Assessment of ecological risk associated with irrigation in the Fitzroy basin

Phase 2 – Analysis and characterisation of risk with emphasis on effects on macroinvertebrates

Project UCQ3

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Final Report

Abstract
This project in the Fitzroy catchment in Queensland was one of three case studies used to illustrate how ERA may be employed in the management of irrigation areas in Australia. In the problem formulation stage of the ERA process community stakeholders identified issues and helped to prioritise research needs for the second phase. These were that the research should focus on the irrigation area scale and the impact of the decline of water quality on macroinvertebrate populations with emphasis on determining the relative contribution of various environmental factors to changes in these populations.

As part of the ERA analysis phase, an initial pilot study was successfully undertaken in 2001/2002 and based on this some changes to the original design were required. The study addressed four key questions in relation to a drain that delivers irrigation runoff to the Dawson river via Gap Creek. Findings of the risk assessment phase were that the magnitude of the effect of water from the drain on macroinvertebrates in the river was too small to be detected, based on the end points used. (Further study at the species level of identification may be warranted.) However, effects (loss of sensitive taxa) were recorded at a site on Gap Creek, where risks from endosulfan exposure were determined to be high from December to February. Statistical analyses suggested that the most important factors explaining differences in the macroinvertebrate structure were discharge variables and levels of the pesticide endosulfan sulphate. Discharge variables that were identified as being of significance are discharge divided by time since that discharge, maximum discharge and discharge > 200 ML/day. Based on a lack of significance of effects on macroinvertebrates at a site 3 km downstream from the impacted site on Gap Creek, it was determined that the risks of runoff from irrigated land on macroinvertebrates were highly localised. Rate of recovery from effects was difficult to assess given the single impacted site where measurable effects were recorded. It is postulated that measured pesticide contamination of stream sediments may hinder such recovery and this may be a useful avenue of future research.

Project results are summarised against the six major objectives listed below:

1. **As part of the larger Ecological Risk Assessment project set up by NPIRD, use the Fitzroy catchment as one of three case studies to produce an ecological risk assessment framework for the irrigation industry in Australia.**

The first phase of the ecological risk assessment process (*the problem formulation phase*) was completed for the Fitzroy in February 2001 (Duivenvoorden *et al.* 2001). Similar work was done for the Ord catchment in northern Western Australia and the Goulburn-Broken Catchment in Victoria as part of the larger ERA project overseen by a Linkage team headed

by Professor Barry Hart. To assist in establishing the framework, the Fitzroy team actively participated in two ERA workshops in Melbourne (June 2002 and August 2003) and contributed information on the ERA process carried out in the Fitzroy. A general overview of the ERA process in the Fitzroy is provided in the Final Technical Report (see Figure 1 of Appendix 1). This technical report describes in detail the analysis and risk determination phases of the ERA process undertaken. *The outcome of this is that the process in the Fitzroy can be used as a guide for the adoption of ERA in other irrigation areas in Australia, particularly where macroinvertebrates are used in the assessment of ecological risk.*

2. Address the priority information gaps identified in Phase I of the ERA project for the Fitzroy Catchment.

In phase 1 of the ERA priority actions to address information gaps and research needs for the second phase of the project were recommended. These were that the research should focus on the irrigation area scale and the impact of the decline of water quality on macroinvertebrate populations with emphasis on determining the relative contribution of various environmental factors to changes in these populations.

Modifications to original design

The analysis phase of the ERA commenced with an initial pilot study successfully undertaken during the 2001/2002 irrigation season to assess site suitability, new methodology and variability in macroinvertebrate populations. As a result of this study some changes to the original design of the main experiment were required owing to the discovery of pesticides in areas that were intended to be control sites. Hence rather than using the MBACI or MBACI (P) approach to analyse the data, a gradient of impact approach was considered more suitable. Hence sites upstream of the irrigation area would constitute "low impact" sites and those downstream were chosen over a length of river where a gradient of impact was likely to occur. Thus four "low impact" sites (1-4) were sampled upstream of the irrigation area and three "impacted" sites (5-7) sampled downstream of it. Additionally, two other sites (5a and 7a), though monitored less frequently, were chosen to gain more detail on the extent of possible effects. Further to feedback from local irrigators, one other change was the provision of a study site (a) on Gap Creek upstream of where runoff from the irrigation area enters via a drain. (Gap creek enters the Dawson river between sites 5 & 6.)

The study addressed four key questions in relation to the drain that delivers irrigation runoff to the Dawson river via Gap Creek:

1. What is the magnitude of the effect that the water from the drain has on the macroinvertebrate assemblage in the river? (see objective 4)
2. What is the relative significance of pesticides compared to other environmental parameters (such as reduced oxygen levels and river discharge) on changes to macroinvertebrate communities? (see objective 6)
3. How does the relationship between pesticides and effect on the assemblage change with distance down the river? (see objective 5)
4. What is the rate of recovery of the assemblage from the effects of disturbance? (see objective 5)

Outcomes for how these priority information gaps were addressed are detailed under objectives 4, 5 and 6.

3. In collaboration with the linkage team, reassess the Phase 1 work to further draw out the process used, the results and the lessons learned

The Phase 1 work was reassessed during the workshop on 17-18 June 2002 with the Linkage team in Melbourne. Further detailed information on the process in the Fitzroy was

provided to workshop participants and this resulted in a report prepared by the Linkage team (Hart *et al.* 2003) that detailed the lessons learnt from the process in the Fitzroy and the other case studies. *Some of the key outcomes of this learnt from the Fitzroy were that for a successful ERA process emphasis should be placed on developing trust between the ERA technical people and irrigators, attention should be paid to how the workshop is facilitated (appropriate mix of people, venue etc.) and that conceptual models (on both a catchment wide and irrigation area scale) worked very well in helping to come to grips with the range of issues of relevance.*

4. Determine the effect of irrigation on the ecology of aquatic systems on an irrigation area scale.

Methodology used to address this objective (based on the research design described under objective 2) was first tested during the pilot study in the 2001/2002 irrigation season (a report of the pilot study is provided at the end of the final technical report – see Appendix 1). **Methodology** for the main experiment included measurement of physical/chemical parameters (including pesticides and flow), macroinvertebrate assemblages and direct toxicity tests in the field. Discharge at three gauging stations and Theodore weir was used in conjunction with discharge determined for Gap Creek by current flow measurements during a flow event in early February 2003. Sampling for physical/chemical and biological parameters was carried out at approximately three weekly intervals. The first significant rains came between 6 and 8 February with cyclone Beni dropping rain on the irrigation area and further north, whereas the low impact sites upstream of the irrigation area were not affected. Further rains came in early April; these were more widespread and affected flows at all sites in the study area except those on Gap Creek.

Physical and chemical parameters at each site were generally recorded on each sampling occasion as per the Monitoring River Health Initiative methodology. Sites were selected as having a similar amount of overhanging vegetation and similar substrate characteristics. To determine a time-integrated concentration of pesticides, at each site, three passive samplers containing 10mL trimethylpentane were deployed as per methodology in Leonard *et al.* 1999. Samples were sent to a NATA certified laboratory for analysis of a range of pesticides. These pesticides were selected based on information regarding recent usage obtained from the main supplier in Theodore. Time integrated pesticides levels in water were estimated from the concentrations in the passive samplers using the equations in Leonard *et al*. (2002). Sediment samples were also analysed for pesticides.

Macroinvertebrate communities were sampled on each sampling occasion by means of hand net sweeps using a 250 μm mesh standard pond net, with three two-minute samples taken at each of 10 m of the open edge habitat at each site. Upon return to the laboratory, each sample was sub-sampled and sorted until either approximately 200 individual invertebrates were counted or the whole sample was sorted (Walsh, 1997). Invertebrates were identified to family level only. To conduct **direct toxicity tests** at each site during each sampling interval, animals were placed in three cages at each site and their mortality monitored. The national Standard Operating Protocols as provided in the ANZECC and ARMCANZ (2000) guidelines were followed for these tests. Death of the organisms was used as the test endpoint. **Statistical analyses:** Linear regression was used to test for a gradient of impact. A correlation matrix was produced from data from all sites and sampling times to initially determine relationships between physical-chemical and biological variables. Using Multi-linear regression analyses, each macroinvertebrate index was then regressed against those abiotic variables that were significantly correlated with it to determine how much of the biological data was explained by the environmental variables. Cluster analysis, MDS, ANOSIM, PCA and BIOENV were then used to assess

similarities between sites and sampling times and to assess the contribution of various environmental parameters to any biological differences between these.
Endosulfan sulphate concentrations estimated from those in the passive samplers were below the ANZECC and ARMCANZ (2000) 99% trigger value for slightly-moderately disturbed systems for all sites, except for the December to February period at site b, when this trigger value was exceeded. **Macroinvertebrate taxa richness and abundance** varied significantly over the study period (Figures 1 and 2, and section 3.4 of Appendix 1). Both taxa richness and abundance tended to increase over the course of the study period, particularly from January onwards for abundance, however the variability in the abundance data was very high. There did not appear to be any marked difference in taxa richness between site 5 and 6, the entry point of Gap Creek, which received the water from the drain. Regression analyses failed to detect a gradient of impact along the river. ANOSIM pair-wise tests found many significant differences between sites (including control sites), and because of this high background variability between sites along the river it was difficult to detect an effect of water from the drain on macroinvertebrates. Cluster analyses and MDS generally supported these results. **Thus based on analyses of both univariate and multivariate parameters, the magnitude of the effect of runoff from the irrigation area into Gap Creek on macroinvertebrates in the river was essentially too small to be detected.**
The endosulfan data were used along with chlorpyrifos and DDT data in a determination of total risk by calculation of risk quotients (Figure 3; and section 4 of Appendix 1). ***The outcome of this was that risk to macroinvertebrate assemblages associated with the pesticides measured in this study is likely to be low for the Dawson River over the 40 km length below Theodore weir. In contrast, the risk is higher for site b on Gap Creek, with risk quotients exceeding one for the 11 November to 22 February period.***
When this risk is compared to the actual data on macroinvertebrate populations, the lack of any significant effect on macroinvertebrates along the river (see Section 3.6.1 in Appendix 1) provides confidence in this risk assessment. The fact that effects did occur at site b provides further confidence in the assessment, even though the risk quotients <1 outside the December to February period did not match the presence of an effect on the macroinvertebrates.

5. Determine the spatial and temporal extent of the effects of storm water runoff from one part of the Theodore Irrigation area on the macroinvertebrate populations and community structure in the receiving waters of the Dawson River.

A gradient of effect on the macroinvertebrate assemblage was not established along the Dawson river in relation to pesticides as described under objective 4. In contrast, significant differences between sites a and b on Gap Creek were found by the ANOSIM analyses. The most significant difference was the absence of the most pollution sensitive taxa from site b over the entire study period (except for one occasion), while site a had at least one member of these types of animals present on most sampling occasions (Figure 4). This effect was limited to the distance between site b and site 6 – approximately 3 km. Differences between

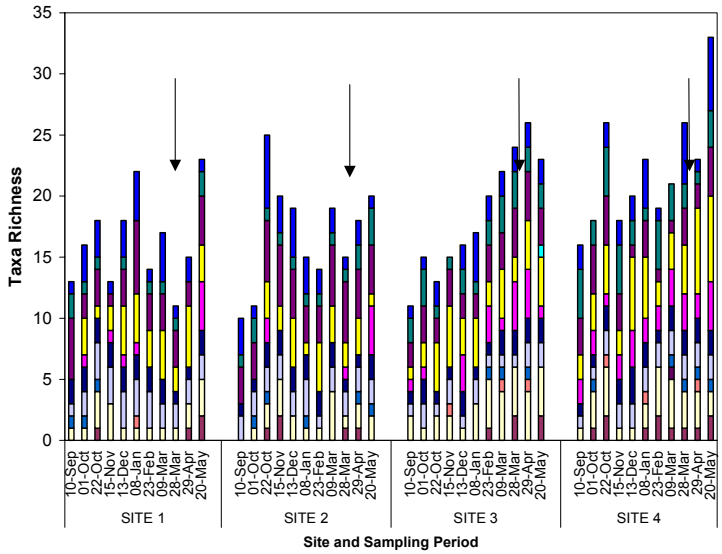

Figure 1. Macroinvertebrate taxa richness at sites upstream of the Dawson Valley Irrigation area between September 2002 and May 2003. Arrows indicate major flow events.

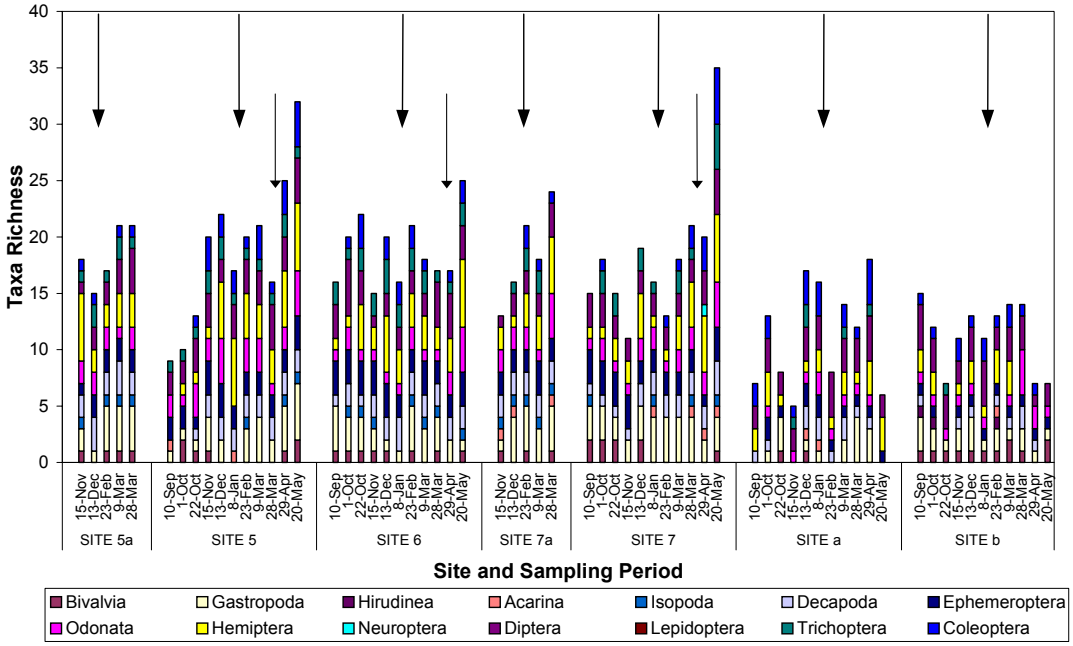

Figure 2. Macroinvertebrate taxa richness at sites 5a, 5, 6, 7a and 7 downstream of the Dawson Valley Irrigation area and at sites a and b on Gap Creek between September 2002 and May 2003. Arrows indicate major flow events.

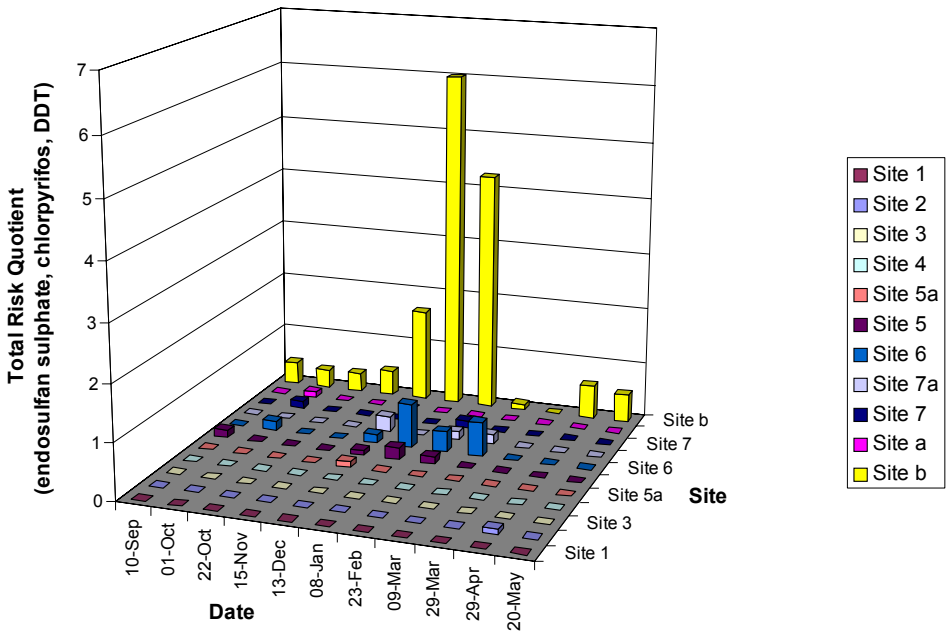

Figure 3. Total Risk Quotient for the estimated concentration of endosulfan sulphate, chlorpyrifos and DDT in water, calculated using the relevant ANZECC and ARMCANZ (2000) trigger values recommended for slightly-moderately disturbed systems as effect concentrations.

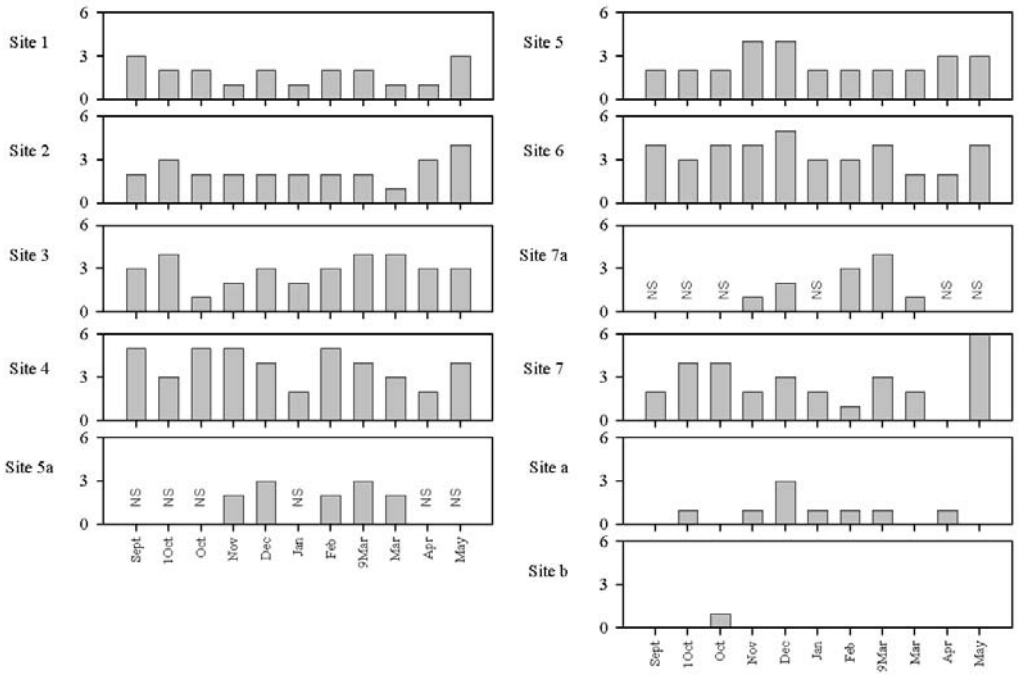

Figure 4. “PET no Baetidae” index for each site over the survey period.
(NS = not sampled).

levels of pesticides at site b and those at site 6 were high – a 10-fold reduction in concentration was observed in both the 2001/2002 and 2002/2003 irrigation seasons. Owing to the lack of an impact gradient along the river and only a single "impacted" site, conclusions about the **rate of recovery from effects** of disturbance related to pesticide exposure are difficult to make. Since "recovery" of sensitive species at site b was not observed, it appears this may take longer than the period of this study, particularly if low levels of endosulfan are present for extended periods of time as was the case for site b (see Figure 10 in Appendix 1). Contamination of the sediments with pesticides as observed at site b in this study may be important to this recovery.

The outcome of this from an overall catchment perspective is that the results of this study suggest that risks to macroinvertebrate assemblages in relation to pesticides are only of highly localised importance. *Information from this study, notably based on only one site with high pesticide levels, suggests that in the Dawson Valley Irrigation Area effects become non-detectable within 3 km of where runoff from irrigated areas enters streams. Critical to this proposal is that there was an observed 10 fold decrease in pesticide concentrations over this distance along Gap Creek in two successive irrigation seasons. This is most likely related to adsorption of pesticides onto substrates and the dilution capacity of the Dawson River. Further work is required to increase the confidence of this assessment.*

6. Determine the relative extent to which various parameters such as flows and pesticides affect variation in macroinvertebrate communities.

Correlation analyses found the environmental variables that significantly correlated with the univariate macroinvertebrate indices were alpha and beta endosulfan, endosulfan sulphate, total endosulfan, Secchi depth, turbidity, minimum discharge, detritus cover, dissolved oxygen and pH. Each biological index was then used as the dependent variable in *multiple linear regression* analyses against the variables that were significantly correlated with it. The maximum variation in these indices they explained was 21.7% of the variation in the biological index "PET no Baetidae" (see Table 2 in Appendix 1). Hence the predictive power of the environmental variables was quite poor and likely related to the range of levels in the environmental gradients being too small to produce significant changes in the biological indices.

Since changes to macroinvertebrate assemblages were not detected along a pesticide gradient in the river, results from site b were included in analyses to gain further insights on the relative significance of environmental variables to changes in the biological assemblages between sites. The approach used was to compare the biological differences between sites as shown by the MDS with principal component analyses on the environmental data. This was done via the BIOENV procedure in PRIMER. The analyses suggested that the most important factors explaining differences in the macroinvertebrate structure were discharge variables and levels of the pesticide endosulfan sulphate. Discharge variables that were identified as being of significance are discharge divided by time since that discharge, maximum discharge and discharge > 200 ML/day. Temperature and detritus cover are also of significance to these communities, but are not as highly correlated with the biological data as the above flow and pesticide variables.

Actions taken and planned to publicise project results and promote their uptake

Pilot study results were presented to key irrigators and a representative from Cotton Australia on 31 May 2002 prior to presenting them to the Annual Dawson Water Quality forum. Due to the high level of interest results were again presented to a group of irrigators on 10 July in relation to a containment strategy for runoff from the irrigation area. Pilot study results were also presented to the international ITERACT conference in 2002 and to

an international workshop on passive samplers in October of that year (Duivenvoorden *et al.* 2002a,b). Results of the main experiment were presented to the Dawson Water Forum on 23 May 2003 and recently to the INTERACT conference at the Goldcoast in July 2004 (Duivenvoorden *et al.* 2004). Another presentation is planned for irrigators in 2004. A number of journal articles are currently in preparation (e.g. Paper on pilot study – see Appendix 1).

To complete this risk assessment process, further communications with stakeholders are required, as detailed in Figure 1 in Appendix 1. Most importantly, appropriate management of the risks by irrigators will minimise risks to the ecological health of the stream systems.

