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Environmental Recovery Monitoring of Tsetse Fly Spraying Impacts in the Okavango Delta – 2003

Final Report

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

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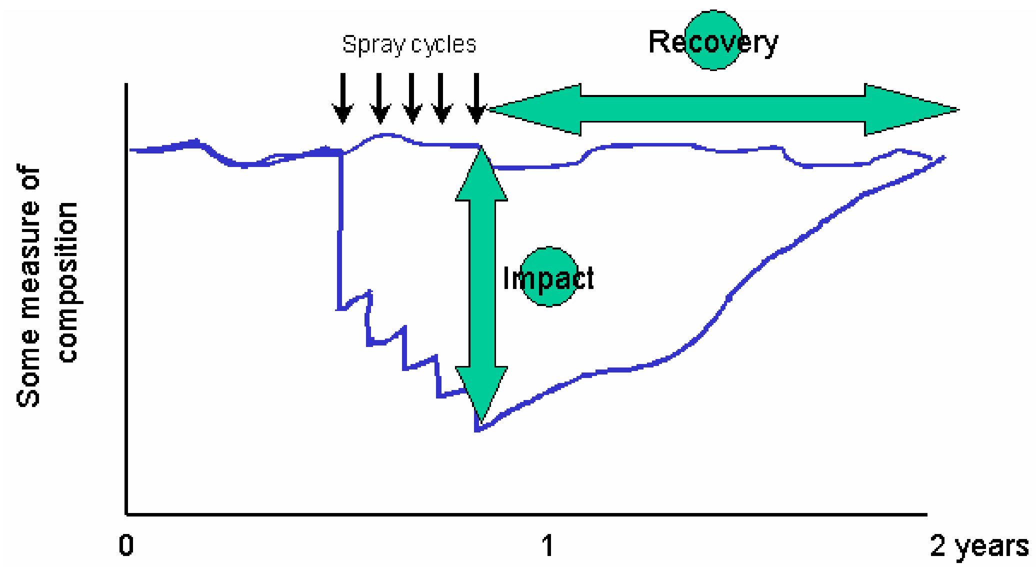

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Executive Summary

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. An extensive environmental monitoring programme accompanied the 2002 spraying.

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.

The 2002 aquatic invertebrate monitoring was repeated in 2003. Species showed different levels of tolerance to the spray, and generally the aquatic invertebrates recovered well. Some of the spray-affected families however remained at reduced levels, notably shrimps and small backswimmers, and 10% of identified species may have been lost due to spraying. Many of the affected families returned to pre-spray abundances and the composition of aquatic invertebrates in the sampled habitats returned approximately to their pre-spray patterns.

Terrestrial invertebrate sampling was conducted using hand-held insecticide foggers on the same trees as in 2002. Abundances and composition returned to pre-spray levels within one year. Some species may be locally reduced but problems of sampling low abundance make this difficult to quantify. Results from the mopane control site suggest that 6% of species losses between years are directly the results of pesticide spraying.

Planning and logistical constraints limited the amount of pre-spray data and information on natural variation in abundance and composition whilst the spraying program gave only limited opportunity for control areas. A single monitoring contract to cover the whole program, with a budget approved alongside the spraying plan, would have allowed for improved planning, better benchmark data and continuity in the monitoring effort.

The Okavango delta ecosystem withstood the 2001 and 2002 spray events and showed good recovery by August 2003. This resilience is through a combination of high aquatic and terrestrial habitat diversity, the chemical properties and specificity of the pesticide to the target organism, and tolerance of many of the organisms to disturbance. Whilst this outcome is encouraging repeated spraying should be undertaken only with care.

Acknowledgements

Many individuals and institutions contributed towards making the 2003 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 Gyps, Nchadi, Messiah and Goots, ran the catering and logistics at Nxaraxa, Xakanaxa and Khwai. Kupaza did an excellent job in helping us to relocate the trees monitored in 2002, while 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.

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1. An Overview of Environmental effects caused by deltamethrin spraying of the Okavango Delta 2001, 2002 and recovery monitoring in 2003.

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 (Figure 1.1). 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 (Figure 1.1), 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

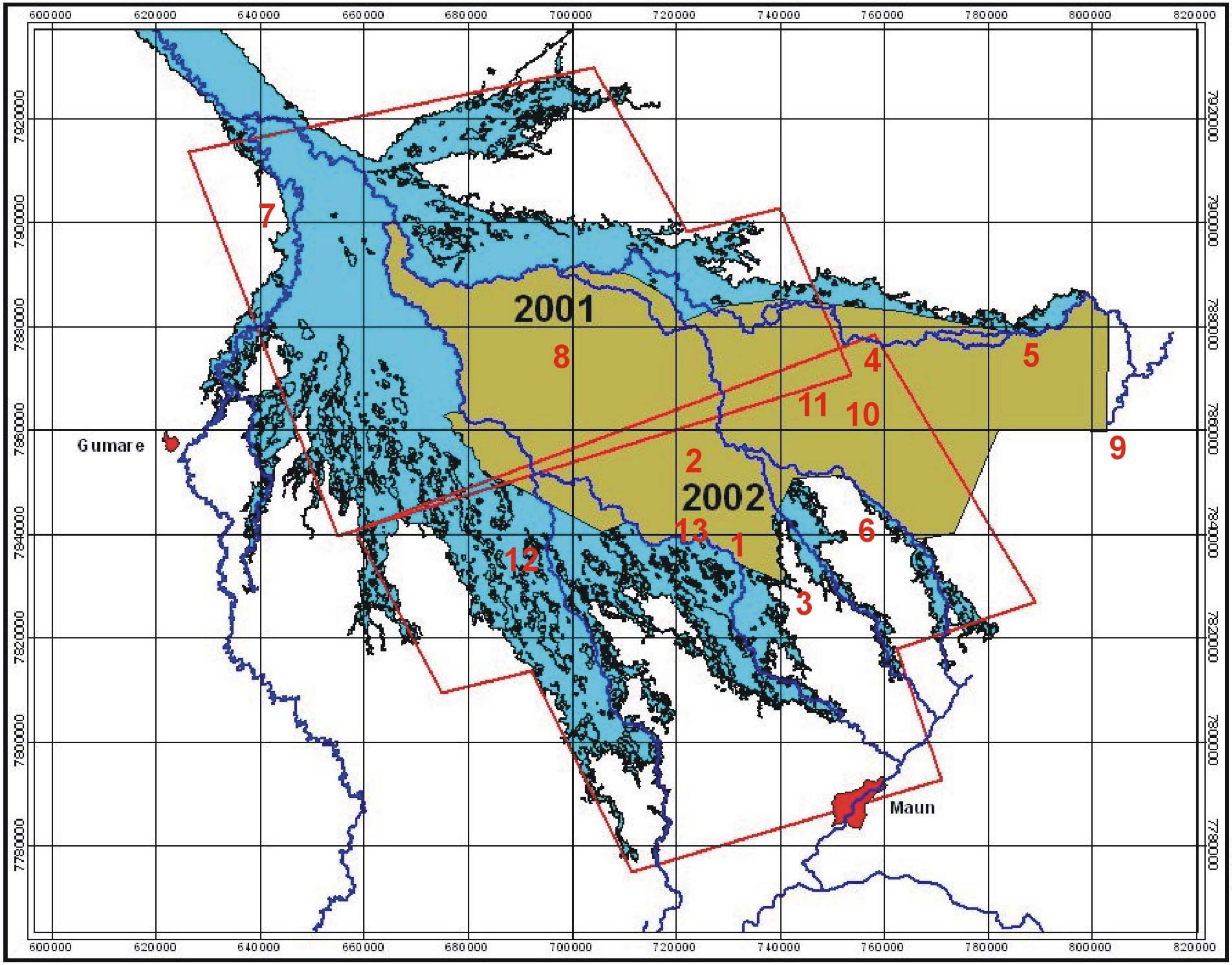

Figure 1.1
Sampling Locations

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

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 caused that these fishes got 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)

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: i.e., 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 (Figure 1.1). 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 (Figure 1.1) - were done both 2001 and 2002 by a research student from the University of California.

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, together with the low wind speed during the 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 65 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.

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 33 families to 31). In lagoons abundances declined by 25% (1230 individuals to 917, and 35 families to 29). Overall the decline was 35% from (2197 individuals to 1437, and 41 families to 36).

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 increased from the benchmark to date of the final cycle 5 spray by 6 % (789 individuals to 839, and 29 to 30 families).

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 20-30% of families absent from the final state. In the initial state lagoon and channels each has a characteristic faunal composition. This difference was less clear in the final state as rarer families, specific to each habitat disappeared. In addition, abundances decreased. The results from the other locations were in accordance with theses changes. Overall, absences were mainly in terms of changed abundances, and families were either locally absent (present at a different site), or rare families that might simply not have been collected.

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.

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

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.

The main results were:

- Different habitats were characterised by recognisably different biota.
- A range of habitats must be sampled to include the aquatic biodiversity of the delta.
- 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.
- Key sensitive taxa were: shrimps, pygmy backswimmers, damselflies, baetid mayflies, water striders creeping water bugs and beetles, flathead mayflies, backswimmers and predaceous beetles.
- 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.

The main 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. 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.

3. 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 2002 by collecting invertebrates that had been knocked down by the spraying, under tree crowns on plastic sheets with an area of just under $3m^2$. 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. Number of flying insects were drecorded to a limited extent by 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 study). 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 (Figure 1.1), which was compared with a site on Chiefs Island that was sprayed 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 3 and 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 dis-appear 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 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 (Figure 1.1). Mombo and Guma in the northern spray block were sprayed 2001 and Chitabe and Nxaraxa in the southern block were sprayed 2002 (Figure 1.1). No suitable not-sprayed reference sites could be found.

Because 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%. This 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 20-30% 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.

1.5 Recovery monitoring in 2003

1.5.1 Aquatic Invertebrates

A spray-correlated effect, and recovery from that, was detected and measured at community, family and morphospecies levels.

The total abundance was after a year still significantly lower: 39% in channels and 60% in lagoons below benchmark. It is however difficult to assign this as a remaining spraying effect as the natural variation from year to year in total abundance is unknown.

At the community level the spraying caused significant changes in number of taxa and abundance of the various families that comprise the community. In the benchmark/initial state, the faunal composition of lagoons and channels were distinctly different. The spraying caused significant reductions in abundance of many taxa and sensitive taxa often specific for respective habitat (lagoons and channels) disappeared altogether. What remained in both habitats was a common group of hardy species. Thus, in the impacted state the compositions overlapped, and in the recovery state they moved towards becoming distinctly different again. This is caused by a re-appearance of the sensitive taxa and out of the six taxa that disappeared during the spraying 2002 all were found again during the 2003 recovery study. The total number of taxa found 2003 is also slightly higher than in the benchmark 2002.

There remain after a year significant differences in the abundance of some taxa. At the family level (table 1.5), the most negatively affected families were Atyidae (shrimps), which are characteristic of areas of permanent flow; and Pleidae (pygmy backswimmers), which are also found in the more seasonal areas. The abundance of Naucoridae (Creeping water bugs) was significantly lower than the benchmark level as well. It is difficult to assign these lower levels to the spraying as the natural variations as stated above are unknown.

Identification to morphospecies of aquatic invertebrates proved in many cases not possible; in particular for larval and nymph stages. This was therefore only done for families with adult stages in water. Out of 39 identified morphospecies four (10%) were classified as "Sensitive" (table 1.6) in that they did not re-appear during the recovery study 2003. Three of these belonged to the family Notonectidae (Backswimmers) and one to Naucoridae (Creeping water bugs). Both these families are generally very sensitive to spraying and this loss of 4 morphospecies (10% of identified ones) may reflect a genuine loss from the system.

Table 1.5 Common families (>8 individuals collected in Cycle 1) from Xakanaxa lagoons (L) and channels (CH), and their responses during the benchmark, impact and recovery states. (Significant = Probability, P, <0.05 and <0.01, very significant = P <0.001.)

FAMILY	BENCHMARK	IMPACT	RECOVERY
Shrimps (Atyidae)	Dominant in L and CH	Very significant decrease in L and CH	Limited recovery but still significantly below benchmark in L , further significant decrease in CH
Pygmy backswimmers (Pleidae)	Dominant in L, common in CH	Very significant decrease in L, and in CH	Significant decrease in L, recovered to benchmark levels in CH
Damselflies (Coenagrionidae)	Dominant in L and CH	Very significant decrease in L, no significant change in CH	Further significant decrease in L, significant decrease in CH
Baetid mayflies (Baetidae)	Common in L, dominant in CH	NO significant change L, very significant decrease in CH	Very significant increase in L, recovery but still significantly below benchmark in CH
Water striders (Veliidae)	Common in L and CH	Significant decrease in L and very significant decrease in CH	Recovered to benchmark in L, still significantly below benchmark in CH
Creeping water bugs (Naucoridae)	Common in L and CH	Very significant decrease in L and CH	Still very significantly below benchmark in L and CH
Caenid mayflies (Caenidae)	Common in L and CH	Significant increase in L, significant decrease in CH	Still significantly more than benchmark in L, CH
Creeping water beetles (Noteridae)	Common in L, rare in CH	Very significant decrease in L, rare in CH	Recovered to benchmark in L, rare in CH

Table 1.6 Changes in morphospecies at Xakanaxa

XAKANAXA MORPHOSPECIES (MSP) PRESENCE/ABSENCE				
Morphospecies category	**Family and number of morphospecies (MSP) identified**		**No. of MSP in response category**	**% MSP in response category**
"Tolerant" = present in benchmark, impact and recovery	Backswimmers	7	1	30%
	Dragonflies	5	0	
	Baetid mayflies	6	4	
	Caenid mayflies	4	4	
	Predatory beetles	13	1	
	Water bugs	4	2	
"Vulnerable" = Present in benchmark, absent from impact, reappear in recovery	Backswimmers	7	1	9%
	Dragonflies	5	2	
	Baetid mayflies	6	0	
	Caenid mayflies	4	0	
	Predatory beetles	13	0	
	Water bugs	4	0	
"Sensitive" – present in benchmark absent after spray, and do not reappear	Backswimmers	7	3	10%
	Dragonflies	5	0	
	Baetid mayflies	6	0	
	Caenid mayflies	4	0	
	Predatory beetles	13	0	
	Water bugs	4	1	
Unrelated to spray: appear first after spray or in recovery	Backswimmers	7	2	51%
	Dragonflies	5	3	
	Baetid mayflies	6	2	
	Caenid mayflies	4	0	
	Predatory beetles	13	12	
	Water bugs	4	1	

1.5.2 Terrestrial invertebrates

In May and August 2003 sampling of terrestrial invertebrates using ground based fogging of tree canopies was completed. The same trees sampled in 2002 at Stanleys Camp and Nxaraxa were sampled again with the objective to establish if abundance and composition had recovered and to try and resample species not seen in the post-spray sampling.

A total of 46,876 specimens were recorded from 216 fogging samples. Average catch per sample in May 2003 was 38% lower than on pre-spray samples in May 2002 and in August 2003 was 31% higher than post-spray August 2002. This general result was consistent in the different locations (near Stanleys Camp and at Nxaraxa) and was more pronounced when considered for each tree species.

On *Kigelia africana* trees all taxa had recovered to pre-spray abundances in both May and August 2003 samples with the exception of beetles which were still well below pre-spray levels. Average invertebrate abundance recovered to pre-spray levels on *Combretum imberbe* and there was no detectable change between years on *Lonchocarpus capassa*.

Looking at each of the key insect groups we see that spider abundance recovered in all cases to pre-spray levels; beetle abundance had not recovered on *K. africana* but recovered on the other tree species; there was no change in fly or ant abundance between years and hemipteran abundance was significantly greater in 2003 than before the spray events. In general there was a strong recovery in insect abundance.

The composition of invertebrate morphospecies in tree canopies changed immediately after aerial spraying. The combinations of species and the relative abundance of each were very different in post-spray than pre-spray samples in 2002. Morphospecies composition of samples taken in 2003 is more similar to pre-spray than to post-spray samples. This suggests that the assemblages within the tree canopies had recovered to their pre-spray composition.

In 2002 there was concern over morphospecies that were recorded in pre-spray samples but not in post-spray samples. These taxa may have been locally depleted as a result of the spray.

In *K. africana* trees near to Stanleys Camp, 50 morphospecies of the 87 "lost" were not recovered in May or August samples in 2003. Fifteen of these were found elsewhere suggesting that 35 (40%) may have been locally depleted. However, 57 morphospecies were found for the first time in 2003 so almost as many species that were potentially lost from 2002 were recorded for the first time in 2003. This shows that there is great variation between years in the composition of invertebrates. More than two thirds of the lost taxa were only recorded initially as single individuals making it difficult to sample them again.

As in 2002 it was possible to compare recovery on mopane trees in two areas, one on Chiefs island that received all five spray cycles and another in Moremi that was outside the spray zone. Spraying altered the composition of invertebrates on mopane trees and increased the variation in composition between samples. In 2003 the same trees were sampled again. There was measurable change in composition between years in both the sprayed and unsprayed mopane, which suggests that species are gained, lost and change in abundance between years even without the application of insecticide.

Around half the morphospecies recorded in pre-spray samples on both sprayed and unsprayed mopane sites were not seen in post-spray sampling in 2002. This means that even without spraying there is a big change in the species that occur on mopane within a year. In 2003, 32% of the morphospecies found in 2002 were not re-sampled from the sprayed site and 26% from the unsprayed site. This suggests that spraying may account for a failure to recover about 6% of the original species complement.

1.6 Conclusions, limitations and improvements

It is likely that the total abundance of invertebrates in both the terrestrial and aquatic habitats recovered into a level of normal variations. Total abundance is however a blunt parameter. More encouraging is the fact that the taxa that were lost after spraying in the aquatic system were found again in the recovery study and that in both systems the number of species and taxa were as high or higher the year after spraying. Ecological functions are therefore likely to be un-disturbed.

Effects on biodiversity and loss of species are more difficult to evaluate. The first problem concerns the large number of rare species and that we do not know the total number of species and their natural variations. This adds wide confidence limits to the possible long lasing species loss of 6% and 10% reported here. Secondly, the appearance of many new species and taxa during the recovery may at least partly be an effect of the spraying itself in that the species negatively affected left open ecological niches that then became occupied by very rare species waiting for their chance. This is a well known function of rare species in biological systems. If this is the case then the long lasting loss of species might have been larger than suggested here. The effect of such changes is that the ecological functions are maintained under present conditions but the system has lost some of its resilience to environmental change.

Planning and logistical constraints limited the amount of pre-spray data and information on natural variation in abundance and composition of non-target taxa. Decisions to go ahead and allocation of funds meant that the environmental monitoring project was initiated only after the first two spray cycles had been completed in 2001. This prevented the collection of pre-spray benchmark data for any taxa within the northern spray block. The design of the spraying, which was optimal for the objective of tsetse eradication, prevented the use of control comparisons in the environmental monitoring as there were no areas of similar habitat available that did not receive the spray.

A single monitoring contract to cover the whole program, with a plan and a budget approved alongside the spraying plan, would have allowed for improved detailed planning, better benchmark data and continuity in the monitoring effort. Any future spraying operations would benefit greatly from this approach, one that allows sufficient time for benchmark data to be collected prior to the first insecticide application.

Despite these constraints the survey teams were able to collect a great deal of high quality data that provided not only a reliable appraisal of impact and recovery from the insecticide application, but also generated inventories and benchmark assemblages of aquatic and terrestrial invertebrate taxa on a scale not previously achieved in the Okavango Delta.

2. Recovery Monitoring of Aquatic Invertebrates

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Morphospecies identification: in collaboration with BioTrack Australia.

2.1 Introduction

The Okavango River Delta extends over an area in excess of 4000km^2 and comprises a network of terrestrial and aquatic ecosystems. In 2001 and 2002 this area was comprehensively sprayed with deltamethrin, in order to control populations of tsetse fly that threaten both human and animal health (Allsop 2003). Tsetse flies are terrestrial, but the entire terrestrial-aquatic system was sprayed, and there were indications that, because of vulnerable organisms, and rapid pesticide breakdown and adsorption onto organic/sediment particles, the effects of deltamethrin on the aquatic ecosystems should be monitored (Scott Wilson 2001).

2.1.1 Study area

The Delta ecosystem is endorheic (a system draining the landscape without reaching the sea), with the Okavango River flowing strongly in the panhandle and with decreasing velocity in the main tributary channels. Water then spreads out into a maze of side channels, pans, pools, flood plains and lagoons. The flat topography, deep sand substrate and lower velocities result in a complex mosaic of seasonally and spatially diverse aquatic habitats. Although described variously in different studies (Hart 1997, AquaRAP 2000, Palmer 2002, Dallas & Mosepele 2003, Mosepele et al. 2003), these aquatic habitats are generally defined and recognized in terms of flow, vegetation and flood-related seasonality. Habitat is a major factor governing the presence, absence and abundance of aquatic invertebrates (Palmer et al. 1991, Kay et al. 1999, Dallas 2002), providing the physical template against which diversity is evaluated. The Delta is strongly seasonal, with a gradient of more permanent to more seasonal flow and inundation from the panhandle, south, south-west and south-east. In all 3 phases of the tsetse spray study, aquatic invertebrate samples were specified by habitat and season.

2.1.2 Monitoring in relation to spray events

The aim of the study has been to monitor and evaluate the effects of the 2001 and 2002 deltamethrin spray events on the composition of aquatic invertebrate communities. Biological composition has been used as an indicator of the functional "health" of the ecosystem (Uys et al. 1996, Downes et al, 2002). Interpretation of invertebrate presence, absence and abundance data has been guided by the knowledge that biological diversity and distribution generally follow particular patterns, including seasonal and inter-annual variability. Interpretation of data will therefore be difficult without the prior knowledge of this seasonal variation. Diversity is also characterized by some abundant taxa, but many more taxa that are rarer; and distribution is frequently patchy in time and space (Begon et al. 1996). Therefore, in an extensive area, with high habitat diversity and strong seasonality, like the Delta, the numbers of rare taxa in a sample will be more variable than of abundant taxa; and the absence of a taxon may indicate absence from the sample rather than absence from the system.

Dangerfield (2002) listed three sets of data on, and descriptions of, biological composition that are required in environmental impact assessment:

1) benchmark or "normal" (pre-spray) state;
2) impacted (post-spray) state; and
3) recovery state (changes towards the benchmark, or an agreed "recovery" state).

In this study each of these states is described by the biological composition of three sets of monitoring samples:

benchmark samples	01APR	August pre-spray 2001
	02MPR	May pre-spray 2002
impact samples	01APO	August post-spray 2001
	02M-APO	May, June, July, August post-spray, within the spray cycle period 2002
	02APO	August post-spray 2002
recovery samples	03MPO	Recovery sampling in May 2003, a year after the initial May 2002 post-spray period
	03APO	Recovery sampling in August 2003, a year after the final August 2002 post-spray period (Table 2.1).

In this study the presence, absence and abundance of aquatic invertebrates was used to describe and compare these states.

2.1.3 Aims of Phase 3: Recovery Monitoring

Three conclusions were drawn by Palmer (in prep.) after family-level analysis of Phase 2 data:

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 tolerant taxa.

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.

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

The aims of the Phase 3, 2003, Recovery Monitoring were to monitor and report on the status of previously monitored sites so as to assess recovery, and to evaluate the Phase 2 conclusions.

Specific objectives were therefore:

- To assess the degree and significance of overall and taxon-specific changes in aquatic invertebrate abundance;
- To assess the degree and significance of overall and taxon-specific changes in aquatic invertebrate composition;
- To assess additional insights provided by the presence and absence of identified morphospecies.
- To make recommendations about further investigations.

The objective were achieved by

- sampling aquatic invertebrates in May and August 2003, with identification of all taxa to family, and of selected families to morphospecies;
- re-sorting samples from 2001 and 2002 appropriately, with identification of and identify Selected families to morphospecies; and
- comparing selected aquatic invertebrate community structures from 2001, 2002 and 2003.

2.2 Study sites and sampling regimes

2.2.1 Study sites

Because continuity was needed for the data collected in 2001 and 2002, the same sites were used for monitoring in 2003. These included the following: sites located within the 2002 spray zone (Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom) and outside of the spray zone (North Gate) (Figure2.1).

Xakanaxa (XA)

- Khwai Main Channel: The KSB1 channel site (Khwai main channel immediately outside Xakanaxa camp). 10 samples for three days (30 samples).
- Khwai South Branch: The KSB1 lagoon and shallow seasonal (2003) sites (Khwai south branch at Paradise Pools). 10 samples per habitat for three days (60 samples).

Bodumatau Bund (BB)

- Bodumatau Bund channel. 10 samples on one day (10 samples).

North Gate (Khwai Bridge) (NG)

- The unsprayed site. Khwai channel, lagoon and shallow seasonal. 10 samples on one day (30 samples).

Pom Pom (PP)

- Pom Pom channel, lagoon and shallow seasonal sites. 10 samples per habitat for one day (30 samples).

Nxaraxa (NX)

- NX channel and shallow seasonal sites. 10 samples per habitat for one day (20 samples).

A total of 180 samples were collected from five sites and three habitats.

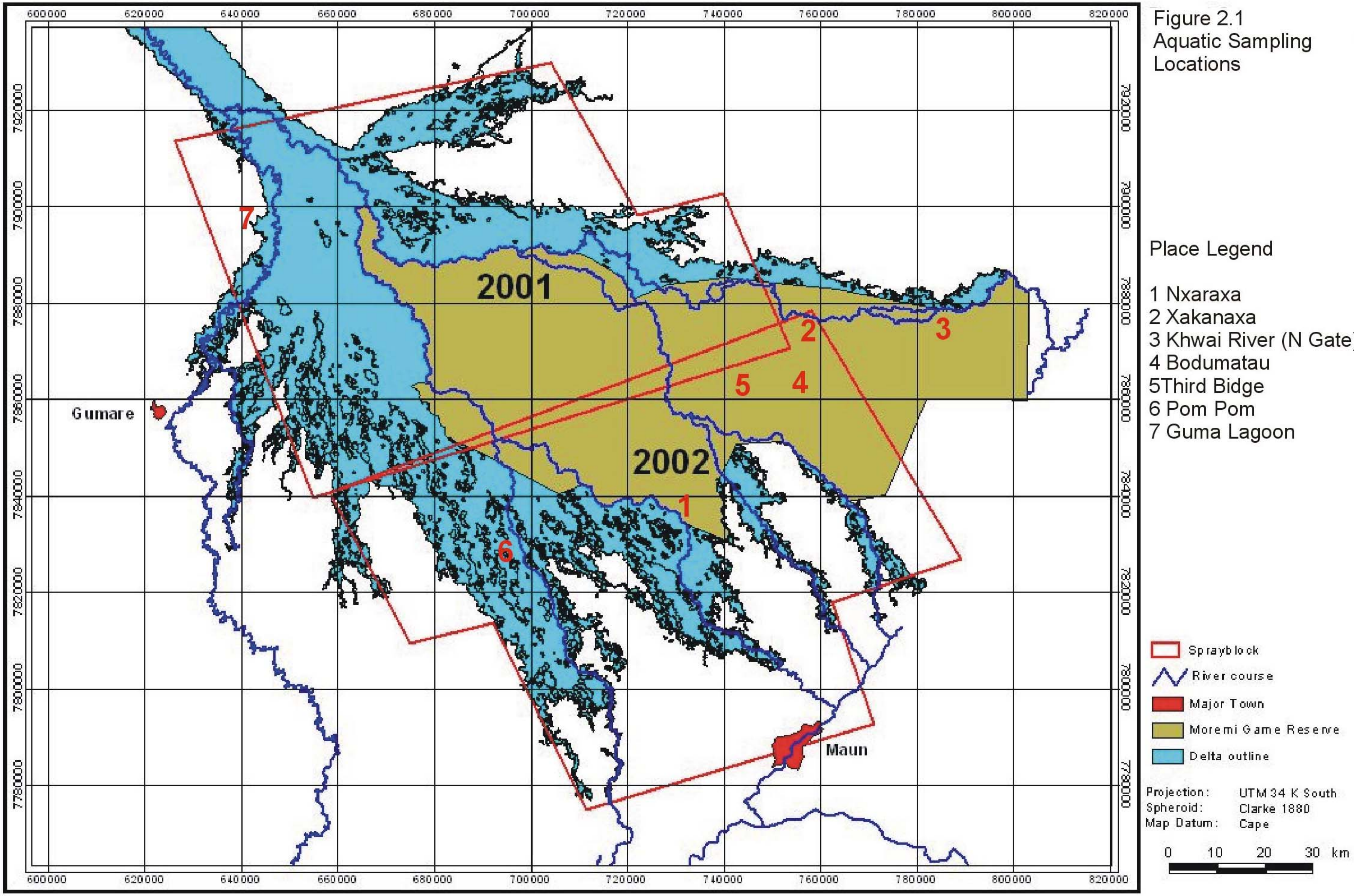


Figure 2.1
Aquatic Sampling
Locations

2.2.2 Tsetse control impact monitoring

The tsetse spray impact monitoring study was based on temporally and spatially stratified sampling, conducted in three phases:

Phase 1 2001 Experimental Monitoring:

Single spray event (August) of a limited, previously unsprayed area in the South (Pom Pom).

Monitored x1 pre- and x1 immediately post-spray.

NOTE: In Phase 1, five cycles of spraying over the North of the Delta were unmonitored.

Phase 2 2002 Spray Monitoring:

Five cycles of spraying over the South of the Delta, including re-spraying of

Pom Pom.

Monitored 1x pre-spray, 1x pre- and post- each spray cycle at 4 sites

[Xakanaxa (XA), North Gate (NG), Bodumatau Bund (BB); Third Bridge (3B)] & monitored post-cycle 5 at Nxaraxa (NX) and Pom Pom (PP).

Phase 3 2003 Recovery Monitoring:

Monitored May (time of 2002 pre-spray) and August (time of 2002 final post-spray) at a range of sites.

May: XA, BB & NG and August, XA, BB, NG, NX, PP.

Wherever possible three main habitats were sampled:

lagoon (L) (marginal vegetation, no flow, permanent water),

channel (CH) (instream vegetation, flow, permanent water), and

shallow seasonal (SS) (rooted vegetation, no flow, seasonal inundation).

Monitoring has been reported: Phase 1 (Perkins and Ramberg 2002), Phase 2 (Perkins and Ramberg 2003), and Phase 3 (this report). For aquatic invertebrates, this report returns to data from Phase 1 and Phase 2 in order to add an extra dimension of analysis: identification of selected families to morphospecies. To facilitate consistent reporting, in this report the results from all sampling occasions were standardized to 5 net-sweeps per sample, and 10 samples per habitat. Sets of sampling occasions have been coded:

01APR	2001	August	Pre-spray
01APO	2001	August	Post-spray
02MPR	2002	May	Pre-spray
02M-APO	2002	May-August	Post-spray within spray cycle periods
02APO	2002	August	Post spray
03MPO	2003	May	Post-spray recovery
03APO	2003	August	Post-spray recovery

The opportunity to collect benchmark data was very limited and some use was made of data collected from the unsprayed, but downstream site, at North Gate (Palmer 2002). There are also varying levels to which organisms can be identified, and the level of identification affects the analysis and interpretation of biological composition. In all three phases of the study, samples were identified to family level and counted. Six families indicative of spray impacts were then identified to morphospecies level from the Pom Pom and Xakanaxa sites, from the benchmark, initial spray and recovery periods. Morphospecies identification of samples from these periods was undertaken until each morphospecies was recorded as “present”. Morphospecies are therefore reported on a presence/absence basis, compared with the abundance information at the family level.

The three-year sampling regime (Table 2.1) provided the opportunity for a variety of spatial and temporal comparisons.

Table 2.1 Record of aquatic invertebrate samples collected during Tsetse control monitoring programme 2001–2003 and used in this report (BB - Bodumatau Bund; L – lagoon, CH – channel, SS – shallow seasonal)

Site:	**North Gate**			**Xakanaxa**			**BB**	**Nxaraxa**			**Pom Pom**		
Habitat:	**L**	**CH**	**SS**	**L**	**CH**	**SS**	**CH**	**L**	**CH**	**SS**	**L**	**CH**	**SS**
01APR											X	X	X
01APO											X	X	X
02MPR	X	X		X	X		X						
02M-APO	X	X		X	X		X						
02APO	X	X		X	X	X	X	X	X	X	X	X	X
03MPO	X	X		X	X		X						
03APO	X	X		X	X	X	X	X	X	X	X	X	X

2.3 Methods

2.3.1 Macroinvertebrate sweep net collection

For the purposes of continuity and data comparison, the same macroinvertebrate sweep net collection methods were used as in 2001 and 2002.

Lagoon, channel margin and shallow seasonal habitats were sampled using a standard SASS net (300x300mm net, pore size 1mm, Chutter 1998). For the 2003 analyses, the contents of 5 "scoops" or "sweeps" of the net over a distance of 5m comprised one sub-sample. After each scoop or sweep the invertebrates were washed down and concentrated into the pit of the net. The contents of the sub-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 sub-samples. In each habitat 10 samples were collected over a wide area.

2.3.2 Processing of 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.

2.3.3 Data analysis

Chi-squared analyses were used to compare the bench-mark condition abundances with 1) the August 2002 post-spray state, and 2) the August 2003 recovery state. In each instance the "expected" was that abundance would have remained similar to the observed, benchmark, state. Analyses were also undertaken to show similarities and differences between years; sites; and habitats, in terms of the presence, absence and abundance of macroinvertebrate families. Both Sigmastat (means, standard errors and deviations), and PRIMER v5 (Clarke and Gorley, 2001) multivariate statistical packages were used. Data entered into PRIMER were analysed on the basis of presence, absence and abundance. Non-metric analyses using Multi-Dimensional Scaling (MDS) plots were produced for each habitat, using untransformed data (which places emphasis of analyses on taxa at either end of each distribution) and transformed (log x+1) data (which places emphasis on taxa with median abundance); using the Bray-Curtis Similarity measure. Results are presented as ordination diagrams showing samples grouped in a 2-dimensional ordination space.

Subsequently, Analysis of Similarities (ANOSIM) procedures, a multivariate equivalent of ANOVA, to test for *a priori* differences in composition between categories, were also computed, and results are recorded as Global R statistics, to look for between-group and within-group similarities. Also presented are the significance levels, which are a probability estimate of the observed outcome. If the ANOSIM was significant, an analysis to assess the contribution of individual taxa to similarities (Similarity Percentages, SIMPER) was performed.

Presence/absence of morphospecies were described and used to gain insight into changes indicated by multi-variate analysis of the community structure.

2.3.4 Morphospecies identification

Morphospecies are taxa that are readily separated into groups based on external morphological differences obvious to a trained technician. These morphological differences are character traits of taxonomic importance and are specific to the group under examination. Morphospecies are considered a surrogate for formal species level classification, and as such offer a more detailed analysis of community structure.

BioTrack Australia Pty Ltd, based in Sydney, Australia, has been responsible for monitoring spray effects on the terrestrial macroinvertebrates in the Delta. The use of morphospecies in determining community changes (developed by BioTrack) is based on a system of integrative imaging and the development of identification keys based on appropriate character traits. Use of barcodes facilitates efficient data capture as each specimen under examination is identified beyond family level.

Those aquatic families identified in 2002 as showing the clearest responses to deltamethrin (Table 2.5) were then selected from all samples collected in 2001, 2002 and 2003, and transported to BioTrack, Sydney, Australia. These selected families had high relative abundances, and included a range of trophic levels (predators, grazers and detritivores). Individuals were identified to morphospecies level, under x20 or x40 magnification. Keys were developed, and, together with multiple images of the morphospecies, were recorded in the web-based databank maintained by BioTrack. Presence or absence of morphospecies was recorded for samples from Pom Pom and Xakanaxa from each sample year, and each habitat.

