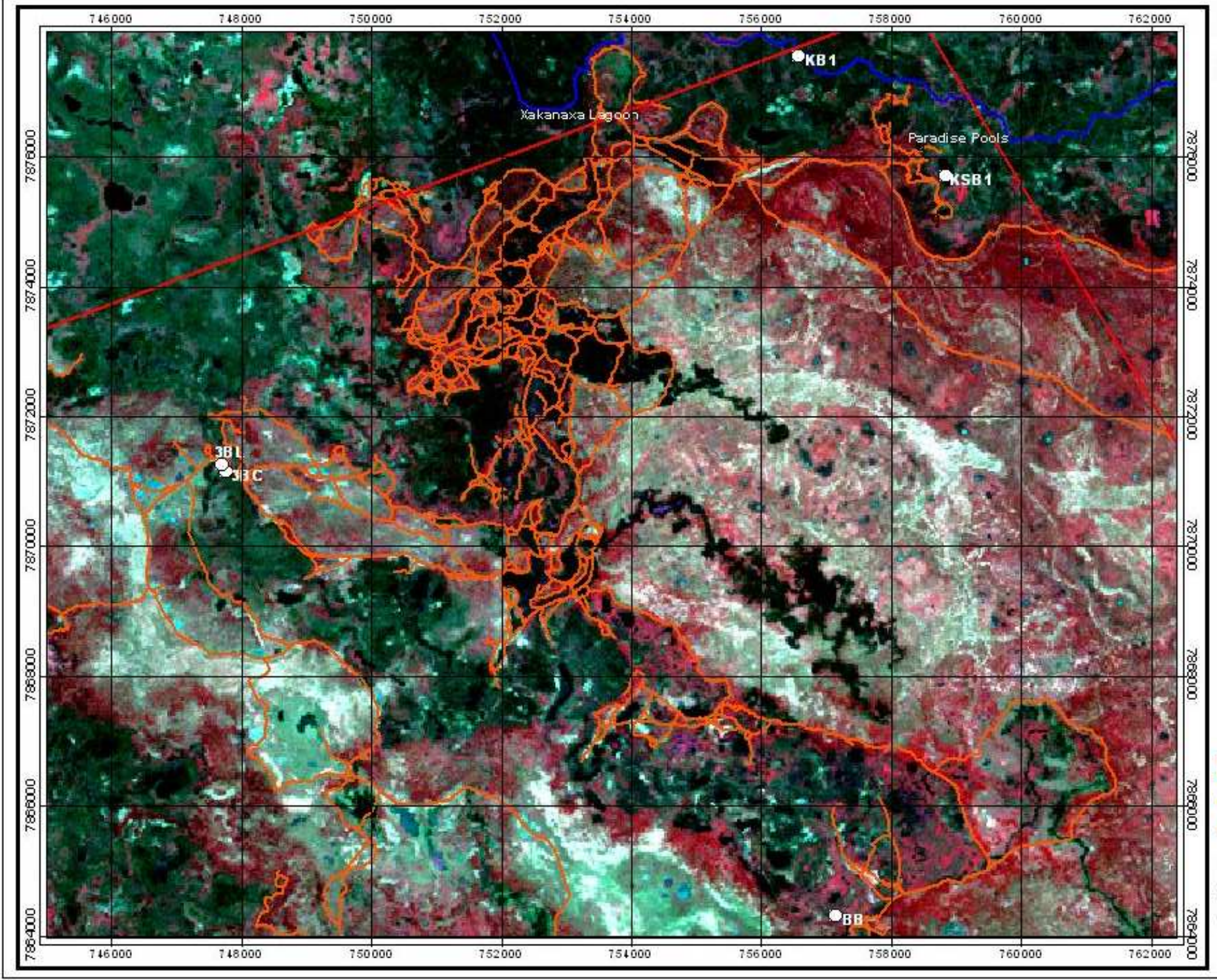


Figure 4.2
Detailed Location of Aquatic Sampling Sites in the Spray Zone

Sampling site
Sprayblock 2002
River course
Track

Projection: UTM 34 K South
Spheroid: Clarke 1880
Map Datum: Cape

0 1000 2000 3000 m

a 100m length of channel. Loose and floating aquatic vegetation including *Salvinia molesta* (Kariba Weed) was caught up against the channel wall particularly in back water sections where the currents were slower. The channel floor was Kalahari sand with very little accumulation of organic rich sediment.

The lagoon was an area 30 X 80 m, <1m deep, with isolated patches of emergent sedges with interspersed water lilies. The Kalahari sand bottom was covered in a layer of organic rich sediment (detritus).

The pool was approximately 200m in diameter and up to 2m deep, surrounded by vegetated wetland with occasional islands.
The water was turbid, due to hippopotamus and fish activity, and the bottom was covered with a thick layer of soft, anoxic organic detritus. Occasional water lilies reached to the surface, particularly on the shallower margins.

4.2.2 Bodumatau Bund

Bodumatau Bund (GR's S 19°18' 0.1", E 23°26' 48. 6") was surveyed once before and after each spray cycle. The site was south west of Xakanaxa, well within the 2002 spray zone. The river channel was narrow in a wider landscape of seasonal swamp inundated by surface-flow and channel seepage with the arrival of floodwaters. Channel margin habitat was sampled.

4.2.3 Third Bridge

This site was south of Xakanaxa (GR. S 19°14' 23", E 23°21' 24"),was well within the 2002 spray zone and was sampled once before and after each spray cycle. Channel habitat was sampled along a clearly defined strongly flowing main channel (2-3m wide by 1.5m deep) and a more diffuse, sedge lined, marginal zone. Dominant marginal vegetation was *Cyperus papyrus* and shorter sedges. The main channel was swift flowing over Kalahari sands with very little organic sediment accumulated. Lagoon habitat was sampled at 3rd Bridge adjacent to the water tank/tower just upstream of the bridge within the campsite (GR. S 19°14' 19.3", E 23°21' 22").

4.2.4 Khwai Bridge (North Gate)

This "control" site (GR's S 19°11' 49.8", E 23°2 7' 41.5") was surveyed once before and after each spray cycle. The site provided samples from outside the spray zone (although its downstream location meant spray contamination was possible) (Figure 4.1). The site comprised a different habitat mosaic from Xakanaxa, Bodumatau Bund and Third Bridge and was used mainly to provide information on natural seasonal patterns in invertebrate community structure.

4.3 Methods

4.3.1 Macroinvertebrate sweep net collection

Lagoon, channel margin and shallow seasonal habitats were sampled using a standard SASS net (300x300mm net, pore size 1mm, Chutter 1998). Each sample comprised the contents of either 10 "scoops" or 3-5 "sweeps" of the net over a distance of 5m. After each scoop or sweep the invertebrates were washed down and concentrated into the pit of the net. The contents of the sample net were tipped into a labelled zip seal plastic bag with approximately 1 litre of water. The net was reversed and flushed clean with water to clean it between samples. In lagoon habitats patches of submerged vegetation, particularly water lilies, were selected. In each habitat, on each sampling occasion, 3-5 replicate samples were collected.

4.3.2 Drift net sampling (Macroinvertebrates and Zooplankton)

Drift nets (300X300mm square aperture, 1000µm (macroinvertebrates) and 80µm (zooplankton) pore size) were staked into the substrate, in a channel current, with the top of the net at the water surface for 3 hours (5.30pm to 8.30pm).

Because large amounts of organic detritus were collected in initial zooplankton samples, from Cycle 3 onwards a separating method was employed. Samples were flushed into a bucket of water, stirred and then left to settle overnight during which some/most of the zooplankton swim upwards. Before sunrise the supernatant was decanted through 80µm mesh and preserved in 4-5% formalin. The drift macroinvertebrate samples were collected and cooled overnight for sorting and identification the following morning.

4.3.3 Zooplankton trawl net

Trawl zooplankton sampling was undertaken in open water, in Paradise Pools at Xakanaxa. An 80µm mesh net was dropped into the water, the boat continued in a forward curve, the net was then pulled directly towards the boat, for 30m, just below the water surface, through water that had not been disturbed by the engine. Two replicates were collected on each occasion. Samples were preserved in 4% formalin.

4.3.4 Processing of field-collected samples

After collection, samples were kept cool in a refrigerator. As soon as possible samples were sorted by picking invertebrates out of the debris with pipettes and/or forceps. A maximum of three quarters of an hour was spent sorting and extracting invertebrates on each sample. Discarded vegetation/debris material was retained on a parallel sorting tray to collect invertebrates that might be hiding. Approximately 10 minutes were spent sorting through this debris tray after sorting the main tray. Quality control was a scan of both trays (sorted and debris tray) by a third party after sorting to determine if any obvious families have been missed before discarding the sample. Samples were identified to family in the field using Gerber and Gabriel (2002) and Davies and Day (1998). All invertebrates were preserved in 75% ethanol.

Zooplankton samples were maintained in formalin for delivery to Prof. Rob Hart in Pietermaritzburg, South Africa, who analysed the contents. Initial inspection revealed that the samples collected in the first two cycles contained excessive amounts of organic detritus that made detection of the zooplankton impossible. The sample procedure introduced from Cycle 3 improved the quality of the samples. Samples were split and a sub-sample counted.

4.3.5 Data notation

Aquatic macroinvertebrate and zooplankton data from each of the five spraying cycles were captured within a spreadsheet environment. Monitoring regime and spray dates for respective locations for each of the five cycles was as presented in Table 4.1.

Table 4.1: Aquatic biomonitoring schedule and spray dates at respective sampling localities for 2002 Tsetse spray operations

	Aquatic biomonitoring		**Spray Evening Dates at:**		
	Start	**End**	**Xakanaxa (KS & KB)**	**Third Bridge (3B)**	**Bodumatau Bund (BB)**
Cycle 1	19-May-02	24-May-02	22-May-02	21-May-02	21-May-02
Cycle 2	07-Jun-02	13-Jun-02	09-Jun-02	08-Jun-02	08-Jun-02
Cycle 3	29-Jun-02	07-Jul-02	02-Jul-02	01-Jul-02	01-Jul-02
Cycle 4	23-Jul-02	30-Jul-02	25-Jul-02	24-Jul-02	24-Jul-02
Cycle 5	10-Aug-02	20-Aug-02	14-Aug-02	13-Aug-02	13-Aug-02

For data analysis the various sampling localities were coded as follows:

KS	Khwai South Branch (lagoon & channel at Paradise Pools, Xakanaxa)
KB	Khwai Branch/Main Channel (drift nets only)
BB	Bodumatau Bund
3B	Third Bridge
NG	North Gate

Mainly Lagoon (L) and Channel (C) habitats were sampled.

There were four distinct sample types, separated into two main biological groupings:

Macroinvertebrate	**Zooplankton**
Macroinvertebrate sweep net (SASS)	Trawl
Drift Net	Drift Net

In the text, the "initial state" refers to samples collected before the first spray event, and the "final state" refers to samples collected after the 5th spray event.

4.3.6 Data analysis

Data were interrogated using multivariate analysis, which uses the presence, absence and abundance of organisms to group samples together. Analyses were undertaken by Biotrack Australia using PRIMER software, and by the Institute for Water Research using Minitab 12.2 software. Ordination analysis was by Multi-dimensional Scaling (MDS) or Principle Components Analysis (PCA), and results are presented as ordination diagrams showing samples grouped in a 2-dimensional ordination space. Samples with more similar composition are closer together. Statistical similarity was indicated by significant R statistics using ANOSIM (Analysis of Similarities) and SIMPER (Similarity Percentages).

4.4 Results

The family is a very coarse level of identification at which to describe biological effects, although it is routinely used in the South African river health programme (Uys et al. 1996, WRC 2002). In the Okavango, aquatic invertebrate fauna are distributed across the delta in a complex mosaic of associations in time and space (AquaRAP 2000, Palmer 2002). In this study, particular assemblages of organisms were characteristic of recognisable habitats, sites, and times of the year. However these assemblages were generally characterised more by different proportions and abundances of organisms than by the presence or absence of particular families.

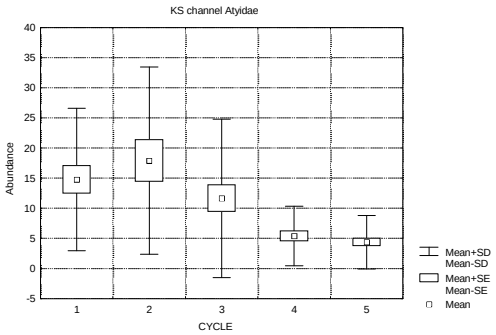
A total of 65 taxa were identified and 95% of the numbers of organisms collected were sampled from either lagoon or channel habitat. In each of those habitats the 10 most common taxa accounted for 99% of the numbers collected. This means that most of the organisms present are there in low numbers and the presence or absence of a family in a sample could be related to the chance of collecting or missing it, rather than to a spray effect. Since it is more likely that a morphospecies would respond to the effects of spraying than a whole family, care must therefore be taken in the interpretation of the data.

The results of the zooplankton analyses are not presented as they were less conclusive than those of the macroinvertebrate drift and sweep samples, and did not reveal any additional information. The results presented are mainly from lagoon and channel habitat samples at Xakanaxa as most samples were collected there. The Xakanaxa trends were checked against the samples from Third Bridge and Bodumatau Bund, and any additional information revealed was noted. Additional samples collected after Spray cycle 5 are not presented, except for an analysis of samples from Naraxa which were used to demonstrate the composition of shallow seasonal habitats. Those samples will be used in any recovery monitoring study that follows.

4.4.1 Spray effects

Figure 4.3 shows a variable response by some of the most common families in Xakanaxa lagoons and channels through the spray cycles.

A



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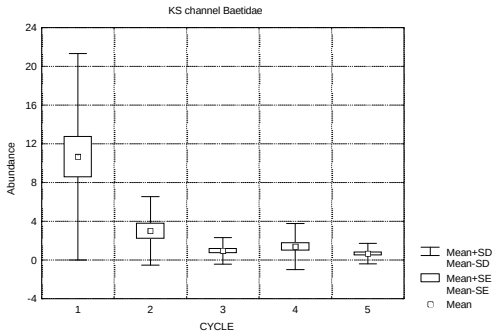
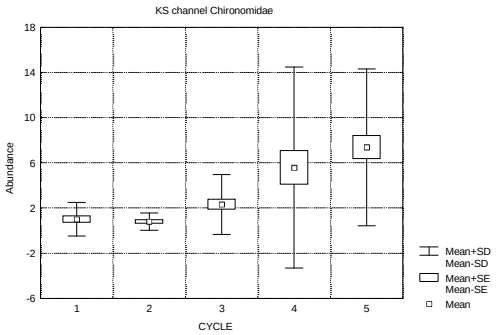


Figure 4.3. Graphs showing changes in aquatic invertebrate abundances in Xakanaxa channels and lagoons, as well as the Shannon index of diversity, through the period of the spray cycles. Channels:

A. Shrimps (Atyidae) increased slightly after Cycle 1, and then decreased over the spray cycles, with variability decreasing after Cycle 3.
B. Baetid mayflies (Baetidae) decreased sharply after Cycle 1, and then remained at a low abundance.
C. Midge (Chironomidae) numbers increased through the spray cycles, possibly because of a decrease in predation, or emergences after spray events or resistance to the spray.
D. The damselfly (Coenagrionidae) response was similar to the shrimps (A.), increasing slightly and then decreasing.

C



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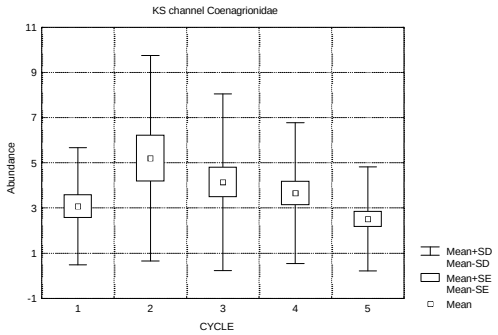

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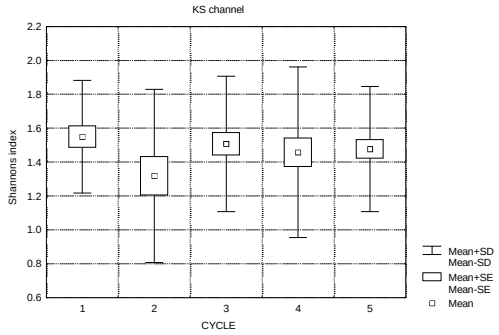

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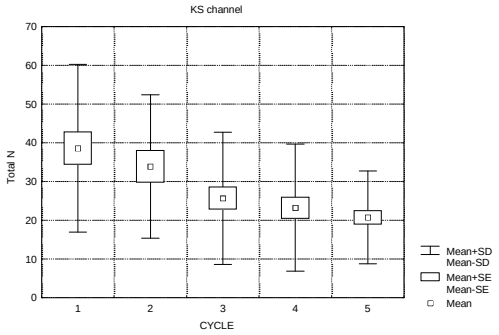

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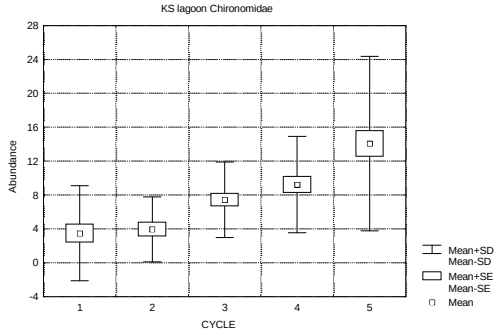

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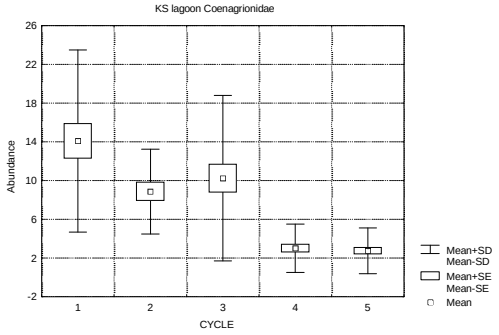


Figure 4.3. continued… Graphs showing changes in aquatic invertebrate abundances in Xakanaxa channels and lagoons, as well as the Shannon index of diversity, through the period of the spray cycles.
Channels:
E. The Shannon index of biodiversity remained similar throughout the spray period.
F. Total numbers of invertebrates in channels decreased through the spray period.
Lagoons:
G. Midges (Chironomidae) also increased in the lagoons.
H. Damselfly numbers decreased more sharply in lagoons than in channels, but mainly after Cycle 3.

Lagoons:
I. Dragonfly (Libellulidae) numbers were variable but not linked to the spray cycles.
J. The total number of pond snails (Lymnaedae) increased in Cycle 5, but numbers per sample were more variable.

I

J

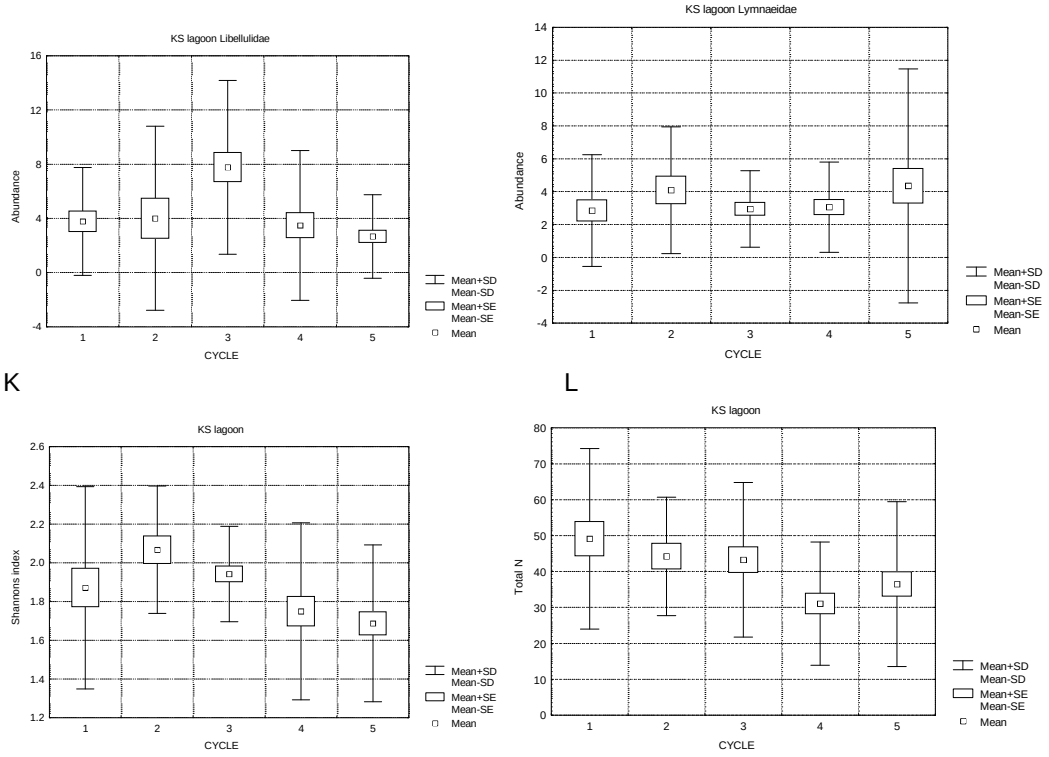
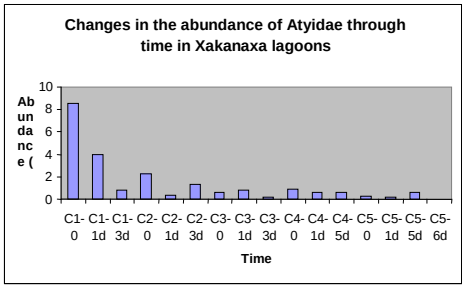
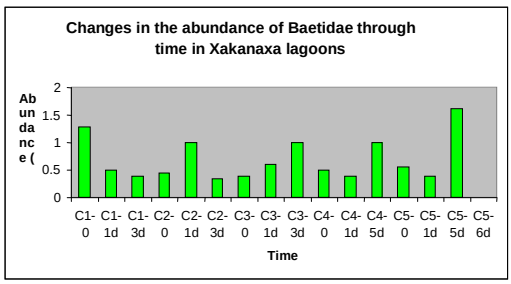
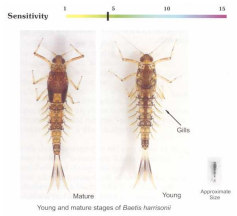


Figure 4.3. continued… Graphs showing changes in aquatic invertebrate abundances in Xakanaxa channels and lagoons, as well as the Shannon index of diversity, through the period of the spray cycles. Lagoons:
K. The Shannon index of biodiversity did not follow a clearly linked pattern with the spray cycles.
L. Total aquatic invertebrate numbers showed a slight decrease over the spray period.

Two of the clearest patterns were of shrimps (Atyidae) and baetid mayflies (Baetidae) in the channels, with shrimps showing a steady decline and the mayflies showing an inter-cycle recovery (Figure 4.4).







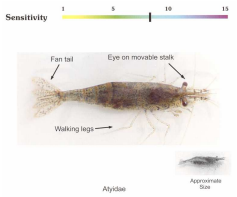


Figure 4.4 Shrimps (Atyidae) and baetid mayflies (Baetidae) in Xakanaxa channels

Tables 4.2 and 4.3. show detailed information on changes in numbers through the spray cycles.

Table 4.2 Changes in numbers in Xakanaxa channel fauna

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Atyidae	Shrimps	384	358	408	189	197
Baetidae	Baetid mayflies	277	60	33	48	29
Coenagrionidae	Damselflies	80	104	145	128	113
Caenidae	Caenid mayflies	42	13	16	25	24
Leptoceridae	Cased caddisflies	36	33	44	52	32
Veliidae	Water striders	28	14	0	0	1
Chironomidae	Midges	26	16	81	195	332
Naucoridae	Creeping water bugs	20	11	9	2	4
Libellulidae	Dragon flies	19	4	29	27	36
Heptageniidae	Flathead mayflies	13	10	2	0	0
Pleidae	Pigmy backswimmers	12	3	5	1	1
Conchostraca	Clam shrimps	8	0	14	4	4
Tricorythidae	Stout crawler mayflies	8	0	0	0	0
Lymnaeidae	Pond snails	5	2	20	17	15
Leptophlebiidae	Prong-gill mayflies	4	15	16	10	3
Nepidae	Water scorpions	4	0	1	2	6
Planorbinae	Orb snails	3	3	18	20	35
Hydracarina	Water mites	3	1	6	16	7
Ceratopgonidae	Biting midges	3	1	3	23	37
Polymitarcyidae	Pale burrowing mayflies	3	1	0	6	1
Hydropsychidae	Net spinning caddisflies	3	0	0	0	0
Corduliidae	Longlegged dragon flies	2	5	1	0	0
Bulininae	Bilharzia snails	2	3	0	13	20
Noteridae	Creeping water beetles	2	1	2	0	0
Aeshnidae	Swimming dragonflies	2	0	2	1	0
Gyrinidae	Whirligig beetles	2	0	1	0	1
Potamonautidae	Crabs	2	0	0	0	0
Lestidae	Slim damselflies	2	0	0	0	0
Gerridae	Pond skaters	2	0	0	1	0
Culicidae	Mosquito larvae	2	0	0	0	1
Hirudinea	Leeches	1	1	14	16	7
Tabanidae	Horsefly larvae	1	1	1	0	1
Nematomorpha	Horsehair worm	1	0	0	11	0
Hydroptilidae	Cased caddisflies	0	11	0	0	0
Pyralidae	Butterfly larvae	0	3	0	0	1
Ecnomidae	Caseless caddisflies	0	2	1	1	0
Chlorocyphidae	Damselflies	0	0	5	0	0
Physidae	Pouch snails	0	0	5	0	0
Dytiscidae	Predatory diving beetles	0	0	4	0	8
Platycnemidae	Damselflies	0	0	3	1	0
Oligochaeta	Water earthworms	0	0	2	0	1
Protoneuridae	Damselflies	0	0	2	0	0
Polycentropodidae	Caseless caddisflies	0	0	2	0	2
Helodidae	Small beetles	0	0	2	1	0
Corixidae	Water boatmen	0	0	1	0	3
Gomphidae	Burrowing dragonflies	0	0	0	1	5
Simuliidae	Blackflies	0	0	0	0	4

Table 4.3 Changes in numbers in Xakanaxa lagoon fauna

		Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5
Coenagrionidae	Damselflies	380	186	358	105	123
Atyidae	Shrimps	141	46	25	28	11
Libellulidae	Dragonflies	102	84	272	122	120
Chironomidae	Midges	94	83	260	323	633
Pleidae	Pigmy backswimmers	93	19	7	2	2
Lymnaeidae	Pond snails	77	86	103	107	196
Dytiscidae	Predatory diving beetles	74	26	7	5	10
Noteridae	Creeping water beetles	68	3	3	1	0
Naucoridae	Creeping water bugs	49	3	0	0	1
Hydracarina	Water mites	40	25	36	41	48
Conchostraca	Clam shrimps	37	4	101	20	50
Baetidae	Baetid mayflies	25	12	21	19	36
Caenidae	Caenid mayflies	20	49	79	123	115
Aeshnidae	Swimming dragonflies	17	2	4	0	0
Polymitarcyidae	Pale burrowing mayflies	16	20	15	13	4
Notonectidae	Back swimmers	15	0	1	0	0
Bulininae	Bilharzia snails	13	36	0	35	49
Veliidae	Water striders	9	1	0	1	0
Planorbinae	Orb snails	8	53	53	38	81
Hirudinea	Leeches	8	10	13	11	19
Sphaeriidae	Pill clams	5	19	6	15	29
Nepidae	Water scorpions	5	3	1	4	3
Culicidae	Mosquito larvae	5	3	0	0	1
Belostomatidae	Giant water bugs	5	2	3	0	1
Ceratopgonidae	Biting midges	4	1	12	22	81
Lestidae	Slim damselflies	3	1	4	0	0
Tabanidae	Horsefly larvae	2	5	2	1	0
Gerridae	Pond skaters	2	1	0	0	0
Thiaridae	Snails	2	0	1	0	0
Nematomorpha	Horsehair worm	2	0	0	14	0
Leptoceridae	Cased caddisflies	1	3	13	13	5
Oligochaeta	Water earthworms	1	1	21	1	13
Corduliidae	Longlegged dragon flies	0	113	6	4	0
Helodidae	Small beetles	0	7	0	2	1
Pyralidae	Butterfly larvae	0	5	5	3	6
Elmidae/Dryopidae	Riffle beetles	0	5	0	0	1
Hydrophilidae	Caseless caddisflies	0	4	0	2	0
Corixidae	Water boatmen	0	2	0	2	1
Haliplidae	Beetles	0	2	0	0	0
Physidae	Pouch snails	0	1	59	2	0
Platycnemidae	Damselflies	0	1	11	4	0
Gomphidae	Burrowing dragonflies	0	1	0	0	0
Ampullariidae	Apple snails	0	1	0	0	0
Leptophlebiidae	Prong-gill mayflies	0	0	4	5	0
Ecnomidae	Caseless caddisflies	0	0	3	0	0
Chlorocyphidae	Damselflies	0	0	2	0	0
Hydroptilidae	Cased caddisflies	0	0	1	0	0
Simuliidae	Blackflies	0	0	1	0	0
Potamonautidae	Crabs	0	0	0	0	1

It is evident that the numbers of some taxa increased, some decreased and some were relatively unchanged. There were taxa that "disappeared" over the spray cycles. However, identification has been to family and rare families are not likely to be effective indicators of spray effects. Table 4.4 indicates the more abundant families that were most affected, and should be the focus of a recovery survey. Subsequent studies could focus on changes at the morphospecies level.

Table 4.4 Key indicators of spray effects

Nature of change	**Taxa**
Restricted to channels and disappeared by Cycle 5.	Flathead mayflies (Heptageniidae) Stout crawler mayflies (Tricorythidae)
Restricted to lagoons and reduced to single individuals.	Creeping water beetles (Noteridae)
Abundant in lagoons and channels. Reduced totally or to sampling of single individuals.	Water Striders (Veliidae) Creeping water bugs (Naucoridae) Pygmy backswimmers (Pleidae)
Dominant taxa that remained common, but were reduced in numbers in lagoons and channels.	Shrimps (Atyidae) Damselflies (Coenagrionidae)
Abundant and affected by spray but morphospecies identification required to discern pattern.	Baetid mayflies (Baetidae)

Results from Bodumatau Bund and Third Bridge provided no extra information, except that creeping water beetles (Noteridae) were found in Bodumatau Bund channels and were less affected than at Xakanaxa (it may be a different species), and baetid mayflies were greatly reduced in numbers after Cycle 1 at Bodumatau Bund, but less affected at Third Bridge. Xakanaxa baetid mayflies showed interesting patterns (Figure 4.4) and undoubtedly the family comprises several species. The family would be a focus of any future study where identification was to morphospecies.

The nature of the effect of deltamethrin spray on aquatic invertebrates is best illustrated by considering the integrated assemblage changes in lagoon and channel habitats (Figure 4.5).

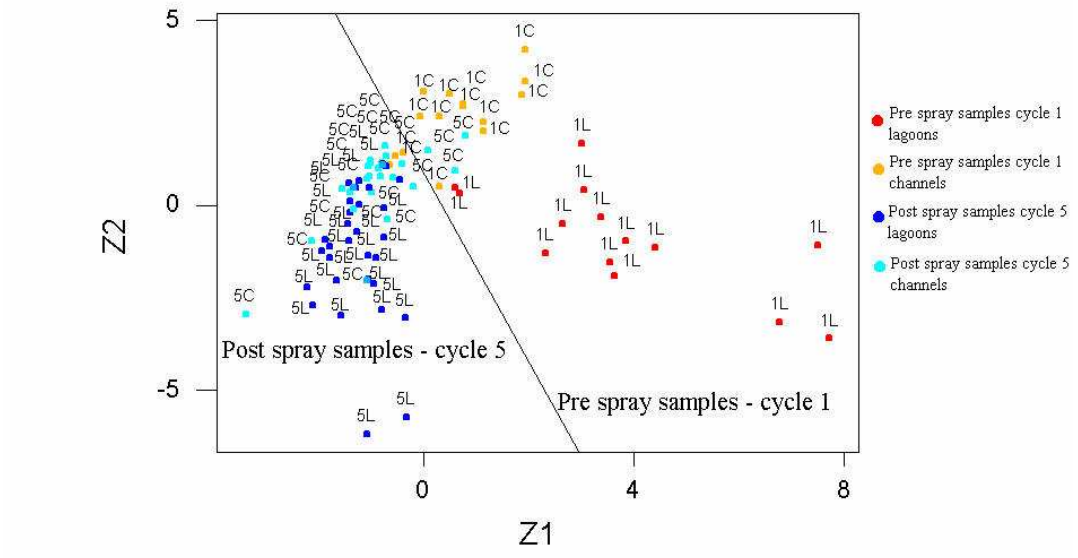


Figure 4.5 PCA ordination of initial and final state abundances in Xakanaxa lagoons and channels. 1-1 pre spray samples, Cycle 1; 5-post spray samples, Cycle 5; C= channel; L= lagoon

Figure 4.5 shows a comparison of the initial and final states. Distinctive channel and lagoon fauna are evident on the right hand side of the ordination diagram, and were characteristic of the pre-spray or initial condition. On the left hand side of the ordination diagram, the post spray samples collected during Cycle 5 from lagoon and channel habitats are much more tightly clustered. There is no spatial separation of samples from the two habitats in the final condition (samples collected after the spray in Cycle 5). It is also clear that the composition of the lagoon had changed more than the composition of the channels. This is indicative of a post spray effect where the numbers of abundant organisms were reduced, and organisms that characterised each habitat (e.g. flathead and stout crawler mayflies on channels and creeping water beetles in lagoons) have either disappeared or become very rare (Table 4.4). The final state fauna comprised more common, resistant fauna.

A further indication of the cumulative impact of spraying on aquatic ecosystem dynamics was visible in results obtained from drift net sampling – specifically for the freshwater shrimps Atyidae (*Caridina* spp) and Leptocerid cased caddis flies from the Khwai main channel site (KB) before and after Deltamethrin spraying (Figure 4.6).

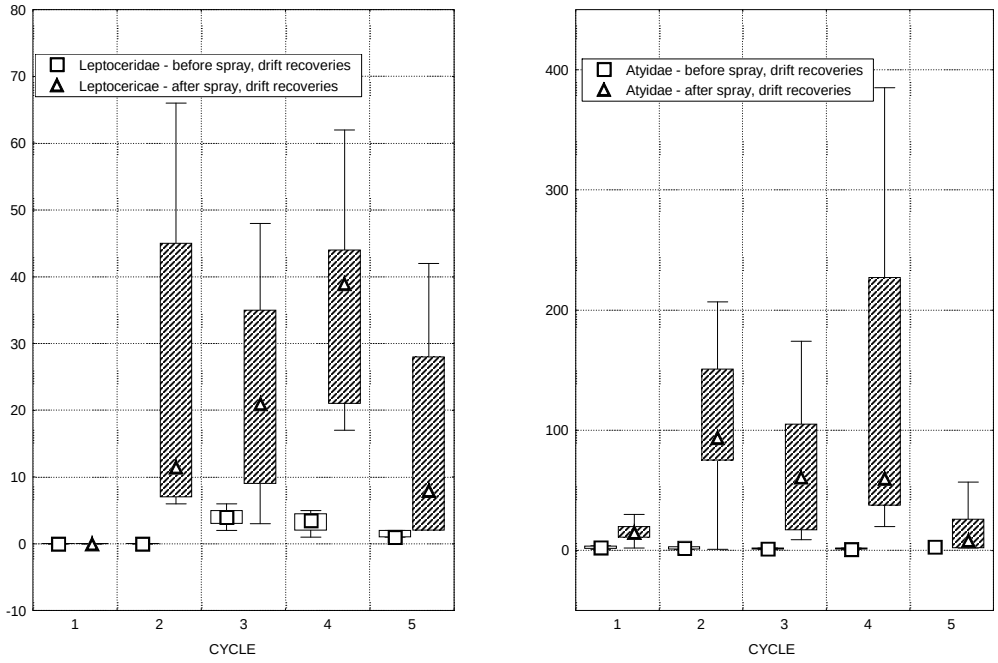


Figure 4.6 Drift net recoveries of fresh water shrimps (Atyidae) and cased caddis fly larvae (Leptoceridae) from the Xakanaxa Khwai River main channel site (KB) before and after Deltamethrin spraying in each spray cycle.

Figure 4.6 shows a clear aquatic macroinvertebrate response to spraying. In all cycles there was a significant increase in the number of shrimps (Atyidae), and larvae of cased caddis flies (Leptoceridae), caught in the drift nets after the spray events. Respectively this represents an approximately 3000% and 660% increase over the "normal" drift of these families within the sample area. It was not possible to compare this response with drift at the unsprayed North Gate site, as shrimps and caddis larvae were never caught in drift nets at that site.

Key Results – spray effects
•1 Key sensitive taxa were: flathead mayflies, stout crawler mayflies, creeping water beetles, water striders, creeping water bugs, pygmy backswimmers, shrimps, coenagrionid damselflies and baetid mayflies. •2 After 5 spray cycles the fauna of lagoons and channel habitats had become more similar, and sensitive taxa were missing or greatly reduced in each habitat.

4.4.2 Patterns of aquatic invertebrate biota in the Okavango delta.

The aim of this section is to provide a description of the patterns of distribution of aquatic biota in time and space in the delta, as this provides essential evidence for the final conclusion: that although there are measurable effects from the spray, they are unlikely to affect the functioning of the ecosystem.

4.4.2.1 Aquatic invertebrates from different habitats

There was a clear, statistically significant (Table 4.5) difference in community composition between the biota of lagoons and channels. This is shown specifically for the Xakanaxa site in Figure 4.7.

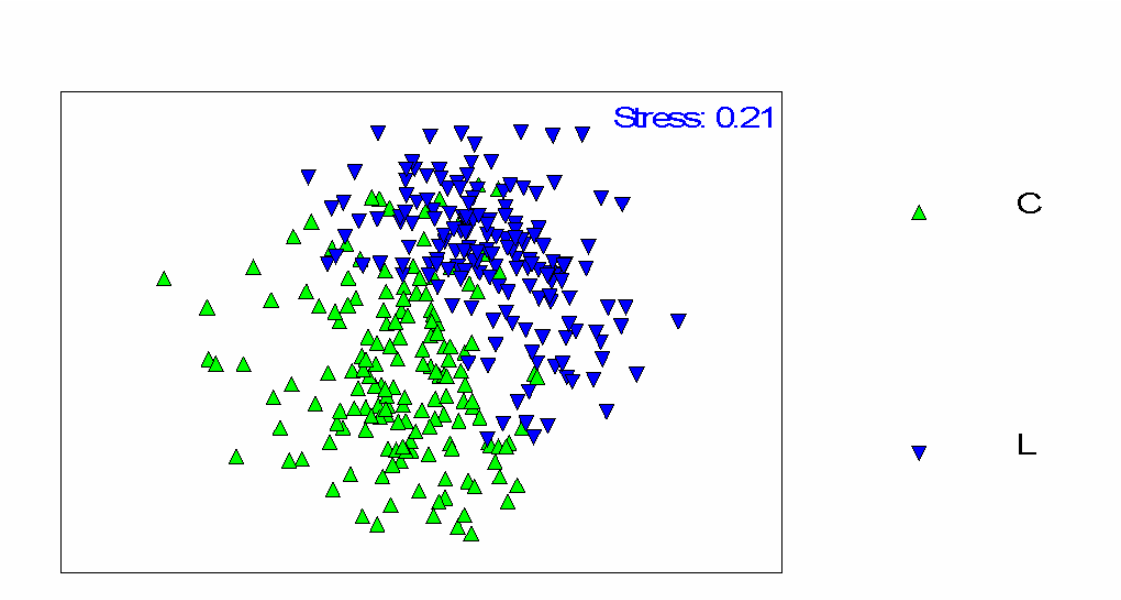


Figure 4.7 MDS ordination of macroinvertebrate samples from Xakanaxa channel (C, green) and lagoon (L, blue) habitats. Clearly the two habitats are inhabited by different organisms.