Future R&D needs

Re-examination of the initial conceptual model indicated areas of the model where further information is required to assist in the ERA process. In particular the examination of the macroinvertebrate samples collected (to the species level of identification) warrants further study, as does the role of pesticide contamination of sediments. Also worthy of study, is the significance of the absence of submerged macrophytes along the Dawson river in relation to the habitat they may provide for fish and macroinvertebrates. Parameters recommended for study in future projects that examine the link between biological and environmental variables (more detailed studies using less sites are recommended) are changes in water height at each site and the impact of cessation of flow.

Acknowledgement

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Publication and reference list

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Appendix 1. Final Technical Report for project UCQ3

National Program for Sustainable Irrigation

Project UCQ3

Project Title: Assessment of ecological risk associated with irrigation in the Fitzroy basin: Phase 2 – Analysis and characterisation of risk with emphasis on effects on macroinvertebrates.

Fitzroy Project Team: Leo Duivenvoorden, Michael Price, Bob Noble, Chris Carroll.

Final Technical Report

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Summary

This is one of three case studies used to illustrate how ERA may be employed in the management of irrigation areas in Australia. The aim is to document the major elements of the risk assessment process undertaken in the Fitzroy Basin. These are the problem formulation phase, analysis phase (including the study design and methodology, the results and their analyses) and the risk assessment phase.

In the problem formulation stage a priority list of six ecological consequences of irrigation within the Fitzroy was developed in consultation with community stakeholders. Future priority actions to address information gaps and research needs for the second phase of the project were recommended. These were that the research should focus on the irrigation area scale and the impact of the decline of water quality on macroinvertebrate populations with emphasis on determining the relative contribution of various environmental factors to changes in these populations.

As part of the analysis phase an initial pilot study was successfully undertaken during the 2001/2002 irrigation season to assess site suitability, new methodology and variability in macroinvertebrate populations. Some changes to the original design were required owing to the discovery of pesticides in areas that were intended to be control sites. The study addressed four key questions in relation to a drain that delivers irrigation runoff to the Dawson river via Gap Creek:

1. *What is the magnitude of the effect that the water from the drain has on the macroinvertebrate assemblage in the river?*
2. *What is the relative significance of pesticides compared to other environmental parameters (such as reduced oxygen levels and river discharge) on changes to macroinvertebrate communities?*
3. *How does the relationship between pesticides and effect on the* macroinvertebrate *assemblage change with distance down the river?*
4. *What is the rate of recovery of the* macroinvertebrate *assemblage from the effects of disturbance?*

Findings of the risk assessment phase were that the magnitude of the effect of water from the drain on macroinvertebrates in the river was essentially too small to be detected, based on the family level of identification end points used. (Further study at the species level of identification may be warranted.) However, effects (loss of sensitive taxa) were recorded at a site on Gap Creek, where risks from endosulfan exposure were determined to be high from December to February. Statistical analyses suggested that the most important factors explaining differences in the macroinvertebrate structure were discharge variables and levels of the pesticide endosulfan sulphate. Discharge variables that were identified as being of significance are discharge divided by time since that discharge, maximum discharge and discharge > 200 ML/day. Based on a lack of significance of effects on macroinvertebrates at a site 3 km downstream from the impacted site on Gap Creek, it was determined that the risks of runoff from irrigated land on macroinvertebrates were highly localised. Rate of recovery from effects was difficult to assess given the single impacted site where measurable effects were recorded. It is postulated that measured pesticide contamination of stream sediments may hinder such recovery and this may be a useful avenue of future research.

This case study is one of three (the others are in the Ord in Western Australia and Goulburn-Broken catchments in Victoria) used to illustrate how ERA may be employed in the management of irrigation areas in Australia. This report updates that produced in August 2003 and provides more detailed information on using macroinvertebrates within the ERA process in relation to irrigation. The aim is to document the major elements of the risk assessment process undertaken in the Fitzroy. These are:
1. Problem formulation Phase
2. Analysis Phase (including the study design and methodology, the results and their analyses)
3. Risk Assessment Phase (Figure 1).

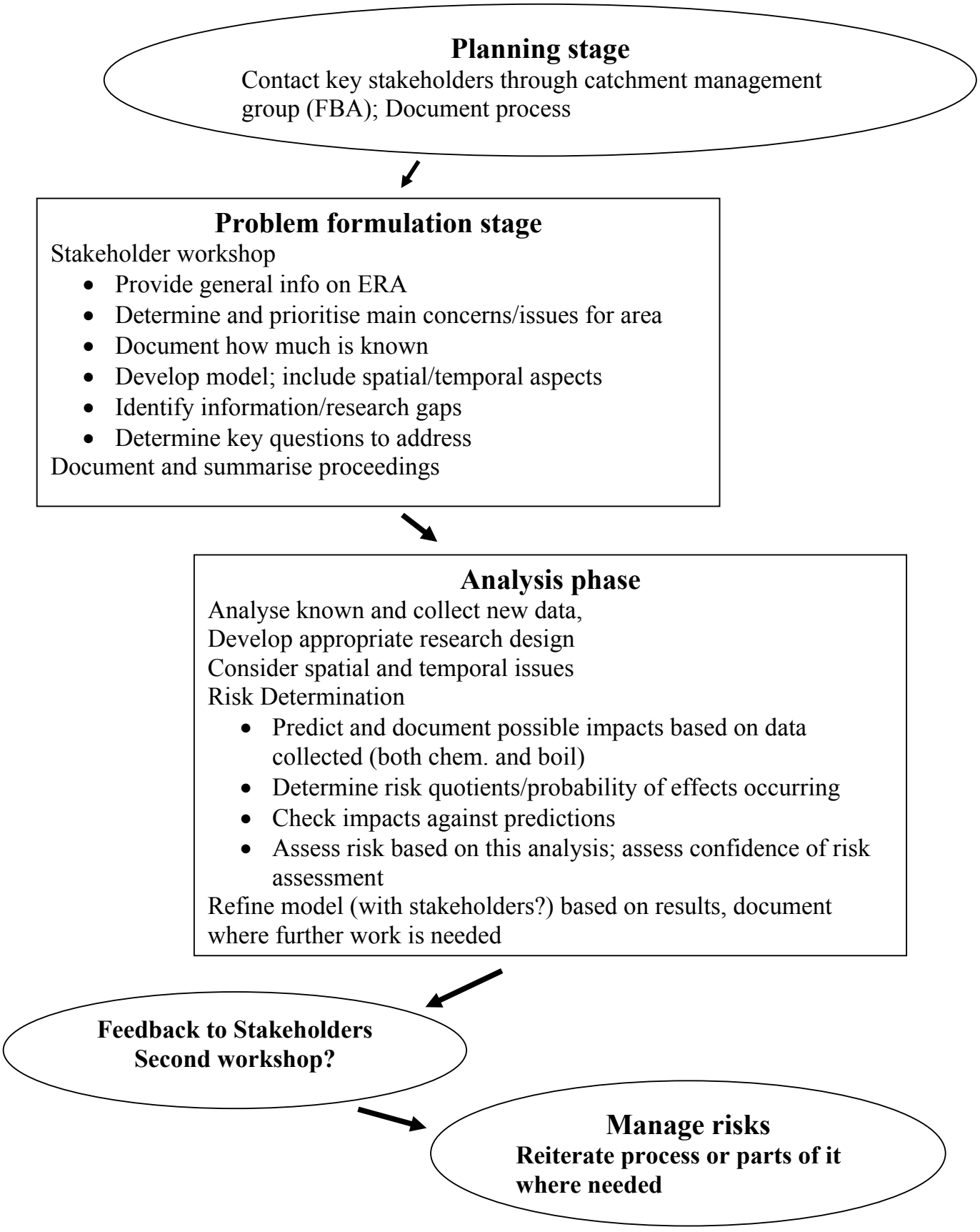
1 Problem Formulation Phase: a brief overview

The main objectives of the first phase of the ERA project in the Fitzroy were: to develop a list of up to six ecological consequences of irrigation within the Fitzroy in consultation with community stakeholders; develop conceptual models containing relevant data for these consequences; complete a table to help establish priorities for future research; provide justification for the rankings within this table; and recommend future priority actions to address information gaps and research needs for the second phase of the project (Duivenvoorden *et al*. 2001).

A community workshop was held in November 2000 and the six ecological effects were identified at each of two different scales – the local irrigation area scale and the entire catchment scale. At the local scale the effects ranked in order of priority were decline in water quality, soil degradation, increase in salinity, changes in composition and decrease in abundance of aquatic macroinvertebrates, changes in nutrient cycles and decreases in desirable fish populations. The effects at the catchment scale were similar though ranked in slightly different order. The most important ecological effect of irrigation at both scales was decline in water quality, since there is wide acknowledgement that nutrient and pesticide concentrations often exceeded water quality guidelines in irrigation areas. When related to the effects on aquatic communities, concern about the influence of this decline in water quality on macroinvertebrate and fish populations were paramount and hence conceptual models of the effect of irrigation and other factors on macroinvertebrate and fish populations were developed.

Detailed assessment of the conceptual models and associated data led to the research team ranking decline in water quality, impacts on macroinvertebrates and fish and soil degradation as the four most important ecological effects in the catchment. Knowledge gaps were identified and current past and future projects briefly described during the process of justifying the ranking obtained by the team (Duivenvoorden *et al*. 2001, p. 16). In summary, effects on macroinvertebrates were ranked most highly of all of the ecological effects of irrigation because of the important ecological function of these organisms within river systems (their role in the food supply of fish to name one) and the high probability that irrigation activities will affect their populations through either release of pesticides, increases in suspended sediments or flow regulation. Macroinvertebrate data collected from irrigation areas in the catchment in earlier studies indicated that taxa richness, for example, significantly decreases during the irrigation season (Duivenvoorden and Roberts 1997,

Figure 1. Flowchart of major elements of the ERA process in the Fitzroy catchment



Duivenvoorden *et al.* 2000). Also of significance is the current awareness of stakeholders in the catchment of the significance of macroinvertebrates as indicators of the health of the river systems. This awareness has been assisted by two projects running over the period 1993 to 1999 that investigated in detail the chemistry and aquatic biota of the Fitzroy River system; this included a very important community involvement process which enabled information on these areas to be widely disseminated around the catchment (Noble *et al.* 1997; Noble 2000). The work of the Waterwatch co-ordinator employed by the Fitzroy Basin Association has also been significant in communicating the significance of macroinvertebrates as indicators of healthy river systems to the general public.

In the final report of Phase 1, three recommendations for future priority actions to address information gaps and research needs for Phase two of the ERA project were provided. In summary these were:

- that Phase 2 of the ecological risk assessment project research should focus more on the effects of irrigation at the local irrigation area scale than on those at the broader catchment scale;
- that the impact of a decline in water quality on macroinvertebrate (and if possible fish) populations should be investigated in the Fitzroy with focus placed on determining the relative contribution of factors such as rapid water level fluctuations and changes in water quality associated with rainfall and irrigation runoff events to these populations; and
- that studies should, where possible, include comparisons between particular land/irrigation management practices to increase our understanding of the impact of these on aquatic ecosystems in the tropics.

2 Analysis Phase

The conceptual models described in the report of Phase 1 list several factors that may affect the abundance and composition of macroinvertebrate communities. These are food supply, increased sedimentation, changed substrate, reduction in submerged plants, fish, pesticides, algal production, reduced oxygen, rapid water level fluctuations, reduced flow variability and reduction in riffle habitats. The significance of rainfall events associated with irrigated cropping was highlighted in the report via their effect on suspended sediments, leading to a decline in water quality. Levels of pesticides in irrigation areas of the Fitzroy often exceed ANZECC and ARMCANZ (2000) trigger levels and the significance of this to macroinvertebrate and fish populations is unknown – illustrating an important information gap (Duivenvoorden *et al*. 2001). Hence of the possible factors affecting macroinvertebrates in the river, assessing the relative significance of pesticides in relation to rainfall events would fill an important gap in our knowledge. Information on the effect of flows is also considered relevant, since recent work has suggested that high flows may be important in reducing the number of taxa at unimpounded riverine sites (Duivenvoorden *et al.* 2000). Information on the spatial and temporal nature of possible effects on macroinvertebrates is also sought.

Of the irrigation areas in the Fitzroy catchment the Dawson Valley Irrigation Area (DVIA) was chosen for study (Figure 2). This area is much older than the Emerald

Figure 2. Location of Dawson Valley Irrigation Area (white box in (A)) in the Fitzroy catchment in north-eastern Australia (B).

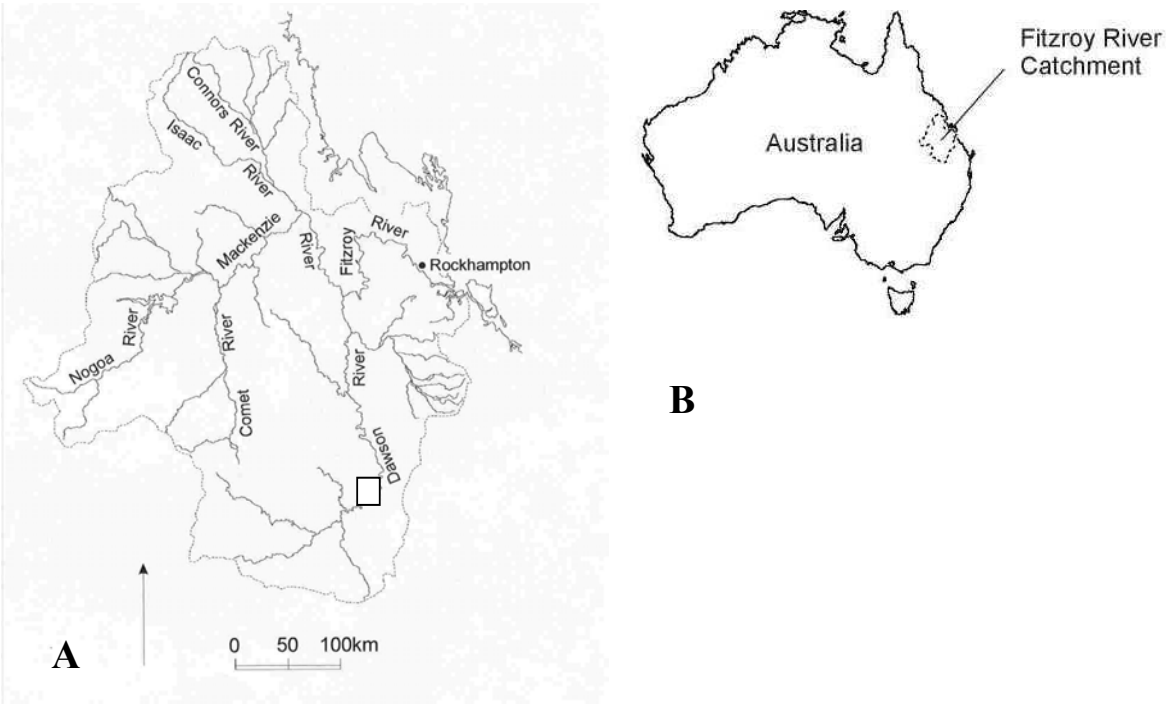


Figure 3. Dawson River Irrigation area in relation to study sites. The drain between sites a and b enters Gap Creek 3.5 km above its junction with the Dawson River. Theodore weir is located approximately 11 km upstream of the Gap Creek entry to the Dawson.

Irrigation Area and does not have the tail water recycling dams of the latter, though irrigators spend considerable effort recycling water from the drains that enter the Dawson or its tributaries. One of these drains enters Gap Creek and the study addressed four key questions in relation this drain and its possible effects on the river (Figure 3).

1. *What is the magnitude of the effect that the water from the drain has on the macroinvertebrate assemblage in the river?*
2. *What is the relative significance of pesticides compared to other environmental parameters (such as reduced oxygen levels and river discharge) on changes to macroinvertebrate communities?*
3. *How does the relationship between pesticides and effect on the assemblage change with distance down the river?*
4. *What is the rate of recovery of the assemblage from the effects of disturbance?*

2.1 Study design and Methodology

An initial pilot study was undertaken to assess site suitability, new methodology and variability in macroinvertebrate populations. Details of this study are presented in Appendix 1.

2.2 Modifications to original experimental design

Further to the pilot study some changes were needed to the design of the main experiment as proposed in the original proposal. The pilot study recorded pesticides at the intended control sites upstream of the DVIA. Although there was a possibility that pesticides may not have been recorded at the control sites during the main experiment, the occurrence of pesticides in this area previously suggested that these sites could not be used as controls. Hence rather than using the Multiple Before After Control Impact or Multiple Before After Control Impact (Paired) MBACI (P) approach to analyse the data, a gradient of impact approach was considered more suitable. Hence sites upstream of the irrigation area would constitute "low impact" sites and those downstream were chosen over a length of river where a gradient of impact was likely to occur. This approach could be analysed by regression techniques.

Originally it was proposed that the main experiment would entail the sampling of four control and two impacted sites along the Dawson river, plus some subsidiary sampling in Gap creek and at three sites downstream of site 6 to provide information on response to a gradient of impact. Further to the Pilot study this was changed so that four "low impact" sites were sampled upstream of the irrigation area and three "impacted" sites sampled downstream of it. The four downstream would include the two previously selected at sites 5 and 6 and one additional one (site 7) further downstream than site 6 (Figure 3). Additionally, sites 5a and 7a, though monitored less frequently, were chosen to gain more detail on the extent of possible effects. Sites 5a, 5, 6, 7a and 7 were located approximately 4, 8, 11.2, 18 and 40 km downstream of Theodore weir, respectively. Gap Creek enters the Dawson river approximately 11 km downstream of the weir. This arrangement of sites was aimed at providing a gradient of response to the expected gradient of pesticide concentration along this length of the river. It was also aimed at providing much needed information about the magnitude of the response to impact, important for later predictive models. It was expected that each site would be sampled 5 times before the first insecticide runoff from the irrigation system and 5 times in the period after this – as per the original proposal.

One other change to the design was the shifting of the original site in the drain entering Gap Creek to a location on Gap creek (site a) upstream of the entry of this drain (Figure 3). Additionally, both this site and site b on Gap Creek would be monitored for macroinvertebrates on 5 occasions before the first runoff event and 5 occasions in the period thereafter. This modification was made so that there was at least one control for site b on Gap Creek. Farmers were particularly interested in having a control site for the caged animals that died at the latter site in the Pilot study, as well as knowing whether macroinvertebrate communities in the Creek are similar to those in the river and how the water coming from the drain might impact them. Inclusion of the impacted site on Gap Creek was also considered useful because it was expected to be the most impacted site, and so should widen the range of response to

potential impact and hence increases our ability to determine the magnitude of the response to impact.

2.3 Methodology

Methods used in the Pilot study were also used in the main experiment, with some minor modifications as detailed below.

2.3.1 Stream discharge and rainfall

In order to assess the possible impact of stream flows on macroinvertebrate populations, river discharge data at three gauging stations (Glebe Weir (Gauging Station 130345), Isla-Delusion (Gauging Station 130358) and Woodleigh (Gauging Station 130317) and also water height data and discharge for Theodore weir was obtained from Sunwater and Queensland Department of Natural Resources and Mines. Discharge data for the sites were obtained from the gauging areas as follows: Glebe Weir (Sites 1 and 2), Isla-Delusion (Sites 3 and 4), Theodore Weir (Sites 5a, 5, 6 and 7a) and Woodleigh (Site 7).

Discharge in Gap Creek was measured to permit quantification of the relative contribution of pesticides from this creek to those in the Dawson River. Discharge from Gap Creek was calculated from a series of current measurements across the stream channel at various water heights during the February flows. Measurements were taken at the causeway on Gap Creek (Sawmill Road) as well as approximately 0.5km downstream from the causeway to check the accuracy of calculations. The stream channel was segmented into a number of areas with each area less than or equal to 10% of total cross section. The velocity of the flow was measured using a Pygmy Current Meter (Model No. OSS-PC1) (Serial No. 02-02) and a Current Meter Counter 20 (Serial No. 01-84) and converted to metre per second. Current profiles were measured at five water levels during the receding limb of the flow in Gap Creek between the 7th and 9th of February 2003. Discharge was then calculated using the midsection method from Gupta (1989). From these data a simple-stage discharge relation (Gupta, 1989) was determined and used with the electronic water height recorder data to produce the hydrograph for flow.

Various components of the discharge were derived for each of the periods between samplings for use in correlations with macroinvertebrate indices.

Sampling was carried out at approximately 3 weekly intervals between August 2002 and 19 May 2003 (Figure 4). More sampling trips than originally expected were undertaken due to the late onset of summer rains. Rainfall affected site access and increased the time between sampling trips on two occasions. The first significant rains came between 6 and 8 February 2003 with cyclone Beni dropping rain on the irrigation area and further north, resulting in flooding of streams in the lower Dawson. Flows upstream of the irrigation area were not affected. Further rains came in early April; these were more widespread and affected flows at all sites in the study area except those on Gap Creek.

2.3.2 Water quality parameters and site characteristics

Physical and chemical parameters at each site were recorded on each sampling occasion as per the Monitoring River Health Initiative methodology (Choy &

Thompson, 1996) with the exception of phenol alkalinity and total alkalinity. Sampling involved spot measurements of dissolved oxygen, pH, conductivity and temperature, using a YSI Sonde 6600. In addition to Secchi depth, turbidity was also measured using the Sonde from December 2002 onwards. For the earlier four sampling times, although the R^2 value was low, turbidity was estimated from a regression of turbidity on Secchi depth (Turbidity = 310.0-4.745 x Secchi; R^2 = 0.276). Sites were selected as having a similar amount of overhanging vegetation and similar substrate characteristics: usually firm, fine (<1 mm) sediment (the only exceptions to this were sites 3 and b, where the substrate was not as well consolidated). Detritus cover (estimated visually or by grab samples when turbidity was high) and current speed were also recorded. Flow rate was measured using a Marsh-McBrirney Model 201D portable water current meter at 5 cm above the substrate, and was recorded in more than 5 points at each site, with the average value calculated. At each site, 3 replicate measurements were taken and the mean of these used in subsequent analyses.

2.3.3 Measurement of pesticide levels

To determine a time-integrated concentration of pesticides, at each site, three passive samplers containing 10mL trimethylpentane were deployed. Further to information gained at a passive sampler workshop in September 2002, tributyl phosphate was also added to the trimethylpentane in the passive samplers. This was done to check for samplers that were not within the "normal range" of membrane permeability (Leonard *et al.* 2002). Each bag was placed in a small metal cage and surrounded by a nylon (0.8 mm mesh) bag. These were tied to large bricks and each secured in the river bed by means of a star picket hammered into the substrate. The passive samplers were replaced on each sampling occasion (at approximately three weekly intervals). Water samples were analysed for the same pesticides at most sites in October 2002 and May 2003, as well as during the flow in February 2003.

For measurement of water samples during flows a refrigerated autosampler and electronic height recorder was installed at the Gap Creek at site b prior to the commencement of the wet season. This enabled samples for pesticide determinations to be collected as soon as flows started in the creek. This sampler successfully took 12 samples at one-hour intervals during the 6-8 February flows. Seven further manually collected samples were taken at various intervals to supplement these samples during the receding limb of the hydrograph. Glass bottles were used to collect grab samples by hand and these as well as those from the autosampler were kept on ice for transport to the testing laboratory. To determine variability in pesticide levels in water samples, three replicates were collected at two sites on one occasion. (Levels were within 10% of each other.)