The success of developing morphospecies keys varied between the families. Coenagrionidae were an initial obvious choice for keying characters, because of their dominance across the habitats and sites and their evident decline after spaying and lack of recovery (Table 2.5). However, morphological features were impossible to observe consistently and the family proved unsuitable for morphospecies identification. The dominance of soft bodied larval stages in aquatic fauna is of concern where rapid identification of morphospecies is necessary. Difficulties in characterizations included hairs that fall out after preservation, and body patterns that change with instars; size cannot be used as this may change with conditions and instar. However, keys were developed for seven families: Notonectidae (backswimmers), Aeshnidae (swimming dragonflies), Baetidae (baetid mayflies), Caenidae (caenid mayflies), Dytiscidae (predatory diving beetles), Noteridae (and Naucoridae (creeping water bugs). Morphospecies were recorded simply as present or absent, and once presence was recorded no further individuals were identified.

Two other taxa recorded at the family level also provide morphospecies level insights. There is only one species of pleid (Pleidae: pygmy back swimmer) in the Delta so all results are for that species. The freshwater shrimps (Atyidae) are also likely to include at most 2 species and their abundances are considered as a single morphospecies.

2.4 Results

2.4.1 Family abundances at Xakanaxa and North Gate

Family abundances and composition are shown in Table 2.2-2.9). A total of 67 taxa were identified in the 2003 recovery state, compared to 65 for the 2002 impact. In each of the habitats and sites the 10 most common taxa generally accounted for more than 85% of the numbers collected. This means that, as found in 2002, most of the taxa present were 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.

In Xakanaxa channels abundances declined by 46% in the impact state, but recovered by 7% in the recovery state (Tables 2.3). In Xakanaxa lagoons there was no such recovery, abundances decreased in the impact state by 25%, and by a further 35% in the recovery state (Table 2.5). This decline was mainly due to a decrease in numbers of shrimps, and the persistent lack of recovery by shrimps and pygmy backswimmers is the most noticeable negative effect related to spraying. Overall, at Xakanaxa there was a decline in abundances, which did not recover in 2003.

Family composition showed less clear spray-related patterns (Tables 2.3 & 2.5). There were Xakanaxa families that disappeared in the impact state (channels - 30%, lagoons - 34%, overall – 31%). However, in lagoons (17%) channels (18%) and overall (15%) at Xakanaxa more than 15% of families that disappeared during the impact, reappeared in recovery. Between 5-10% of the family changes were unrelated to the spray (families appeared for the first time during the impact or recovery states).

However, the apparent spray-impact on families at Xakanaxa (e.g. 15% disappearing after spraying) may simply be natural seasonal patterns. The same pattern was evident at North Gate, the unsprayed site, even taking in account that North Gate could still have been slightly impacted by downstream drift of pesticide. There, 21% of families disappeared after the spray, 14% were collected again in the recovery period and 38% appeared for the first time during and after the spray (Tables 2.6 & 2.7)

Tables 2.8 and 2.9 summarise the main changes in family abundances in relation to spray exposure.

2.4.2 Family abundances across sites, habitats and through the study phases

More detailed comparative abundances of selected families are presented for the families for which there are subsequent morphospecies data (Figures 2.2-2.7). Abundances of pygmy backswimmers (Pleidae) (Figure 2.8) and freshwater shrimps (Atyidae) (Figure 2.9), are also presented (one pleid species and two shrimp species). At a similarly detailed level, the relative abundances of additional common families for which morphospecies data are not available are given in Appendix 1 (Figures 2A.1-2A.7). Figures 2.2-2.9 and 2A.1-2A.7 should be viewed using information from Table 2.1. The comparative graphs indicate sites and habitats from which no samples were collected, but within some of the graphs, samples were absent on particular sampling occasions. Taking this into account, information relevant to spray effects has been synthesised (Table 2.10).

Table 2.2 Changes in the relative abundance (adjusted to 10 samples) of taxa (Cycles 1-5, 2002; and 2003) in the Xakanaxa channels.

Family	Common name	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	May 2003	Aug 2003
Atyidae	Shrimps	369	448	291	135	109	78	62
Baetidae	Baetid mayflies	266	75	24	34	16	118	179
Coenagrionidae	Damselflies	77	130	104	91	63	54	37
Caenidae	Caenid mayflies	40	16	11	18	13	27	111
Leptoceridae	Cased caddisflies	35	41	31	37	18	6	15
Veliidae	Water striders	27	18	0	0	1	21	5
Chironomidae	Midges	25	20	58	139	184	36	66
Naucoridae	Creeping water bugs	19	14	6	1	2	8	2
Libellulidae	Dragonflies	20	11	22	19	20	11	15
Heptageniidae	Flat-head mayflies	13	13	1	0	0	3	7
Pleidae	Pigmy backswimmers	12	4	4	1	1	13	8
Conchostraca	Clam shrimps	8	0	10	3	2	3	3
Tricorythidae	Stout crawler mayflies	8	0	0	0	0	0	1
Lymnaeidae	Pond snails	5	3	14	12	8	7	12
Leptophlebiidae	Prongill mayflies	4	19	11	7	2	1	1
Nepidae	Water scorpions	4	0	1	1	3	3	1
Planorbinae	Orb snails	3	4	13	14	19	7	9
Hydracarina	Water mites	3	1	4	11	4	0	1
Ceratopogonidae	Biting midges	3	1	2	16	21	0	14
Polymitarcyidae	Pale burrowing mayflies	3	1	0	4	1	0	0
Hydropsychidae	Net spinning caddisflies	3	0	0	0	0	0	0
Bulininae	Bilharzia snails	2	4	0	9	11	0	1
Noteridae	Creeping water beetles	2	1	1	0	0	2	3
Aeshnidae	Swimming dragonflies	2	0	1	1	0	0	1
Gyrinidae	Whirligig beetles	2	0	1	0	1	3	0
Potamonautidae	Crabs	2	0	0	0	0	0	1
Lestidae	Slim damselflies	2	0	0	0	0	0	0
Gerridae	Pond skaters	2	0	0	1	0	3	4
Culicidae	Mosquito larvae	2	0	0	0	1	2	1
Hirudinea	Leeches	1	1	10	11	4	0	1
Tabanidae	Horsefly larvae	1	1	1	0	1	0	1
Lepidostomatidae	Cased caddisflies	1	0	0	1	0	0	0
Nematomorpha	Horsehair worm	1	0	0	8	0	0	0
Hydroptilidae	Cased caddisflies	0	14	0	0	0	1	1
Pyralidae	Butterfly larvae	0	4	0	0	1	1	1
Ecnomidae	Caseless caddisflies	0	3	1	1	0	1	1
Sphaeriidae	Pill clams	0	1	0	0	0	1	0
Chlorosyphidae	Damselflies	0	0	4	0	0	0	0
Physidae	Pouch snails	0	0	4	0	0	0	1
Dytiscidae	Predatory diving beetles	0	0	3	0	4	4	2
Platycnemid	Damselflies	0	0	2	1	0	0	0
Oligochaeta	Water earthworms	0	0	1	0	1	1	1
Protoneuridae	Damselflies	0	0	1	0	0	0	0
Polycentropodidae	Caseless caddisflies	0	0	1	0	1	0	0
Helodidae	Small beetles	0	0	1	1	0	0	1
Corixidae	Water boatmen	0	0	1	0	2	0	2
Gomphidae	Burrowing dragonflies	0	0	0	1	3	0	0
Psychodidae	Moth flies	0	0	0	0	1	0	3
Simuliidae	Blackflies	0	0	0	0	2	1	17

Table 2.3 Chi-squared analyses of the total abundances and individual families abundances in the Xakanaxa channels, comparing Cycle 1 (benchmark) with Cycle 5 (impact), and Cycle 1 (benchmark) with August 2003 (recovery) samples; and summary channel comparisons.

Ns = not significant; P = probability values, P <0.05 and <0.01 = significant, P <0.001 = very significant. N/a = not applicable calculation.

Family	Common name	Cycle 1	Cycle 5	Chi^2 (Cycle 1 v Cycle 2)	P	Aug 03	Chi^2 (Cycle 1 v Aug 03)	P
Atyidae	Shrimps	369	109	141.42	<0.001	62	218.67	<0.001
Baetidae	Baetid mayflies	266	16	221.63	<0.001	179	17.01	<0.001
Coenagrionidae	Damselflies	77	63	1.4	Ns	37	14.03	<0.001
Caenidae	Caenid mayflies	40	13	13.75	<0.001	111	33.38	<0.001
Leptoceridae	Cased caddisflies	35	18	5.45	<0.05	15	8	<0.01
Veliidae	Water striders	27	1	24.14	<0.001	5	15.12	<0.001
Chironomidae	Midges	25	184	120.96	<0.001	66	18.47	<0.001
Naucoridae	Creeping water bugs	19	2	13.76	<0.001	2	13.76	<0.001
Libellulidae	Dragonflies	20	20	0	Ns	15	0.71	Ns
Heptageniidae	Flat-head mayflies	13	0	13	<0.001	7	1.8	Ns
Pleidae	Pygmy backswimmers	12	1	9.3	<0.01	8	0.8	Ns
Conchostraca	Clam shrimps	8	2	3.6	Ns	3	2.27	Ns
Tricorythidae	Stout crawler mayflies	8	0	8	<0.01	1	5.44	<0.05
Lymnaeidae	Pond snails	5	8	0.69	Ns	12	2.88	Ns
Leptophlebiidae	Prongill mayflies	4	2	0.676	Ns	1	1.8	Ns
Nepidae	Water scorpions	4	3	0.14	Ns	1	1.8	Ns
Planorbinae	Orb snails	3	19	11.63	<0.001	9	3	Ns
Hydracarina	Water mites	3	4	0.14	Ns	1	1	Ns
Ceratopogonidae	Biting midges	3	21	13.5	<0.001	14	7.11	<0.01
Polymitarcyidae	Pale burrowing mayflies	3	1	1	Ns	0	3	Ns
Hydropsychidae	Net spinning caddisflies	3	0	3	Ns	0	3	Ns
Bulininae	Bilharzia snails	2	11	6.23	<0.05	1	0.33	Ns
Noteridae	Creeping water beetles	2	0	2	Ns	3	0.2	Ns
Aeshnidae	Swimming dragonflies	2	0	2	Ns	1	0.33	Ns
Gyrinidae	Whirligig beetles	2	1	0.33	Ns	0	2	Ns
Potamonautidae	Crabs	2	0	2	Ns	1	0.33	Ns
Lestidae	Slim damselflies	2	0	2	Ns	0	2	Ns
Gerridae	Pond skaters	2	0	2	Ns	4	0.67	Ns
Culicidae	Mosquito larvae	2	1	0.33	Ns	1	0.33	Ns
Hirudinea	Leeches	1	4	1.8	Ns	1	0	Ns
Tabanidae	Horsefly larvae	1	1	0	Ns	1	0	Ns
Lepidostomatidae	Cased caddisflies	1	0	1	Ns	0	1	Ns
Nematomorpha	Horsehair worm	1	0	1	Ns	0	1	Ns
Hydroptilidae	Cased caddisflies	0	0	N/A	N/a	1	1	Ns
Pyralidae	Butterfly larvae	0	1	1	Ns	1	1	Ns
Ecnomidae	Caseless caddisflies	0	0	N/A	N/a	1	1	Ns
Sphaeriidae	Pill clams	0	0	N/A	N/a	0	N/A	N/a
Chlorosyphidae	Damselflies	0	0	N/A	N/a	0	N/A	N/a
Physidae	Pouch snails	0	0	N/A	N/a	1	1	ns

Table 2.3 continued

Family	Common	Cycle 1	Cycle 5	Chi^2 (Cycle 1 v Cycle 2)	P	Aug 03	Chi^2 (Cycle 1 v Aug 03)	P
Dytiscidae	Predatory diving beetles	0	4	4	ns	2	2	ns
Platycnemid	Damselflies	0	0	N/A	N/a	0	N/A	N/a
Oligochaeta	Water earthworms	0	1	1	ns	1	1	ns
Protoneuridae	Damselflies	0	0	N/A	N/a	0	N/A	N/a
Polycentropodidae	Caseless caddisflies	0	1	1	ns	0	N/A	N/a
Helodidae	Small beetles	0	0	N/A	N/a	1	1	ns
Corixidae	Water boatmen	0	2	2	ns	2	2	ns
Gomphidae	Burrowing dragonflies	0	3	3	ns	0	N/A	N/a
Psychodidae	Moth flies	0	1	1	ns	3	3	N/a
Simuliidae	Blackflies	0	2	2	ns	17	17	<0.001
TOTALS		967	520	134.7	<0.001	592	90.2	<0.001

SUMMARY FOR XAKANAXA CHANNELS

	Benchmark / Initial Pre-spray State	Impact / Final Post-spray State	Recovery State
Numbers of individuals adjusted to 10 samples	967	520	592
% Decline		**46%**	**39%**
% Recovery			**7%**
Number of Families	**33**	**31**	**37**
	Number of Benchmark Families		**% benchmark families**
Benchmark condition	33		100
Present in benchmark, disappeared during spray cycles	10		30
Disappeared only during spray cycles – reappeared in recovery	6		18
Appeared for the first time during spray cycles	10		30
Appeared for the first time in recovery	3		9

Table 2.4 Changes in the relative abundance (adjusted to 10 samples) of taxa (Cycles 1-5, 2002; and 2003) in the Xakanaxa lagoons

Family	Common name	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	May 2003	August 2003
Coenagrionidae	Damselflies	352	221	256	75	68	97	5
Atyidae	Shrimps	131	55	18	20	6	41	26
Libellulidae	Dragonflies	94	235	198	90	67	53	50
Chironomidae	Midges	87	99	186	231	352	106	73
Pleidae	Pigmy backswimmers	86	23	5	1	1	42	31
Lymnaeidae	Pond snails	71	102	74	76	109	8	31
Dytiscidae	Predatory diving beetles	69	31	5	4	6	106	7
Noteridae	Creeping water beetles	63	4	2	1	0	3	62
Naucoridae	Creeping water bugs	45	4	0	0	1	4	4
Hydracarina	Water mites	37	30	26	29	27	4	7
Conchostraca	Clam shrimps	34	5	72	14	28	7	12
Baetidae	Baetid mayflies	23	14	15	14	20	33	54
Caenidae	Caenid mayflies	19	58	56	88	64	12	64
Aeshnidae	Swimming dragonflies	16	2	3	0	0	8	1
Polymitarcyidae	Pale burrowing mayflies	15	24	11	9	2	2	1
Notonectidae	Back swimmers	14	0	1	0	0	7	1
Bulininae	Bilharzia snails	12	43	0	25	27	0	2
Veliidae	Water striders	8	1	0	1	0	10	2
Planorbinae	Orb snails	7	63	38	27	45	7	16
Hirudinea	Leeches	7	12	9	8	11	1	0
Sphaeriidae	Pill clams	5	23	4	11	16	0	1
Nepidae	Water scorpions	5	4	1	3	2	1	2
Culicidae	Mosquito larvae	5	4	0	0	1	6	2
Belostomatidae	Giant water bugs	5	2	2	0	1	13	1
Ceratopogonidae	Biting midges	4	1	9	16	45	1	10
Lestidae	Slim damselflies	3	1	3	0	0	0	0
Tabanidae	Horsefly larvae	2	6	1	1	0	1	1
Gerridae	Pond skaters	2	1	0	0	0	13	7
Thiaridae	Snails	2	0	1	0	0	0	0
Nematomorpha	Horsehair worm	2	0	0	10	0	0	0
Leptoceridae	Cased caddisflies	1	4	9	9	3	0	4
Oligochaeta	Water earthworms	1	1	15	1	7	2	2
Turbellaria	Flatworms	1	0	0	0	0	0	0
Hydrometridae	Marsh treaders	1	0	0	0	0	1	0
Hydropsychidae	Net spinning caddisflies	1	0	0	0	0	0	0
Helodidae	Small beetles	0	8	0	1	1	0	0
Pyralidae	Butterfly larvae	0	6	4	2	3	3	1
Elmidae/Dryopidae	Riffle beetles	0	6	0	0	1	0	4
Hydrophilidae	Scavenger beetles	0	5	0	1	0	1	1
Corixidae	Water boatmen	0	2	0	1	1	19	8
Haliplidae	Beetles	0	2	0	0	0	0	0
Physidae	Pouch snails	0	1	42	1	0	1	1
Platycnemid	Damselflies	0	1	8	3	0	0	0
Gomphidae	Burrowing dragonflies	0	1	0	0	0	0	0
Ampullariidae	Apple snails	0	1	0	0	0	0	0
Leptophlebiidae	Prongill mayflies	0	0	3	4	0	3	1
Ecnomidae	Caseless caddisflies	0	0	2	0	0	1	1
Chlorosyphidae	Damselflies	0	0	1	0	0	0	0
Polycentropodidae	Caseless caddisflies	0	0	1	0	1	0	0
Hydroptilidae	Cased caddisflies	0	0	1	0	0	0	0
Simuliidae	Blackflies	0	0	1	0	0	0	1
Potamonautidae	Crabs	0	0	0	0	1	0	1

Table 2.5 Chi-squared analyses of the total abundances and individual families abundances in the Xakanaxa lagoons, comparing Cycle 1 (benchmark) with Cycle 5 (impact), and Cycle 1 (benchmark) with August 2003 (recovery) samples; summary lagoon and overall comparisons; and morphospecies presence/absence. Ns = not significant; P = probability values, P <0.05 and <0.01 = significant, P <0.001 = very significant. N/a = not applicable calculation.

Family	**Common name**	**Cycle 1**	**Cycle 5**	**Chi^2 (Cycle 1 v Cycle 5)**	**P**	Aug 03	**Chi^2 (Cycle 1 v Aug 03)**	**P**
Coenagrionidae	Damselflies	352	68	192.03	<0.001	5	337.28	<0.001
Atyidae	Shrimps	131	6	114.05	<0.001	26	70.22	<0.001
Libellulidae	Dragonflies	94	67	4.52	<0.05	50	13.44	<0.001
Chironomidae	Midges	87	352	159.97	<0.001	73	1.23	ns
Pleidae	Pygmy backswimmers	86	1	83.05	<0.001	31	25.85	<0.001
Lymnaeidae	Pond snails	71	109	8.02	<0.01	31	15.69	<0.001
Dytiscidae	Predatory diving beetles	69	6	52.92	<0.001	7	50.58	<0.001
Noteridae	Creeping water beetles	63	0	63.00	<0.001	62	0.01	ns
Naucoridae	Creeping water bugs	45	1	42.09	<0.001	4	34.31	<0.001
Hydracarina	Water mites	37	27	1.56	ns	7	20.45	<0.001
Conchostraca	Clam shrimps	34	28	0.58	ns	12	10.52	<0.01
Baetidae	Baetid mayflies	23	20	0.21	ns	54	12.48	<0.001
Caenidae	Caenid mayflies	19	64	24.40	<0.001	64	24.40	<0.001
Aeshnidae	Swimming dragonflies	16	0	16.00	<0.001	1	13.24	<0.001
Polymitarcyidae	Pale burrowing mayflies	15	2	9.94	<0.01	1	12.25	<0.001
Notonectidae	Back swimmers	14	0	14.00	<0.001	1	11.27	<0.001
Bulininae	Bilharzia snails	12	27	5.77	<0.05	2	7.14	<0.01
Veliidae	Water striders	8	0	8.00	<0.01	2	3.60	ns
Planorbinae	Orb snails	7	45	27.77	<0.001	16	3.52	ns
Hirudinea	Leeches	7	11	0.89	ns	0	7.00	<0.01
Sphaeriidae	Pill clams	5	16	5.76	<0.05	1	2.67	ns
Nepidae	Water scorpions	5	2	1.29	ns	2	1.29	ns
Culicidae	Mosquito larvae	5	1	2.67	ns	2	1.29	ns
Belostomatidae	Giant water bugs	5	1	2.67	Ns	1	2.67	ns
Ceratopogonidae	Biting midges	4	45	34.31	<0.001	10	2.57	ns
Lestidae	Slim damselflies	3	0	3.00	Ns	0	3.00	ns
Tabanidae	Horsefly larvae	2	0	2.00	Ns	1	0.33	ns
Gerridae	Pond skaters	2	0	2.00	Ns	7	2.78	ns
Thiaridae	Snails	2	0	2.00	Ns	0	2.00	ns
Nematomorpha	Horsehair worm	2	0	2.00	Ns	0	2.00	ns
Leptoceridae	Cased caddisflies	1	3	1.00	Ns	4	1.80	ns
Oligochaeta	Water earthworms	1	7	4.50	Ns	2	0.33	ns
Turbellaria	Flatworms	1	0	1.00	Ns	0	1.00	ns
Hydrometridae	Marsh treaders	1	0	1.00	Ns	0	1.00	ns

Table 2.5 continued

Family	Common name	Cycle 1	Cycle 5	Chi^2 (Cycle 1 v Cycle 5)	P	Aug 03	Chi^2 (Cycle 1 v Aug 03)	P
Hydropsychidae	Net spinning caddisflies	1	0	1.00	ns	0	1.00	Ns
Helodidae	Small beetles	0	1	1.00	ns	0	N/a	N/a
Pyralidae	Butterfly larvae	0	3	3.00	ns	1	1.00	Ns
Elmidae/Dryopidae	Riffle beetles	0	1	1.00	ns	4	4.00	Ns
Hydrophilidae	Scavenger beetles	0	0	N/A	N/a	1	ns	Ns
Corixidae	Water boatmen	0	1	1.00	ns	8	ns	Ns
Haliplidae	Beetles	0	0	N/A	N/a	0	N/a	N/a
Physidae	Pouch snails	0	0	N/A	N/a	1	1.00	Ns
Platycnemid	Damselflies	0	0	N/A	N/a	0	N/a	N/a
Gomphidae	Burrowing dragonflies	0	0	N/A	N/a	0	N/a	N/a
Ampullariidae	Apple snails	0	0	N/A	N/a	0	N/a	N/a
Leptophlebiidae	Prongill mayflies	0	0	N/A	N/a	1	1.00	ns
Ecnomidae	Caseless caddisflies	0	0	N/A	N/a	1	1.00	ns
Chlorosyphidae	Damselflies	0	0	N/A	N/a	0	N/A	N/a
Polycentropodidae	Caseless caddisflies	0	1	1.00	ns	0	N/A	N/a
Hydroptilidae	Cased caddisflies	0	0	N/A	N/a	0	N/A	N/a
Simuliidae	Blackflies	0	0	N/A	N/a	1	1.00	ns
Potamonautidae	Crabs	0	1	1.00	ns	1	1.00	Ns
TOTALS		1230	917	45.23	<0.001	498	310.08	<0.001

SUMMARY FOR XAKANAXA LAGOONS

	Benchmark / Initial Pre-spray State	Impact / Final Post-spray State	Recovery State
Numbers of individuals adjusted to 10 samples	1230	917	498
% Decline		**25**	**60**
% further decline (mainly shrimps)			**35**
Number of Families	**35**	**29**	**37**
	Number of Benchmark Families		**% benchmark families**
Benchmark condition	35		100
Present in benchmark, disappeared during spray cycles	12		34
Disappeared only during spray cycles – reappeared in recovery	6		17
Appeared for the first time during spray cycles	6		17
Appeared for the first time in recovery	5		14

Table 2.5 continued

SUMMARY FOR COMBINED XAKANAXA LAGOON AND CHANNEL PRESENCE / ABSENCE

	Benchmark / Initial Pre-spray State	Impact / Final Post-spray State	Recovery State
Numbers of individuals adjusted to 10 samples	2197	1437	1490
% Decline		**35%**	**50%** > decline - shrimps
% further decline (mainly shrimps)	**56**	**39**	47
Number of Families	**41**	**36**	**43**
	Number of Benchmark Families		**% of benchmark**
Benchmark condition	41		100
Present in benchmark, disappeared during spray cycles	13		31
Disappeared only during spray cycles – reappeared in recovery	6		15
Appeared for the first time during spray cycles	10		24
Appeared for the first time in recovery	4		10

XAKANAXA MORPHOSPECIES (MSP) PRESENCE/ABSENCE

Morphospecies category	**Family and number of morphospecies (MSP) identified**		**No. of MSP in response category**	**% MSP in response category**
"Tolerant" = present in benchmark, impact and recovery	Backswimmers	7	1	30%
	Dragonflies	5	0	
	Baetid mayflies	6	4	
	Caenid mayflies	4	4	
	Predatory beetles	13	1	
	Water bugs	4	2	
"Vulnerable" = Present in benchmark, absent from impact, reappear in recovery	Backswimmers	7	1	9%
	Dragonflies	5	2	
	Baetid mayflies	6	0	
	Caenid mayflies	4	0	
	Predatory beetles	13	0	
	Water bugs	4	0	
"Sensitive" – present in benchmark absent after spray, and do not reappear	Backswimmers	7	3	10%
	Dragonflies	5	0	
	Baetid mayflies	6	0	
	Caenid mayflies	4	0	
	Predatory beetles	13	0	
	Water bugs	4	1	
Unrelated to spray: appear first after spray or in recovery	Backswimmers	7	2	51%
	Dragonflies	5	3	
	Baetid mayflies	6	2	
	Caenid mayflies	4	0	
	Predatory beetles	13	12	
	Water bugs	4	1	

Table 2.6 Changes in the relative abundance of taxa (2002 and 2003) in the North Gate channels

Family	Common name	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	May 2003	Aug 2003
Baetidae	Baetid mayflies	352	317	480	156	205	245	183
Caenidae	Caenid mayflies	86	362	210	148	191	13	95
Coenagrionidae	Damselflies	68	117	58	33	39	29	77
Conchostraca	Clam shrimps	51	60	16	0	4	117	14
Chironomidae	Cased caddisflies	30	118	200	74	73	79	102
Libellulidae	Dragonflies	26	0	8	20	44	8	63
Aeshnidae	Dragonflies	23	45	8	15	6	13	0
Bulininae	Bilharzia snails	21	20	0	14	40	0	2
Dytiscidae	Predatory diving beetles	14	13	16	14	33	116	4
Notonectidae	Black swimmers	13	7	0	0	0	7	1
Pleidae	Pigmy backswimmers	13	25	14	40	19	22	12
Veliidae	Water striders	13	15	0	0	1	0	0
Sphaeriidae	Pill clams	13	2	4	0	6	2	10
Atyidae	Shrimps	11	17	19	8	9	8	5
Corixidae	Water boatmen	10	3	1	0	4	4	0
Gerridae	Pond skaters	7	2	0	6	3	33	1
Lymnaeidae	Pond snails	6	10	8	3	3	31	18
Planorbinae	Orb snails	5	0	4	10	28	17	52
Culicidae	Mosquito larvae	5	0	0	1	1	10	2
Belostomatidae	Giant water bugs	4	8	1	1	0	8	4
Nepidae	Water scorpions	4	3	3	4	2	0	1
Hirudinea	Leeches	4	25	4	9	12	8	10
Hydracarina	Water mites	3	8	3	6	4	8	1
Naucoridae	Creeping water bugs	2	0	0	3	0	0	1
Noteridae	Creeping water beetles	1	20	0	4	4	24	94
Gyrinidae	Whirligig beetles	1	0	0	0	0	0	2
Hydrophilidae	Scavenger beetles	1	8	2	1	1	4	2
Polymitarcyidae	Pale burrowing mayflies	1	8	0	1	1	0	0
Pyralidae	Butterfly larvae	1	0	1	1	0	1	4
Ceratopogonidae	Biting midges	0	12	14	10	38	18	27
Elmidae/Dryopidae	Riffle beetles	0	0	1	0	0	1	4
Hydroptilidae	Cased caddisflies	0	0	0	0	0	1	1
Corduliidae	Longlegged dragonflies	0	33	5	0	0	0	0
Simuliidae	Blackflies	0	7	202	36	48	0	25
Lestidae	Slim damselflies	0	2	0	0	0	0	0
Tipulidae	Crane flies	0	2	0	0	0	0	1
Physidae	Pouch snails	0	0	16	0	0	1	1
Oligochaeta	Water earthworms	0	0	4	0	4	9	7
Leptophlebiidae	Prongill mayflies	0	0	2	0	0	1	0
Haliplidae	Beetles	0	0	1	0	0	0	1
Potamonautidae	Crabs	0	0	0	0	1	0	0
Platycnemid	Damselflies	0	0	0	0	3	0	0
Tabanidae	Horsefly larvae	0	0	0	0	1	1	0

Table 2.7 Chi-squared analyses of the total abundances and individual families abundances in the North Gate channels, comparing Cycle 1 [benchmark] with Cycle 5 periods, and Cycle 1 with August 2003 samples. Ns = not significant; P = probability values, P <0.05 and <0.01 = significant, P <0.001 = very significant. N/a = not applicable calculation.

Family	Common name	Cycle 1	Cycle 5	Chi-squared (Cycle 1 v Aug 03)	P	Aug 03	Chi-squared (Cycle 1 v Aug 03)	P
Baetidae	Baetid mayflies	352	245	19.18	<0.001	183	53.39	<0.001
Caenidae	Caenid mayflies	86	13	53.83	<0.001	95	0.45	ns
Coenagrionidae	Damselflies	68	29	15.68	<0.001	77	0.56	ns
Conchostraca	Clam shrimps	51	117	25.93	<0.001	14	21.06	<0.001
Chironomidae	Cased caddisflies	30	79	22.03	<0.001	102	39.27	<0.001
Libellulidae	Dragonflies	26	8	9.53	<0.01	63	15.38	<0.001
Aeshnidae	Dragonflies	23	13	2.78	Ns	0	23.00	<0.001
Bulininae	Bilharzia snails	21	0	21.00	<0.001	2	15.70	<0.001
Dytiscidae	Predatory diving beetles	14	116	80.03	<0.001	4	5.56	<0.05
Notonectidae	Black swimmers	13	7	1.80	Ns	1	10.29	<0.01
Pleidae	Pygmy backswimmers	13	22	2.31	Ns	12	0.04	ns
Veliidae	Water striders	13	0	13.00	<0.001	0	13.00	<0.001
Sphaeriidae	Pill clams	13	2	8.07	<0.01	10	0.39	ns
Atyidae	Shrimps	11	8	0.47	Ns	5	2.25	ns
Corixidae	Water boatmen	10	4	2.57	Ns	0	10.00	<0.01
Gerridae	Pond skaters	7	33	16.90	<0.001	1	4.50	<0.05
Lymnaeidae	Pond snails	6	31	16.89	<0.001	18	6.00	<0.05
Planorbinae	Orb snails	5	17	6.55	<0.05	52	38.75	<0.001
Culicidae	Mosquito larvae	5	10	1.67	Ns	2	1.29	ns
Belostomatidae	Giant water bugs	4	8	1.33	Ns	4	0.00	ns
Nepidae	Water scorpions	4	0	4.00	<0.05	1	1.80	ns
Hirudinea	Leeches	4	8	1.33	Ns	10	2.57	ns
Hydracarina	Water mites	3	8	2.27	Ns	1	1.00	ns
Naucoridae	Creeping water bugs	2	0	2.00	Ns	1	0.33	ns
Noteridae	Creeping water beetles	1	24	21.16	<0.001	94	91.04	<0.001
Gyrinidae	Whirligig beetles	1	0	1.00	Ns	2	0.33	ns
Hydrophilidae	Scavenger beetles	1	4	1.80	Ns	2	0.33	ns
Polymitarcyidae	Pale burrowing mayflies	1	0	1.00	Ns	0	1.00	ns
Pyralidae	Butterfly larvae	1	1	0.00	Ns	4	1.80	ns
Ceratopogonidae	Biting midges	0	18	18.00	<0.001	27	27.00	<0.001
Elmidae/Dryopidae	Riffle beetles	0	1	1.00	Ns	4	4.00	ns
Hydroptilidae	Cased caddisflies	0	1	1.00	Ns	1	1.00	ns
Corduliidae	Longlegged dragonflies	0	0	N/A	N/a	0	N/A	N/a
Simuliidae	Blackflies	0	0	N/A	N/a	25	25.00	<0.001
Lestidae	Slim damselflies	0	0	N/A	N/a	0	N/A	N/a
Tipulidae	Crane flies	0	0	N/A	N/a	1	1.00	ns
Physidae	Pouch snails	0	1	1.00	Ns	1	1.00	ns

Table 2.7 continued

Family	Common name	Cycle 1	Cycle 5	Chi^2 (Cycle 1 v Aug 03)	P	Aug 03	Chi^2 (Cycle 1 v Aug 03)	P
Oligochaeta	Water earthworms	0	9	9.00	Ns	7	7.00	Ns
Leptophlebiidae	Prongill mayflies	0	1	1.00	Ns	0	N/A	N/a
Haliplidae	Beetles	0	0	N/A	N/a	1	1.00	Ns
Potamonautidae	Crabs	0	0	N/A	N/a	0	N/A	N/a
Platycnemid	Damselflies	0	0	N/A	N/a	0	N/A	N/a
Tabanidae	Horsefly larvae	0	1	1.00	Ns	0	N/A	N/a
TOTALS		789	839	1.54	ns	827	0.89	Ns

SUMMARY FOR NORTH GATE CHANNELS

	Benchmark / Initial Pre-spray State	Impact / Final Post-spray State	Recovery State
Numbers of individuals adjusted to 10 samples	789	839	827
% Increase		**6%**	**5%**
Number of Families	**29**	**30**	**33**
	Number of Benchmark Families		**% benchmark families**
Benchmark condition	29		100
Present in benchmark, disappeared during spray cycles	6		21
Disappeared only during spray cycles – reappeared in recovery	4		14
Appeared for the first time during spray cycles	8		28
Appeared for the first time in recovery	3		10

Table 2.8 Key indicators of spray effects on common taxon abundance (>8 individuals collected in Cycle 1) in Xakanaxa lagoons and channels, compared to North Gate channels (refer to Tables 2.2 to 2.7 and graphs 2.2 to 2.9). Lagoons = L, Channels = CH. Significant = Probability, P, <0.05 and <0.01, very significant = P <0.001.