Table 4.5 Summary results of multivariate analysis of variance (ANOVA) for Xakanaxa sites, highlighting the distinction between channel (C) and lagoon (L) biotopes

Statistic	**Result**
Sample Statistic (Global R)	0.423
Significance Level of Sample Statistic (%)	0.1

The particular families characterising the groupings identified in the ordination (Figure 4.6) were identified as follows:

Channel Group
Average similarity: 34.51

Organism	**Families**	**Contrib%**	**Cum.%**
Shrimps	Atyidae	45.79	45.79
Damselflies	Coenagrionidae	19.93	65.72
Midges	Chironomidae	17.07	82.79
Baetid mayflies	Baetidae	5.52	88.31
Cased caddis flies	Leptoceridae	3.51	91.81

Lagoon Group
Average similarity: 36.25

Organism	Families	Contrib%	Cum.%
Midges	Chironomidae	33.29	33.29
Damselflies	Coenagrionidae	22.43	55.71
Pond snails	Lymnaeidae	11.43	67.14
Dragonflies	Libellulidae	10.55	77.69
Caenid mayflies	Caenidae	5.79	83.47
Seed mussels	Conchostraca	2.82	86.29
Water ticks	Hydracarina	2.16	88.46
Bilharzia snails	Planorbinae	2.04	90.50

Lagoon samples are thus marginally more similar with each other than are the channel samples.

The following families characterised the dissimilarity between the lagoon and channel biotopes:

Groups: Channel & Lagoon
Average dissimilarity = 76.28

Organisms	Families	Contrib%	Cum.%
Shrimps	Atyidae	17.28	17.28
Midges	Chironomidae	16.20	33.48
Damselflies	Coenagrionidae	11.96	45.44
Dragonflies	Libellulidae	7.69	53.13
Pond snails	Lymnaeidae	6.75	59.87
Baetid mayflies	Baetidae	5.09	64.96
Caenid mayflies	Caenidae	4.73	69.69
Bilharzia snails	Planorbinae	2.96	72.65
Seed mussels	Conchostraca	2.66	75.31
Cased caddis flies	Leptoceridae	2.57	77.88
Water ticks	Hydracarina	2.31	80.19
Biting midges	Ceratopogonidae	2.06	82.25
Orb snails	Bulininae	1.93	84.18
Long-legged dragonflies	Corduliidae	1.66	85.85
Predatory diving beetles	Dytiscidae	1.45	87.29
Pigmy backswimmers	Pleidae	1.43	88.72
Leeches	Hirudinae	1.10	89.83
Creeping water bugs	Naucoridae	1.03	90.85

Shallow seasonal habitats also had a specific biota, characterised by culicid mosquito larvae ephydrid shore fly larvae, and at Nxaraxa, also by very abundant chironomid midge larvae (Figures 4.4 and 4.5). The abundance of shore fly larvae in this habitat is interesting as they were very rare in the channel habitat at Bodumatau Bund. This is a further indication of the resilience offered by the mosaic of habitats.

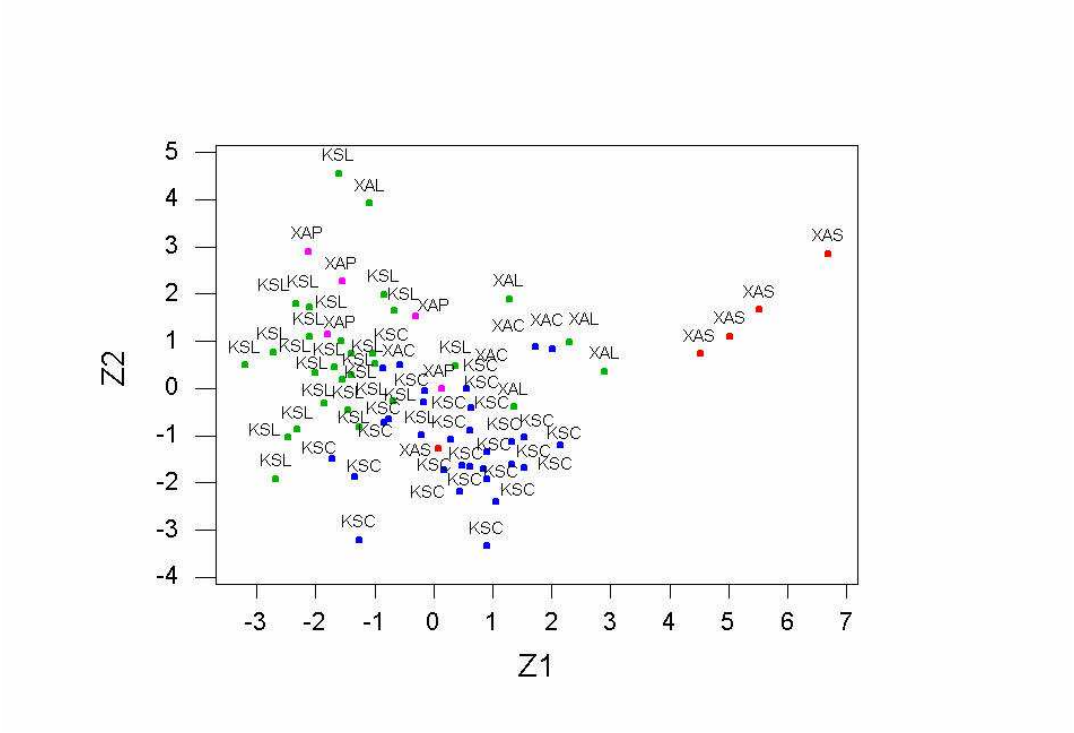


Figure 4.8 PCA ordination of final state macroinvertebrate samples from Xakanaxa (XA and KS) in lagoons (L, green), channels (C, blue), shallow permanent (P, pink) and shallow seasonal habitats (S, red). The shallow seasonal habitat was characterised by the presence of mosquito larvae and shore fly larvae.

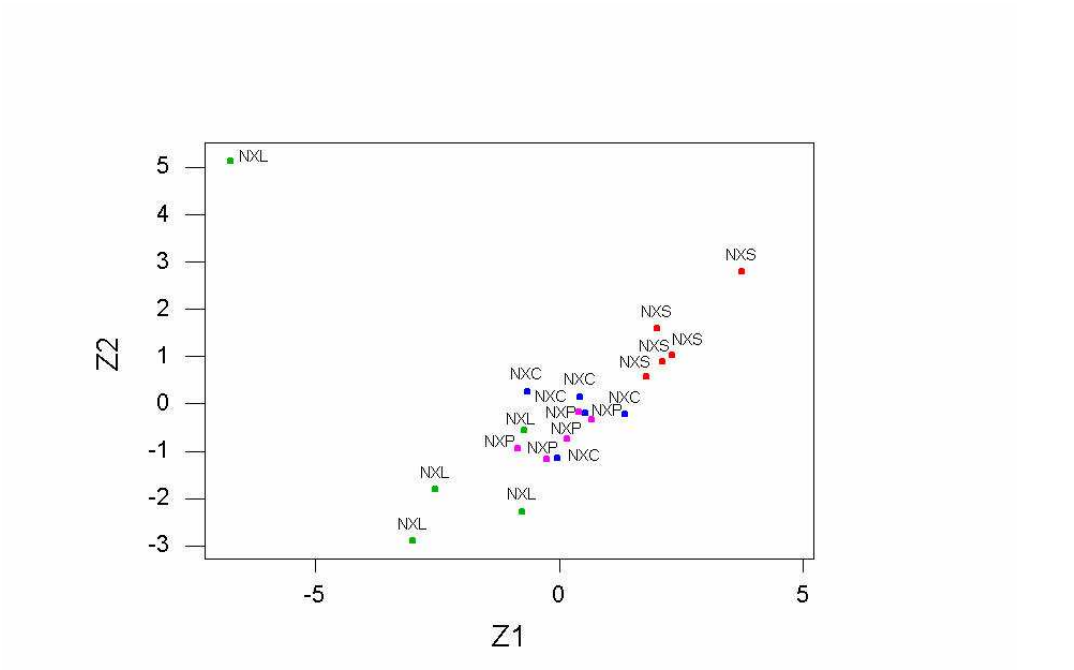


Figure 4.9 PCA ordination of final state macroinvertebrate samples from Nxaraxa (NX) in lagoons (L, green), channels (C, blue), shallow permanent (P, pink) and shallow seasonal habitats (S, red). The shallow seasonal habitat was characterised by the presence of mosquito larvae, shore fly larvae and by very abundant midge larvae.

4.4.2.2 Aquatic invertebrates from different sites

There was a clear, statistically significant difference in community composition between the biota from different sites. This is shown in a comparison of the channel biota from Xakanaxa, Bodumatau and Third Bridge (Figure 4.10, Table 4.6) and a comparison of Xakanaxa and North Gate (Figure 4.10, Table 4.7).

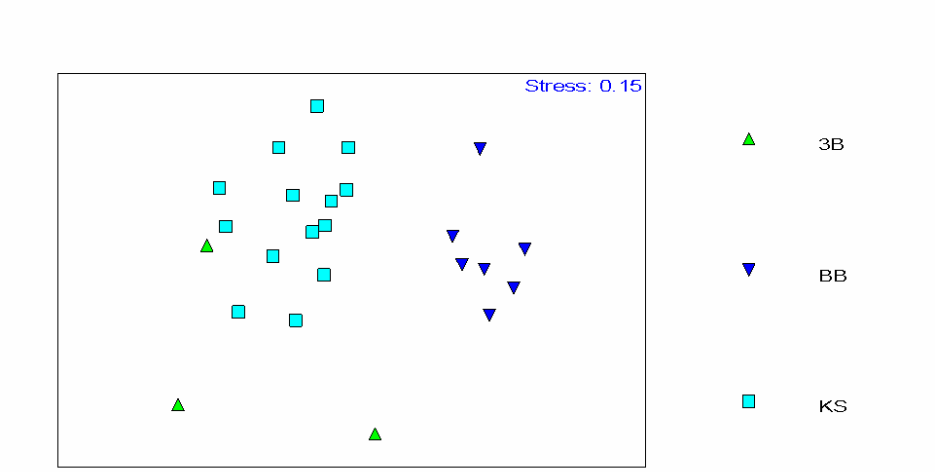
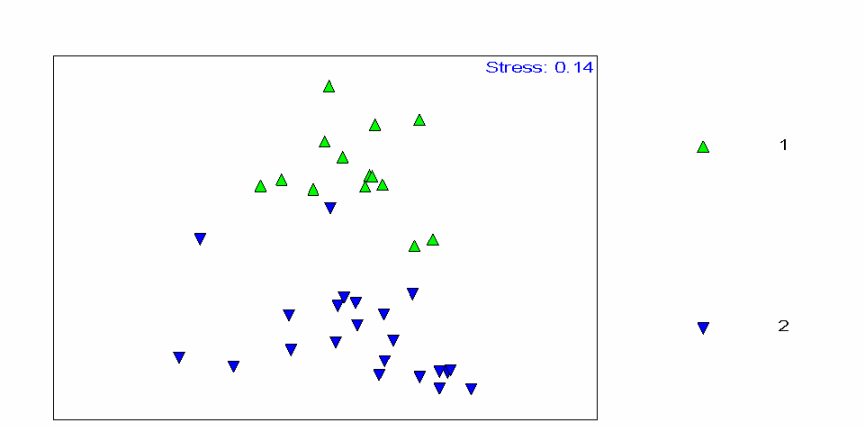


Figure 4.10 MDS ordination of macroinvertebrate samples collected from the channel biotope for the Paradise Pools (KS), Bodumatau Bund (BB) and 3rd Bridge (3B) sites during Cycle 1, and before Deltamethrin spraying.

Table 4.6 Summary results of multivariate analysis of similarity (ANOSIM) for KS, BB and 3B sites during Cycle 1 and before Deltamethrin spraying

Statistic/Test	**R Statistic**	**Significance (%)**	**Average Dissimilarity (%)**
Sample Statistic (Global R) (KS, 3B & BB sites)	0.822	0.1	
KS, 3B	0.648	0.6	76
KS, BB	0.892	0.1	79
BB, 3B	0.921	0.8	88



The family composition of aquatic invertebrates from Third Bridge is more similar to Xakanaxa than to Bodumatau Bund, and Xakanaxa is different from Bodumatau Bund.

Figure 4.11 MDS ordination of macroinvertebrate samples collected from channel habitats at Xakanaxa (1, green) and North Gate (2, blue) before Cycle 1 deltamethrin spraying.

Table 4.7 Summary results of multivariate analysis of similarity (ANOSIM) for Paradise Pools (KS) and North Gate (NG) sites during Cycle 1, and before Deltamethrin spraying.

Statistic/Test	R Statistic	Significance (%)
Sample Statistic (Global R) (NG vs KS sites)	0.639	0.1

The family composition of aquatic invertebrates from Xakanaxa is different from North Gate.

4.4.3 Seasonal changes

The "control" site at North Gate provided the best opportunity of describing a natural change in biota during the period of the spray. The magnitude and nature of these natural seasonal changes could then be compared to changes in the biota of sprayed sites.

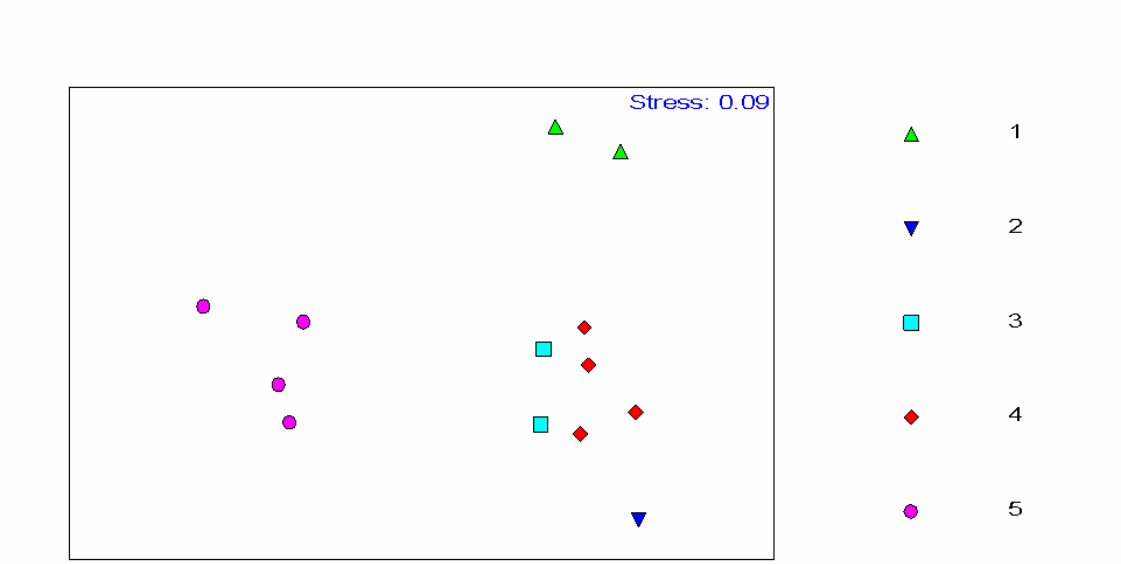


Figure 4.12 shows that the greatest change in the composition of drift net sample composition occurred between cycles 4 and 5, which coincided with the arrival of the floodwaters.

Figure 4.12 MDS ordination of macroinvertebrate drift net samples from the North Gate "control" site for all 5 spray cycles.

Key Results
•1 Different habitats are characterised by recognisably different biota. •2 A range of habitats must be sampled to include the aquatic biodiversity of the delta. •3 A range of habitats should be sampled, and monitored for immediate and longer term spray effects. •4 Different sites are characterised by a recognisably different suite of organisms. •5 A range of habitats at different sites should be sampled to include the aquatic biodiversity of the delta. •6 The effects of spraying should therefore be reported by habitat and site. •7 There are natural seasonal changes and these will account for some of changes through the spray cycle. It unlikely that all of the changes described are seasonal effects.

4.5 Conclusions

The main concluding statements are:

1. There was a measurable impact on aquatic invertebrate biota as a result of 2002 deltamethrin spraying.

   The main impacts were: the numbers of some of the most abundant organisms were reduced; some common, but less abundant, taxa that were specifically characteristic of either the channel or lagoon habitat "disappeared"; and the assemblage composition of the lagoon and channel habitats became more similar and were characterised by fewer, more resistant taxa.

2. The impact of the 2002 deltamethrin spraying is unlikely to damage ecosystem processes and it is most likely that the aquatic invertebrate biota will recover.

   The mosaic of biotopes and occurrence of refugia, as well as the complex seasonal emergent and life cycles of the biota probably mean there is good recovery potential.

3. It is likely that identification to morphospecies would considerably enhance the information to be gained from the samples collected.

4.6 References

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5. Monitoring of Terrestrial Invertebrates

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5.1 Background

The intention of the Government of Botswana in eradicating tsetse flies from northern Botswana is to improve public health whilst minimizing environmental impact. Previous documents have outlined the rationale for tsetse fly eradication using aerial applications of insecticide (Allsopp & Phillemon-Motsu, 2001) backed by independent environmental impact assessment recommendations (Scott Wilson Consultants, 2001).

There are three critical requirements for effective environmental monitoring of management impacts:

1. knowledge of the current or pre-management state and normal variation in that state (benchmark)

2. magnitude of change in response to the management practice (impact)

3. rates and duration of recovery to either the benchmark levels or a target level that is deemed acceptable (recovery).

These requirements are summarized graphically in Figure 5.1. Clearly the dependent variable (or variables) on such a graphic must be selected carefully to be a reliable and realistic measure of the environment. The ideal design to monitor such effects is a Before-After-Control-Impact approach (Underwood, 1994), which involves an ANOVA type experimental design using control sites to provide baseline information for trends in the metric without the management intervention as well as trends on impacted treatment sites. This requirement for adequately replicated control and treatment sites is complicated by the blanket spraying approach of the proposed intervention in the Okavango Delta.

In the case of non-target organism responses to aerial spraying of insecticides there are additional issues in defining the benchmark and so assessing the impact. It is possible to use loss of species as the impact measure but the problem then is how to assess, with inevitably limited sampling effort, if a species has become locally extinct. Alternatively the benchmark can be some measure of composition in all or selected taxa and relative changes in composition used as the measure of impact. The advantage to this is that artifacts of incomplete sampling can be reduced. Also it is much easier to cover the known problems of change in species composition with distance.

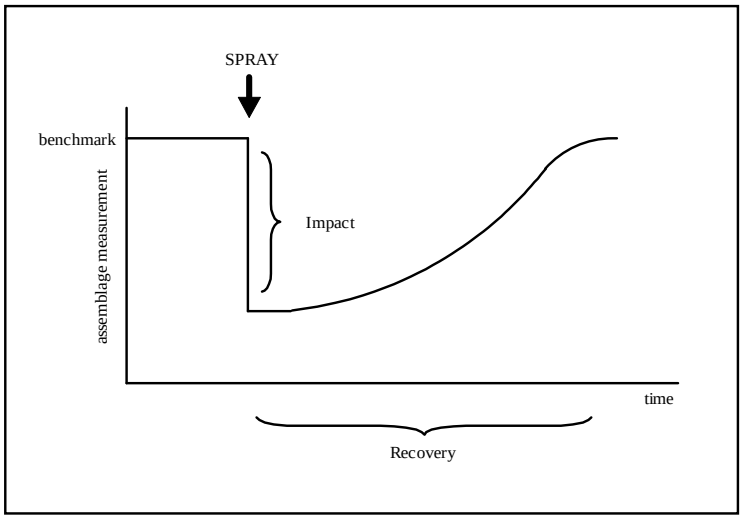


Figure 5.1 A stylised model of patterns in an environmental measurement in response to an impact or management intervention. In the case of the aerial spraying for tsetse fly the spray impact is a multiple one that occurs over several months.

Recovery is easier to assess in that a clear target composition can be determined from control areas or pre-management benchmarks. Then, the likelihood of a sample achieving the target can be given a definite probability. Recoveries take time and so monitoring must include sampling at intervals that reflect this. Recovery of a population within a year may not occur but this does not imply extinction. Dispersal may be slow and so recovery may take several years but so long as the species does not make critical contributions to system processes within that time on its return it negates any management intervention.

Invertebrates in the tree canopies, especially species in certain beetle, fly, spider and Hemipteran families are most vulnerable to aerial deltamethrin applications. This is to be expected as the application of fine aerosols is targeted at this component of the habitat to give best results on resting tsetse flies. Data from the 2001 monitoring program in the Okavango Delta also identified these taxa as potentially vulnerable and is consistent with the literature on the effects of aerial applications. A focus on these taxa for the 2002 monitoring was proposed for the intrinsic risk to these taxa but also as a method for accurate appraisal of impact and recovery.

The approach focused on catches of invertebrates that fall from the canopy into plastic trays. Other sampling methods were used sparingly to provide data on any unusual or unexpected effects on ground fauna (pitfall traps) and flying invertebrates (malaise trap).

It is important to establish the pattern of change in abundance and composition of these sensitive taxa throughout each of the five spray cycles. This requires an estimate of their abundance immediately prior to the first application, the impact of the insecticide on these populations at the spray event and then an estimate of what remains in the canopy following the spray. This combination of pre-spray / spray / post-spray information was repeated throughout the five cycles to assess the cumulative impact of successive aerosol applications. An estimate of what remains alive in tree canopies after an aerosol application could only be achieved by collecting knockdown from chemical fogging of individual canopies before and after spray events.

The August 2001 surveys of terrestrial invertebrates at Pom Pom and the additional knockdown data from Guma Lagoon and Mombo suggest that the key groups likely to be affected by deltamethrin are beetles, flies, Hemiptera and selected spiders, and that the major impact will be in habitats with a tree canopy. In the delta these are either riparian habitats associated with channels and permanent lagoons or on islands originating from termite

mounds, especially in the seasonal floodplains. Whilst the Okavango Delta is structurally complex and spatially dynamic the vegetation composition is relatively predictable and a small number of canopy tree species dominate. In the seasonally inundated areas typical of the southern spray zone, *Lonchocarpus capassa, Kigelia africana* and *Combretum imberbe* are frequent in fringing vegetation, especially in frequently flooded areas. Isolated individuals of these species can also be selected which minimizes the variance in invertebrate composition typical of collections under mixed tree canopies.

The approach was therefore to target selected families of beetles, flies, spiders (see table) together with Hemiptera using a combination of knockdown traps and malaise trap (singular).

Target Families		
Beetles	Flies	Spiders
Curculionidae Chysomelidae Staphylinidae	Muscidae Phoridae	Oxyopidae Theridiidae Salticidae

This approach also provided a benchmark for a reliable indicator assemblage of organisms. Using several taxa will overcame the problems of indicator species and reduced the sampling and taxonomic effort needed.

At selected sites individuals from three tree species, *Kigelia africana, Combretum imberbe* and *Lonchocarpus capassa*, were selected and fogged with deltamethrin prior to any aerial spray applications. This provided a **benchmark** estimate of canopy invertebrates before the spray. Then knockdown samples from the aerial spray were collected from beneath these and non-fogged trees during each spray cycle. Some trees were fogged with backpack sprays 48 hours after each spray cycle to assess what was still alive in the canopy following aerial application. This gave an estimate of **impact**. The sites should be revisited 12 months later and assessed for **recovery** with both fogging and malaise trap samples, and clearly the number of replicates and tree species that can be handled in this design depends on available budget and logistics. It should however provide a true measure of impact and recovery, together with a baseline for future monitoring of tsetse fly management activities**.**

5.2 Methods

5.2.1 Study areas

A study area typical of the broader 2002 spray zone including a mixture of dry and indundated floodplains, islands, fringing vegetation and larger mopane woodland patches was selected (Figure 5.2). This area, within a 10km radius of the HOORC research camp at Nxaraxa Lagoon, was selected as it was in the centre of the spray zone, had representation of all the dominant habitats and allowed a spread of sampling approaches (Figure 5.3). Specifically it allowed for canopy fogging to be separated from knockdown sampling from aerial applications to avoid cross contamination, a problem identified by the independent reviewer. During site selection it also became clear that fires were a likely hazard necessitating a wider geographical spread of sampling sites. Although this created some logistical problems it was necessary insurance against fire effects confounding the impact of spray cycles.

An area outside the spray zone was selected to sample *Colophospermum mopane* trees that were not exposed to the spray cycles (Figure 5.2)

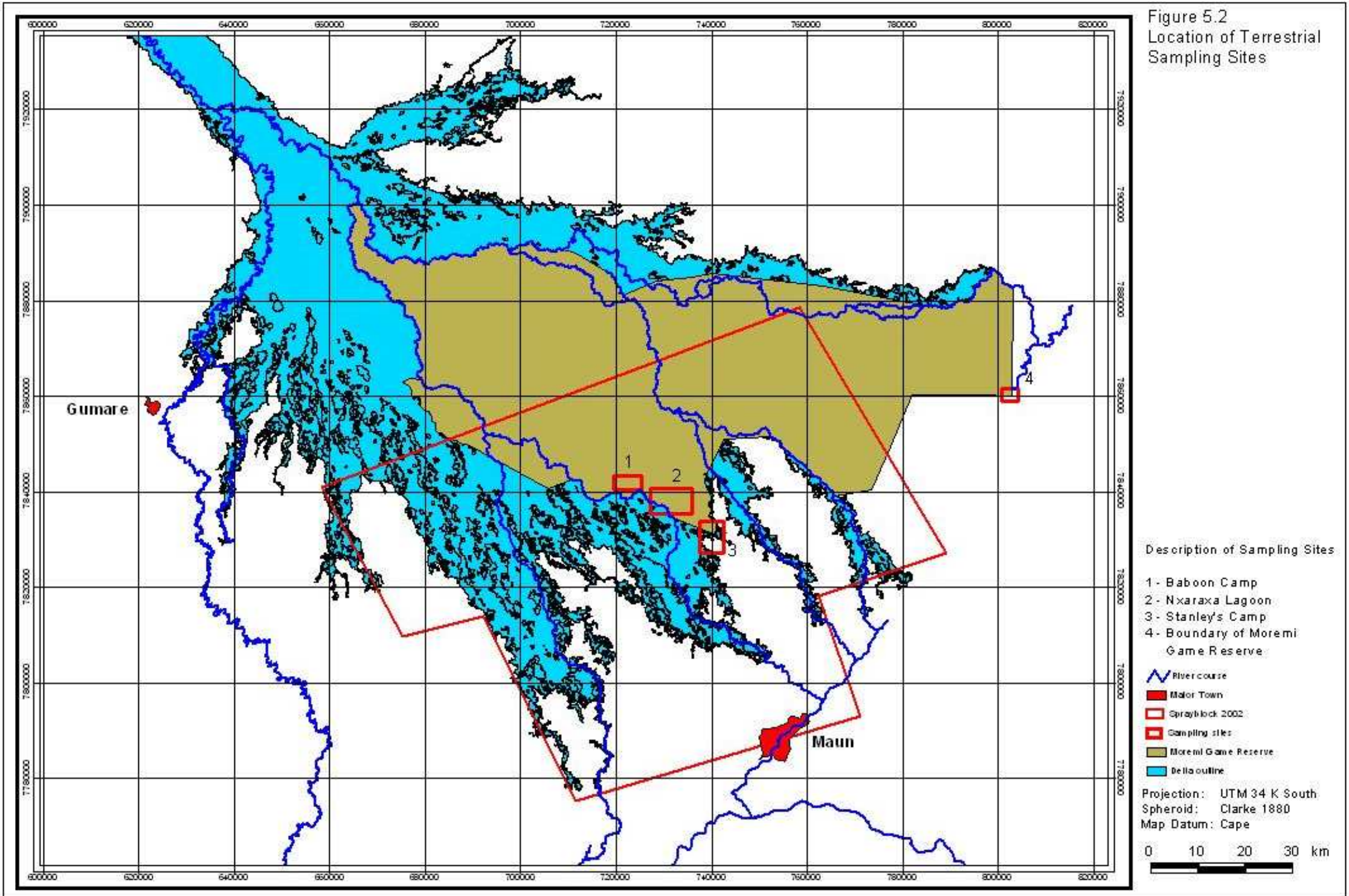
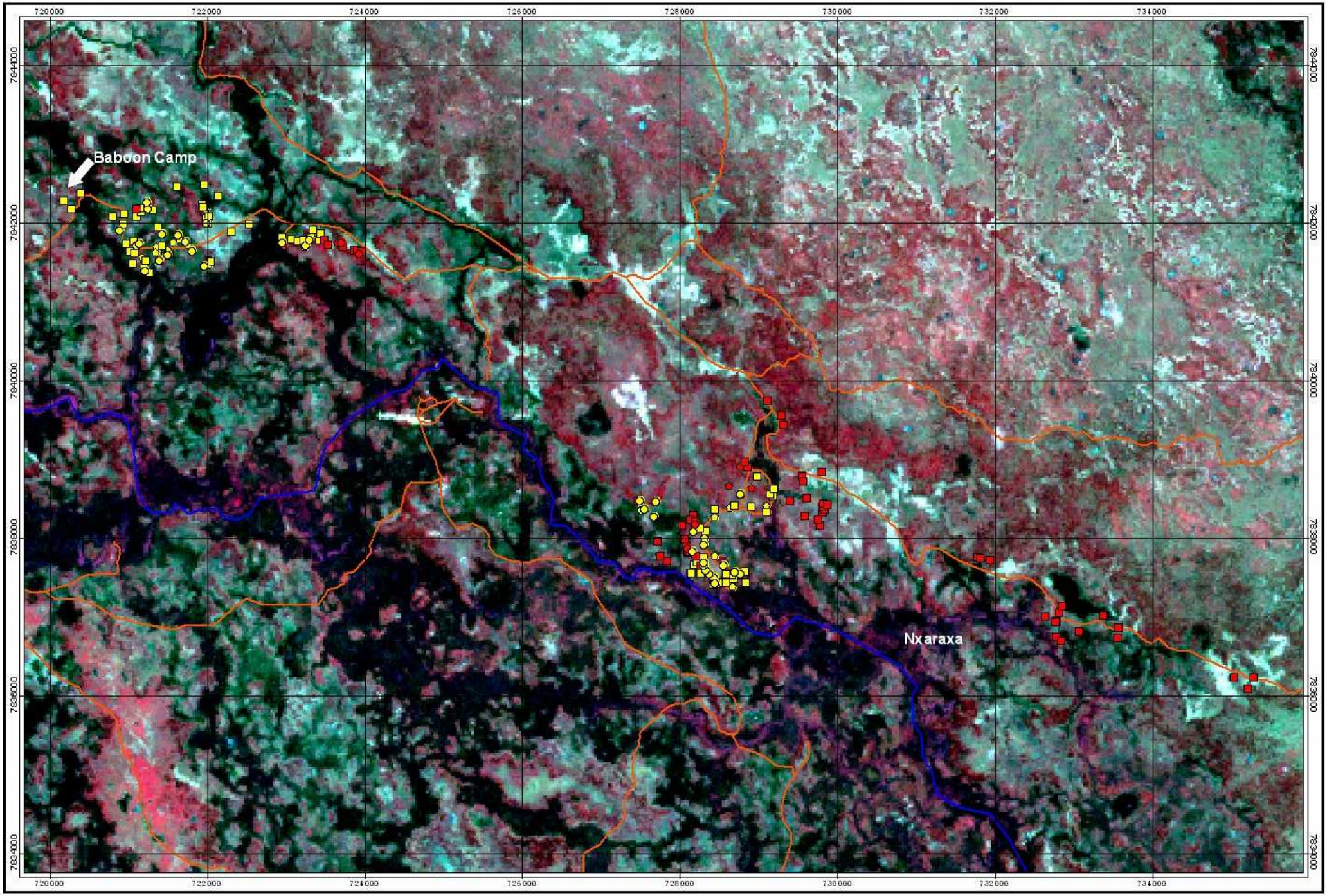


Figure 5.2
Location of Terrestrial Sampling Sites

Figure 5.3 Location of terrestrial sampling sites at Nxaraxa and Baboon Camp



5.2.2 Spray cycle canopy knockdown

When the insecticide aerosol is released from the aircraft and drifts through the canopy any insects exposed to the droplets are at risk. The probability that an insect will suffer a lethal dose will be a function of its position in the canopy, the number and volume of spray droplets that impinge upon it and its topical tolerance distribution to the pesticide formulation (Jepson et al., 1990). Even though not all individuals suffer a lethal dose many become disorientated or lethargic and fall from the canopy. This "knockdown" begins almost immediately after the application and continues at a declining rate for up to 48 hours. Data from the 2001 monitoring program suggested that around 100 individuals m^{-2} were collected in knockdown trays after 12 hours, declining to less than 5 m^{-2} after 72 hours (Figure 10.2 in Perkins & Ramberg 2002).

Sheets measuring 150cm x 240cm were cut from a roll of industrial quality UV resistant plastic. Ropes attached to each corner together with cross-hair string stanchions fashioned an effective tray with a catch area of around 2.86m^2. Suspended off the ground with string attached to 1m wooden stakes each tray was effective in catching knockdown and secure from foraging invertebrate predators, especially ants. Each tray was weighted with a *Kigelia africana* pod placed in the centre of the tray.

One tray was sited approximately halfway between the tree bole and the edge of the canopy for each tree sampled to generate a representative sample and to be consistent with the 2001 design (Perkins & Ramberg, 2002). Where the tree canopy was uneven the tray was placed beneath the most significant bough. Trays were installed on the day of the spray to minimise the opportunity for damage by large animals. In the morning following the spray event all invertebrates in the trays were carefully swept to one corner of the tray and transferred into a barcoded plastic bag.

Over the next 48 hours the specimens were transferred into vials containing ethanol and prepared for shipment to Sydney.

5.2.3 Mortality in knockdown

Not all the invertebrates that fall from the trees as a result of the spray are dead. Many recover and it is important to estimate the proportion of insects that survive. Two cotton bed sheets were established as knockdown trays under the same tree, one of the trays was treated with a contact insecticide to kill any invertebrates that landed on the tray. The other was not treated which allowed animals time to recover and crawl away. Three *K. africana* and one *L. capassa* were selected close to the Nxaraga Camp and recovery trays constructed. Collections were made from these trays 18-24 hours after the spray event.

5.2.4 Canopy fogging

Knowledge of the assemblages of invertebrates inhabiting the canopy prior to insecticide application is critical to interpretations of any impact and forms benchmark against which to measure recovery. The approach used was to infiltrate the canopy with deltamethrin from the ground using hand-held foggers (Swingfog). A petrol engine generates sufficient heat to volatilize the insecticide and its oil based solution into a smoke that rises readily through the tree canopy from the ground. This technique is very effective if used at the correct time of day when temperature and wind conditions allow the hot fog to rise and envelop the canopy and has been used successfully in tropical environments (e.g. Basset 1991).

5.2.4.1 Pre-cycle canopy fogging

A total of 28 isolated individual *Kigelia africana*, 10 *Lonchocarpus capassa* and 10 *Combretum imberbe* trees were selected in an approximately 4km^2 area south of Stanleys

Camp and west of Chiefs Island (Figure 5.4). This area was assessed as suitable during a field visit on 27th May 2002 and target trees marked and geo-referenced in advance. This exercise took three days due to awkward logistics, familiarization and maintenance of the fogging equipment.

Ten individual *Colophospermum mopane* trees at a site on Chiefs Island near the boundary marker of Moremi Game Reserve were fogged prior to the first spray cycle (Figure 5.3). Samples were collected from trays the following morning. A second mopane site north-east of South Gate on the boundary of Moremi Game Reserve was selected and 10 trees fogged (Figure 5.2). This area is outside the eastern limit of the spray zone.

5.2.4.2 Within-cycle canopy fogging

Impact can only be known if the composition of organisms living in the canopies is established **before** each aerial spray and what remains in the canopy **after** the spray event. Canopy fogging with deltamethrin from a backpack fogging machine can provide samples to obtain this information. An area 10km north of Nxaraxa Lagoon was surveyed and 30 *Kigelia africana* trees suitable for fogging were identified (Figure 5.3). These trees were fogged in sequence through the five cycles and invertebrates collected in plastic trays as for pre-spray knockdown samples.

Six trees were selected at each cycle and separated into three pairs. The first tree in each pair was fogged 24 hours before the aerial spray and an invertebrate sample collected in a knockdown tray. After the aerial application of deltamethrin an invertebrate sample was collected from both trees with one knockdown tray per tree. 24 hours after the aerial application the second tree in the pair was fogged and a sample taken. This sequence, repeated for each of three tree pairs for each spray cycle, provided replicated data to establish which invertebrates were in the canopy before the aerial spray and those that were still alive in the canopy immediately after the spray.

Method	*Kigelia africana*	*Lonchocarpus capassa*	*Combretum imberbe*	*Colophospermum mopane*	Island fringe at Nxaraxa
Prespray fogging	✓	✓	✓	✓	
Cycle knockdown (1-5)	✓	✓	✓		
Within cycle fogging (2-5)	✓				
Malaise trap cycles 1-5					✓
Pitfall traps cycles 1-5					✓
Postspray fogging after cycle 5	✓	✓	✓	✓	

Schedule of sampling from each tree species and the fringing vegetation at Nxaraxa.

5.2.3 Pitfall trapping

At the Nxaraxa site pitfall traps were installed in mixed island fringe vegetation adjacent to the main HOORC field camp. Three transects of 5 traps consisting of plastic honey jars with approximately 50ml of antifreeze and ethanol mixture were sited parallel to the fringe, 20m from the outer edge of the tree canopy, with traps 20m apart. Installed five days before the spray event the traps were collected on the day of the spray, replaced and left for a further 5 days. The same trap positions were used in each cycle.