Water and trimethylpentane samples were sent to Scientific Services, Queensland Health at Coopers Plains (a NATA certified laboratory) for analysis of a range of organophosphate and organochlorine pesticides (profenofos, α- and β- endosulfan, endosulfan sulphate, total DDT(s), and chlorpyrifos, and selected pyrethroids (cyhalothrin, cypermethrin and deltamethrin). Samples were also tested for two relatively new pesticides, emamectin and spinosad, for which methods had to be developed by the testing laboratory. (This involved evaporating the tri-methyl pentane to 1mL and exchanging the solvent with methanol, which was further concentrated to 0.5mL before adding 0.5 mL of deionised water to give a final volume

of 1 mL. LCMSMS instrumentation was used for the measurement of the emamectin B1a and B1b compounds in the samples, while for spinosad, spinosyn A and D and the metabolites K, B and B of D were determined.) These pesticides were selected based on information regarding recent usage obtained from the main supplier in Theodore. Trimethylpentane in the samplers were analysed directly by GC-Electron Capture Detection, (organochlorines) GC-Flame Photometric Detection (Organophosphates) and LC-MS (emamectin, spinosad) and results confirmed using GC-MS. Time integrated pesticides levels in water were estimated from the concentrations in the passive samplers using the equations in Leonard *et al*. (2002).

Sediment samples were also analysed for the same pesticides examined in the water samples, except for the emamectin and spinosyn. These samples were collected from sites 1, 2, 3, a and b and also at four sites over an 18 km length of the Dawson immediately downstream of Theodore weir. The latter four samples were pooled to gain an overall average pesticide concentration for this area. Further to an acetone/hexane (50:50 by vol) solvent extraction these samples were analysed for pesticides by the same laboratory using the same techniques as above.

2.3.4 Macroinvertebrate communities

On each sampling occasion macroinvertebrate communities were sampled by means of hand net sweeps using a 250 μm mesh standard pond net, with three two-minute samples taken at each of 10 m of the open edge habitat at each site. Samples were then combined to make one sample per site and immediately preserved with ethyl alcohol. Upon return to the laboratory, each sample was sub-sampled and sorted until either approximately 200 individual invertebrates were counted or the whole sample was sorted (Walsh, 1997) with, however, a time limit of three hours sorting in the latter case. Invertebrates were identified to family level only, as several studies in Central Queensland (and elsewhere) have shown that this level identification is sufficient to detect the magnitude of the changes expected at sites in the proposed study (such as a reduction in family taxa richness of 50%) (Duivenvoorden *et al*., 2000; Duivenvoorden & Roberts, 1997; Duivenvoorden, 1995; Faith *et al*., 1995). Some taxonomic groups were sent to specialists for verification of identification.

2.3.5 Direct toxicity tests

To conduct toxicity tests at each site during each sampling interval, animals were placed in three cages at each site and their mortality monitored. Results of the Pilot study suggested that animals for the tests would be better sourced from an area independent of the study sites. Attempts at finding appropriate numbers of *Macrobrachium intermedium* (as used in the Pilot study) for this purpose failed. The snail *Thiara balonnensis* was therefore used instead, since it was readily available in large numbers from a site in Rockhampton. Ten animals were randomly assigned to each of the 45 x 25 x 25cm cages (3mm mesh size) in accordance with the national Standard Operating Protocols provided in the ANZECC and ARMCANZ (2000) guidelines. Death of the organisms was used as the test endpoint.

In relation to these tests a Greenspan dissolved oxygen sensor Model DO 300 was deployed at site b to continuously monitor oxygen levels at the site. This was done because results of the pilot study suggested that low oxygen levels may have been responsible for the high mortality of animals in the cages at this site during the 2001/2002 irrigation season.

2.3.6 Statistical analyses

A correlation matrix was produced using data from all sites and sampling times to initially determine relationships between physical-chemical and biological variables. Correlates included temperature, dissolved oxygen, pH, conductivity, current speed, detritus cover (%), Secchi depth, turbidity (NTU), average discharge, minimum discharge, maximum discharge, maximum discharge over time since that discharge, maximum discharge above 200 ML/day, the latter over time since that discharge, alpha and beta endosulfan, endosulfan sulphate, total endosulfan, chlorpyrifos and six macroinvertebrate indices: taxa richness, abundance, PET index, PET index without the Ephemeropteran family Baetidae, the SIGNAL2 index and the graded SIGNAL2 index using abundance data (Chessman, 2003). To determine how much of the biological data was explained by the environmental variables Multiple Linear Regression analysis was employed. Prior to using this technique abiotic variables correlated with each other were removed from the analyses and each macroinvertebrate index was then regressed against those abiotic variables that were significantly correlated with it. The correlation matrix and the multi-linear regression analyses were produced using SigmaStat version 3.0 (SigmaStat 2003).

To assess similarities in the macroinvertebrate data between sites and times, taxa richness and abundance data were run through the cluster analyses program in PRIMER (Clarke and Warwick, 1994) following determination of similarities using the Bray-Curtis index of similarity. Abundance values were log (x+1) transformed for these analyses to reduce the dominating effect on calculated similarities of the counts for the very abundant Diptera in some samples (Clarke and Warwick 1994). To get a clearer picture of relationships between the sites, cluster analyses were also run on pooled data – averaged over each season and averaged over all sampling times. For the seasonal data, sampling times in September, October and November were pooled to give the spring data, December to February for the summer data and March to May for autumn. Data were then analysed via the MDS program in the same software package to illustrate how sampling times for each site clustered together. To reduce the high stress levels in the MDS analyses data from each site were pooled to provide a better illustration of the relationship between sites. The ANOSIM program was also employed to test for differences between sites, including pair-wise tests. P values of 10% (= 0.1) were used to determine differences between sites to help reduce the frequency of occurrence of any Type II error (Zar, 1999).

Principal Components Analysis was used on the physical-chemical parameters to map similarities between sites and sampling times and to determine the main parameters responsible for differences between these. Data were normalised for the analysis and some variables were transformed before analysis. All discharge data was log (x+1) transformed, while turbidity was log transformed. The BIOENV procedure in PRIMER (Clarke and Warwick 1994) was then used to find the set of environmental variables in the data that best explain the community structure observed. Pooled data were used for these analyses. Environmental parameters that were highly correlated (correlation >0.95) were first excluded from the analyses and the procedure then run using up to seven combinations of variables.

3 Results and discussion

3.1 Flow data

Discharge data for the Dawson river is provided in Figure 4 and for Gap Creek during the February flow in Figure 5. These data show that stream flows in February were restricted to that part of the river adjacent to and downstream of the irrigation area and that flows along the entire length of the river did not occur until early April. A small flow in Gap Creek that occurred immediately after Christmas was not recorded by the instrumentation at Gap Creek due to a problem with the electronic height recorder, though information from locals indicated this was a very small flow. Flows in Gap Creek during the February discharge lasted for about 5-6 days, peaking at 1275 ML/day and entering the Dawson River while the Theodore weir was still filling. It was determined that on 6 February 570 ML of water entered the Dawson from Gap creek while on the same day 160ML flowed over the weir. The timing of flows in the Dawson River relative to Gap Creek is important to the risk assessment since the river can dilute concentrations of pesticides coming from the creek.

3.2 Physical/chemical data

Physical-chemical parameters measured during the main experiment are provided in summary form in Figure 6. Mean temperatures for each site ranged between 23.1 and 29.1 ^{o}C, with highest temperatures occurring between January and February. Dissolved oxygen levels were below 30% saturation on at least two occasions at sites 1 and 2 and a and b during the study. This is reflected in the averages in Figure 6. Conductivity was generally less than 300 µSiemens/cm at sites 1 to 4, while at sites 5 & 6 the range often exceeded 500 µS/cm. At most sites Secchi depth was usually less than 50 cm, with depths of less than 10 cm commonly occurring, particularly at sites 1-4 where turbidity values were often very high. In contrast, Secchi depth was greatest at sites a and b, at which turbidity was also low. Detritus was generally highest at sites a and b and decomposition of this material may have been responsible for the slightly lower mean oxygen values at these sites. Similarly, the higher turbidity at sites 1 and 2 would have influenced photosynthesis in the water at these sites and is likely to have been responsible for the apparently lower oxygen values at these sites.

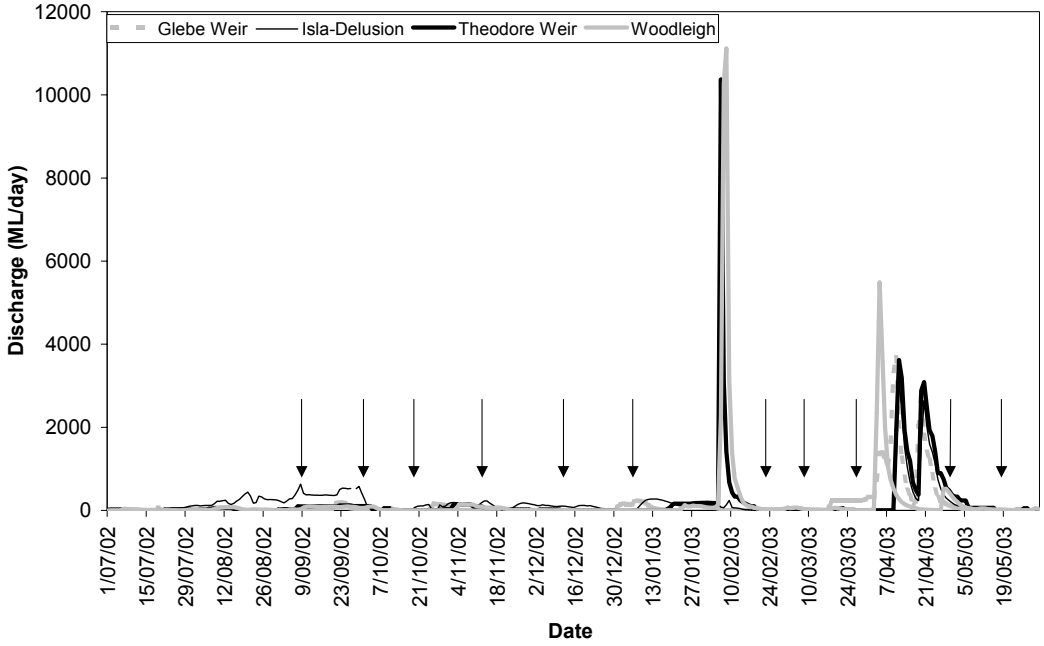


Figure 4. Flows in the Dawson River at four gauging stations over the course of the study period. The Glebe Weir station is adjacent to sites 1 & 2, Isla-Delusion adjacent to sites 3 & 4, Theodore Weir adjacent sites 5a, 5, 6 & 7a and Woodleigh is at site 7. Arrows indicate sampling times.

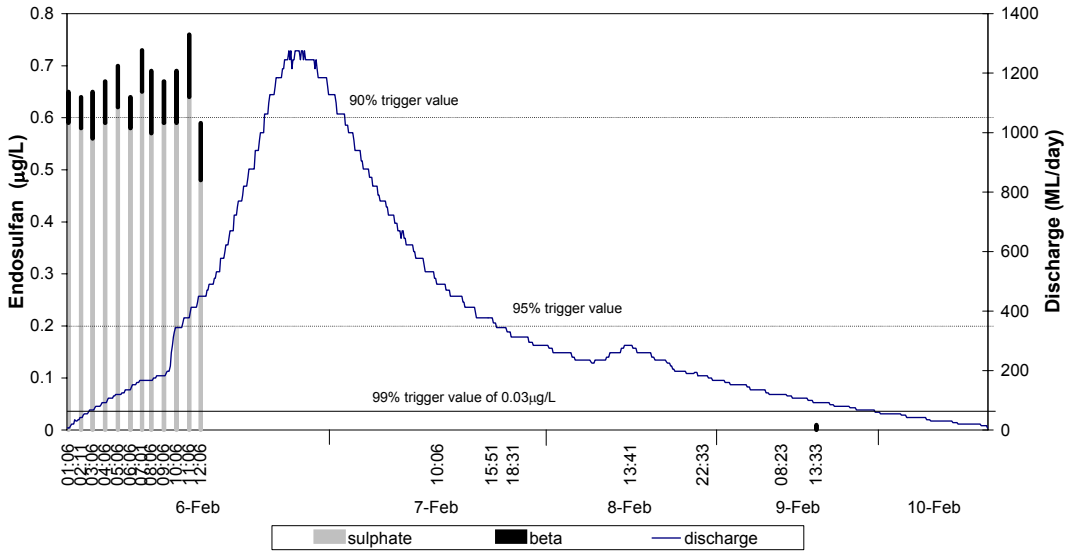


Figure 5. Discharge from Gap Creek and concentrations of beta endosulfan and endosulfan sulphate in water samples collected from site b on Gap Creek during the flow event in February 2003. Also shown are the ANZECC and ARMCANZ (2000) trigger values for endosulfan sulphate recommended for slightly-moderately disturbed systems.

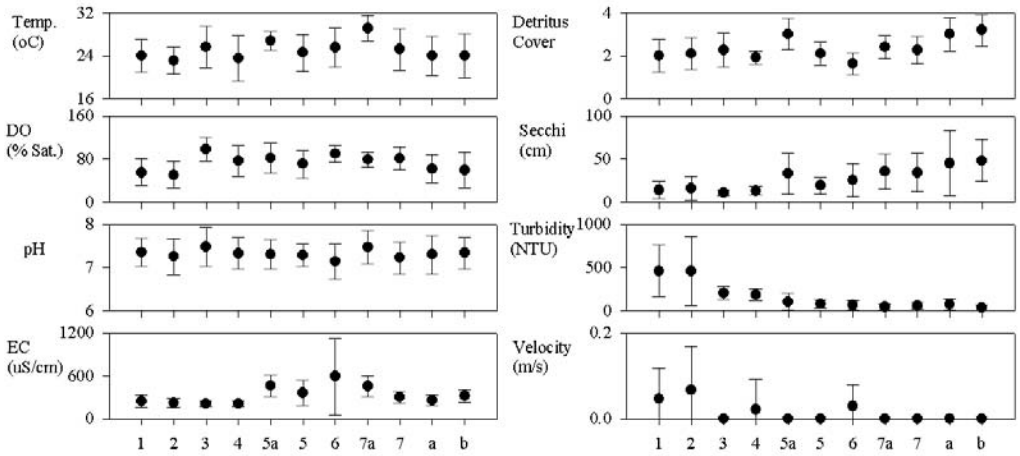


Figure 6. Mean physical-chemical parameters (± standard deviation) for the sites sampled during the survey.

3.3 Pesticide data

Pesticides measured during the flow event in February were beta endosulfan and endosulfan sulphate and DDT, chlorpyrifos, Pesticide concentrations estimated from those in the passive samplers are provided in Figures 7 to 11. Concentrations were highest at site b and diminished with distance downstream (Figures 7 and 8). The fraction of alpha-endosulfan decreased between December and February, reflecting use of this pesticide up until end of December 2002. Results further indicate that the small flow that occurred over Christmas brought some pesticides down to site 6 on the river but flows were not large enough for these pesticides to be brought further downstream. This is not the case however for the flows that occurred over the 6 – 8 February, where pesticides were recorded some 40 km downstream of the Theodore weir at site 7 (Figure 8). (See Figure 5 for endosulfan levels during the flow in early Feb). Low levels of endosulfan were found at site b from August through to November 2002, increasing over the summer months and then decreasing from March 2003 onwards (Figure 9). Levels at this site were higher than those recorded at other sites over the study period (Figure 9). Endosulfan sulphate concentrations estimated from those in the passive samplers exceeded the ANZECC and ARMCANZ (2000) 99% trigger value for slightly-moderately disturbed systems for the December to February period at site b, but were below this trigger value for the other sites (Figure 10). Estimated concentrations for chlorpyrifos were below the 95% trigger value of 0.01ug/L recommended for slightly –moderately disturbed systems for all sites and times (Figure 11). Figure 10 also shows that endosulfan was not detected at any of the control sites 1 to 4 during the course of the study. Concentrations of DDT, recorded in some passive samplers during the study, could not be estimated in water because equations for entry of this pesticide into the samplers are not yet available.

Levels of endosulfan in the sediments may be of importance to macroinvertebrate assemblages. Concentrations of endosulfan sulphate in the sediments at site b in May 2003 were 9 μg/kg on a dry mass basis, while concentrations in the Dawson for a

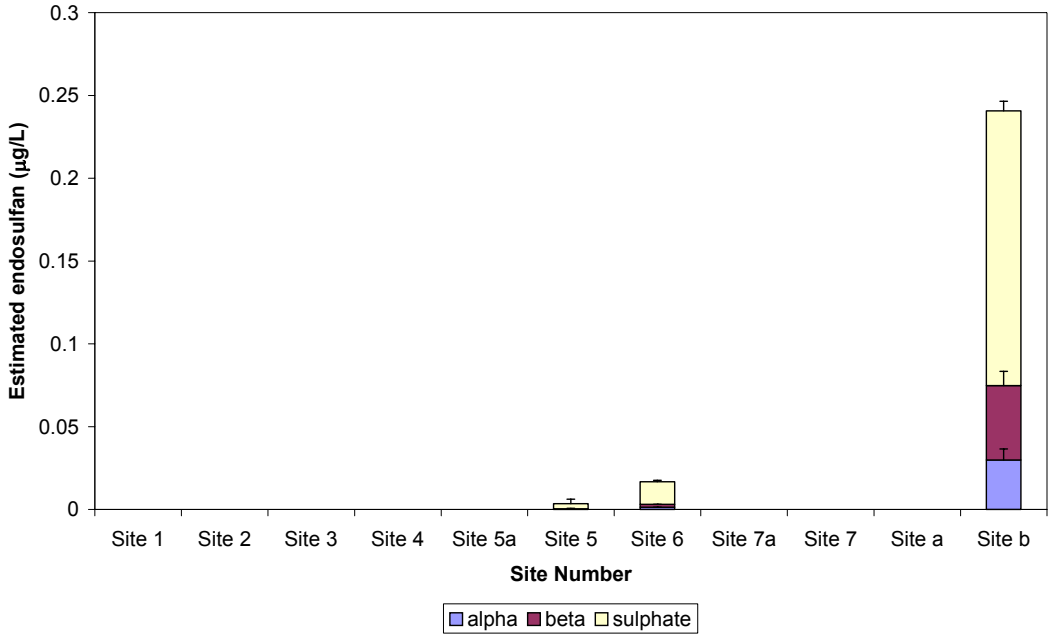


Figure 7. Different forms of endosulfan estimated from concentration in passive samplers at field sites between December 13th 2002 and January the 8th 2003. Error bars are standard deviation of the mean of three samples.

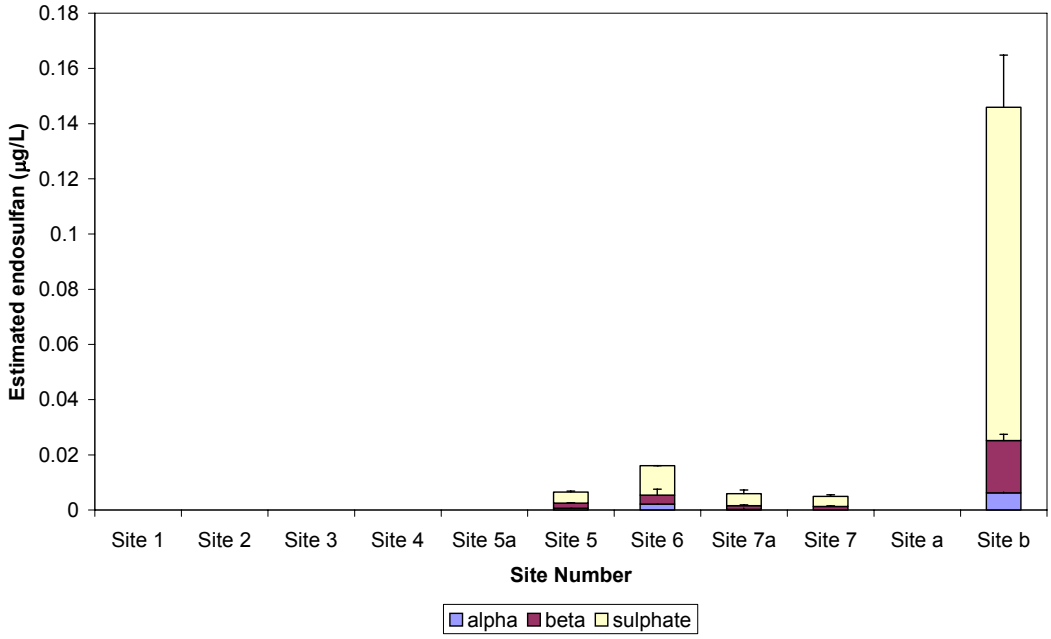


Figure 8. Different forms of endosulfan estimated from concentration in passive samplers at field sites between January 8th and February the 23rd 2003. Error bars are standard deviations of the mean of three samples.

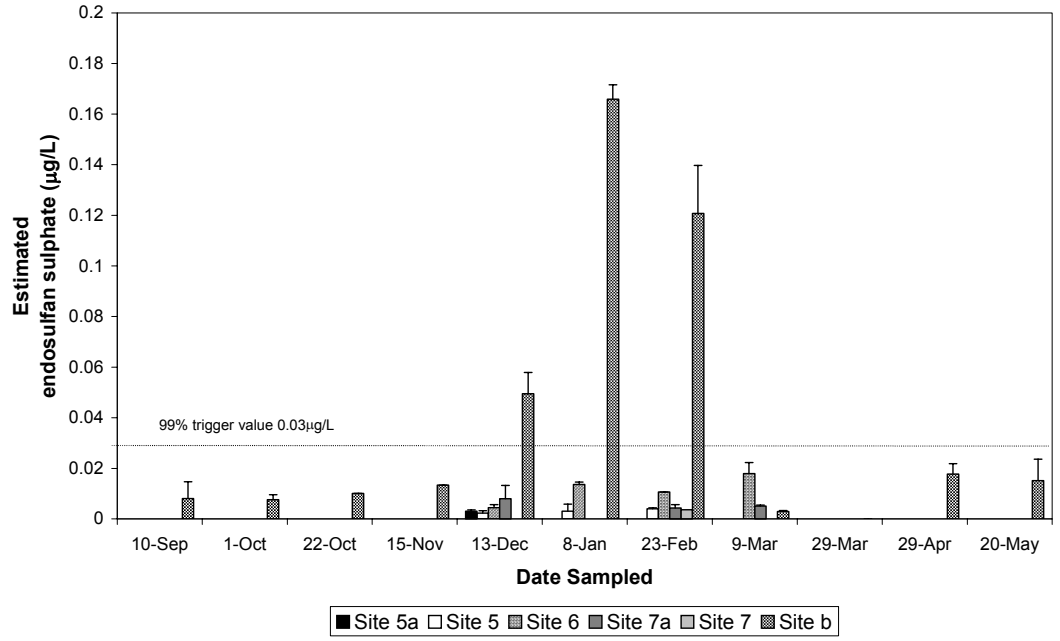


Figure 9. Estimated concentration of endosulfan sulphate in water, calculated using the concentration in passive samplers. Sites without endosulfan sulphate were not included. Also shown is the 99% ANZECC and ARMCANZ (2000) trigger value for endosulfan sulphate recommended for slightly-moderately disturbed systems. Error bars are standard deviations of the mean of three samples.