FAMILY	**XAKANAXA: (SPRAYED)**			**NORTH GATE: (UNSPRAYED)**		
	BENCHMARK	**IMPACT**	**RECOVERY**	BENCHMARK	**IMPACT PERIOD**	**RECOVERY PERIOD**
Shrimps (Atyidae)	Dominant in L and CH	Very significant decrease in L and CH	Limited recovery but still significantly below benchmark in L , further significant decrease in CH	Present in small numbers	No significant changes	No significant difference to benchmark
Pygmy backswimmers (Pleidae)	Dominant in L, common in CH	Very significant decrease in L, and in CH	Significant decrease in L, recovered to benchmark levels in CH	Present in small numbers	No significant changes	No significant difference to benchmark
Damselflies (Coenagrionidae)	Dominant in L and CH	Very significant decrease in L, no significant change in CH	Further significant decrease in L, significant decrease in CH	Dominant	Very significant decrease	No significant difference to benchmark
Baetid mayflies (Baetidae)	Common in L, dominant in CH	NO significant change L, very significant decrease in CH	Very significant increase in L, recovery but still significantly below benchmark in CH	Dominant	Very significant decrease	Further significant decrease
Water striders (Veliidae)	Common in L and CH	Significant decrease in L and very significant decrease in CH	Recovered to benchmark in L, still significantly below benchmark in CH	Present in small numbers	Absent	Absent
Creeping water bugs (Naucoridae)	Common in L and CH	Very significant decrease in L and CH	Still very significantly below benchmark in L and CH	Rare	No significant changes	No significant difference to benchmark
Caenid mayflies (Caenidae)	Common in L and CH	Significant increase in L, significant decrease in CH	Still significantly more than benchmark in L, CH	Dominant	Significant decrease	No significant difference to benchmark
Creeping water beetles (Noteridae)	Common in L, rare in CH	Very significant decrease in L, rare in CH	Recovered to benchmark in L, rare in CH	Common	Very significant increase	Present in small numbers

TABLE 2.8 CONTINUED						
	XAKANAXA: (SPRAYED)			**NORTH GATE: (UNSPRAYED)**		
	BENCHMARK	**IMPACT**	**RECOVERY**	BENCHMARK	**IMPACT PERIOD**	**RECOVERY PERIOD**
Backswimmers (Notonectidae)	Common in L, absent from CH	Absent from L, CH	Very significantly below benchmark in L, absent from CH	Common	No significant changes	Significant decrease below benchmark
Swimming dragonflies (Aeshnidae)	Common in L, rare in CH	Absent from L, CH	Very significantly below benchmark n L, rare in CH	Common	No significant changes	Absent
Burrowing mayflies (Polymitarcyidae)	Common in L, rare in CH	Significant decrease in L, rare in CH	Significantly below benchmark in L, absent in CH	Rare	No significant changes	No significant difference to benchmark
Flathead mayflies (Heptageniidae)	Absent from L, present in small numbers in CH	Absent from L, CH	Absent from L, no significant difference from benchmark in CH	Absent	Absent	Absent
Prongill mayflies** (Leptophlebiidae)	Absent from L, present in small numbers in CH	Absent from L, rare in CH	Rare in L, CH	Absent	Rare	Absent
Cased caddisflies (Leptoceridae)	Absent from L, common in CH	Absent from L, significant decrease in CH	Absent from L, still significant decrease from benchmark in CH	Absent	Absent	Absent
Dragonflies (Libellulidae)	Dominant in L, common in CH	Significant decrease in L, no change in CH	Very significantly below benchmark in L, no change in CH	Common	Significant decrease	Very significant increase from benchmark
Midges (Chironomidae)	Dominant in L, common in CH	Very significant increase in L and CH	Recovery to benchmark in L, still significantly higher than benchmark in CH	Common	Very significant increase	Very significant increase from benchmark
Pond snails (Lymnaeidae)	Common in L, present in small numbers in CH	Significant increase in L, no significant change in CH	Significant decrease below benchmark in L, no significant change in CH	Present in small numbers	Very significant increase	Significant increase from benchmark

TABLE 2.8 CONTINUED						
	XAKANAXA: (SPRAYED)			**NORTH GATE: (UNSPRAYED)**		
BENCHMARK	**IMPACT**	**RECOVERY**	BENCHMARK	**IMPACT PERIOD**	**RECOVER Y PERIOD**	
Water mites (Hydracarina)	Common in L, present in small numbers in CH	No significant change in L, CH	Very significant decrease below benchmark in L, no significant change in CH	Rare	Common	Rare
Clam shrimps (Conchostraca)	Common in L, present in small numbers in CH	No significant change in L, CH	Significant decrease below benchmark n L, no significant change in CH	Dominant	Very significant increase	Very significant decrease below benchmark
Bilharzia snails (Bulininae)	Common in L, present in small numbers in CH	Significant increase in L, CH	Significant decrease below benchmark in L, no significant change from benchmark in CH	Common	Absent	Rare

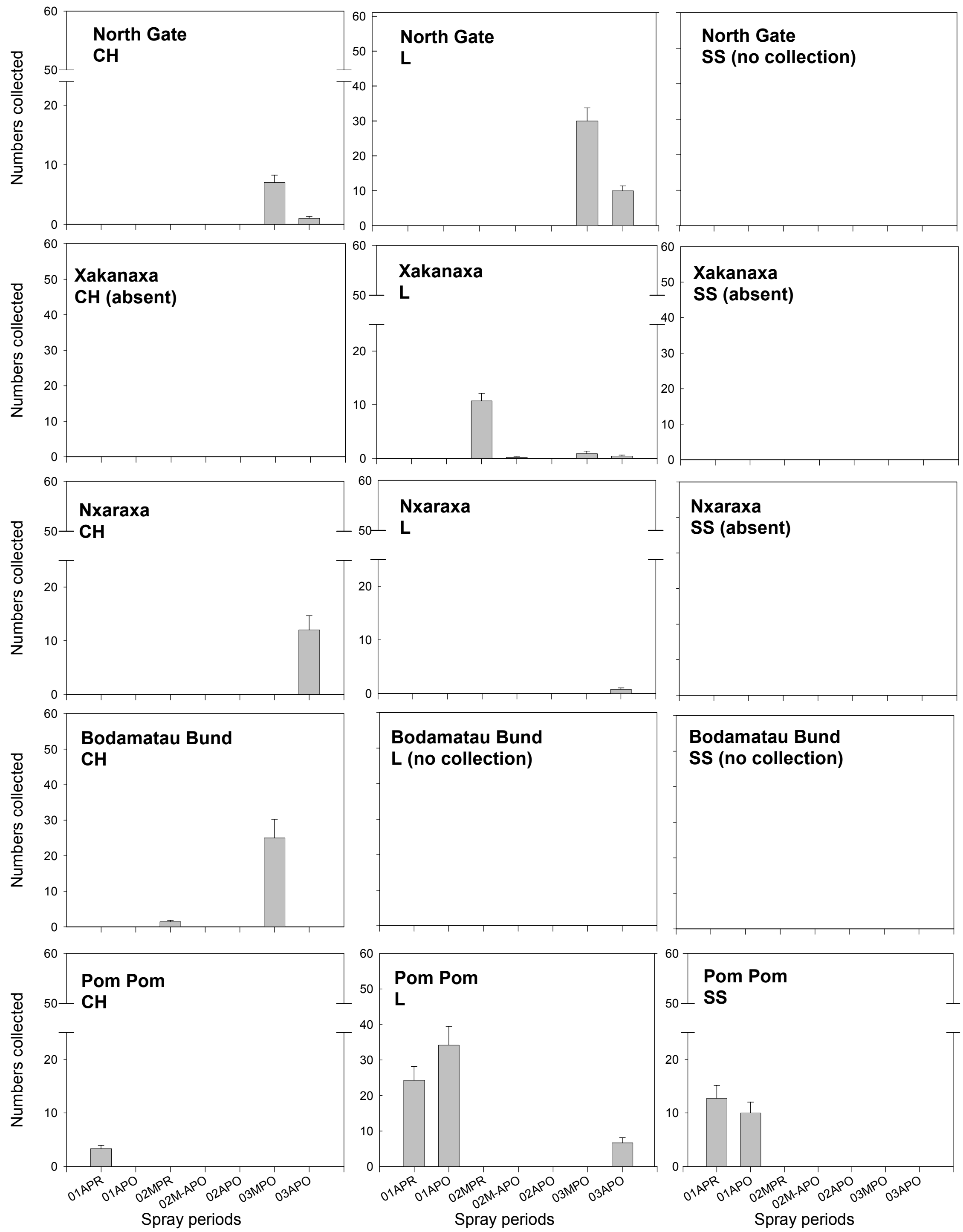


Figure 2.2 Abundances of NOTONECTIDAE (backswimmers) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

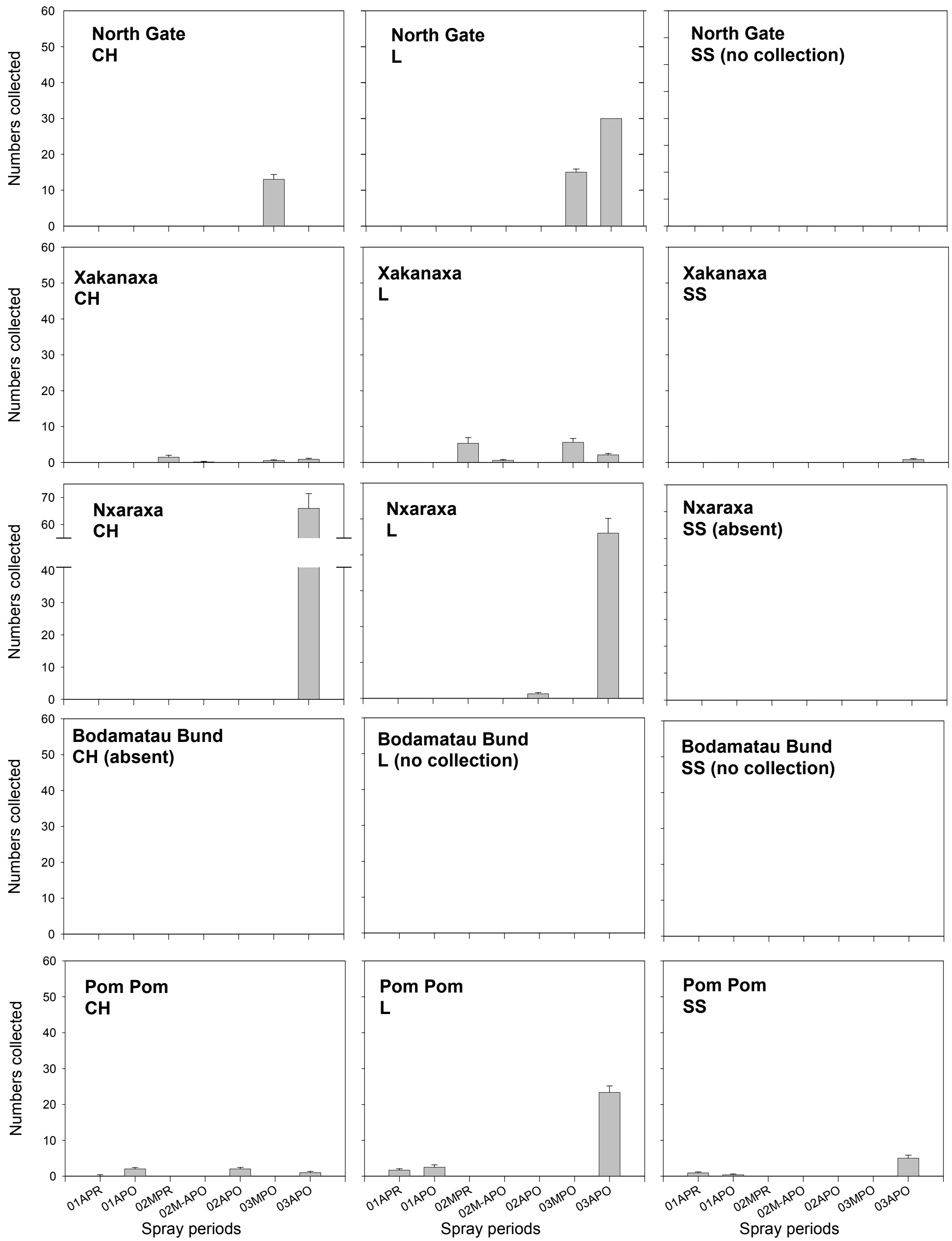


Figure 2.3 Abundances of AESHNIDAE (swimming dragonflies) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

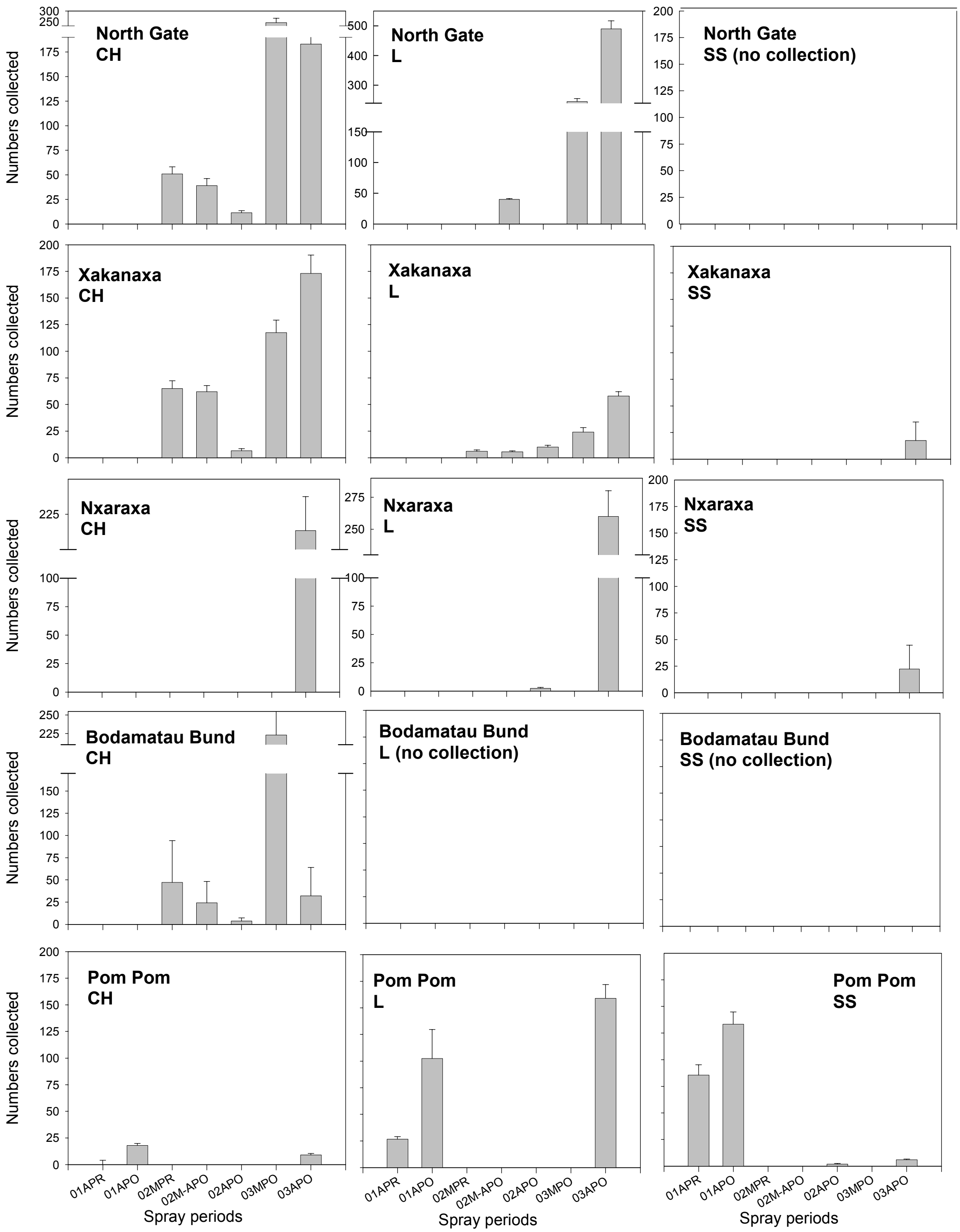


Figure 2.4 Abundances of BAETIDAE (baetid mayflies) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

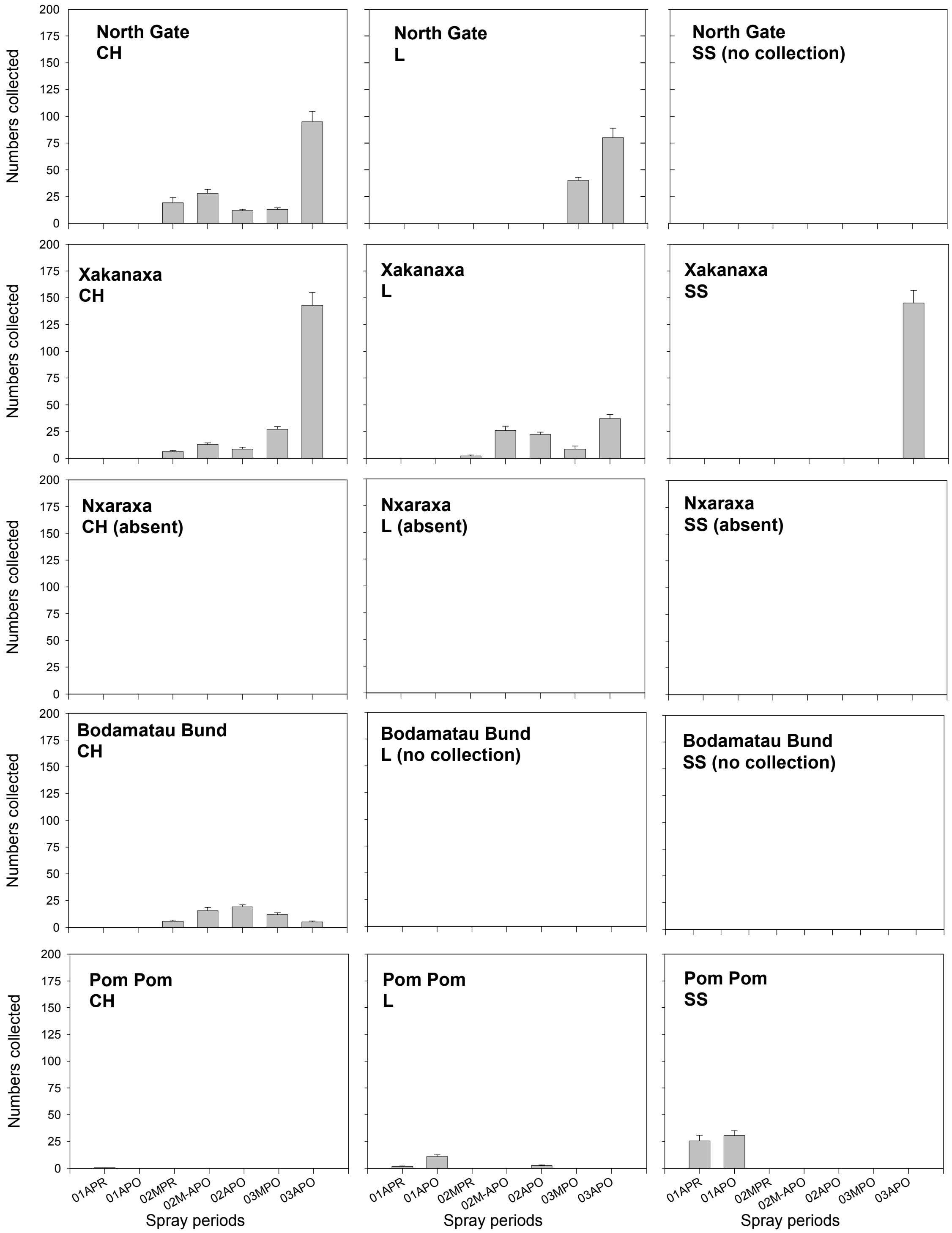


Figure 2.5 Abundances of CAENIDAE (caenid mayflies) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

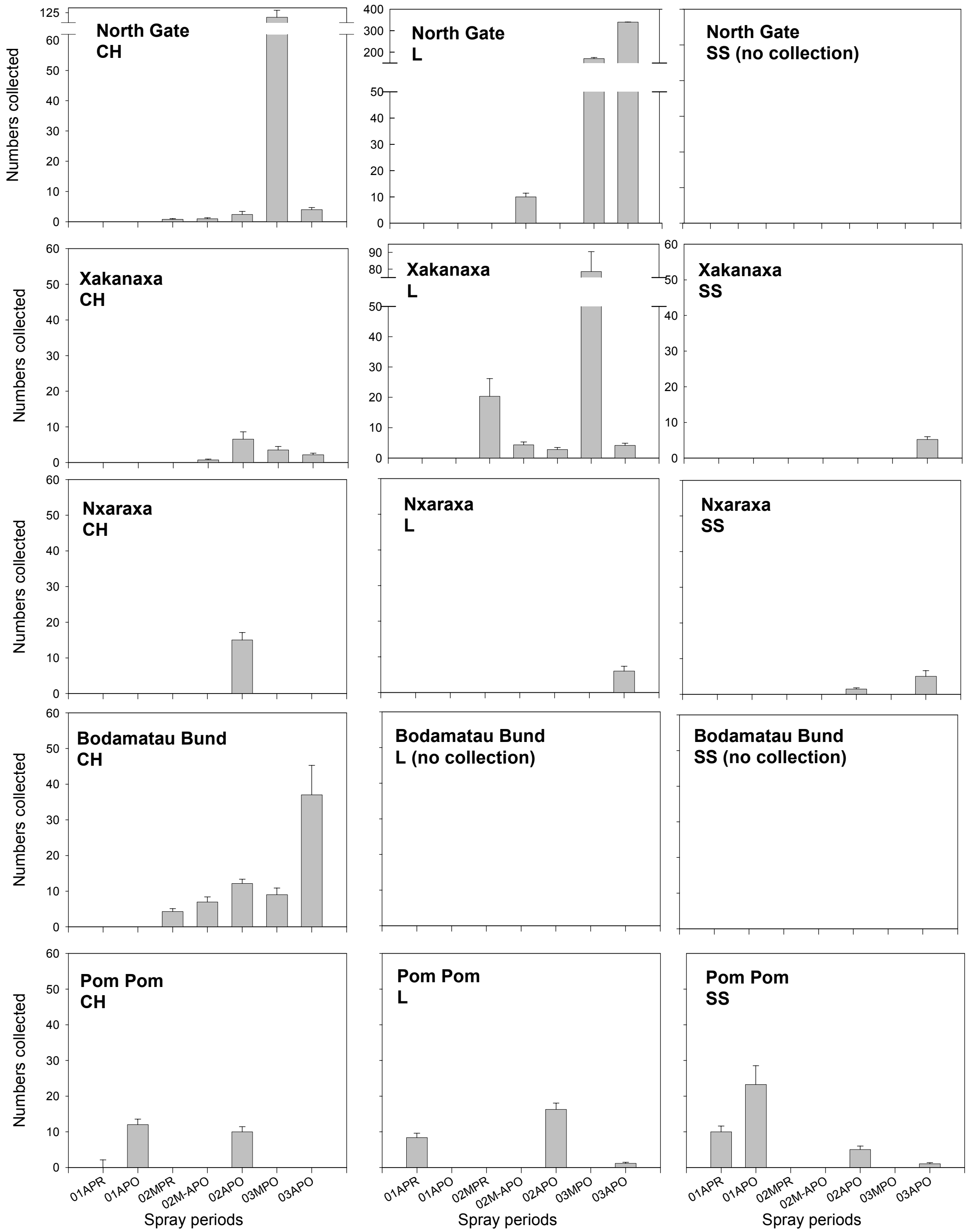


Figure 2.6 Abundances of DYTISCIDAE (predatory diving beetles) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

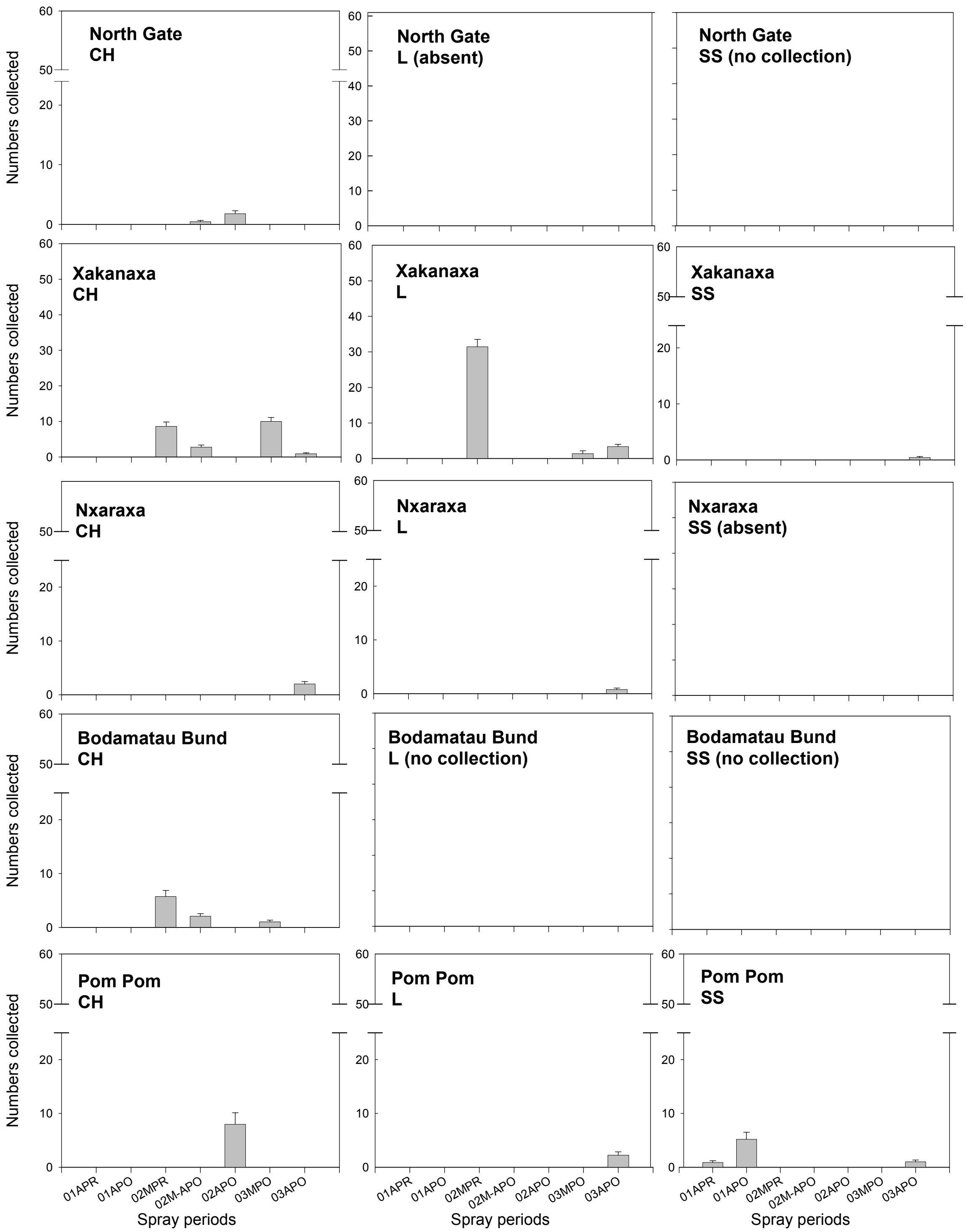


Figure 2.7 Abundances of NAUCORIDAE (creeping water bugs) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

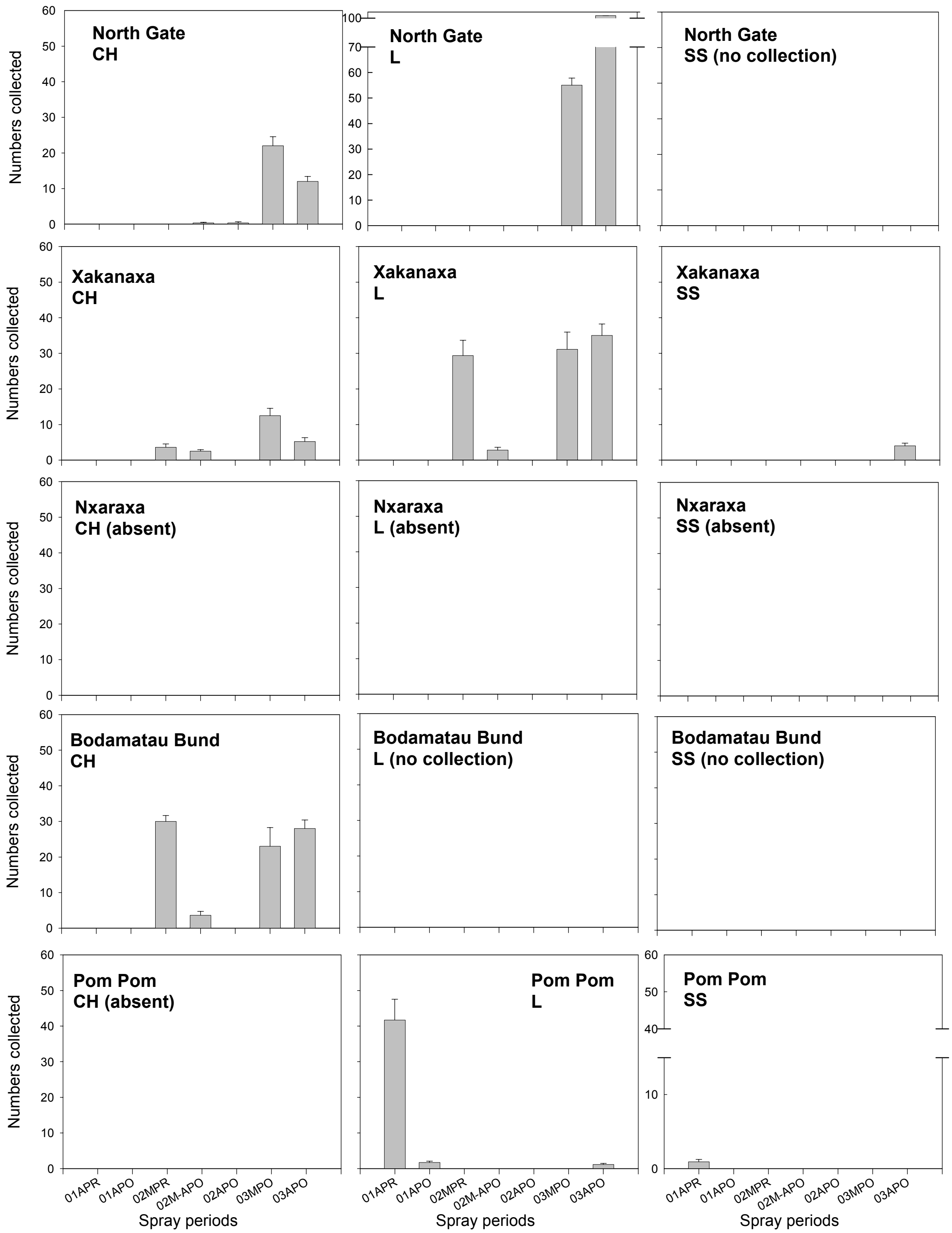


Figure 2.8 Abundances of PLEIDAE (pygmy backswimmers) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

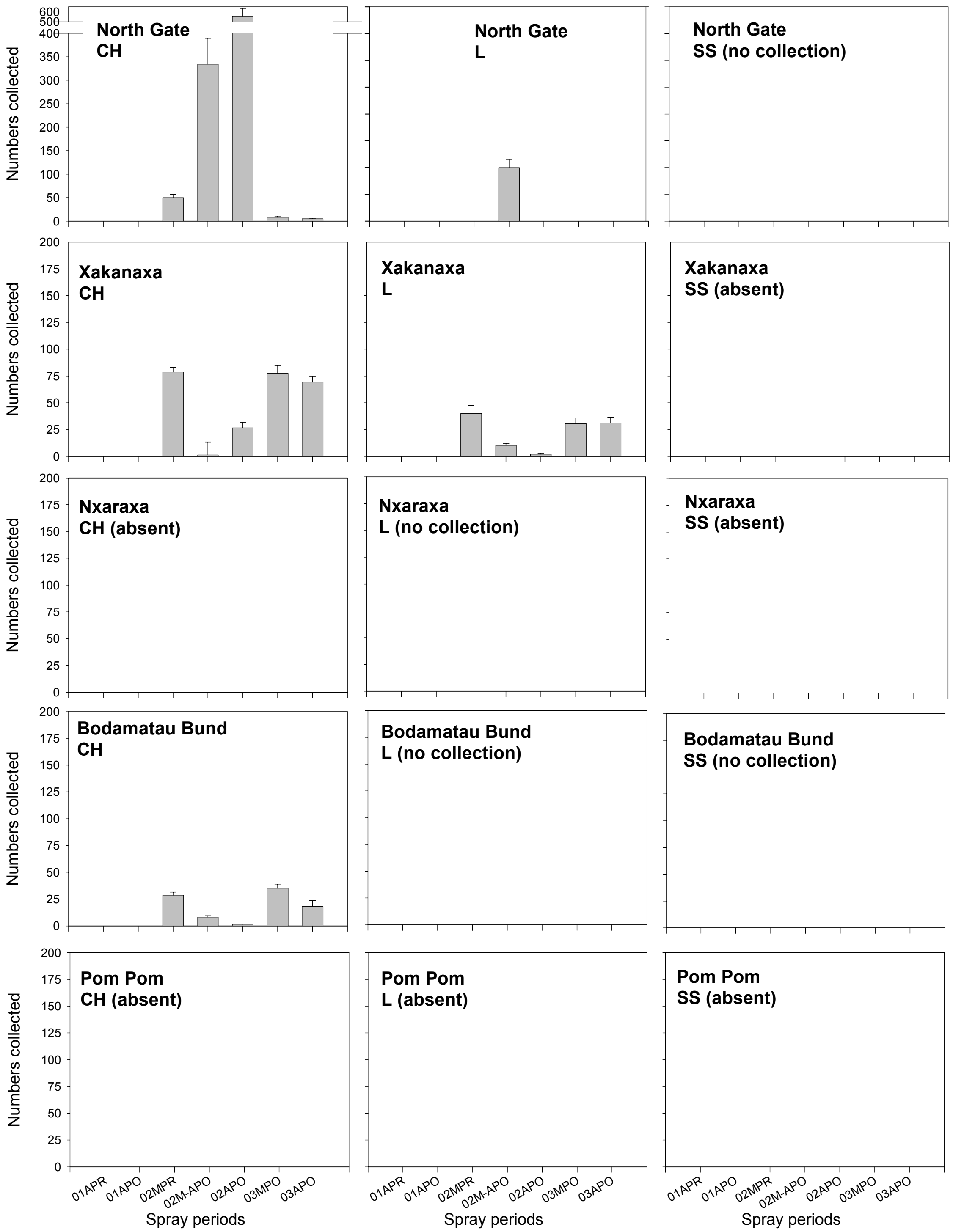


Figure 2.9 Abundances of ATYIDAE (shrimps) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

2.4.3 Summary of family and morphospecies responses to spray events

Table 2.8 relates the summary of Xakanxa morphospecies responses to familiy-level responses. Table 2.9 shows the presence and absence of morphospecies in each of 6 families in samples collected from 3 habitats (lagoon, channel and shallow seasonal) at Pom Pom and Xakanaxa (the two sites for which the most comprehensive spatial and temporal data were available). The broad (Tables 2.3, 2.5 & 2.7) and detailed (Figures 2.2-2.9) family abundance data, together with the morphospecies presence/absence data (Table 2.9) were used to summarise family and morphospecies level responses in the 3 Phases of the study (Table 2.10).

Table 2.9 Presence and absence of morphospecies at Pom Pom (PP) and Xakanaxa

	2001		**2002**			**2003**	
	Pre-spray August (01APR)	**Post-spray August (01APO)**	**Pre-spray May (02MPR)**	**Cycles 1 - 5 (02M-APO)**	**Post-spray August (02APO)**	**Recovery May (03MPO)**	**Recovery August (03APO)**
Notonectidae (backswimmers)							
0001		PP (4)					
0002		PP (4)	XA (1)	XA (1)			XA (1)
0003		PP (4)					
0004		PP (4)	XA (3)				
0005		PP (4)				XA (5)	
0006	PP (2)	PP (2)	XA (2)			XA (2)	XA (2) PP (2)
0007		PP (4)					
0008							PP (5)
0009			XA (3)				
0010			XA (3)				
0012						XA (5)	
Aeshnidae (swimming dragonflies)							
0001		PP (4)		XA (4)	XA (4)		XA (4)
0002		PP (4)		XA (4)			PP (4)
0003		PP (4)		XA (4)		XA (4)	PP (4)
0004		PP (4)	XA (2)			XA (2)	XA (2) PP (4)
0005		PP (4)	XA (2)			XA (2)	PP (4)
Baetidae (baetid mayflies)							
0001		PP (4)	XA (1)	XA (1)	XA (1)	XA (1)	XA (1)
0002	PP (6)	PP (6)	XA (1)	XA (1)	XA (1)		XA (1)
0003				XA (4)	XA (4)		
0004		PP (4)	XA (1)	XA (1)	XA (1)		XA (1)
0006	PP (3)		XA (1)	XA (1)			XA (1)
0007				XA (4)			

Table 2.9 continued							
Caenidae (caenid mayflies)							
0001	PP (6)	PP (6)	XA (1)	XA (1)	XA (1)	XA (1)	XA (1)
0002	PP (6)	PP (6)	XA (1)	XA (1)	XA (1) PP (6)	XA (1)	XA (1)
0003		PP (4)	XA (1)	XA (1)		XA (1)	XA (1) PP (4)
0005		PP (4)	XA (1)		XA (1)		XA (1)
Dytiscidae (predatory diving beetles)							
0001		PP (4)			XA (4)	XA (4)	XA (4)
0002	PP (6)	PP (6)		XA (4)	XA (4)	XA (4)	XA (4)
0004	PP (1)	PP (1)	XA (1)	XA (1)	XA (1)	XA (1)	XA (1) PP (1)
0005		PP (4)					
0006	PP (6)	PP (6)				XA (5)	XA (5)
0007					XA (4)		
0008				XA (4)			XA (4)
0009				XA (4)			
00010				XA (4)	XA (4)		
00012					XA (4)		
00015					XA (4)		
00017					XA (4)		XA (4)
00018		PP (4)					XA (5)
00019		PP (4)					XA (5)
Naucoridae (creeping water bugs)							
0001							XA (5) PP (5)
0002		PP (4)	XA (1)	XA (1)	XA (1) PP (4)		XA (1)
0003		PP (4)	XA (1)	XA (1)	XA (1) PP (4)		XA (1) PP (4)
0004		PP* (4)	XA (3)				
0005		PP (4)					PP (4)
0006							PP (5)

(XA) 2001-2003. Assessment states: Pre-spray = Benchmark; Post-spray and cycles = Impacted; Recovery.
(1) = Morphospecies present in benchmark, through spray periods, and recovery
(2) = Morphospecies present in benchmark, absent through spray periods, re-appearing in recovery
(3) = Morphospecies disappear after spraying and no re-appearance
(4) = Morphospecies appear for first time after spraying
(5) = Morphospecies appear for first time in 2003 (recovery)
(6) = Morphospecies present in pre-spray/benchmark samples and after one spray cycle.