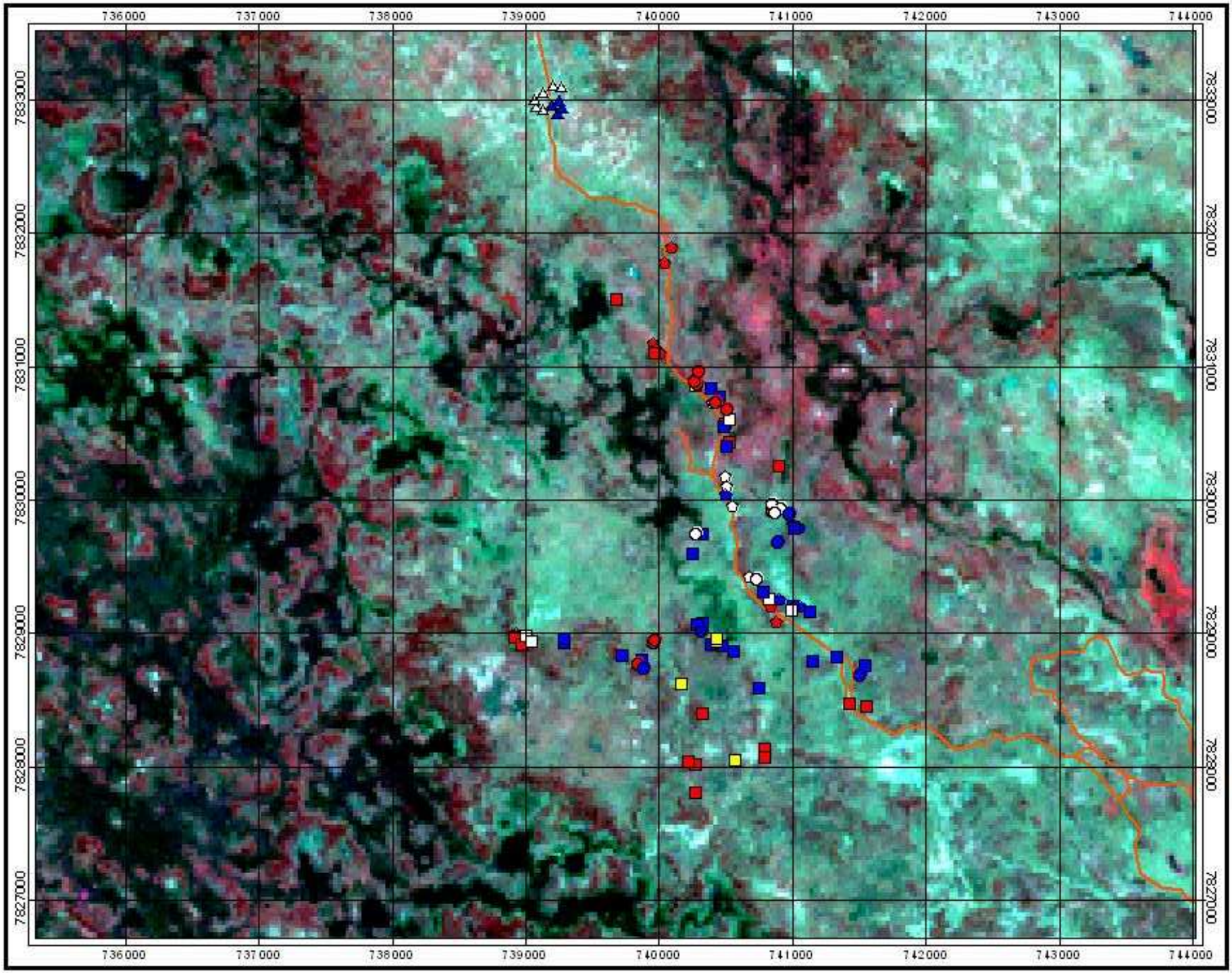


Figure 5.4
Detailed location of terrestrial sites at Stanley's Camp

5.2.4 Malaise trap samples

In order to monitor any unusual activity or impact on flying invertebrates a single malaise trap of standard design was erected near to the Nxaraxa camp. Specimens were collected at dawn and dusk for two days prior to and two days post the spray event on each cycle. In cycle one the trap was erected outside the vegetation fringe on the floodplain. A significant grass fire that eventually burnt up to the camp forced a shift in the trap position and loss of two sampling days. Samples were collected without incident through the remaining 4 cycles.

5.2.5 Implementation

The sampling activities detailed above were implemented as follows.

5.2.5.1Pre-spray fogging

A total of 69 trees were successfully sampled prior to the first spray cycle. The full compliment of 30 isolated individual *Kigelia africana*, 9 *Lonchocarpus capassa* and 10 *Combretum imberbe* trees were sampled from the area south of Stanleys Camp and west of Chiefs Island. One sample from a *L. capassa*, while fogged, returned no specimens, subsequently the sample was assigned a dummy collection code and entered as a sample event with a return of 0 specimens.

Pre-spray fogging from ten *Colophospermum mopane* trees on Chiefs Island and ten trees in Moremi Game Reserve was also completed prior to the first spray cycle (Table 5.1).

5.2.5.2Cycle 1

Knockdown samples were successfully collected from 46 trees. Pitfall trap samples were collected successfully on all the cycles. Only one trap was consistently disturbed by baboons.

The knockdown fogging sampling was hampered by difficulties with fogging equipment and transport to the fogging site which prevented implementation of the desired design. The malaise trap samples were affected by a local grass fire which required a relocation of the trap, however, some pre-cycle and post-cycle samples were obtained.

5.2.5.3 Cycle 2-5

The knockdown and pitfall trapping was completed successfully. The knockdown recovery was successful thanks to an improvement in the trap design to retain specimens.

Access to the fogging sites became difficult during cycle 3, 4 and 5 as the flood advanced and caused significant logistical constraints which were compounded slightly by changes to the spray dates.

Occasional pitfall traps were lost to baboons otherwise these and the mailaise trap samples were successfully collected.

5.2.5.4 Post cycle fogging

A total of 136 trees were successfully sampled by fogging the canopy with deltamethrin from hand-held foggers. Individual *Kigelia africana*, *Lonchocarpus capassa* and *Combretum imberbe* trees were sampled from the area south of Stanleys Camp, west of Chiefs Island and also from Nxaraxa. Most of these trees had not been sampled before but around 10% had been sampled throughout the spray cycles or in pre-spray fogging.

Fifteen *Colophospermum mopane* trees on Chiefs Island and ten trees on the boundary of Moremi Game Reserve were successfully fogged (Table 5.1).

Table 5.1 Summary of terrestrial invertebrate sampling

	Spray Dates	Date received In Sydney	Number and type of samples expected	Samples received
Pre-spray	9 -16 May	5 June	60 canopy fogging	68 canopy fogging
Cycle 1	16 -23 May	5 June	50 knockdown 12 knockdown fogging 8 recovery knockdown 30 pitfall 8 malaise	46 knockdown 21 knockdown fogging 0 recovery knockdown 29 pitfall 4 malaise + 6 knockdown fogging + 6 C. mopane at Xaxanaxa
Cycle 2	6 -13 June	2 July	50 knockdown 12 knockdown fogging 8 recovery knockdown 30 pitfall 8 malaise	53 knockdown 19 knockdown fogging 8 recovery 30 pitfalls 8 malaise + 10 *C.mopane* at Xaxanaxa + 8 3-day post spray recovery
Cycle 3	30 June -8 July	24 July	50 knockdown 12 knockdown fogging 8 recovery knockdown 30 pitfall 8 malaise	53 knockdown 20 knockdown fogging 8 recovery 27 pitfalls 10 malaise + 10 mopane Xaxanaxa + 8 3-day post spray recovery
Cycle 4	21–26 July	15 August	50 knockdown 12 knockdown fogging 8 recovery knockdown 30 pitfall 8 malaise	53 knockdown 20 knockdown fogging 8 recovery 29 pitfalls 10 malaise + 10 mopane Xaxanaxa + 8 3-day post spray recovery
Cycle 5	7-16 August	3 Sept	50 knockdown 12 knockdown fogging 8 recovery knockdown 30 pitfall 8 malaise	53 knockdown 18 knockdown fogging 8 recovery 2 pitfalls 10 malaise + 10 mopane Xaxanaxa + 8 3-day post spray recovery
Post-spray	19-25 August	3 Sept	136 canopy fogging	136 canopy fogging

5.3 Results

5.3.1 Overall captures

A total of 102,248 terrestrial invertebrates were captured from 746 samples of various types. In all 26 higher taxa, mostly at the taxonomic level of Order, were recorded. This was a good sampling return and emphasizes the richness of the terrestrial invertebrate fauna even when sampled during the dry winter months when invertebrate activity is generally reduced.

Beetles (34%), flies (28%), ants (22%), Hemiptera (6%) and wasps (3%) were the most abundant taxa, collectively accounting for 93% of the total sampled and as with most samples of biodiversity the majority of the remaining taxa were infrequent. A breakdown of captures by sampling method (Table 5.2), shows that flies, beetles and ants were usually the most abundant taxa in samples, irrespective of the sampling method.

Method	Samples	Specimens	Orders	Five most abundant taxa
Fogging	203	55,761	23	Beetles, ants, flies, Hemiptera, wasps
Knockdown	276	22,860	21	Flies, ants, beetles, Hemiptera, wasps
Knock- fog	55	6,650	18	Flies, ants, beetles, termites, Hemiptera
Pitfalls	140	5,796	18	Beetles, ants, Collembola, flies, termites
Malaise	40	7,636	14	Flies, wasps, Hemiptera, Lepidoptera, beetles
Recovery	32	3,545	19	Flies, ants, wasps, Hemiptera, beetles
Totals	**746**	**102,248**	**26**	Beetles, flies, ants, Hemiptera, wasps

Table 5.2 Summary of sampling returns for terrestrial invertebrates by sampling method for all tree species during the 2002 spray cycles.

Canopy fogging with deltamethrin from the ground returned most canopy invertebrates with an average of 400 individuals per sample prior to the spray cycles. Trees fogged in this way after the five aerial application cycles produced on average 140 specimens per sample. The sampling trays had a capture area of just under 3 m^2, hence the density of invertebrates falling to the ground directly beneath the tree canopies was around 140 m^{-2} in the pre-cycle fogging and 49 m^{-2} in the post-cycle samples

Canopy fogging from the ground produced on average 30 to 80% more specimens per sample than the aerial application in cycle 1. These samples all came from separate trees and the average differences were significant (ANOVA, $F_{1,88}$=6.6, P=0.012). Fogged trees were at least 10km from the main sampling area at Nxaraxa and this geographic separation may have affected abundance differences. It seems likely, however, that the intensity of the first spray cycle was rather less than ground based fogging with the same insecticide formulation. The same insecticide formulation was used in the foggers as that delivered from the planes but the intensity and mode of delivery suggested a much larger dose was delivered by canopy fogging than in the aerial sprays.

Beetles were abundant in the canopy fogging samples and may be especially susceptible to deltamethrin applied in this manner (Table 5.3). Ants and flies were sampled consistently across all methods. Hemiptera, wasps and spiders were also consistently sampled across all methods but at low numbers per sample.

Orders	Fogging	Knockdown	Recovery	Knockdown fogging	Pitfall	Malaise
Coleoptera	29708	1691	89	683	2426	35
Formicidae	10172	7431	435	1929	2011	33
Diptera	5478	9923	2731	3106	261	7201
Hemiptera	4198	1424	95	199	31	66
Hymenoptera	1666	875	115	205	42	190
Araneae	1148	543	28	102	193	9
Isoptera	777	14	8	216	212	0
Thysanoptera	602	185	12	35	0	1
Neuroptera	540	33	3	10	0	0
Pseudoscorpionida	313	35	3	45	3	0
Larvae	243	134	5	26	80	0
Acarina	240	284	7	22	25	12
Lepidoptera	218	111	5	31	22	55
Blattodea	161	32	2	11	64	11
Psocoptera	110	40	1	1	0	13
Mantodea	77	30	1	33	7	0
Thysanura	41	21	1	0	3	0
Orthoptera	30	20	1	1	39	4
Solifugae	17	0	0	0	2	0
Ephemeroptera	13	32	3	1	0	0
Collembola	7	0	0	0	372	0
Scolopendrida	1	0	0	0	0	0
Scorpionida	1	1	0	0	0	0
Odonata	0	1	0	0	0	5
Siphonaptera	0	0	0	0	0	1
Spirostreptida	0	0	0	0	3	0

Table 5.3 Rank abundance of higher taxa in samples using different sampling methods

Key Results
• 102,248 specimens were recorded from 746 samples • A total of 26 higher taxa were identified • Taxa sampled were those recognised as being potentially vulnerable to the aerial applications • Canopy fogging from hand-held foggers are more effective in knocking invertebrates from the canopy than aerosol applications of the same insecticide formulation from aircraft

5.3.2 Higher taxa responses from spray cycle and canopy fogging

This section describes data generated from invertebrates captured in knockdown trays beneath *Kigelia africana, Lonchocarpus capassa* and *Combretum imberbe* trees. Samples taken in pre-cycle and post-cycle canopy fogging and all five aerial spray cycle applications are included.

5.3.2.1 Number of specimens sampled

The average number of specimens caught per tray from all tree species declined by 44% from cycle 1 to cycle 5 and the catches from canopy fogging prior to the spray cycles were up to three times those in post-cycle fogging (Table 5.4). This suggests that the aerial application of deltamethrin significantly reduced invertebrate abundance in tree canopies.

Closer examination of these results show a large amount of this trend is due to changes in the abundance of beetles, which decline from an average of 114 in Cycle 1 to 39 per tray by Cycle 5. The average catch rates without beetles decline by only 16% through the cycles, however, the decline is moderated to some extent by a steady increase in catches of flies through the cycles from an average around 6 to more than 30 per sample by cycle 5. This increase in capture rates for flies is most likely a result of seasonal patterns in emergence of adults and activity patterns in response to the arrival of the floodwaters and seasonal increases in temperatures through the cycles.

	Pre-cycle fogging	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Post-cycle fogging
All taxa	**316**	**217**	**154**	**134**	**125**	**122**	**114**
Beetles	206	114	73	57	46	39	33
Flies	6	6	7	13	25	33	31
Ants	67	67	51	43	37	35	36
Hemiptera	13	11	8	6	5	4	4
Wasps	5	5	4	4	4	4	4
Spiders	9	8	6	4	4	3	3
Total without beetles	110	103	81	76	79	83	81

Table 5.4 Average number of specimens per knockdown tray of invertebrate taxa sampled by canopy fogging and from aerial spraying through five cycles from all tree species

Average number of specimens without beetles and flies declines steadily from 110 per tray in cycle 1 to 35 in cycle 5, a drop of 68%. The general trend through the cycles is for a steady decline in catch rate to around one third of the catches in cycle 1.

Ants maintained average numbers through the cycles but Hemiptera and spiders showed steady declines to around on third of their initial rates by cycle 5.

When these data are separated by tree species the declines follow similar patterns to the total (Tables 5.5, 5.6, 5.7). By cycle 5 beetles had declined to 37% of their cycle 1 average numbers on *K. africana* and *C. imberbe*. Declines without beetles were 16 and 23% respectively and were again moderated by significant increases in numbers of flies through the cycles. Removing beetles and flies overall number of specimens through the cycles declined by 44% on *K. africana* and by 43% on *C. imberbe*.

Comparisons between pre-cycle and post cycle fogging samples suggested an overall decline of 73% on *K. africana* and 68% on *C. imberbe* again largely due to a sharp difference in the numbers of beetle specimens. Without beetles the declines were 43 and 36% of the pre-cycle averages.

	Pre-cycle fogging	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Post-cycle fogging
All taxa	**358**	**255**	**190**	**168**	**156**	**150**	**95**
Beetles	246	137	93	74	60	50	23
Flies	6	7	7	16	30	37	29
Ants	66	79	64	57	48	46	31
Hemiptera	13	11	8	6	5	5	1
Wasps	7	6	5	5	5	5	6
Spiders	9	8	6	5	4	4	1
Total without beetles	112	118	97	94	97	99	72

Table 5.5 Average numbers of specimens per knockdown tray of invertebrate taxa sampled by canopy fogging and from aerial spraying through five cycles from beneath *Kigelia africana* trees

	Pre-cycle fogging	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Post-cycle fogging
All taxa	**380**	**231**	**177**	**144**	**130**	**124**	**120**
Beetles	238	133	92	70	57	49	39
Flies	7	6	6	9	18	23	22
Ants	96	60	48	40	35	34	43
Hemiptera	17	15	11	8	7	6	5
Wasps	10	7	6	5	4	3	3
Spiders	10	7	6	5	4	3	3
Total without beetles	142	97	85	74	73	75	81

Table 5.6 Average numbers of specimens per knockdown tray of invertebrate taxa sampled by canopy fogging and from aerial spraying through five cycles from beneath *Combretum imberbe* trees

In comparison samples from *L. capassa* trees actually showed an overall increase in average catch per tray through the cycles and only a 30% decrease between pre-cycle and post-cycle fogging samples (Table 5.7). Mostly this was due to a large increase in the number of flies through to cycle 5 and a decline in the number of beetles.

	Pre-cycle Fogging	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Post-cycle fogging
All taxa	**80**	**69**	**20**	**28**	**64**	**98**	**56**
Beetles	16	12	3	4	2	3	9
Flies	3	4	7	16	54	82	20
Ants	35	33	4	3	4	5	17
Hemiptera	8	7	1	1	1	2	1
Wasps	2	2	2	2	2	3	3
Spiders	7	5	2	1	0	1	1
Total without beetles	64	58	17	25	62	95	48

Table 5.7 Average numbers of specimens per knockdown tray of invertebrate taxa sampled by canopy fogging and from aerial spraying through five cycles from beneath *Lonchocarpus capassa* trees

Key Results
• Number of individual specimens in trays across all taxa declined by more than 40% overall through the cycles • Without beetles and flies numbers of specimens declined by 68% • The number of fly specimens increased through the cycles • Changes in the numbers of specimens differed between tree species • Overall the average number of beetle specimens declined by 63% through the cycles

5.3.2.2 Changes in higher taxa diversity

A total of 23 higher taxa were sampled from the canopy fogging and 21 from the knockdown during the aerial application cycles. The majority of these are at Ordinal rank but the Hymenoptera are split into wasps and ants as two categories and a general category of larvae is included which groups some Lepidoptera, Diptera and Coleoptera together, however, these taxa were also all present as adults.

One dragonfly was recorded in the knockdown but not in the canopy fogging. One centipede (Scolopendrida) and a handful of springtails (Collembola) were sampled by fogging but not in knockdown. Only the sun spiders (Solifugae) were recorded reasonably consistently (17 individuals) in fogging samples but not at all in the knockdown.

Combining data from the three tree species, all higher taxa were represented in at least one sample within each cycle and in the pre-cycle and post-cycle fogging. No higher taxa appear to have been lost immediately as a result of the spraying.

Average higher taxa richness (number of taxa) per sample did decline steadily from cycle 1 through to cycle 4 then picked up again in cycle 5 (Figure 5.5). This suggests that as the number of individuals sampled declined then the number of taxa in each sample also declined. As the majority of the taxa are relatively infrequent then lower catches inevitably result in fewer taxa.

Average number of higher taxa per sample in the pre-cycle and post-cycle samples were not significantly different.

Diversity indices combine the number of individuals and the number of taxa in various ways to allow comparisons between categories or over time. Shannons diversity index (H') was computed for each sample. Average values for H' were steady from the pre-cycle sample through to cycle 3 then declined by up o 50% in cycle 4 only to pick up again close to initial values in cycle 5 and the post-cycle fogging.

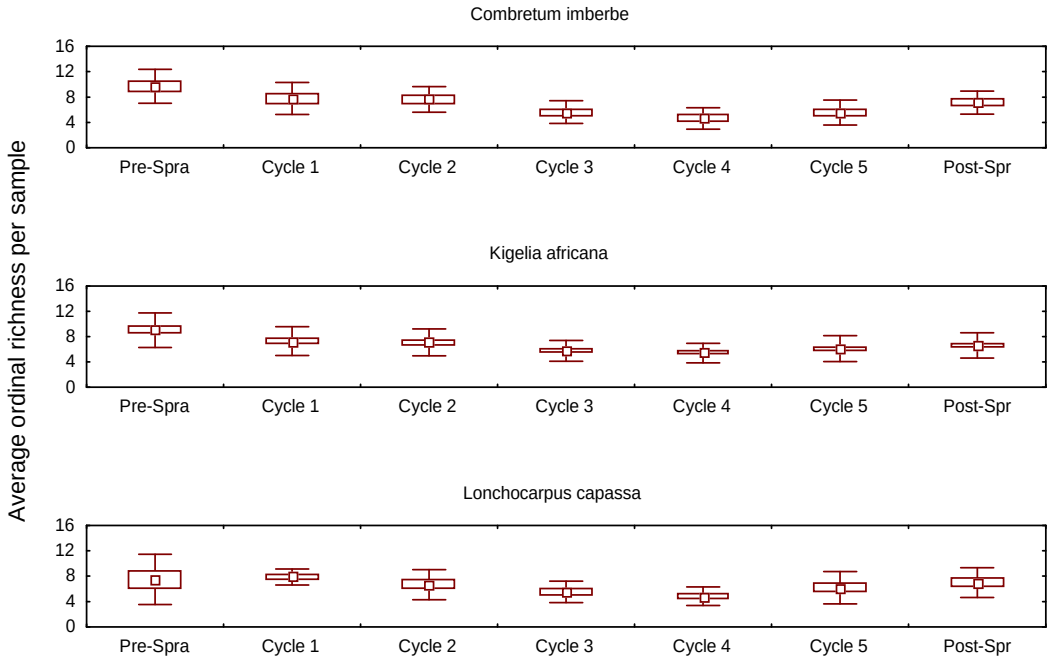


Figure 5.5 Average number of higher taxa per sample in pre-cycle fogging, through the 5 aerial applications and post-cycle fogging on all three tree species.

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5.3.2.3 Compositional change

Another way of visualizing patterns in compositional change is to plot ordinations of the multivariate compositional data in two dimensions. A full description of the ordination approach and hypothesis testing using permutation procedures is given in chapter 3. A non-metric Multi-Dimensional Scaling (nmMDS) plot for higher taxa composition through the cycles sampled from *K. africana* shows that samples from each cycle form quite discrete clusters, particularly for cycle 1 and cycle 2 (Figure 5.6). The pattern form *C. imberbe* is similar with discrete groupings continuing through to cycle 5 (Figure 5.7). Groupings are still apparent for *L. capassa* but the separation is less clear than for the other tree species (Figure 5.8).

The statistical hypothesis that samples from each cycle form discrete clusters on the MDS, i.e. they are, on average, more similar to each other (closer together on the plot) than would be expected if they were distributed randomly, can be tested with the Analysis of Similarities procedure (ANOSIM). On all three tree species, and in almost all cases, samples from each cycle formed a significant group (Table 5.7). In other words samples from the same cycle were more similar to each other in composition than expected by chance. In addition, only a handful of pairwise comparisons were not significant, mostly between the later cycles. This means that the spray events were having a significant influence on the abundance and composition in subsequent samples.

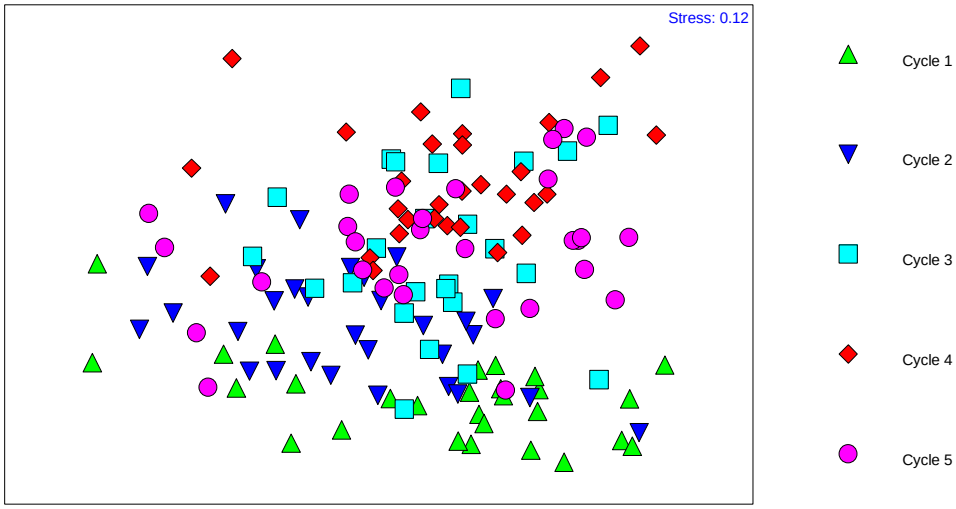

Figure 5.6 nmMDS plot of untransformed ordinal abundance and composition in knockdown samples from *Kigelia africana* trees through the five spray cycles. Each symbol represents one sample from one tree and distance between the samples reflects difference in composition.

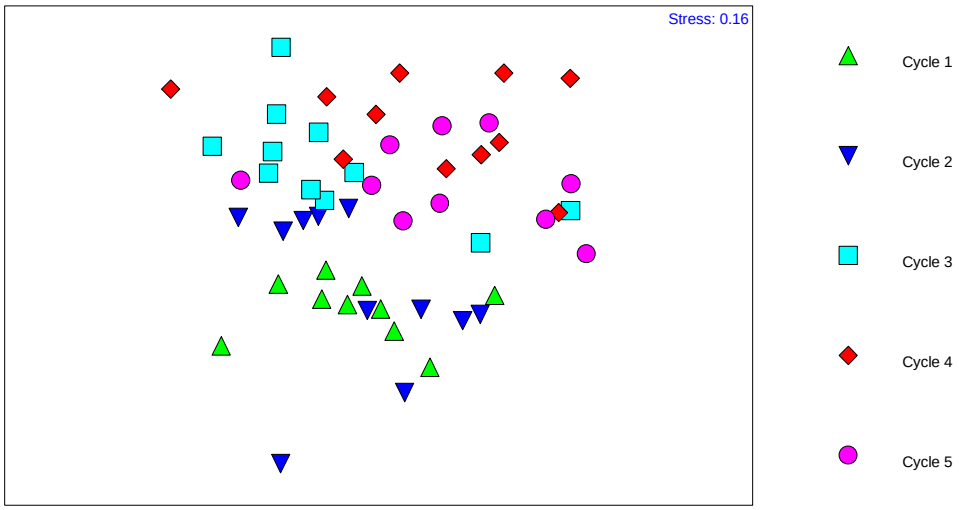

Figure 5.7 nmMDS plot of untransformed ordinal abundance and composition in knockdown samples from *Combretum imberbe* trees through the five spray cycles.

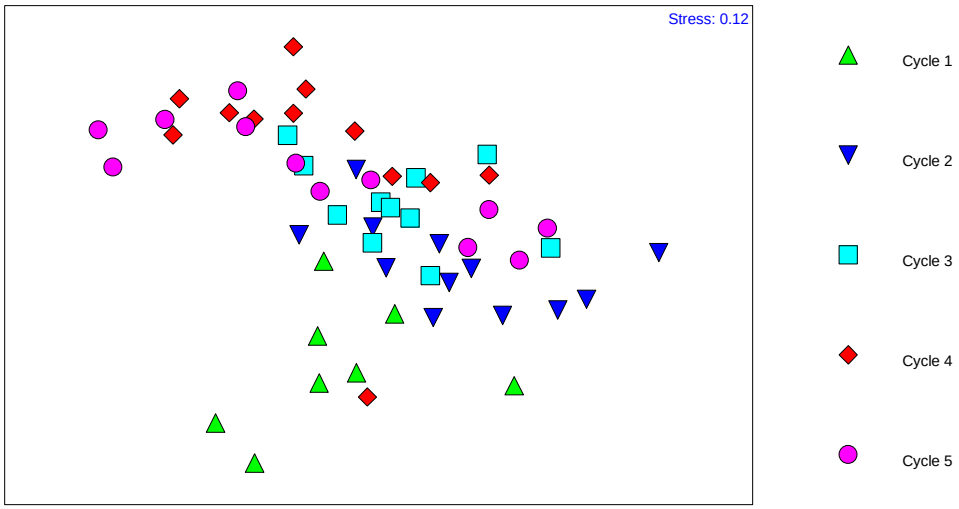

Figure 5.8 nmMDS plot of untransformed ordinal abundance and composition in knockdown samples from *Lonchocarpus capassa* trees through the five spray cycles.

Transformation of the data to presence-absence removes the effect of the number of individuals on the ordination analysis and focuses exclusively on changes in composition. When this is done the ordination results for *K. africana* and *C. imberbe* are similar to those for the raw data but with more pairwise comparisons showing non-significant dissimilarity between them, especially for later cycle comparisons (Table 5.8). In *L. capassa* this effect was strong enough to result in no significant separation of groups overall. The interpretation is that the effect of aerial applications on ordinal diversity patterns is strongest on abundance but composition also changes especially between cycle 1, cycle 2 and the later cycles.

	Analysis of Similarities		
	Global R	Probability	Non-significant comparisons
Raw data			
Kigelia africana	0.237	0.001	C3+C5
Combretum imberbe	0.301	0.001	C1+C2, C4+C5
Lonchocarpus capassa	0.241	0.001	C2+C3, C3+C5, C4+C5
Presence-absence			
Kigelia africana	0.077	0.001	C1+C2, C3+C4, C3+C5, C4+C5
Combretum imberbe	0.118	0.001	C1+C2, C2+C5, C3+C5, C4+C5
Lonchocarpus capassa	0.033	0.134	C1+C2, C1+C5, C2+C3, C2+C4, C2+C5, C3+C4, C4+C5

Table 5.8 Analysis of Similarity (ANOSIM) results testing for overall and pairwise differences in composition between samples from the same cycle on all three tree species using both raw and presence-absence data. Global R is the permutation statistic used to establish if the composition is significantly different between the cycles. The non-significant comparisons are where the composition between two specific cycles is not significantly different.

It is also possible to determine which taxa are responsible for these patterns of change using the SIMPER procedure in PRIMER. On *K. africana*, flies, ants and beetles combine to best characterize similarity within each cycle. These are the taxa that show the largest changes in abundance. The differences (dissimilarity) between cycles, especially between cycle 1 and the remaining cycles, is a result of abundance changes in ants, flies and beetles but also spiders and Hemiptera.

The data for *C. imberbe* show a similar pattern with a slightly stronger contribution of spiders to similarity within cycles. On *L. capassa* wasps and Hemiptera also contribute to similarity within the cycles and a much larger proportion of the total taxa contribute to dissimilarity between the cycles including spiders, wasps, butterflies and mites. These taxa then continue to contribute to dissimilarity between later cycles.

Key Results
• No higher taxa are lost during the cycles • Higher taxa richness per sample declines through cycle 4 then increases slightly in cycle 5 • Diversity changes through the cycles, especially after cycle 2 • Patterns in diversity change are dominated by changes in abundance • Ants, flies and beetles contribute most to differences in composition between cycles

5.3.3 Morphospecies responses from spray cycle and canopy fogging

Morphospecies are not true taxonomic species in the Linnean sense but recognisable taxonomic units at the species level based on easily distinguishable taxonomic features. In most cases and for the majority of invertebrate taxa, morphospecies show more than 90% congruence with true species (Oliver & Beattie, 1996).

5.3.3.1 Beetles

More than 30,000 beetles were identified and represented 22 Families (Table 5.9). The Chrysomelidae and Curculionidae were by far the most abundant taxa making up 73% of the total sorted. At the other end of the abundance spectrum seven families were represented by less than 10 specimens. Morphospecies were identified from 13 of the most abundant Families

< 10 specimens sampled	Between 10 and 300 specimens sampled	> 500 specimens sampled
Elateridae Nitidulidae Ptiliidae Bostrichidae Ptiliidae Zopheridae	***Histeridae*** ***Coccinellidae*** ***Anthribidae*** ***Staphylinidae*** ***Carabidae*** ***Anthicidae*** ***Buprestidae*** Tenebrionidae Melyridae ***Cerambycidae*** ***Scarabaeidae***	***Chrysomelidae*** ***Curculionidae*** ***Brentidae*** ***Anobiidae***

Table 5.9 Infrequent, moderately abundant and abundant Families of beetles sampled from fogging and aerial cycles on the three tree species. Specimens from Families in bold italics were further sorted into morphospecies.

	Pre-cycle fogging	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Post-cycle fogging
Species richness (S)	**74**	**60**	**46**	**27**	**26**	**33**	**46**
Average species per sample	7	6	3	3	2	3	3
Average abundance per sample	208	15	5	5	3	4	15

Table 5.10 Total number of beetle morphospecies (richness), average richness per sample and average number of individuals per sample across all three tree species and cycles.

Across all samples and tree species there were 133 beetle morphospecies recorded from 13 Families sorted. Between 56 and 20% of the total species were recorded from any one spray cycle, which suggests a very high temporal turnover in species composition (Table 5.10). Recall that the cycle data comes from the same trees, method and tray position (see section so to record less than 50% of the total species in any one cycle is surprising.

One explanation for this high turnover in composition between cycles is that new morphospecies are being sampled for the first time in later cycles. Combining the pre-spray and cycle 1 samples generates 97 species meaning that 36 new morphospecies, more than a quarter of the total, are added from cycle 2 through to post-cycle fogging. Obviously some new species would be added as a result of the cumulative increase in sampling effort (more samples generates more species, especially for invertebrate taxa) and the majority of the trees in the post-cycle fogging were not previously sampled and so might be expected to generate some new species. However, for 27% of species to be added in this way was unexpected. Eleven of the beetle morphospecies (8%) were first recorded in the post-cycle fogging. .

Equally likely is that morphospecies are not sampled. A total of 45 morphospecies, 34% of the total, were sampled in either the pre-cycle fogging or in cycle 1 but not thereafter. A further 8 morphospecies were not sampled after cycle 3, 4 morphospecies after cycle 4 and 2 morphospecies in the post-cycle fogging after cycle 5. In all 59 morphospecies (44%) were sampled up to cycle 4 but not in cycle 5 or the post-cycle fogging. It would be important to establish if these species will recover and be sampled again in subsequent years.

On all three tree species, and for both raw and presence absence data, beetle morphospecies from each cycle formed a significant group on MDS plots (Figure 5.9, 5.10 & 5.11). This suggests that overall samples from the same cycle were more similar to each other in composition than expected by chance positioning in the ordination space (Table 5.11). On *K. africana*, where more data was available, these groupings were strong and the only non-significant pairwise comparison was between cycle 3 and cycle 4. On *C. imberbe* and *L. capassa* the differences were mostly between cycle 1, cycle 2 and the remaining samples suggesting that changes were the result of the impact of the first two spray cycles and corresponded to a major decrease in beetle abundance.

On *K. africana*, *Curculionidae*0003 and *Chrysomelidae*0007 were important in characterization of similarity within each cycle. Both these morphospecies showed dramatic declines in abundance from pre-cycle fogging to cycle 2 but remained at low numbers through the other cycles. *Histeridae*0001 and *Histeridae*0002 were at low proportions in the early cycles but persisted at similar abundances through the cycles and became important in group classifications in later cycles. Between 8 and 13 morphospecies were needed to explain 75% of cumulative dissimilarity between cycles.

The data for *C. imberbe* show a similar pattern only with *Chrysomelidae*0001 and *Chrysomelidae*0002 being the important morphospecies. *Histeridae*0002 was important for characterizing cycle 5 and fewer morphospecies (6-9) were needed to explain 75% of cumulative dissimilarity between cycles. On *L. capassa, Histeridae*0002 and *Brentidae*0002 and *Brentidae*0003 were important for group characterization and 6-9 morphospecies were needed to explain 75% of cumulative dissimilarity between cycles.

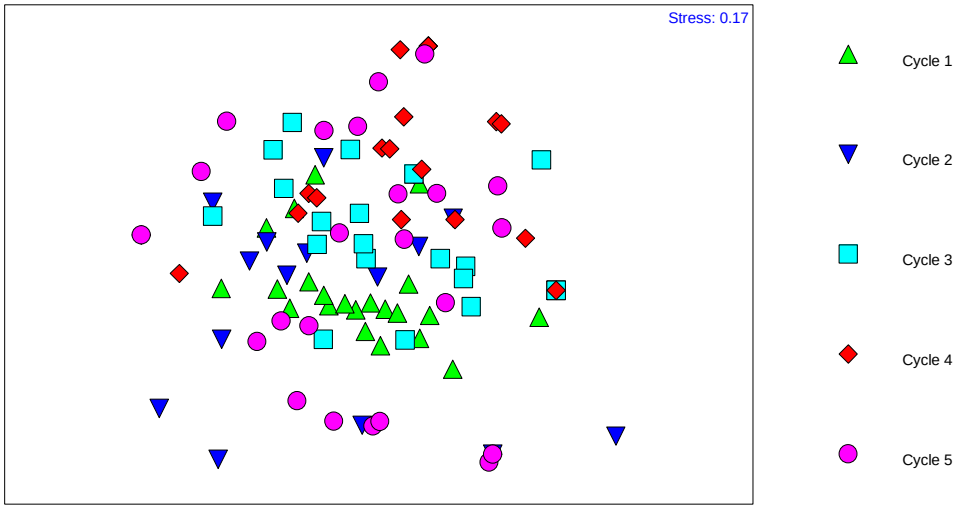

Figure 5.9 nmMDS plot of untransformed beetle morphospecies composition in knockdown samples from *Kigelia africana* trees through the five spray cycles. Each symbol represents one sample from one tree and distance between the samples reflects difference in composition

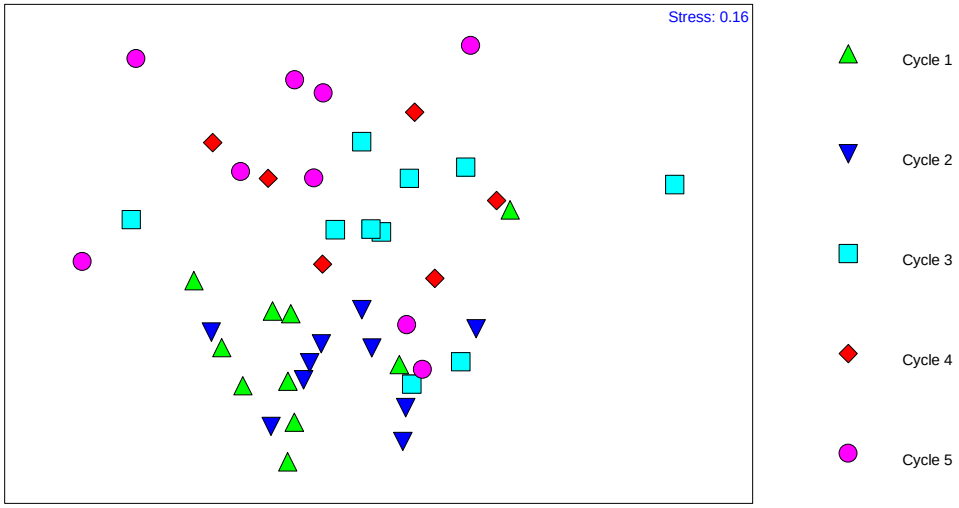

Figure 5.10 nmMDS plot of untransformed beetle morphospecies abundance and composition in knockdown samples from *Combretum imberbe* trees through the five spray cycles.

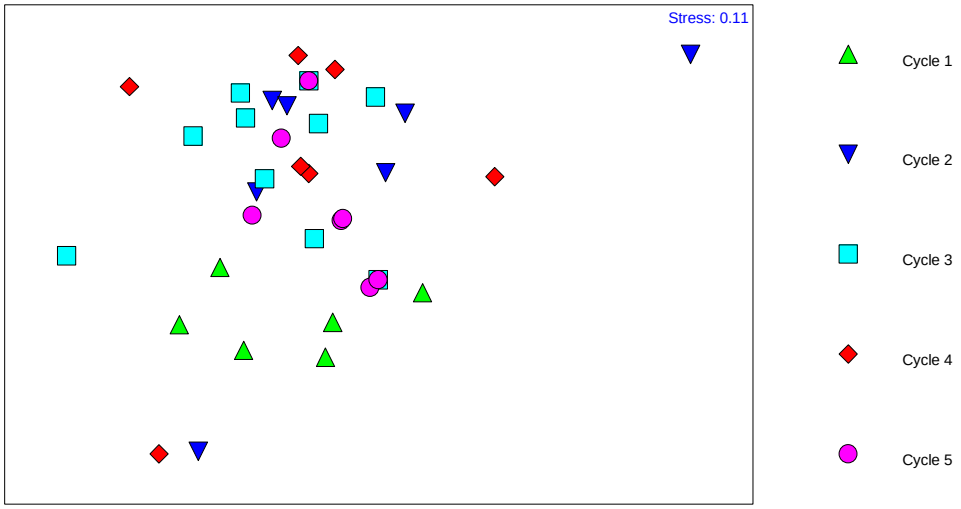

Figure 5.11 nmMDS plot of untransformed beetle morphospecies composition in knockdown samples from *Lonchocarpus capassa* trees through the five spray cycles.

	Analysis of Similarities		
	Global R	Probability	Non-significant comparisons
Raw data			
Kigelia africana	0.160	0.001	C3+C4
Combretum imberbe	0.254	0.001	C3+C5, C3+C4, C4+C5
Lonchocarpus capassa	0.133	0.012	C2+C3, C2+C4, C2+C5, C3+C4, C3+C5,C4+C5
Presence-absence			
Kigelia africana	0.158	0.001	C3+C4
Combretum imberbe	0.172	0.001	C1+C3, C1+C4, C3+C4, C3+C5, C4+C5
Lonchocarpus capassa	0.144	0.005	C2+C3, C2+C4, C2+C5, C3+C4, C3+C5,C4+C5

Table 5.11 Analysis of Similarity (ANOSIM) results testing for overall and pairwise differences in beetle morphospecies composition between samples from the same cycle on all three tree species using both raw and presence-absence data.