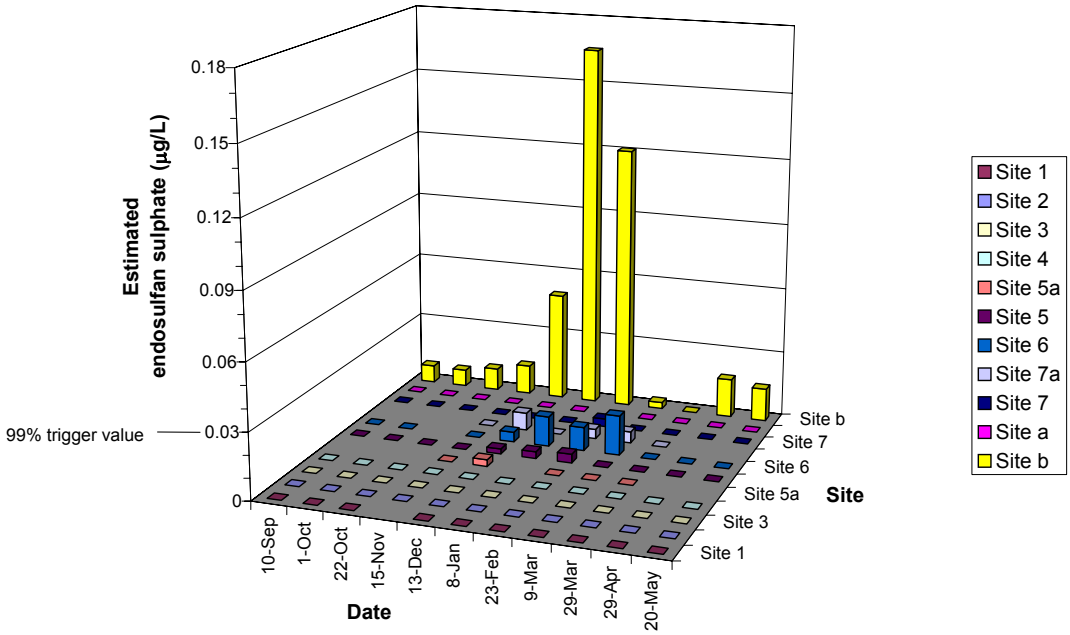


Figure 10. Estimated average concentration of Endosulfan sulphate in water (n=3), calculated using the concentration in passive samplers. Also shown is the 99% ANZECC and ARMCANZ (2000) trigger value for endosulfan sulphate recommended for slightly-moderately disturbed systems.

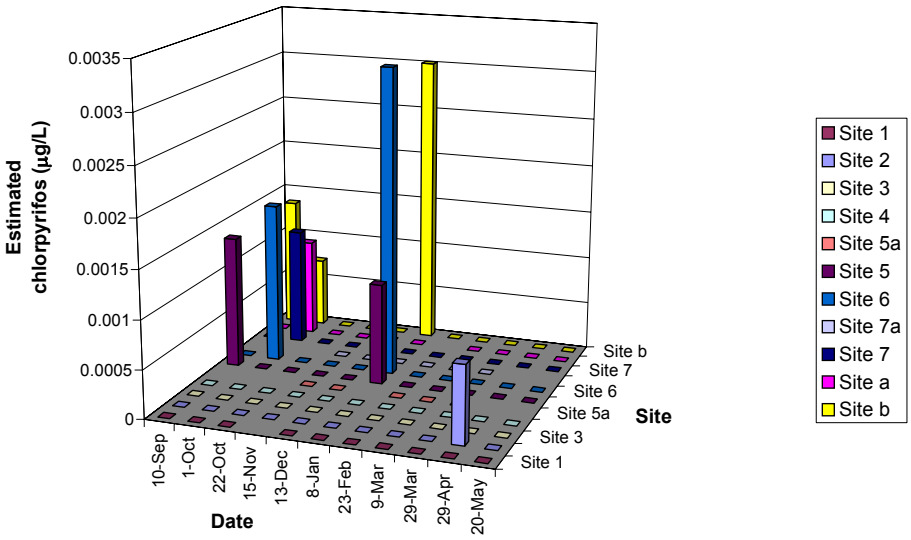


Figure 11. Estimated average concentration of chlorpyrifos in water (n=3), calculated using the concentration in passive samplers. Levels were below the 0.01µg/L 95% ANZECC and ARMCANZ (2000) trigger value recommended for slightly-moderately disturbed systems.

distance of 18 km downstream of Theodore weir were not detected (<1 µg/kg dry mass). The latter was also true of sites 1 to 3 upstream and site a on Gap Creek.

3.4 Macroinvertebrate data

Macroinvertebrate taxa richness and abundance varied significantly over the study period (Figures 12 – 15). Both taxa richness and abundance tended to increase over the course of the study period, particularly from January onwards for abundance, however the variability in the abundance data was very high (Figures 14 and 15). Diptera were dominant components of the population at sites 1 to 3 and at sites a and b on Gap Creek. There did not appear to be any marked difference in taxa richness between the control sites upstream of the irrigation area (Figure 12) and those downstream (Figure 13) and in particular between site 5 and 6, the entry point of Gap Creek. Sites a and b on Gap Creek, however, generally showed lower richness than sites on the Dawson river (Figure 13). Further, there did not appear to be any marked change in abundance or richness associated with the major flow events during the study, the timing of which is illustrated by the arrows in the figures.

Sensitive taxa, as indicated by the PET index, were not very well represented at site b. The only Ephemeropteran family present at this site was Baetidae, which has been regarded as pollution tolerant taxa in other studies (e.g. Leonard *et al.* 1999). When Baetidae are removed from this index (forming the "PET no Baetidae" index) results showed that these sensitive taxa were only recorded once at site b during the sampling period (Figure 16). Site a also did not score well on this index, though at least one of these sensitive taxa were present most of the time. Significantly there were no marked

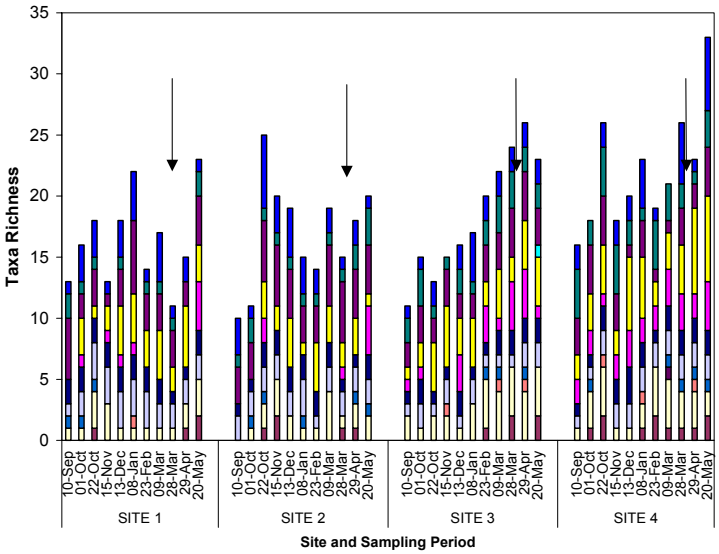


Figure 12. Macroinvertebrate taxa richness at sites upstream of the Dawson Valley Irrigation area between September 2002 and May 2003. Arrows indicate major flow events.

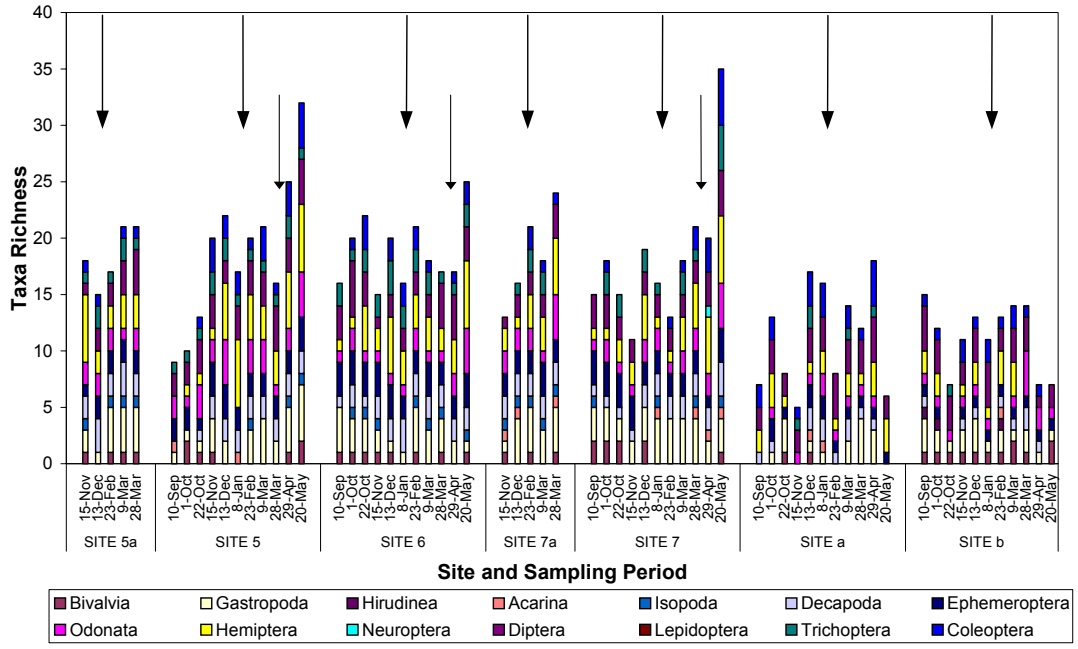


Figure 13. Macroinvertebrate taxa richness at sites 5a, 5, 6, 7a and 7 downstream of the Dawson Valley Irrigation area and at sites a and b on Gap Creek between September 2002 and May 2003. Arrows indicate major flow events.

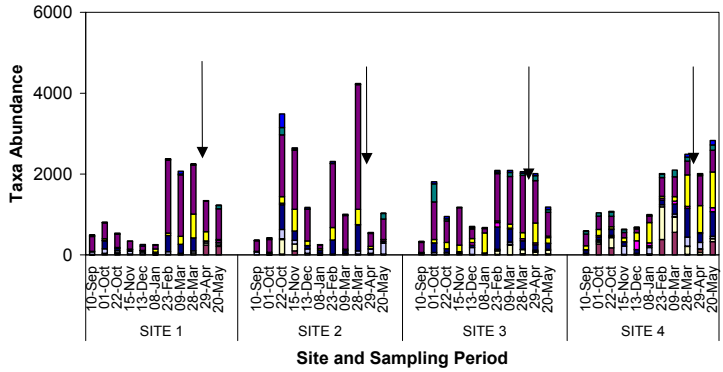


Figure 14. Abundance of aquatic macroinvertebrate taxa at sites 1, 2, 3 and 4 upstream of the Dawson Valley Irrigation area, between September 2002 and May 2003. Arrows indicate major flow events.

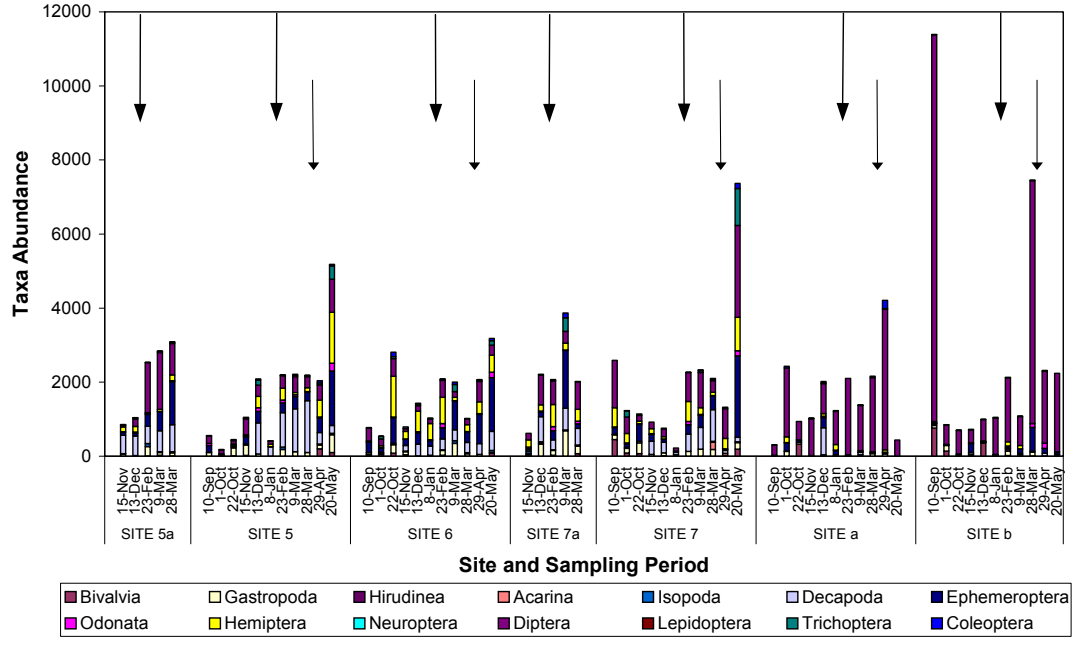


Figure 15. Macroinvertebrate abundance at sites 5a, 5, 6, 7a and 7 downstream of the Dawson Valley Irrigation area and sites A and B on Gap Creek, between September 2002 and May 2003. Arrows indicate major flow events

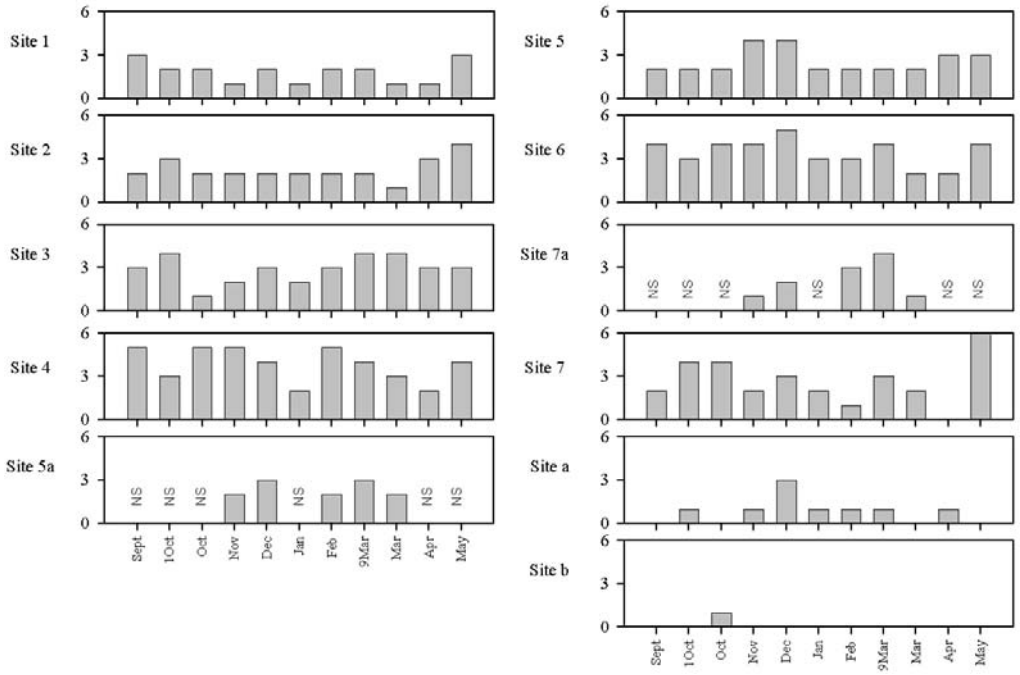


Figure 16 "PET no Baetidae" index for each site over the survey period.
(NS = not sampled).

changes in this index at site 6 during the period that endosulfan was recorded there (Figure 16 and 10) and the numbers of these sensitive taxa at this site compared favourably with those at site 5 and control sites 3 and 4 upstream. The SIGNAL2 index did not show any marked variation between sites or times, having a mean of 3.6 and a range of 2.4 to 4.4. The SIGNAL2 graded index (incorporating abundance) had a similar distribution.

3.5 Direct toxicity tests

Results from these tests show that significant mortality of test animals occurred at sites both upstream and downstream of the irrigation area (Figures 17 and 18). They show that there was no clear differentiation between the mortality of these organisms between these two stream reaches. High mortality at control sites 1 and 2 occasionally occurred when pools in which the cages were placed dried up. Also the high turbidity at these sites may have restricted algal food sources for these animals. Problems with calibration of the meter that measured dissolved oxygen continuously at site b prevented adequate assessment of levels at the site. Spot measurements however on each sampling occasion revealed levels occasionally dropped to less than 30%. Hence low oxygen levels may have impacted on the survival of animals in the cages at this site. Although mortality was usually highest at site b, results of the direct toxicity tests overall were inconclusive, factors other than pesticide exposure clearly being important to mortality.

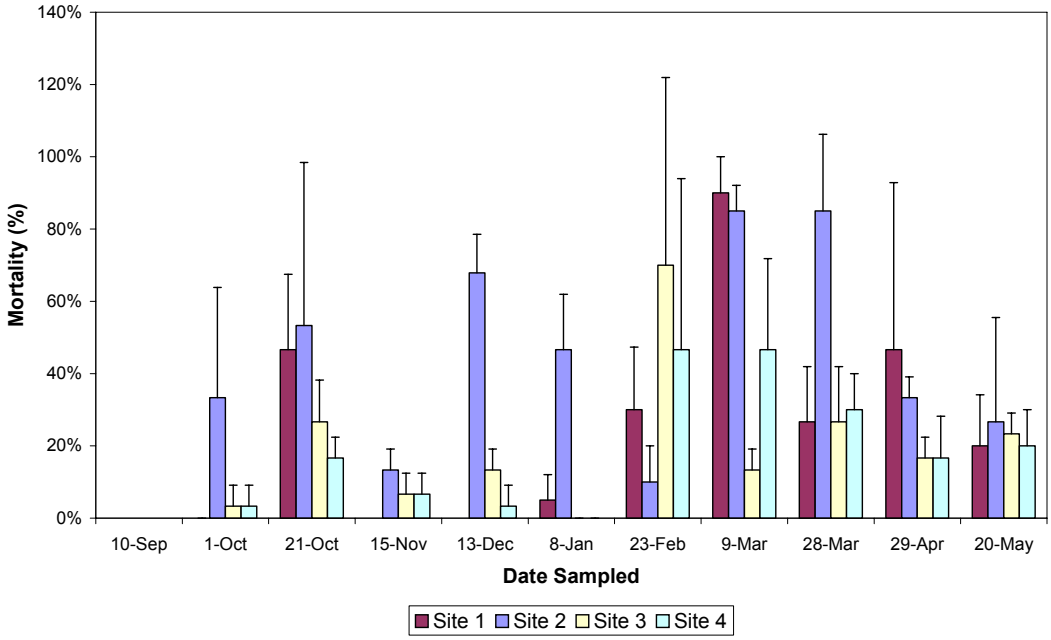


Figure 17. Mortality of the snail *Thiara ballonensis* in cages at sites 1, 2, 3 and 4 upstream of the Dawson Valley Irrigation area, between September 2002 and May 2003. Error bars are standard deviations of the mean of three samples.

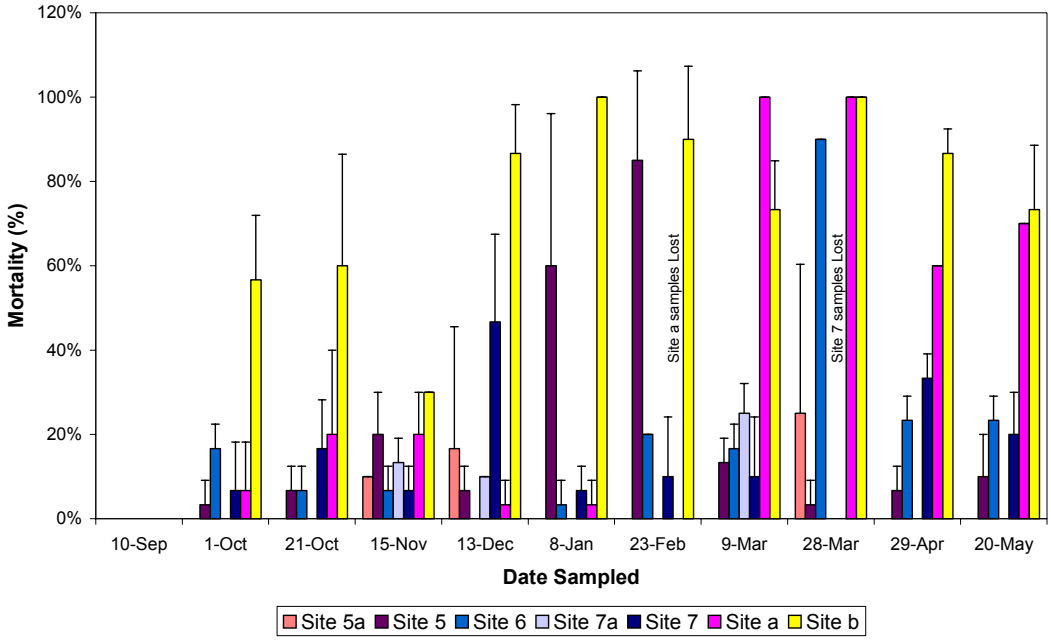


Figure 18. Mortality of the snail *Thiara ballonensis* in cages at sites 5a, 5, 6, 7a and 7 downstream of the Dawson Valley Irrigation area and at sites a and b on Gap Creek between September 2002 and May 2003. Error bars are standard deviations of the mean of three samples.

3.6 Data analyses and discussion

A gradient of pesticide concentration was present along the river between December 2002 and March 2003, as shown by endosulfan levels decreasing from site 6 immediately below Gap Creek, to site 7a and site 7 further downstream (Figures 6, 7 and 8). Regression of the six univariate biological variables against distance downstream over this period did not result in any of the regression coefficients being significantly different from zero (all $P>0.11$, $F_{1,8}<3.20$ and $R^2<0.2858$). When the biological indices were regressed against total endosulfan for the same sites over this period significant relationships were not found (e.g. for PET no Baetidae $P = 0.754$, $F_{1,8} = 0.1049$, $R^2 = 0.0129$). Inclusion of data from sites 5a and 5 for the same period in these regressions also resulted in a non-significant relationship ($P>0.462$, $F_{1,15}<0.567$, $R^2<0.0364$). And the same was true if data from all river sites and sampling times was included in the regression. There was no indication in any of these responses of non-linear responses to the concentration gradient. *Hence a gradient of impact on the macroinvertebrates was not detected with respect to the endosulfan gradient along the river.* This is likely due to the magnitude of the pesticide gradient not being high enough or possibly that the macroinvertebrate indices used (based on family level identification) were not sensitive enough to detect any significant change. More detailed study of the macroinvertebrate samples that have been collected may be warranted to check whether an effect may be present at the species level of identification.