The presence/absence of morphospecies during particular phases of spray and recovery has been generally interpreted as follows:

(1) = Morphospecies present in benchmark, through spray periods, and recovery
'tolerant' [no detectable spray effect]

(2) = Morphospecies present in benchmark, absent through spray periods, re-appearing in recovery
'vulnerable' [apparent spray effect and recovery]

(3) = Morphospecies disappear after spraying and no re-appearance
'sensitive' [apparent spray effect and no recovery]

(4) = Morphospecies appear for first time after spraying AND

(5) = Morphospecies appear for first time in 2003 (recovery)
'unrelated to spray' [presence apparently unrelated to spray]

(6) = Morphospecies present in pre-spray/benchmark samples and after one spray cycle, not present in 2003 recovery period.

Table 2.10 Details of selected family responses (based on numerical data) and morphospecies responses (based on presence/absence data); descriptions of data presented in Figures 2.2 – 2.9, and Table 2.9. XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Condition	**Date**	**Response**
Notonectidae (backswimmers) (Figure 2.2)		
Ecology: Predators, therefore less numerous than most consumers. Return to surface to renew air supply, therefore susceptible to surface effects such as the oil-based insecticide carrier.		
Bench mark	August 01	Present in PP channels, abundant in PP lagoons and shallow seasonal
	May 02	Common in XA lagoon Present in BB channels
Impact	August 01	Absent from PP channels – suggesting a spray effect Still abundant in PP lagoons and shallow seasonal
	May – August 02	Absent from XA lagoons – suggesting a spray effect Absence from PP channels, lagoon and shallow seasonal – suggesting a spray effect Present in unsprayed NG lagoons and channels, NX channels and BB channels only July and August 03 – no spray-linked pattern.
Recovery	August 03	Present PP lagoons – indicating some recovery Absent PP channels and shallow seasons – suggesting a spray effect
Family Comments		*Indications of both seasonal and spray-linked effects*

Morphospecies MSP) MSP0001 MSP0002 MSP0003 MSP0004 MSP0005 MSP0006 MSP0007 MSP0008 MSP0009 MSP0010 MSP0012	11 morphospecies identified, and were present as follows: PP post-spray 01 – never again, may be spray effect PP post-spray 01, XA during spray cycles, but absent by August 02, and present again in 03 - suggesting spray effect and recovery at XA PP post-spray 01 – never again, may be spray effect PP post-spray 01, XA pre-spray 02, then absent – suggesting spray effect PP post-spray 01, XA recovery - no clear linkage PP pre- & post-spray 01, absent 02, present 03 – suggesting spray effect and recovery PP post-spray 01 – never again, may be spray effect PP recovery only - no clear linkage XA pre-spray then absent 03 – suggesting spray effect XA pre-spray then absent 03 – suggesting spray effect XA recovery only - no clear linkage
Morphospecies comments	*The dominant MSP0006 showed reduction in numbers, absence and then recovery at PP & XA – suggesting a spray-related response. MSP0004,MSP0009 & MSP0010 indicated an effect by their initial post-spray absence, and no apparent recovery in 03, but were fewer in number in the benchmark pre-spray period. MSP0001, MSP0003, MSP0007 were present PP post-spray 01, but never again. It is unclear if this indicates a spray effect or not.*

Table 2.10 continued. Summary of selected family responses (based on numerical data) and morphospecies responses (based on presence/absence data); descriptions of data presented in Figures 2.2 – 2.9, and Table 2.9. XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Condition	Date	Response
Aeshnidae (swimming dragonflies) (Figure 2.3)		
Ecology: Large predators, therefore less numerous than most consumers. Possibly longer-living therefore with longer population recovery capacity.		
Bench mark	August 01	Present in Pom Pom lagoons and shallow seasonal
	May 02	Present in XA channels Common in XA lagoons Absent from NG, NX and BB
Impact	August 01	No discernable impact
	May – August 02	Absent from XA lagoons and channels – suggesting a spray effect
Recovery	August 03	Limited recovery at XA: present again in channels, lower numbers in lagoons Good recovery at PP: common in lagoons and shallow seasonal
Family Comments		***Not present at NX before August 03, and then very common in lagoon and channels in that "recovery" period. This response cannot be termed recovery – probably a seasonal occurrence. Also common at NG (unsprayed) July and August 03 only – indicative of seasonality***
Morphospecies (MSP)		5 morphospecies identified. All 5 present at Pom Pom post-spray August 2001 and:
MSP0001		XA during spray cycles and recovery 03
MSP0002		XA during spray cycles and PP recovery 03
MSP0003		XA during spray cycles and recovery 03 and PP recovery 03
MSP0004		XA during spray cycles and recovery 03 and PP recovery 03
MSP0005		XA during spray cycles and recovery 03 and PP recovery 03
Morphospecies comments		*Each morphospecies was present in at least one set of recovery samples. Therefore, although only limited recovery of numbers was indicated at XA 2003, 4 of the 5 morphospecies were present. Recovery of numbers and morphospecies (4 of the 5) was also evident at PP. PP and XA are distant from one another, with different seasonal habitat mosaics. The data indicate recovery potential.*

Table 2.10 continued. Summary of selected family responses (based on numerical data) and morphospecies responses (based on presence/absence data); descriptions of data presented in Figures 2.2 – 2.9, and Table 2.9. XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Condition	**Date**	**Response**
Baetidae (baetid mayflies) (Figure 2.4)		
Ecology: This family of small swimming mayflies includes predators, algal grazers and detritivores. No predators were sampled in the Delta, and all these baetids were consumers and were very abundant. Baetidae are frequently cited as "sensitive" taxa.		
Bench mark	August 01	Present in PP lagoons and abundant in PP shallow seasonal
	May 02	
Impact	August 01	No discernable impact - present or abundant in all PP habitats
	May – August 02	Decline in NG channels through spray cycles - no clear linkage (possibly seasonal, but concern about downstream effects) Sharp decline in XA channel - suggesting a spray effect Decline in channels at BB
Recovery	August 03	Increased abundance at NG – inter-annual differences Recovery evident in XA channels, higher than benchmark abundance in XA lagoons and shallow seasonal Recovery in channels at BB
Family Comments		*Indications of seasonal, inter-annual and spray-linked effects*
Morphospecies MSP)		6 morphospecies identified, and were present as follows:
MPS0001		PP post-spray, XA through spray cycles and recovery – suggesting tolerance
MSP0002		PP pre-&post spray (but not recovery), XA through spray cycles and recovery – suggesting tolerance
MSP0003		XA only during spay cycles, no clear linkage
MSP0004		PP post-spray (but not recovery), XA through spray cycles and recovery – suggesting some tolerance
MSP0006		PP pre-spray, absent post spray and recovery, XA pre-spray and during spray cycles, but absent post spray. Present again in 03 – suggesting spray effect and recovery.
MSP0007		XA present only during spray cycles, no clear linkage
Morphospecies comments		*Presence of apparently spray-tolerant (MSP0001, MSP0002) and a spray-sensitive (MSP0006) morphospecies. This mixture, together with species with insufficient information, explains lack of clarity at the family level. Overall Baetids were abundant and appeared resilient.*

Table 2.10 continued. Summary of selected family responses (based on numerical data) and morphospecies responses (based on presence/absence data); descriptions of data presented in Figures 2.2 – 2.9, and Table 2.9. XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Condition	**Date**	**Response**
Caenidae (caenid mayflies) (Figure 2.5)		
Ecology: small crawling mayflies, generally detritivores but some filterers. Abundant consumers.		
Bench mark	August 01	PP common in shallow seasonal, present in lagoons
	May 02	NG common in channels XA common in lagoons and channels
Impact	August 01	PP unchanged in shallow seasonal, more abundant in lagoons
	May – August 02	NG – seasonal and inter annual differences, more abundant in 03 and in August. XA unaffected in lagoons, reduced in channels – possible spray effect
Recovery	August 03	Increased in abundant in lagoons and very much increased in channels Abundant in shallow seasonal for the first time
Family Comments		*Weak evidence of spray effect in XA channels. Caenids never collected at NX which is quite unexpected, as is their sudden abundance in XA shallow seasonal. Indications of seasonal and/or inter-annual patterns.*
Morphospecies (MSP) MPS0001 MSP0002 MSP0003 MSP0005		4 morphospecies identified, and were present: PP pre-&post spray (not recovery), XA through spray cycles and recovery – suggesting tolerance PP pre-&post spray, & recovery, XA through spray cycles and recovery – suggesting tolerance PP pre-spray and recovery (absent in post-spray) - suggesting spray effect and recovery, XA absent post spray, present recovery - suggesting spray effect and recovery PP post-spay, XA pre-spay, during spray and recovery – no clear linkage
Morphospecies comments		*Again, a mixture of tolerance (MSP0001, MSP0002) and sensitivity (MSP0003), but with recovery. This explains weak family level interpretation. Generally Caenids were abundant and resilient.*

Table 2.10 continued. Summary of selected family responses (based on numerical data) and morphospecies responses (based on presence/absence data); descriptions of data presented in Figures 2.2 – 2.9, and Table 2.9. XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Dytiscidae (predatory diving beetles) (Figure 2.6)		
Ecology: Predatory diving beetles have fully aquatic larvae which were present but rare in samples. Adults have to return to surface to renew air supply, therefore susceptible to surface effects such as the oil-based insecticide carrier.		
Bench mark	August 01	Common in PP lagoons and shallow seasonal
	May 02	NG large inter-annual differences, much more abundant in 03 XA Common in lagoons, absent from channels BB common in channels
Impact	August 01	Common in channels, absent from lagoons more abundant in shallow seasonal – no clear linkage
	May – August 02	XA lagoons decline in numbers post spray (possible spray effect) – but increase in channels and no pattern in shallow seasonal. NX no pattern, BB inter-annual BB increase in channels
Recovery	August 03	XA sharp increase then decrease – may be recovery or inter-annual variability
Family Comments		*When only XA lagoons and channels were considered, decline in lagoons seemed quite clear. Consideration of 3-year data set provides less clarity.*
Morphospecies (MSP)		14 morphospecies identified, and were present:
MPS0001		PP post-spray (no recovery), XA post spray and recovery, no clear linkage
MSP0002		PP pre- & post spray (no recovery), XA through spray cycles and recovery – suggesting tolerance
MSP0004		PP pre-&post spray, & recovery, XA through spray cycles and recovery – suggesting tolerance
MSP0005		PP post-spray only, no clear linkage
MSP0006		PP pre- & post spray (no recovery), XA recovery only, no clear linkage
MSP0007		XA post-spray only, no clear linkage
MSP0008		XA through spray cycle, absent post spray, present August 03 – some indication of effect and recovery.
MSP0009		XA through spray cycle then absent, absent from recovery, indication of spray effect
MSP0010		XA through cycle & post-spray, absent from recovery, no clear linkage
MSP0012		XA post-spray only, no clear linkage
MSP0015		XA post-spray only, no clear linkage
MSP0017		XA post-spray and recovery, no clear linkage
MSP0018		PP post-spray, XA recovery, no clear linkage
MSP0019		PP post-spray, XA recovery, no clear linkage
Morphospecies comments		*The morphospecies data indicate high diversity; patterns of presence and absence with no clear links to season, inter-annual variation or spray effects; and some spray-tolerance. The variability of the morphospecies data explains the ambiguous family level result.*

Table 2.10 continued. Summary of selected family responses (based on numerical data) and morphospecies responses (based on presence/absence data); descriptions of data presented in Figures 2.2 – 2.9, and Table 2.9. XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Naucoridae (creeping water bugs) (Figure 2.7)		
Ecology: Predatory bugs strongly linked to still water and vegetation.		
Bench mark	August 01	
	May 02	XA common in lagoons and channels BB common in channels
Impact	August 01	
	May – August 02	XA reduces in lagoons and channels BB absent from channels
Recovery	August 03	XA increased numbers - evidence of recovery BB present again in channel – evidence of recovery
Family Comments		*Not a consistently abundant family, but some evidence of spray effect and recovery*
Morphospecies (MSP)		5 morphospecies identified, and were present:
	MPS0001	PP & XA recovery only, no clear linkage
	MSP0002	PP pre-&post spray, & recovery, XA through spray cycles and recovery – suggesting tolerance
	MSP0003	PP pre-&post spray, & recovery, XA through spray cycles and recovery – suggesting tolerance
	MSP0004	PP post spray only, XA pre-spray only –suggesting possible spray effect
	MSP0006	PP recovery only, no clear linkage
Morphospecies comments		*A mixture of tolerance and sensitivity.*
Pleidae (pygmy backswimmers) (Figure 2.8)		
Ecology: Tiny predatory bugs strongly linked to still water and vegetation. Entire body covered with an air bubble when swimming and have to return to surface to renew air supply, therefore susceptible to surface effects such as the oil-based insecticide carrier.		
Bench mark	August 01	Dominant in PP lagoons, present in shallow seasonal
	May 02	Abundant in BB channels and XA lagoons
Impact	August 01	Reduced in PP lagoons
	May – August 02	Reduced in BB channels and XA lagoons
Recovery	August 03	Abundances high again in BB channels and XA lagoons NG indicates inter-annual variation.
Family/species Comments		*Pleidae seem susceptible to spray events but recover well. These ubiquitous organisms are resilient.*

Table 2.10 continued. Summary of selected family responses (based on numerical data) and morphospecies responses (based on presence/absence data); descriptions of data presented in Figures 2.2 – 2.9, and Table 2.9. XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Atyidae (freshwater shrimps) (Figure 2.9)		
Ecology: Shrimps are detritivores, able to cope with some current, and strongly associated with marginal vegetation. They have a long complex life cycle involving change in gender with maturing. The pesticide may have longer term sub-lethal effect on shrimp populations, such as endocrine disruption. This would be a worthwhile research area to persue.		
Bench mark	August 01	Shrimps were not found at Pom Pom
	May 02	Dominant in XA lagoons and channels and BB channels, present at NG
Impact	August 01	Shrimps were not found at Pom Pom
	May – August 02	Reduced in XA lagoons and channels and BB channels – suggesting spray impact , abundant at NG –suggesting seasonal variability Dominant at NG
Recovery	August 03	Abundance increased again XA lagoons & channels and BB channels Much reduced at NG
Family Comments		*Shrimps were never collected from shallow seasonal habitats and were restricted to sites with more permanent flow, where they were a dominant part of the macroinvertebrate fauna. They were negatively affected by spray event, and have been slower than some taxa to recover. The very low 2003 numbers at NG may be inter-annual variation – or may be a longer term downstream effect of the spray. In parts of the delta where they occur, shrimps are an excellent indicator of ecosystem status.*

The main families which appeared, showed localized spray effects; with the morphospecies compositions indicating mixtures of tolerant and sensitive indicators, and morphospecies apparently unaffected by the sprays.

Fauna that can be earmarked as being '*sensitive'* to spray events [show apparent spray effect and no recovery]:

Families: Coenagrionidae; Leptoceridae; Naucoridae; Atyidae (channels); Hydracarina (lagoons); Polymitarcidae (lagoons).
Morphospecies: Notonectidae 0004, 0009, and 0010 all found at Xakanaxa;
Baetidae 0006 found at Pom Pom;
Naucoridae 0004 found at Xakanaxa.

Fauna that can be earmarked as being '*vulnerable*' to spray events [show apparent spray effect and some or complete recovery]:

Families: Pleidae; Veliidae; Aeshnidae; Gerridae; Corixidae; Atyidae (lagoons); Dytiscidae (lagoons); Libellulidae (lagoons); Notonectidae (lagoons); Lymnaeidae (lagoons); Baetidae (channels).
Morphospecies: Notonectidae 0006, found at Pom Pom and Xakanaxa;
Aeshnidae 0004, 0005, found at Xakanaxa.

Fauna that can be earmarked as being *'tolerant'* to spray effects [no detectable spray effect shown]:

Families: Libellulidae (channels).
Morphospecies: Notonectidae 0002 found at Xakanaxa;
Baetidae 0001, 0002, 0004 and 0006 found at Xakanaxa;
Caenidae 0001, 0005 at Xakanaxa;
Dyticsidae 0004 found at Pom Pom and Xakanaxa;
Naucoridae 0002, 0003 found at Xakanaxa.

All other families and morphospecies sampled were either abundant in small numbers within the one habitat, and abundance did not change after spray events; or appeared after the first spray event but not present in 2003 recovery period.

2.4.4 Community-level responses to spray cycles

The two study sites with the most comprehensive spatial and temporal data in relation to the spray event were Xakanaxa and Pom Pom (Table 4.1). Xakanaxa was first sampled in 2002 before spraying was initiated (benchmark condition), through five spray cycles, (May, June, July 1, July 2 and August) (impact condition) and again in May and August 2003 (recovery condition). Xakanaxa represents the part of the delta where there is more permanent water. Pom Pom was experimentally sampled pre-spray (benchmark condition) and post-spray (impact condition 1) a single spray event in 2001, and again in August 2002 (impact condition 2) and August 2003 (recovery condition).

2.4.4.1 Community level responses at Xakanaxa

Figure 2.10A (XA 02MPR) indicates the clear benchmark condition of characteristic fauna in lagoons and channels (Lagoon fauna were characterized by Coenagrionidae, Pleidae, Atyidae, Libellulidae, Naucoridae and Noteridae; and the channels characterized by Atyidae, Baetidae, Coenagrionidae and Chironomidae). Distinguishing factors were relative abundances rather than presence/absence. Differences between channels and lagoons were attributed to a number of families with approximately equal dissimilarity weightings. Figure 2.10.B (XA 02APO) indicates that although the fauna of channel and lagoon were still significantly different ($P<0.01$) within the August 02 post spray (impacted condition), there was an observable overlap between the fauna from the two habitats. Differences were based more on the relative abundances of common taxa rather than distinguishing taxa (channels were characterized by Chironomidae, Atyidae and Coenagrionidae; and lagoons by Chironomidae, Lymnaeidae, Libellulidae, Caenidae and Coenagrionidae).

Overall, the August 02 impacted state was characterized by decreases in the numbers of shrimps (Atyidae) and damselflies (Coenagrionidae) and increases in the numbers of midges (Chironomidae) and pond snails (Lymnaeidae).

Figure 2.11A (XA 03MPO) indicates that in the May recovery state there was a return to showing little overlap between the lagoon and channel fauna. Characteristic similarities and dissimilarities were mainly shifts in the relative abundances of common taxa. It is notable that Leptoceridae (cased caddisflies) were only characteristic in the channel benchmark condition. Figure 2.11B (XA03APO) indicates continued distinction between channels and lagoons, Leptoceridae were still not characteristic of faunal composition, and differences were mainly based on shifts in the relative abundances of common taxa. An impact on Leptocerid numbers, and a lack of recovery, are also shown in Table 2.5.

When all the Xakanaxa data were considered by date (Figure 2.12) each sampling occasion and therefore each condition (benchmark, immediate impact, spray cycle impact and recovery) was significantly different from the others in community composition. Differences were ascribed to the relative abundances of 9 of the most common taxa (Atyidae, Coenagrionidae, Baetidae, Dytiscidae, Pleidae, Chironomidae Libellulidae, Caenidae and Lymnaeidae.) The details of the responses of these individual taxa have been described (Figs 2.2-2.9, Tables 2.2-2.8 and Appendix 1).

When all the Xakanaxa data were considered by habitat (Figure 2.13) each of the habitats had a significantly distinctive fauna, again characterized by variations in abundances of the common taxa.

2.4.4.2 Community level responses at Pom Pom

When all the Pom Pom data were considered by date (Figure 2.14), as with Xakanaxa, each condition had a siginificantly different faunal association. The Pom Pom dominant fauna were somewhat different from Xakanaxa. The benchmark and impact states of 2001 were not significantly different –indicating a single spay cycle had little impact. The 2001 fauna were somewhat similar to Xakanaxa in

that Baetidae, Chironomidae, Coenagrionidae, Caenidae Noteridae and Dytiscidae were characteristic. In 2002, snails were important together with Chironomidae, and in 2003, Corixidae and Gerridae were characteristic. It is notable that Corixidae and Gerridae are surface dwelling/breathing organisms that were present in 2001 but not dominant, virtually disappeared in 2002 during heavy spraying, reappearing in 2003.

Consideration of the Pom Pom data by habitat (Figure 2.15) indicated significant differences between habitat fauna, but again the same major groups featured.

2.4.4.3 General community level responses

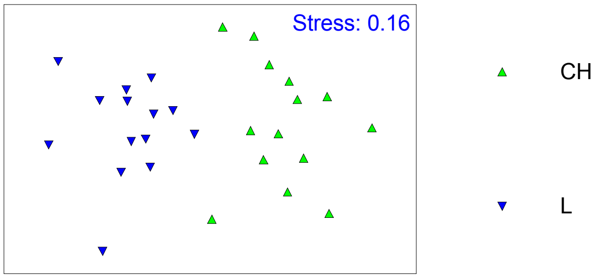
It is clear from the community level data that there are shifts through time and space in the relative abundances of the main aquatic invertebrate families. The common families are widespread and appear and disappear according to seasonal cycles, and other, unknown cues.

Multi-dimensional scaling (MDS) plots with the levels of significance between sample sets (ANOSIM) and the relative weight of components (SIMPER) are given in Figures 2.10 – 2.13 (Xakanaxa) and 2.14 - 2.15 (Pom Pom).

Note: All samples have been grouped and analysed according to the codes:

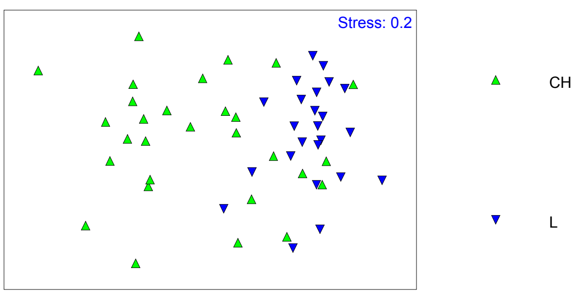
01APR	2001	August	Pre-spray
01APO	2001	August	Post-spray
02MPR	2002	May	Pre-spray
02M-APO	2002	May-August	Post-spray within spray cycle periods
02APO	2002	August	Post spray
03MPO	2003	May	Post-spray recovery
03APO	2003	August	Post-spray recovery

A.



Group CH	Group L	Groups CH & L
Average similarity: 49.49	Average similarity: 56.52	Average dissimilarity = 66.06
Species / Contrib%	Species / Contrib%	Species / Contrib%
Atyidae 38.01	Coenagrionidae 19.81	Pleidae 9.62
Baetidae 23.41	Pleidae 15.78	Coenagrionidae 8.25
Coenagrionidae 16.23	Atyidae 10.71	Baetidae 7.02
Chironomidae 5.12	Libellulidae 10.15	Noteridae 6.99
	Naucoridae 9.57	Libellulidae 6.45
	Noteridae 6.30	Atyidae 6.21
		Dytiscidae 5.81
		Naucoridae 5.44

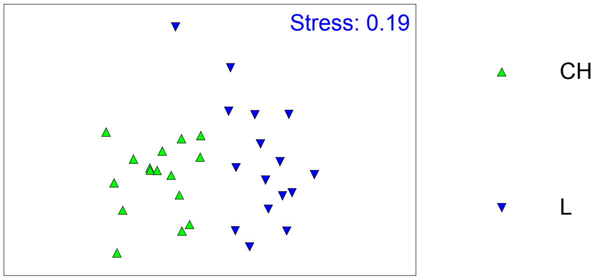
B.



Group CH	Group L	Groups CH & L
Average similarity: 36.67	Average similarity: 47.64	Average dissimilarity = 68.08
Species / Contrib%	Species / Contrib%	Species / Contrib%
Chironomidae 42.37	Chironomidae 53.29	Chironomidae 30.48
Atyidae 28.03	Lymnaeidae 12.70	Atyidae 12.70
Coenagrionidae 14.02	Libellulidae 7.67	Lymnaeidae 11.70
	Caenidae 5.89	Planorbinae 6.39
	Coenagrionidae 5.88	Libellulidae 5.98
		Coenagrionidae 5.51

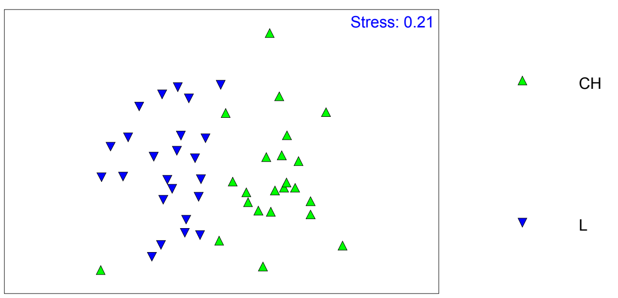
Figure 2.10. Distribution of families within the Xakanaxa 2002 May pre-spray (02MPR) **[A]** and August post-spray (02APO) **[B]** periods between channels and lagoons. Data were transformed using log (x+1). Included in the table are the SIMPER group similarities and dissimilarities with percentage family contributions. ANOSIM Global R = 0.749 (02MPR) and 0.339 (02APO) where $p < 0.01$.

A.



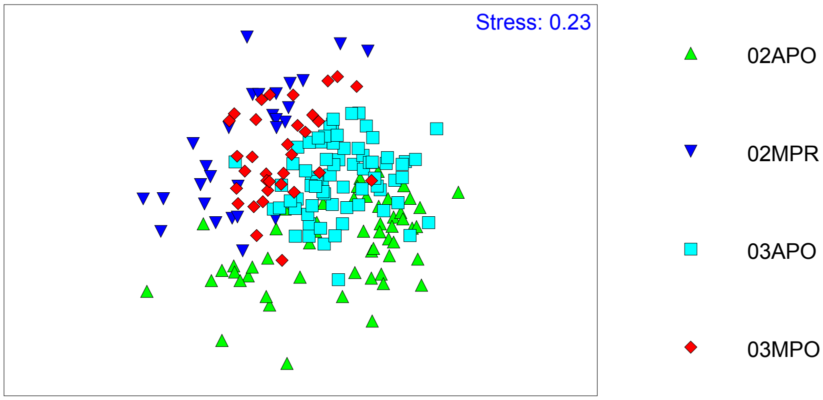
Group CH		Group L		Groups CH & L	
Average similarity: 60.25		Average similarity: 52.92		Average dissimilarity = 54.94	
Species	Contrib%	Species	Contrib%	Species	Contrib%
Baetidae	23.96	Coenagrionidae	18.33	Dytiscidae	11.34
Atyidae	20.58	Chironomidae	18.01	Baetidae	7.97
Coenagrionidae	17.91	Dytiscidae	17.52	Pleidae	6.46
Chironomidae	13.33	Libellulidae	8.55	Atyidae	6.24
Caenidae	8.42	Atyidae	8.18	Libellulidae	6.17
		Pleidae	7.87	Caenidae	6.13
		Baetidae	7.40	Chironomidae	5.44

B.



Group CH		Group L		Groups CH & L	
Average similarity: 58.65		Average similarity: 61.17		Average dissimilarity = 49.93	
Species	Contrib%	Species	Contrib%	Species	Contrib%
Baetidae	21.35	Noteridae	17.07	Noteridae	12.17
Chironomidae	18.55	Coenagrionidae	16.45	Caenidae	9.06
Caenidae	18.23	Chironomidae	16.24	Atyidae	7.24
Atyidae	12.84	Baetidae	12.35	Baetidae	6.73
Coenagrionidae	10.10	Lymnaeidae	8.21	Pleidae	6.05
		Caenidae	6.22	Libellulidae	5.77
		Libellulidae	5.90	Leptoceridae	5.58
		Pleidae	5.57	Coenagrionidae	5.40
				Lymnaeidae	5.11

Figure 2.11. Distribution of families within the Xakanaxa 2003 May post-spray (03MPO) **[A]** and August post-spray (03APO) **[B]** periods between channels and lagoons. Data were transformed using log (x+1). Included in the table are the SIMPER group similarities and dissimilarities with percentage family contributions. ANOSIM Global R = 0. 525 (03MPO) and 0.607 (03APO) where $p < 0.01$.



Group 02APO

Average similarity: 45.90

Species	Contrib%
Chironomidae	38.85
Coenagrionidae	13.61
Libellulidae	8.93
Lymnaeidae	7.16
Caenidae	7.14
Atyidae	7.05

Group 02MPR

Average similarity: 43.12

Species	Contrib%
Atyidae	26.08
Coenagrionidae	20.24
Baetidae	12.29
Naucoridae	7.24
Pleidae	6.51
Libellulidae	5.78

Group 03APO

Average similarity: 52.9

Species	Contrib%
Chironomidae	22.00
Baetidae	18.14
Caenidae	14.30
Coenagrionidae	10.16
Libellulidae	7.38
Lymnaeidae	5.0

Group 03MPO

Average similarity: 50.12

Species	Contrib%
Coenagrionidae	20.34
Chironomidae	17.30
Baetidae	15.11
Atyidae	14.70
Dytiscidae	6.19
Libellulidae	6.14
Pleidae	5.48

Groups 02APO & 02MPR

Average dissimilarity = 70.62

Species	Contrib%
Chironomidae	10.94
Atyidae	9.61
Baetidae	7.43
Coenagrionidae	7.32

Groups 02APO & 03APO

Average dissimilarity = 59.49

Species	Contrib%
Baetidae	12.64
Caenidae	10.00
Atyidae	7.10
Noteridae	6.70
Chironomidae	6.53
Coenagrionidae	6.09
Libellulidae	5.89
Lymnaeidae	5.86

Groups 02MPR & 03APO

Average dissimilarity = 63.00

Species	Contrib%
Chironomidae	10.00
Caenidae	9.18
Baetidae	7.61
Atyidae	7.40
Noteridae	5.89
Coenagrionidae	5.76

Groups 02APO & 03MPO

Average dissimilarity = 64.00

Species	Contrib%
Baetidae	10.37
Atyidae	8.62
Coenagrionidae	7.64
Dytiscidae	7.38
Chironomidae	6.10
Pleidae	6.01
Caenidae	5.52
Libellulidae	5.45

Groups 02MPR & 03MPO

Average dissimilarity = 57.45

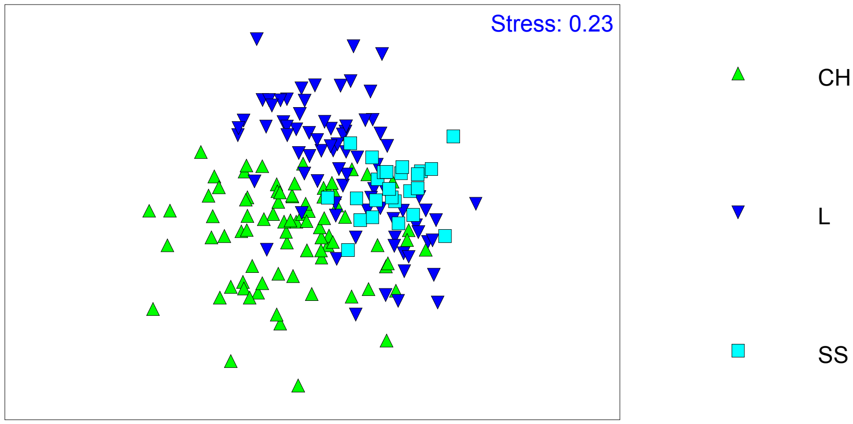
Species	Contrib%
Chironomidae	8.47
Baetidae	7.76
Dytiscidae	7.45
Pleidae	6.22
Atyidae	6.14
Coenagrionidae	6.03
Libellulidae	5.71

Groups 03APO & 03MPO

Average dissimilarity = 54.91

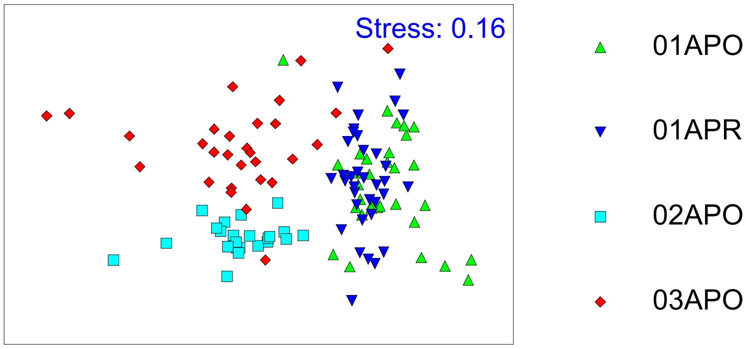
Species	Contrib%
Caenidae	8.28
Atyidae	7.25
Baetidae	6.86
Dytiscidae	6.62
Coenagrionidae	5.73
Noteridae	5.62
Chironomidae	5.48
Libellulidae	5.46
Pleidae	5.38

Figure 2.12. Distribution of families within the channels, lagoons and shallow seasonal biotopes of Xakanaxa 2002 and 2003 relative to the spray periods (02MPR, 02APO, 03MPO and 03APO). Data were transformed using log (x+1). Included in the table are the SIMPER group similarities and dissimilarities with percentage family contributions. ANOSIM Global R = 0. 521 where $p < 0.01$.