Key Results
• 30,000 specimens were recorded representing 22 families • 133 morphospecies were identified from 13 most abundant families • 36 morphospecies (25% of total) were first recorded after cycle 1 • 45 morphospecies (34%) were sampled in pre-cycle canopy fogging and/or cycle 1 but not thereafter • there was a change in abundance and composition after cycle 2 • a large number of beetle morphospecies that were present in low numbers contributed to the characterization of a group within the ordinations

5.3.3.2 Ants

Across all samples and tree species there were 35 ant morphospecies recorded. Between 71% and 56% of the total species were recorded from any one cycle, significantly less turnover than for beetles. Only three or four species on average were recorded in any one sample (Table 5.12).

Four morphospecies, *Polyrhachis*0004 (3 individuals), Tetramorium0005 (4), *Cataulacus*0002 (7) and *Acropyga*0002 (5) were first sampled after cycle 1. None of these were recorded in significant numbers.

Only 4 morphospecies, 11% of the total, were sampled in either the pre-cycle fogging or in cycle 1 but not thereafter. *Acantholepis*0001 (379 individuals), *Crematogaster*0007 (51) and *Camponotus*0018 (23) were initially in significant numbers and samples in more than one sample, whilst *Acantholepis*0002 (5) was infrequent.

	Pre-cycle fogging	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Post-cycle fogging
Species richness (S)	**23**	**25**	**22**	**21**	**21**	**22**	**29**
Average species per sample	4	4	3	3	3	4	3
Average abundance per sample	66	70	26	22	12	29	38

Table 5.12 Total number of morphospecies (richness), average richness per sample and average number of individuals per sample of ant morphospecies across all three tree species and cycles.

Ant morphospecies from each cycle formed significant groupings on MDS plots for *K. africana* (Figure 5.12), for *C. imberbe* but only for presence-absence data (Figure 5.13) and for *L. capassa* (Figure 5.14). This suggests that overall samples from the same cycle were more similar to each other in composition than expected by chance positioning in the ordination space (Table 5.13).

The biggest differences between cycles were between cycle 1 and the remainder with cycle 2, cycle 3 and cycle 4 having few pairwise differences on any of the tree species.

In samples from *K. africana*, one morphospecies (*Cataulacus*0001) contributed most to within group similarity and three morphospecies (*Cataulacus*0001, *Camponotus*0015, *Phiedole*0009) contributed consistently to between group dissimilarity. Only 3 to 5 morphospecies were needed to contribute 75% of between group dissimilarity. A similar pattern was apparent on *L. capassa* but with the same three species (*Camponotus*0015, *Phiedole*0009, *Melissotarsus*0001) characterising similarity within cycle groups and dissimilarity between groups.

In samples from *C. imberbe* untransformed data showed no significant groupings and the presence-absence data transformation resulted in 7 to 10 morphospecies being needed to account for 75% of between group dissimilarity.

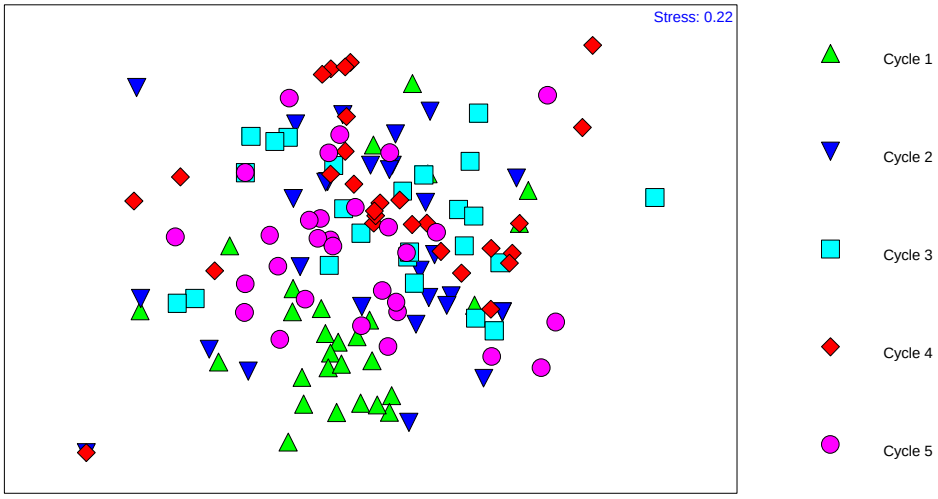

Figure 5.12 nmMDS plot of untransformed ant morphospecies abundance and composition in knockdown samples from *Kigelia africana* trees through the five spray cycles.

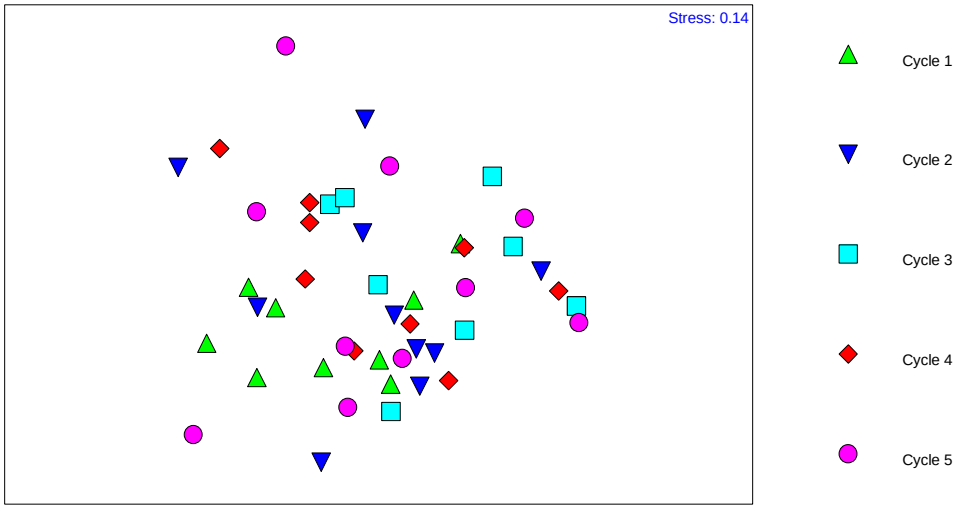

Figure 5.13 nmMDS plot of untransformed ant morphospecies abundance and composition in knockdown samples from *Combretum imberbe* trees through the five spray cycles.

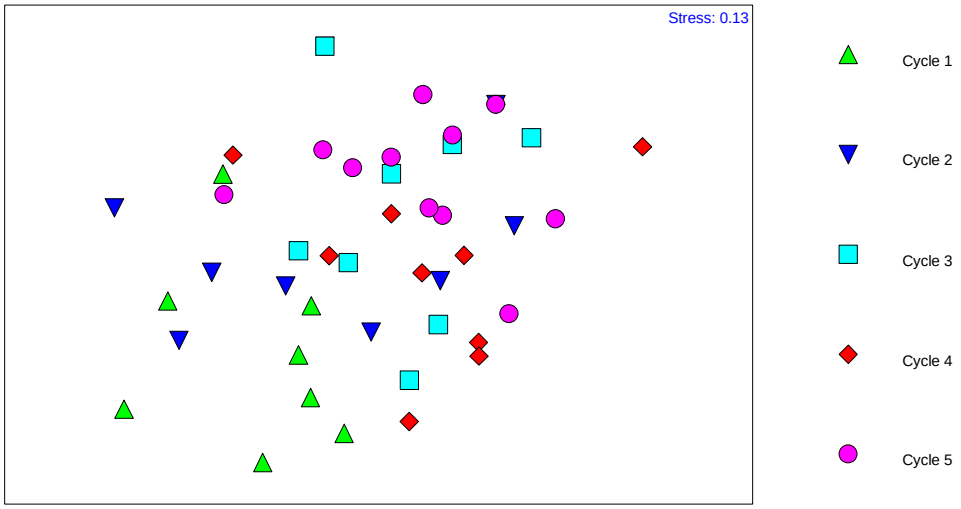

Figure 5.14 nmMDS plot of untransformed ant morphospecies abundance and composition in knockdown samples from *Lonchocarpus capassa* trees through the five spray cycles.

	Analysis of Similarities Global R	Probability	Non-significant comparisons
Raw data			
Kigelia africana	0.095	0.001	C2+C3, C2+C4, C2+C5, C3+C4, C3+C5
Combretum imberbe	0.018	0.306	All
Lonchocarpus capassa	0.160	0.004	C1+C2, C2+C3, C2+C4, C3+C4, C3+C5, C4+C5
Presence-absence			
Kigelia africana	0.069	0.001	C1+C2, C1+C5, C2+C3, C2+C5, C3+C4, C3+C5
Combretum imberbe	0.051	0.087	C1+C5, C2+C3, C2+C4, C2+C5, C3+C4, C3+C5, C4+C5
Lonchocarpus capassa	0.147	0.001	C1+C2, C2+C3, C2+C4, C2+C5, C3+C4, C3+C5

Table 5.13 Analysis of Similarity (ANOSIM) results testing for overall and pairwise differences in ant morphospecies composition between samples from the same cycle on all three tree species using both raw and presence-absence data.

Key Results
• 17,603 specimens were sampled and identified to 35 morphospecies • 4 morphospecies (11%) were sampled in pre-cycle canopy fogging and/or cycle 1 but not thereafter • The biggest change in composition occurred after cycle 2

5.3.3.3 Spiders

A total of 23 spider morphospecies were identified from the knockdown and fogging samples on *K. africana, C. imbrebe* and *L. capassa*. Spiders are difficult to identify to morphospecies unless they are adults and preferably adult males. Only the target families suspected to be vulnerable to deltamethrin applications were identified to morphospecies.

In the knockdown samples many of the spiders were juveniles and very difficult to determine even for experienced specialist taxonomists. Adults were also recorded in low numbers, averaging only one per sample through the middle cycles, hence species numbers were inevitably low. Between 52 and 19% of the total species were recorded from any one cycle, suggesting significant turnover in composition between cycles.

Overall richness declined by one half during the cycles (Table 5.14) and seven morphospecies sampled in the pre-cycle fogging or in cycle 1 were not sampled again. Two more were not recorded after cycle 4 and another after cycle 5 (Table 5.15).

Five morphospecies, close to one fifth of the total, *Oxyopidae*0008 (2), *Salticidae*0013 (1), *Salticidae*0014 (4), *Theridiidae*0002 (4) and *Theridiidae*0003 were first sampled after cycle 1.

	Pre-cycle Fogging	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Post-cycle fogging
Species richness (S)	**12**	**11**	**4**	**4**	**4**	**5**	**7**
Average species per sample	2	1	1	1	1	1	2
Average abundance per sample	3	2	1	1	1	1	2

Table 5.14 Total number of morphospecies (richness), average richness per sample and average abundance per sample of spider morphospecies across all three tree species and cycles.

Not sampled after Cycle 1		Not sampled after Cycle 4		Not sampled after Cycle 5	
Salticidae0001	8	Thomisidae0001	1	Oxyopidae0006	4
Thomisidae0009	3	Oxyopidae0005	2		
Salticidae0001	2				
Oxyopidae0004	1				
Theridiidae0001	2				
Oxyopidae0007	1				
Thomisidae0002	1				

Table 5.15 Spider morphospecies sampled in either pre-cycle fogging or in cycle 1 knockdown but not sampled thereafter. Numbers are total catches for that morphospecies.

Only samples from *K. africana* generated sufficient data for MDS and ANOSIM analyses. Even though data were sparse the overall groupings were significant (ANOSIM, Global $R=0.329$, $P<0.001$) mostly due to a strong cluster in cycle 1 (Figure 5.15). Between group comparisons other than with cycle 1 were not significant. Comparisons between pre-cycle and post-cycle canopy fogging samples were not significant ($R=-0.131$, $P=0.826$, Figure 5.16).

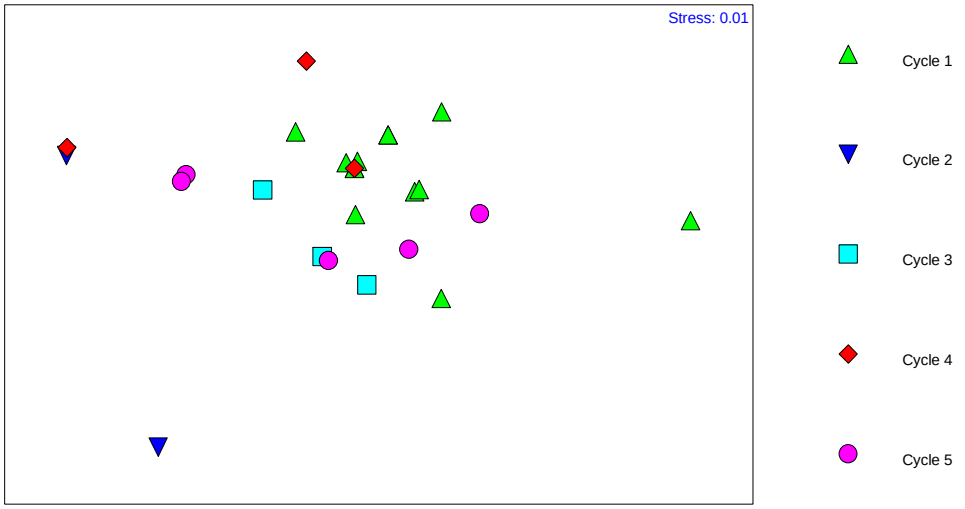

Figure 5.15 nmMDS plot of untransformed spider morphospecies abundance and composition in knockdown samples from *Kigelia africana* trees through the five spray cycles.

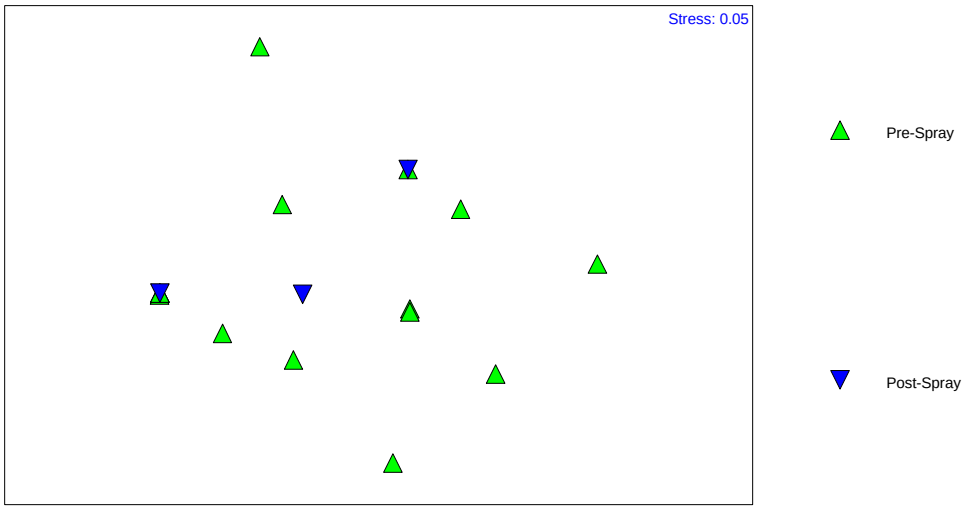

Figure 5.16 nmMDS plot of untransformed spider morphospecies abundance and composition in pre-cycle and post-cycle canopy fogging samples from *Kigelia africana* trees.

Key Results
• 1691 specimens from 4 Families known to be vulnerable were identified to 23 morphospecies • 7 morphospecies (30%) were sampled in pre-cycle canopy fogging and/or cycle 1 but not thereafter • Richness declined by a half through the cycles • Most change in composition and abundance occurred between cycle 1 and cycle 2.

5.3.3.4 Flies

A total of 649 fly specimens from 32 families (Table 5.16) were identified to morphospecies. This is a rich fauna at Family level and generated 117 morphospecies. Families with the largest number of individuals were not identified to morphospecies. Between 36% and 21% of the total morphospecies were recorded from any one cycle, however, only two or three morphospecies on average were recorded in any one sample (Table 5.17).

Families of flies identified to morphospecies		
Acroceridae	Stratiomyiidae	Scatopsidae
Lauxaniidae	Calliphoridae	Tanyderidae
Sepsidae	Platypezidae	Empididae
Anthomyiidae	Syrphidae	Scenopinidae
Muscidae	Cryptochetidae	Tephritidae
Simuliidae	Psychodidae	Ephydridae
Silidae	Tabanidae	Sciaridae
Phoridae	Culicidae	Therevidae
Sphaeroceridae	Sarcophagidae	Tipulidae
Bombyliidae	Tachiniade	
Pipunculidae	Dolichopodidae	

Table 5.16 Families of flies identified to morphospecies

	Pre-cycle Fogging	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Post-cycle fogging
Species richness (S)	**40**	**42**	**36**	**24**	**26**	**37**	**40**
Average species per sample	3	3	2	2	2	3	1
Average abundance per sample	4	3	3	2	2	3	1

Table 5.17 Total richness, average richness per sample and average abundance per sample of fly morphospecies across all three tree species and cycles.

Even though not all fly families that contributed to the increase in abundance through the cycles were identified to morphospecies there was considerable turnover in composition through the cycles. 30 morphospecies were not sampled after cycle 1, 18 morphospecies after cycle 2, 6 morphospecies after cycle 3, 5 morphospecies after cycle 4 and 18 morphospecies after cycle 5 (Table 5.18).

Some 16 morphospecies were sampled for the first time after cycle 2, 12 after cycle 3, 8 after cycle 4 and 11 after cycle 5 (Table 5.19). In most cases, however, these losses and additions were for morphospecies represented by just one specimen. This is an ongoing problem of assessment when the bulk of the diversity is represented by taxa with low overall abundance.

Not sampled after Cycle 1		Not sampled after Cycle 2		Not sampled after Cycle 3	
Phoridae0007	14	Sarcophagidae0002	14	Acroceridae0005	1
Calliphoridae0011	11	Dolichopodidae0002	14	Dolichopodidae0007	1
Muscidae0010	6	Sarcophagidae0001	7	Ephydridae0009	1
Calliphoridae0001	4	Muscidae0024	6	Phoridae0005	1
Acroceridae0004	3	Muscidae0021	5	Tachinidae0009	1
Stratiomyiidae0002	2	Muscidae0019	3	Tephritidae0010	1
Dolichopodidae0005	2	Calliphoridae0013	3		
Muscidae0011	2	Tephritidae0009	2		
Phoridae0008	2	Lauxaniidae0004	2		
Sepsidae0003	2	Sarcophagidae0005	2	6	
Calliphoridae0007	2	Bombyliidae0002	1		
Calliphoridae0010	2	Ephydridae0005	1		
Muscidae0001	2	Muscidae0012	1		
Acroceridae0002	1	Phoridae0001	1		
Asilidae0005	1	Platypezidae0001	1		
Dolichopodidae0004	1	Tabanidae0002	1		
Muscidae0017	1	Tachinidae0001	1		
Muscidae0025	1	Tachinidae0010	1		
Sepsidae0002	1				
Tachinidae0005	1				
Calliphoridae0012	1	18			
Lauxaniidae0002	1				
Muscidae0022	1				
Pipunculidae0002	1				
Psychodidae0001	1				
Sarcophagidae0003	1				
Stratiomyiidae0004	1				
Syrphidae0007	1				
Tephritidae0004	1				
Tipulidae0001	1				
30					

Not sampled after Cycle 4		Not sampled after Cycle 5	
Tephritidae0003	7	Muscidae0018	18
Sarcophagidae0004	8	Ephydridae0007	15
Calliphoridae0014	1	Muscidae0029	14
Syrphidae0009	1	Syrphidae0008	13
Tachiniade0008	1	Culicidae0002	10
		Tephritidae0002	7
		Tachinidae0006	5
		Bombyliidae0003	5
		Sarcophagidae0006	4
		Sciaridae0001	4
		Stratiomyiidae0003	4
		Scenopinidae0002	3
		Scenopinidae0001	2
		Asilidae0011	1
		Bombyliidae0008	1
		Bombyliidae0012	1
		Tephritidae0001	1
		Tephritidae0005	1
5		18	

Table 5.18 Fly morphospecies not sampled again after each cycle. Numbers at the bottom of each column are the total number for that cycle.

First sampled Cycle 2	First sampled Cycle 3	First sampled Cycle 4	First sampled Cycle 5
Culicidae0001	Simuliidae0004	Therevidae0001	Muscidae0029
Simuliidae0003	Simuliidae0001	Scenopinidae0006	Sarcophagidae0006
Muscidae0029	Tephritidae0003	Scenopinidae0002	Ephydridae0011
Culicidae0002	Bombyliidae0003	Tabanidae0003	Stratiomyiidae0007
Stratiomyiidae0006	Stratiomyiidae0007	Scenopinidae0001	Pipunculidae0001
Sarcophagidae0006	Acroceridae0005	Calliphoridae0014	Stratiomyiidae0005
Tephritidae0009	Dolichopodidae0007	Syrphidae0009	Asilidae0011
Scatopsidae0001	Ephydridae0009	Tachiniade0008	Bombyliidae0008
Bombyliidae0002	Phoridae0005		Bombyliidae0012
Ephydridae0005	Tachinidae0009		Tephritidae0001
Muscidae0012	Tephritidae0010		Tephritidae0005
Phoridae0001			
Platypezidae0001			
Tabanidae0002			
Tachinidae0001			
Tachinidae0010			
16	12	8	11

Table 5.19 Fly morphospecies sampled for the first time **after** each cycle.

Fly morphospecies from each cycle formed significant groupings on MDS plots for all three tree species (Figure 5.17, 5.18, 5.19). This suggests that overall samples from the same cycle were more similar to each other in composition than expected by chance positioning in the ordination space (Table 5.20).

Except for the middle cycles 3 and 4, most of the pairwise comparisons were significant, although cycle 1 and cycle 2 also showed some similarity. Because of the generally low numbers of individuals per morphospecies the presence-absence data transformation produced very similar results to the untransformed data. Pre-cycle and post-cycle fogging comparisons were significant for *K. africana* (ANOSIM, Global R=0.049, P=0.005), *C. imberbe* (R=0.100, P=0.023) but not *L. capassa* (R=0.121, P=0.078)

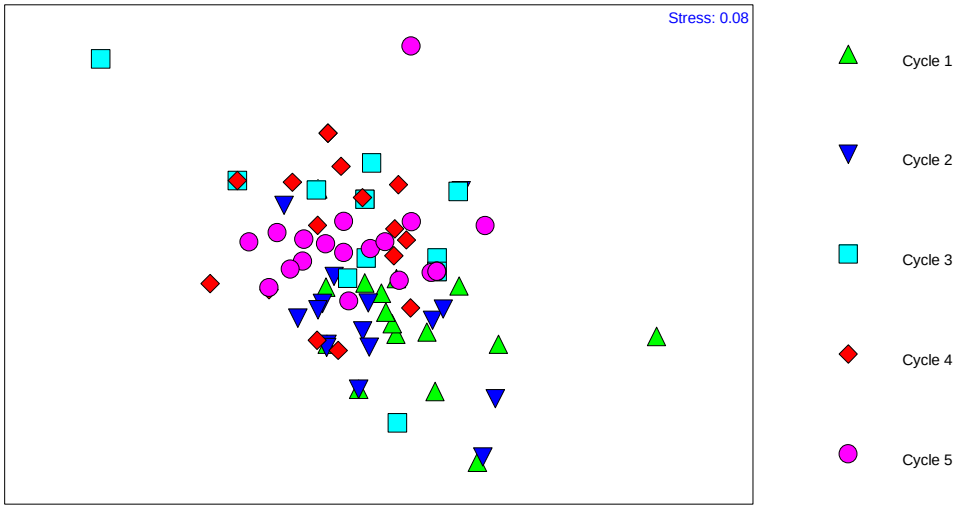

Figure 5.17 nmMDS plot of untransformed fly morphospecies abundance and composition in knockdown samples from *Kigelia africana* trees through the five spray cycles.

	Analysis of Similarities		
	Global R	Probability	Non-significant comparisons
Raw data			
Kigelia africana	0.075	0.001	C1+C2, C3+C4, C4+C5
Combretum imberbe	0.192	0.015	C1+C2, C2+C3, C1+C5, C2+C3, C3+C5, C4+C5
Lonchocarpus capassa	0.477	0.001	C1+C3, C3+C4
Presence-absence			
Kigelia Africana	0.077	0.001	C1+C2, C3+C4, C3+C5, C4+C5
Combretum imberbe	-	-	-
Lonchocarpus capassa	0.477	0.001	C1+C3, C3+C4

Table 5.20 Analysis of Similarity (ANOSIM) results testing for overall and pairwise differences in fly morphospecies composition between samples from the same cycle on all three tree species using both raw and presence-absence data.

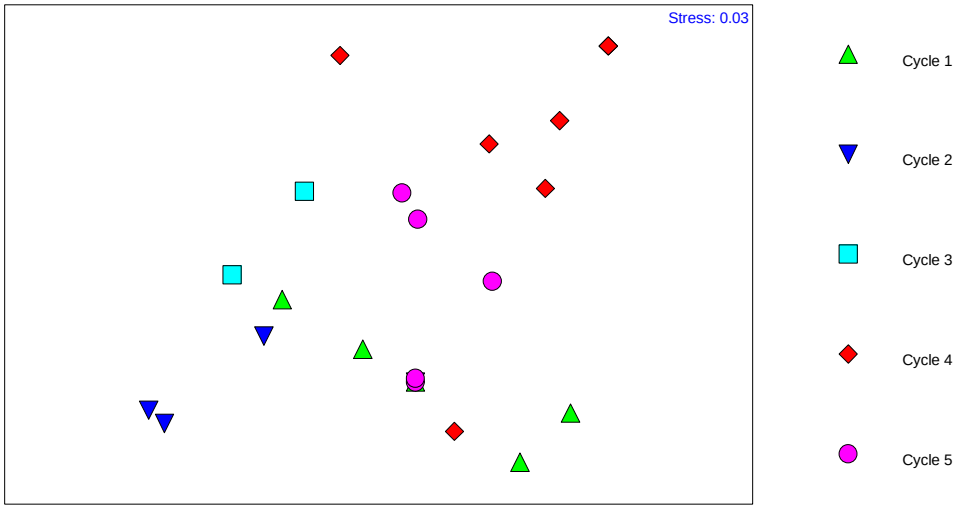

Figure 5.18 nmMDS plot of untransformed fly morphospecies abundance and composition in knockdown samples from *Combretum imberbe* trees through the five spray cycles.

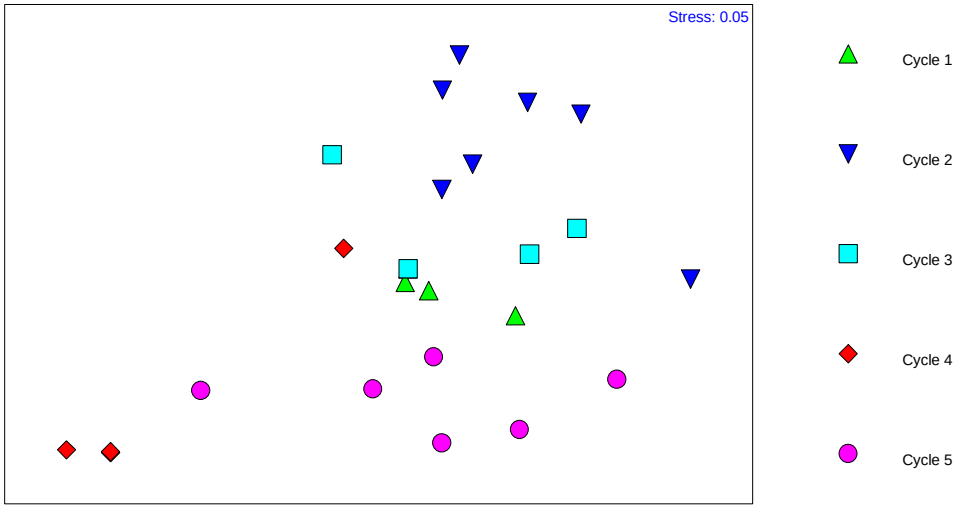

Figure 5.19 nmMDS plot of untransformed fly morphospecies abundance and composition in knockdown samples from *Lonchocarpus capassa* trees through the five spray cycles.

Key Results
• 117 morphospecies were identified from 649 specimens from 32 Families • 30 morphospecies (26%) were sampled in pre-cycle canopy fogging and/or cycle 1 but not thereafter and 77 morphospecies were only sampled occasionally hence the problem of small sample size is especially important for flies • 47 morphospecies (40%) were first sampled after cycle 1 • There were large differences in composition between most cycles

5.3.3.5 Hemiptera

A total of 774 specimens of Hemiptera from 12 families were identified to 59 morphospecies. Between 41% and 14% of the total morphospecies were recorded from any one cycle, however, only one or two morphospecies on average were recorded in any one sample after cycle 1 (Table 5.21).

More than one third of the morphospecies (22) representing 10 of the 12 Families were present in almost all the cycles including the post-cycle fogging. 16 of the morphospecies declined significantly in numbers of individuals (abundance) through the cycles losing on average 57% of their combined pre-cycle fogging and cycle 1 abundance. There were 6 morphospecies that increased in abundance through the cycles by an average of 182%.

	Pre-cycle Fogging	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Post-cycle fogging
Species richness (S)	**27**	**24**	**22**	**8**	**15**	**19**	**14**
Average species per sample	2	3	1	1	1	2	1
Average abundance per sample	9	9	2	1	1	2	1

Table 5.21 Total number of morphospecies (richness), average richness per sample and average number of individuals (abundance) per sample of Hemiptera morphospecies across all three tree species and cycles.

There was considerable turnover in morphospecies composition through the cycles due to 15 morphospecies that were not sampled after cycle 1, 12 morphospecies after cycle 2, 3 morphospecies after cycle 3, 5 morphospecies after cycle 4 and 10 morphospecies after cycle 5 (Table 5.22). There were also 22 morphospecies that were sampled for the first time after cycle 1 (Table 5.23). In most cases, however, these losses and additions were for morphospecies represented by just one specimen.

Not sampled after Cycle 1	Not sampled after Cycle 2	Not sampled after Cycle 3
Membracidae0002 Membracidae0003 Cicadellidae0022 Reduviidae0006 Reduviidae0001 Membracidae0001 Reduviidae0005 Cicadellidae0004 Cicadellidae0005 Cicadellidae0012 Reduviidae0004 Reduviidae0001 Triozidae0003 Membracidae0004 Reduviidae0007 15	Aphididae0002 Cicadellidae00013 Saldidae0001 Tingidae0002 Delphacidae0004 Cicadellidae0033 Cicadellidae0019 Cicadellidae0003 Cicadellidae0031 Membracidae0005 Reduviidae0012 Tingidae0005 12	Saldidae0002 Cicadellidae0006 Cicadellidae0002 3

Not sampled after Cycle 4	Not sampled after Cycle 5
Cicadellidae0009 Cicadellidae0034 Cicadellidae0035 Triozidae0001 Triozidae0002 5	Tingidae0001 Derbidae0001 Tingidae0003 Cicadellidae0015 Reduviidae0003 Cicadellidae0008 Cicadellidae0026 Delphacidae0001 Cicadellidae0025 Saldidae0003 10

Table 5.22 Hemiptera morphospecies not sampled again after each cycle. Numbers at the bottom of each column are the total number for that cycle.

First sampled Cycle 2	
Cydnidae0001	Reduviidae0002
Cydnidae0002	Membracidae0005
Cicadellidae0006	Flatidae0001
Delphacidae0001	Cicadellidae0035
Cicadellidae0026	Cicadellidae0034
Triozidae0002	Cicadellidae0031
Triozidae0001	Cicadellidae0003
Tingidae0005	Cicadellidae0002
Tingidae0004	Cicadellidae0019
Saldidae0003	Cicadellidae0017
Reduviidae00012	Cicadellidae0009

Table 5.23 Hemiptera morphospecies sampled for the first time **after** each cycle.

Hemiptera morphospecies from each cycle formed significant groupings on MDS plots for all three tree species (Figure 5.20). This suggests that overall samples from the same cycle were more similar to each other in composition than expected by chance positioning in the ordination space (Table 5.24).

Except for the middle cycles 3 and 4, most of the pairwise comparisons were significant, although cycle 1 and cycle 2 also showed some similarity. Because of the generally low numbers of individuals per morphospecies the presence-absence data transformation produced very similar results to the untransformed data. Pre-cycle and post-cycle fogging comparisons were significant for *K. africana* (ANOSIM, Global R=0.049, P=0.005), *C. imberbe* (R=0.100, P=0.023) but not *L. capassa* (R=0.121, P=0.078)

	Analysis of Similarities		
	Global R	Probability	Non-significant comparisons
Raw data			
Kigelia africana	0.330	0.001	C2+C3, C2+C5, C3+C4, C3+C5, C4+C5
Combretum imberbe	0.072	0.183	All
Lonchocarpus capassa	0.128	0.079	All
Presence-absence			
Kigelia africana	0.316	0.001	C2+C3, C2+C5, C3+C4, C3+C5, C4+C5
Combretum imberbe	0.049	0.278	All
Lonchocarpus capassa	0.116	0.101	All

Table 5.24 Analysis of Similarity (ANOSIM) results testing for overall and pairwise differences in Hemiptera morphospecies composition between samples from the same cycle on all three tree species using both raw and presence-absence data.

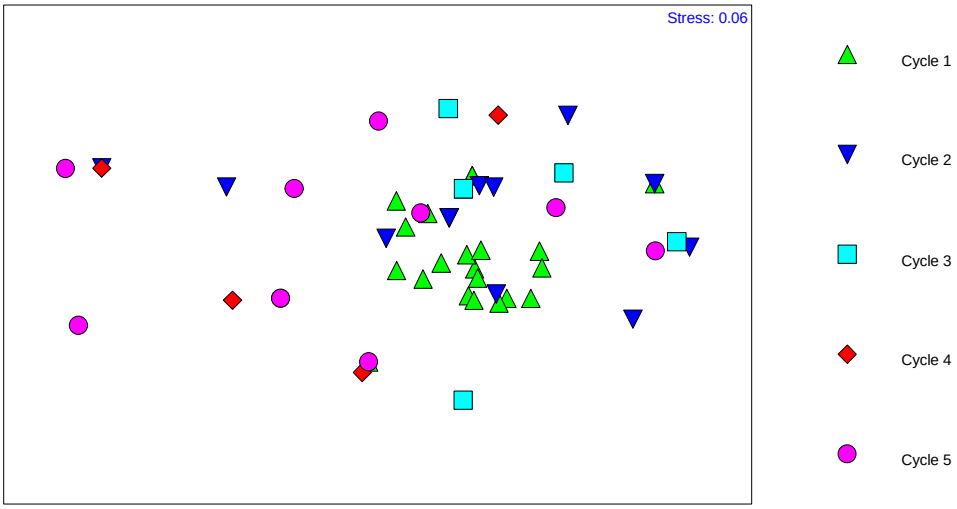

Figure 5.20 nmMDS plot of untransformed Hemiptera morphospecies abundance and composition in knockdown samples from *Kigelia africana* through the five spray cycles.

Key Results
• 59 morphospecies were identified from 774 specimens from 12 Families • 30 morphospecies (51%) were sampled in pre-cycle canopy fogging and/or cycle 1 but not thereafter and 22 morphospecies (37%) were first sampled after cycle 1 • There were large differences in composition between cycle 1 and the remaining cycles on *K. africana* and no differences in composition through the cycles on the other tree species

5.3.4 Knockdown–fogging within the spray cycles

The idea of sampling using canopy fogging within the spray cycles was to determine if the aerial application of deltamethrin actually knocked down all the invertebrates in a tree canopy. Three key comparisons are possible with the design:

1. invertebrates knocked down by the spray from a tree fogged 24 hours earlier
2. invertebrates knocked down by canopy fogging 24 hours after a spray application and
3. invertebrates knocked down by the spray from a tree previously fogged or not fogged

Unfortunately difficulties with logistics prevented the fogging of trees prior to cycle 1 so Table 5.25 represents total number of individuals (abundance) of all invertebrates for cycle 2 to 5. Data in this table represent the abundance of all invertebrates sampled in knockdown trays enabling comparisons between individual trees to be made down the columns of the table. For example, the first column for cycle 2 shows a return of 11 specimens from fogging and 16 specimens from subsequent spraying of the same tree and 14 and 30 specimens from a nearby tree. In this manner pairwise comparisons can be made throughout the tables.

Cycle 2			
Fogged	11 14	Not fogged	
Sprayed	16 30	Sprayed	22 43 7
Not fogged		Fogged	16 13 36

Cycle 3			
Fogged	152 218 128	Not fogged	
Sprayed	103 45 17	Sprayed	21 39 20
Not fogged		Fogged	29 67 31

Cycle 4			
Fogged	415 419 464	Not fogged	
Sprayed	203 53 240	Sprayed	48 83 38
Not fogged		Fogged	381 371 121

Cycle 5			
Fogged	79 202	Not fogged	
Sprayed	152 99	Sprayed	82 20 293
Not fogged		Fogged	75 50 188

Table 5.25 Number of individuals (abundance) of all invertebrates sampled beneath *K. africana* trees in a study area near Baboon Camp. Each data point is one sample and each column of figures represents one tree so that comparisons of numbers down the table are the most relevant.

Again the results are complicated somewhat by the increase in abundance of flies as the season progressed but the general trend is for canopy fogging to be a more efficient sampling method. Catches are generally greater for canopy fogging catches even when the tree had received several aerial sprays.

Comparison of catches from aerial sprays were either similar or slightly less than in trees that had not been fogged 24 hours before. This is the reverse of the expectation that canopy fogging might seriously deplete the invertebrates exposed to aerial applications.

Comparisons of compositional differences show similar results. ANOSIM testing for differences in composition between samples taken as knockdown from aerial applications on trees that had previously been fogged versus adjacent trees that had not been fogged were not significant for higher taxa (Global R=0.013, P=0.331). There was also no significant difference between samples from fogging and the subsequent spray cycle (R=-0.023, P=0.588). There was, however, a significant change in composition between samples from the spray cycle and post-cycle canopy fogging (R=0.134, P=0.022, Figure 5.21). Changes in the abundance of flies, ants and beetles accounted for the bulk of this difference. Much of this may be because canopy fogging appears to be a more efficient sampling method.

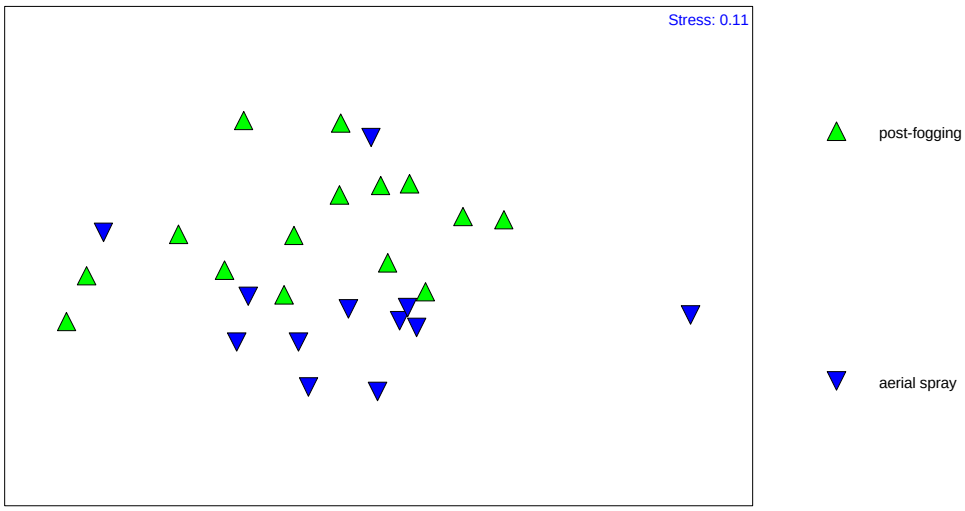

Figure 5.21 nmMDS plot of untransformed higher taxa composition in immediate post-cycle fogging and knockdown samples from aerial applications from *Kigelia africana* trees near Baboon Camp.