Cluster analyses of the macroinvertebrate data showed that for taxa richness (data analysed as presence/absence), site b clustered separately from all of the other sites, none of which clustered together as site b did (Figure 19). Site a clustered mainly amongst the Dawson river sites 1 and 2, while the rest of the sites were intermingled in this analysis. The relationship between the sites is shown more clearly in Figure 20, for which data from each site were pooled. These results show that the composition of the macroinvertebrate data at site b was markedly different from that at the other sites. For the abundance data, a similar plot emerged, with site b in one cluster that included about half of the site a sampling times and the rest of site a times intermingled with sites 1 and 2 (Figure 21). Apart from some site 3 sampling times, the other river sites formed a large cluster at a similar level of about 55% (Figure 21). Averaging the abundance data showed this more clearly, with all sites apart from a and b and 1 and 2 forming a separate cluster (Figure 22). This was also apparent when data from sampling times were averaged for each of three seasons (Figure 23).

An initial *MDS (Multidimensional Scaling) analysis* based on all sampling times from all sites resulted in plots having a stress level of 0.25 for both the taxa richness and abundance data (e.g. Figure 24). Although the pattern in this figure needs to be interpreted with a great deal of scepticism, it appears to show that for the presence/absence data site b sampling times are grouped towards one side of the diagram, mostly outside the main central group containing most sites. When data from the different sampling times for sites were averaged for each season, the resultant stress level was reduced to 0.18 (Figure 25). In this plot site b is clearly distinct from those of the other sites, as it is when data were averaged for each site, resulting in a stress level of 0.05 (Figure 26). Site a is more similar to the other sites, particularly sites 1 and 2, the most upstream reference sites. These results indicate that the macroinvertebrate populations at site b, and to a lesser extent site a, are

distinct from those of sites along the Dawson River, most likely a reflection of differences between them in stream discharge and other variables including turbidity, detritus cover and possibly oxygen levels (Figure 6).

Pair-wise tests in the ANOSIM program in PRIMER using presence/absence and abundance data from each MDS showed there were many significant differences between the sites. Results using the presence/absence data were similar to those using the abundance data, with the latter generally finding more differences between the sites. The tests confirmed the significant difference (P<10%) between site b and all of the river sites, with all of the pair-wise R values of these comparisons exceeding 0.50 (Table 1). Many significant differences were found between the control sites and the other sites (e.g. 3 and 6), as well as between control sites (e.g. 3 and 4), though importantly, the R values for many of these comparisons were less than 0.5 (and several less than 0.25) indicating a degree of overlap between the pairs. Of interest is that although sites 5 and 6 (those immediately upstream and downstream respectively of where Gap Creek enters the Dawson River) are significantly different, the R value for this comparison was the lowest recorded for those sites that were significantly different (0.116, Table 1). Given the relatively higher magnitude of the differences between the other river sites, a significant effect of the entry of the drain into Gap Creek and then into the Dawson River is difficult to detect. The results indicate that the magnitude of the effect is not as high as that resulting in the differences between other sites. Sites a and b were significantly different (P=0.2%) but the R value for this comparison was only 0.175, indicating these sites were barely separable (see Figure 24 & 25). The differences between the control sites 1 and 2 and the other river sites may be related to the higher turbidity, or the tendency for oxygen levels to be slightly lower at sites 1 and 2 (Figure 6). The dendogram of the cluster analysis (Figures 20 & 22) showing the grouping of sites and the MDS based on taxa abundance (Figure 24) generally support these comparisons.

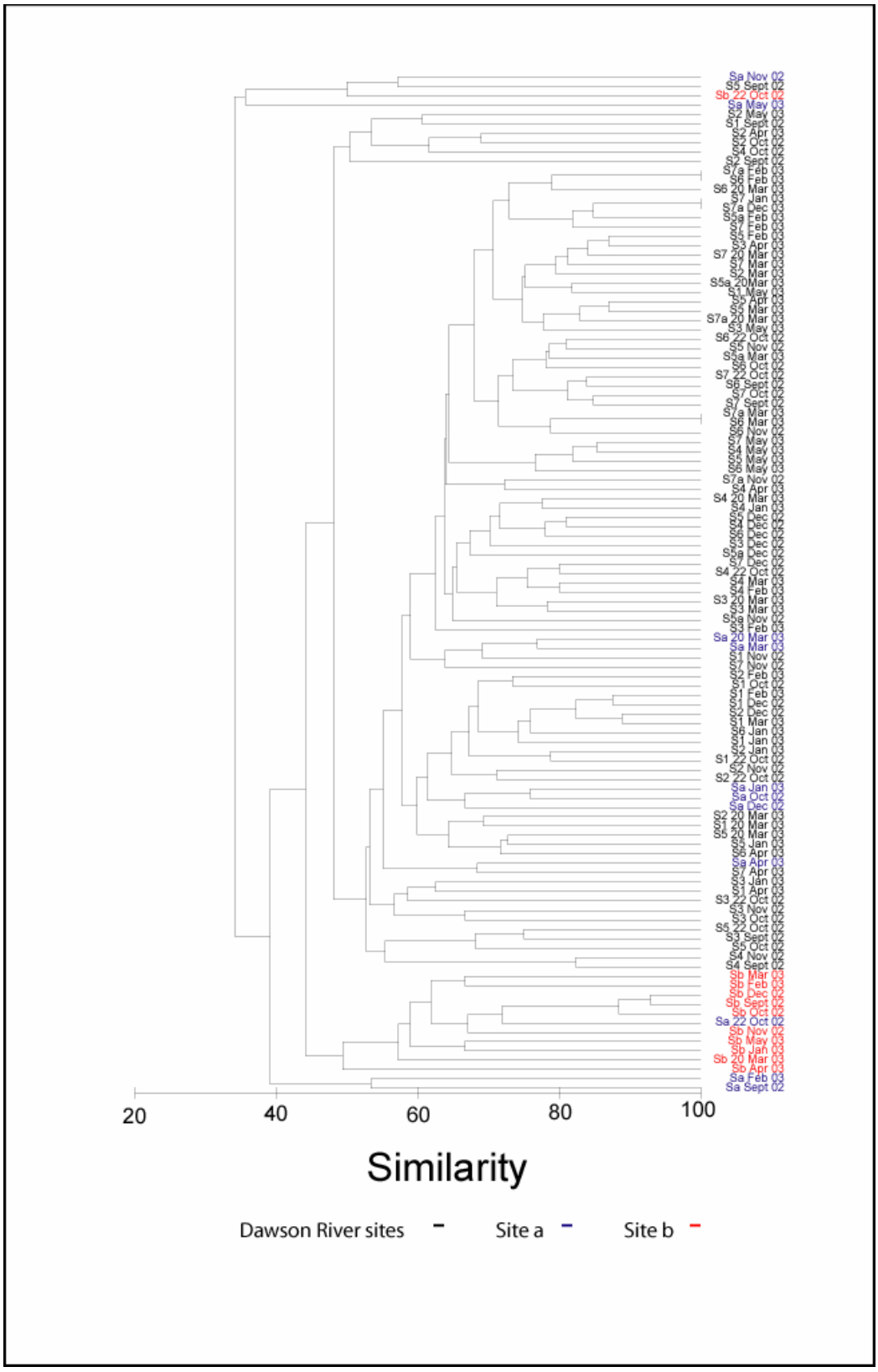


Figure 19. Cluster analysis using Bray-Curtis index of similarity with presence/absence data from all sites and sampling times.

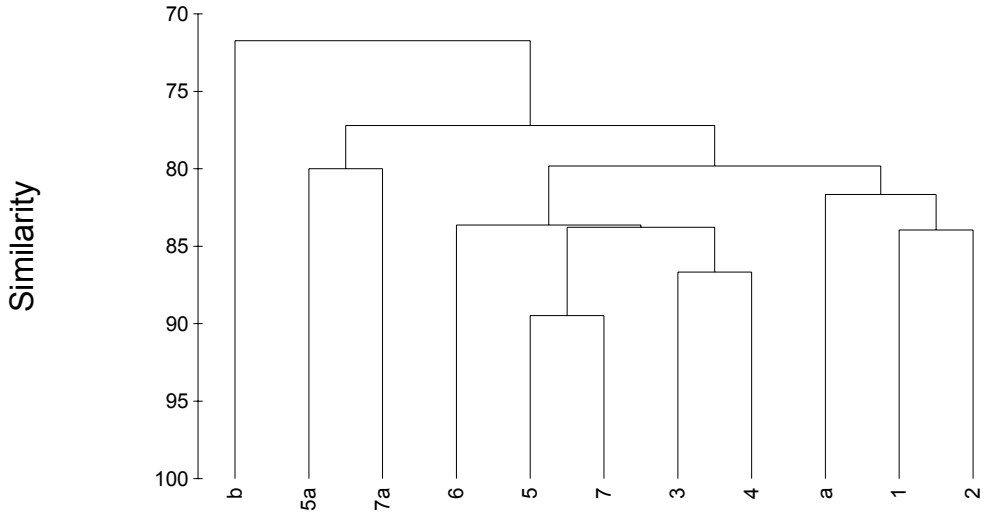


Figure 20. Cluster analysis using Bray-Curtis index of similarity based on presence/absence data averaged for each site.

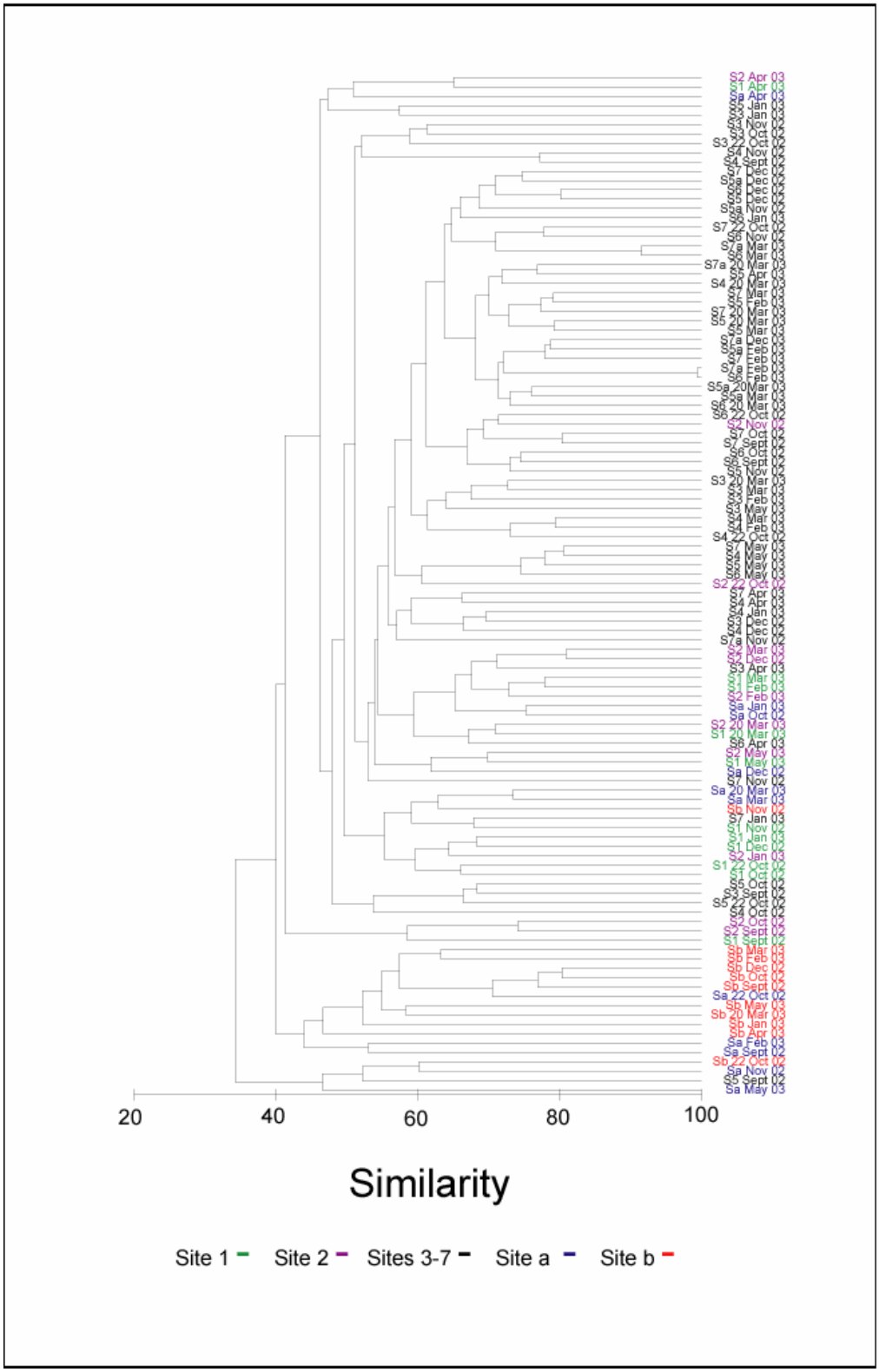


Figure 21. Cluster analysis using Bray-Curtis index of similarity with (log (x+1)) abundance data from all sites and sampling times.

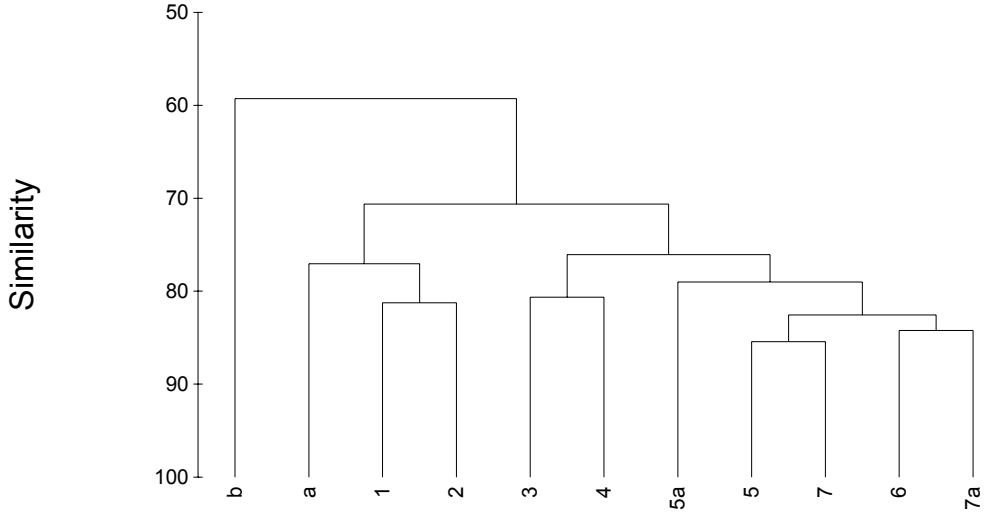


Figure 22. Cluster analysis using Bray-Curtis index of similarity of (log (x+1)) abundance data averaged for each site.

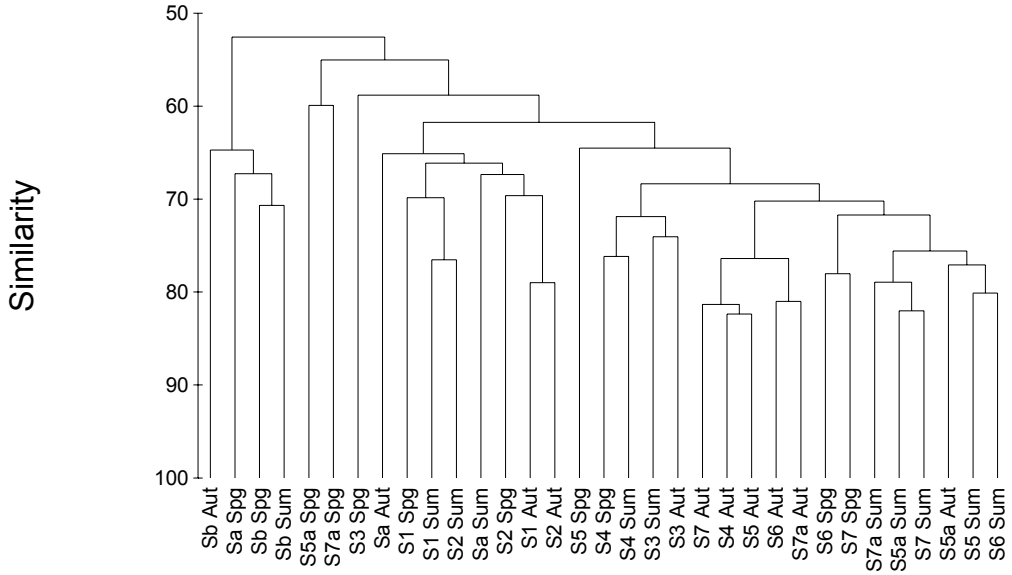


Figure 23. Cluster analysis using Bray-Curtis index of similarity of (log (x+1)) abundance data averaged for each of three seasons.

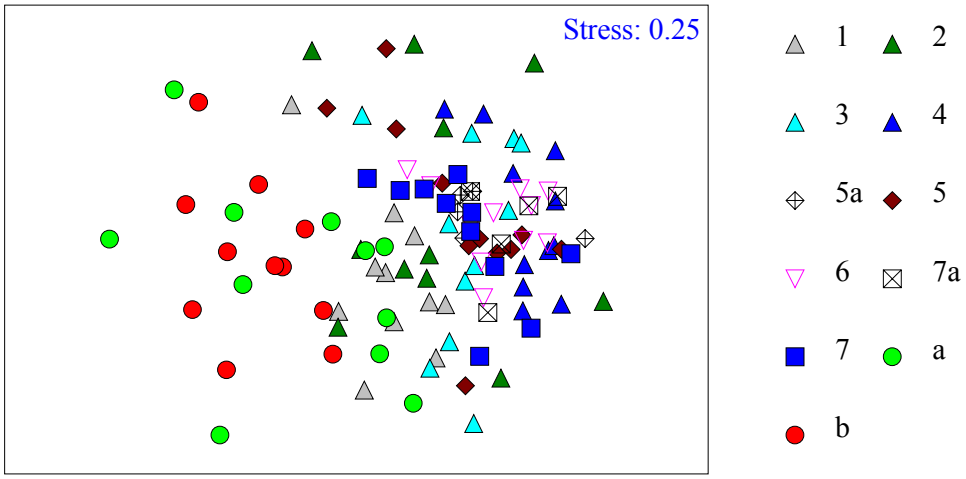


Figure 24. MDS using Bray-Curtis index of similarity with (log (x+1)) abundance data from all sites and sampling times.

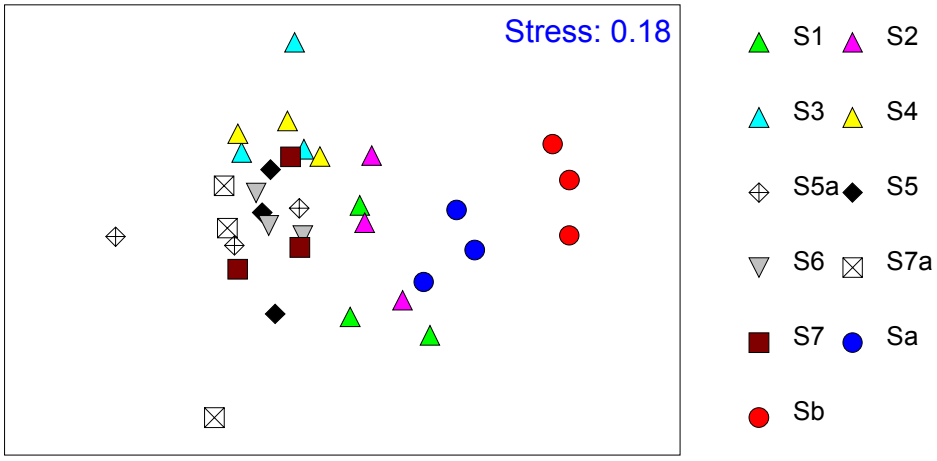


Figure 25. MDS using Bray-Curtis index of similarity with (log (x+1)) abundance data with data averaged for each of three seasons.

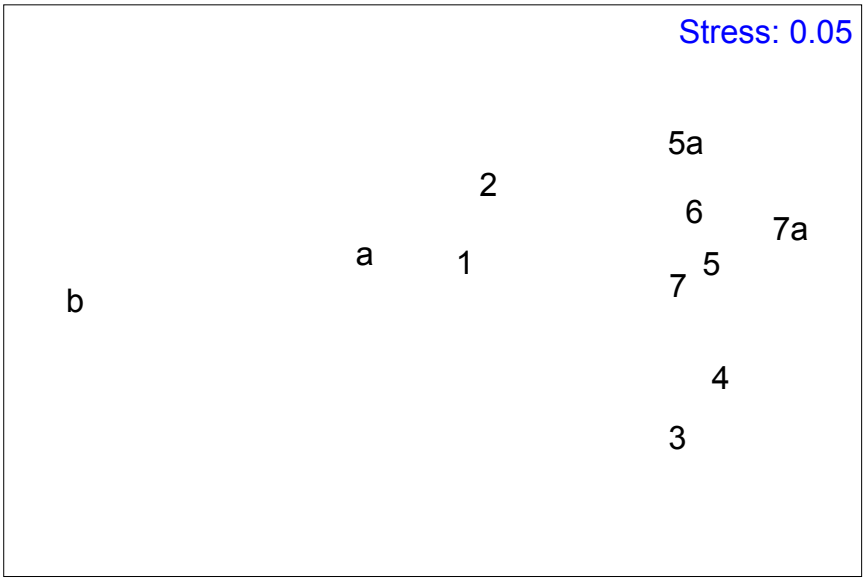


Figure 26. MDS of (log (x+1)) abundance data averaged for each site.

Table 1 Summary of the ANOSIM Pair-wise tests of the (log+1) macroinvertebrate abundance. Values in the table represent the probability (%) that the null hypothesis of "no difference between sites" is true, with pair-wise R values below each probability. Values of R >0.75 indicate sites are well separated, values >0.5 and <0.75 indicate sites are overlapping but clearly different. Global R = 0.349.

	Site 1	Site 2	Site 3	Site 4	Site 5a	Site 5	Site 6	Site 7a	Site 7	Site a
Site 1										
Site 2	37.8 (.011)									
Site 3	0.1 (.434)	0.1 (.375)								
Site 4	0.1 (.564)	0.1 (.486)	0.6 (.174)							
Site 5a	10.4 (.182)	5.0 (.195)	5.6 (.221)	7.1 (.18)						
Site 5	0.1 (.269)	0.2 (.264)	0.5 (.202)	2.4 (.127)	68.4 (-.079)					
Site 6	0.1 (.442)	0.1 (.415)	0.1 (.533)	0.1 (.395)	41.4 (.03)	3.0 (.116)				
Site 7a	0.1 (.443)	1.9 (.347)	0.5 (.363)	4.4 (.211)	54.0 (-.008)	38.4 (.019)	11.3 (.155)			
Site 7	0.1 (.284)	0.1 (.36)	0.1 (.293)	0.7 (.199)	74.6 (-.103)	32.2 (.015)	13.9 (.059)	61.4 (-.052)		
Site a	1.2 (.149)	0.2 (.245)	0.1 (.485)	0.1 (.638)	4.3 (.269)	0.1 (.356)	0.1 (.575)	1.2 (.354)	0.1 (.371)	
Site b	0.1 (.638)	0.1 (.681)	0.1 (.811)	0.1 (.9)	0.1 (.759)	0.1 (.728)	0.1 (.889)	0.2 (.801)	0.1 (.708)	0.2 (.175)
Number below p (p=0.1%)	7	5	4	3	1	2	2	0	2	0
Number below p (p=1%)	7	7	7	6	1	2	2	1	2	1

3.6.1 What is the magnitude of the effect that the water from the drain has on the macroinvertebrate assemblage in the river?