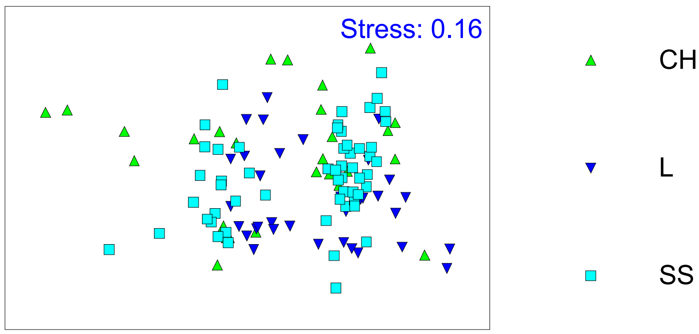
Group CH Average similarity: 45.46 Species Contrib% Atyidae 23.80 Chironomidae 21.70 Coenagrionidae 16.76 Baetidae 14.14 Caenidae 8.14	*Group L* Average similarity: 45.37 Species Contrib% Chironomidae 21.53 Coenagrionidae 17.28 Libellulidae 10.71 Lymnaeidae 9.94 Baetidae 6.77 Caenidae 5.54 Pleidae 5.09	*Group SS* Average similarity: 56.55 Species Contrib% Chironomidae 26.16 Caenidae 17.76 Baetidae 17.42 Libellulidae 11.03 Planorbinae 6.53 Lymnaeidae 5.36
Groups CH & L Average dissimilarity = 61.74 Species Contrib% Atyidae 8.43 Baetidae 7.91 Chironomidae 7.24 Coenagrionidae 6.48 Caenidae 6.45 Lymnaeidae 6.12 Libellulidae 5.94 Pleidae 5.40	**Groups CH & SS** Average dissimilarity = 60.00 Species Contrib% Caenidae 10.45 Baetidae 10.09 Atyidae 9.55 Chironomidae 8.92 Libellulidae 6.68 Planorbinae 6.23 Lymnaeidae 5.33	*Groups L & SS* Average dissimilarity = 56.67 Species Contrib% Baetidae 9.80 Caenidae 9.75 Coenagrionidae 7.04 Chironomidae 6.66 Planorbinae 5.81 Noteridae 5.50

Figure 2.13. Distribution of families within the Xakanaxa 2002 and 2003 spray periods (02MPR, 02APO, 03MPO and 03APO), relative to channels, lagoons and shallow seasonal biotopes. Data were transformed using log (x+1). Included in the table are the SIMPER group similarities and dissimilarities with percentage family contributions. ANOSIM Global R = 0. 452 where p < 0.01.



Group 01APO Average similarity: 38.45 Species Contrib% Baetidae 32.77 Coenagrionidae 22.66 Noteridae 20.14 Chironomidae 8.19 **Caenidae 7.44**	*Group 01APR* Average similarity: 42.64 Species Contrib% Baetidae 36.94 Chironomidae 19.77 Coenagrionidae 15.50 Caenidae 9.24 **Dytiscidae 7.50**	*Group 02APO* Average similarity: 53.28 Species Contrib% Planorbinae 34.46 Lymnaeidae 33.34 Chironomidae 18.90	*Group 03APO* Average similarity: 31.47 Species Contrib% Corixidae 23.04 Gerridae 17.78 Baetidae 13.42 Planorbinae 13.33 Chironomidae 7.19 Lymnaeidae 6.57
Groups 01APO & 01APR Average dissimilarity = 60.58 Species Contrib% Baetidae 17.60 Noteridae 14.69 Chironomidae 13.82 Coenagrionidae 12.68 Caenidae 10.86 Dytiscidae 9.02 Notonectidae 8.64 **Libellulidae 6.26**	*Groups 01APO & 02APO* Average dissimilarity = 87.91 Species Contrib% Planorbinae 19.20 Lymnaeidae 17.30 Baetidae 10.61 Chironomidae 8.66 Noteridae 7.61 Coenagrionidae 7.25	*Groups 01APR & 02APO* Average dissimilarity = 83.45 Species Contrib% Planorbinae 19.77 Lymnaeidae 17.77 Baetidae 11.49 Chironomidae 8.06 Coenagrionidae 6.72 Dytiscidae 5.92	*Groups 01APO & 03APO* Average dissimilarity = 86.48 Species Contrib% Baetidae 11.39 Corixidae 10.36 Noteridae 8.94 Coenagrionidae 8.29 Gerridae 7.25 Planorbinae 6.50 Chironomidae 6.34
Groups 01APR & 03APO Average dissimilarity = 84.25 Species Contrib% Baetidae 10.98 Corixidae 10.39 Chironomidae 8.31 Coenagrionidae 7.18 Gerridae 7.13 Planorbinae 6.51 Caenidae 5.79	*Groups 02APO & 03APO* Average dissimilarity = 77.12 Species Contrib% Planorbinae 15.44 Lymnaeidae 14.50 Corixidae 8.91 Chironomidae 8.54 Baetidae 6.42 Gerridae 5.98		

Figure 2.14. Distribution of families within the channels, lagoons and shallow seasonal biotopes of Pom Pom 2002 and 2003 relative to the spray periods (02MPR, 02APO, 03MPO and 03APO). Data were transformed using log (x+1). Included in the table are the SIMPER group similarities and dissimilarities with percentage family contributions. ANOSIM Global R = 0. 62 where $p < 0.01$.



Group CH Average similarity: 20.78 Species Contrib% Baetidae 31.74 Chironomidae 21.89 Lymnaeidae 9.27 Dytiscidae 9.08 Gerridae 7.39 Coenagrionidae 5.68 Planorbinae 5.06	*Group L* Average similarity: 29.27 Species Contrib% Chironomidae 21.60 Coenagrionidae 20.36 Baetidae 16.43 Noteridae 12.18 Planorbinae 7.34 Notonectidae 5.14	*Group SS* Average similarity: 29.75 Species Contrib% Baetidae 31.57 Chironomidae 17.47 Coenagrionidae 12.47 Caenidae 10.71 Dytiscidae 5.47 Planorbinae 5.17
Groups CH & L Average dissimilarity = 79.52 Species Contrib% Chironomidae 9.98 Baetidae 9.96 Coenagrionidae 9.58 Noteridae 9.36 Planorbinae 9.35 Lymnaeidae 7.73 Corixidae 6.50 Notonectidae 5.44	*Groups CH & SS* Average dissimilarity = 77.54 Species Contrib% Baetidae 13.77 Chironomidae 9.67 Lymnaeidae 9.49 Planorbinae 9.33 Coenagrionidae 7.86 Caenidae 7.55 Dytiscidae 6.39 Noteridae 5.13	*Groups L & SS* Average dissimilarity = 72.49 Species Contrib% Baetidae 11.34 Planorbinae 9.28 Chironomidae 8.69 Noteridae 8.46 Coenagrionidae 8.25 Lymnaeidae 6.13 Corixidae 6.10 Caenidae 5.83 Notonectidae 5.53

Figure 2.15. Distribution of families within the Pom Pom 2002 and 2003 spray periods (02MPR, 02APO, 03MPO and 03APO), relative to channels, lagoons and shallow seasonal biotopes. Data were transformed using log (x+1). Included in the table are the SIMPER group similarities and dissimilarities with percentage family contributions. ANOSIM Global R = 0. 262 where $p < 0.01$.

2.5 Conclusions

The Conclusions are presented in 3 sections:

- an evaluation of the 2002 conclusions in the context of the 2003 recovery data,
- the final conclusions of the 2003 Recovery study, and
- recommendations for further sampling as a basis for future management.

2.5.1 Evaluation of 2002 conclusions in relation to 2003 recovery

CONCLUSION ONE 2002

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

This conclusion has been confirmed by consideration of the whole three-year data-set. After the recovery monitoring, the main family of concern was the Atyidae (freshwater shrimps). Numbers were reduced in post-spray conditions and did not return to benchmark levels. There is evidence of low numbers at the downstream, unsprayed site, which could be ascribed either to inter-annual variability or possibly to sub-lethal effects. Shrimps are confined to the more permanent waters of the Delta, but there, they are a dominant part of the macroinvertebrate fauna. Shrimps would be a good taxon for long-term monitoring in permanent Delta waters, and experimental studies of shrimp tolerances to deltamethrin would be valuable. Of the 20 families from Xakanaxa lagoons and channels for which more than 8 individuals were collected in the benchmark condition, 12 showed a significant ($p<0.05$) decrease in abundance in at lease one of the habitats.

At a morphospecies level, five “sensitive” morphospecies were identified. They were sampled in the benchmark state, not in the impacted state and not in the recovery state:

Notonectidae MSP0004, MSP0009 and MSP0010

Baetidae MSP0006

Naucoridae MSP 0004.

The true bugs (Notonectidae & Naucoridae) are air breathers and it is suggested the pesticide carrier would have exacerbated the negative effect. Baetid mayflies are ubiquitous and abundant in the Delta, and although Baetidae MSP0006 did not recover at Pom Pom, it did recover at Xakanaxa, giving a good example of how spatial heterogeneity may add to the resilience of the overall macroinvertebrate community.

Other "vulnerable" morphospecies, showing some spray effect (present in benchmark, absent from impact and reappearing in recovery samples) were:

Notonectidae MSP0006

Aeshnidae MSP0004, MSP0005

CONCLUSION TWO 2002

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 Xakanaxa channel or lagoon habitat "disappeared"; and the assemblage composition of the lagoon and channel habitats became more similar and were characterised by fewer, more tolerant taxa.

In the benchmark state at Xakanaxa, lagoon and channel fauna were significantly different. In the post-spray, recovery state there was visually, but not statistically, less of a difference in faunal composition. In the recovery state the difference increased again. The recovery analysis also indicated that the decreased numbers of Leptoceridae in the Xakanaxa channels was a concern.

CONCLUSION THREE 2002

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.

There is evidence of recovery at the community, family and morphospecies levels. Macroinvertebrate resilience to impact was facilitated by widespread distribution patterns and temporal and spatial heterogeneity. Each mode of response contributed to macroinvertebrate recovery capacity. The community analyses revealed a fauna of relatively low diversity, but high resilience – the same families dominated the faunal composition in a complex pattern that included spray, seasonal and inter-annual effects, although in different proportions. The common fauna do not appear to be at risk.

It is important to note shifts in community composition along the permanent-seasonal gradient. Snails were abundant in the Delta and showed marked shifts in abundance – these increases were not clearly linked to spray events and additional information on snail biology and seasonality of abundance would be valuable. The shrimps were of concern in the more permanent parts of the Delta, and the corixid and gerrid bugs of concern in the more seasonal areas. Any future monitoring should include aquatic invertebrate sites both of the “Xakanaxa” and “Pom Pom/Nxaraxa” types. At the community response level, the relatively low diversity of the macroinvertebrate fauna means:

- each taxon affected has a large role in the ecosystem, but
- most taxa are widespread and individuals can be expected to survive in either spatial or temporal refugia.

At the family level, Pleidae, Velidae, Caenidae, Noteridae and Leptophlebiidae showed good recovery; Coenagrionide, Baetidae, Naucoridae, Notonectidae, Aeshnidae, and Heptageniidae showed intermediate recovery and only the Atyidae and the Polymitarcidae showed limited recovery. It would be worth monitoring shrimps at Xakanaxa in the future.

At the morphospecies level, recovery was enhanced by those morphospecies which appeared to be tolerant (found in benchmark, impacted and recovery samples):

Notonectidae MSP0002
Baetidae MSP0001, MSP0002, MSP0003, MSP0004
Caenidae MSP0001, MSP0002, MSP0003, MSP0005
Dytiscidae MSP0004
Naucoridae MSP0002 MSP0003;

There were also morphospecies showing no apparent spray-related pattern.

CONCLUSION FOUR 2002

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

The main contribution of morphospecies identifications is an increase in the confidence of conclusions indicated by family level information.

Identification to morphospecies level has demonstrated that individual morphospecies varied considerably in their distribution in time and space. However the overall patterns of aquatic invertebrate distribution indicate that although the three main habitats identified in this study (lagoons, channels, and shallow seasonal) extend over the broad expanse of the Delta, and there is considerable overlap in the biotic composition and diversity of these, most morphospecies are likely to be found in a similar habitat at a different site, either in the same or a different season. Although most taxa appear to be generalists rather than specialists, there is simply not enough known of the biology of the Delta macroinvertebrate fauna to attempt explanations of natural distribution patterns.

2.5.2 Final conclusions

1. A spray-correlated effect was measurable at community, family and morphospecies levels.
2. The most negatively affected families were Atyidae (shrimps), which are characteristic of areas of permanent flow; and Pleidae (pygmy backswimmers), which are also found in the more seasonal areas. The negative effect was in terms of reduced numbers, and a lack of recovery of numbers.
3. The most negatively affected morphospecies were two Notonectidae (backswimmers)
4. Negative spray-related effects included localised absences and reduced numbers.
5. Recovery was measurable and was in terms of increased numbers, re-appearance, or both.
6. The system is resilient, and has high levels of spatial and temporal variability
7. Limits to interpretation are due to the repeated evidence of spatial and temporal variability. Taxa appear, and are absent, from sites and at times that could be related to season, inter-annual variation, or to patterns simply not apparent at this scale of sampling. Aquatic invertebrate responses at the unsprayed, North Gate site indicate the scale of seasonal and inter-annual variation.

8. The measurable localized number reductions absences would increase in area with increases in exposure, and sub-lethal effects would ultimately affect reproductive success of sensitive and vulnerable taxa. This would become apparent as changed community structure. Repeated spraying is likely to have serious negative effects.

2.5.3 Recommendations

1 The results of the recovery study indicate good recovery, except for a return to some of the levels of abundance, and since most invertebrates have rapid reproduction and life-cycles, It was not considered necessary to further monitor recovery.

2 Tsetse management will continue, and it is not yet clear as to the frequency, if any, of re-spraying. Since there were insufficient benchmark data for a highly confident description of the benchmark condition it would a sound investment in the quality of future management decisions if routine monitoring of aquatic invertebrate fauna were to be initiated.

3. Routine monitoring should be undertaken at least seasonally (pre- and post-flood), and should include a site in a region of permanent flow (such as as Xakanaxa), a site with large seasonally inundated areas (such as Nxaraxa or Pom Pom), and any sites at which future spraying is likely.

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2.7 APPENDIX 1

Appendix 1 has been included in this report because the data presented in the main sections of the report are sufficient to demonstrate the characteristics of the responses of aquatic invertebrates to the deltamethrin spray events in 2001 and 2002. These data, however, provide additional information that supports the conclusions drawn, and in view of the paucity of data on aquatic invertebrates in the Delta, it is important to include as full a description as possible.

2.7.1. Appendix 1: Abundances of families for which there are no morphospecies data.

Selected family abundance data are presented for ecologically and numerically important families.

Table 2A.1 Descriptions of the abundances (Figures 2A.1 – 2A.6) of selected families for which there are no morphospecies data.
XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Condition	Date	Response
Coenagrionidae (damselflies) (Figure 2A.1)		
Ecology: Damselflies are closely related to dragonflies, and the larvae are aquatic predators. Damselfly larvae are closely associated with marginal vegetation and were overall the most abundant invertebrate family at the main sample sites. This family was a prime candidate for morphospecies identification but preservation and identification methods did not provide results, so only family level data can be described.		
Bench mark	August 01	Common in PP lagoons and shallow seasonal habitats.
	May 02	Common in NG and XA channels and XA lagoons; present in BB channels.
Impact	August 01	Increased abundance in PP lagoons and shallow seasonal habitats, and present on channels. No apparent spray effect.
	May – August 02	Evidence of strong seasonal variation with low numbers at the unsprayed NG site, Sharp decline in numbers in XA lagoons and channels – could be spray-related.
Recovery	August 03	Evidence of strong inter-annual variation, with increased numbers at NG, possible lack of recovery in XA lagoons and slight recovery in XA channels (both these responses are within the NG pattern on unsprayed variation)
Family Comments		Taken on their own, the XA abundances indicate spray impact and a slow or minimal recovery. NG indicates high possible natural variation.

Table 2A.1 continued… Descriptions of the abundances (Figures 2A.1 – 2A.6) of selected families for which there are no morphospecies data.
XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Condition	Date	Response
Chironomidae (midges) (Figure 2A.2)		
Ecology: Midges fill as variety of ecological niches and component species feed in a wide variety of ways. It would be time-consuming but valuable to establish morphospecies for this group. They breed rapidly and are abundant.		
Bench mark	August 01	Dominant in XA lagoons and common in shallow seasonal.
	May 02	Abundant in NG channels, absent from NG lagoons. Present in XA lagoons and channels abundant in BB channels.
Impact	August 01	Reduced in PP lagoons, increased in PP channels.
	May – August 02	Reduced in NG channels, present in NG lagoons – seasonal effect; increase in XA lagoons and channels – possible increase with decrease in predation. Abundant, and no spray effect at BB.
Recovery	August 03	Steady increase in NG channels – indicating either seasonal and inter-annual variation, or, possibly, a delayed spray effect by downstream drift. High abundance in Shallow seasonal habitat. Increasing abundance at BB.
Family Comments		Midges typically increased in the post-spray period indicating tolerance to spray, and rapid reproductive responses, possibly because of reduced numbers of predators.
Ceratopogonidae (biting midges) (Figure 2A.3)		
Ecology: These midge larvae are predatory.		
Bench mark	August 01	Absent from all PP habitats.
	May 02	Absent from all PP habitats. Present in XA lagoons and channels, and BB channels.
Impact	August 01	Still absent from all PP habitats. Absent from NG; increased in XA lagoons and channels, decreased in BB channels.
	May – August 02	Further increase in XA lagoons, decrease in channels, return to May benchmark in BB channels, present for the first time in all PP habitats.
Recovery	August 03	Increases in NG (unsprayed site); sharp increase in BB and PP shallow seasonal, increase in all XA habitats.
Family Comments		No clear link between abundance and spray events.

Table 2A.1 continued… Descriptions of the abundances (Figures 2A.1 – 2A.6) of selected families for which there are no morphospecies data.
XA – Xakanaxa, PP – Pom Pom, NG – North Gate, NX - Nxaraxa, BB - Bodumatau Bund.

Condition	Date	Response
Noteridae (creeping water beetles) Figure 2A.4		
Ecology: Predatory, closely related to Dytiscidae – predatory diving beetles.		
Bench mark	August 01	Absent from PP channels, common in PP lagoons and shallow seasonal
	May 02	Absent from all PP habitats Common in XA lagoons and BB channels
Impact	August 01	Present in PP channels, increased in lagoons and shallow seasonal.
	May – August 02	Reduced in BB channels and XA lagoons (possible spray effect) Slight recovery, but not to pre-spray levels in BB channels, no recovery (absent) from XA lagoons.
Recovery	August 03	Abundant in NG channels. Abundant in XA lagoons present in XA channels and shallow seasonal and lagoon. Present in PP lagoons and shallow seasonal.
Family Comments		Few responses that can be spray-linked, but in view of additional information given by variable morphospecies response in Dytiscidae, spray response in some species cannot be excluded. Seasonal effects evident.
Planorbinae (orb snails) (Figure 2A.5)		
Ecology: Grazers		
Bench mark	August 01	Absent from all PP habitats
	May 02	Present in NG channels, XA channels and lagoons, BB channels
Impact	August 01	Still absent from all PP habitats Increased numbers in NG Channels, XA channels and lagoons. BB channels Abundant, and present for the first time at all NX habitats.
	May – August 02	Abundant in all PP habitats Decreased in NG channels, common for the first time in lagoons, further increases in XA channels and lagoons, and BB channels.
Recovery	August 03	Increases at NG, common in XA channels and lagoons, abundant in shallow seasonal. Abundant in all NX habitats and PP lagoons and shallow seasonal.
Family Comments		No apparent link between abundances and spray events.
Lymnaeidae (pond snails)(Figure 2A.6)		
Ecology: Grazers		

Bench mark	August 01	Absent from all PP habitats
	May 02	Present in NG channels, XA channels and lagoons, BB channels
Impact	August 01	Still absent from all PP habitats Increased numbers in NG Channels, XA and BB, increased to abundant levels in XA lagoons.
	May – August 02	Further increases in XA lagoons and channels. Abundant, and present for the first, time in all NX habitats. Abundant in all PP habitats
Recovery	August 03	Abundant at NG, XA NX and common at PP – widespread.
Family Comments		Similar pattern to the orb snails – but no apparent link to spray events.

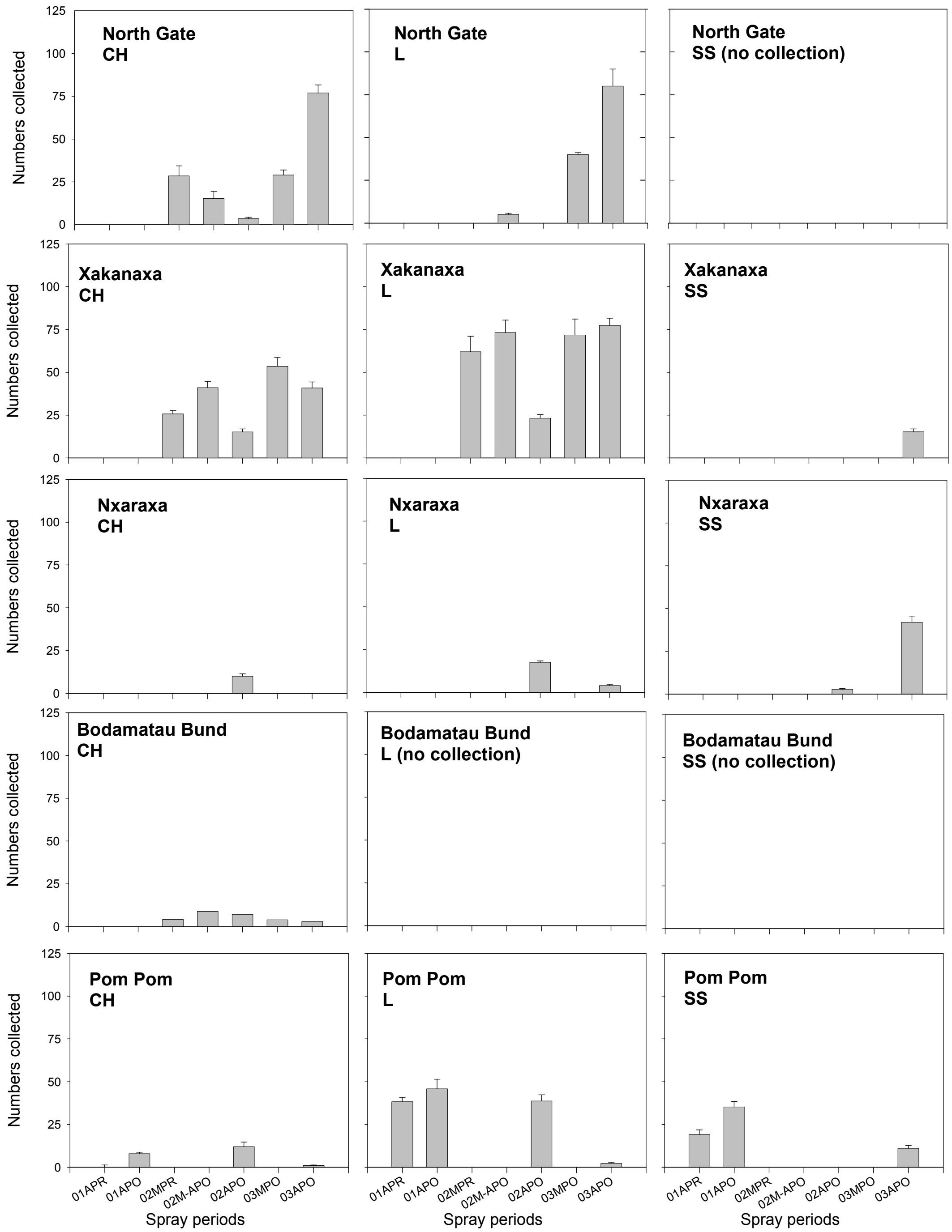

Figure 2A.1 Abundances of COENAGRIONIDAE (damselflies) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

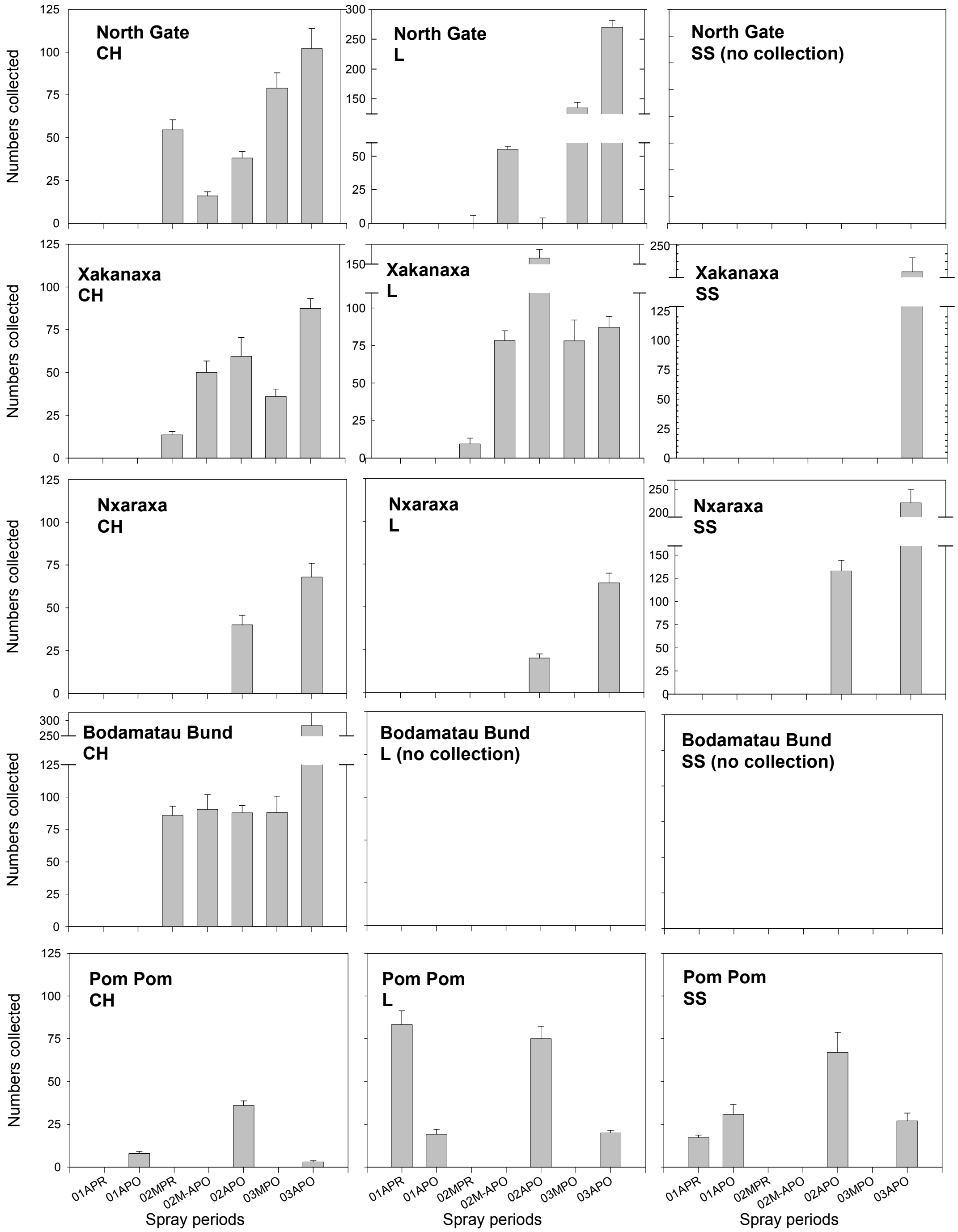

Figure 2A.2 Abundances of CHIRONOMIDAE (midges) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

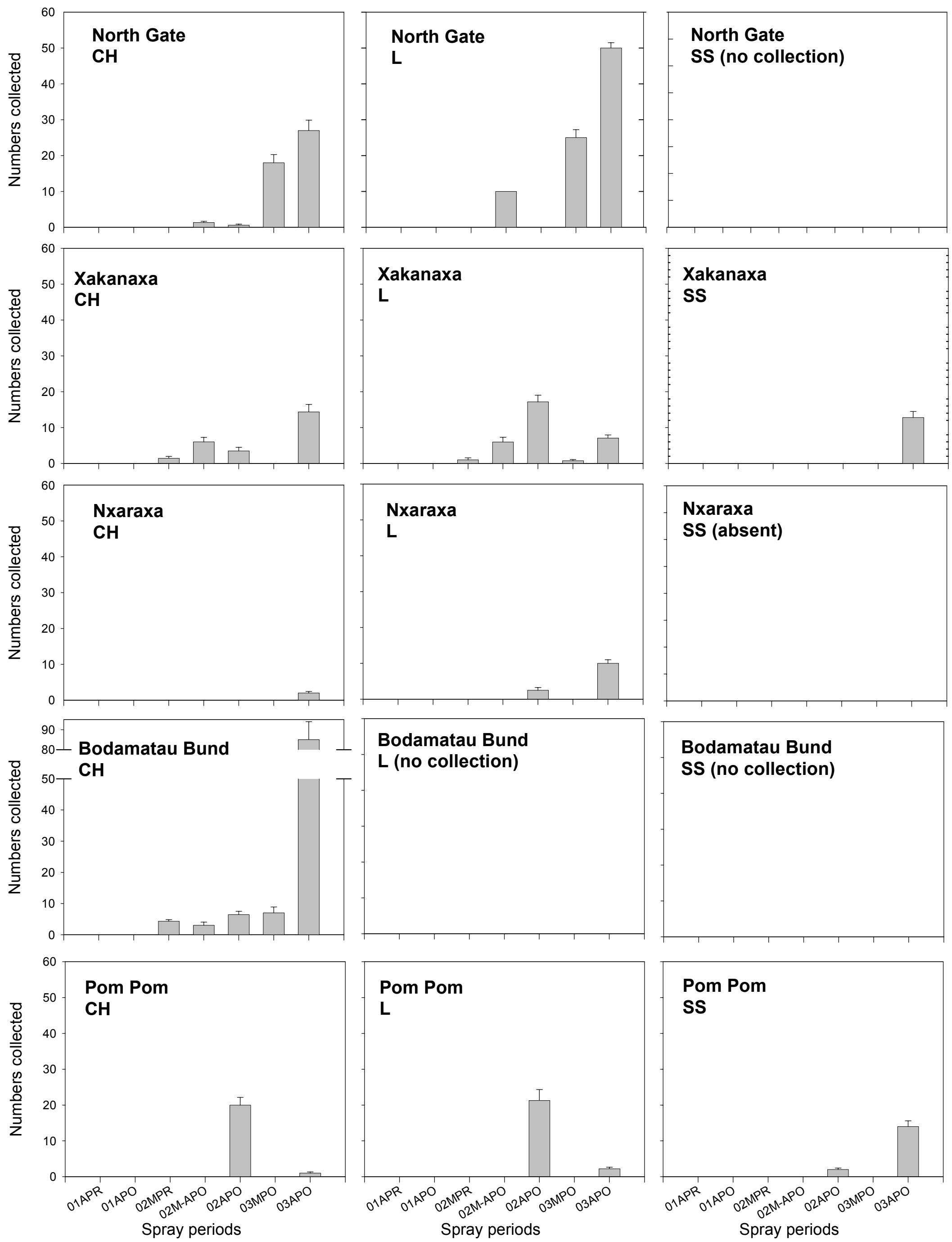

Figure 2A.3 Abundances of CERATOPOGONIDAE (biting midges) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

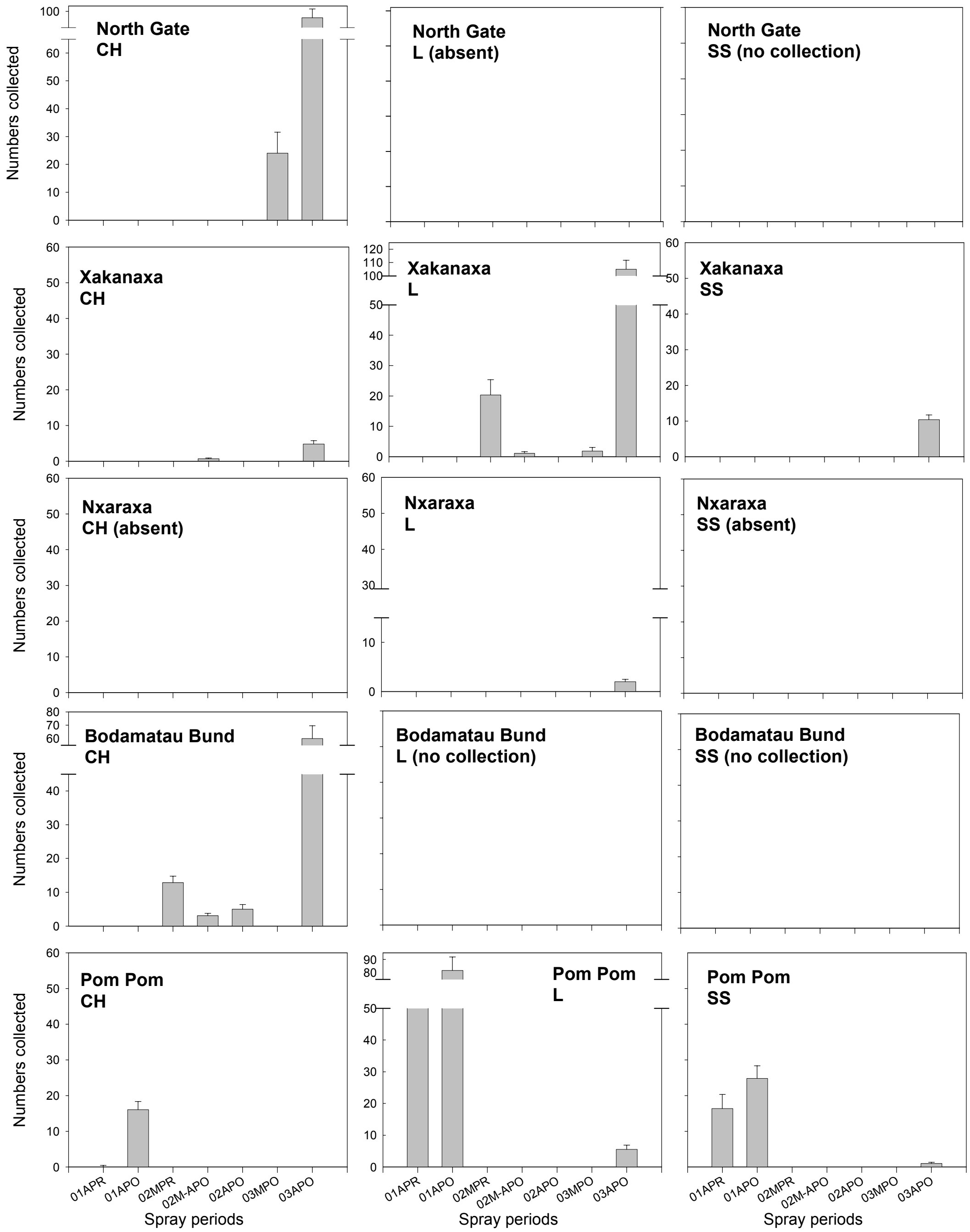

Figure 2A.4 Abundances of NOTERIDAE (creeping water beetles) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

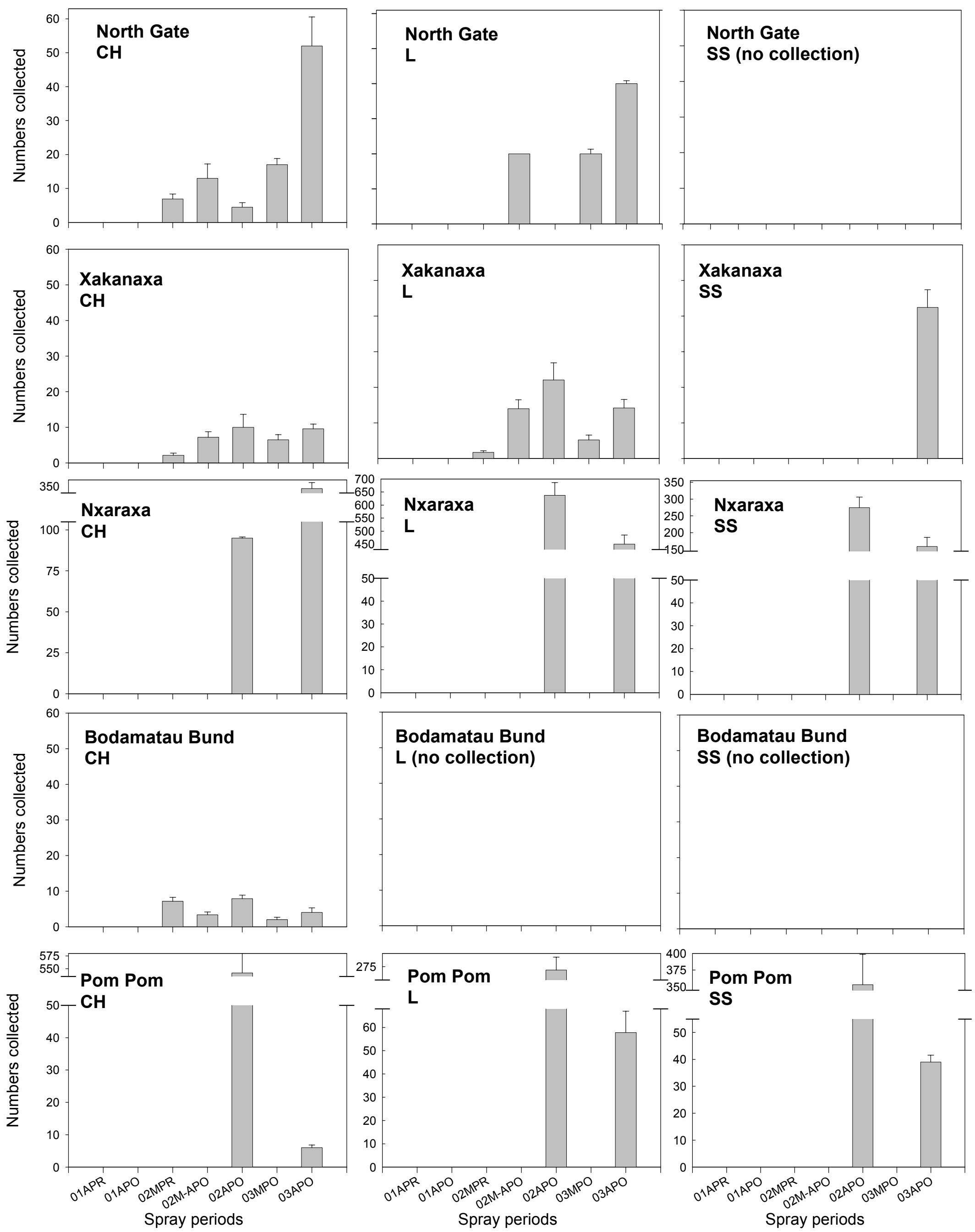

Figure 2A.5 Abundances of PLANORBINAE (orb snails) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.