Key Results
• Canopy fogging with deltamethrin appears to be a more efficient sampling method than aerial application • When a tree is fogged not all the invertebrates are killed and there were still invertebrates in the tree canopies after the five spray cycles • Canopy fogging does not reduce subsequent catches from aerial applications • The composition of higher taxa is not affected by pre-cycle fogging

5.3.5 Invertebrate mortality in knockdown

Not all the invertebrates that are knocked down are dead, many recover and can return to the canopy. Comparison of samples collected on cotton sheets impregnated with insecticide allowed a field-based estimation of the proportion of individuals that might recover. Strong winds during many of the cycles disturbed the cotton sheets and an unknown number of specimens were lost.

There were no significant differences in the average number of individuals between treated and untreated sheets for total catches or for any of the five most abundant orders (ANOVA, F values all < 3.0, P>0.08). The only significant pattern was the increase in the number of individual flies caught through the cycles.

The average number of higher taxa sampled from the treated sheets was generally greater than from the untreated (Figure 5.22). Four taxa, Mantodea, Neuroptera, Orthoptera and Pseudoscorpionida were only sampled from the treated sheets.

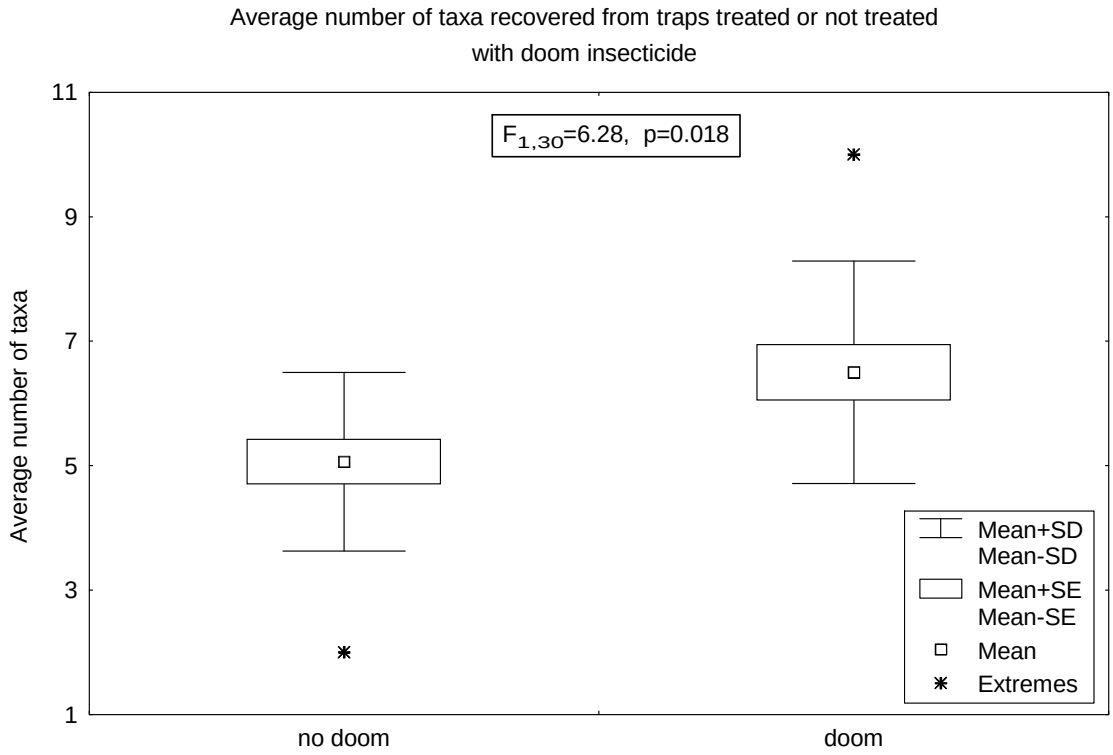

Figure 5.22 Average number of higher taxa per sample from cotton tray with and without insecticide.

More beetles were sampled from treated sheets than untreated but the same morphospecies were sampled on treated and untreated sheets. Eleven fly morphospecies were only recorded from treated trays, nearly half the total identified from this sampling method (Table 5.26). Three of these morphospecies (in bold italic in Table 5.26) were not recorded in any other knockdown samples. The reverse pattern was seen for ants with eight morphospecies (44% of total) not sampled from treated sheets.

Fly morphospecies sampled from insecticide treated sheets
Calliphoridae0001 ***Culicidae0003*** Muscidae0017 ***Muscidae0029*** Muscidae0028 Pipunculidae0001 Syrphidae0008 Tabanidae0003 ***Empididae0005*** Empididae0003 Muscidae0018

Table 5.26 Fly morphospecies recorded from knockdown sheets treated with insecticide (Doom). Morphospecies in bold were only recorded with this method.

Key Results
• Insecticide treated trays captured on average 20% higher taxa richness than untreated trays • Mantodea, Neuroptera, Orthoptera and Pseudoscorpionida were only sampled from the treated trays • 11 fly morphospecies were only sampled from treated trays and three of these were not sampled in any other method

5.3.6 Malaise trap samples

Only one malaise trap was available and this had to be moved during cycle 1 due to a bush fire. Specimens were captured for three days prior to and three days after the spray event on all other cycles.

More than 7,500 individuals, mostly flies, were sampled and there was a significant difference in average total catches between cycles (ANOVA, $F_{3,28}$=5.9, P=0.003) and generally fewer caught after the spray event than before ($F_{1,28}$=7.9, P=0.009). However, the increase in fly activity through the season meant that abundance prior to a subsequent spray event was greater than in the days following the current event (Figure 5.23). There were no significant trends in any of the other taxa.

A total of 72 fly morphospecies from selected Families were identified from malaise trap samples. Average richness per sample declined from 7 in pre-spray to 5.5 in post-spray samples but this decline was not significant (ANOVA, F=1.4, P>0.1).

The higher taxa composition changed significantly over the cycles (ANOSIM, R=0.316, P<0.001) almost entirely due to increases in the abundance of flies (Figure 5.24). The decline in catches of flies after each spray resulted in a significant effect on composition (R=0.155, P=0.044).

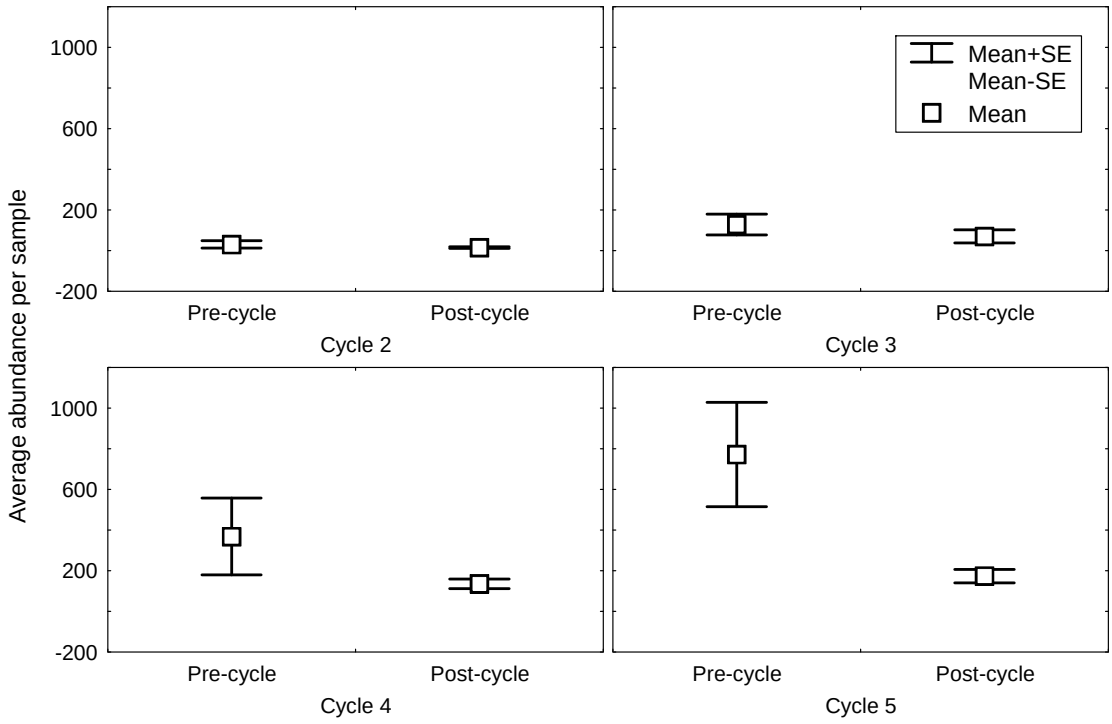

Figure 5.23 Average number of individual invertebrates in malaise trap samples.

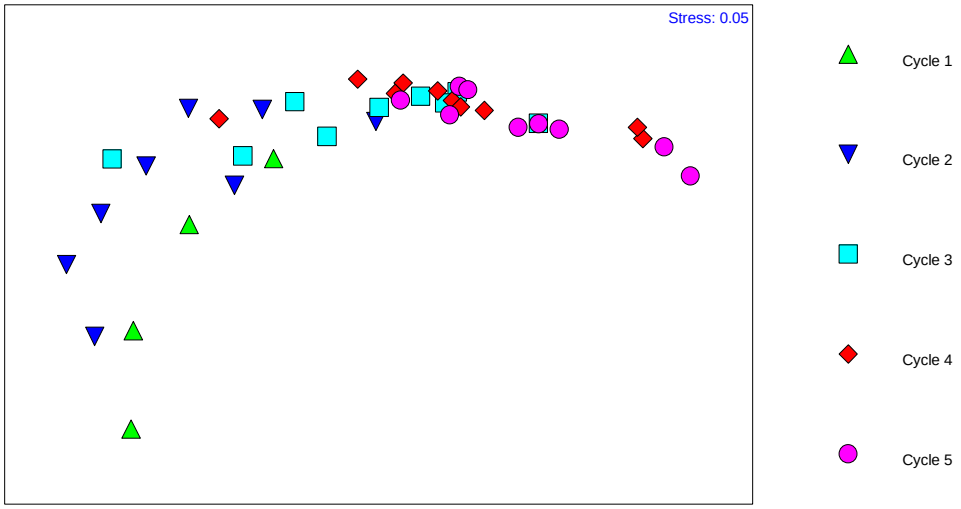

Figure 5.24 nmMDS plot of untransformed higher taxa composition in malaise trap sample across five spray cycles

Key Results
• 7,500 individuals, mostly flies, were sampled • Within each cycle catches were lower in the days after the spray event but then increased between the spray cycles to be much greater in subsequent pre-cycle catches • Composition of samples changed over the cycles mostly due to the increase in the abundance of flies

5.3.7 Pitfall trap samples

Over 5,700 individuals from 18 higher taxa were sampled in pitfall traps. The samples were dominated by beetles and ants but also included significant numbers of springtails (Collembola) and termites (Isoptera) not sampled extensively by other methods.

Around 40 individuals per trap were recorded in each cycle and although post-cycle samples generally trapped fewer individuals this trend expressed as mean values was not significant (ANOVA on logN+1 transformed values, $F_{1,130}$=2.90, P=0.09, Figure 5.25).

Average richness of orders per trap of around 5.5 did not differ significantly between the cycles ($F_{4,130}$=0.38, P=0.82) but did decline significantly between pre-cycle and post-cycle samples ($F_{1,130}$=5.01, P=0.03), especially in cycle 1 and cycle 3 (Figure 5.26).

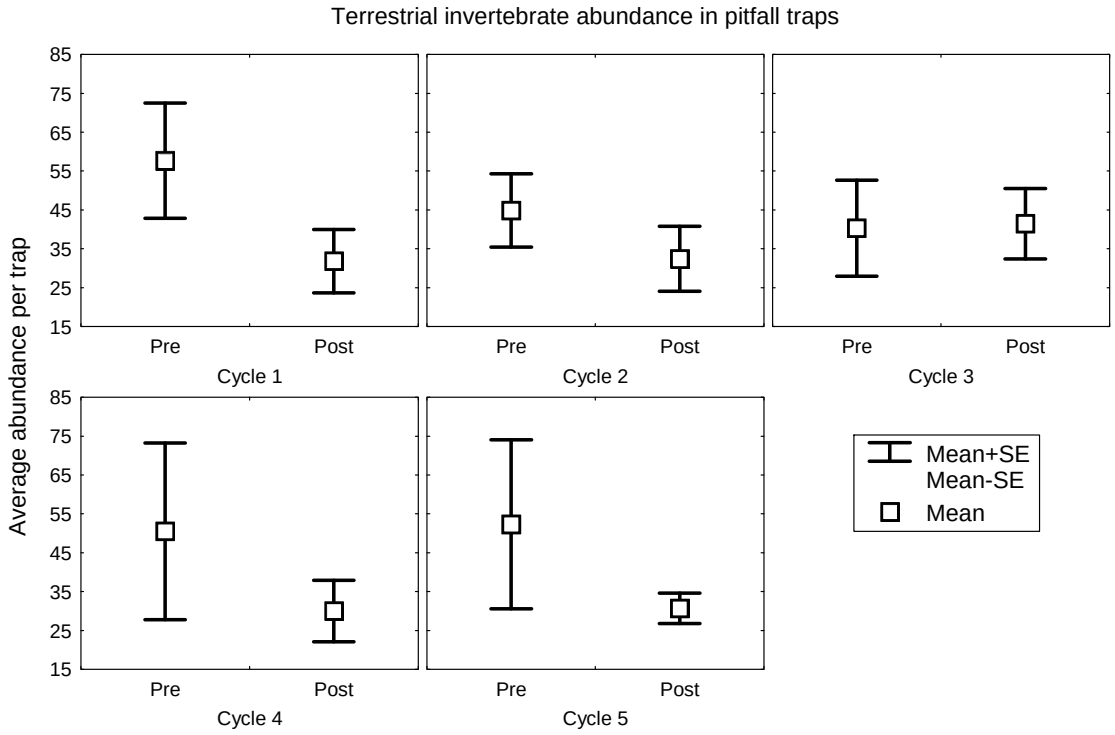

Figure 5.25 Average number of individual invertebrates in pitfall traps set for 5 days pre-cycle and 5 days post-cycle

There was no significant change in higher taxa composition based on ANOSIM tests between the cycles for pre-cycle (Global R=0.022, P=0.052) or post-cycle samples (R=0.031, P=0.119). Similarly there were no significant differences in higher taxa composition for samples taken before and after the spray within each cycle (R values all <0.047, P>0.150).

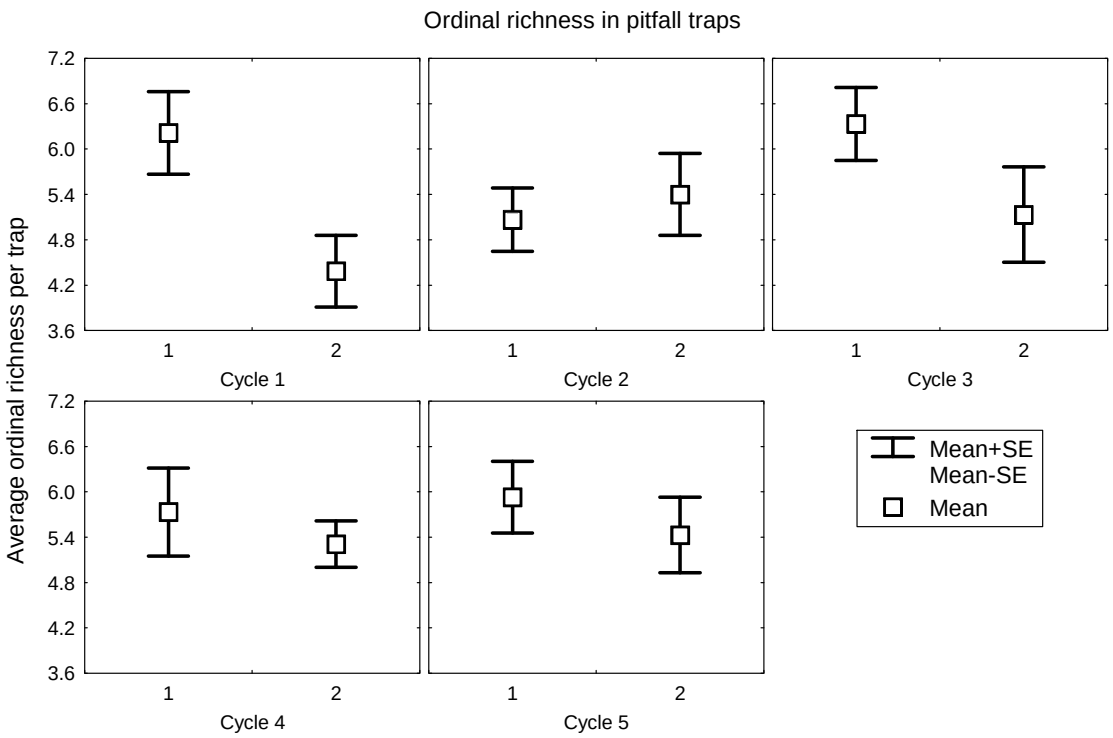

Figure 5.26 Average number of higher taxa (richness) sampled in pitfall traps set for 5 days pre-cycle and 5 days post-cycle

Key Results
• 5,700 individuals from 18 higher taxa were sampled • There were no significant trends in abundance or richness were apparent through the cycles or between pre-cycle and post-cycle samples • There was no significant change in higher taxa composition within or between cycles

5.3.8 Fogging and knockdown samples from *Colophospermum mopane*

As the objective of the aerial applications was eradication of tsetse flies over the entire delta there were no suitable control habitats that contained the dominant tree species of the island and floodplain mosaic typical of the southern part of the delta. However, extensive stands of *Colophospermum mopane* existed within and outside the spray zone. A site was selected on Chiefs Island which was within the spray zone and a second site on the edge of Moremi Game Reserve which was more than 40km from the edge of the sprayed zone (Figure 5.2)

A total of 31,184 specimens were sampled from the fogging of mopane trees. This was from only 51 samples to give significantly higher catch rates than for the other tree species. Even on the Chiefs Island site which received all five spray cycles catches were greater than 300 per sample in post-cycle fogging despite a highly significant decline over pre-cycle rates (Figure 5.27).

Beetles accounted for 58% of the individuals sampled and only ants and flies were close in overall abundance (Table 5.27). The majority of the 22 higher taxa sampled were infrequent and in low numbers. The 737 termites (Isoptera) sampled in the Moremi pre-cycle fogging may have appeared due to dead wood being used to weight down the trays and 677 termites emerging from this wood in one of the samples.

Overall richness of higher taxa per sample also declined significantly in the samples from Chiefs Island but increased slightly in Moremi (Figure 5.28).

	Moremi Not sprayed		Chiefs Island Sprayed	
	Pre-cycle Fogging	Post-cycle fogging	Pre-cycle fogging	Post-cycle fogging
Coleoptera	4892	8523	4592	55
Isoptera	737	6	7	4
Formicidae	275	456	1181	1184
Hemiptera	263	1750	371	1091
Araneae	129	191	226	39
Thysanoptera	35	244	65	43
Blattodea	29	35	17	6
Hymenoptera	22	576	199	133
Acarina	21	69	18	10
Pseudoscorpionida	17	26	73	88
Diptera	12	84	87	2388
Lepidoptera	11	34	52	11
Larvae	6	69	15	10
Psocoptera	4	94	9	1
Collembola	2	3	0	1
Mantodea	2	6	11	2
Neuroptera	2	479	0	24
Orthoptera	2	10	7	0
Thysanura	1	3	8	13
Ephemeroptera	0	1	5	0
Scorpionida	0	0	0	0
Solifugae	0	2	1	14

Table 5.27 Total catches of higher taxa in pre-cycle and post-cycle fogging of *Colophospermum mopane* trees on Chiefs Island and in Moremi Game Reserve

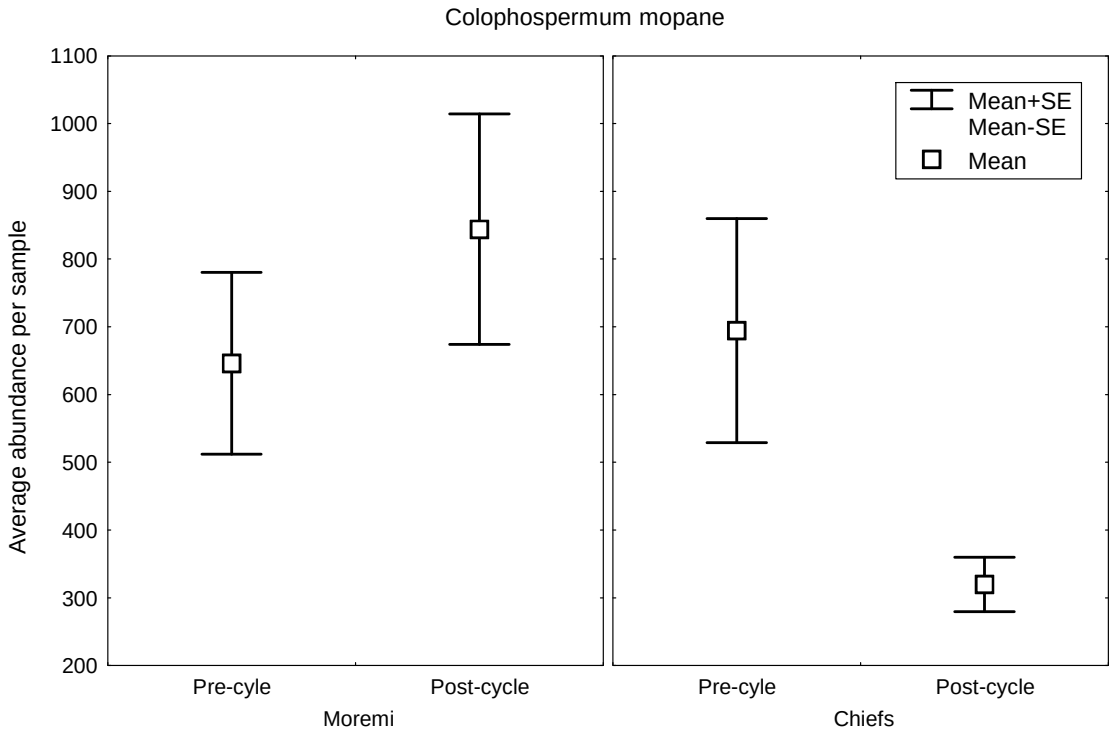

Figure 5.27 Average catch rates of all invertebrates per sample from pre-cycle and post-cycle fogging of *Colophospermum mopane* trees on Chiefs Island (sprayed) and in Moremi Game Reserve (not sprayed).

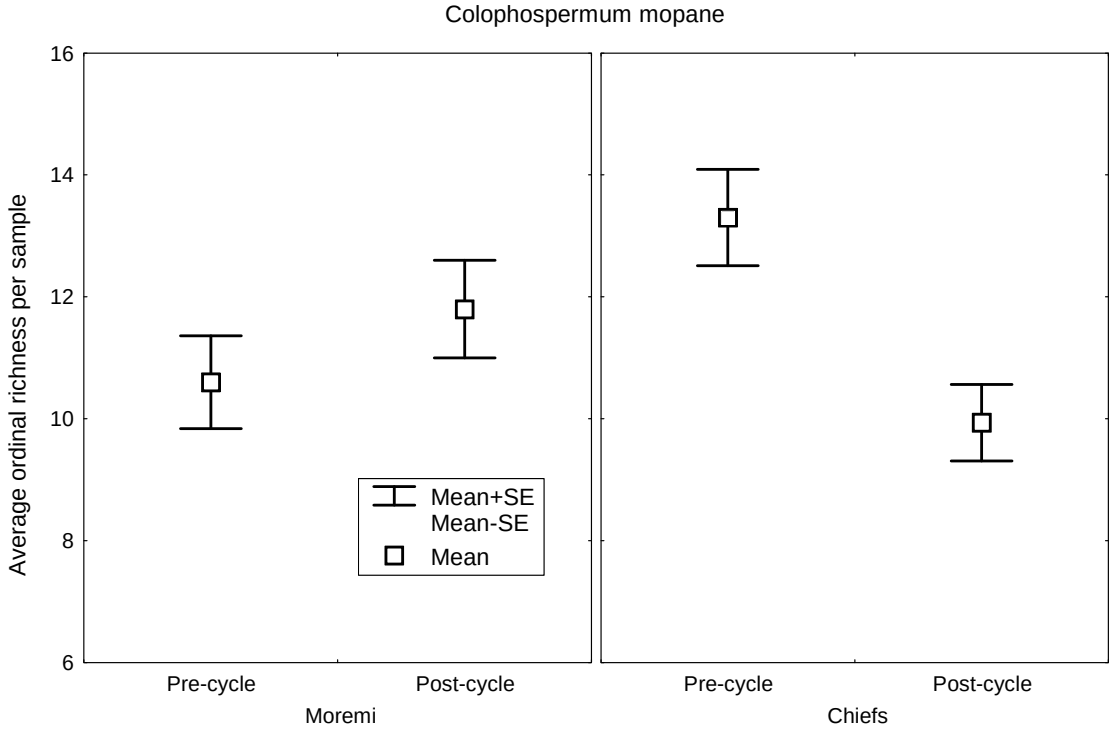

Figure 5.28 Average number of higher taxa per sample from pre-cycle and post-cycle fogging of *Colophospermum mopane* trees on Chiefs Island (sprayed) and in Moremi Game Reserve (not sprayed).

Compositional change in higher taxa between pre-cycle and post-cycle samples was highly significant on Chiefs Island (ANOSIM, R=0.940, P<0.00, Figure 5.29) almost 50% of which was attributable to the drop in beetle abundance and a further 19% due to an increase in fly abundance. Compositional change at the Moremi site was not significant (ANOSIM, R=0.071, P=0.136).

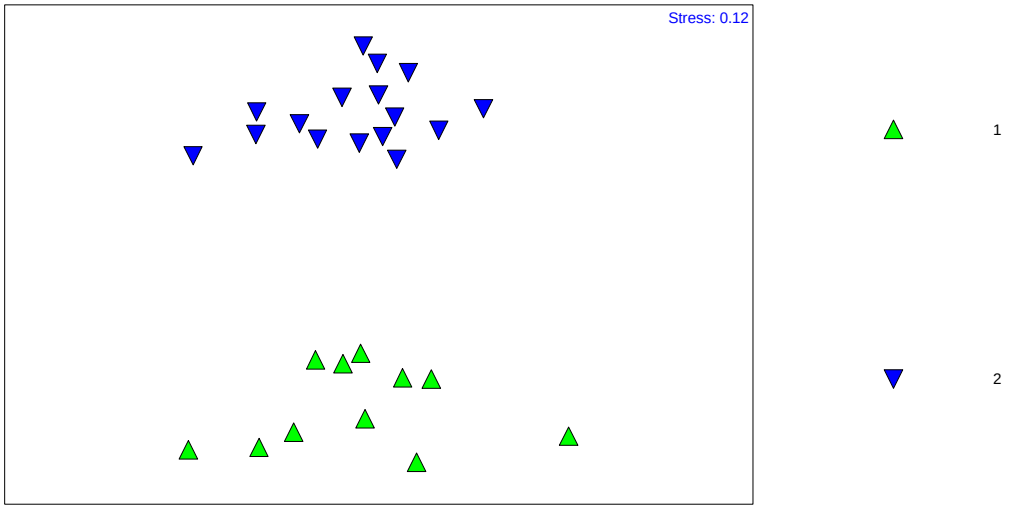

Figure 5.29 nmMDS plot of untransformed higher taxa abundance and composition from pre-cycle (1) and post-cycle (2) canopy fogging of *Colophospermum mopane* trees inside the spray zone on Chiefs Island.

Beetles declined dramatically between pre-cycle and post-cycle samples on Chiefs Island but almost doubled in abundance at the Moremi site. The decline was almost certainly due to the impact of the spray applications being consistent with patterns in beetle abundance from the other tree species. The increase in the Moremi samples may be confounded somewhat by patterns of leaf fall on the trees. Only a small number of trees at the Moremi site still had a reasonable canopy of green leaves, the majority had lost leaves prior to spring flush. The well leaved trees were chosen preferentially for fogging but may well have received immigration from adjacent trees in the weeks prior to sampling.

In all 51 morphospecies were recorded from mopane but two of these, *Chrysomelidae*0001 and *Brentidae*0001 accounted for 98% of all individuals. These two morphospecies also declined dramatically in post-spray samples from Chiefs Island and contributed to a significant difference in beetle composition (R=0.419, P<0.001). At Moremi where there was no aerial application of insecticide there was no significant change in abundance or composition (R=-0.071, P=0.848).

Moremi (not sprayed)		Chiefs Island (sprayed)	
Sampled only in pre-cycle fogging	Sampled only in post-cycle fogging	Sampled only in pre-cycle fogging	Sampled only in post-cycle fogging
Anthribidae0001 Anthribidae0007 Brentidae0002 Buprestidae0006 Buprestidae0009 Carabidae0009 Carabidae0015	Curculionidae0017 Coccinellidae0004 Curculionidae0003 Curculionidae0002 Chrysomelidae0001 Chrysomelidae0005 Chrysomelidae0007 Chrysomelidae0001 Chrysomelidae0018 Coccinellidae0003 Coccinellidae0007 Curculionidae0006 Staphylinidae0002	Brentidae0001 Chrysomelidae0011 Curculionidae0018 Anthribidae0004 Staphylinidae0015 Buprestidae0009 Carabidae00015 Chrysomelidae0007 Chrysomelidae0018 Coccinellidae00014 Curculionidae0007 Curculionidae0017	Curculionidae0001 Curculionidae0006 Anobiidae0002 Curculionidae0002 Carabidae0009 Chrysomelidae0014 Histeridae0001 Histeridae0002 Staphylinidae0002 Staphylinidae0012

Table 5.28 Beetle morphospecies unique to pre-cycle and post-cycle samples from canopy fogging of *Colophospermum mopane* trees inside (Chiefs Island) and outside the spray zone (Moremi)

Given the numerical dominance of two morphospecies the remainder were generally in very low numbers. This contributed to a large number of morphospecies being sampled in only the pre-cycle or the post-cycle samples in both locations (Table 5.28).

A total of 27 ant morphospecies were sampled from mopane trees in canopy fogging with some geographic effects resulting in eight of these being unique to the Chiefs Island samples and six unique to the Moremi samples. Average abundance increased slightly in post-cycle samples in Moremi but there was no significant change in composition ($R=0.077$, $P=0.119$) even though four species were only sample in the pre-cycle and nine species in the post-cycle samples (Table 5.29). Ant abundance did not change in the Chiefs Island samples but there was a significant change in composition ($R=0.623$, $P<0.001$) due mainly to both increases and decreases in abundance of *Crematogaster* morphospecies.

A total of 31 fly morphospecies were recorded from canopy fogging of mopane trees but as with most of the beetles these were in very small numbers. The increase in fly abundance is mostly due to families that were not targeted for identification to morphospecies hence the data for fly morphospecies were too sparse for meaningful ANOSIM analyses. The most significant result for flies is a two order of magnitude increase in abundance between the pre-cycle and post-cycle samples on Chiefs Island that was not apparent in the unsprayed area in Moremi. The sampling site in Moremi was also dry, being many kilometers from any influence of seasonal flooding. In both sites, however, there were many morphospecies recorded only in the post-cycle samples (Table 5.30)

Moremi (not sprayed)		Chiefs Island (sprayed)	
Sampled only in pre-cycle fogging	Sampled only in post-cycle fogging	Sampled only in pre-cycle fogging	Sampled only in post-cycle fogging
Crematogaster0011 Camponotus0016 Crematogaster0009 Tetraponera0002	Crematogaster0010 Camponotus0018 Technomyrmex0002 Acantholepis0001 Monomorium0018 Camponotus0019 Monomorium0019 Pheidole0010 Tetraponera0001	Crematogaster0011 Technomyrmex0002 Acantholepis0002 Monomorium0017 Crematogaster0007 Camponotus0017 Camponotus0009 Monomorium0016	Myrmicaria0001 Crematogaster0008 Tetraponera0002 Monomorium0020 Leptothorax0001 Pheidole0010 Cataulacus0001

Table 5.29 Ant morphospecies unique to pre-cycle and post-cycle samples from canopy fogging of *Colophospermum mopane* trees inside (Chiefs Island) and outside the spray zone (Moremi)

Moremi (not sprayed)		Chiefs Island (sprayed)	
Sampled only in pre-cycle fogging	Sampled only in post-cycle fogging	Sampled only in pre-cycle fogging	Sampled only in post-cycle fogging
Dolichopodidae0005 Muscidae0007	Calliphoridae0009 Anthomyiidae0001 Asilidae0011 Bombyliidae0003 Bombyliidae0004 Asilidae0005 Asilidae0010 Bombyliidae0007 Calliphoridae0015 Muscidae0023 Tephritidae0008	Stratiomyiidae0003 Dolichopodidae0003 Sciaridae0001 Cryptochetidae0001 Dolichopodidae0005 Empididae0004 Ephydridae0005 Sepsidae0001 Sphaeroceridae0005	Asilidae0009 Culicidae0001 Bombyliidae0003 Bombyliidae0009 Empididae0005 Simuliidae0003

Table 5.30 Fly morphospecies unique to pre-cycle and post-cycle samples from canopy fogging of *Colophospermum mopane* trees inside (Chiefs Island) and outside the spray zone (Moremi)

Key Results
• 31,184 individuals recorded from only 51 samples representing a much greater capture rate than for the other tree species. • Catches were dominated by beetles, particularly two morphospecies, and between the pre-cycle and post-cycle sampling more than doubled on the unsprayed site compared to a significant decline on the sprayed site. • There was no significant change in morphspecies composition at the unsprayed site • Fly abundance increased by two orders of magnitude in the sprayed site compared to only a slight increase at the unsprayed site that was distant from the influence of the annual flood

5.4 Discussion

5.4.1 Changes in abundance

The consequence of aerial spraying of deltamethrin in the Okavango Delta on non-target terrestrial invertebrate taxa appears to be a significant reduction in abundance as measured by reduced catches in knockdown samples from the canopies of trees. This reduction is confounded by increases in some taxa, particularly flies, that appear to be the result of seasonal patterns of adult emergence in response to temperature increases and the arrival of the annual floods to the study areas.

Beetles make up a large percentage of the overall decrease in catches declining by two orders of magnitude in many cases. At the time of spraying, the probability that an insect will suffer a lethal dose will be a function of its position in the canopy, the number and volume of spray droplets that impinge upon it and its topical tolerance distribution to the pesticide formulation (Jepson et al., 1990). Clearly deltamethrin applications at these doses fits these criteria for beetles. However, more than 85% of the declines in beetle abundance on *K. africana, C imberbe* and *C. mopane* was due to reductions in three morphospecies. Abundance declines in the remaining morphospecies were less dramatic. In many respects abundance changes reflect the specific patterns of behaviours that put certain morphospecies in the canopy at the time of spray application and in such positions as to expose them to the spray.

Overall abundance declined by 68% through the cycles when beetles and flies were not included in the summations. This decline was both significant and consistent across the range of higher taxa sampled. The bulk of this change occurred after cycle 1 or cycle 2 suggesting that the impacts are the result of one, or at most two, deltamethrin applications. Importantly, however, the impact is not total removal even after later applications because specimens from all higher taxa were still being sampled during these later cycles, even if in reduced numbers.

While no higher taxa were lost as a consequence of the applications there was a significant reduction in average higher taxa richness in samples. Much of this will be due to the sampling consequences of low numbers. When the frequency of observations are unevenly distributed among categories (in this case higher taxa) in the classical negative binomial pattern and sample size decreases, the less frequently observed categories have a much reduced probability of being recorded in any one sample. This problem also applies to the data on morphospecies because the majority of these were represented by one, or at most, a handful of specimens through all samples. For example, 39 of the 77 fly morphospecies not sampled again after one of the cycles were recorded as singletons. It also makes it difficult to decide if taxa have been lost or are simply not detected by the sampling effort due to reduced numbers.

It is possible to overcome some of this problem by comparing sprayed with control sites. However the extensive scale of this spraying exercise precluded any control options for similar habitats to the seasonal floodplains and islands. Fortunately it was possible to make a control comparison by canopy fogging *Colophospermum mopane* trees within the spray zone (Chiefs Island) and well outside the spray zone on the boundary of Moremi Game Reserve. Outside the spray zone captures of invertebrates in post-cycle fogging samples were 96% greater than in the pre-cycle fogging. Most of this was due to a doubling of the beetle numbers and an order of magnitude increase in catches of Hemiptera, wasps, lacewings (Neuroptera) and thrips (Thysanoptera). Catches of flies did not change significantly, a contrast to major increase in catches within the spray zone study sites that were influenced by the annual flood.

Whilst these results suggest that invertebrate numbers increase over the season on mopane patterns of leaf fall meant that the trees fogged in the post-cycle samples in Moremi were a

select few that were still in leaf. Many insects may have gravitated to these trees for food and shelter at the time of sampling. It was decided, however, that an equally biased result would accrue if trees without leaves had been fogged.

On Chiefs Island within the spray zone invertebrate abundance on mopane trees decreased by 26% in post-cycle fogging samples over pre-cycle estimates. This was despite an order of magnitude increase in the abundance of flies. If flies were removed then the decline was 61%, very similar to the estimates for overall declines on the other tree species within the spray zone.

The problem in impact assessment is attempting to pick out a significant signal from the background noise. This may only happen if the perturbation is large, perhaps even by an order of magnitude (Grant, 1988). Even though sampling was limited prior to the spray cycles and precluded any estimate of background noise, the decline in overall abundance is up to two orders of magnitude for some higher taxa (beetles). This change is readily detectable overall and on individual tree species and verified to some extent on mopane trees by comparison with a control area.

Whilst patterns in abundance are complicated by natural history and natural environmental changes in temperature and the arrival of the flood during the sampling period, the evidence is that aerial application of deltamethrin in repeated cycles depletes invertebrate abundance by approximately two-thirds to 30-40% of pre-cycle levels.

5.4.2 Changes in composition

Changes in abundance, especially for flies, beetles and ants, was sufficient for compositional change at higher taxa level to be significant between the cycles. Most of this significance was due to changes in composition between cycle 1, cycle 2 and the later cycles which reinforces the observation that the effect of application happens early in the sequence of cycles. It is not clear, however, if there is an additive or cumulative effect of repeat applications on non-target taxa although the lack of compositional difference between later cycles suggests this may be trivial. If higher taxa compositional change is considered just on presence or absence of taxa then changes are no longer significant, reaffirming the early conclusion that as no higher taxa are lost the consequence of application are not acute at this taxonomic resolution.

A total of 367 morphospecies were identified from selected families of beetles, spiders, flies, all Hemiptera and ants in samples taken from *Kigelia africana, Combretum imberbe* and *Lonchocarpus capassa* trees within the spray zone. Whilst this does not represent dramatic richness compared to samples from rainforest or even moist temperate forests it is not, nor was it intended to be, a full inventory. Not all higher taxa or families were identified to morphospecies and only two sampling methods (canopy fogging and aerial applications) were used. Despite this, 367 morphospecies provides a very robust biological signature of the canopy invertebrate assemblages and more than sufficient data intensity to assess impact.

Around 28% of these morphospecies were recorded in pre-cycle fogging and cycle 1 but not thereafter. Of these 101 morphospecies, only around 30 were sampled in any significant numbers prior to the spray cycles the rest being recorded as singletons or just a handful of specimens, hence the problem of low numbers and limited sampling effort applies again. Proportionately beetles (34%), spiders (30%), flies (26%) and Hemiptera (25%) had the most morphospecies not sampled after cycle 1 and ants (11%) the least. Despite the sampling efficiency problem these taxa represent an important group for future monitoring of recovery.