Based on the results of the above analyses, which broadly compare the composition of the macroinvertebrate assemblages between all sites, it is difficult to detect an effect of water from the drain on macroinvertebrates in the river because of the high background variability between sites along the river. Only the ANOSIM analysis indicated that the composition changed in the river between sites 5 and 6, the entry point for Gap Creek that receives water from the drain. When taken in the context of the high background variability in composition between sites (including control sites) along the river, as well as the magnitude of the differences compared to that between sites 5 and 6, the results suggest that the magnitude of the effect is not as great as the differences between other sites (including control sites) that were significantly different. Essentially, the effect was not detectable. This is supported by the lack of apparent change in the univariate indices taxa richness and "PET no Baetidae" as well

as the lack of a gradient of impact along the river from site 6 to site 7 (from the regression analyses). Hence*, based on analyses of both univariate and multivariate parameters, significant effects of runoff from the irrigation area into Gap Creek on macroinvertebrate populations in the river essentially could not be detected.*

If it can be accepted that changes in the macroinvertebrate assemblages *along the river* in the area where pesticides have been recorded are small compared to the differences between other sites, then it appears that environmental factors other than pesticides are more important than pesticides as determinants of changes in invertebrate assemblages. Hence to address the question – to determine the relative significance of pesticides and other factors in explaining changes to invertebrate assemblages - data from all sites, particularly site b, were included in the analyses. Inclusion of information from site b was of interest to maximise the chances of determining the significance of pesticides relative to other factors, since pesticide concentrations were higher at site b than any other site, and the more sensitive macroinvertebrate taxa were largely absent from this site (Figure 16).

The first step in this analysis was a correlation matrix between all environmental and biological parameters (Appendix 2) (and bivariate scatter plots to investigate their relationships). Significant negative correlations were found between beta endosulfan, endosulfan sulphate and total endosulfan and the biological indices PET, "PET no Baetidae", SIGNAL2 and SIGNAL2 graded. From this matrix, the variables significantly ($P<0.05$) correlated to the univariate biological indices were run through a *multiple linear regression* and these explained a maximum of 21.7% of the variation in the biological index SIGNAL2 graded (Table 2). Hence the predictive power of the environmental variables was quite poor and likely related to the range of levels in the environmental gradients being too small to produce significant changes in the biological indices.

Table 2. Biological variables and environmental predictors used in multi-linear regressions analyses

Dependent variable	Independent variable	R Square
Taxa richness:	pH	0.0797
Abundance:	DO, Secchi depth	0.103
Pet index:	Secchi depth, endosulfan sulphate	0.155
PET (no Baetidae)	Secchi depth, endosulfan sulphate	0.202
SIGNAL2	DO, endosulfan sulphate, min. discharge	0.194
SIGNAL2 graded	DO, endosulfan sulphate, min. discharge	0.217

Prior to using the BIOENV procedure (Clarke and Warwick 1994) to further investigate the significance of various environmental parameters to biological data, two PCA analyses were run to examine the environmental factors accounting for most of the variation between sites and sampling times. In the first, all environmental parameters not significantly correlated with each other were used (Figure 27). The first two principal components explained 37.3% of the variation in the data and the highest factor scores for these axes included maximum discharge, Secchi depth/turbidity, discharge > 200ML/day, detritus and discharge divided by time since

that discharge (Table 3). Notably, site b sampling times were not distinct from those of other sites in this figure. Hence in the second PCA only those environmental parameters that were significantly related to the biological indices were used (Figure 28). For this Figure, the first two principal components explained 55.7% of the variability in the data and the environmental variables with the largest factor scores included Secchi depth/turbidity, detritus, minimum discharge, dissolved oxygen and pH. The largest factor score on the third principal component (which explained a further 14.2% of the variability) was endosulfan sulphate (Table 4). In this figure several of the site b sampling times are located towards the lower left hand side of the diagram and are separated from the others largely on the basis of high Secchi depth and high detritus as well as low minimum discharge. This provides evidence that factors other than pesticides are important in the biological differences between site b and the other sites.

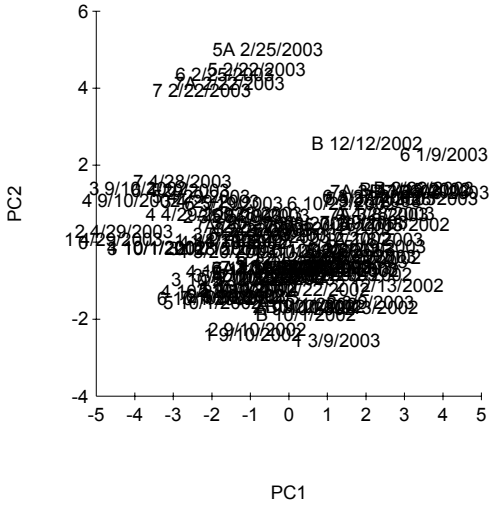


Figure 27. PCA of all measured environmental parameters not significantly correlated with each other

Table 3. Using all variables that were not highly correlated (product moment correlations of pair-wise comparisons not greater than 0.95).

Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	3.62	24.1	24.1
2	1.97	13.2	37.3
3	1.77	11.8	49.1
4	1.40	9.3	58.5
5	1.12	7.5	66.0

Table 3 (continued)
Eigenvectors
(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3	PC4	PC5
Temp	0.219	0.309	0.092	-0.064	-0.175
DO	-0.191	-0.011	0.588	-0.145	0.033
EC	0.244	0.238	0.223	0.077	-0.278
pH	0.010	-0.141	0.434	-0.359	0.068
Secchi	0.346	0.313	0.006	-0.218	0.013
Turbid	-0.352	-0.371	-0.046	0.156	0.018
Velocity	-0.170	0.021	-0.154	0.185	-0.559
Detritus	0.315	0.091	-0.078	-0.021	0.440
sulphate	0.165	0.140	0.137	0.569	0.249
Chlorpyr	0.063	0.010	0.343	0.629	0.013
Max Disch	-0.409	0.332	-0.054	-0.020	-0.015
Min Disch	-0.313	-0.032	0.257	-0.007	0.352
Disch/t since flow	-0.266	0.479	0.009	-0.075	0.218
Disch200	-0.330	0.438	-0.197	0.082	0.106
Moved Sample	0.095	-0.176	-0.361	0.032	0.367

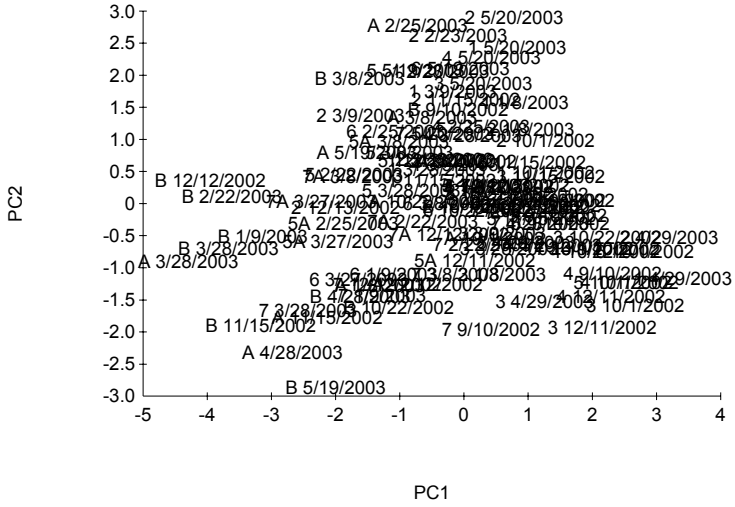


Figure 28. PCA of environmental parameters significantly correlated to the biological indices.

Table 4. Using only those variables that were significantly correlated to the biological indices

Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	2.50	35.8	35.8
2	1.39	19.9	55.7
3	0.99	14.2	69.9
4	0.85	12.1	82.0
5	0.67	9.5	91.5

Eigenvectors
(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3	PC4	PC5
DO	0.328	-0.572	-0.132	0.147	-0.163
pH	0.146	-0.615	0.094	-0.596	-0.156
Secchi	-0.512	-0.327	0.237	0.260	0.011
Turbid	0.516	0.317	-0.176	-0.323	0.092
Detritus	-0.388	-0.106	-0.313	-0.431	0.685
sulphate	-0.231	-0.028	-0.862	0.081	-0.395
Min Disch	0.373	-0.272	-0.212	0.508	0.562

Since the pattern obtained by the MDS of the biological data from all sampling times had a high stress level, the BIOENV procedure was run on the abundance data averaged for each season (MDS stress level 0.18) and then those averaged for each site (MDS level 0.05). For the data based on season, the best combination of variables to explain the biological data was the combination of maximum discharge and endosulfan sulphate, with a weighted Spearman rank correlation ρ_w value of 0.423. Higher correlations were obtained with the data averaged for each site. For this analysis results showed that the single abiotic variable that best described the biological data was maximum discharge (ρ_w =0. 749), followed by discharge divided by the time since that discharge (ρw =0.672) and discharge > 200 ML/day (ρ_w =0.630) (Table 5). The best 2-variable set was discharge/time since discharge and endosulfan sulphate (ρ_w =0.835), with discharge/time since discharge and discharge >200 ML/day next at ρ_w =0.832. The optimum variable set however was one with 3 variables - discharge/time since discharge, discharge >200 ML/day and endosulfan sulphate with a ρ_w value of 0.90 (Table 5).

When the data on taxa richness (presence/absence) averaged for each site was run through the procedure a similar result was obtained, though the optimum combination of variables that best explained the biological data comprised 6 variables. These were (in order of descending importance): Maximum discharge, discharge/time since that discharge, endosulfan sulphate, detritus cover, temperature and minimum discharge. The maximum correlation value obtained for this combination was ρ_w =0.845.

Table 5. Best combinations of variables explaining the biotic community structure (k= number of variables in combination) using the BIOENV procedure based on abundance data averaged over all sampling times and environmental parameters measured during the study.

k	ρ_w
1	maximum discharge (.749), discharge/time since discharge (.672), discharge >200ML/day (.630)
2	discharge/time since discharge, endosulfan sulphate (.835), discharge/time since discharge, discharge>200ML/day (.832)
3	**discharge/time since discharge, discharge>200ML/day, endosulfan sulphate (.907)**
4	discharge/time since discharge, discharge>200ML/day, endosulfan sulphate, maximum discharge (.893)
5	discharge/time since discharge, discharge>200ML/day, endosulfan sulphate, maximum discharge, minimum discharge (.885)

3.6.2 What is the relative significance of pesticides compared to other environmental parameters (such as reduced oxygen levels and river discharge) on changes to macroinvertebrate communities?

To summarise the results above to address this question, the analyses above suggest that endosulfan sulphate is one of the top three variables that best explain the changes in the macroinvertebrate communities on a broad scale. Discharge parameters, notably discharge/time since that discharge and discharge >200 ML/day resulted in higher correlations than pesticides using the BIOENV procedure. Temperature and detritus cover are also of significance to these communities, but are not as highly correlated with the biological data as the above flow and pesticide variables.

3.6.3 How does the relationship between pesticides and effect on the assemblage change with distance down the river?

A gradient of impact on the macroinvertebrate assemblage was not detected along the Dawson river in relation to pesticides (section 3.6), presumably either because the pesticide gradient was not of significance or because the biological parameters measured were not sensitive enough to detect any impact. In contrast, differences between sites a and b were found by the ANOSIM analyses and were quite clear in the cluster analyses and the MDS. Perhaps the most significant difference was the absence of the most pollution sensitive taxa from site b over the entire study period (except for one occasion), while site a had at least one member of these types of animals present on most sampling occasions (Figure 16). Hence the question is perhaps best addressed in relation to the effects measured in Gap Creek.

Since a reduction in the number of sensitive taxa was not observed for site 6, the site immediately downstream of Gap Creek on the river, this suggests that for Gap Creek at least, the effect is limited to the distance between site b and site 6 – approximately

3 km. It is suggested that processes along this creek such as adsorption of pesticides onto sediment and organic material are important in the reduction of the effect to non-detectable levels. Differences between levels of pesticides at site b and those at site 6 were high – a 10-fold reduction in concentration was observed during this study and a similar reduction was noted during the pilot study in the previous irrigation season (Appendix 1).

3.6.4 What is the rate of recovery of the assemblage from the effects of disturbance?

Effects of disturbance related to pesticides were not present or not detectable at any sites along the river. At site b, where sensitive macroinvertebrate taxa were largely absent, there were no apparent changes over time in the macroinvertebrate indices (e.g. Figures 13, 15 and 16) and signs of recovery as might be indicated by the reappearance of sensitive taxa at the site were not found. Hence owing to the lack of an effect gradient along the river and only a single "impacted "site, conclusions about the rate of recovery from effects of disturbance related to pesticide exposure are difficult to make. Since "recovery" of sensitive species at site b was not observed, it appears this may take longer than the period of this study, particularly if low levels of endosulfan are present for extended periods of time as was the case for site b (Figure 10). Contamination of the sediments with pesticides as observed at site b in this study may be important to this recovery.

4 Risk Assessment

The first step in the risk determination stage of the ERA process (Figure 1) was to produce risk quotients for the pesticides studied in the Dawson Valley Irrigation Area. A risk quotient may be calculated as the measured values of pesticides divided by an appropriate "effect concentration" such as the relevant ANZECC and ARMCANZ (2000) trigger value or LC_{50} of a pesticide for the system being studied. Results of these calculations for endosulfan sulphate using the 99% trigger value recommended by ANZECC and ARMCANZ (2000) for this pesticide for slightly-moderately disturbed systems are provided in Figure 29. This shows that the only site and times that the risk of pesticide exposure is greater than one was site b for the period 11 November to 22 February. Errors involved in these estimates are expected to be relatively small based on the error bars of the estimated levels of the pesticide (Figure 9). Of pesticides used in the pest management strategy for cotton, endosulfan is ranked as having the greatest impact on the aquatic system (Leonard *et al.* 1999).

For chlorpyrifos, estimated concentrations in water did not exceed the ANZECC and ARMCANZ (2000) recommended 95% trigger value of 0.01 μg/L and hence the risk quotient for this pesticide did not exceed 1.0 at any site studied.

Levels of DDT in water could not be estimated from concentrations in the passive samplers because an equation relating the entry of this chemical into the TRIMPS (Trimethylpentane samplers) and time deployed is not yet available. However, if we assume that the uptake kinetics of DDT is similar to that of trifluralin (based them having similar K_{ow} values (5.8 vs 5.2 respectively), and the equation for this pesticide is used to estimate time-integrated concentrations in the water, then risk quotients for DDT would not exceed one at any site. Water samples collected during the flow event

in February at Gap Creek revealed that DDT levels ranged from <0.01 µg/L (detection limit) up to 0.10 µg/L with an average of 0.038 µg/L during the first 12 hours of the event. The relevant trigger value for DDT is 0.006 µg/L and hence the risk quotient during this time would on average have been about 6.3. A comparative value for endosulfan for this same period is about 33.3. This supports the idea above that the risk quotient for DDT would not exceed one at any site based on time-integrated measurements and provides some confidence in this risk assessment.

Many of the other pesticides measured in this study were not detected. These included the relatively new pesticides emamectin and spinosyn, the pyrethroids deltamethrin, cypermethrin and cyhalothrin and profenofos.

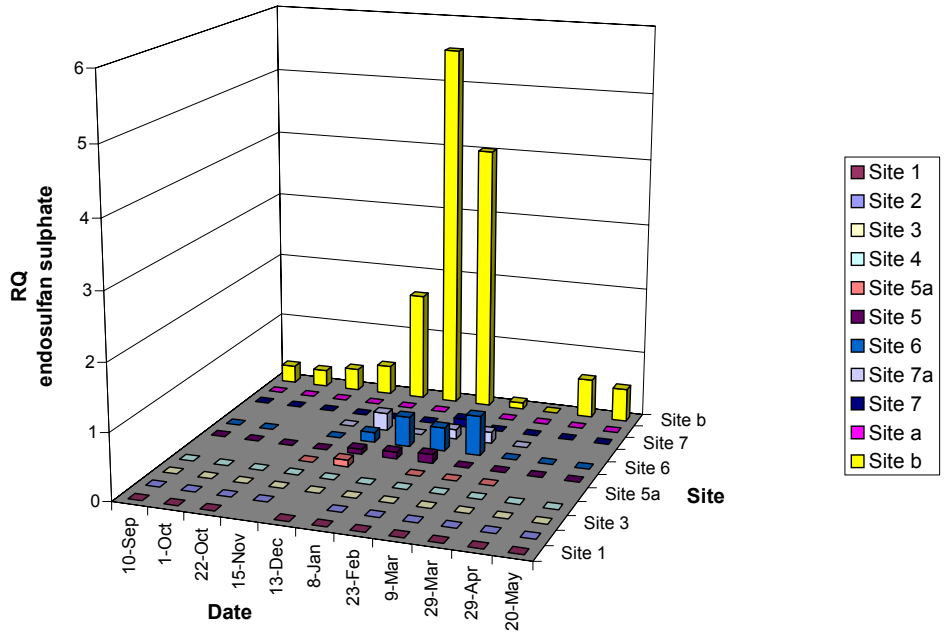


Figure 29. Risk Quotient for the estimated concentration of endosulfan sulphate in water, calculated using the 99% ANZECC and ARMCANZ (2000) trigger value (0.03µg/L) recommended for slightly-moderately disturbed systems.

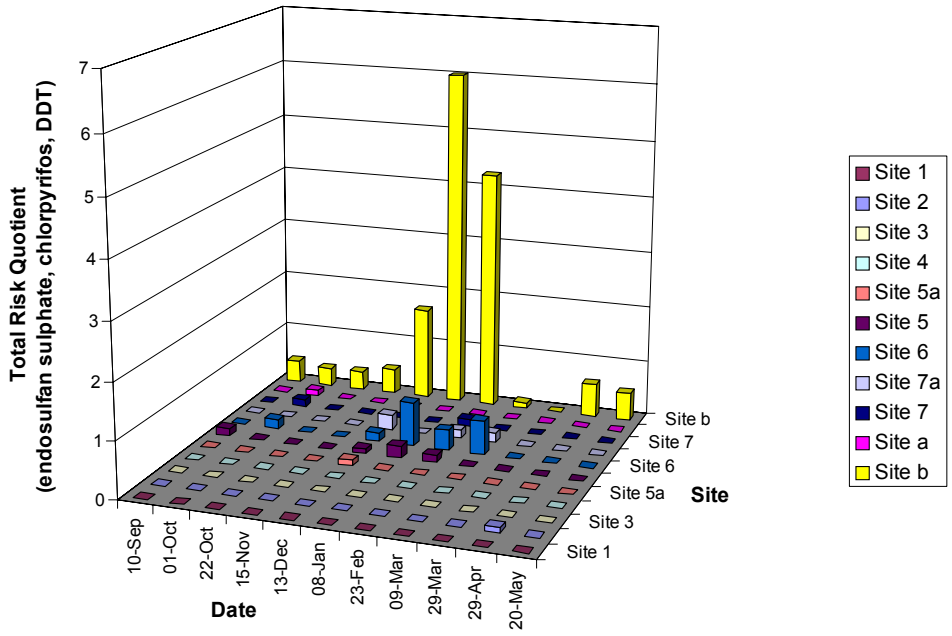


Figure 30. Total Risk Quotient for the estimated concentration of endosulfan sulphate, chlorpyrifos and DDT in water, calculated using the relevant ANZECC and ARMCANZ (2000) trigger values recommended for slightly-moderately disturbed systems as effect concentrations.

To determine a total risk quotient for sites in the study, the Risk Quotients for endosulfan sulphate, chlorpyrifos and DDT were summed, assuming additive effects of these pesticides. The basis of this assumption is that there is evidence that even for those chemicals that have different modes of action their interactions are commonly additive or near additive (USEPA 1998). This increased the risk quotient for some sampling times, but did not increase the level to above 1 for any more sites than had been found already with respect to endosulfan (Figures 29 and 30).

From this analysis, the outcome is that risk to macroinvertebrate assemblages associated with the pesticides measured in this study is likely to be low for the Dawson River over the 40 km length below Theodore weir. In contrast, the risk is higher for site b on Gap Creek, with risk quotients exceeding one for the 11 November to 22 February period.

When this risk is compared to the actual data on macroinvertebrate populations, the lack of any significant effect on macroinvertebrates along the river (Section 3.6.1) provides confidence in this risk assessment. The confidence of the risk assessment for sampling times within Gap Creek is not as high, since although sensitive taxa data (the "PET no Baetidae" index) provide evidence that macroinvertebrates are impacted at this site compared to the control site a upstream, this effect is not restricted to the months of December to February, when the risk quotients for this site were greater than 1. However the fact that effects did occur at site b provides further confidence in the risk assessment, even though the risk quotients <1 outside the December to February period did not match the presence of an effect on the macroinvertebrates. Factors other than pesticide concentrations may have affected macroinvertebrate

assemblages outside the December to February period. It is likely that these factors together with the risk associated with pesticide concentration (RQs <1 in Figure 30) may have resulted in the lack of sensitive taxa at site b. Also, there may have been a time lag effect, macroinvertebrates in the stream after February may still have been recovering from the pesticide levels recorded over the December to February period, and effects prior to December may have been the result of pesticide exposure from the previous summer irrigation period (that is, they may take a long time to recover).

Apart from the factors measured in this study that distinguish site b from the river sites and hence may explain why the site is different biologically, other unmeasured factors may also be involved. For example some evidence for sediment contamination by pesticides was found and this may be an important factor explaining the virtual lack of sensitive taxa at the site. Conclusions however are difficult to draw given that there was only one site in this study that had high levels of pesticides in both sediments and the water column. In hindsight, it may be useful in future studies to initially assess the level of contamination in sediments as a prelude to site selection for a design in which a range of pesticide exposures is to be examined.

The nature of the effects seen at site b – the loss of the most sensitive types of macroinvertebrates – is cause for some concern and may be worthy of further investigation, depending on interest of stakeholders. To put the results into a regional perspective, the intensity of the effects are not as extreme as the more than 80% reduction in total taxa richness at the most polluted sites along the Dee River at Mount Morgan, the result of acid mine drainage (Duivenvoorden 1995). Important too is the extent of the effects: this study suggests that the effects do not extend down to site 6 on the Dawson river, some 3 km downstream.