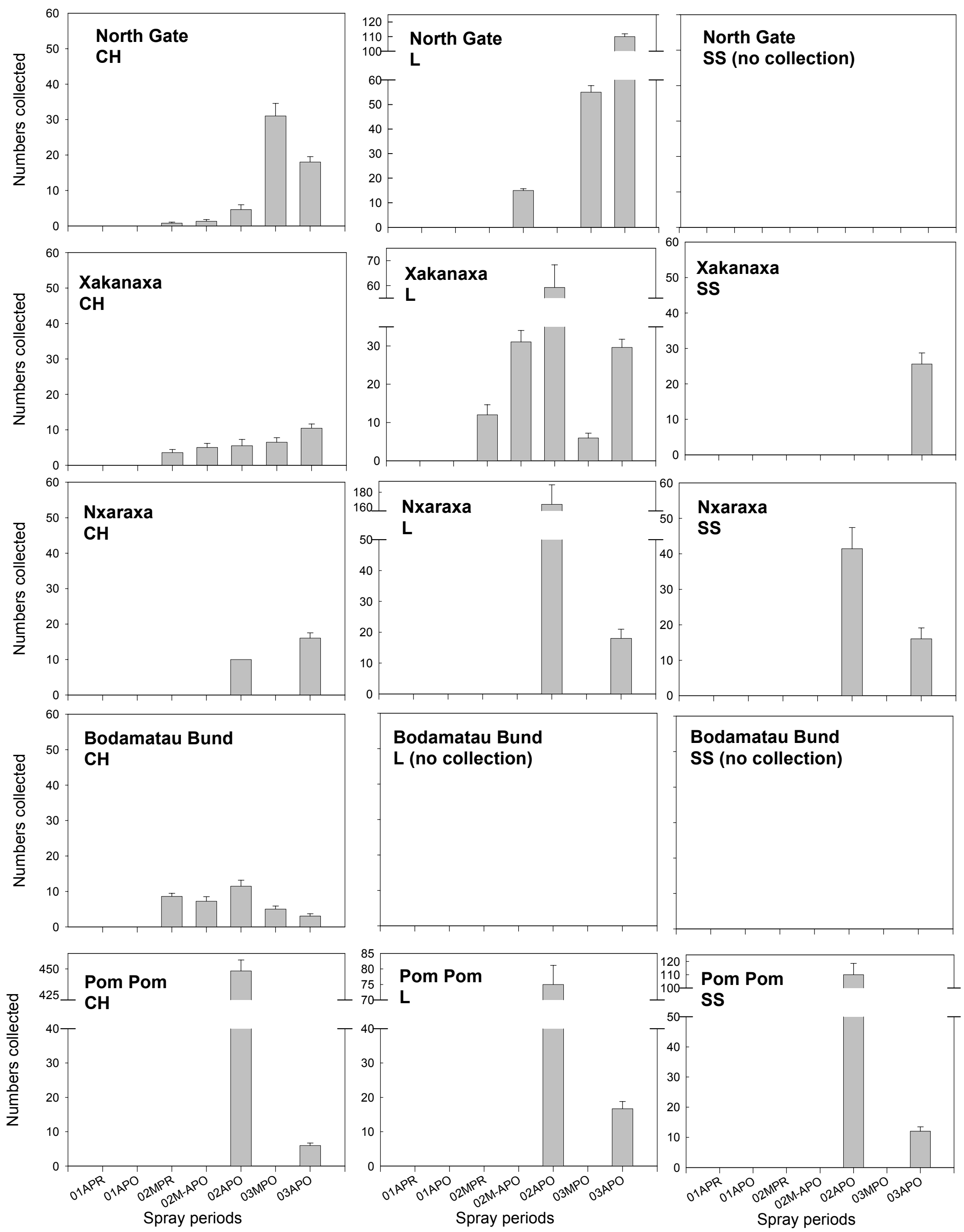

Figure 2A.6 Abundances of LYMNAEIDAE (pond snails) at each site [North Gate, Xakanaxa, Nxaraxa, Bodumatau Bund and Pom Pom] within each biotope sampled [channel = CH, lagoon = L, shallow seasonal = SS]. The spray periods refer to sets of sampling occasions [01APR = 2001 August pre-spray, 01APO = 2001 August post-spray, 02MPR = 2002 May pre-spray, 02M-APO = post-cycle spray, 02APO = 2002 August post spray, 03MPO = 2003 May post-spray recovery, 03APO= 2003 August post-spray recovery]. Refer to Table 2.1 for individual spray periods not sampled.These data support the patterns of spray response indicated by Figures 2.2-2.9 and Table 2.7, and substantiate the final conclusions (Section 2.5.2):

3. Monitoring of Terrestrial Invertebrate Recovery

Report prepared by

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Biotrack Australia Pty Ltd

Specimen identifications

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Data analyses

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Project management

Eleanor Azzi

3.1 Background

Aerial spraying of deltamethrin by the Government of Botswana to eradicate tsetse flies from northern Botswana has been a success. No tsetse flies have been recorded since the end of the spraying in August 2002. Assessment of the impacts of spraying on non-target terrestrial invertebrates was completed by Biotrack Australia in 2002. The outcome was a reduction in invertebrate abundance, especially of beetles, and the potential loss of some species. Sampling was completed in May and August 2003, one year on from the spray applications, to measure the recovery of these invertebrates and the results are the subject of this report.

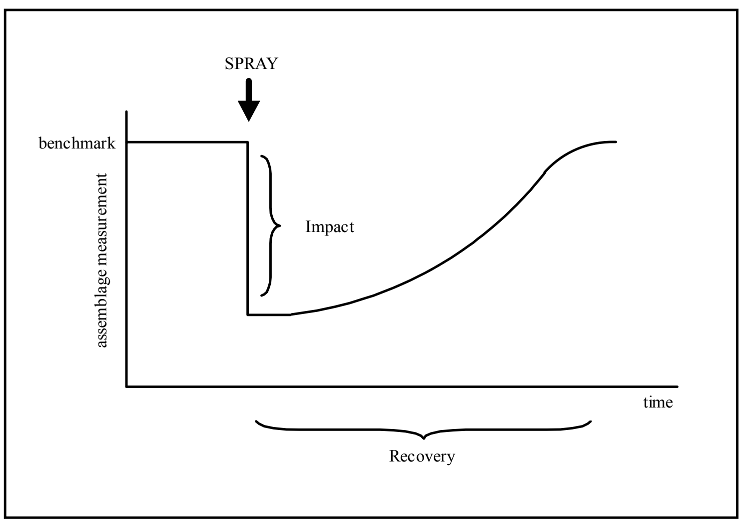
The true impact of deltamethrin applications for tsetse fly control on non-target organisms in the Okavango Delta requires knowledge of

1) the abundance and composition of potentially sensitive taxa prior to the application,
2) changes in abundance and composition as a result of the spray and
3) recovery time.

These requirements are summarised graphically below.

The preliminary sampling in 2001 and the extensive monitoring program during the 2002 spray cycles have provided data to benchmark the pre-application abundance and composition and subsequent changes during, and at the end of the spray cycles but not recovery.

Habitats sampled during the 2002 spray cycles were re-sampled in 2003 to determine the recovery in terms of both abundance and composition of terrestrial invertebrates to the levels recorded prior to the spray cycles.



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

Impact is only part of the answer because a speedy recovery from even significant impacts is possible in many resilient systems. A large change in abundance may only be temporary if the organisms can respond by increasing their numbers in subsequent seasons, or they have life stages not affected by the application.

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 impact.

The focus for 2003 was the tree canopies which were fogged with deltamethrin from hand-held foggers at two areas near Nxaraxa, on Chiefs Island and east of the spray zone just outside Moremi National Park. Sampling was conducted during two events: May 2003 one year on from pre-spray sampling and in August 2003 one year on from post-spray sampling. Particular attention was paid to morphospecies that were not recorded after the first cycle of spraying in 2002 with data analysis and management following the protocols used in the impact monitoring program in 2002.

3.2 Impact assessment in 2002

In 2002, a total of 102,248 terrestrial invertebrates were captured from 746 samples of various types. In all, 26 higher taxa, mostly at Ordinal level, were recorded. 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 expected, the majority of the remaining taxa were infrequent. A total of 367 morphospecies were identified from selected families of beetles, spiders, flies, Hemiptera and ants in samples taken from *Kigelia africana, Combretum imberbe, Lonchocarpus capassa* and *Colophospermum mopane* trees within the spray zone.

Aerial spraying of deltamethrin in the Okavango Delta on non-target terrestrial invertebrates resulted in a significant reduction in the abundance of spiders, ants, hemipterans and, in particular, beetles. The abundance of flies actually increased, presumably due to seasonal patterns of adult emergence and behaviours triggered by the arrival of the annual floods to the study areas.

A significant reduction in the richness of higher taxa was also detected. This phenomenon, however, may be due to undersampling, given that the abundance of specimens had been reduced as a result of the spraying.

While changes in the abundance of flies, beetles and ants led to significant compositional changes between spray cycles at the higher taxa level, these changes lose their significance if compositional changes are considered just on the presence or absence of taxa. This led to the conclusion that the consequences of application are not acute at this taxonomic resolution.

In summary, the evidence from the 2002 monitoring showed that some taxa are affected by repeated aerial applications of deltamethrin and that these effects may be acute for some morphospecies but apparently not across higher taxa.

3.3 Methods

3.3.1 Number of trees to sample

The approach taken to monitor recovery was to resample a subset of the trees sampled in 2002, in May and August 2003. These times were one year on from the pre-spray benchmark samples obtained by fogging trees from the ground and similar sampling undertaken in August 2002 after the last of the five aerial applications.

The sampling in 2002 was split between an area around the Okavango Research Centre field camp at Nxaraxa and an area some 20km to the south near Stanleys Camp (Figure 3.2). This separation was to prevent contamination of water habitats at Nxaraxa with deltamethrin from the fogging.

In the area south of Stanleys camp 30 *K. africana* trees were fogged prior to the spray cycles and a further 24 fogged at the end of the spray cycles in 2002. No data were collected from each spray cycle in this area. Four of these trees were fogged on both occasions. In May 2003 these four trees were resampled together with 14 trees from the

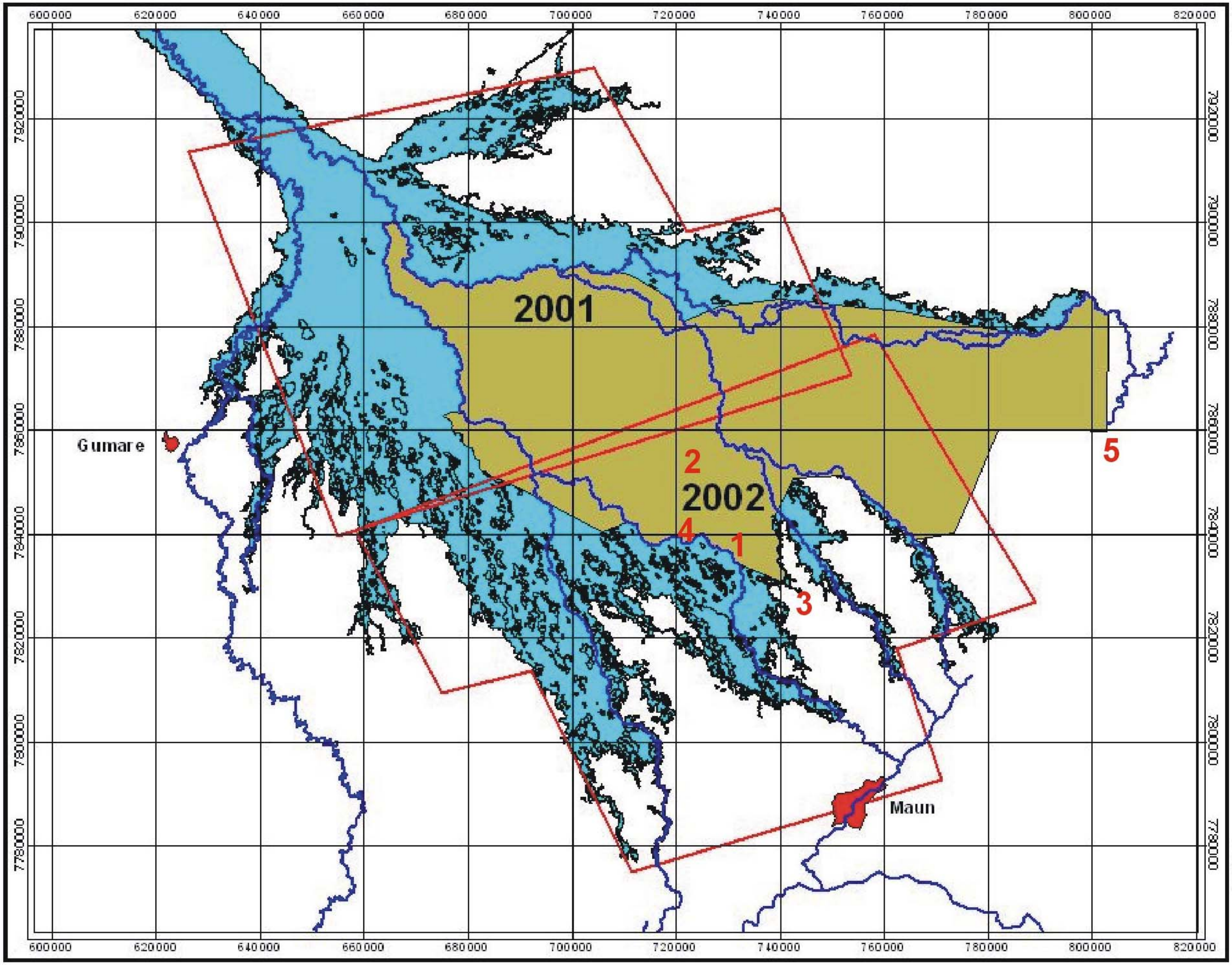


Figure 3.1
Terrestrial Sampling Locations

pre-spray. A further 6 new trees were sampled that received the spray but had not been fogged in 2002.

Data from these samples provided comparisons between trees that were a) fogged in both years, b) fogged twice and those fogged only once, c) fogged in 2002 and not fogged in 2002. These samples will also generate specimens in which to search for the lost morphospecies.

In August 2003 the four *K. africana* trees that were fogged on both occasions in 2002 were fogged for the fourth time to provide an idea of the relative impact of repeated application of deltamethrin. A further 14 of the trees from the 2002 post-spray fogging were fogged again, along with another 6 trees not previously fogged.

Across the two sampling events, a total of 48 *K. africana* trees were sampled from the area south of Stanleys Camp.

At Nxaraxa there was no pre-spray fogging to avoid contamination of the site for freshwater sampling but trees were sampled during each spray event and by post-spray fogging. A total of 22 *K. africana* trees were sampled through all 5 cycles and 6 of these were fogged together with a further 51 trees that were not monitored during the cycles. In May 2003 these 6 trees were resampled together with 18 trees monitored during the cycles but not previously fogged. In August 2003 the 6 trees sampled through the cycles and fogged in 2002 were fogged for the fourth time together with 8 cycle trees (including 4 that were also fogged in May 2003) and 10 trees that were fogged in the post-spray sampling in 2002.

Also at Nxaraxa, 10 *C. imberbe* and 10 *L. capassa* trees sampled through the spray cycles but not fogged in 2002 were fogged in May 2003 and 5 of these trees for each species fogged again in August 2003. A further 5 trees of each species that were fogged during post-spray sampling in 2002 were fogged in August 2003 (Table 3.1)

Status from 2002	*Kigelia africana*		*Lonchocarpus capassa*		*Combretum imberbe*	
	May	August	May	August	May	August
Nxaraxa						
Sampled in spray cycles	18	8	10	5	10	5
Post-spray fogged	-	10	-	-	-	-
Cycles & post-spray fogged	6	6	-	5	-	5
Stanleys						
Pre-spray fogged	14	-	-	-	-	-
Post-spray fogged	-	14	-	-	-	-
Pre & Post-spray fogged	4	4	-	-	-	-
Not fogged	6	6	-	-	-	-

Table 3.1 Summary of the numbers of trees sampled in each category and location

3.3.2 Sampling of Colophospermum mopane

At a site on the southern end of Chiefs Island within the spray zone, 18 *C. mopane* trees were fogged in May 2002 and again in August 2002. In May and again in August 2003 10 of these trees were fogged and a further 10 trees selected that have not previously been fogged.

On the Moremi cutline site, which was outside the spray zone, 20 *C. mopane* trees were fogged in May and a further 20 in August 2003. In the August samples 10 of the trees were fogged in May and 10 were trees not previously fogged.

3.3.3 Selection of trees

The number of trees to be sampled was selected to maximize the information on insect composition and abundance for meaningful comparisons of invertebrate assemblages. Specifically sufficient replication was needed to detect differences between pre-spray assemblages and those one year later. A non-significant difference in assemblage composition would be strong evidence for recovery.

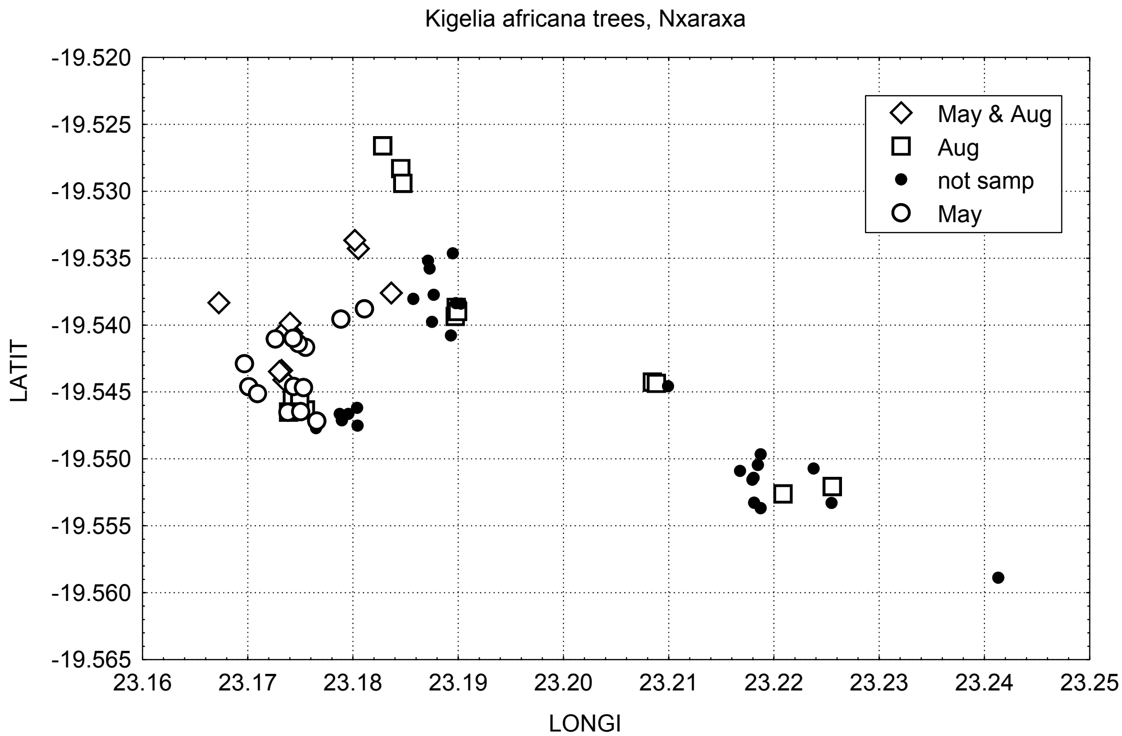
Equally important was the sampling of those morphospecies that were "lost" from samples after the first few spray cycles in 2002. It was therefore important in the selection of specific trees that, wherever possible, the trees on which these lost species were initially recorded were resampled. Finding these 'lost' morphospecies in the same trees would clearly be excellent evidence of successful recovery.

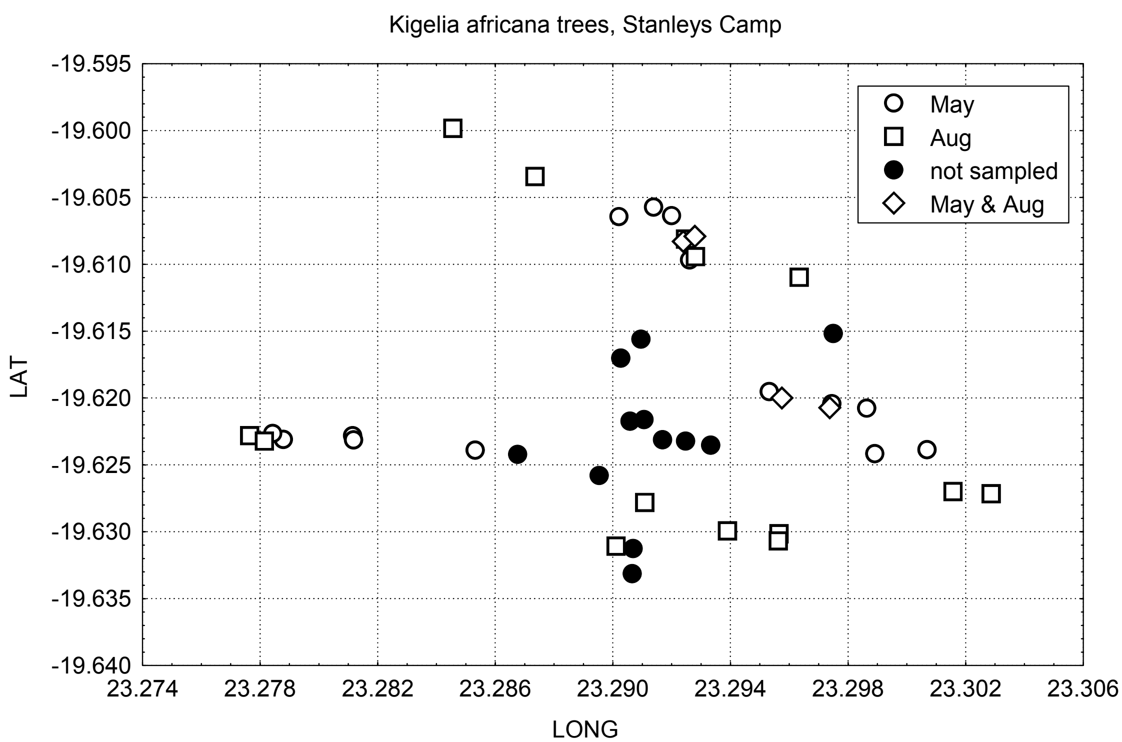
In the case of *C. imberbe* and *L. capassa,* almost all the trees sampled through the cycles were fogged in 2003 so tree selection was not an issue. However, for *K. africana* around half the trees sampled in 2002 at Stanleys and Nxaraxa were sampled in 2003, requiring some selection.

Unfortunately the lost morphospecies were widely spread among the trees sampled. For example, there were 7 spider morphospecies not sampled after cycle 1 but specimens were recorded from 12 of the 55 trees (22%) on which spiders were sampled throughout the 2002 program and on 55% of the trees on which spiders were sampled in the pre-spray fogging and cycle 1. Overall there are 84 morphospecies not recorded after pre-spray fogging and cycle 1 on *K. africana* alone, so following the pattern for spiders ideally all trees should be sampled. Selections included the trees that had most morphospecies and were logistically most efficient to sample.

There was a significant risk of fire in the Nxaraga Lagoon area and in the site identified for fogging during the spray cycles. Contingencies to replace trees used in the monitoring lost to fire included moving some 5km to the south and east of Nxaraxa onto floodplains that were wetter and less likely to burn. Fortunately, fire did not prove to be a problem during the sampling period.

The following maps display the specific trees to be sampled in May and August in the area around Stanleys Camp and at Nxaraxa.


Kigelia africana trees, Nxaraxa
May & Aug
Aug
not samp
May
LATIT
LONGI
-19.520
-19.525
-19.530
-19.535
-19.540
-19.545
-19.550
-19.555
-19.560
-19.565
23.16
23.17
23.18
23.19
23.20
23.21
23.22
23.23
23.24
23.25


Kigelia africana trees, Stanleys Camp
May
Aug
not sampled
May & Aug
LAT
LONG
-19.595
-19.600
-19.605
-19.610
-19.615
-19.620
-19.625
-19.630
-19.635
-19.640
23.274
23.278
23.282
23.286
23.290
23.294
23.298
23.302
23.306

3.3.4 Sampling

Sampling followed the same fogging method and equipment used in 2002. Each tree was fogged with deltamethrin in early evening and invertebrates knocked down by the fog collected in 2.5m x 1.5m plastic tray after 12 hours. One tray per tree was suspended 50cm above the ground and positioned half way between the tree bole and the edge of the canopy.

Specimens were then transferred to plastic bags, with a barcode applied to the bag and a pre-printed data sheet. A small amount of insecticide was sprayed into the plastic bag to kill any stunned animal.

Date	Actions
5-10 May 2003	Reassembly and maintenance of knockdown traps
	Fieldwork logistics and equipment supplies
17 May	Travel to field site at Nxaraxa
18 May	Reconnoitre of sites to relocate trees
19-21 May	Fogging of Ka at Stanleys
	Sorting of samples
22-25 May	Fogging of Ka, Lc & Ci trees at Nxaraxa
	Sorting of samples
26 May	Fogging of Cm on Chiefs Island
27 May	Fogging of Cm on Moremi cutline
1 Aug	Travel to field site at Nxaraxa
2 Aug	Reconnoitre of sites to relocate trees
3-5 Aug	Fogging of Ka at Stanleys
	Sorting of samples
6-8 Aug	Fogging of Ka, Lc & Ci trees at Nxaraxa
	Sorting of samples
9 Aug	Fogging of Cm on Chiefs Island
10 Aug	Fogging of Cm on Moremi cutline

Table 3.2 Fieldwork timetable

3.3.5 Sorting procedures

At the field camp specimens were sifted from litter debris and placed into vials of ethanol and the barcode transferred to the vial. Preliminary sorting of the material was completed in the field under the supervision of Biotrack staff. Beetles, flies, ants, hemiptera and spiders were separated and counted, while the abundance for all remaining specimens were recorded. The five taxa were then packaged and shipped to the Biotrack laboratory in Sydney for morphospecies identifications which followed the same protocols and images used in 2001 and 2002.

The remaining specimens were packaged for storage at the HOORC.

Beetles	**Hemiptera**	**Spiders**
Anobiidae	Aphididae	Oxyopidae
Anthicidae	Cicadellidae	Salticidae
Anthribidae	Cydnidae	Theridiidae
Bostrichidae	Delphacidae	Thomisidae
Brentidae	Derbidae	
Buprestidae	Flatidae	
Carabidae	Membracidae	
Cerambycidae	Plataspididae	
Chrysomelidae	Reduviidae	
Coccinellidae	Saldidae	
Curculionidae	Tingidae	
Elateridae	Triozidae	
Histeridae		
Melyridae		
Nitidulidae		
Ptiliidae		
Scarabaeidae		
Staphylinidae		
Tenebrionidae		
Zopheridae		

Table 3.3 Selected families that were processed

Unfortunately, ant and fly specimens collected in August were lost during air transit from Johannesburg to Sydney.

Morphospecies sorting was conducted on the remaining taxa (beetles, hemiptera and spiders) in the Sydney laboratory. Only selected families from these groups were identified (Table 3.3). These families were found in the 2002 study, and are generally the most abundant and ecologically important taxa from each group.

3.3.6 Data Analysis

Descriptions of average abundance and richness and comparisons between categories were made using ANOVA routines in Statistica®.

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*". On an ordination diagram, the distance between two points reflects the dissimilarity in assemblage composition. If points are far apart they either share few taxa in common, or they share some taxa but at very different levels of abundance.

There are many different types of ordination procedures. Multi-dimensional scaling (MDS) was used for its simplicity, best use of sample information and general applicability (Clarke & Warwick 1994). MDS also makes fewer assumptions regarding the form of the data and the inter-relationships of the samples and more efficiently preserves distance relationships in low-dimensional ordination space (Clarke & Warwick, 1994). The graphical presentation of the MDS is prepared using similarity matrixes, based on similarity coefficients that measure the similarity (S) between sample pairs. Here, the Bray-Curtis measure of association was used.

Matrices of abundance data for taxa by sample were exported from Biota®. 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.

An 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. The specific procedure used was Analysis of Similarities (ANOSIM), which is a multivariate analogue of one-way analysis of variance. The null hypothesis tested is that there is no assemblage difference 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).

3.4 Results

3.4.1 Overall captures

A total of 46,876 terrestrial invertebrates were captured from 213 fogging samples. This was a good sampling return and shows immediately that the aerial spraying did not have devastating medium term effects on the abundance of canopy invertebrates in the Okavango Delta. More than 200 specimens per sample tray is considerable even though samples were taken during the dry winter months when invertebrate activity is generally reduced.

In 2002 beetles (34%), flies (28%), ants (22%), Hemiptera (6%) and wasps (3%) were the most abundant taxa, collectively accounting for 93% of the total sampled. In 2003 these taxa accounted for 76% of specimens in May and 80% in August, still a large majority but not as dominating (Table 3.4). This was mostly due to lower catches of beetles which made up only 16% and 7% of specimens compared to 34% in 2002.

	May-03		Aug-03	
	Numbers	% of total	Numbers	% of total
Hemiptera	7110	26	3051	15
Formicidae	6277	23	2617	13
Coleoptera	4346	16	1486	7
Araneae	1721	6	831	4
Diptera	1112	4	7967	40
Other taxa	6393	24	3965	20
Total	**26959**		**19917**	

Table 3.4 Total number of specimens sampled in May and August 2003.

Canopy fogging with deltamethrin from the ground returned most canopy invertebrates with an average of 400 individuals per sample prior to the spray cycles in May 2001. Trees fogged in this way after the five aerial application cycles produced on average 140 specimens per sample in August 2002. By May 2003 samples contained an average of 250 specimens and 184 in August 2003 samples.

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, 83 m^{-2} in May 2003 and 61 m^{-2} in August 2003.

As in 2002 there were considerable differences in average abundance per sample between tree species (Table 3.5). More insects were sampled from *C. mopane* trees and *Kigelia africana* than the taller and more open canopy *Lonchocarpus capassa* and *Combretum imberbe*.

	Kigelia africana		*Lonchocarpus capassa*		*Combretum imberbe*		*Colophospermum mopane*	
	May	Aug	May	Aug	May	Aug	May	Aug
Araneae	8	8	3	4	6	7	24	16
Coleoptera	8	25	3	4	36	15	70	11
Diptera	6	85	4	92	4	100	10	13
Formicidae	61	64	20	7	10	10	44	19
Hemiptera	21	21	10	7	42	21	99	86
Others	34	26	21	12	30	15	47	131
Total	**139**	**229**	**62**	**125**	**127**	**169**	**295**	**276**

Table 3.5 Average number of specimens per sample from four tree species in May and August 2003.

Key Results

- 46,876 specimens were recorded from 216 fogging samples
- Average catch per sample in May 2003 was 38% down on pre-spray samples in May 2002
- Average catch per sample in August 2003 was 31% higher than post-spray August 2002
- There were differences in average catches between tree species

3.4.2 Insect abundance on *Kigelia africana*

3.4.2.1 Fogged trees near Stanleys Camp

Kigelia africana trees near Stanleys were fogged with deltamethrin from hand held foggers in all four sampling periods.

The average number of specimens in pre-spray samples in May 2002 was 358 compared to 90 in the post-spray sampling in August 2002. A significant decline of 75%, with declines of up to 90% for specific pairwise comparisons of individual trees.

K. africana trees in the same area yielded an average of 199 individuals in May 2003, a 44% decline on the previous year. However, there was considerable variation between trees so this difference in average values was not statistically significant (non-parametric comparison of means using Mann-Whitney statistic, U=396, P=0.420).

Average abundance in the August 2003 samples was 189, a decline of 44% over the pre-spray estimate from May 2002 but more than double the immediate post-spray sample in August 2002. (Table 3.6). There was a significant between year difference in mean total abundance (two-way ANOVA on log transformed data; $F_{1,107}$=5.3, P=0.023) and a significant effect of month ($F_{1,107}$=7.8, P=0.006). *Post hoc* tests showed no significant difference in mean abundance between May 2002 and May 2003 (SNK test, P=0.748) but a strong increase between August 2002 and August 2003 (P=0.001).

Eighteen of the trees were sampled in both years allowing for specific pairwise comparisons. Six of these trees showed an increase in abundance in 2003 over 2002 by an average of 122 specimens (330%), whilst 12 trees showed a decline of nearly 500 individuals per trap, equivalent to around 63%. This percentage decrease is around 54% if three trees with a high proportion of ants in the sample are removed.

Six *K. africana* trees within 1km of the pre-spray trees were fogged for the first time in May 2003. The average of 122 specimens per trap from these trees was not significantly different to the average of 140 for the previously fogged trees suggesting that the fogging of trees in 2002 did not overtly affect the numbers of specimens sampled from those trees in 2003. Similarly in August 2003, the average of 170 per sample from new trees was not significantly different to, and was lower than, the 190 per sample from previously fogged trees.

	2002		2003		% change	
	May	Aug	May	Aug	May to May	Aug to Aug
Araneae	9	1	7	7	-27	509
Coleoptera	246	20	25	37	-90	89
Diptera	6	22	16	57	149	161
Formicidae	66	35	95	49	43	41
Hemiptera	13	1	30	14	132	1148
Others	17	10	26	25	52	158
Total	**358**	**89**	**199**	**189**	**-44**	**114**

Table 3.6 Average number of specimens per sample from *Kigelia africana* trees fogged near Stanleys in May and August 2002 and 2003 (total n=111)

In general abundance was up compared with the levels seen at the end of the spray cycles in August 2002 but lower than the pre-spray estimates from May 2002.