Spiders are considered useful indicators because their behaviours, including web formation, are interrupted by deltamethrin (Stark et al., 1995). The evidence here is that the effects on the sensitive families are apparent almost immediately in that both spider abundance and diversity decline after cycle 1. It would be important to know if there were longer term effects that it was not possible to estimate during this impact study. Failure to sample seven spider morphospecies after the first aerial application is an important result as only Families known to be potentially sensitive to deltamethrin were identified to morphospecies. It cannot be

concluded that these species have been lost or become locally extinct, merely that they were not sampled. They do, however, form an ideal target group on which to focus efforts in the monitoring of recovery as it would be important to see a number of these taxa in samples taken using canopy fogging in the future.

A further 28% of the total recorded morphospecies were first sampled in cycle 2 or later cycles, that is after at least one aerial application of deltamethrin. Proportionately Hemiptera (37%), flies (32%), beetles (27%) had the most morphospecies that were first sampled after cycle 1, whilst spiders (14%) and ants (11%) relatively few. The most likely explanation for these patterns is a combination of chance sampling effects, that is as the absolute sampling effort accumulates over the cycles more taxa will inevitably be sampled, and temporal change in composition resulting from natural histories of individual species. Some species will not appear in samples until they become active in the tree canopies, perhaps as a result of the arrival of floodwaters, and are exposed to the spray during later cycles.

The consequence of these losses and gains in morphospecies is that for each taxon composition of samples changes significantly between the cycles and particularly between cycle 1, cycle 2 and the remaining cycles. It should be noted that catches during any one cycle sampled only between 20 and 50% of the total morphospecies recorded throughout the study. This means that statistical tests of compositional change were likely to be significant.

5.4.3 Other sampling methods

The estimates of mortality in knockdown samples showed that there was greater taxon richness in trays treated with an insecticide suggesting some taxa do recover some time after the aerial applications. Abundance differences between treated and untreated trays were not significant, however, which may have been due to constraints on sampling efficiency generated by windy conditions at the time of sampling. Overall it is not easy to draw significant conclusions from this method.

Results from the pitfall trap samples revealed no obvious differences in abundance or higher taxon richness or composition between samples taken before and after each spray event. This is similar to results from the 2001 pitfall trap survey at Pom Pom. Sorption (the process by which one substance takes up or holds another) and degradation processes can restrict the toxicity of many synthetic pyrethriods to soil organisms (Elliot et al., 1978). This effect is also prevalent as the aerosol applications are stratified through vegetation or crop canopies and penetrate to the ground presenting a wide range of chemical concentrations to the invertebrates that inhabit different layers of the habitat (Jepson, 1993). At higher taxa level the evidence from pitfall trap data is that both abundance and diversity of ground active invertebrates are not affected by any one cycle application nor cumulatively over the five cycles.

Fewer flying insects were captured in the malaise trap in the days following compared with those immediately before the aerial applications. This was a consistent result through all five cycles, however, abundance prior to a cycle was always greater than the post spray catches from the preceding cycle. This suggests that flight activity is depressed temporarily as a result of the application but this is a relatively short-lived effect.

5.4.4 Consequences of aerial application of deltamethrin on terrestrial invertebrates

The evidence presented here is that some taxa are affected by repeated aerial applications of deltamethrin, that these effects may be acute for some morphospecies but apparently not across higher taxa. Several morphospecies from each of the higher taxa sorted were sampled in the pre-cycle fogging and/or in cycle 1 but not thereafter (Table 5.31). It is also clear that these effects are confounded by geography and seasonal changes to the habitat especially as the flood arrives. The effects of sampling effort, especially relative sample size as abundance declined, and the ubiquitous pattern of most taxa occurring naturally in low

Morphospecies not sampled again after pre-cycle fogging and cycle 1				
Beetles	Ants	Spiders	Flies	Hemiptera
Anobiidae0005 Anthribidae0003 Anthribidae0005 Brentidae0004 Brentidae0005 Buprestidae0001 Buprestidae0002 Buprestidae0003 Buprestidae0004 Buprestidae0008 Carabidae0002 Carabidae0003 Carabidae0004 Carabidae0007 Carabidae0010 Cerambycidae0001 Cerambycidae0003 Chrysomelidae0005 Chrysomelidae0017 Chrysomelidae0019 Coccinellidae0006 Coccinellidae0008 Coccinellidae0009 Coccinellidae0013 Curculionidae0005 Curculionidae0014 Curculionidae0015 Curculionidae0023 Curculionidae0025 Curculionidae0026 Curculionidae0027 Curculionidae0028 Curculionidae0029 Curculionidae0030 Elateridae0001 Elateridae0002 Elateridae0003 Scarabaeidae0001 Scarabaeidae0006 Scarabaeidae0007 Staphylinidae0002 Staphylinidae0003 Staphylinidae0010 Staphylinidae0023 Staphylinidae0024	Acantholepis0001 Crematogaster0007 Camponotus0001 Acantholepis0002	Salticidae0001 Thomisidae0009 Salticidae0001 Oxyopidae0004 Theridiidae0001 Oxyopidae0007 Thomisidae0002	Phoridae0007 Calliphoridae0011 Muscidae0010 Calliphoridae0001 Acroceridae0004 Stratiomyiidae0002 Dolichopodidae0005 Muscidae0011 Phoridae0008 Sepsidae0003 Calliphoridae0007 Calliphoridae0010 Muscidae0001 Acroceridae0002 Asilidae0005 Dolichopodidae0004 Muscidae0017 Muscidae0025 Sepsidae0002 Tachinidae0005 Calliphoridae0012 Lauxaniidae0002 Muscidae0022 Pipunculidae0002 Psychodidae0001 Sarcophagidae0003 Stratiomyiidae0004 Syrphidae0007 Tephritidae0004 Tipulidae0001	Membracidae0002 Membracidae0003 Cicadellidae0022 Reduviidae0006 Reduviidae0001 Membracidae0001 Reduviidae0005 Cicadellidae0004 Cicadellidae0005 Cicadellidae0012 Reduviidae0004 Reduviidae0001 Triozidae0003 Membracidae0004 Reduviidae0007

Table 5.31 Morphospecies sampled in either the pre-cycle fogging or cycle 1 but not in any subsequent samples during the 2002 monitoring program. These taxa would be appropriate for inclusion as focal taxa in a recovery monitoring program.

numbers, also make it difficult to decide if failure to sample some taxa in the later cycles and post-cycle fogging is because they are depleted or have become locally extinct.

Much of the literature on screening for side effects against non-target invertebrates comprises a combination of laboratory, semi-field and field levels (Brown et al., 1990) with various methods for standardizing the measurement of these effects (Sotherton et al., 1988). It has been recognized, however, that there is major difficulty in simulating the scale of treatment in many cases (Duffield & Aebischer, 1994). There is clearly a huge difference between the applications of insecticide for crop protection which involves precise applications to a crop that spares field margins, fallow and other non-crop habitats within the landscape, and blanket applications. In many crop situations perhaps only 30% of the habitat will be treated and contiguous areas of several km^2 treated simultaneously (Aebischer, 1990).

An example of the consequence of large scale applications is available for ground beetles where the duration of significant reduction in ground beetle populations increases as the spatial scale of a single insecticide application increases (e.g. (Jepson & Thacker, 1990). The proposed explanation is that as recruitment is external via reinvasion larger scale applications reduce the number and increase the distance from source populations. In many cases, however, some taxa may be able to recover from residual populations within treated areas and quickly if they are released from predator impacts (Duffield & Aebischer, 1994). These mechanisms of reinvasion are significantly restricted in the present case because of the wide geographic extent of the spray applications and the drastic change in habitat types around the periphery of the spray zone. Reinvasion is only likely along the Linyanti-Kwando system in the east and, perhaps more slowly, from southern Angola along the pan handle. The relative remoteness and point nature of these sources might mean if population recovery from survivors is not possible then reinvasion as a mechanism for recovery may be slow. A recovery monitoring program would need to consider these longer time scales of recovery as a consequence of the large extent of aerial applications.

It has been suggested that a metapopulation approach, where patterns of change are considered for several geographically separate populations of the same species, is needed if the long-term side effects of pesticides are to be properly evaluated (Sherratt & Jepson, 1993). That is recovery should be assessed at more than one location representing several populations of each taxon under evaluation. Recovery is also dependent on many factors both intrinsic and extrinsic to the organisms. Prey species will recover quickly if their predators have been depleted and faster where the reinvasion of those predators is less likely (Duffield & Aebischer, 1994). The data provided in this survey would allow such a design to be implemented in a cost-effective manner in the future.

Terrestrial invertebrates were not extirpated by the application of deltamethrin in 2002. There were, however, significant declines in the abundance of several taxa and a potential loss of some species. Consequently biological composition did change significantly through the spraying cycles. The true impact of sequential aerosol applications of deltamethrin in the Okavango Delta can only be fully evaluated by monitoring recovery of these invertebrate assemblages over time.

5.5 Bibliography

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6. Monitoring of Birds

Data Analysis and Report by
Frank Pendleton

Data collection
Frank Pendleton, Tracy Hart, and Kathy Hageman

6.1 Background

In order to assess the immediate short term effects of the spraying operation on bird populations, sites in the 2001 and 2002 spray blocks were monitored before, during and after spraying operations. The goal of this monitoring program was to collect baseline data, and to monitor effects on bird populations.

The three critical requirements for effective monitoring of management impacts were emphasized in chapter 5 (section 5.1) as :-

1. knowledge of the current or pre-management state and normal variation in that state (benchmark)

2. magnitude of change in response to the management practice (impact)

3. rates and duration of recovery to either the benchmark levels or a target level that is deemed acceptable (recovery)

While environmental impacts can affect birds in such ways as changing foraging behavior and reducing reproductive success, information on the effects on populations is the most vital. In order to provide the most valuable benchmark for birds in general, it is necessary to collect sufficient amounts of data on as many species as possible to be able to detect population declines. To meet this goal, population monitoring techniques were chosen over intensive single species monitoring techniques, such as studying foraging behavior or nesting success.

Point counts and transects were used to collect population level data on many species at the same time. While these techniques are the best choice for developing a benchmark for a broad species base and detecting large scale bird declines, they can not pin point the mechanism of a population decline (emigration, reduced breeding success), or the ultimate cause of a population decline (did the spraying or the fires cause the decline).

6.1.1 Factors affecting bird populations.

The spraying was just one of many factors affecting the number of birds detected, and it is rarely possible to attribute change in wildlife populations to a single factor. Several of the factors making it difficult to assign changes in bird populations to the spraying are described here.

There are 2 main complications which arise out of the spraying protocol. First, deltamethrin is highly unlikely to kill birds directly due to its low toxicity to vertebrates (Hudson *et al.,* 1984; Elliot *et al*., 1978). Therefore, we are looking for the effects on bird populations caused by a reduction in food supplies, not as the result of direct death by poisoning. Reductions in food supplies can lead to immediately population declines by causing increased emigration out of the spray block, but reductions in food supplies can also cause reductions in breeding success, which would not be detectable the year of the spraying.

Second, it takes several months to complete a spray block, and bird populations change naturally from season to season, and year to year. For this study, surveys began in March, which is the end of the rainy season. During the rainy season many migrants come to the Okavango. Many of these migrants, and most of the residents, breed during the rainy season. This means that there are more birds in the area, and that any given bird is likely to be more vocal. Therefore, it would be expected that more birds would be detected in March than in July even if there was no spraying. So the question “Did the spraying affect bird population?” cannot be answered by simply looking at the number of birds detected before and after the spraying within a given year. The question is, “Did detections drop off faster in the year of the spraying than the year before?” And declines in populations can only be detected if they are immediate, (caused by death or emigration). Effects on breeding success may not be detected for several years.

Abiotic factors such as weather also affect bird populations, and the Okavango received above average rains in 2001 and below average rains in 2002. Good rains mean more food for herbivores as well as insectivores, which means higher survival and higher breeding success. And one last factor is the fact that both Chitabe and Nxaraxa burned after the last pre-spray survey, and before the first post-spray survey (May 2002). Therefore, there may be changes in bird populations unrelated to the spraying.

With all these factors as part of the equation, it is unlikely the smaller effects of the spraying can be teased out from other factors. But catastrophic effects, such as the large declines in species or guilds (groups of species with similar niche characteristics), would readily be detected by these surveys, and would be a cause for serious concern. The results from this study provide a benchmark for bird populations which will be vital to future monitoring efforts.

6.1.2 Acknowledgments

This study would not have been possible with out the help of Okavango Wilderness Safaris, and Guma Lagoon Campground. They provided access to study areas, transportation and accommodation, and I am very thankful to both.

6.2 Methods

6.2.1 Study sites

Two study sites that were sprayed in 2001 (Mombo and Guma), and two that were sprayed in 2002 (Chitabe and Nxaraxa) were utilized (Figure 6.1). Two control areas were tested, but neither proved satisfactory. The Maun Education Reserve was monitored in 2001, but was dropped in 2002 because it was added to the 2002 spray block. Khwai was monitored 3 times in 2002, but was dropped because it was not similar enough to the other sites to be a fair comparison.

6.2.2 Data collection techniques

Because of the variety of habitat types and bird species several monitoring techniques were used at each site. Circular point counts and calling stations were used for monitoring forest birds (Bibby et al. 1992), transects were used for monitoring acacia thornveld species, and boat surveys were used to monitor water dependent species (Table 6.1).

6.2.3 Point counts

Forest point count stations were located a minimum of 300 m apart, and were surveyed for 10 minutes. The number of each bird species seen or heard, and the distance from point center (0-20 m, 20-50 m, 50 -100 m and >100 m) (Buckland et al. 1993, Buckland 1987) was recorded.

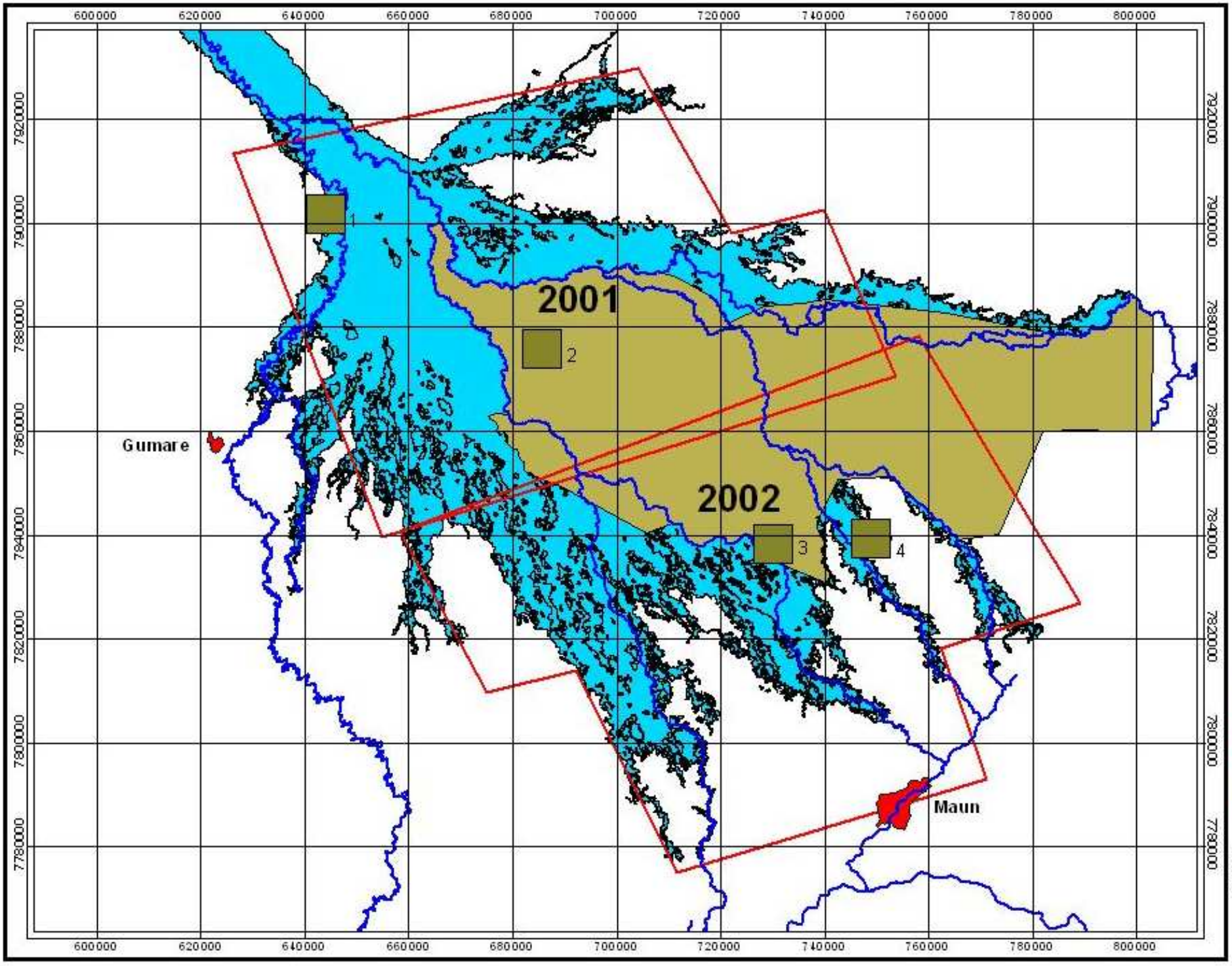


Figure 6.1
Location of Bird
Monitoring Sites

Description of Sampling Sites

1 - Guma
2 - Mombo
3 - Nxaraxa
4 - Chitabe

Sampling site
River course
Major Town
Sprayblock
Moremi Game Reserve
Delta outline

Projection: UTM 34 K South
Spheroid: Clarke 1880
Map Datum: Cape

0 10 20 30 km

Point counts were surveyed between 6:00 – 10:00 AM, and in the same order each time to minimize variance due to time of day effects. Point counts were digitally recorded using a directional microphone making it possible to verify questionable songs and calls later.

6.2.4 Acacia thornveld bird surveys (Road transects)

Road transects were conducted with a driver and myself as an observer in the back of an open safari vehicle. The same stretch of road was followed, going as slowly as possible without stalling the vehicle (5 k/hr), and any bird that was seen was recorded. These transects were tried at all sites, but only yielded enough data to be worthwhile at Mombo. The Mombo transect was 4 km long, with 9 surveys conducted in 2001 and 8 in 2002.

6.2.5 Water bird surveys

Water surveys were problematic due to changes in habitats caused by the coming and going of the flood. While various water surveys were conducted at different sites, only the Guma Lagoon boat survey is analyzed here. The river transect was conducted at Guma by driving a boat approximately 5 km along the edge of the lagoon and counting all birds that were seen. The driver had a hand held GPS to help him maintain 5 km/h. A distance of about 30 meters from the edge of the lagoon was maintained, allowing most birds to be detected while minimizing the number of birds flushed. Care was taken to avoid repeat counts of birds that flushed and flew forward along the transect.

A walking river transect was conducted at Mombo, but the amount of area covered by water changed drastically with the coming of the floods. This change in habitat could have had a serious effect on bird numbers, so the data were not analyzed.

Table 6.1 The number of surveys of various methods used at each site.

Location	**Point Counts**	**Water Surveys**		
		Type of Survey	**Distance**	**# of Points**
Guma	10	Boat survey of Lagoon	5 km	
Mombo	10	Walking river survey	600 m*	
Nxaraxa	10	Pool point counts		5**
Chitabe	10			

* The Mombo river transect was canceled due to habitat change caused by the flooding.
** The Nxaraxa pool surveys were canceled because the pools dried up.

6.2.6 Diet Guilds

Because deltamethrin is more likely to affect birds indirectly by reducing insect food supplies than by killing birds directly (Hudson *et al.,* 1984; Elliot *et al*., 1978), the effects of the spraying can be monitored by looking at the effects on insectivorous birds versus the effects on other birds. To make this analysis possible, each species was assigned to one of 4 diet groups; Herbivore, Carnivore, Omnivore, and Insectivore (Table 6.2). Each of the 4 groups was further divided into 3 or 4 secondary groups based on food preferences (Maclean 1993). The secondary grouping emphasizes preferences within the primary grouping. So a species which ate mostly insects, but also ate seeds, berries and carrion, would have a primary classification of Omnivore and a secondary classification of insects. Birds which ate mostly insects were considered insectivorous in all diet analyses, while other birds were considered non-insect-dependent.

Table 6.2 Diet categories for birds detected in the Okavango Delta. Birds in the shaded diet groups were considered insectivores in all diet analyses.

Primary Group	Secondary Group	Description of Diet
Herbivore	General	Herbivore with a varied diet.
	Aquatic	Eats mostly aquatic plants.
	Fruits	Eats mostly fruits.
Carnivore	General	Vertebrates, insects, carrion, mollusks.
	Aquatic	Fish, frogs, tadpoles.
	Vertebrates	Small mammals, birds.
	Insects	Carnivore that includes insects as a large part of the diet.
Omnivore	General	Very general diet.
	Aquatic	Water plants, fish, mollusks, aquatic insects.
	Herbivore	Varied diet but concentrates on plant material.
	Insects	Varied diet but concentrates on insects.
Insectivore	General	General insect eater.
	Larvae	Larvae specialist.
	Terrestrial	Flying and crawling insects, not aquatics.

6.2.7 Statistics

All statistical comparisons were made with 2 tailed t-tests using the standard critical value of p = 0.05. While p = 0.05 may be the standard cut off point for statistical comparisons, any value less than p = 0.1 is of interest, even if it is not "statistically significant".

Presence absence analyses were conducted by looking at species that were detected on at least 2 surveys, and counting the number of species in the following categories:

Present in 2001 and absent in 2002 = lost species
Absent in 2001 and present in 2002 = gained species
Present in 2001 and present in 2002 = unchanged species

Only counting species that were seen at least 2 times reduces the number of incidental species causing inflated numbers of species gained or lost in the second year.

6.3 Implementation

Since 2001 was the first season of the project there was considerable set up time. Once sites were established they were surveyed once per visit before going on to the next site. This was changed early in the 2001 season and each site was then surveyed 3 times per visit in order to reduce travel time and expense. Under ideal conditions this took 3 days, but wind and other factors regularly caused surveys to be abandoned before all points were completed. Tables 6.3 and 6.4 summarize the survey effort for forest birds and water birds respectively.

Table 6.3 The number of forest point counts conducted at each site.

Location	# of Days Surveyed		# of Point Counts	
	2001	2002	2001	2002
Guma	7	10	54	79
Mombo	7	11	44	85
Nxaraxa	8	17	56	115
Chitabe	10	17	70	116

Table 6.4 The number of water surveys conducted at each study site.

Location	Pool Point Counts		River Surveys	
	2001	2002	2001	2002
Guma	--	--	11	9
Mombo	--	--	4*	10*
Nxaraxa	14**	6**	--	--

* Mombo was dropped because the floodplain around the river was too variable.
** The Nxaraxa pool surveys were canceled because the pools dried up.

Not all survey techniques were well suited for all study sites.

Road surveys were tested at Guma, Chitabe, Nxaraxa and Mombo, but only yielded enough data to be worthwhile at Mombo.

The Nxaraxa water survey consisted of a series of 5 pools, but water levels were highly variable, and 4 of the pools dried up during 2002. Therefore the Nxaraxa water survey was dropped and no analysis completed. The Mombo river survey was also dropped, due to the drastic changes in the amount of flooded habitat throughout the year.

Two control sights were tested but neither location was suitable. The Maun Education Reserve was not in the original spray plan, so I collected pre-spray data there in 2001, however, the reserve was later added to the 2002 spray block. The habitat at Khwai was too different from the rest of the sights to be a suitable control. Therefore, sights were compared to themselves before and after spraying. This situation is not ideal, but it is better than using a control which does not have similar conditions to the other sites.

6.4 Results

6.4.1 Forest birds (Point counts)

A total of 605 point counts were undertaken, detecting a total of 21,045 birds from 162 species. There were another 18 species groups which included birds that were detected and identified at a taxonomic level higher than species, such as hornbill, dove, babbler, or woodpecker. Birds were detected from 110 genera, 53 families, and 20 orders.

6.4.1.1 Diet guild population analyses

The purpose of the diet guild analyses are to look for obvious trends related to diet. If the spraying is having an effect on birds, it would be expected that insectivorous birds would suffer greater declines than non-insect-dependent birds.

Birds were classified as insectivorous and non-insect dependent, depending on the amount of insects in their diet. Because to the large part of insects in their diet, species in the Omnivore / insects and Carnivore / insects groups were classified as insectivorous for analyses. It is not surprising that the majority of birds are in the Omnivore general category, as many common birds are generalists (Table 6.5).

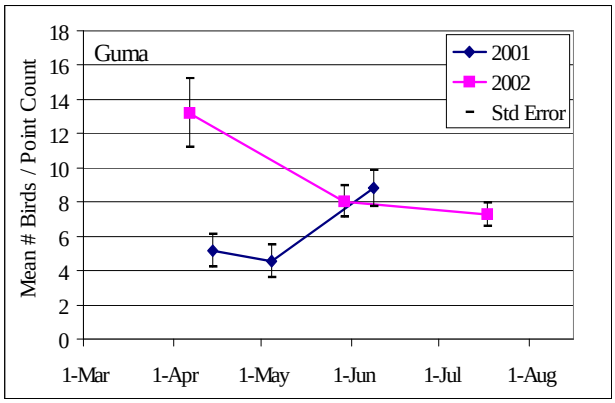
Table 6.5 Number of birds detected by diet grouping. Shaded areas are for birds considered insectivorous in analyses.

Primary Diet	Secondary Diet	Number	Percent
Carnivore	Aquatic	417	2.0%
	General	172	0.8%
	Insects	750	3.6%
	Vertebrates	88	0.4%
	Total	**1427**	**6.8%**
Herbivore	Aquatic	161	0.8%
	Fruits	781	3.7%
	General	109	0.5%
	Total	**1051**	**5.0%**
Insectivore	General	131	0.6%
	Larvae	124	0.6%
	Terrestrial	2003	9.5%
	Total	**2258**	**10.7%**
Omnivore	Aquatic	17	0.1%
	General	12447	59.1%
	Herbivore	1279	6.1%
	Insects	2566	12.2%
	Total	**16309**	**77.5%**
Insectivore Total		**5574**	**26.5%**
Grand Total		**21045**	**100.0%**

In order to look for trends, results were graphed with both years on the same axes, making it easy to see trends within a year as well as between years. Means displayed in the graphs are for visits, not dates. In each case a site was visited for 3 or more days. Such that if a site had 10 point count stations and was monitored for 3 days in a row, the mean would be based on 30 point counts.

Guma and Mombo were sprayed in 2001. Mombo was sprayed after my field season had ended, and Guma was sprayed during the final field visit in June 2001. Consequently, most of the 2001 data for these sites are pre-spray, and all 2002 data are the year after the spraying. The results of the diet guild analysis of these sites are not what would be expected if the spraying had a significant impact on insectivorous species (Figures 6.2 and 6.3, Table 6.6). Non-insect-dependent species showed no significant change between years in Guma (P = 0.403) and declined in Mombo (P = 0.0001), while insectivorous birds showed an increase at both Guma (P = 0.003) and Mombo (P = 0.039).

Figure 6.2 Mean number of **insectivorous** birds detected in the 2001 spray block (Guma and Mombo).



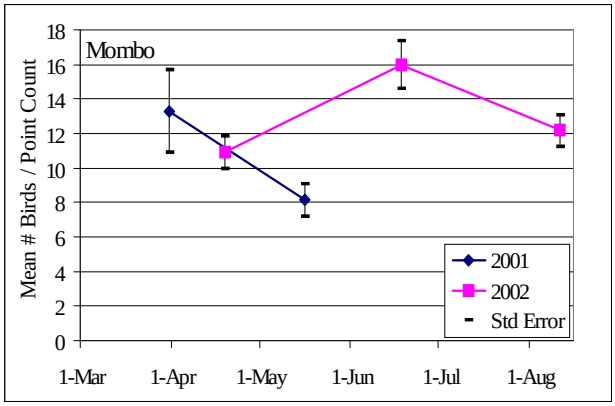
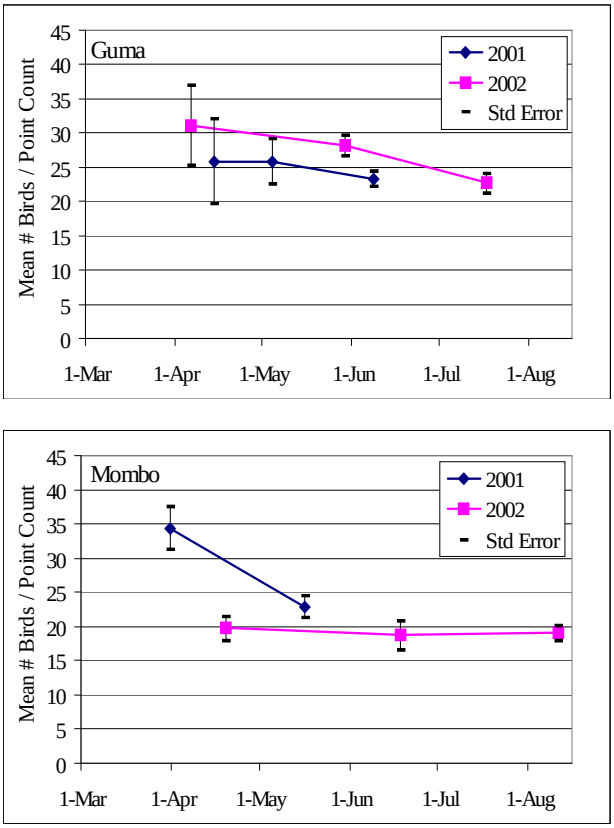


Figure 6.3 Mean number of **non-insect-dependent** birds detected in the 2001 spray block.



The Guma insectivorous bird population remained constant from April to May 2001, but rose rather sharply between May and June. This may have been the result of the spraying taking place during the June surveys, possibly sparking a period of increased activity due to the abundance of sick and dead insects providing an easy food source. The 2002 population follows an expected seasonal decline (as migrant birds leave the area), and is mostly above the 2001 population, demonstrating an increase rather than a decline following the spraying. During the same period non-insect-dependent birds remained constant (Figure 6.3).

The Mombo insectivorous bird population declined as expected from April to May 2001, but a third visit in 2001, to check whether the trend continued, was . The 2002 detections unexpectedly increased from April to June and maintained fairly high numbers through August 2002, resulting in the increase which was detected between years (Table 6.6).

Nxaraxa and Chitabe were in the 2002 spray block. Spraying began May 16th 2002, and ran until August 16th 2002. I concentrated on the southern spray block in 2002 and managed to visit Chitabe 4 times and Nxaraxa 5 times. Because of this I was able to make before and after spray comparisons using only 2002 data, or combining the 2001 data with the 2002 before spray data. Either way, there was no significant change to insectivorous or non-insect-dependent species at Nxaraxa. Chitabe non-insect-dependent birds showed no change, while insectivorous birds declined (Figures 6.4 and 6.5 and Table 6.6). The decline is statistically significant when 2001 data is combined with 2002 before data ($P = 0.0005$), and note worthy when only 2002 data is analyzed, even if it is not statistically significant ($P = 0.069$).

Figure 6.4 Mean number of **insectivorous** birds detected in the 2002 spray block.

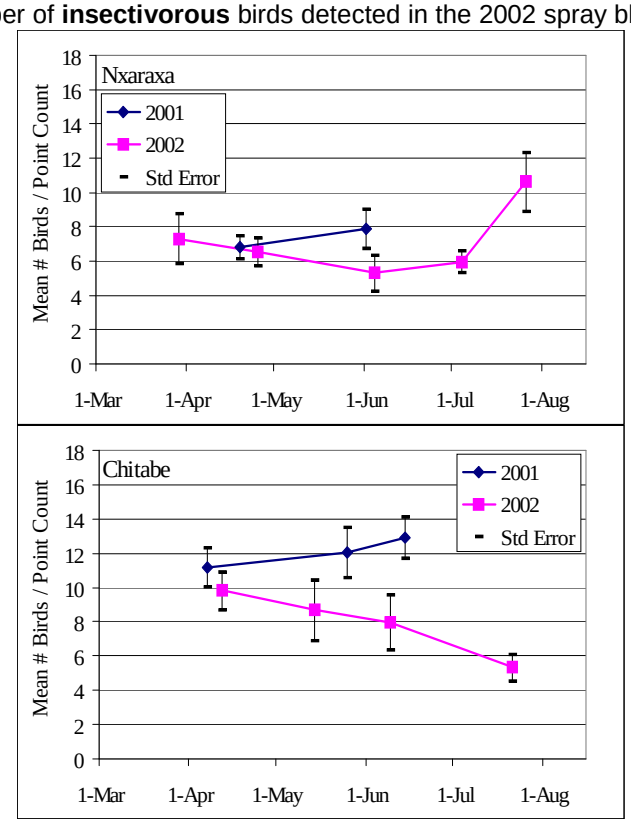
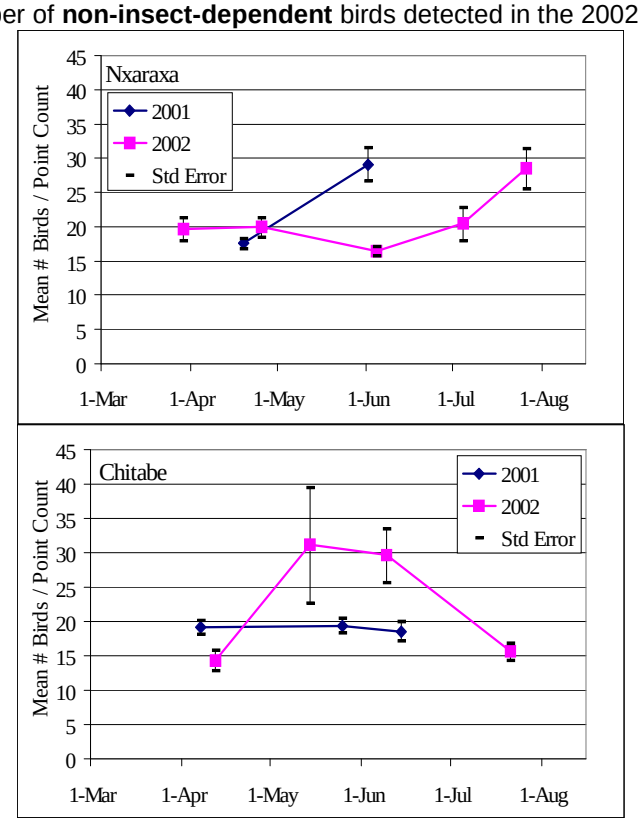


Figure 6.5 Mean number of **non-insect-dependent** birds detected in the 2002 spray block.



The 2001 Nxaraxa insectivorous bird data shows no change. The rise in the 2002 data is also reflected in Nxaraxa non-insect-dependent birds, and may be related to the fires that took place in May. However, Chitabe burned at the same time and showed very different results.

The rise in 2001 Chitabe insectivorous bird detections is unexpected, but it is not too surprising as it is rather small scale. But when compared with 2002 declines were detected. By itself, the Chitabe data is evidence for a decline in insectivorous species during the spraying, but when it is looked at with the other sites it is unlikely that there was a catastrophic effect on insectivorous birds as a group.

The peak in Chitabe 2002 non-insect-dependent bird data is due to large numbers of doves detected at some point counts. Because these flocks were detected at some point counts and not at others, they caused large standard errors.

Table 6.6 Results of 2 tailed t-tests comparing bird numbers for insectivorous and non-insect-dependent birds detected at point counts before and after the spraying.

Data sets compared	**P-value**	
	Insectivorous birds	**Non-insect dependent birds**
(Chitabe 2001) to (Chitabe 2002b*)	(P = 0.0427) down	P = 0.388
(Chitabe 2001 & 2002b) to (Chitabe 2002a*)	(P = 0.0005) down	P = 0.570
(Chitabe 2002b) to (Chitabe 2002a)	P = 0.069	P = 0.939
(Nxaraxa 2001) to (Nxaraxa 2002b)	P = 0.779	P = 0.215
(Nxaraxa 2001 & 2002b) to (Nxaraxa 2002a)	P = 0.786	P = 0.576
(Nxaraxa 2002b) to (Nxaraxa 2002a)	P = 0.719	P = 0.250
(Guma 2001) to (Guma 2002)	(P = 0.003) up	P = 0.403
(Mombo 2001) to (Mombo 2002)	(P = 0.039) up	(P = 0.0001) down

* 2002b is 2002 data before spraying started.
* 2002a is 2002 data after spraying started.
P-value in parentheses ( ) are statistically significant.

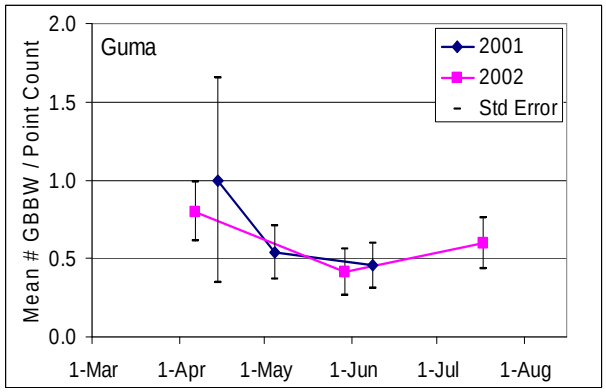
Key Results
• 21,045 birds were detected from 162 species at 605 point count surveys. • Chitabe showed declines in insectivorous birds when comparing (2001) to (2002 before the spraying), and when comparing (2001 & 2002b) to (2002a), but Chitabe non-insect-dependent birds showed no changes. • Nxaraxa showed no changes. • Guma and Mombo showed increases in insectivorous birds when comparing (2001) to (2002), while non-insect-dependent birds remained constant at Guma and declined at Mombo.

6.4.1.2 Greybacked Bleating Warblers

Greybacked bleating warblers are a good species for monitoring change in insectivorous birds for 3 reasons. First, they call regularly throughout the year, so detections are not likely to decline as a result of calling behavior. Second, they do not form flocks, avoiding the statistical problem of dependent observations. And third, they are residents, making it unlikely for numbers to change due to factors outside the Delta.

Chitabe showed a statistically significant difference in the number of Greybacked bleating warblers detected when 2001data was included in the before spray data set (P = 0.023) (Figures 6.6 and 6.7, Table 6.7). When only looking at 2002 data, the difference was not significant (P = 0.079), but it is worth noting. This difference is due to the drop off in the number of detections in the July 2002 surveys (Figure 6.7). None of the data from the other sites comes close to having a statistically significant difference (Table 6.7). The combined means used in the t-test are displayed graphically in figure 6.8.

Figure 6.6 Mean number of Greybacked bleating warblers detected in the 2001 spray block.



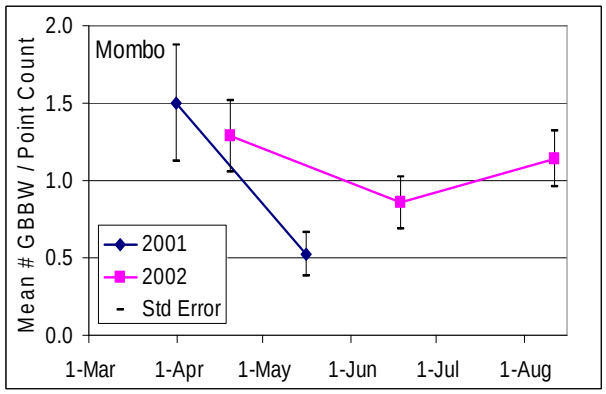


Figure 6.7 Mean number of Greybacked bleating warblers detected in the 2002 spray block.