With respect to the extent of the effects, of interest is how effectively flows in the Dawson River might dilute pesticide concentrations coming from Gap Creek. The worst-case scenario might be that flows from the irrigation area entered Gap Creek and there was no corresponding flow in the Dawson to dilute any pesticide concentrations that may be present. This is more likely to occur when water levels in the Theodore weir are low. A scenario similar to this occurred during the February 2003 flows in this study, as described in section 3.1. The concentration of pesticides in the Dawson river can be predicted using a mass-balance approach if the relative concentrations and discharge volumes of Gap Creek and the Dawson upstream of Gap creek are known. One example of such predictions is shown in Figure 31. For this figure, maximum endosulfan concentrations in Gap Creek during the February 2003 flows and values estimated via the TRIMPS for site 5 for the Dawson River were used, along with the discharge information gained in this study. Hence for the figure endosulfan levels used were 0.65µg/L and 0.0039 µg/L in Gap Creek and the Dawson upstream of Gap Creek, respectively. **From this figure it is clear that the Dawson has a high diluting capacity and this will act to reduce the risk of high pesticide concentrations downstream of Gap Creek, even under a near worst-case scenario.**

As discussed in section 3.6.5 the potential recovery from the effects on macroinvertebrates is difficult to assess from the information in this study. The recovery of the macroinvertebrate assemblages at site b (to a state at least similar to that at site a) may occur following a very high flow event in this system. Such an

event would need to be of sufficient magnitude to scour the system of much of its detrital material and this may also decrease the amount of contaminated sediment at the site. Sufficient time then needs to elapse for more sensitive taxa to recruit to the site. Without specific information on the life-history strategies of such taxa, predicting the time to recovery is not really possible. Again, further investigation following such an event may provide useful information on recovery processes for macroinvertebrates in this system.

Putting the results of this study into an overall catchment perspective suggests that risks to macroinvertebrate assemblages in relation to pesticides are only of highly localised importance. Information from this study, notably based on only one site with high pesticide levels, suggests that in the Dawson Valley Irrigation area effects become non-detectable within 3 km of where runoff from irrigated areas enters streams. Critical to this proposal is that there was an observed 10 fold decrease in pesticide concentrations over this distance along Gap Creek in two successive irrigation seasons. Further work, incorporating other sites within areas close to where runoff from irrigation enters streams, is required to increase the confidence of this assessment.

To complete this risk assessment process, further communications with stakeholders are required, as detailed in the flow chart of the process currently being undertaken in the Fitzroy catchment (Figure 1). Most importantly, appropriate management of the risks by irrigators will ensure that risks to the ecological health of the stream systems are minimised.

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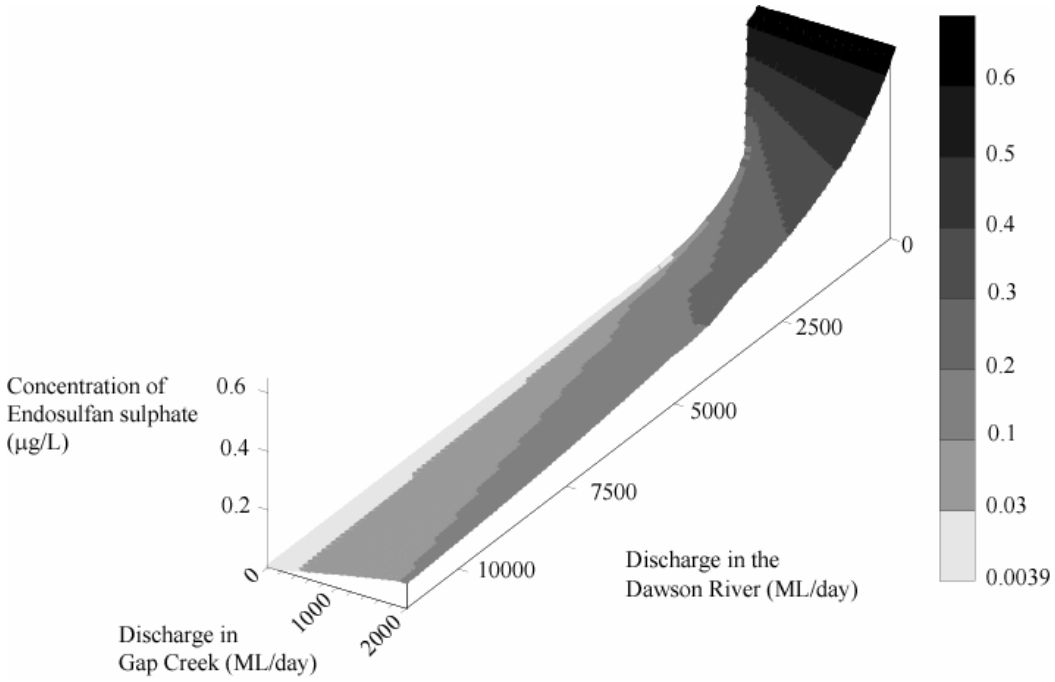

Figure 31. Predicted values of endosulfan sulphate in the Dawson River downstream of Gap Creek for various discharge rates based on concentrations of 0.65 µg/L in water in Gap Creek and 0.0039 µg/L in the Dawson river upstream of the creek.

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Appendix 1 of Final Technical Report for NPSI Project UCQ3

Ecological risk associated with irrigation: using passive samplers, direct toxicity tests and macroinvertebrate assemblages in the Dawson Valley Irrigation Area, Central Queensland, Australia – a pilot study.

Appendix 2

Correlation matrix of all biological and environmental parameters measured at nine sites along the Dawson river and at two sites on Gap Creek. Note: Shaded areas indicate a negative correlation

	DO (% saturation)	Conductivity	pH	Turbidity	Current speed	Detritus Cover	Richness	Abundance	PET	alpha endo	beta endo	endo sulfan	Total endo	Chlorpyrifos	Secchi Depth	No Baetidae PET Index	SIGNAL 2	SIGNAL 2 Graded	Av Disch	Max Disch	Min Disch	Disch/t since flow	Disch200	Disch200/t since flow	Moved Sample
Temperature		2E-05		0.008		0.001									0.01					0.042	4E-05		0.0442		
DO (% saturation)			1E-05					0.01									5E-04	0.009							
Conductivity				2E-04										0.0032	0.003						0.035				
pH							0.003	0.004									0.026								
Turbidity					7E-04	0.002		0.008							6E-11						0.014				
Current speed						0.005													0.022						
Detritus Cover									0.006	0.03	0.0105	0.0062	0.0063		0.001	8E-04	0.003	9E-04	5E-04		0.399				
Richness								8E-04	5E-14							2E-11									
Abundance															0.013										
PET											0.0394	0.0127	0.0182		3E-04	1.E-66	2.E-11	1E-10							
alpha endo											1.E-63	4.E-23	3.E-34	7.E-13				0.005							
beta endo												2.E-38	3.E-57	3.E-11		0.02	0.044	0.001							
endo sulfan													5.E-86	5E-06		0.005	0.025	7E-04							
Total endo														5E-08		0.008	0.028	6E-04							
Chlorpyrifos																									
Secchi Depth																2E-05			0.044		0.028				
No Baetidae PET Index																	1.E-12	5E-11			0.031				
SIGNAL 2																		4.E-34			0.004				
SIGNAL 2 Graded																					0.002				
Av Disch																				2E-15	1.E-05	1.E-12	2.E-15	2.E-13	
Max Disch																						4.E-31	7.E-174	2.E-32	
Min Disch																						6.E-04		0.005	
Disch/t since flow																							4.E-31	3.E-87	
Disch200																								1.E-32	
Disch200/t since flow																									

Appendix 1 of Final Technical Report for NPSI Project UCQ3

Ecological risk associated with irrigation: using passive samplers, direct toxicity tests and macroinvertebrate assemblages in the Dawson Valley Irrigation Area, Central Queensland, Australia.

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ABSTRACT

A pilot study was conducted during the 2001/02 cotton irrigation season in the Fitzroy Catchment in Central Queensland in order to assess the levels of pesticides entering local waterways and the potential impact of this pesticide contamination on macroinvertebrate communities inhabiting them. A number of new methodologies were adopted during this study, including the use of trimethlypentane-containing passive samplers (TRIMPS) for the continuous detection of pesticide concentrations and the use of caged shrimp for complementary field direct toxicity tests. Endosulfan and profenofos compounds were detected in high levels during the study, with trends in levels in water estimated from concentrations in the TRIMPS found to be consistent with those found in water grab samples. Macroinvertebrate richness did not vary markedly during the course of the study, but may have been influenced by runoff from irrigated areas prior to the commencement of the study. Further information on these communities prior to the first post-winter flows is required to adequately determine the magnitude of potential impact on these communities. 100 % mortality of *Macrobrachium intermedium* during *in situ* direct toxicity tests at two heavily contaminated sampling sites suggest contamination by pesticides may have been responsible, but moderately high mortality at reference sites indicate that more detailed investigation of factors other than pesticides is warranted.

Extra keywords: ecotoxicity, endosulfan, passive samplers, profenofos, macroinvertebrates, semi-permeable membrane devices, SPMDs, *Macrobrachium*.

INTRODUCTION

The Dawson Valley Irrigation Area (DVIA) centred near Theodore in Central Queensland is a predominately cotton growing region and as such, crops grown in the area require the application of multiple pesticides for the effective control of the *Heleothis* boll predator. Pesticides commonly used include organochlorines (e.g. endosulfan, others) early in the season (December) followed by application of organophosphates such as profenofos and Dominex in later months (January - February). The present study, which forms part of an ecological risk assessment project being undertaken for the National Program for Sustainable Irrigation (NPSI), focussed on measuring levels of pesticide contamination in local waterways in the DVIA, and exploring the potential for negative environmental impacts resulting from this contamination on macroinvertebrate communities. The DVIA was considered a region with a high likelihood of producing adverse ecological effects given that three major drains allow potentially contaminated runoff to enter the Dawson River directly, despite the considerable efforts of local farmers to reduce this runoff by pumping from these drains back onto farms.

Macroinvertebrate populations play vital functional roles in aquatic systems. However, due to their habitat in benthic sediments, many macroinvertebrate populations are located where high concentrations of pesticides residues such as endosulfan accumulate due to a strong pesticide distribution bias towards the sediments (Leonard *et al*. 1999; Peterson & Batley 1993). Not surprisingly, highly mobile taxa may appear to have increased resistance to pesticide contaminants as they can escape areas where high levels accumulate. The toxic impacts of pesticides, particularly endosulfan, on aquatic communities potentially include direct mortality to

macroinvertebrates (Woods *et al*., 2002; Lombardi *et al*., 2001) and to fish (Cengiz & Ünlü, 1999; Sunderam *et al*., 1992). A wide range of sublethal effects are also apparent, including histopathological alterations to gills in fish and invertebrates (Cengiz & Ünlü, 2002; Bhavan & Geraldine, 2000), reduced population densities (Leonard *et al*., 2000; Leonard *et al*., 1999; Hyne *et al*., 1998), larval emergence (Schulz & Liess, 1995), and tadpole survivorship (Broomhall, 2002), change in community structure (Barry & Logan, 1998), reduced or changed growth and reproductive capabilities (Wirth *et al*., 2002; Wirth *et al*., 2001; Barry, 1996) and increases in macroinvertebrate drift (Hose *et al*., 2002; Brooks, 1999; Davies *et al*. 1994). Recently, the immunosuppressive effects of organochlorines on fish have also been noted (Galloway & Handy, 2003). The acute and chronic toxicity of endosulfan, along with its potential for bioaccumulation was comprehensively reviewed by Naqvi & Vaishnavi (1993). The authors reported endosulfan to be capable of being absorbed via inhalation, ingestion and dermal routes in marine and freshwater animals. They also identified several factors influencing the toxicity of endosulfan to aquatic organisms, including temperature, salinity and life stage and the type of bioassay technique used to determine toxicity (Naqvi & Vaishnavi, 1993). This multitude of effects indicates a clear need to determine the possible wider ecological effects associated with pesticide contamination in freshwater aquatic environments. As much toxicity testing has been carried out via laboratory based ecotoxicity studies, reports of *in situ* studies are particularly valuable. This study aimed to investigate the possible effects of combination pesticide exposure in a field environment.

In addition, as part of this study, new methodology for the continuous detection of pesticides in waterways using passive samplers was trialed. The use of semi-

permeable membrane devices (SPMDs) for the continuous measurement of pesticides in aquatic environments is a relatively newly-established methodology, but has already been adopted successfully in other parts of Australia and elsewhere (e.g. Leonard *et al*., 2002; Leonard *et al*., 2000; Petty *et al*., 2000; Muschal, 1999). These devices generally involve the use of polyethylene bags containing small amounts (~ 10mL) of trimethlypentane, which are then often enclosed in a protective nylon mesh bag to prevent tearing and loss of solvent from the exposure chamber. Passing toxicants in the water diffuse through the polyethylene membrane and are absorbed in the solvent, which is later withdrawn and analysed for toxicant concentrations (Sabaliūnas & Södergren 1996). The development and use of such collection devices is aimed at circumventing problems faced by using hand-grab sample collections, which primarily include difficulty in capturing peak pesticide concentrations, especially during peak flows (Leonard *et al*. 2002) and the detection of low-concentration compounds which would be below detection limits in hand-collected samples (Shertzer 1995). In this study, the samplers were deployed for continuous measurements of a range of organochlorine, organophosphate and selected pyrethroid compounds.

MATERIALS AND METHODS

The study was conducted between 7 December 2001 and 8 February 2002 in the DVIA of the Fitzroy Catchment in Central Queensland (Figs. 1 & 2). Four sampling sites (1 – 4) were chosen upstream of the irrigation area on the Dawson River as reference sites, in addition to two sites downstream (sites 5 & 6, Fig. 2), which receive runoff from the irrigation area. A further two sampling sites were also chosen; one,

(site d) on a drain receiving water from the irrigation area, and a second, (site b) on Gap Creek, approximately 100m downstream of where the drain enters the creek. This creek then enters the Dawson River (Fig. 2). The aim of sampling at these two sites determine the gradient of pesticide concentration between the point at which runoff exits the irrigation area and the river downstream. All sites chosen were pool sections, as problems with low flow in the river were anticipated for later studies. Sampling commenced on 7 December 2001, following the first significant rainfall (and presumed runoff) in the November growing season. Data collection included measurement of water quality parameters, pesticide levels (both grab and passive samplers) sampling of the macroinvertebrate communities, and direct *in situ* toxicity testing. At sites d & b, sampling was restricted to collection of water samples, deployment of passive samplers for pesticide analysis and insertion of cages for direct toxicity tests.

Water quality parameters and site characteristics

Measurement of water quality parameters at all sampling sites in the DVIA was conducted to allow for the relative significance of pesticides versus other environmental factors to be determined via statistical analyses. Physical and chemical parameters at each site were recorded as per the Monitoring River Health Initiative methodology (Choy & Thompson, 1996) with the exception of phenol alkalinity and total alkalinity. Sampling involved spot measurements of dissolved oxygen, pH, conductivity and temperature, using a YSI Sonde 6600. Sites were selected as having a similar amount of overhanging vegetation and similar substrate characteristics: usually firm, fine sediment (the only exceptions to this were sites 3 and b, where the substrate was not as well consolidated). Detritus cover and current speed were also

recorded. Flow rate was measured using a Marsh-McBrirney Model 201D portable water current meter at 5 cm above the substrate, and was recorded in more than 5 points at each site, with the average value calculated. The 6 study sites on the Dawson River (sites 1 – 6) were sampled on 6-7 December, 19-20 December, 16-17 January and 6-7 February, however sampling did not occur at sites 1 and 2 on the first sampling occasion. At each site, 3 replicate measurements were taken and the mean of these used in subsequent analyses.

Measurement of pesticide levels

Water samples for pesticide analysis were collected from 8 sites (sites 1 to 6, Dawson River; sites a & b, Gap Creek) on 18-19 December, 16-17 January and 6-7 February. Sites a and b were not included during December sampling. Samples were collected by hand grabs using glass bottles and were kept on ice for transport to the testing laboratory. Also, at each site, three passive samplers containing 10mL trimethylpentane were deployed. Each bag was placed in a small metal cage and surrounded by a nylon (800 μm mesh) bag. These were tied to large bricks and secured in the river bed by means of a star picket hammered into the substrate. Samplers were deployed from 19th December and collected on 16th January, at which time the samplers were replaced and re-deployed for a further 3 weeks before the second retrieval on 7 February.

Samples were sent to Scientific Services, Queensland Health at Coopers Plains (a NATA certified laboratory) for analysis of a range of organophosphate and organochlorine pesticides (profenofos, α- and β- endosulfan, endosulfan sulphate,

total DDT(s), and chlorpyrifos, and selected pyrethroids (cyhalothrin, cypermethrin and deltamethrin). These pesticides were selected based on information regarding recent usage obtained from the main supplier in Theodore. Solvents in the samplers were analysed directly by GCECD, GCFPD and GCMS. Time integrated pesticides levels in water were estimated from the concentrations in the passive samplers using the equations in Leonard *et al*. (2002). Data were compared against available pesticide information from Sunwater and the DNRM Ambient Water Quality monitoring program. This allowed a systematic analysis to be made of those chemicals posing the greatest risk to the environment.

Macroinvertebrate communities

Macroinvertebrate communities along the Dawson River were sampled on 4 occasions: on 8 & 19-20 December 2002; 15-16 January 2003 and 6-7 February 2003 (sites 1 – 6). Sites 1 and 2 were omitted from sampling on 8 December. Sampling was conducted by means of hand net sweeps using a 250 μm mesh pond net, with three two-minute samples taken at each of 10 m of the open edge habitat at each site. Samples were then combined to make one sample per site and immediately preserved with ethyl alcohol. Upon return to the laboratory, each sample was subsampled and sorted until either approximately 200 individual invertebrates were counted or the whole sample was sorted (Walsh, 1997) with, however, a time limit of three hours sorting in the latter case. Invertebrates were identified to family level only, as several studies in Central Queensland (and elsewhere) have shown that this level identification is sufficient to detect the order of magnitude of differences expected between sites in the proposed study (Duivenvoorden *et al*., 2000; Duivenvoorden &

Roberts, 1997; Duivenvoorden, 1995; Faith *et al*., 1995). Some taxonomic groups were sent to specialists for verification, identification and review.

Direct toxicity tests

Use of caged shrimp in mortality trials in conjunction with passive samplers and assessment of community assemblages is a novel approach to measure the possible ecological effects of stormwater runoff from the irrigation drain into Gap Creek. Individuals of the decapod *Macrobrachium intermedium* were collected from control site 4 for use in the trials. These were then randomly assigned to 45 x 25 x 25cm cages (3mm mesh size) that were placed at sites 1 – 6 and D and b, from 15 January to 7 February 2002. The toxicity tests consisted of 3 replicates each of 10 caged animals, in accordance with the national Standard Operating Protocols as provided in the ANZECC and ARMCANZ (2000) guidelines. Death of the organisms was used as the test endpoint.

RESULTS

Significant rainfall events occurred in the irrigation area prior to and during the study period (Fig. 3). Significant runoff occurred on 17-18 December 2001, and 1 January and 5 February 2002. Such events are considered to have the potential to increase pesticide concentrations due to localised run-off and subsequent transport of contaminated sediments into nearby rivers and streams (Hose *et al*., 2002; Leonard *et al*., 1999; Simpson *et al*. 1998). Figure 3 also depicts times when pesticides were applied in the section of the irrigation area drained by the drain entering Gap Creek.

Water quality parameters

Water quality data collected during the study are presented in Fig 4 (a-e) and in Table 1. Variability between sites was minimal, and most parameters did not vary dramatically over the study period. Temperatures ranged between 26.2 and 31.8^{0}C; pH between 6.9 and 8.1; conductivity between 164 and 281 μS cm^{-1}, and oxygen between 38.7 and 105.3 % saturation.

River water during the sampling period was very turbid, with Secchi depths ranging between 5 to 15cm (Fig. 4d). Water velocity just above the streambed was generally higher at sites 1 and 2, probably owing to the smaller cross sectional area of the water column at these sites (Table 1). Water depth varied depending on flows down the system, with sampling undertaken at depths of 1.5m on one occasion. Detritus cover was difficult to estimate, particularly when depth of the water or turbidity was high.

Pesticide concentrations

Results of pesticide concentrations in water samples between December and February are provided in Figure 5. Levels of several pesticides were higher than the relevant trigger values for protection of aquatic ecosystems (ANZECC and ARMCANZ, 2000), although some pesticides sampled for have not yet had guideline values derived. Chlorpyrifos exceeded the 95 % trigger value (0.01 μg L^{-1}), recommended for slightly-moderately disturbed systems in 50 % of samples collected in both December and February (Fig. 7). Endosulfan contamination (α- and β-endosulfan and

endosulfan sulphate) in water samples was only recorded for sites 5, 6, D, and b and exceeded the 99% trigger value of 0.03 μg L-^{1} recommended for slightly-moderated disturbed systems. Profenofos peaked towards the end of sampling, with the highest values recorded being 7.2 μg L^{-1} (January) and 9.2 μg L^{-1} (February) for site a and 5.7 μg L^{-1} (January) and 5.6 μg L^{-1} (February) at site b.

Passive samplers were successfully deployed with 100% having a recovery volume >80% of the initial volume. Pesticide levels in water estimated from concentrations in passive samplers (Figs. 6 & 7) served to give a time-integrated (average) level over the three-week deployment time. Passive sampler results were generally in agreement with manually collected water samples in that endosulfan was recorded in highest concentrations during early sampling (December – January) whilst profenofos featured high values later in the season (January – February). During this latter period, peak levels of profenofos (up to 2.7 μg L^{-1}) were estimated to be 7 times the magnitude of peak endosulfan levels (up to 0.38 μg L^{-1}).

For both deployment periods, levels of endosulfan sulphate in the passive samplers at site D were approximately halved at site b (located about 300m downstream), and were less that one-tenth at site 6 (approximately 3km downstream, Figs. 6 and 7). This was also true for chlorpyrifos. The use of passive samplers also highlighted the presence of trace amounts of endosulfan in areas where manually collected samples had not previously detected it. For example, a small level of endosulfan (0.002 μg L^{-1}) was detected at sites 1 and 2, despite these being upstream of the irrigation area.

Impact on macroinvertebrate communities in the DVIA

The results of macroinvertebrate sampling in the Dawson River are provided in Figs. 8 & 9. Analysis of macroinvertebrate richness data showed sites 1 & 2 to have the lowest number of taxa overall, ranging from 11 –14 taxa. Sites 3 – 6 appeared to be similar with respect to both number of taxa and the composition of major orders. All sites featured dominance by gastropods, and to a lesser extent Odonatans and Trichopterans (Fig. 8). Coleopterans and hemipterans were noticeably absent from the most upstream sites (sites 1 and 2), excepting on the 15 January. No oligochaetes were identified from any sites during the study.

Significant differences in macroinvertebrate abundance were not evident between the six sampling sites (Fig. 9). In addition, there were no consistent trends discernable between sampling dates. Overall maximum and minimum abundances were both recorded at site 6 with almost 4,500 individuals recorded on 16 January, but only around 600 on 19 December (Fig. 9).