Key Results
• Average invertebrate abundance from fogging *K. africana* trees near Stanleys in May 2003 was 44% down on May 2002 mean abundance • Average invertebrate abundance August 2003 was 114% higher than in August 2002 • Fogging of specific trees in 2002 did not affect abundances in those same trees in 2003 • Overall abundance appeared to have recovered to near pre-spray levels

3.4.2.2 Fogged trees at Nxaraxa

At the Okavango Research Centre site at Nxaraxa there was no fogging of trees prior to the spray cycles in 2002 to avoid possible contamination of the site prior to water sampling. Consequently comparisons for the May 2003 sampling are with the cycle 1 knockdown data. So we are comparing catches from the same trees at a similar time of year but two different sampling methods.

Rather than use the direct knockdown data the May 2002 abundances were adjusted with percentage differences calculated from comparisons between fogging and knockdown samples at Stanleys and Naxaraxa for 2002. As fogging was slightly more efficient than knockdown (see 2002 report) most of the adjustments are to higher average values. All adjusted values are given in italics.

The average number of specimens in pre-spray samples in May 2002 was 182 compared to 100 in the post-spray sampling in August 2002, a significant decline of 45%. *K. africana* trees in the same area yielded an average of 139 individuals in May 2003, a 24% decline on the previous year (Table 3.7).

	2002		2003		% change	
					May to	Aug to
	May	Aug	May	Aug	May	Aug
Araneae	*9*	2	8	11	*-4*	634
Coleoptera	*24*	25	8	15	*-68*	-41
Diptera	*6*	35	6	180	*-1*	420
Formicidae	*75*	28	61	43	*-18*	54
Hemiptera	*10*	1	21	20	*123*	1771
Others	*59*	9	34	26	*-43*	179
TOTAL	***182***	**100**	**139**	**295**	***-24***	**196**

Table 3.7 Average number of specimens per sample from *Kigelia africana* trees fogged at Nxaraxa in May and August 2002 and 2003 (total n=106)

Comparisons between the August samples showed a trebling from 100 to 295 individuals per sample, largely due to high fly numbers.

There was a significant between year difference in mean total abundance (two-way ANOVA on log transformed data; $F_{1,102}$=7.2, P=0.008) but no significant effect of month ($F_{1,102}$=0.1, P=0.778). *Post hoc* tests showed no significant difference in mean abundance between May 2002 and May 2003 (SNK test, P=0.927) but a large increase between August 2002 and August 2003 (P=0.002).

Key Results
• Average invertebrate abundance from fogging *K. africana* trees at Nxaraxa in May 2003 was 24% down on May 2002 mean abundance • Average invertebrate abundance August 2003 was 196% higher than in August 2002 • Overall abundance appeared to have recovered beyond pre-spray levels

3.4.3 Insect abundance on Lonchocarpus capassa and Combretum imberbe

Fogging of *Combretum imberbe* and *Lonchocarpus capassa* was completed around Nxaraxa in August 2002 and May and August 2003. Data for May 2002 again came from adjusted values of knockdown data with fogging being 39% more efficient than aerial spraying for *C. imberbe* and 14% more efficient for *L. capassa*.

On *Combretum imberbe* trees at Nxaraxa the average adjusted abundance in May 2002 was 92 compared to 131 from fogging in 2003, an increase of 42%. August 2002 values were high, mostly a consequence of many ants (Table 3.8), despite being immediately post aerial spraying. Comparisons with August 2003 showed an overall decline of 48% but a 21% increase without ants.

There were no significant differences in mean total abundance either between years (two-way ANOVA on log transformed data; $F_{1,31}$=0.01, P=0.953) or months ($F_{1,27}$=2.58, P=0.118).

	2002		2003		% change	
	May	Aug	May	Aug	May to May	Aug to Aug
Araneae	*5*	5	6	7	*6*	46
Coleoptera	*27*	6	40	15	*51*	173
Diptera	*5*	39	4	100	*-16*	155
Formicidae	*30*	261	10	10	*-68*	-96
Hemiptera	*14*	1	42	21	*202*	1990
Others	*7*	15	30	15	*314*	3
TOTAL	***92***	**327**	**131**	**169**	***42***	**-48**

Table 3.8 Average number of specimens per sample from *Combretum imberbe* trees fogged at Nxaraxa in May and August 2002 and 2003 (total n=35)

These values would suggest that aerial spraying had no detectable effect on invertebrate abundance in *C. imberbe* canopies, however, the flood had arrived at the study site during the 2003 sampling, some six weeks earlier than in 2002. Data from May 2002 were collected in the absence of the flood, which is known to have a strong positive influence on the abundance of key taxa, especially flies.

On *Lonchocarpus capassa* trees at Nxaraxa the average abundance was lower than the other tree species. This was expected given this is an open canopy species with small leaves. Fogging these trees was also tricky given that many were tall.

There was a 5% decline in average abundance from May 2002 to May 2003 and a 91% increase between August 2002 and August 2003 (Table 3.9). However, there was no significant statistical differences in mean total abundance either between years (two-way ANOVA on log transformed data; $F_{1,27}$=0.07, P=0.785) or months ($F_{1,27}$=0.33, P=0.569).

Much of the observed differences were due to increased fly abundance in August samples, attributed to greater fly activity following the arrival of the annual flood. Adjusting the totals without flies shows that average abundance fluctuated very little between the four sampling periods.

	2002		2003		% change	
	May	Aug	May	Aug	May to May	Aug to Aug
Araneae	*5*	1	3	4	*-36*	170
Coleoptera	*9*	7	3	4	*-63*	-50
Diptera	*3*	41	4	92	*54*	126
Formicidae	*32*	6	20	7	*-39*	13
Hemiptera	*6*	1	10	7	*61*	440
Others	*10*	9	21	12	*117*	34
TOTAL	***65***	**65**	**62**	**125**	***-5***	**91**

Table 3.9 Average number of specimens per sample from *Lonchocarpus capassa* trees fogged at Nxaraxa in May and August 2002 and 2003 (total n=23)

Key Results
• Average abundance recovered on *C. imberbe* • Average abundance on *L.capassa* was lower than the other tree species but showed no detectable change after spraying

3.4.4 Patterns in abundance of specific taxa

3.4.4.1 Spiders

Near Stanleys camp a single knockdown trap collected, on average, 9 spiders from beneath a *K. africana* in May 2002, 7 in May and 7 in August 2003, a slight but non-significant increase. This compares to a decline to an average of 1 spider per trap from fogging in August 2002.

At Nxaraxa on *K. africana* spider catches were 9 per trap pre-spray and 8 and 11 in May and August 2003; this followed a decline to 2 per trap after the spraying in August 2002. Average catches stayed between 5 and 7 specimens per trap throughout on *C. imberbe.* Average catches decreased from 5 to 1 per trap from *L. capassa* in 2002 but returned to 3 and 4 per trap in 2003.

Spider abundance appears to be at similar levels to those prior to the spray in 2002. This is an encouraging result.

3.4.4.2 Beetles

Near Stanleys camp, a single knockdown trap collected, on average, 246 beetles from beneath a *K. africana* prior to the aerial spraying in May 2002. Post-spray fogging produced only 20 beetles per trap, a decline that did not show any significant recovery in 2003 with an average of 25 in May and 37 in August.

At Nxaraxa on *K. africana* beetle catches were 24 per trap pre-spray and 25 in the post-spray samples. In 2003 the averages were 8 and 15 in May and August. Average catches of beetles declined from 27 to 6 in 2002 but recovered to 40 and 15 in 2003 on *C. imberbe.* Average catches remained in single figures per trap throughout from *L. capassa*.

The large decline in beetle abundance on *K. africana* near Stanleys appears not to have recovered. However, at Nxaraxa abundances on *K. africana* were less affected by the spray. The apparent increase on *C. imberbe* may well be due to the earlier arrival of the flood at this site. The patterns of beetle abundance on mopane described later, sheds more light on the apparent contradiction.

3.4.4.3 Flies

Near Stanleys camp a single knockdown trap collected, on average, 6 flies from beneath a *K. africana* in May 2002 and 16 in May 2003, a significant increase of more than 166% (U=189, P=0.001). This compares to a 228% increase to an average of around 22 flies per trap from fogging in August 2002. The increase between May and August 2003 was even greater at more than 250%. Overall fly numbers were higher in August 2003 than in August 2002 and prior to the spray events.

At Nxaraxa on *K. africana* fly catches were 6 per trap in 2002 and 6 per trap in 2003. Again the increase in fly numbers in August 2003 was greater than the increase in 2002.

Average catches increased from 5 to 39 per trap from *C. imberbe* from May to August in 2002 and 4 to 100 in 2003. Average catches increased from 3 to 41 per trap from *L. capassa* from May to August in 2002 and 4 to 92 in 2003.

Fly abundance on all trees at Nxaraxa increased to higher levels in 2003 than in 2002 suggesting successful recovery; however, this pattern may also be due to the fact that the habitats around Nxaraxa were dry prior to May 2003. Even the main channel of the Boro River did not receive flood water until the May sampling.

3.4.4.4 Ants

Ant abundance can be highly variable due to activity rates and proximity of nests to the sampled area. Near Stanleys camp average ant abundance per trap declined slightly between May and August in both years but numbers were around 40% higher in 2003 than in 2002. The same pattern occurred at Nxaraxa on *K. africana* and *L. capassa.* The trend on *C. imberbe* was confused by a high average in the August 2002 samples.

There continues to be high variability between individual trees in catches of ants and generally lower catches in August compared to May but no apparent effect of the spray.

3.4.4.5 Hemiptera

Hemipteran numbers were greater in 2003 than 2002 on *K. africana* near Stanleys and at Nxaraxa. This was despite a significant decline to an average of 1 specimen per sample post-spray in 2002 at both sites.

On *L. capassa* and *C. imberbe* average abundance also declined to 1 per sample post-spray but recovered to pre-spray levels in 2003.

If anything, Hemipteran abundance was greater in 2003 than in 2002 suggesting no detrimental effect of the spray despite low catch rates in August 2002.

Key Results
• Spiders abundance has recovered to pre-spray levels • Beetle abundance declined and has not recovered but this is only a strong effect on *K. Africana* • Beetles may show wide natural fluctuations in abundance • Fly abundance did not appear to be affected by the spray at all • Ant abundance did not change significantly between years • Hemipteran abundance in 2003 was generally up on 2002 pre-spray values

3.4.5 Changes in composition of morphospecies

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 on abundance to 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.

Unfortunately fly and ant specimens from August 2003 sampling were lost by the airline during transit to Australia. Analyses were carried out using all five taxa for three time periods (May and August for 2002 and May 2003) as well as using the remaining three taxa (Hemiptera, beetles and spiders) for all four time periods (May and August 2002 and 2003).

3.4.5.1 Changes on *K. africana*

Morphospecies composition in post-spray samples was significantly different to pre-spray samples (Figure 3.2). While the samples taken in 2003 also differ in composition from the pre-spray samples, the 2003 samples are more similar to the pre-spray than to the post-spray. Sample points closer together on the ordination diagram are more similar in composition than those that are further apart and tight clusters of points suggest a consistency in composition between samples (Figure 3.2).

When taxa are examined individually, a similar pattern is present across transformations. Firstly, while beetle composition changes as a result of the spray, the composition of samples generally returns to the pre-spray pattern, with points tightly clustered around centroids that are located close to each other.

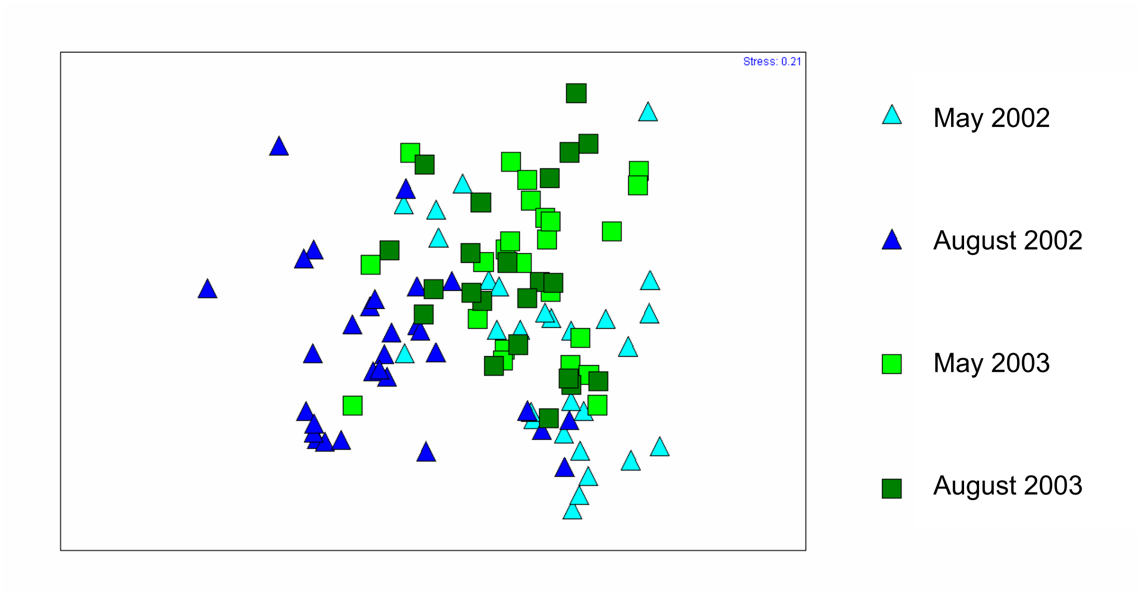
Spiders and Hemiptera, however, are more variable. Changes in composition with aerial spraying was seen with these taxa, often by a complete absence in samples. Centroids of samples also changed, indicating a change in assemblage composition.

The significance of the patterns seen in the MDS ordinations was tested using the ANOSIM procedure in PRIMER version 5. Overall, a large difference in the composition of samples between years was found, with $P<0.01$ for Global R values for all taxa individually and combined (Table 3.10 and Table 3.11). This pattern was consistent for both untransformed and binary (presence/absence) data (Table 3.10 and Table 3.11).

With the exception of spiders, spraying in 2002 resulted in significant shift in the composition of taxa (Tables 3.10 and 3.11). The lack of a significant difference seen with spider morphospecies is likely due to low number problems and under-sampling.

Yearly comparisons between May and August also revealed significant differences in the composition of samples (Tables 3.10 and 3.11).

(a) Untransformed



(b) Binary

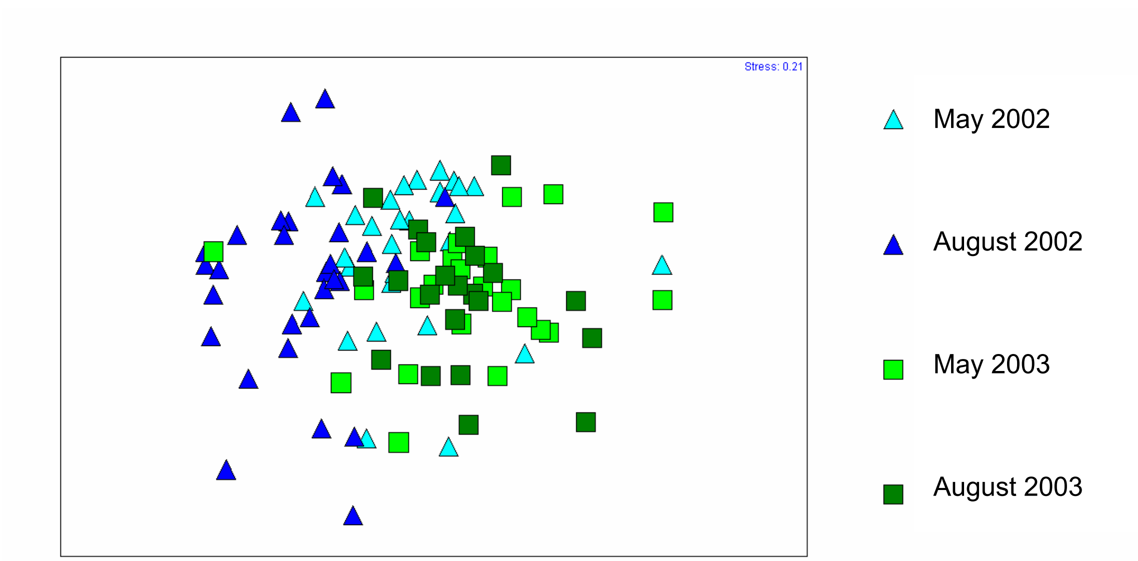


Figure 3.2 MDS ordination of morphospecies composition (Hemiptera, spiders and beetles) on *K. africana* at Stanleys (a) untransformed data; (b) binary data. Untransformed data reflects the pattern in composition and abundance while binary (or presence/absence) data is a composition index.

Thus, while significant differences were seen between the clusters in Figure 4.1 after spraying had occurred, the centroid of these clusters generally returned to pre-spraying patterns. That is, while composition initially changed after spraying, the 2003 compositional effects were close to what was initially found in May 2002. This result is further demonstrated by the similarities in composition between May and August 2003 (Tables 4.7 and 4.8).

	Global R	2002 May v Aug	2003 May v Aug	Recovery May v May	Aug v Aug
Araneae	0.199***	-0.003^{NS}	0.027^{NS}	0.265***	0.427**
Coleoptera	0.134***	0.247***	0.04^{NS}	0.098**	0.127**
Hemiptera	0.132***	0.257**	0.006^{NS}	-0.001^{NS}	0.413***
Combined taxa	0.172***	0.234***	0.033^{NS}	0.123**	0.174***

Table 3.10 Analysis of similarities results for untransformed morphospecies abundance data on *K. africana* near Stanleys. Statistic is global R and the significance levels are *** *P*<0.01, ** *P*<0.01, * *P*<0.05, ^{ns} *P*>0.05.

	Global R	2002 May v Aug	2003 May v Aug	Recovery May v May	Aug v Aug
Araneae	0.205***	0.01^{NS}	0.018^{NS}	0.269***	0.447**
Coleoptera	0.105***	0.135***	0.042^{NS}	0.101**	0.104*
Hemiptera	0.11**	0.226**	-0.008^{NS}	0.007^{NS}	0.385**
Combined taxa	0.175***	0.157***	0.012^{NS}	0.178***	0.224***

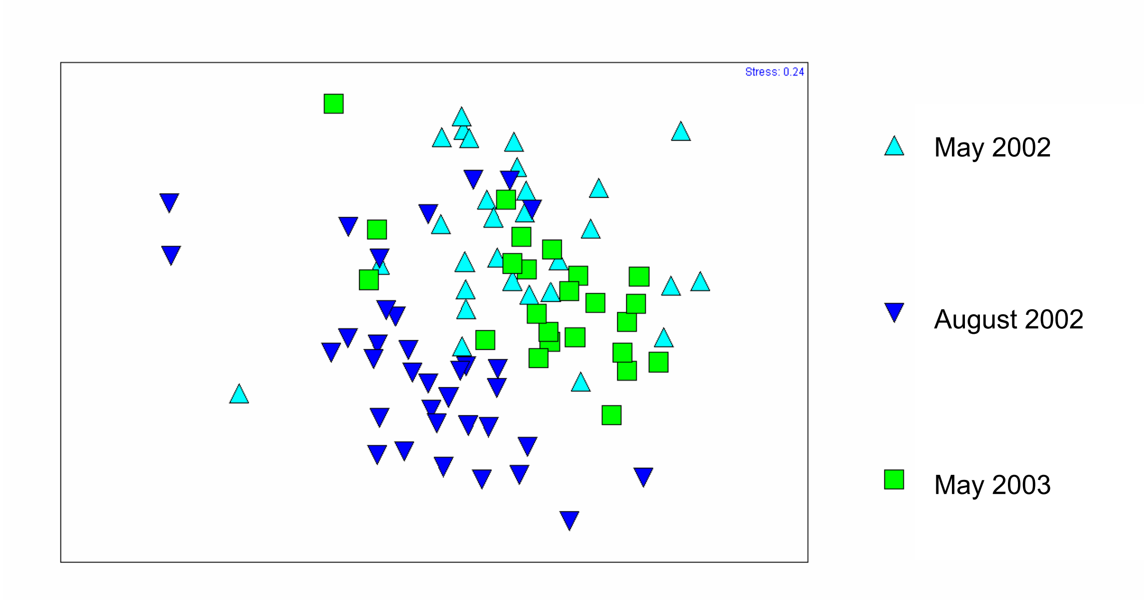
Table 3.11 Analysis of similarities results for binary transformed morphospecies abundance data on *K. africana* near Stanleys. Statistic is global R and the significance levels are *** *P*<0.01, ** *P*<0.01, * *P*<0.05, ^{ns} *P*>0.05.

An examination of compositional effects using the combination of all five of the key taxa revealed similar patterns to those displayed in Figure 3.3. Despite the lack of the August 2003 samples, the general compositional trends found were an initial disruption caused by the spraying, followed by a return of the 2003 samples to the general patterns of May 2002. This configuration was seen across transformations (Figure 3.3).

A closer look at individual taxa showed that beetles followed the trend demonstrated when taxa were combined. In comparison, compositional changes were detected in ants and flies, with the centroid of samples shifting over time. Changes were seen in Hemipteran communities after spraying, but patterns of composition were similar in May 2002 and May 2003. Finally, spider communities showed high levels of variability both between and within sampling times, with individual sampling events only loosely clustered together.

A large difference in the composition of samples between years was found, with $P<0.01$ for Global R values for all taxa individually and combined (Table 3.12 and Table 3.13). This pattern was consistent for both untransformed and binary (presence/absence) data (Table 3.12 and Table 3.13). Most taxa experienced compositional change after spraying. High variability was seen with spiders, while Hemiptera communities in May 2003 were not significantly different to the communities found in May 2002.

(a) Untransformed



(b) Binary

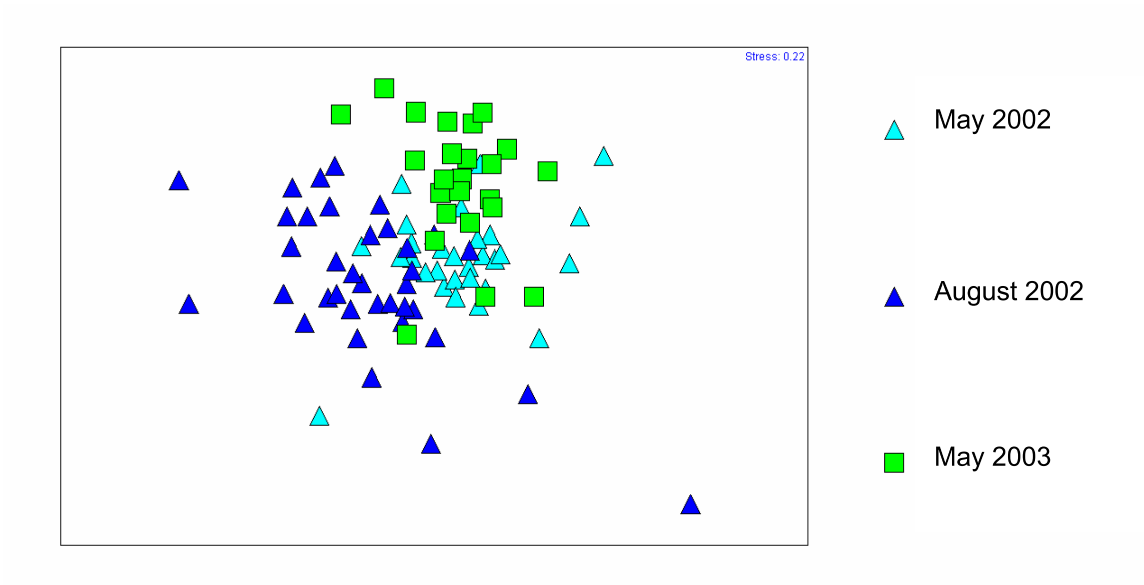


Figure 3.3 MDS ordination of morphospecies composition (Hemiptera, spiders, beetles, ants and flies) on *K. africana* near Stanleys (a) untransformed data; (b) binary data.

	Global R	2002 May v Aug	Recovery May v May
Araneae	0.24***	-0.003^{NS}	0.265***
Coleoptera	0.172***	0.247***	0.098**
Diptera	0.089***	0.081**	0.129***
Formicidae	0.156***	0.084***	0.146***
Hemiptera	0.17***	0.257**	-0.001^{NS}
Combined taxa	0.229***	0.251***	0.119***

Table 3.12 Analysis of similarities results for untransformed morphospecies abundance data on *K. africana* near Stanleys. Statistic is global R and the significance levels are *** P<0.01, ** P<0.01, * P<0.05, ^{ns} P>0.05.

	Global R	2002 May v Aug	Recovery May v May
Araneae	0.251***	0.01^{NS}	0.269**
Coleoptera	0.124***	0.135***	0.101**
Diptera	0.089***	0.082**	0.129***
Formicidae	0.186***	0.101***	0.212***
Hemiptera	0.159**	0.226**	31.4^{NS}
Combined taxa	0.248***	0.185***	0.213***

Table 3.13 Analysis of similarities results for binary transformed morphospecies abundance data on *K. africana* near Stanleys. Statistic is global R and the significance levels are *** P<0.01, ** P<0.01, * P<0.05, ^{ns} P>0.05.

Key Results
▪ Assemblages on insects changed in composition immediately after aerial spraying ▪ Clusters in subsequent samples are discrete ▪ Morphospecies composition of samples taken in 2003 are more similar to pre-spray than to post-spray samples.

3.4.5 Morphospecies losses

In the report of terrestrial invertebrate monitoring in 2002 several morphospecies were identified that were recorded in pre-spray fogging samples and the first knockdown samples from the aerial spraying but not in later samples. These species were recognized as being potentially vulnerable and lost from the system.

At Stanleys 229 morphospecies were recorded on *K. africana*, slightly fewer in 2003 than in 2002 as fewer specimens were recorded and samples of flies and ants from August were not processed (Table 3.14).

In all there were 133 morphospecies recorded in pre-spray samples and 87 of these species were not sampled in post-spray fogging (Table 3.15). These were the taxa that may have been vulnerable or lost as a consequence of the aerial spraying. They are also listed in Table 4.13. More than 42% of these species were sampled again in 2003. This percentage would have been higher but for the loss of ant and fly samples for August 2003. However, 50 morphospecies (58%) were not sampled again in 2003. Half of these losses were beetles.

	total msp sampled	total msp in 2002	total specimens in 2002	total msp in 2003	total specimens in 2003
Spiders	24	12	47	18	116
Beetles	81	67	7845	36	1362
Flies	*57*	36	90	*26*	*37*
Ants	*36*	35	3118	*22*	*2265*
Hemiptera	31	20	258	23	308
Total	*229*	170	*11358*	*125*	*4088*

Table 3.14 Number of morphospecies recorded from fogging of *K. africana* trees near Stanleys in 2002 and 2003. Numbers in italics are data for May samples only as August samples were lost in transit.

	# msp recorded pre-spray	# msp not found in post-spray	# msp resampled in 2003	# msp not found in 2003
Spiders	**13**	**8**	**6**	**2**
Beetles	**55**	**34**	**8**	**26**
Flies	**25**	**20**	*6*	*14*
Ants	**24**	**14**	*10*	*4*
Hemiptera	**16**	**11**	**7**	**4**
Total	**133**	**87**	*37*	*50*

Table 3.15 Number of morphospecies recorded from fogging of *K. africana* trees near Stanleys in 2002 and 2003. Numbers in italics are data for May samples only as August samples were lost in transit.

As table 3.16 shows 34 of these 50 unrecovered morphospeicies were originally recorded as single individuals. This means that they were initially very infrequent in the habitat at the time of pre-spray sampling and although the sampling effort remained consistent throughout could easily have been missed. Absence with such low initial numbers does not imply local extinction.

Although not recorded on *K. africana* near Stanleys in 2003, 1 spider (50% of lost morphospecies), 4 beetle (15%), 6 fly (43%), 3 ant (75%) and 1 hemipteran (25%) morphospecies were recorded from *K. africana* at Nxaraxa or other tree species in 2003. Sampling inefficiencies might therefore account for at least 15 morphospecies or 30% of those "lost" from Stanleys.

Whilst there were 50 morphospecies not recovered in 2003 samples there were 57 sampled for the first time in May or August 2003 samples (Table 3.17). These species were spread among the taxa and 30 of them were again represented by just one specimen.

Overall the "lost" morphospecies were balanced by morphospecies captured for the first time in 2003 .

Spiders	
Thomisidae0009	55
Thomisidae0001	15
Salticidae0001	7
Thomisidae0006	5
Salticidae0009	4
Salticidae0010	2
Oxyopidae0004	1
Oxyopidae0006	1

Ants	
Anoplolepis0001	1414
Cataulacus0001	586
Crematogaster0006	217
Acantholepis0001	197
Camponotus0018	93
Technomyrmex0003	65
Semonius0001	61
Pheidole0010	55
Leptothorax0001	41
Camponotus0017	41
Crematogaster0011	14
Crematogaster0010	9
Plagiolepis0001	4
Tetraponera0002	1

Hemiptera	
Cicadellidae0022	8
Reduviidae0001	5
Tingidae0003	5
Reduviidae0003	4
Reduviidae0006	3
Derbidae0001	2
Reduviidae0010	2
Cicadellidae0005	1
Cicadellidae0013	1
Cicadellidae0024	1
Saldidae0001	1

Beetles	
Cerambycidae0001	24
Curculionidae0025	16
Curculionidae0010	10
Coccinellidae0003	7
Curculionidae0005	6
Anthicidae0001	6
Staphylinidae0010	5
Chrysomelidae0017	4
Chrysomelidae0013	4
Chrysomelidae0005	4
Carabidae0005	3
Curculionidae0024	2
Curculionidae0004	2
Coccinellidae0008	2
Brentidae0003	2
Brentidae0001	2
Staphylinidae0022	1
Staphylinidae0017	1
Staphylinidae0015	1
Elateridae0001	1
Curculionidae0029	1
Curculionidae0023	1
Curculionidae0007	1
Coccinellidae0006	1
Coccinellidae0001	1
Chrysomelidae0008	1
Chrysomelidae0002	1
Carabidae0007	1
Brentidae0005	1
Brentidae0002	1
Anthribidae0005	1
Anthribidae0001	1
Anobiidae0006	1
Anobiidae0005	1

Flies	
Ephydridae0007	14
Phoridae0007	10
Calliphoridae0009	5
Muscidae0010	4
Muscidae0021	4
Muscidae0019	3
Sarcophagidae0004	3
Calliphoridae0011	3
Stratiomyiidae0002	2
Acroceridae0004	2
Tephritidae0002	2
Acroceridae0002	1
Calliphoridae0001	1
Calliphoridae0013	1
Dolichopodidae0002	1
Dolichopodidae0004	1
Dolichopodidae0005	1
Empididae0005	1
Phoridae0008	1
Sepsidae0002	1

Table 3.16 Morphospecies and total number of specimens recorded from pre-spray fogging of *K. africana* trees near Stanleys in 2002. Species in bold are those that were sampled again in 2003.

Spiders	
Thomisidae0010	13
Theridiidae0002	5
Salticidae0018	4
Thomisidae0004	3
Thomisidae0013	3
Thomisidae0014	2
Thomisidae0011	2
Salticidae0015	2
Thomisidae0016	1
Oxyopidae0005	1
Salticidae0011	1

Ants	
Tetraponera0003	17

Beetles	
Staphylinidae0029	10
Anobiidae0004	7
Carabidae0002	4
Chrysomelidae0014	3
Carabidae0003	2
Curculionidae0021	2
Carabidae0018	2
Coccinellidae0005	1
Histeridae0003	1
Histeridae0006	1
Staphylinidae0026	1
Staphylinidae0031	1
Staphylinidae0032	1
Staphylinidae0036	1

Hemiptera	
Reduviidae0007	10
Reduviidae0013	5
Cicadellidae0020	6
Reduviidae0014	6
Cicadellidae0008	1
Cicadellidae0036	1
Cicadellidae0045	1
Flatidae0002	1
Cicadellidae0039	1
Flatidae0003	1
Reduviidae0015	1

Flies	
Muscidae0013	4
Acroceridae0006	2
Muscidae0011	2
Muscidae0016	2
Muscidae0017	2
Phoridae0026	2
Psychodidae0001	2
Bombyliidae0003	1
Culicidae0002	1
Dolichopodidae0003	1
Ephydridae0004	1
Ephydridae0016	1
Muscidae0015	1
Muscidae0018	1
Muscidae0020	1
Muscidae0022	1
Phoridae0004	1
Psychodidae0005	1
Tabanidae0001	1
Tephritidae0010	1

Table 3.17 Morphospecies and total number of specimens from pre-spray fogging of *K. africana* trees near Stanleys sampled for the first time in 2003.

Key Results
• 50 morphospecies of the 87 “lost” were not recovered from K. africana trees at Stanleys in 2003 • 15 of these were found elsewhere suggesting that 35 (40%) may have been locally depleted • 57 morphospecies were found for the first time in 2003 • More than two thirds of the lost taxa were only recorded initially as single individuals making it difficult to sample them again

3.4.7 Collections from sprayed and unsprayed *Colophospermum mopane*

3.4.7.1 Abundance on 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. These sites were visited again in May and August 2003 and identical fogging procedures completed.

Overall there was a significant decline in abundance both in the sprayed (Table 3.18) and unsprayed (Table 3.19) between years and as a consequence of the spray. This result for overall abundance was significant for effects of spray (MANOVA, $F_{1,122}$=5.22, P=0.024) and year ($F_{1,122}$=35.5, P>0.001).

However, the effect is almost entirely due to changes in the numbers of beetles, which declined dramatically on the sprayed site. Remove beetles and total abundance changes by just a few percentage points between years on the sprayed site and increases by 43% on the unsprayed control.

A key result is that beetle abundance at the unsprayed control site in Moremi also declines but over a longer time period. The August 2003 abundance was only 16 per trap compared with 589 at the end of the spray cycles in the previous year, a 97% decline.

On the sprayed site at Chiefs ants declined, hemiptera increased and spiders changed little through the four sample periods. Flies showed a peak immediately after the spray events in August 2002. On the unsprayed site in Moremi the average abundance of each taxa, except beetles, fluctuated slightly but were consistent through the four sampling times.

	Chiefs Island (Sprayed)					
	2002		2003		% change	
					May	Aug
	May	Aug	May	Aug	to May	To Aug
Araneae	23	2	24	15	7	505
Coleoptera	459	3	9	6	-98	76
Diptera	9	149	18	23	102	-85
Formicidae	118	74	42	15	-64	-80
Hemiptera	37	68	87	100	134	46
Others	49	23	53	127	10	463
TOTAL	**694**	**320**	**233**	**285**	**-66**	**-11**
TOTAL (no beetles)	235	316	224	279	-5	-12

Table 3.18 Average number of specimens per sample from *Colophospermum mopane* trees fogged at Chiefs Island within the spray zone in May and August 2002 and 2003

	Moremi Cutline (Not sprayed)					
	2002		2003		% change	
					May	Aug
	May	Aug	May	Aug	to May	to Aug
Araneae	13	13	24	16	88	29
Coleoptera	489	568	132	16	-73	-97
Diptera	1	6	2	2	54	-60
Formicidae	28	30	46	24	69	-21
Hemiptera	26	117	112	72	326	-38
Others	89	110	41	135	-54	22
TOTAL	**646**	**844**	**357**	**267**	**-45**	**-68**
TOTAL (no beetles)	157	276	225	251	43	-9

Table 3.19 Average number of specimens per sample from *Colophospermum mopane* trees fogged along Moremi Cutline outside the spray zone in May and August 2002 and 2003

3.4.7.2 Changes in composition on mopane

Samples from Chiefs Island that received the spray showed a shift and greater variance in composition between May and August 2002 (Figure 3.4). This was clear for both untransformed and binary representations suggesting that the main effect was in composition and not abundance. Samples from 2003 showed tight clusters that overlap and are closer to the pre-spray composition than the post-spray (Figure 3.4).