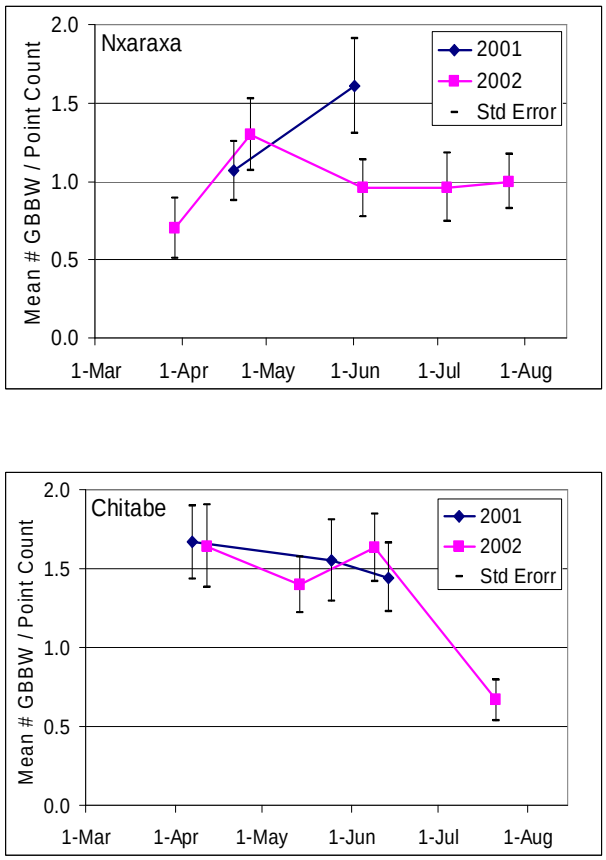
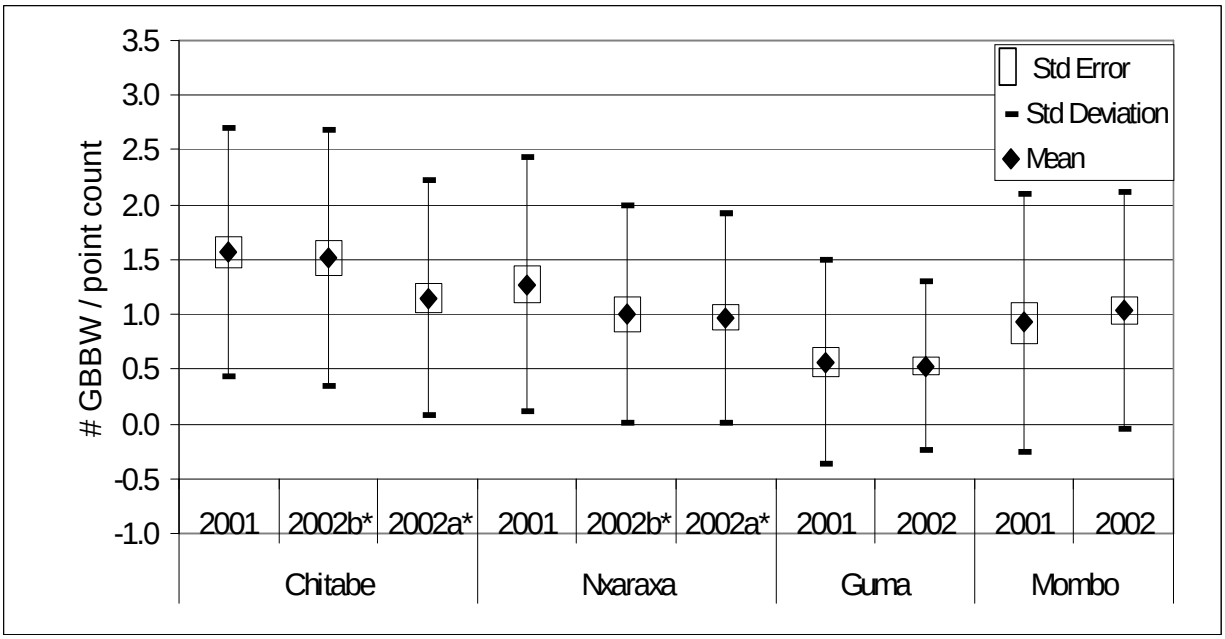


Table 6.7 Results of 2 tailed t-tests comparing Greybacked bleating warbler numbers at all 4 sites before and after the spraying.

Data sets compared	P-value
(Chitabe 2001) to (Chitabe 2002b*)	P = 0.786
(Chitabe 2001 & 2002b) to (Chitabe 2002a*)	(P = 0.023) down
(Chitabe 2002b) to (Chitabe 2002a)	P = 0.079
(Nxaraxa 2001) to (Nxaraxa 2002b)	P = 0.240
(Nxaraxa 2001 & 2002b) to (Nxaraxa 2002a)	P = 0.278
(Nxaraxa 2002b) to (Nxaraxa 2002a)	P = 0.890
(Guma 2001) to (Guma 2002)	P = 0.919
(Mombo 2001) to (Mombo 2002)	P = 0.469

* 2002b is 2002 data before spraying started.
* 2002a is 2002 data after spraying started.
P-value in parentheses ( ) are statistically significant.

Figure 6.8 Mean number of Greybacked Bleating Warblers detected at different sites.



* 2002b is 2002 data before the spraying started.
* 2002a is 2002 data after the first spray event.

Key Results
• Chitabe showed declines in greybacked bleating warblers when comparing (2001 & 2002b) to (2002a), but not when comparing (2002b) to (2002a). • No other site showed a significant change in the number of greybacked bleating warblers.

6.4.2 Acacia thornveld birds (Mombo driving transect)

6.4.2.1 Mombo acacia thornveld presence / absence analysis

Based on 5 surveys in 2001 and 6 surveys in 2002, species in the Mombo thornveld were lost or gained at similar rates regardless of diet (Tables 6.8 and 6.9). Insectivorous birds were lost at a lower rate than non-insect-dependent birds, but due to the small sample size of insectivorous birds (n=19) this slight difference should not be viewed as significant.

Regardless of diet, more species appeared for the first time in 2002 than disappeared. The loss of a species in this survey should not to be taken as a species going locally extinct, but as an artifact of sample effort. As the number of surveys increases, so does the number of species detected. If far more species disappeared than appeared in the second year there would be cause for concern. In this case there is not cause for concern.

Table 6.8 Summary of species presence / absence data from Mombo acacia thornveld (driving surveys).

	# of species Present both years		Absent 2001 and present 2002 (gained)		Present 2001 and absent 2002 (lost)		Total
Non-Insectivorous	21	60.0%	8	22.9%	6	17.1%	35
Insectivorous	11	57.9%	6	31.6%	2	10.5%	19
Total	32	59.3%	14	25.9%	8	14.8%	54

Table 6.9 Birds that were detected in one year but not both years in Mombo acacia thornveld surveys. Shaded areas are for birds considered insectivorous in analyses.

Absent in 2001 and present in 2002 (up)	**Present in 2001 and absent in 2002 (down)**
Blackwinged Stilt	Pearlspotted Owl
Whitebacked Vulture	Gabar Goshawk
Doublebanded Sandgrouse	Laughing Dove
Redfaced mousebird	Ostrich
Glossy Starling	Eastern Paradise Whydah
Helmeted Guineafowl	Whitebellied Sunbird
Melba Finch	Pied Babbler
Crested Francolin	Arrowmarked Babbler
Bennett's Woodpecker	
Little Bee-eater	
Oxpecker	
Chestnutvented Titbabbler	
Blackcrowned Tchagra	
Striped Kingfisher	

6.4.2.2 Mombo acacia thornveld diet guild population analysis

There was no significant change in the number of insectivorous birds between 2001 and 2001 (P = 0.157), but there was a significant reduction in non-insect-dependent species (P = 0.012) (Table 6.10).

Table 6.10 Results of 2 tailed t-tests comparing insectivorous and non insect dependent bird numbers in acacia thornveld (Mombo driving transect) before and after the spraying.

Data sets compared	**P-value**	
	Insectivorous birds	**Non-insect-dependent birds**
Mombo 2001 to Mombo 2002	P = 0.157	(P = 0.012) down

P-value in parentheses ( ) are statistically significant.

Key Results
• The majority of species were detected in both years (>57%) regardless of diet guild. • Nearly twice as many species were gained (only sighted in 2002) than lost (species only sighted in 2001). • There was a significant decrease in the number of non-insect-dependent birds (P = 0.012), but not in the number of insectivorous birds.

6.4.3 Water birds (Guma Lagoon surveys)

6.4.3.1 Guma Lagoon presence / absence analysis

Based on 11 surveys in 2001 and 9 surveys in 2002, species in Guma Lagoon were lost or gained at similar rates regardless of diet (Tables 6.11 and 6.12). Insectivorous birds were lost at a higher rate than non-insect-dependent birds, but due to the small sample size of insectivorous birds (n=14) this slight difference should not be viewed as significant.

For all species combined and non-insect-dependent species, more species gained than lost in 2002. For insectivorous species, more species were lost than gained in 2002. Due to the small sample size, the slightly higher loss of insectivorous species is no cause for concern.

Table 6.11 Summary of presence absence data from Guma Lagoon (boat surveys).

	# of species present both years		Absent 2001 and present 2002 (gained)		Present 2001 and absent 2002 (lost)		Total
Non-Insectivorous	21	70.0%	6	20.0%	3	10.0%	30
Insectivorous	9	64.3%	2	14.3%	3	21.4%	14
Total	30	68.2%	8	18.2%	6	13.6%	44

Table 6.12 Birds that were detected in one year but not both years in Guma Lagoon surveys. Shaded areas are for birds considered insectivorous in analyses.

Absent in 2001 and present in 2002 (up)	Present in 2001 and absent in 2002 (down)
Dabchick	Blacksmith Plover
Little Egret	Purple Heron
Rufousbellied Heron	Redshouldered Widow
White Winged Tern	Tawneyflanked Prinia
African Marsh Harrier	Copperytailed Coucal
Redeyed Bulbul	Cisticola
Redwinged Pratincole	
Warbler	

6.4.3.2 Guma Lagoon diet guild population analysis

There was no significant change in the number of insectivorous birds (P = 0.238) or non-insect-dependent birds (P = 0.76) between 2001 and 2002 (Table 6.13). So while there was some change in species composition, there was not a drop in the number of birds regardless of diet guild.

Table 6.13 Results of 2 tailed t-tests comparing insectivorous and non insect dependent bird numbers along Guma Lagoon (Boat survey) before and after the spraying.

Data sets compared	P-value	
	Insectivorous birds	Non-insect-dependent birds
Guma 2001 to Guma 2002	P = 0.238	P = 0.076

P-value in parentheses ( ) are statistically significant.

Key Results
• The majority of species were detected in both years (>64%) regardless of diet guild. • More insectivorous species were lost (only sighted in 2001) than gained (only sighted in 2002), but the magnitude of this loss is no cause for concern. • There was not a significant decrease in the number of insectivorous or non-insect-dependent birds.

6.5 Discussion

The basic premise of many environmental monitoring programs is to look for changes in a dependent variable in response to an independent variable. In this case the dependent variable is bird numbers and the independent variable is the spraying of deltamethrin for tsetse fly control. The question being asked is, “Are bird numbers changing in response to the spraying?” The situation is complicated by the fact that deltamethrin sprayed at the concentrations used in this operation is highly unlikely to kill birds. If bird numbers do change, it is in response to reductions in their insect food supply caused by the spraying. But regardless of how direct or indirect the connection is, the spraying is having an effect if bird populations change in ways that would not have taken place without the spraying.

Anything aside from the spraying that causes bird numbers to change is background noise which makes it more complicated to pick out changes caused by the spraying. The Okavango Delta is a very dynamic area, with many factors that cause background noise in a bird population study. There are seasonal factors, such as temperature, rainfall, and flooding, as well as such factors as fire, that take place at more random intervals. And there are also natural population cycles, which may be in response to predator and prey numbers or some other combination of factors. Taking background noise into account, a population change would have to be substantial and correlated with the spraying, in order to be convincing evidence that it was caused by the spraying. Even if a change is statistically significant it is not necessarily caused by the spraying. For the evidence to be convincing, several bird populations or guilds would have to show declines which could be correlated with the spraying.

As detailed in chapter 5, the spraying affected non-target insects in several way. The most important to bird populations would be the reduction in insect abundance. A decline in the number of insects means less food for birds. If this reduced food supply does affect bird numbers, insectivorous birds would be expected to decline more than non-insect-dependent birds. Because insects have been reduced, and not eliminated, the mechanisms of a population decline would likely be decreased reproduction and emigration, rather than the immediate death of birds. Surveys can detect changes in bird populations caused by immediate large scale die offs or emigration of birds, but effects of small scale emigration or reductions in reproductive success will likely take years to happen and be difficult to detect.

With this in mind, a short term catastrophic decline in bird populations caused by the spraying of deltamethrin was not detected. Out of 14 t-test on insectivorous bird populations comparing pre and post-spray datas, 2 showed a decrease, 2 showed an increase and 10 showed no change. Out of 8 t-test on non-insect-dependent bird populations, 2 showed a decrease, none showed an increase, and 6 showed no change.

While these analyses provide evidence that the spraying did not have an immediate catastrophic effect on insectivorous birds as a group, they do not prove that there were no short term effects. There is substantial variation from what was expected, and this variation could have been caused by many factors.

The food guild analysis from the point count data is the most powerful test of the effects of the spraying on bird populations in general, because it is based on the largest data set and includes the broadest range of species of all the techniques. The increases in insectivorous birds at Mombo and Guma a year after the spraying, is strong evidence that there was no immediate catastrophic effect. What actually caused the increases remains unknown. While it is doubtful that the increases were related to the spraying, there is the possibility that insects more commonly eaten by birds had higher resistance to deltamethrin had population increases as their competitors suffered population decreases. Staff at Mombo reported increased numbers of flies after the spraying, but this in not verified by any hard data. The increase in birds is more likely in response to a variable which was not measured or just natural fluctuations in local populations.

Whatever the cause of the increases, they are definitely not evidence of a decrease in local populations.

Chitabe showed a significant decline in insectivorous birds during the year of the spraying, but there was also a significant decline from 2001 to 2002 before the spraying. So the population showed a decline before the spraying, and that decline continued through the spraying. This is a case of a significant decline, which can not simply be assigned to the spraying. Whether or not the spraying hastened the decline is unknown. Nxaraxa, which was in the same spray block, showed no such decline, making it difficult to assign the Chitabe decline to the spraying.

Chitabe and Nxaraxa both burned between the last pre-spray surveys and the first post-spray surveys. This could have caused increases and declines in local populations as birds migrated in or out of the area in response to changes in the habitat. Possibly, birds were emigrating from Chitabe in response to the spraying, the fires, or a combination of these and other factors. There were fires all over the Okavango Delta during the 2002 spray season which could have cause local as well as large scale movement of birds.

The only significant change in Greybacked bleating warbler numbers was found at Chitabe when comparing the 2001 and 2002 before spray data with the 2002 after spray data. It is worth noting that the comparison of Chitabe 2002 before spray data with 2002 after spray data yielded a difference that was almost significant ($P = 0.079$). This difference is clearly related to the large drop off in July 2002 detections. If this decline is related to the spraying it was not immediate since the June 2002 numbers were similar to all the other visits. It would be difficult to argue that this decline was in response to the spraying since Nxaraxa, which was in the same spray block showed no such decline.

The fact that the acacia thornveld insectivorous birds at Mombo did not decline, while the non-insect-dependent birds did decline is a bit of a mystery. It is unlikely that it was due to the spraying since the number of insectivorous birds was not reduced. The presence / absence data do not demonstrate a change related to the spraying either, as more insectivorous birds were gained in 2002 than lost. This data should not be taken as a real gain in the number of insectivorous species since the data set is pretty small and new detections are an expected artifact of increasing sample size. So while it is not evidence of an increase, it surely does not demonstrate a decrease.

Neither insectivorous nor non-insect-dependent water birds showed a reduction in numbers at Guma Lagoon. The presence / absence data did not show any substantial deviations from what would be expected if the spraying had no effect. Ultimately there were some local changes in populations during the spraying, but nothing catastrophic was detected, and there is no convincing data that the changes were caused by the spraying. But due to the problems of delayed response to changes in insect numbers, and background noise in the populations it cannot be said that the spraying had no effect. Effects of the spraying could be too small to be statistically significant, or they may not have happened yet. It is strongly suggested that populations continue to be monitored in upcoming years so that longer term changes in populations can be tracked. As each year goes by it will become more difficult to attribute population changes to the spraying operation, but valuable insight into the cycles of Okavango Delta bird populations will be gained.

There is also the possibility of the spraying causing behavioral changes which were not studied. One camp manager who had lived in the Delta for 10 years pointed out that he had seen behavioral changes in some species. In particular he had noticed forktailed drongos hawking insects over the river near Nxaraxa just after the spraying started. This change in foraging behavior may have been caused by a decrease in canopy insect species, or by a increase in insects above the water. If it is the former case the drongos may be stressed and suffer from decreased reproductive success. If it is the latter case, the drongos should not suffer any declines.

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7. Salvinia Weevils

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7.1 Executive summary

An insecticide, deltamethrin was sprayed at a dosage rate of 0.3 grams per hectare in the 1st and 2nd cycles and 0.26 grams/hectare in the subsequent three cycles in the southern part of Okavango Delta in the winter 2002 to control tsetse fly, Glossina morsitans Westwood. The effect of deltamethrin spray was investigated on the beneficial non-target weevil, Cyrtobagous salviniae Calder & Sands, which has been biologically controlling the floating salvinia weed, Salvinia molesta Mitchell in Moremi Game Reserve.

Static short-term bioassay methods were adapted in the studies. Three sets of experiments were carried out and they are Basin-bank, Basin-water and Field-water. In the Basin-bank studies, the materials in the plastic basins were placed in the banks of Khwai River water bodies whereas the Basin-water studies include the perforated basins kept in shallow water to expose their rims. In the third set, field salvinia samples at various sites were collected to determine the impact of spray on weevils in pre spray and post spray periods. The materials in the plastic containers include sediments, river water, half a kg of salvinia and 50 adult weevils. Similarly control basins were maintained at 30km outside the spray block. The weevil mortalities were determined in the first sample after 12 hours of the spray and at 24, 48, and 72 hours from the 12 hours sampling period. Temperature, pH, electrical conductivity and dissolved oxygen determined from the three sets of experimentals were compared under pre spray and post spray conditions.

The adult weevils were susceptible to deltamethrin spray and the mortalities during 72-hour time span of the experiment were statistically significant at 5% and 1% levels. The death of weevils exhibited no difference between Basin-bank and Basin-water and the % mortality was in the range of 5.5 and 46.8. Weevils extracted from the field salvinia mat did not show decrease in the weevil number between the pre and post spray periods. No differences existed in pH, dissolved oxygen except in the conductivity, which was relatively higher in the closed Basin-bank compared to Basin-water and Field-water. Cold and warm temperature factor did not increase the toxicity of deltamethrin on the weevil populations.

The aluminium foil sheets that spread across the bottom of the plastic basins were placed along with salvinia containers to collect the spray drift. The quantity of insecticide deposit was uneven and related to the toxicity to weevils in basins and in the field. Drift sprays less than 1 $\mu g/m^2$did not decrease the survival of weevils as compared to controls.

The mortality rates of salvinia weevils could be due to deltamethrin toxicity drifted into the closed systems as demonstrated in basins. The insecticide toxicity in the field conditions could be transient and the effects might be minimal.

7.2 Introduction

The populations of tsetse fly, *Glossina morsitans* Westwood increased alarmingly in the Northwest district particularly in Okavango Delta in the later periods of 19th century. It was reported that more than 300 cattle died in the year 2000 due to *nagana* disease caused by *Trypanosome* (tsetse fly is the vector to the parasite, *Trypanosome*) around Beetsha and other areas of the delta. The reasons for such steady increase in tsetse fly densities were attributed to 1. Aerial spraying had been discontinued since 1991, 2. Problems in the adequate deployment of Odour Baited Targets (OBTs) and their maintenance in the field and 3. Inaccessibility to the areas during flood seasons. The increase in tsetse fly populations

would also reduce the visitors into Moremi Game Reserve, a hub of tourist active region in the country. Considering these threats, Botswana Department of Tsetse-fly Control Division (TCD) has launched an aerial spraying operation to eradicate the tsetse fly in Okavango Delta in two phases. The first phase covering Seronga on the north and a part of Moremi Game Reserve was sprayed in the winter of the year 2001 (Figure 7.1). The major part of the lower ephemeral channels in Moremi Game Reserve were sprayed in 2002 winter (Figure 7.1).

7.2.1 Distribution and Biological Control of Salvinia

Salvinia molesta Mitchell is commonly called as salvinia, kariba weed and african payal. Salvinia weed is a floating water fern and listed as "the worst weed in the world" by the 1985 Guinness World Book of Records. The weed's native range is south-eastern Brazil. It invaded Botswana via Zambezi and discovered for the first time in Kazungula ($S17^0 42'$; $E25^0 14'$) in 1948 (Edwards & Thomas 1977). Currently the weed occurs in Khwai river, Xini and Mogogelo systems in Moremi Wildlife Reserve of Okavango Delta and Kwando/Linyanti/Chobe river systems bordering Botswana and Namibia on the northeast of the country. The host-specific biological control agent, *Cyrtobagous salviniae* Calder & Sands (salvinia weevils) has been actively involved since 1986 in controlling the *Salvinia* weed in most of the infested waters in the country (Forno & Smith 1999, Naidu et al 2000)

Low temperatures in winter reduce the breeding rate of C*. salviniae* while the adult populations survive in the cold periods. This results in the lowest adult densities in winter periods and the breeding picks up in favourable summer and rain seasons.

7.2.2 Description and Life Cycle of Salvinia Weevils

Adult weevils are sub-aquatic and are found on or beneath young leaves, within the leaf buds or among the roots of salvinia weed. Mating occurs more than once, between five and 26 days after emergence. Eggs are deposited singly in rhizome cavities produced by adult feeding, within buds or in the root mass beneath the young rhizome. Newly emerged larvae are white. Feeding commences on or in the young leaf buds and after 3 to 14 days, larvae enter the base of the leaf bud, tunnel inside the rhizomes where they complete their development. Development inside rhizome is temperature dependent. The average period of larval development at 25.5^0 C was 23 days. Fully-grown larvae are about 2.6 mm long. Pupation takes place within a cocoon spun by the larva beneath the surface of the water in close contact with living plant tissue. The cocoons mostly attach below the leaves. Inside the cocoon, the development takes approximately 12 days to complete the adult stage (Forno et al 1983, Sands & Schotz 1984).

Adult weevils feed on buds and terminal leaves of the salvinia plant. Larvae feed on the roots, buds and in most cases tunnel into the rhizomes where they progressively destroy the nodes, internodes and thus proved to be the biological control agent of salvinia weed.

7.2.3 Insecticide Toxicity on *Salvinia* Weevils

Pyrethroids are among the most potent insecticides known. They are synthetic compounds structurally derived from pyrethrin I, one of the six active components of pyrethrum, which is an extract from the dried flower heads of *Chrysanthemum cinerariaefolium* belongs to the family, Compositae of flowering plants. The natural pyrethrins have excellent insecticidal properties and low mammalian toxicity, but are of limited use because of their low photostability and high biodegradability (Wouters & Van den Bercken 1978).

Under laboratory conditions, pyrethroids are highly toxic to wide range of aquatic invertebrates with most LC_{50} values being less than 1.0 ppb and the most sensitive organisms are surface dwelling insects, mayfly nymphs and larger crustaceans (Smith & Stratton 1986). Deltamethrin is a broad-spectrum insecticide and sequential drift sprays of 0.1 to 0.25 g. a. i./ha "knock down" a wide range of arthropods in large numbers (Games 1981). Sprays of 0.25 g a.i/ha in Zimbabwe (Grant & Crick 1987, Ertz 1988) increased the rates of downstream

drift in a wide range of aquatic invertebrates, but effects were transient and no population declines resulted.

Not much research work exists on the impact of deltamethrin on salvinia weevils. When Botswana Tsetse Control Division (BTCD) decided to spray a mixture of endosulfan and deltamethrin in 1987 in Kwando/Linyanti, *C. salviniae* was actively present in the salvinia infestations of the region. There was considerable concern at that time regarding the potential effects of the mixture on the weevils. Semple and Forno (1990) studied the effects of deltamethrin and endosulfan on salvinia weevils in lab conditions by dipping them in respective insecticides and concluded that the weevil was very susceptible to deltamethrin and less susceptible to endosulfan. Schlettwein and Giliomee (1990) carried out field studies in Namibia in 1987 to determine the effect of endosulfan and alphamethrin mixture on survival of *C. salviniae* in mats of salvinia growing in artificial ponds. The insecticide mixture was applied aerial from fixed wing aircraft and they concluded that adult weevil mortality occurred at the concentration as low as 6g/ha of endosulfan and 0.1g/ha of alphamethrin.

Peter Smith, the officer in Aquatic Vegetation Control Unit (AVC Unit) in the Dept. of Water Affairs, was the pioneer in introducing integrated control methods for salvinia control in Botswana. The correspondence between the AVC Unit and Tsetse Control Division in the country revealed that Smith was against the tsetse spraying operation in 1987 as the chemicals might eradicate the weevils introduced for the first time in Kwando/Linyanti Rivers.

Attempts were made again to monitor the insecticide droplet dispersion impinging on salvinia plant and the weevils' response to insecticide spraying in Okavango Delta in 1991. The tsetse research team visited the areas of infestations with Smith prior to the spraying cycles. They had the opinion that the low dosage of deltamethrin would have minimal mortality on the weevils' eggs, larvae and pupae in view of their position within the salvinia plant. The efforts of undertaking the experiments in the field during spraying cycles were curtailed before embarking on the project. However, the research team felt that the effect of chemical spray on weevils must be monitored in future tsetse fly spraying operations (Merron 1991)

As stated earlier, the TCD division sprayed deltamethrin in 2001 winter covering 7160 square km in the upper Okavango delta. The Aquatic Vegetation Control Unit in the Department of Water Affairs conducted bioassay experiments with plastic containers containing 500 grams of salvinia, river water and 50 weevils placed in the banks of Dasakao channels to determine the impact of the insecticide drift on the mortality of the salvinia weevils. The preliminary results indicate that the adult weevils were susceptible to deltamethrin sprays and the maximum rate of deltamethrin drift struck to the ground was 7.39 $\mu g/m^2$. It was suspected that the temperatures of higher degree in the experimental basins compared to field water could have had higher mortality in combination with the insecticide drift (Naidu 2001).

7.2.4 Objectives

The research entails monitoring the impact of deltamethrin aerial spray on salvinia biological control agent and to make direct observation on mortalities. The research protocol is designed in such a way that the temperature factor should not have influence on the weevil mortality during the insecticide spray under field conditions. The present work is the expansion of 2001 program and much attention is focussed on the effects of deltamethrin alone on the non-target beneficial salvinia weevils.

7.3 Materials and Methods

7.3.1 Site Description

In 2002 Tsetse spraying program, a small area (Figure 7.1) of Khwai River in the downstream of Xaxanaxa Lagoon, the stagnant backwater Paradise Pools and MGR 6 HATAB sites (Figure 7.2) falling in the spray block were selected for experimental sites. The Khwai channels consist of several bays, backwater streams and hippo paths. Salvinia weed is known to occur in the margins, bays and hippo-paths, which are associated with few wetlands

along the flowing watercourses. The dominant associated plants include *Miscanthus junceus* (Stapf) Pilg., *Phragmites australis* (Cav.) Steud*., Typha latifolia* L *., Ficus verruculosa* Warb. as emergent fringe vegetation and *Nymhea lotus* L. *N. caerulea* Savigny, *Brasenia Schreberi* J.F. Gmelin*, Nesaea crassicaulis* (Guil. & Perr.) Koehne, *Lagerosiphon ilicifolius* Oberm. as rooted and floating water plants. The emergent *Leersia hexandra* Swartz is the dominant grass in Paradise Pools and the sedge *Cyperus articulatus* L. in MGR 6 HATAB.

7.3.2 Bioassay experiments

"Bioassay signifies a test in which a living tissue, organism or group of organisms is used as a reagent for the determination of the potency of any physiologically active substance of unknown activity" (Reish and Cshida 1987). Bioassays or toxicity tests are conducted to measure the effect of one or more toxicants on one or more species of organisms.

The number of adult weevils is crucial in the success of biological control of salvinia weed in a given water body. Given the varying distribution of salvinia and the large variation in weevil populations in the field, there is rather no practical alternative than to try exposing weevils on their host at the time of spraying. It is also impossible to replicate the physical, chemical and hydrological factors of natural ecosystem under lab conditions. In such situations, static short-term bioassay methods are handy using compartmentalised containers with test materials, can be placed in the field. In this type of bioassay method, the organism is kept in the same solution and on the host for the entire experimental period (Reish & Cshida 1987).

The experiments were conducted at or close to the co-ordinates in Khwai River (Figure 7.2) sites namely KB 1 (19^0 10' 50.9" S 23^0 26' 22.7" E), KBS 4 (19^0 11' 12.3" S 23^0 27' 49.4" E), MGR 6 HATAB (Hotel and Tourism Association of Botswana) pool (19^0 11' 35.0" S 23^0 27' 28.7" E) and Paradise Pools (KPP 4;19^0 11' 35.0" S 23^0 27' 28.7" E). Experiments representing three categories of habitats were designed to determine the impact of deltamethrin spray drift on the weevils.

Twenty-four plastic basins (each 50 cm diameter and 20 cm depth) at six random sites were placed in the banks along the watercourses of KB 1, KBS 4, MGR 6 HATAB and Paradise Pools. Thus each site had four basins. Each basin was filled with river water and handful of sediments weighing *ca*. 300 grams collected in the riverbanks. Half a kilogram green salvinia devoid of weevils was placed in each basin. Fifty weevils of different age groups were released onto the salvinia mat in each basin two days before the spray for insect acclimatization and establishment. This set of basins on the banks is herein after referred to as 'Basin-bank". Tall *Miscanthus* grass and *Phragmites* reeds were cleared to expose the basins directly to the aerial spraying. The containers were at less than four-meter distance in the banks from the river bodies.

Simultaneously, twenty-four perforated basins were used in the second set of experiments. Six perforations of 1 cm diameter were made in the sidewall at the bottom of the basins so as to dip them directly into the water to enable the basin's flat bottom to sit on the sediment in water while the rim of the basin is exposed to the atmosphere. These basins here after called 'Basin-water', contained similar materials as those of Basin-bank. In this way the temperature variations between the basins on the bank and the basins in water can be minimised.

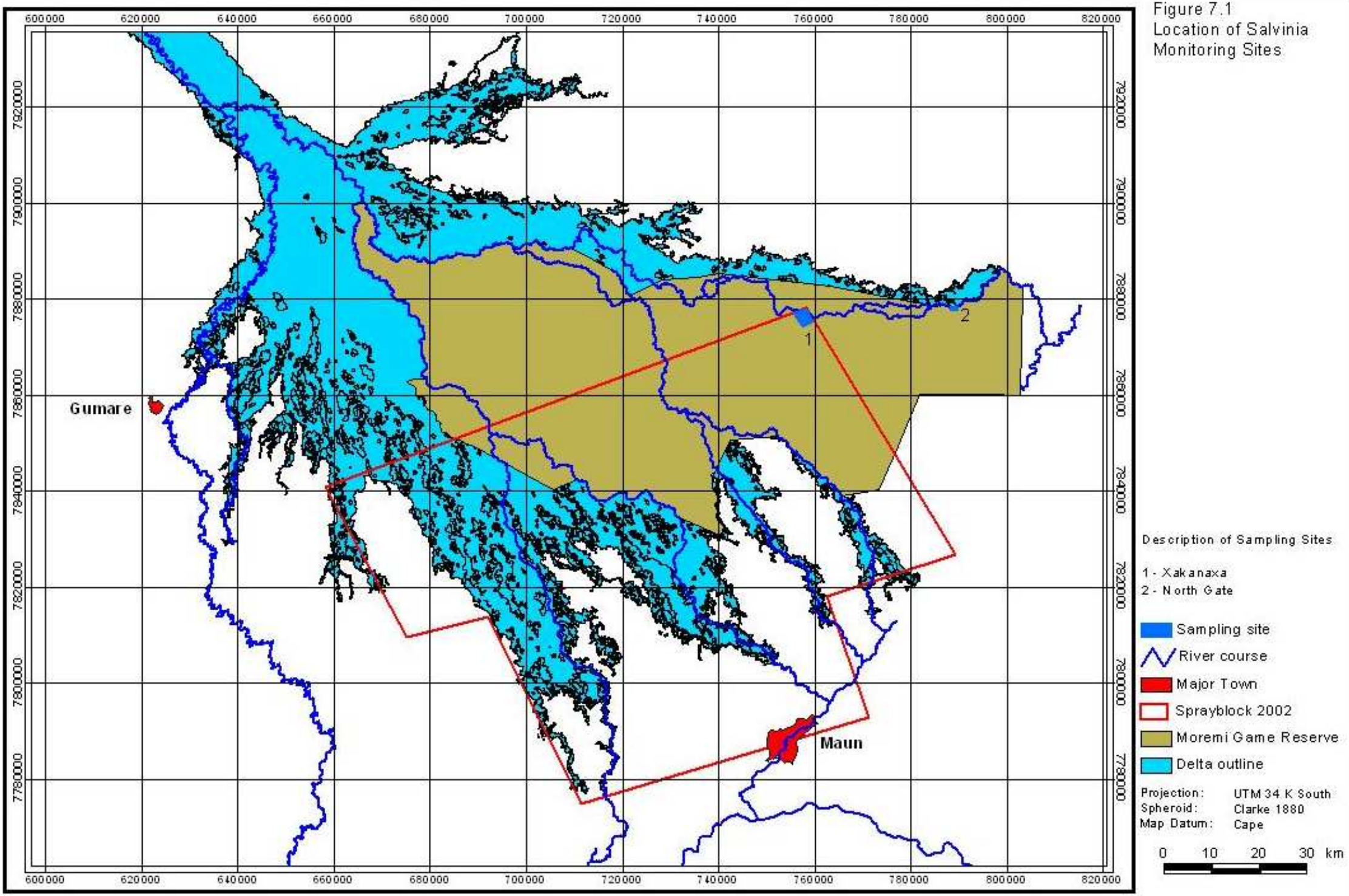


Figure 7.1
Location of Salvinia
Monitoring Sites
600000
620000
640000
660000
680000
700000
720000
740000
760000
780000
800000
820000
7920000
7900000
7880000
7860000
7840000
7820000
7800000
7780000
Gumare
Maun
1
2
Description of Sampling Sites
1 - Xakanaxa
2 - North Gate
Sampling site
River course
Major Town
Sprayblock 2002
Moremi Game Reserve
Delta outline
Projection: UTM 34 K South
Spheroid: Clarke 1880
Map Datum: Cape
0 10 20 30 km

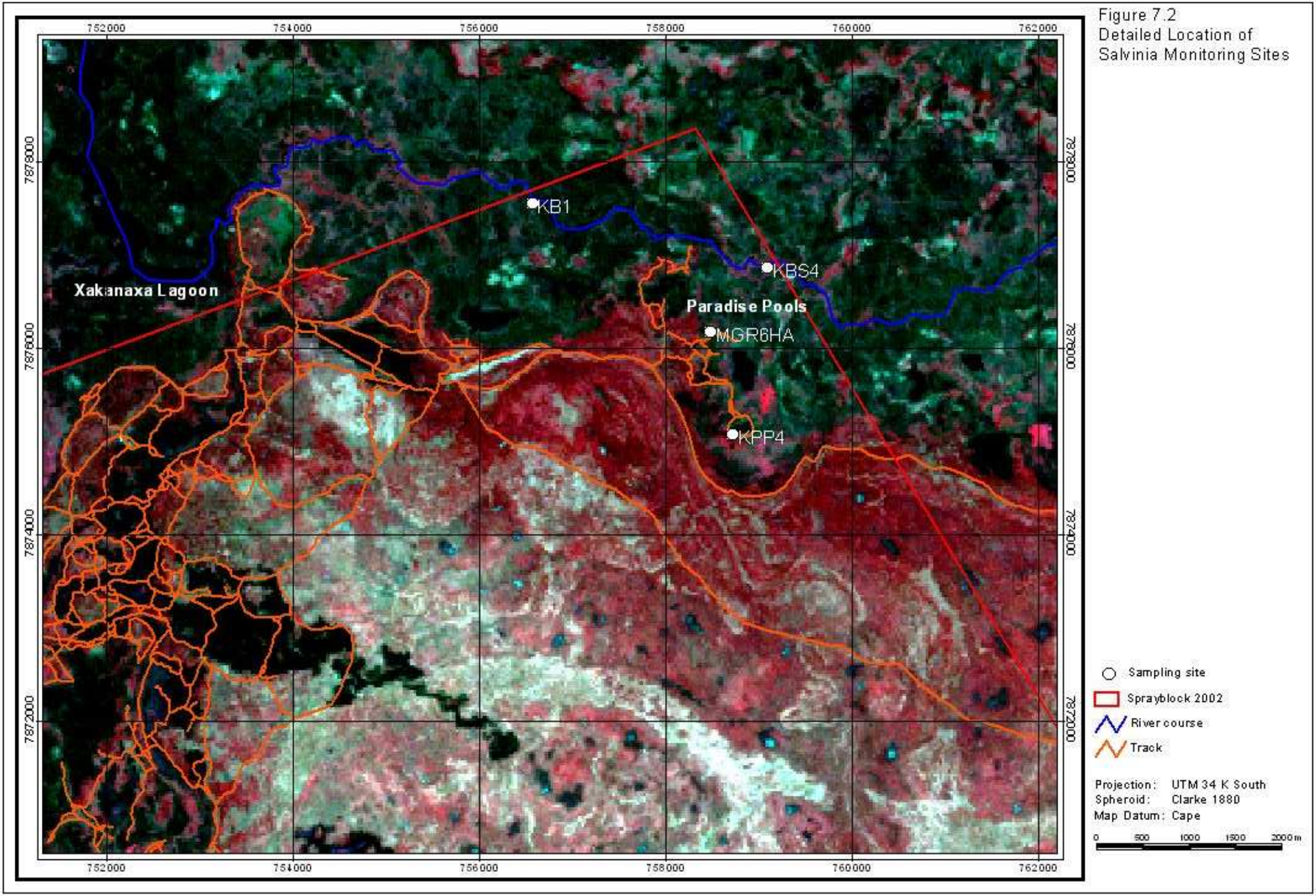


Figure 7.2
Detailed Location of
Salvinia Monitoring Sites

In the third category of 'Field-water' experiments, one kilogram of fresh salvinia mat was sampled in six sites from the field infestations two days prior to the spray and three days after the spray. Salvinia mat was sampled from the same selected sites in each cycle even though the sites might vary from cycle to cycle. This is to measure/determine the variation in population densities in response to deltamethrin spraying in the pre spray and post spray periods.

Twelve representative controls consisting of Basin-bank, Basin-water in a similar manner were maintained at about 30 km outside the spray block at North Gate (Figure 7.2) of Khwai River near Khwai village(19^0 10' 14.6" S 23^0 44' 59.2" E).

The plot was sprayed for five sequential cycles covering 8600 square kilometres (Figure 7.1) in each cycle. Deltamethrin at a dosage rate of 0.3 grams per hectare was sprayed in the first and second cycles and 0.26 grams in the subsequent three cycles. Spraying was done with winged aircraft in the early night hours in each cycle at the following dates with the intervals of 15 to 20 days. The Khwai river area where the experiments had been in progress was sprayed on the last day of every cycle. The experiment was conducted for all the five sequential sprays.

1st cycle	21 May 2002
2nd cycle	04 June 2002
3rd cycle	06 July 2002
4th cycle	26 July 2002
5th cycle	14 August 2002

7.3.3 Sampling

Samples of *Salvinia* with introduced weevils from six basins of Basin-bank as well as in Basin-water at six respective sites were hand-collected on each sampling time. The first sampling was done after 12 hours of the spray between 0600 and 0800 hrs. Subsequent samples were recovered similarly after 24, 48, and 72 hours of the first sample. The knock down/ floated weevils in the basins at the time of sampling ('0' hours) were picked up carefully with hand-fingers and released onto a small fragment of salvinia mat in water taken in small plastic cups for testing their survival after 12 hours. The other weevils in the salvinia mat were completely extracted in 20 hours using Berlese funnels (Boland & Room 1983). After extraction, the weevils were transferred onto salvina mat as described above into another set of plastic cups for determining their death rate after 12 hours. Weevils were also extracted from 1 kg salvinia mat sampled at six field sites (Field-water) in the spray zone two days in the pre spray period followed by three days in the post spray period. Same method was followed for sampling salvinia, the weevil extraction and determination of mortality from three control basins at each sampling time in Khwai North Gate campsite.

7.3.4. Temperature, pH, Electrical Conductivity (EC) and Dissolved Oxygen (DO)

Temperature, pH, EC and DO profiles in Basin-bank, Basin-water and in Field-water were measured one day prior to the spray and three days in the post spray period. Temperature was measured at 7 cm depth from the surface water at 0800, 1300 and 1800 hours and expressed in ^{0}C. A portable pH meter (WTW 330 model) calibrated with pH 4 buffer solution was used to determine the pH while EC was measured with WTW LF 340 model meter on daily basis in the experimental period. Surface water samples were collected from the three categories of experimentals at 0800, 1300 and 1800 hours to determine the dissolved oxygen following the modified azide method (APHA 1992). The mean values of three observations thus obtained were taken into consideration for the content of dissolved oxygen in water.

7.3.5. Mortality determination

Only those weevils that remained alive for more than 12 hours after emerging from the funnels and the weevils that survived after 12 hours of collection from the basins at '0' hours were considered to have survived the spray treatment (Schlettwein and Giliomee 1990). Controls were also subjected to the same timings. Mortality was assessed considering the factors such as crawling on the mat and the response of weevils to cold and warm conditions. The best method to determine the mortality is to wait and observe if they respond to stimuli when the weevils on the host plant are exposed to sunlight.

7.3.6. Preparation of Target Aluminium Foils

Aluminium foil sheets of each with 42X42 cm (1764 cm^2) dimensions were used to assess the deposition of deltamethrin by the aerial spraying method over a period of 12 hours. Six aluminium foils were spread across the bottom of empty plastic basins and placed with salvinia containers in all the five sequential cycles. Three control samples for each spray cycle were similarly prepared.

7.3.7. Collection of Target Samples

The targeted foils were collected and folded once in the early hours of the following day after 10-12 hours of the spray and kept immediately in a plastic box over the ice to preserve the samples. Hand gloves were used to avoid contamination from one sample to the other. The samples were transported to Maun after three hours of collection for storage in the fridge until shipping them for analysis. The targeted aluminium foils were encased in padded envelope and sent to the Natural Resource Institute (NRI), University of Greenwich, UK through DHL courier service for analysis. They arrived within two days of dispatch so the opportunity for degradation of the non- volatile deltamethrin was minimal.

Transport breakdowns and communication was the major constraints to carry out the time schedule duties and required tasks in the field.

7.3.8. Extraction and Analysis

The outline procedure for extraction and quantification of deltamethrin in the aluminium target foils employed by NRI is given below (NRI 1995). Each aluminium foil was unfolded and then carefully cut with scissors into 2-3 cm squares. The cut pieces of foil were transferred into a wide-necked 500 ml Erlenmeyer flask and 250 ml of acetone/hexane in 1:1 ratio was added. The flask was stoppered, placed on a wrist-action shaker and shaken for one hour to ensure that the sample was exposed to the moving solvent.

100ml of the sample extract was then measured and transferred to a 250ml round-bottomed flask and the solvent volume reduced to approximately 5ml using a rotary vacuum evaporator at 40^0C. Deltamethrin extract was determined by Gas Liquid Chromatography with Electron Capture Detector (ECD HP 6890 Instrument). Deltamethrin reference solutions were prepared in acetone at concentrations of 0.01, 0.02, 0.05 and 0.10 μg/ml. The lowest limit of detection on each sample reported by NRI is 0.05μg deltamethrin.

7.3.9 Data Analysis

Students' t-test comparing two groups of unequal and equal size samples (Snedecor & Cochran 1967) was applied to determine whether the weevil mortality was significant after 12 hours in controls and experimental groups (exposed). Percent mortality was calculated by pooling the weevils emerged from the funnels and the weevils survived in the basins with reference to controls.

7.4 Results

7.4.1. Wind

In all the cycles, the wind was stable and blowing slightly from east to west (TCD Division). However, it was observed that the wind velocity was slightly higher at the experimental site in the 3rd cycle.

7.4.2. Water Temperature, pH, Electrical Conductivity and Dissolved Oxygen

Mean water temperatures in Basin-bank were always lower at 0800 hours and higher at 1300 and 1800 hours than in the Basin-water and Field-water (Table 7.1). Temperatures in Basin-bank ranged from 9.8 to 29^0C. Mean minimum and maximum temperatures in Basin-water were 10.9 and 26.8^0C respectively whereas they were 11.6 and 25^0C in Field-water sites (Table 7.1).

Deltamethrin drift did not influence the hydrogen ion concentration, and pH was slightly acidic to above neutral in all the observations ranging between 6.4 to 7.8 units (Figure 7.3A). Moderate to significant electrical conductivity was observed in Basin-bank compared to Basin-water and Field-water samples (Figure 7.3B). The EC in Basin-bank was in between 96 and 156 $\mu S\ cm^{-2}$ whereas EC in Basin-water ranged from 58 to 96 $\mu S\ cm^{-2}$ and 48 to 87 $\mu S\ cm^{-2}$ in field water. However, there was no marked difference in the dissolved oxygen content of waters in the experimentals between the pre-spray and the post-spray periods and the range was in between 4.4 to 8.6 mg/liter (Figure 7.3C). As these parameters did not differ from reference controls, hence the data for controls are not provided here.

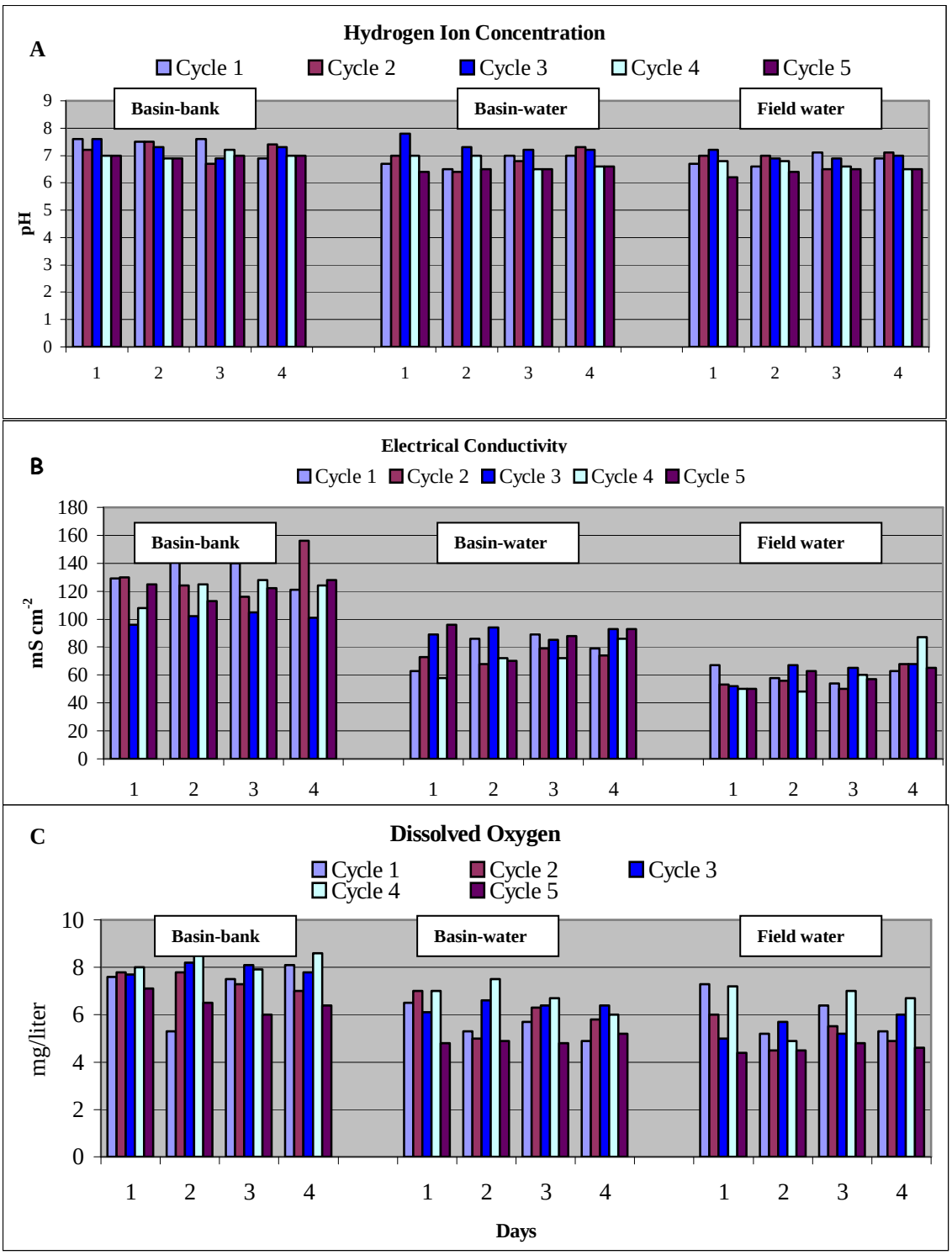
Table 7.1. Mean water temperatures in the experimental period. ±SE =*sd/√n*

Days	Basin-bank			Basin-water			Field-water		
	0800hrs.	1300hrs.	1800hrs	0800hrs.	1300hrs.	1800hrs	0800hrs.	1300hrs.	1800hrs
Cycle 1									
1	14.7 ±0.8	24.0 ±0.1	25.3 ±0.8	17.6 ±0.6	22.0 ±0.0	25.2 ±0.4	17.8 ±0.2	20.5 ±0.6	22.3 ±0.7
2	15.0 ±0.7	23.5 ±0.6	25.1 ±0.3	17.8 ±0.9	21.8 ±0.4	26.2 ±0.1	17.1 ±0.9	19.5 ±0.3	24.9 ±0.3
3	12.9 ±0.1	26.0 ±0.3	26.4 ±0.4	14.7 ±0.2	22.0 ±0.0	26.3 ±0.1	18.0 ±0.3	20.0 ±0.0	25.0 ±0.0
4	13.2 ±0.6	26.3 ±1.0	27.0 ±0.1	15.0 ±0.6	22.0 ±0.2	26.2 ±0.4	17.6 ±0.8	21.0 ±0.6	24.6 ±0.3
Cycle 2									
1	13.1 ±0.1	24.8 ±0.1	24.1 ±0.2	14.4 ±0.1	18.6 ±0.3	22.8 ±0.4	15.3 ±0.3	19.6 ±0.3	24.0 ±0.2
2	12.3 ±0.3	24.3 ±0.3	22.9 ±0.8	**10.9** ±**0.3**	20.6 ±0.2	22.0 ±0.0	11.6 ±0.3	20.0 ±0.0	23.2 ±0.2
3	10.0 ±0.1	24.6 ±0.3	25.5 ±0.3	12.8 ±0.2	22.3 ±0.3	23.0 ±0.0	13.5 ±0.3	23.3 ±0.3	22.6 ±0.3
4	11.6 ±0.3	25.3 ±0.3	25.3 ±0.3	13.3 ±0.3	23.3 ±0.3	23.3 ±0.3	15.6 ±0.4	23.6 ±0.3	23.3 ±0.3
Cycle 3									
1	**9.8** ±**0.1**	21.0 ±0.6	22.7 ±0.4	12.1 ±0.0	19.6 ±0.2	21.0 ±0.6	13.0 ±0.6	21.0 ±0.0	22.0 ±0.0
2	13.6 ±0.3	20.0 ±0.0	22.4 ±0.3	13.3 ±0.3	21.6 ±0.3	22.0 ±0.0	15.3 ±0.3	19.7 ±0.1	20.8 ±0.2
3	10.7 ±0.2	20.4 ±0.3	25.0 ±0.0	14.6 ±0.3	21.0 ±0.3	24.6 ±0.2	16.0 ±0.0	20.3 ±0.3	23.0 ±0.0
4	13.6 ±0.3	22.0 ±0.6	23.5 ±0.3	13.4 ±0.3	21.0 ±0.0	22.8 ±0.4	15.9 ±0.3	19.6 ±0.3	21.7 ±0.1
Cycle 4									
1	14.3 ±0.3	24.3 ±0.2	26.6 ±0.3	13.3 ±0.3	17.3 ±0.3	24.6 ±0.3	12.3 ±0.3	18.5 ±0.3	23.6 ±0.3
2	14.0 ±0.0	23.6 ±0.3	28.2 ±0.2	13.6 ±0.3	19.6 ±0.3	26.9 ±0.3	**11.6** ±**0.3**	18.6 ±0.3	24.9 ±0.3
3	13.0 ±0.3	24.6 ±0.0	**29.0** ±**0.0**	14.0 ±0.1	22.6 ±0.3	26.6 ±0.3	12.0 ±0.0	21.0 ±0.0	24.9 ±0.3
4	12.8 ±0.1	24.6 ±0.3	28.9 ±0.3	14.2 ±0.1	22.1 ±0.1	**26.8** ±**0.0**	13.0 ±0.2	21.3 ±0.3	**25.0** ±**0.1**
Cycle 5									
1	14.7 ±0.3	25.2 ±0.2	25.9 ±0.0	16.3 ±0.3	22.0 ±0.0	22.0 0.6	16.0 ±0.3	19.0 ±0.3	22.0 ±0.0
2	14.2 ±0.2	23.8 ±0.1	25.8 ±0.2	14.5 ±0.0	21.3 ±0.3	23.3 ±0.3	15.0 ±0.0	19.3 ±0.3	23.3 ±0.2
3	11.6 ±0.2	19.5 ±0.3	23.4 ±0.3	12.7 ±0.1	17.4 ±0.1	21.5 ±0.2	13.0 ±0.0	18.6 ±0.2	20.9 ±0.1
4	17.1 ±0.1	22.6 ±0.1	24.9 ±0.0	17.6 ±0.3	20.9 ±0.3	20.3 ±0.3	15.0 ±0.0	21.0 ±0.0	21.3 ±0.3

Table 7.2

Table 7.3

Figure 7.3. Hydrogen ion concentration (pH), Electrical Conductivity (EC) and Dissolved Oxygen (DO) in the experimental period

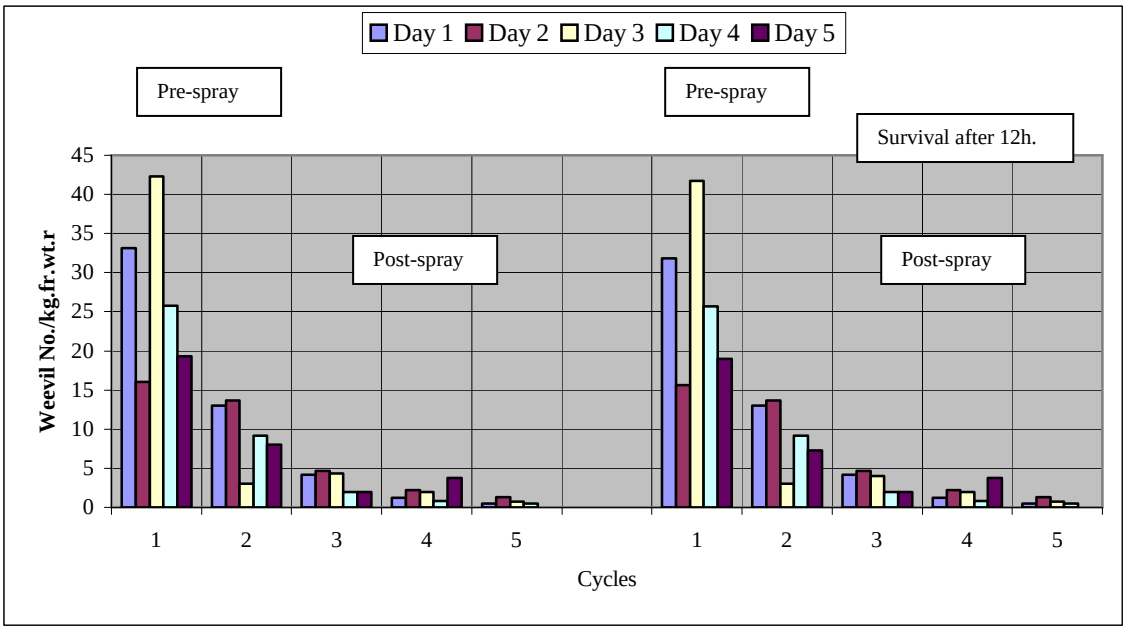


7.4.3. Mortality

Death of the weevils after 12 hours is the criterion used to evaluate the toxicity of deltamethrin. Mortality was not significant in the controls and exposed basins in all cycles from the extraction time to after 12 hours (Table 7.2 and 7.3). However, a significant mortality at 5% among the weevils that initially floated in the basins on the bank was noticed at 12 hours of the spray in 5th cycle only (Table 7.2).

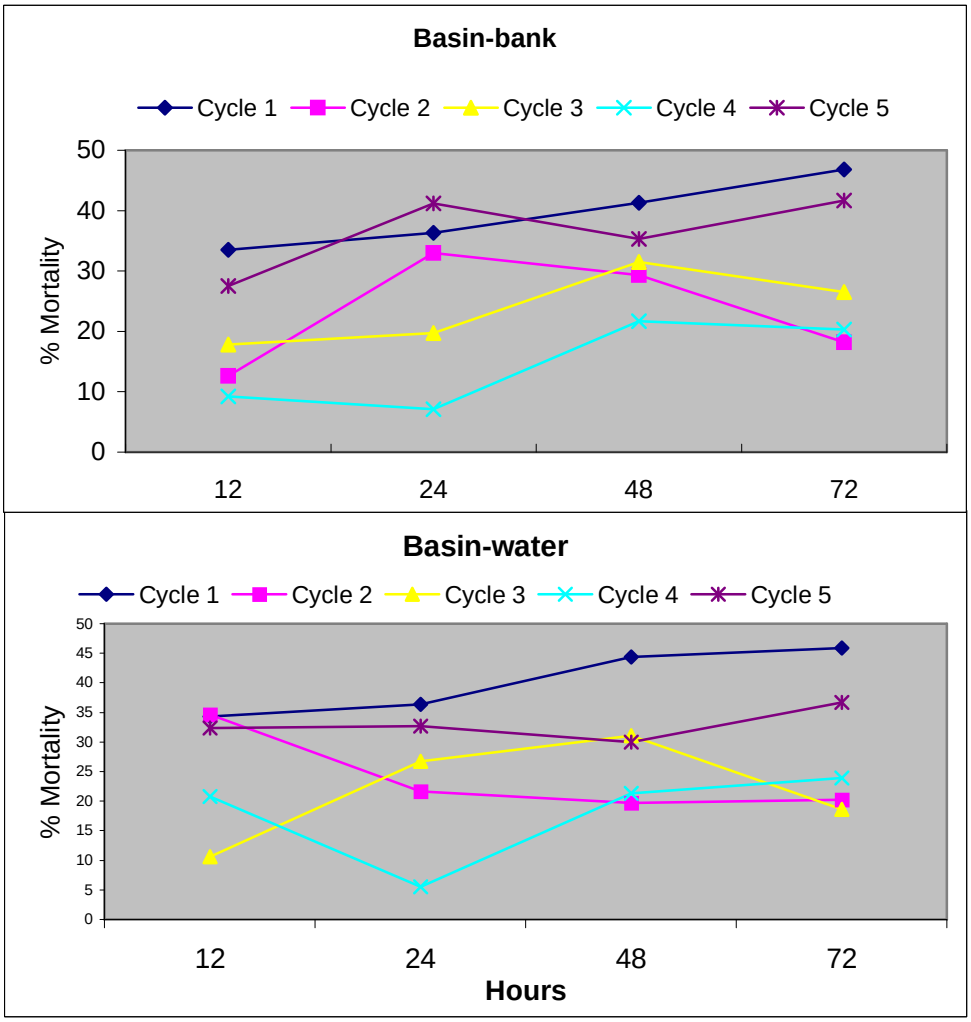
A significant mortality at 5% and 1% levels was observed when t-test was applied to the pooled data between the weevils in controls and weevils in spray exposed basins of 1st and 5th cycles. Nevertheless, the mortalities at 5% level were observed in 24 hours of cycle 2 Basin-bank, and at 1% significance of Basin-water of the same cycle at 24 hours (Table 7.2 and 7.3). Interestingly, death rates were not noticed in 3rd and 4th cycles with reference to controls both in Basin-bank and Basin-water. The weevil mortalities were not observed evidently between the pre-spray and post-spray field samples and the number of weevils obtained from one kg salvinia was highly variable from time to time (Figure 7.4).

Figure 7.4. Weevils' variability and survival in response to deltamethrin spray drift in Field Water



In general, the percent survival of the weevil population in controls was more than 90% in each cycle (Table 7.2 and 7.3). The range of % mortality in the experimental Basin-bank (Table 7.2) conditions with reference to controls was 33.5 to 46.8 in the 1st cycle, 12.6 to 33.0 in the 2nd cycle, 17.8 to 31.5 in the 3rd cycle, 9.2 to 21.7 in the 4th cycle and 27.5 to 41.7 in the fifth cycle. Similarly, the range of % mortality in the experimental Basin-water (Table 7.3) conditions was 34.3 to 45.9 in cycle 1, 19.7 to 34.6 in cycle 2, 10.6 to 31.1 in cycle 3, 5.5 to 23.9 in cycle 4 and 32.4 to 36.7 in cycle 5. Nevertheless, the overall range of mortalities in the five cycles of Basin-bank was in between 9.2 and 46.8 and, it was 5.5 and 45.9 in Basin-water. The % weevil mortalities are presented in Figure 7.5.

Figure 7.5 Percent weevil mortalities in Basin-bank and Basin-water



7.4.4 Insecticide Residue Analysis

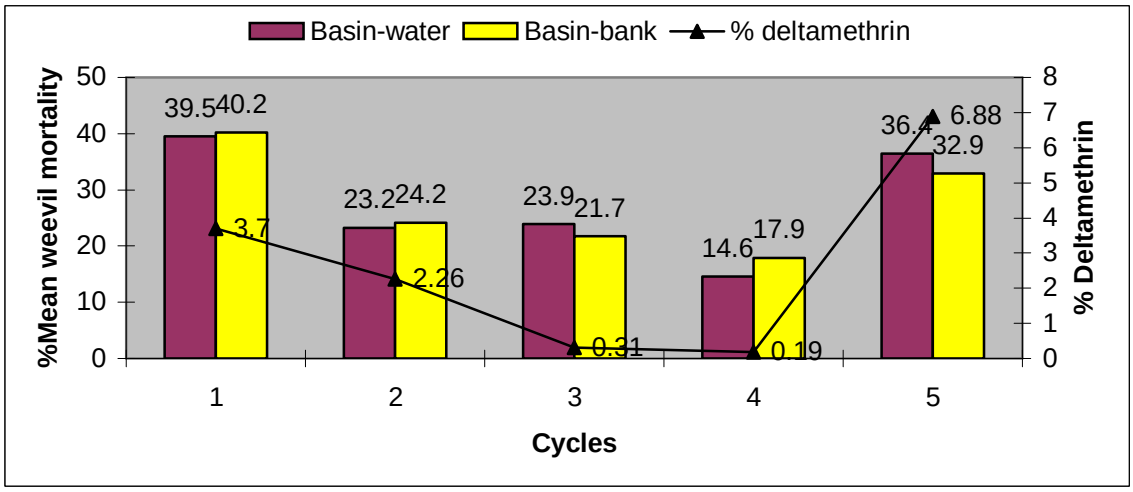
The deltamethrin residual results supplied by NRI, UK are presented in the Table 4. The insecticide in the exposed target foils varied significantly between the independent samples and between the cycles. The insecticide impingement on target Aluminium foils in 5th cycle was higher at the rate of 1.79µg/m^2 followed by 1.11µg/m^2 in 1st cycle in response to the application at the rate of 0.26 and 0.3g/ha respectively. Evidently, the fourth cycle showed least insecticide drift (0.05µg/m^2) where as the 2nd cycle recorded 0.68µg/m^2 and 3rd cycle showed 0.08µg/m^2. The percent insecticide struck to the ground was higher in the 5th cycle (6.88%) and the least in the 4th cycle in relation to the rate of application. The mean % mortality of weevils in the five cycles of Basin-bank and Basin-water in response to the drift is presented in Figure 7.6.

Table 7.4. Residues of deltamethrin from target aluminium foils in µg/m^2 and striking rate in % with respect to total application.

Samples	Cycle 1 (0.3g/ha)		Cycle 2 (0.3g/ha)		Cycle 3 (0.26g/ha)		Cycle 4 (0.26g/ha)		Cycle 5 (0.26g/ha)	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
1	<0.05	1.58	<0.05	0.34	<0.05	<0.05	<0.05	<0.05	<0.05	2.49
2	<0.05	0.74	<0.05	0.62	<0.05	<0.05	<0.05	<0.05	<0.05	1.30
3	<0.05	1.08	<0.05	0.86	<0.05	<0.05	<0.05	<0.05	<0.05	1.30
4		2.38		0.62		<0.05		<0.05		3.19
5		0.62		1.08		<0.05		0.30		1.13
6		0.28		0.57		0.51		<0.05		1.36
Mean .±SE=$sd/\sqrt{n}$	*<0.05 ±0.0	1.11 ±0.31	*<0.05 ±0.0	0.68 ±0.25	*<0.05 ±0.0	0.08 ±0.08	*<0.05 ±0.0	0.05 ±0.04	*<0.05 ±0.0	1.79 ±0.34
% Insecticide	-	3.7	-	2.26	-	0.31	-	0.19	-	6.88

* Not detectable.

Figure 7.6 Mean % mortality with respect to drift spray in the five cycles



7.5 Discussion

Pyrethroids are relatively stable, have a high toxicity to a wide range of insects (Elliott 1976) and are relatively non-toxic to mammals (Elliott 1976). Moreover, pyrethroids are much less persistent than the organochlorine insecticides, such as DDT and dieldrin, and apparently do not accumulate in the environment (Tara et al 1986).

DDT in 1960s, a cocktail of endosulfan (6g/ha) and deltamethrin (0.1g/ha) in 1980s and later a sole synthetic pyrethroid, deltamethrin have been used to control tsetse fly. There has been a remarkable progress in tsetse fly control research to minimize the dosage application of insecticide and 0.26g deltamethrin per hectare is the lowest application ever applied in Botswana.

Temperature measurements provide clear evidence of diurnal surface heating, wind-induced mixing and nocturnal cooling in the Basin-bank, Basin-water and in field waters. Surface heating and cooling was particularly pronounced in closed systems of Basin-bank where the minimum temperature was 9.8^{0}C in the early hours and increased to a maximum of 29^{0}C at 1800 hours compared to the Basin-water and Field-water. It explains that less turbulence of

water molecules is the determinant factor in closed still waters of Basin-bank as evidenced in 2001 spray drift (Naidu 2001).

Dissolved oxygen measurements were in agreement with the earlier observations (UNDP 1997) and the spray drift did not show significant changes in the DO content. Cronberg et al (1996) stated that DO content was below, or substantially below saturation and reflected stagnated conditions through the wetland. The variations in field water possibly reflect the relative stages of flood progression in the Delta.

Slight acidic to mild alkaline conditions, consistent with dystrophic nature of the water are comparable with the earlier research (UNDP 1997) and deltamethrin spray drift did not have any influence on the pH of water in variable experimental conditions.

Ionic conductivity of surface waters in the experimentals was invariably within the range of 48 and 156 $\mu S\ cm^{-2}$ recorded for Okavango Delta. Higher levels of EC in Basin-bank might be attributed to the accumulation of cations in the unique closed biotic conditions. Moreover, the ability of water nutrients to exchange cations for another on leaching into a medium is a common observation in plant growth studies (Alan Wild 1988). In contrast, an ionic gradient could be established between the basins in water and field water through perforations in the basins, as there was no significant difference in EC between Basin-water and Field-Water.

The % mortality with reference to controls clearly shows that adult weevils are susceptible to deltamethrin applied at 0.26g/ha under field conditions. Semple & Forno (1990) found in lab experiments that adult weevils were highly susceptible to deltamethrin (LC_{50} = 0.0000038 mg/l) than endosulfan (LC_{50} = 0.00014 mg/l). The results indicate that the floating behaviour of weevils observed in basins at the time of sampling ('0' hours) was due to the initial knock down followed by recovery or death in response to pyrethroids (Hill 1985).

The growth conditions for salvinia and the weevils were identical with the differences being that only experiment basins received the spray as evidenced from target aluminium foils. Even though the striking rate of deltamethrin to the ground (foil) was higher in the 5th cycle (Table 7.4) than in the 1st cycle, there was not much variation in the mortalities between these two cycles. The spray drift at 0.08 $\mu g/m^2$ (0.31%) or below (0.05 $\mu g/m^2$ = 0.19%) in 3rd and 4th cycles respectively did not show significant weevil mortalities (Figure 7.6). The mortalities showed in the 2nd cycle at 24 hours in Basin-bank (Table 7.2) and at 12 to 24 hours in Basin-water (Table 7.3) may be regarded as the transient period between the knockdown and subsequent recovery of some the weevils, at the striking rate of 0.68 $\mu g/m^2$ (2.26%). This explains the fact that the knocking down of wide range of arthropods was observed in sequential drift sprays of 0.1 g.a.i/ha (Games 1981).

The rate of weevil mortalities in the present experiments clearly showed that the insecticide did not increase its toxicity in cold and warm temperatures recorded for Basin-bank compared to Basin-water and Field-water as suspected in 2001 spray program (Naidu 2001). This is in contrast to the observations that Pyrethroids increase the toxicities at lower temperatures (Cesida 1980). However, the effectiveness of an insecticide may increase when an agent is stressed simultaneously by other adverse factors such as climate, and other natural enemies.

Adult weevils normally hide in buds, roots and beneath the leaves in cold nights that could minimise the surface contact of insecticide directly during the spray period. Even then, Deltamethrin should have had surface toxicity at the water surface zone, as there was no chance for the escape of the insecticide that drifted into the containers. In the environment, deltamethrin could be diluted, partitioned and adsorbed to various organic sediments (Muir et al 1985), which could reduce the toxicity effects on the weevils in field infestations compared to in the containers as obtained in the present investigations.

The rate of application at higher and lower doses of O.3g/ha in the 1st and 2nd cycle and 0.26g/ha in the subsequent three cycles respectively does not have any bearing on the spray drift as reflected in the residue recoveries from the target aluminium foils. Relatively less rate of deltamethrin drift (1.79$\mu g/m^2$; 6.88%) as against in the 2001 spray period (7.39$\mu g/m^2$; 28.42%) has been attributed to three factors. 1. The experimental site was not well placed

inside the spray block and it was close to the buffer zone and the north-eastern corner of the spray block (Figure 7.2). The wind that blew slightly in higher velocity especially during cycle 3 could carry away maximum insecticide drift to the westward direction into the spray block.

It should be recalled that the weevils deposit the eggs in buds and underneath the leaves and newly emerging larvae normally feed inside the rhizome. It would appear therefore that eggs and larvae were well protected against contact with the insecticide (Schelwettein & Giliomee 1990).

The significant percentage (47%) of weevils affected in the present experiments shows the inherent drawbacks of bioassay methods that they do not emulate field conditions completely. This % mortality is almost parallel to that obtained in 2001 spray programme in the country (Naidu 2001). The deltamethrin spraying to control tsetse fly is only for one year in Moremi Game Reserve where the weevils have been active in most of the weed infestations. If the same area is to be sprayed on an annual basis, then there might be a decline in population despite some protection. Therefore, it is unlikely that the weevils would be affected in large numbers as reflected in the field sampling studies. The far fewer number of weevils obtained from 2^{nd} to 5^{th} cycles in the range of 0-13 compared to the cycle 1 (Figure 7.4) reflects the low breeding rate of weevils in winter and is a common feature in natural infestations (Forno et al 1983, Naidu 2000). However, meaningful and comparable results in response to the insecticide spray drift could be obtained in the winter sprays provided the field infestations have a sufficiently large number of weevils.

Long-term tests involving adult density, egg laying or larval development will be more helpful for comparisons of the deltamethrin toxicity in future spraying programs in the country as well as elsewhere. It is pointed out that the response of any organism to the toxicant may differ in closed systems as demonstrated in the containers, from the open field conditions.

Precautions to be followed:

A. Salvinia insects are to be counted and recounted for two to three times before releasing into the small plastic cups that contain standard salvinia plants in water. This way the materials are ready to transfer to basins in the field.

B. At the time of sampling salvinia, each basin must be thoroughly checked for the floating weevils to collect them with hand-fingers.

C. After putting the salvinia mat into funnels, the plastic bags should be overturned to search for insects so as to recover them slowly with hand.

D. When the mat is placed in the funnels, a plastic cup should be placed at the bottom of the hood to recover the running knock down weevils through drops of water.

Acknowledgements

I dedicate this report to Late L. Manga who had been an excellent and dedicated field worker in the AVC Unit. I am grateful to Prof. Ian Grant, Research Director, Natural Resources Institute, UK for his valuable suggestions and comments in preparing the report. The Department of Water Affairs acknowledges Ministry of Agriculture & Tsetse Control Division, Botswana for the financial assistance.

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Table 7.2. Weevils' mean survival and % mortality in response to deltamethrin spray drift in Basin-bank.

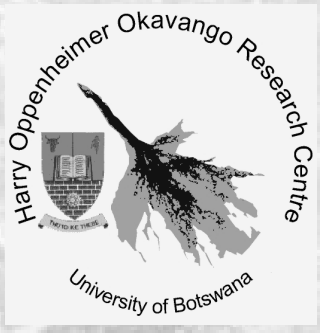
Hours	Extraction 20 hours Control	Survival 12 hours Control A	Extraction 20 hours Sprayed	Survival 12 hours Sprayed B	Basins 0 hours Control	Survival 12 hours Control C	Basins 0 hours Sprayed	Survival 12 hours Sprayed D	A+C Control	B+D Sprayed	% Mortality
Cycle 1											
12	46.0	45.7	34.8	28.7	0	0	3.0	1.7	45.7	**30.4	33.5
24	46.3	46.0	26.8	26.2	0	0	4.7	3.1	46.0	**29.3	36.3
48	46.6	46.0	29.0	24.0	0	0	4.8	3.0	46.0	**27	41.3
72	47.0	46.7	25.7	22.8	0	0	2.5	2.0	46.7	**24.8	46.8
				% Mean mortality							39.5
Cycle 2											
12	48.3	48.3	45.4	42.2	0	0	0	0	48.3	42.2	12.6
24	48.7	48.7	32.8	31.6	0	0	1.4	1.0	48.7	*32.6	33.0
48	49.0	48.0	30.0	29.8	0	0	0	0	46.0	32.5	29.3
72	47.3	47.3	42.7	38.7	0	0	0	0	47.3	38.7	18.2
				% Mean mortality							23.2
Cycle 3											
12	46.7	46.3	38.7	25.7	0	0	2.2	1.1	46.0	37.8	17.8
24	46.7	46.7	36.5	35.5	0	0	3.5	2.0	46.7	37.5	19.7
48	47.7	47.0	31.0	30.2	0	0	2.2	1.8	47.0	32.2	31.5
72	48.7	48.3	33.8	33.5	0	0	4.5	2.0	48.3	35.5	26.5
				% Mean mortality							23.9
Cycle 4											
12	49.0	49.0	44	43.5	0	0	1.3	1.0	49.0	44.5	9.2
24	45.3	45.0	41.3	39.6	0.3	0	2.3	2.2	45.0	41.8	7.1
48	48.0	45.7	34.3	34.2	0.6	0	2.3	1.6	45.7	35.8	21.7
72	47.7	47.7	37.3	35.5	0.3	0	3.5	2.5	47.7	38.0	20.3
				% Mean mortality							14.6
Cycle 5											
12	48.7	48.7	35.3	33.5	0	0	4.5	*1.8	48.7	*35.3	27.5
24	49.3	49.3	25.8	24.5	0	0	4.0	3.5	49.3	**29.0	41.2
48	45.3	45.3	28.8	26.5	0	0	4.5	2.8	45.3	**29.3	35.3
72	45.3	45.3	25.5	24.7	0	0	2.0	1.7	45.3	*26.4	41.7
				% Mean mortality							36.4

*5% significance, **1% significance

Table 7.3. Weevils' mean survival and % mortality in response to deltamethrin spray drift in Basin-water.

Hours	Extraction 20 hours Control	Survival 12 hours Control A	Extraction 20 hours Sprayed	Survival 12 hours Sprayed B	Basins 0 hours Control	Survival 12 hours Control C	Basins 0 hours Sprayed	Survival 12 hours Sprayed D	A+C Control	B+D Sprayed	% Mortality
Cycle 1											
12	48.7	48.7	35.8	31.3	0	0	0.8	0.7	48.7	**32	34.3
24	48.0	48.0	29.5	28.5	0.3	0	4.0	2.0	48.0	*30.5	36.4
48	45.0	45.0	20.3	19.2	0.7	0	8.2	5.8	45.0	**25.0	44.4
72	49.0	49.0	27.3	25.0	0	0	2.5	1.5	49.0	**26.5	45.9
				% Mean mortality							40.2
Cycle 2											
12	48.3	48.3	33.2	31.6	0	0	0	0	48.3	*31.6	34.6
24	48.7	48.7	42.2	38.2	0	0	0	0	48.7	**38.2	21.6
48	48.3	48.3	40.0	38.8	0	0	0	0	48.3	38.8	19.7
72	46.0	46.0	39.3	36.7	0	0	0	0	46.0	36.7	20.2
				% Mean mortality							24.2
Cycle 3											
12	47.3	47.3	43.2	41.8	0	0	1.7	0.5	47.3	42.3	10.6
24	45.7	45.7	33.7	32.8	0.3	0	1.8	0.7	45.7	33.5	26.7
48	45.0	45.0	30.5	29.3	0.3	0	1.3	0.7	45.0	31.0	31.1
72	46.7	46.7	38.5	36.2	0	0	2.0	1.0	46.7	38.0	18.6
				% Mean mortality							21.7
Cycle 4											
12	48.3	46.7	35.7	33.8	0.3	0	3.0	1.3	46.7	37.0	20.8
24	47.3	47.3	41.7	41.2	0.7	0	4.7	3.5	47.3	44.7	5.5
48	48.7	47.0	35.3	34.5	0.3	0	3.0	2.5	47.0	37.0	21.3
72	47.3	47.3	33.8	33.3	0	0	2.7	1.7	47.3	36.0	23.9
				% Mean mortality							17.9
Cycle 5											
12	45.0	45.0	33.2	29.2	0.6	0	2.0	1.2	45.0	**30.4	32.4
24	45.3	45.3	25.8	24.2	0.3	0	9.3	6.3	45.3	**30.5	32.7
48	46.0	46.0	30.7	29.0	0	0	4.0	3.2	46.0	*32.2	30.0
72	48.7	48.7	31.5	28.7	0	0	2.7	2.2	48.7	*30.8	36.7
				% Mean mortality							32.9

*5% significance, **1% significance



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