When taxa are examined individually, Hemiptera and spiders both demonstrate a shift in community composition after spraying. In comparison, after an initial compositional change post-spray, the composition of beetles is close to that of May 2002.

The ANOSIM results demonstrate that, with the exception of beetles, the composition of the communities of key taxa pre-spray was significantly different to the communities present in 2003 (Tables 3.20 and 3.21).

		2002	2003	Recovery	
	Global R	May v Aug	May v Aug	May v May	Aug v Aug
Araneae	0.478***	0.402**	0.099**	0.83***	0.597***
Coleoptera	0.391***	0.352***	0.062^{NS}	0.756***	0.418***
Hemiptera	0.414***	0.171*	0.199***	0.851***	0.336***
Combined taxa	0.531***	0.202**	0.131**	0.975***	0.554***

Table 3.20 Analysis of similarities results for untransformed morphospecies abundance data on *C. mopane* near Chiefs Island. Statistic is global R and the significance levels are *** P<0.01, ** P<0.01, * P<0.05, ^{ns} P>0.05.

	Global R	2002 May v Aug	2003 May v Aug	Recovery May v May	Aug v Aug
Araneae	0.452***	0.325**	0.099**	0.829***	0.593***
Coleoptera	0.26***	0.341***	0.062^{NS}	0.137^{NS}	0.419***
Hemiptera	0.417***	0.137^{NS}	0.199***	0.818***	0.338**
Combined taxa	0.477***	0.169*	0.131**	0.892***	0.549***

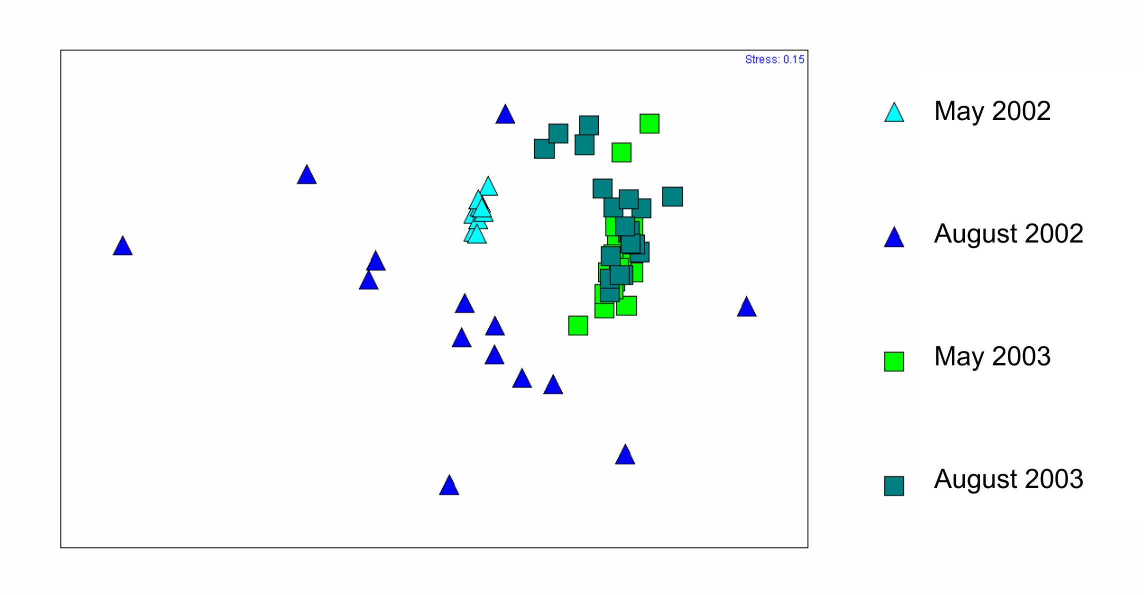
Table 3.21 Analysis of similarities results for binary transformed morphospecies abundance data on *C. mopane* near Chiefs Island. Statistic is global R and the significance levels are *** P<0.01, ** P<0.01, * P<0.05, ^{ns} P>0.05.

The compositional patterns of beetles, however, demonstrate that after an initial compositional change post-spray, the community patterns in 2003 are close to that of May 2002.

MDS maps of the invertebrate composition of unsprayed *C. mopane* on the Moremi cutline shows greater variation between, rather than within, years. This is particularly the result for binary data, when the effects of abundance have been removed (Figure 3.5). The post-spray period overlaps with the pre-spray and does not show the shift or greater between sample variance seen in the sprayed site on Chiefs Island.

The same patterns of compositional change are also found when spiders and beetles are analysed individually. Hemiptera, however, demonstrate no particular yearly effects, with the centroids of monthly clusters apart and positioned regardless of the sampling year.

(a) Untransformed



(b) Binary

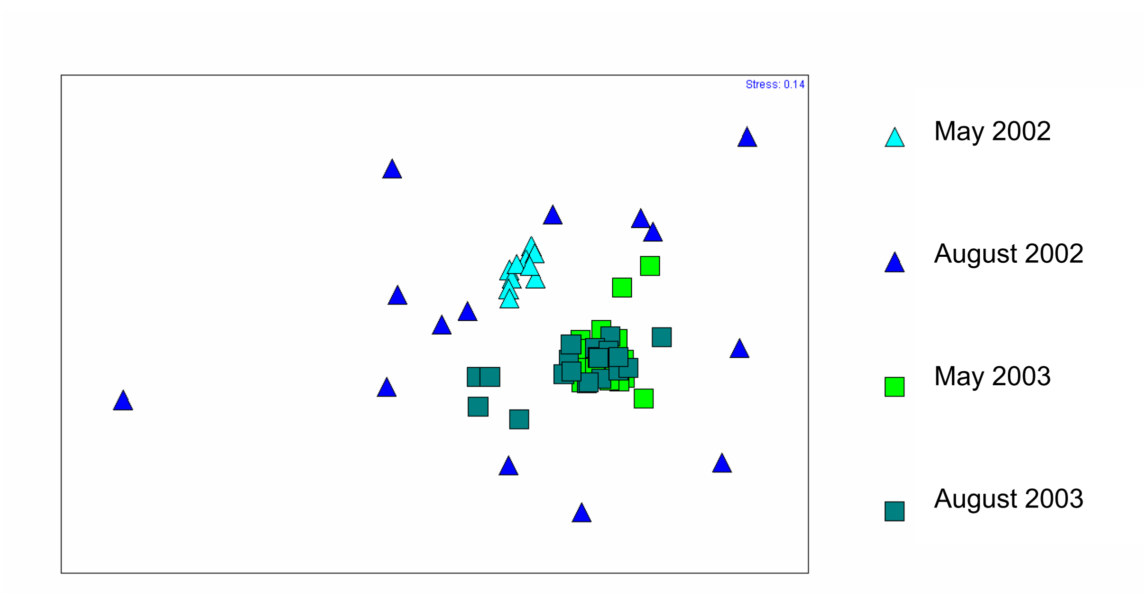


Figure 3.4 MDS ordination of morphospecies composition (Hemiptera, spiders and beetles) on sprayed *C. mopane* at Chiefs Island (a) untransformed data; (b) binary data.

Global R values (Tables 3.22 and 3.23) mirror the large difference in composition between years seen in Figure 3.5. This pattern holds across transformations and for analyses carried out using taxa individually and combined. Interestingly, all taxa displayed no significant differences in composition within the year 2002, a phenomenon also seen in Figure 3.5. All taxa also displayed significant differences in compositional patterns within 2003, although for beetles, this difference was less pronounced (Tables 3.22 and 3.23).

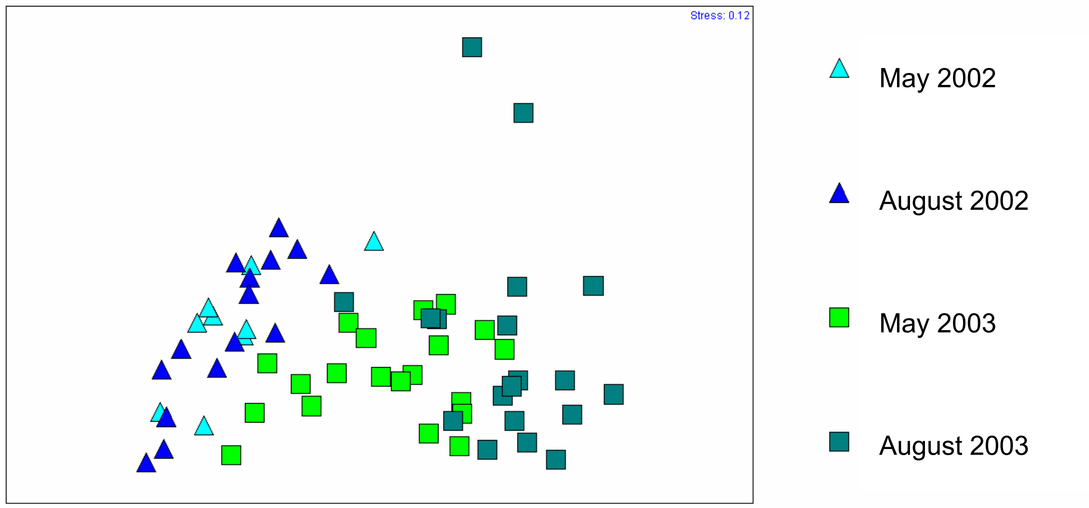
		2002	2003	Recovery	
	Global R	May v Aug	May v Aug	May v May	Aug v Aug
Araneae	0.527***	0.111^{NS}	0.106**	0.924***	0.702***
Coleoptera	0.348***	0.116^{NS}	0.083*	0.422**	0.23***
Hemiptera	0.102**	-0.04^{NS}	0.219***	0.047^{NS}	0.091*
Combined taxa	0.446***	0.19*	0.186***	0.899***	0.722***

Table 3.22 Analysis of similarities results for untransformed morphospecies abundance data on *C. mopane* at Moremi cutline. Statistic is global R and the significance levels are *** P<0.01, ** P<0.01, * P<0.05, ^{ns} P>0.05.

		2002	2003	Recovery	
	Global R	May v Aug	May v Aug	May v May	Aug v Aug
Araneae	0.535***	0.111^{NS}	0.106**	0.924***	0.702***
Coleoptera	0.209***	0.116^{NS}	0.083*	0.422**	0.23***
Hemiptera	0.127**	-0.04^{NS}	0.219***	0.047^{NS}	0.091*
Combined taxa	0.561***	0.19*	0.186***	0.899***	0.722***

Table 3.23 Analysis of similarities results for binary transformed morphospecies abundance data on *C. mopane* at Moremi cutline. Statistic is global R and the significance levels are *** P<0.01, ** P<0.01, * P<0.05, ^{ns} P>0.05.

(a) Untransformed



(b) Binary

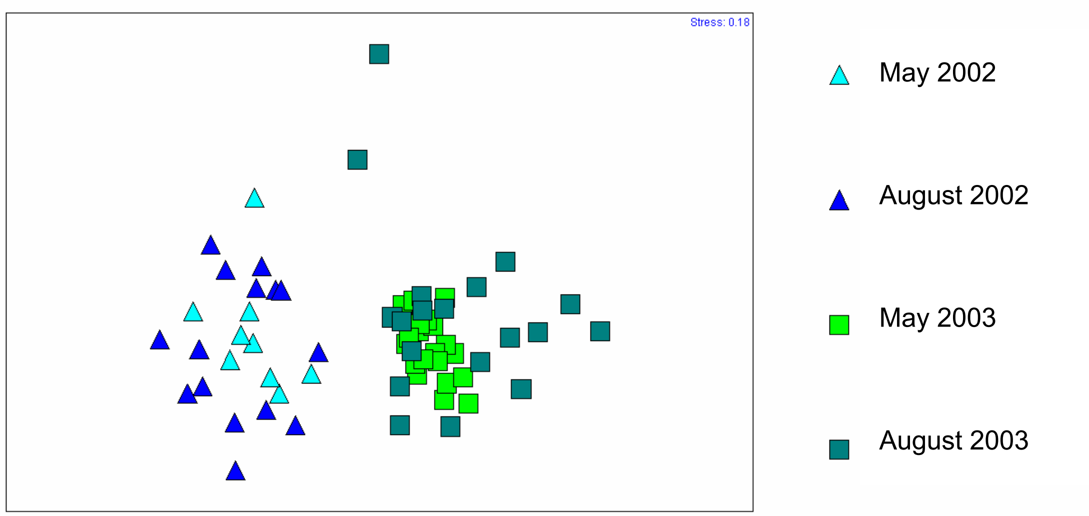


Figure 3.5 MDS ordination of morphospecies composition (Hemiptera, spiders and beetles) on unsprayed *C. mopane* at Moremi cutline (a) untransformed data; (b) binary data.

Key Results
▪ There was a clear effect of spraying on composition on mopane ▪ Spaying changed the composition and increased the variation in composition between samples ▪ Unsprayed mopane showed shifts in composition between years but not within a year

3.4.7.3 Morphospecies losses on mopane

The mopane sites also contained species which were recognized as being potentially vulnerable and lost from the system. In the sprayed site on Chiefs Island 57 morphospecies were recorded in pre-spray samples and 35 (61%) were not sampled in the post-spray samples (Table 3.24). In either May or August 2003 sampling 17 of these “missing” species were recorded again and 18 of these, 32% of the original species richness, were not resampled (normal font in Table 3.24).

Morphospecies with only one specimen recorded accounted for 13 of the18 losses.

If spraying was the primary cause of species “losses” then fewer species would be lost in the unsprayed site on the Moremi cutline. In the pre-spray samples on this site, 38 morphospecies were recorded and 19 of these (50%) were not recorded in the post-spray sampling in August 2002 despite the fact that this area did not receive the spray.

In 2003, 9 of these “missing” species were sampled again and 10 of these, 26% of the original species richness, were not resampled (normal font in Table 3.24).

A quarter of the original species compliment was not sampled in 2003 even though the site was not sprayed, abundances were similar and the sampling effort was equivalent to that in 2002.

On strict percentages, and bearing in mind that there is no site replication in this design which severely limits the statistical power, spraying of deltamethrin may have accounted for an additional 6% of failures to recover morphospecies. That is 32% of species not recovered in the sprayed minus the 26% not recovered in the unsprayed site. In the mopane site on Chiefs Island, 6% of this is 3 species.

Recall also that flies and ants were not processed from the August 2003 samples which would have recovered several more species.

Key Results
▪ Around half the morphospecies recorded in pre-spray samples on both sprayed and unsprayed sites were not seen in post-spray sampling in 2002 ▪ 32% of the original species were not resampled from the sprayed site in 2003 and 26% from the unsprayed site ▪ Spraying may account for a failure to recover about 6% of the original species complement

Chiefs Island, Sprayed

Spiders		Beetles		Flies	
Thomisidae0006	9	**Brentidae0001**	2511	**Sepsidae0001**	6
Salticidae0001	8	**Curculionidae0018**	27	**Stratiomyiidae0003**	6
Ants		**Chrysomelidae0011**	26	**Stratiomyiidae0002**	4
		Anthribidae0004	4	**Dolichopodidae0003**	3
Crematogaster0011	**384**	**Chrysomelidae0007**	4	**Cryptochetidae0001**	2
Technomyrmex0002	**40**	**Buprestidae0009**	3	Sciaridae0001	2
Acantholepis0002	**20**	**Coccinellidae0014**	3	Dolichopodidae0005	1
Monomorium0017	3	Staphylinidae0015	2	Empididae0004	1
Crematogaster0007	2	Carabidae0015	1	Ephydridae0005	1
Camponotus0017	1	Chrysomelidae0018	1	Sphaeroceridae0005	1
Camponotus0019	1	Curculionidae0007	1		
Monomorium0016	1	Curculionidae0017	1		
Hemiptera					
Reduviidae0009	4				
Cicadellidae0009	1				
Cicadellidae0017	1				

Moremi cutline, Not Sprayed

Spiders		Beetles		Flies	
Oxyopidae0005	1	Curculionidae0018	6	Dolichopodidae0005	3
		Curculionidae0020	4	**Muscidae0007**	**2**
Ants		**Curculionidae0017**	2		
Crematogaster0009	**76**	Buprestidae0006	1		
Crematogaster0011	48	Coccinellidae0007	1		
Camponotus0016	**5**	Elateridae0003	1		
Monomorium0017	1	Staphylinidae0007	1		
Tetraponera0002	1				
Hemiptera					
Cicadellidae0020	**36**				
Reduviidae0010	**17**				
Tingidae0002	**2**				
Cicadellidae0028	**7**				

Table 3.24 Morphospecies and total number of specimens recorded from pre-spray fogging of *C. mopane* trees on Chiefs Island (Sprayed) and the Moremi cutline (Not Sprayed) but not in post-spray samples in 2002. Species in bold are those that were sampled again in 2003.

3.5 Discussion

3.5.1 Changes in abundance

The conclusion from analysis of samples taken in 2002 was that aerial spraying of deltamethrin in the Okavango Delta reduced the abundance of non-target terrestrial invertebrate taxa. Samples taken by fogging of tree canopies immediately post-spray aerial spraying in August 2002 contained only one third the number of specimens recorded in pre-spray samples. Beetles appeared to be especially vulnerable with average abundance declining by two orders of magnitude in many cases. However, more than 85% of the declines in beetle abundance on *K. africana, C imberbe* and *C. mopane* were due to dramatic reductions in three morphospecies.

The reduction in abundance was confounded by increases in some taxa, particularly flies, which appeared 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. When beetles and flies were excluded overall invertebrate abundance declined by 68%. This decline was both significant and consistent across the range of higher taxa sampled.

In 2003 across all tree species fogging generated 46,876 specimens from 216 samples, an average of 200 specimens per sample. Average catches per sample in May 2003 were 38% down on pre-spray samples in May 2002 and were 31% higher in August than post-spray August 2002. This means that abundance levels have recovered from the trough immediately post-spray, but not to the levels seen in pre-spray samples.

The general pattern, while encouraging, is only a partial answer. Closer examination of the data from fogging *K. africana* trees near Stanleys Camp shows that average abundance in May 2003 was 44% down on May 2002, but in August 2003 was 114% **higher** than in August 2002. When beetles are taken away the abundance of remaining taxa was 152 per sample in August 2003 compared to 112 per sample in pre-spray May 2002 samples, an increase of 36% and strong evidence that the abundance of most invertebrates had recovered completely.

At Nxaraxa there was no pre-spray fogging, so comparisons were made with adjusted abundance values from knockdown samples. On *K. africana* trees overall abundance was 295 per sample in August 2003, 62% up on the 182 per sample recorded pre-spray 2002. At Nxaraxa there was not the pre-spray peak in beetle abundance seen at Stanleys and on mopane and a much more even abundance pattern between samples was recorded.

Comparisons within sites of trees that were fogged more than once showed that fogging did not affect abundances in those same trees. This is further evidence that whilst the insecticide does kill some insects in tree canopies and knocks others to the ground, recovery is rapid. Most likely this recovery is a combination of immigration from neighbouring vegetation, some individuals escaping the fog and, over time, life stages emerging to replace lost individuals.

Overall abundance appears to have recovered to near pre-spray levels on *K. africana*.

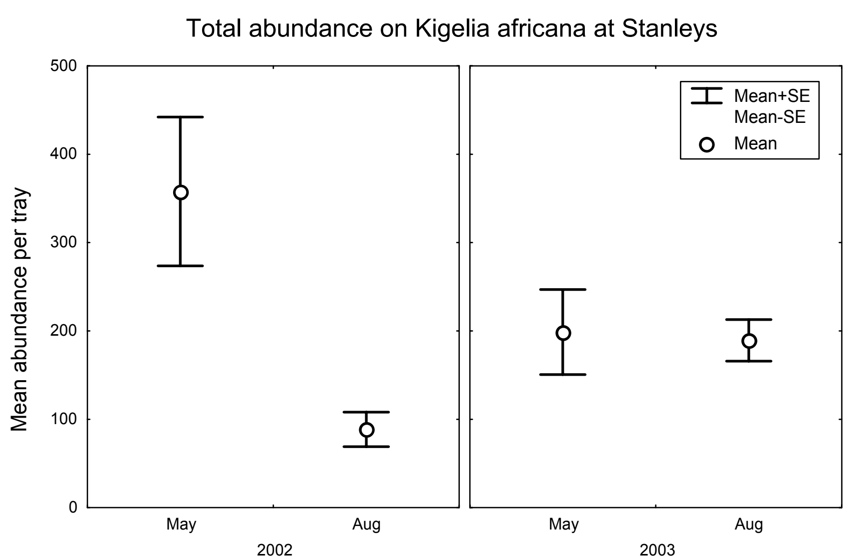
In 2002 the comparison of canopy fogging *C. mopane* trees within the spray zone (Chiefs Island) and well outside the spray zone on the boundary of Moremi Game Reserve showed that outside the spray zone captures of invertebrates in post-cycle fogging samples were 96% greater than in the pre-cycle fogging. 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.

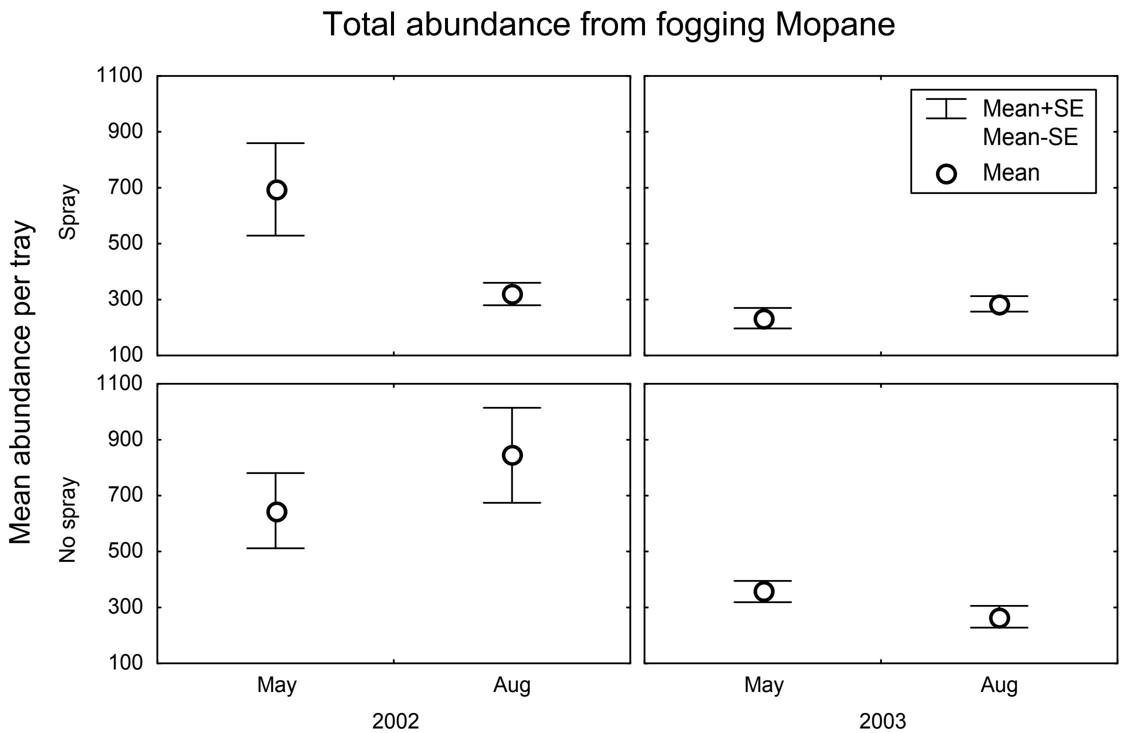
On Chiefs Island within the spray zone invertebrate abundance on mopane trees decreased by 26% in post-cycle fogging samples over pre-cycle estimates. This was despite an order of magnitude increase in the abundance of flies. If flies were removed then the decline was 61%, very similar to the estimates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

In 2003 the average abundance per trap was similar on both the sprayed and unsprayed sites. Both considerably down on 2002 numbers. In the unsprayed site on the Moremi cutline, beetle numbers declined to 132 per sample in May and 16 in August. Recalling that 99% of the 568 beetles per sample in August 2002 were from two morphospecies (*Bruchidae001* and *Chrysomelidae001*), the simplest explanation for the change is a natural population outbreak of these particular species.

This is the common ecological pattern in insects of adult emergence at high densities for only short periods of time to maximize gene flow in mating events and/or to minimize individual probabilities of predation. This appears to be the observation here. The spray did affect these species, hence the more dramatic decline at Chiefs in 2002, but the changes on the control site shows that abundance would have declined in time in any case. Both these morphospecies were recorded again in 2003, so the declines were not losses.

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 initially depletes invertebrate abundance by approximately two-thirds to 30-40% of pre-cycle levels. The observations in 2003 suggest that recovery from this decline is rapid, probably within the year, and average catches are soon within the limits of variability to be expected in a dynamic and heterogeneous system.





Key Conclusion
The impact of aerial spraying of deltamethrin on invertebrate abundance in the Okavango Delta appears to be significant but short lived. Abundance recovers to near pre-spray levels within one year

3.5.2 Changes in composition

There were clear changes in insect composition on all tree species between pre-spray and post-spray samples in 2002. The pattern was for overall composition to shift and for the variation between samples to be greater in post-spray samples. This was true in comparisons of untransformed data where the emphasis is on composition and abundance and with presence-absence data where the comparison is entirely based on composition. This result suggests a strong effect of the aerial application of deltamethrin on composition. Recovery of composition would be seen if samples in 2003 returned to similar positions on the ordination diagrams as those of the pre-spray samples, that is became more similar in composition to pre-spray.

Whilst there was not an exact overlap in any of the cases, the general trend was for 2003 samples to be more similar to pre-spray than the post-spray samples. This is strong evidence for recovery of assemblage composition, especially considering that the taxa used to record these compositional shifts (beetles, flies, Hemiptera, spiders and ants) are, with the exception of ants, known to be vulnerable to deltamethrin. Although there were changes in species and potentially some species losses, the overall insect assemblage was resilient to the impact of deltamethrin.

The data from the mopane control sites demonstrate that the shift and greater variance in composition from post-spray samples was due to the spray as the non-sprayed site in Moremi produced consistent overlap between May and August 2002 samples. There were, however, big between year differences in composition on the unsprayed site. So we can expect composition to change between years and locations and tree species. We can also expect there to be a change as a result of spraying but that this will be a temporary effect and absorbed into the general variation that heterogeneous and disturbed systems are likely to show.

Key Conclusion
Insect composition does change as a result of aerial application of deltamethrin but soon recovers Changes in composition are probably within the tolerance limits of the system which shows resilience to this particular disturbance

3.5.3 Morphospecies losses

A critical issue in the application of insecticides is the possible loss of species. Local extirpation of tsetse flies was the aim of the spraying project and it seems unlikely that any blanket application of insecticide can be specific to just one species. In the 2002 report we highlighted more than 100 morphospecies that were recorded in pre-spray fogging or initial knockdown samples but were not recorded thereafter. A key purpose of the recovery monitoring was to assess what would happen to these species.

On *K. africana* trees at Stanleys there were 87 species not recorded in post-spray samples and 50 of these were not recovered in 2003. 15 of the 50 were found elsewhere on other tree species or locations suggesting that 35, or 40% of the 87, may have been lost or failed to return within the year. The difficulty in interpretation of these data is that 34 of the 50 morphospecies that were not sampled in 2003 were originally recorded as single specimens. This means that they were not abundant in 2003 samples and would be subject to the problem of sampling low abundance and potentially dispersed species. This problem is that a large sampling effort is needed to resample them. Real concern would be for the handful of species that may have originally been relatively common.

Then there is the added problem for interpretation that is again a product of sampling species with low overall abundance. In 2003, 57 morphospecies were sampled from *K. africana* trees at Stanleys that were not recorded at all in 2002. In other words the number of species "losses" was more than balanced by new records of taxa not seen in 2002. This may also mean that the system is naturally dynamic, making specific statements about species losses impossible without longer term data.

The comparisons on the mopane sites were also instructive. Around 50% of the morphospecies recorded in the pre-spray samples were not seen in the post-spray samples in 2002 on both the sprayed and the unsprayed site. On the Chiefs Island site that did receive the aerial applications of deltamethrin, 32% of these species were not resampled in 2003. This was very similar to the 40% on *K. africana* at Stanleys. However, 26% of the original species "lost" were not recovered in the unsprayed site on the Moremi cutline. On both sites there were also many morphospecies recorded for the first time in 2003.

This result from the unsprayed control site on mopane suggests that it would be natural to see considerable between year variation in the presence and absence of species in tree canopies. So much so that perhaps a quarter of species might turnover in this way. This is consistent with the normal pattern for insect biodiversity where the majority of species are locally rare and may undergo dynamic patterns of local extinction and immigration. Taking this result and that from the sprayed mopane and we see that perhaps 6%, that is 32% minus 26%, of morphospecies "losses" could be directly attributed to the spraying of deltamethrin.

Key Conclusion
Whilst some morphospecies may be locally reduced, problems of sampling low abundance make this difficult to quantify. Results from the mopane control site suggests that 6% of species losses between years are directly the result of the aerial application of deltamethrin.

3.5.4 Consequences of aerial application of deltamethrin on terrestrial invertebrates

The evidence from 2002 showed that some terrestrial invertebrate taxa in tree canopies are affected by repeated aerial applications of deltamethrin; and that these effects may be acute for some morphospecies but apparently not across higher taxa. Whilst the absolute numbers are difficult to establish because of natural variation between years, within season and between trees, average abundance appeared to have successfully recovered in 2003.

Whilst a short term decline was recorded in 2002, there is little evidence for a medium term effect of spraying on insect abundance.

The impact on most species is probably well within the natural patterns of stress generated by the changing flood regimes, drought and storm events common in the Okavango Delta. The addition of more disturbance to an already dynamic system may not be as dramatic as disturbing a more stable environment.

In abundance terms the insect abundance in the Okavango appears to show resilience, bouncing back quickly from the spraying, but weaker resistance in some taxa that did decline in abundance as an immediate result of the spraying.

The same appears to be true of composition. Changes were apparent immediately after the aerial applications. Composition of samples were almost universally more variable and show an overall shift compared to pre-spray samples, with the exception of the control site on mopane. Here there was no compositional shift in 2002. In 2003 the composition of samples were of similar consistency to those from pre-spray and in most cases were closer to the pre-spray values than the post-spray. There was also, in the absence of insecticide application, no real shift or increase in variance of composition between May and August samples in 2003.

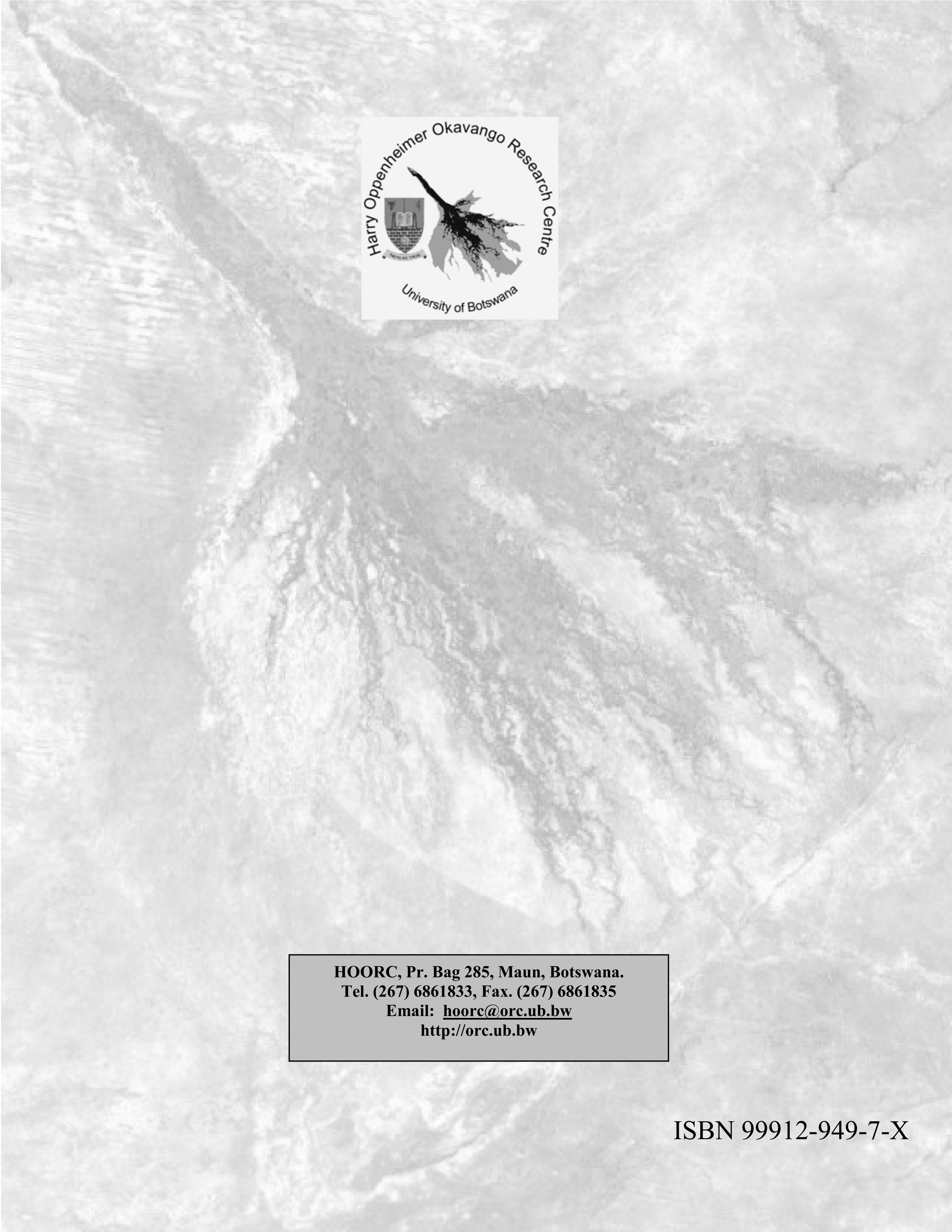
Previous reports in this project have discussed the literature on assessment of insecticide applications. Much of this literature on screening for side effects against non-target invertebrates comprises a combination of laboratory, semi-field and field levels and recognition that it is difficult to simulate the scale of treatment in many cases. This was particularly the case in the Okavango Delta, yet the data presented here represent a very detailed appraisal of the effects on four tree species, in three discrete locations and across five sensitive taxa. This is a truly landscape level appraisal of impact and one that is comprehensive across a full range of sensitive taxa. We are not aware of any studies that have been able to assess non-target canopy taxa so effectively.

In addition, the Harry Oppenheimer Okavango Research Centre now has a data bank of digital images for more than 400 morphospecies together with voucher specimens for each one. This is a valuable inventory for any future monitoring of the Okavango delta and represents the most comprehensive alpha diversity assessment of invertebrates ever undertaken in Botswana.

Terrestrial invertebrates were not extirpated by the application of deltamethrin in 2002. The significant declines in abundance of several taxa were balanced by strong recovery in 2003. Biological composition did change significantly through the spraying cycles but again had recovered by May 2003. Some specific species may have been locally affected but this appears to be only around 6% of the total species sampled, which themselves were from vulnerable taxa.

These data not only provide a comprehensive appraisal of impact and recovery but a valuable benchmark against which any future insecticide application or land use change in the Okavango Delta can be assessed.

Key Conclusions
Whilst aerial application of deltamethrin decreases insect abundance and shifts composition this effect is short lived. Abundance and composition recover within one year. Less than 10% of the less abundant morphospecies in sensitive taxa may become locally depleted and take longer to recover.



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