Direct toxicity tests

Results of direct toxicity tests using *Macrobrachium intermedium* are presented in Fig. 10. At three sites (1b, 2a and 3c), difficulties with the cages were experienced and results for these have been omitted. Records of mortality in the shrimp were based on the presence of live or dead shrimp. However, when some cages were checked following their deployment in the field for a three-week period, dead shrimp were not found, although the cages no longer had shrimp in them. Since shrimp placed in the

cages were considered too large to escape and no holes were found in the cages, presumably the missing shrimp had died and decomposed.

Significant mortality (> 10%) was recorded at upstream sites 1 and 3, and mortality was quite variable within each site. Mortality at sites 5 and 6 was also significant, but highest levels (100%) were recorded at sites D and b. A noteworthy result was the 100% survival of shrimp at site 4, the site from which the experimental animals had been originally collected.

DISCUSSION

Water quality parameters

Overall, water quality data at the study sites were similar to those gathered in previous studies of the Dawson and other local catchments (Noble & Rummenie, 1997). Secchi depth levels, although being quite low due to turbidity, are comparative with others reported for the Dawson River and wider Fitzroy Catchment (Noble & Rummenie, 1997). The presence of high amounts of suspended particulate matter (and consequent low Secchi depths, see Fig. 4) may have a twofold effect on pesticide levels and their impacts in the Dawson River. Firstly, suspended matter causing decreased Secchi depths during 19 December may evidence the recent transport of pesticides into the river in conjunction with storm run-off occurring from rainfall events two days immediately prior to sampling, thus increasing pesticide contamination. However, reduced pesticide toxicity caused by sorption to sediments and subsequent reductions in bioavailability has been recorded for profenofos (Leonard *et al*. 2001) although not necessarily for endosulfan (Hose *et al*. 2002).

Assessment of levels of heavy metals in water collected by Sunwater and DNRM for the DVIA suggested that these were not high enough to be of significant ecological concern.

Detection of pesticide contamination

A significant result was the magnitude of the drop in pesticide contamination (particularly for endosulfan) in sampling areas immediately downstream of the joining of the irrigation drain with Gap Creek. This may be due to a high level of macrophyte cover observed in the irrigation channel itself. Peak pesticide values occurring at sampling sites a and b are directly associated from pesticide runoff into the irrigation drain and consequent input into the Gap Creek tributary. The engineering design of Queensland drainage channels has been reported by Simpson *et al*. (1998) to allow for significant transport of contaminated sediments into nearby waterways.

Use of passive samplers

The results for passive sampling of pesticides were in general agreement with those of water samples. Factors influencing the uptake and release rates of pesticides into or from passive samplers include variability in suspended solids, temperature, time deployed in the field and biofouling (Leonard *et al*., 2002; Muschal, 1999). The impact of temperature on the release of solvent from the passive samplers is likely to be minimal, as temperature measurements taken during sampling showed the water to fluctuate between approximately 26 – 32^{0}C. Leonard *et al*. (2002) has reported that up to 5 ^{0}C variation in river temperature did not have a significant impact on the release of solvents from passive samplers, although other studies have shown

increases in sampling rates to occur when temperature fluctuations are significant (> 10 ^{0}C) for triolein-containing samplers (SPMDs) (e.g. Huckins *et al*., 1999 in Leonard *et al*., 2002). Several studies reviewed by Leonard *et al*. (2002) have shown changes in flow rate to have minimal (if any) impact on pesticide uptake into passive sampling devices, especially where external protective mesh bags were used in conjunction with deployment of the samplers.

The passive samplers detected trace amounts of endosulfan sulphate (ranging from 0.0007 – 0.002 μgL^{-1}) in the upstream reference sites (sites 1 – 4) during January and February 2002. This shows the usefulness of these samplers in being able to detect pesticides that are intermittently make their way into streams – results of grab samples had never before recorded endosulfan in this section of the Dawson. Subsequent discussions with irrigators revealed that a very small amount of cotton is occasionally grown upstream of site 1. This suggested that sites 1 to 4 may not be suitable as reference sites for the planned detailed study of the area. The much higher peak values of profenofos in comparison to endosulfan in the passive samplers collected on 7 February may be related to the application of this chemical just prior to the rainfall that occurred on 5 February (Fig. 3).

Impact to macroinvertebrate communities in the DVIA

Macroinvertebrate richness in samples collected along the Dawson River did not vary markedly over time, despite variations in pesticide contaminant levels being recorded. Macroinvertebrate data, however, are not available for the period prior to the runoff that occurred following the rain in November (Fig. 3). Hence, it would be premature

to suggest that impact on macroinvertebrate populations as a result of pesticide contamination is not evident. Richness was generally lower at the two most upstream sites (1 and 2). This may be related to habitat characteristics such as reduced flows in this area. Macroinvertebrate dominance patterns in the samples compared favourably with studies of other rivers in the Fitzroy Catchment, where gastropods were noted amongst the most dominant orders and odonatans and trichopterans frequented samples (Duivenvoorden & Roberts, 1997; Duivenvoorden *et al*., 2000).

Hyne *et al*. (1998) studied the ecotoxicology of endosulfan on macroinvertebrates using stream mesocosm testing, however no detectable response was observed with respect to richness or abundance after 9h exposure to up to 5.0 μg L^{-1} technical grade endosulfan. However, some responses became evident after 4 day's exposure to lower levels of endosulfan. By contrast, at short lengths of exposure to higher levels (50 μg L^{-1}), treatments significantly differed to controls, probably due to the contributions of the most sensitive taxa (mayflies and trichopterans). As maximum pesticide contamination levels at river sites in the current study were < 1 μg L^{-1} in water samples (Fig. 5c), and endosulfan sulphate levels estimated from concentrations in passive samplers were just below the 99% ANZECC and ARMCANZ (2000) trigger value (Fig. 6), it is possible that pesticide levels were not of high enough concentration to exert an effect. However, Hyne *et al*.'s (1998) study dealt with the effect of just one pesticide, whilst the macroinvertebrate communities of the Dawson River must deal with the potential additive and synergistic effect of several pesticides throughout the season.

Future studies in the DVIA may benefit from including sampling of fish populations for effects from pesticide contamination, as fish collected from the Gwydir River (New South Wales, Australia) during the cotton growing season were found to contain endosulfan residues, and a negative correlation was found between fish size and endosulfan contamination (Novak & Ahmad, 1989).

Direct toxicity tests

The consistent 100 % mortality of *Macrobrachium* sp. occurring at sites D and b on the Gap Creek tributary is not surprising given the total pesticide concentrations in these areas during testing. However, other factors that may have influenced mortality cannot be discounted. Studies of *Macrobrachium rosenbergii* have reported 24 h LC_{50} values for laboratory tests to be 1.64 μg L^{-1} for endosulfan (Lombardi *et al*. 2001), whilst 24 h LC_{50}s for caged *P. australiensis* exposed to endosulfan for 12h was calculated at 2.8 μg L^{-1} (Hyne *et al*. 1998). Total endosulfan levels (α-, β-endosulfan and endosulfan sulphate combined) in water samples collected at sites D and b peaked at 0.58 μg L^{-1}, and 0.49 μg L^{-1} on 6 February for sites D and b respectively. However, it is very likely that additive or synergistic effects of the other contaminants present occurred at this time, with 9.2 and 5.6 μg L^{-1} of profenofos also occurring during February at sites D and b (respectively). Woods *et al*. (2002) reported that LC_{50} values for endosulfan, chlorpyrifos and profenofos were significantly lower for *Ceriodaphnia* when exposed to combinations of those pesticides rather than singularly. Wirth *et al*. (2002) found grass shrimp populations to decrease by nearly one-third after exposure to endosulfan in laboratory trials, as a result of decreased reproductive capacity.

In direct contrast to the results at sites D and b, *Macrobrachium intermedium* at site four all survived during the test period. This was most likely directly related to this site being the original source site for the experimental animals.

CONCLUSION

Results of this preliminary study show that pesticide concentrations in the some parts of the DVIA consistently exceed ANZECC and ARMCANZ (2000) trigger values for the protection of aquatic systems. This was determined by taking grab samples of water and by estimating time-integrated levels using passive samplers. There was an approximate ten-fold decrease in pesticide concentration between the drain leaving the irrigation area and the river, approximately 3km downstream.

The study highlighted the value of passive sampling in detecting trace amounts of pesticides in aquatic systems. The samplers were successful in concentrating some pesticides in the exposure chamber in areas where these compounds had not previously been recorded by intermittent water grab samples.

Macroinvertebrate taxa richness and abundance did not show any marked declines during the course of the study. However, since runoff from the DVIA is likely to have occurred in November, prior to the study, it cannot be concluded that irrigation runoff did not have an significant effect on the macroinvertebrate richness and abundance. Though factors other than pesticide contamination may have contributed to 100% mortality of caged shrimp at the sites with the highest levels of pesticides, results

suggest that further investigations of sites using direct toxicity testing would be useful.

ACKNOWLEDGEMENTS

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Table 1. Physical-chemical parameters at Dawson Valley Irrigation Area sites sampled during the 2001/2002 irrigation season. Values are the mean of 3 replicate samples. ns – not sampled

Parameter	Date	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Velocity (m s^{-1})	6 – 7 Dec	ns	ns	ns	ns	ns	Ns
	19 - 20 Dec	0.20	0.20	0.11	0.13	0.11	0.33
	16 - 17 Jan	0.15	0.00	0.00	0.03	0.03	0.01
	6 - 7 Feb	0.02	0.05	0.00	0.01	0.00	0.02
Gauge Height (m)	6 – 7 Dec				1.37	ns	ns
	19 - 20 Dec				1.31	ns	ns
	16 - 17 Jan				1.56	ns	ns
	6 - 7 Feb				1.47	ns	ns
Mean depth (m)	6 – 7 Dec	ns	Ns	0.4	0.6	0.6	0.5
	19 - 20 Dec	1.0	1.0	0.6	1.3	1.4	1.2
	16 - 17 Jan	1.4	1.0	1.0	1.4	1.4	1.1
	6 - 7 Feb	1.2	0.5	0.6	1.3	1.5	0.5
Detritus cover (%)	6 – 7 Dec	ns	ns	10-35%	35-65%	ns	ns
	19 - 20 Dec	35-65%	35-65%	10-35%	35-65%	35-65%	ns
	16 - 17 Jan	10-35%	10-35%	10-35%	35-65%	10-35%	10-35%
	6 - 7 Feb	10-35%	10-35%	10-35%	35-65%	10-35%	10-35%

LIST OF CAPTIONS

Figure 1. (a) Location of the Fitzroy Catchment in Queensland and (b) Fitzroy Catchment showing the Dawson Valley Irrigation Area (□).

Figure 2. Location of study sites along the Dawson River and Gap Creek tributary.

Figure 3. Rainfall (mm) and time of application of selected pesticides in the Gibber-gunya section of the Dawson Irrigation Area. Arrows indicate when stream sampling was undertaken.

Figure 4. Water quality parameters measured at 6 sites along the Dawson River between December 2002 and February 2003. *Note*: oxygen measurements on 16 Jan were considered unreliable due to equipment malfunction.

Figure 5. Pesticide concentrations measured by hand-grab samples at six sites along the Dawson River and 2 sites on Gap Creek. (a) 19-20 December 2001 (b) 16-17 January 2002 (c) 6-7 February 2003. Values for January and February are the average of three grab samples.

Figure 6. Estimated concentrations of profenofos, endosulfan sulphate and chlorpyrifos in water between December 2001 and January 2002. Error bars are the standard deviations of the mean of three samples.

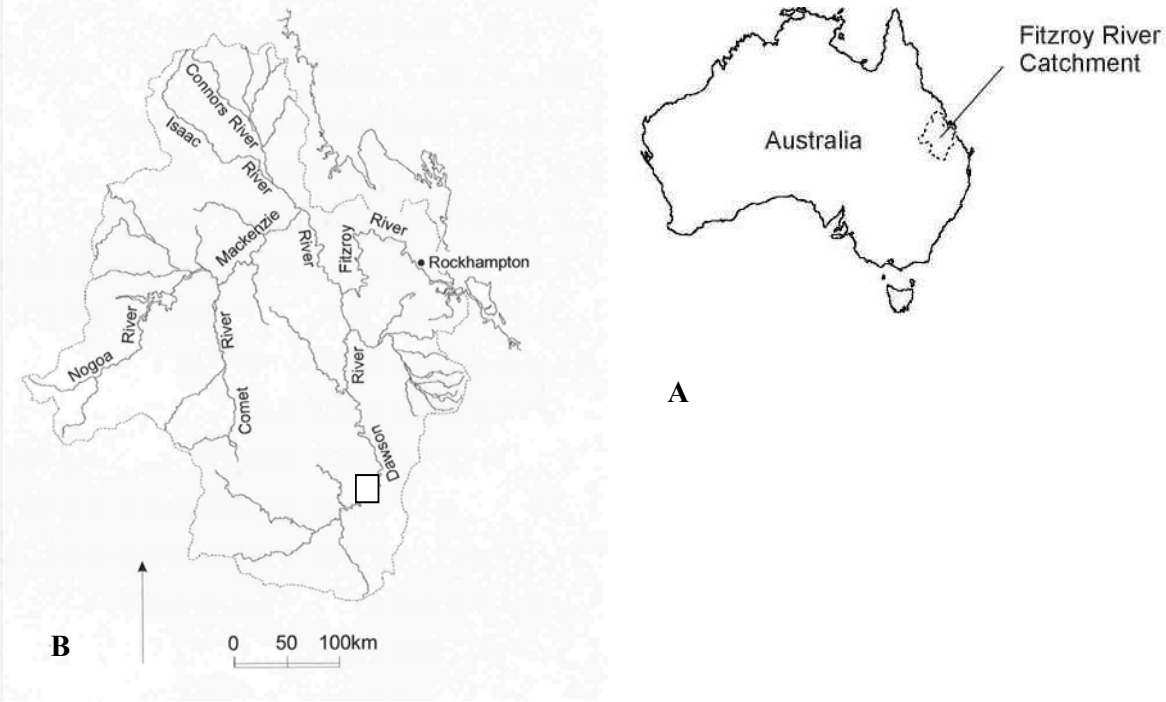
Figure 7. Estimated concentrations of profenofos, endosulfan sulphate and chlorpyrifos in water between January and February 2002. Error bars are the standard deviations of the mean of three samples.

Figure 8. Macroinvertebrate richness sampled at six sites in the Fitzroy catchment between 7 December 2001 and 8 February 2002.

Figure 9. Macroinvertebrate abundance measured in samples collected at 6 sites in the Fitzroy catchment between 7 December 2001 and 8 February 2002.

Figure 10. Mortality of *Macrobrachium* sp. in each of three cages deployed at sites in the Fitzroy catchment.

Figure 1.



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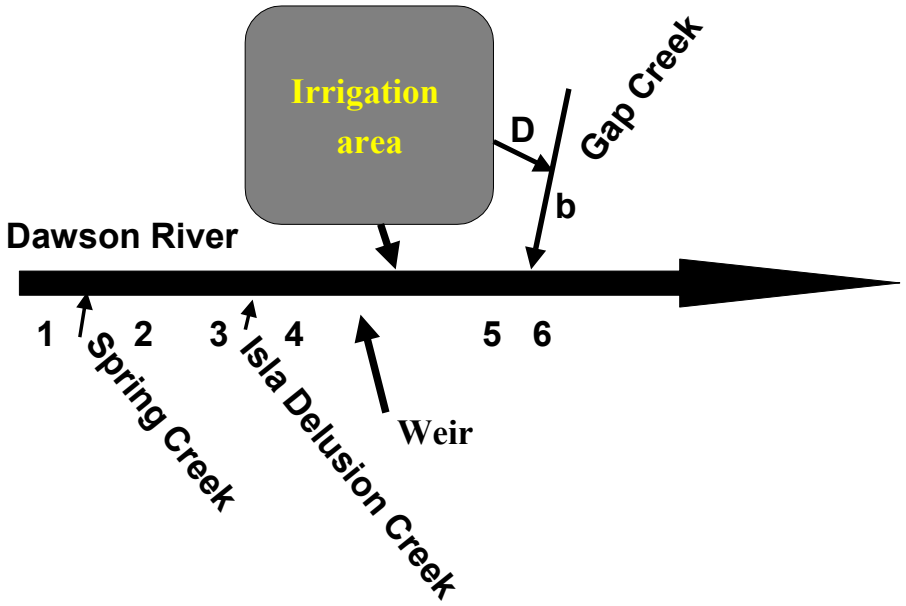
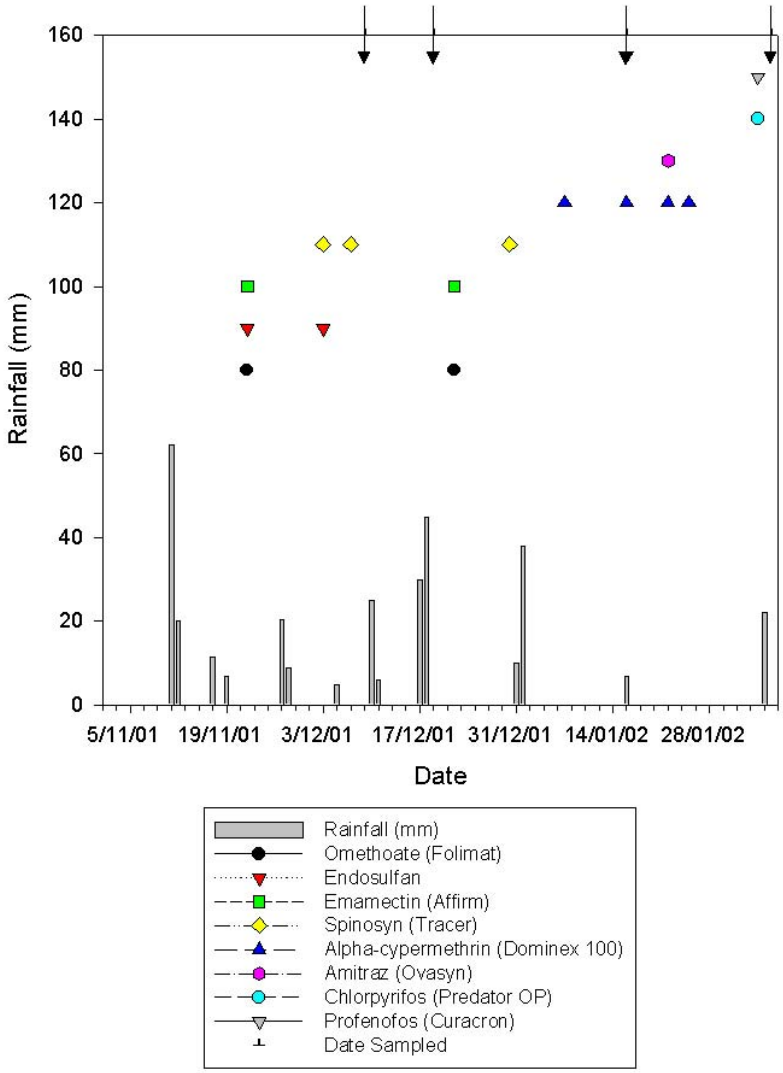
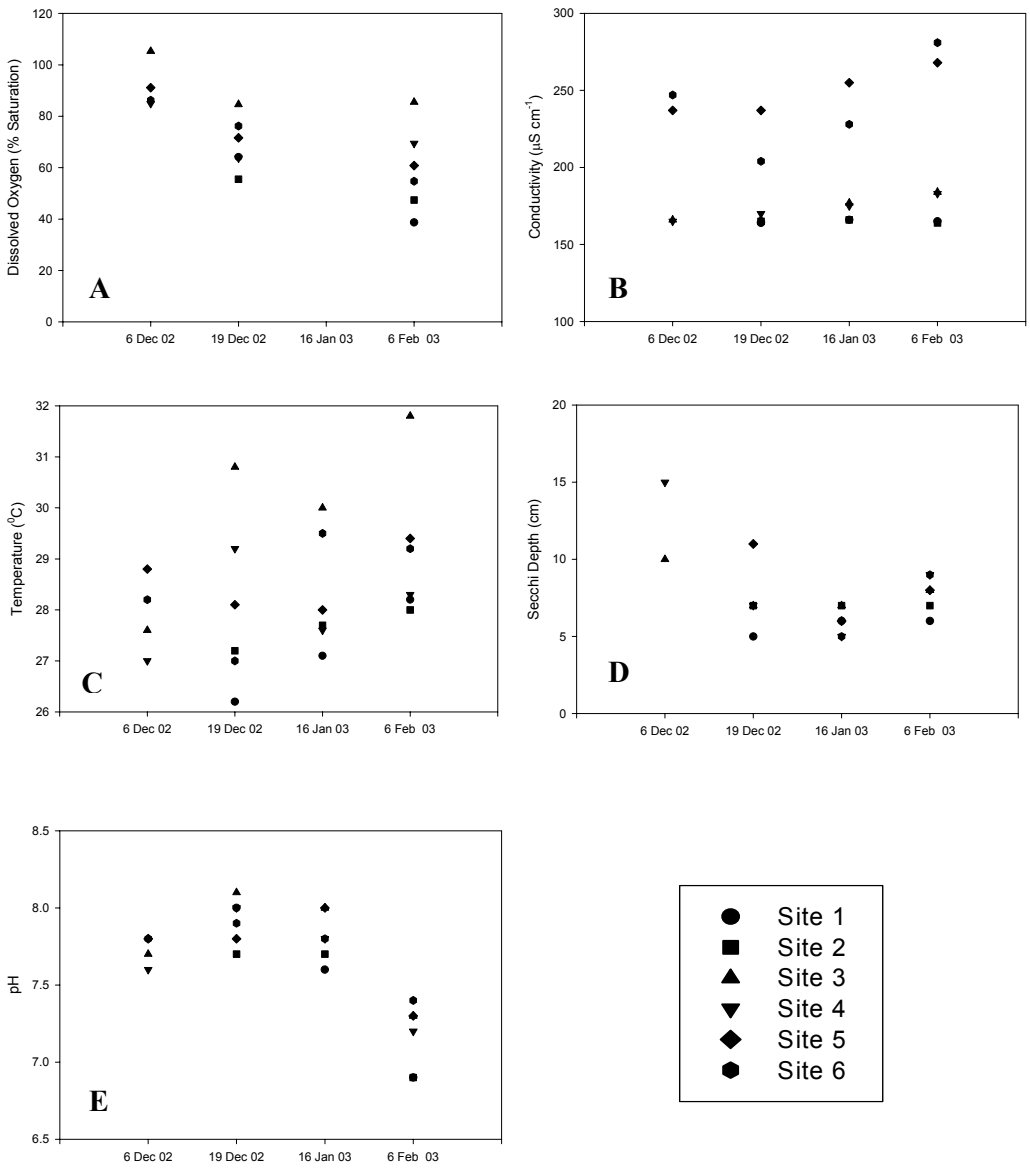



160
140
120
100
80
60
40
20
0
Rainfall (mm)
5/11/01
19/11/01
3/12/01
17/12/01
31/12/01
14/01/02
28/01/02
Date
Rainfall (mm)
Omethoate (Folimat)
Endosulfan
Emamectin (Affirm)
Spinosyn (Tracer)
Alpha-cypermethrin (Dominex 100)
Amitraz (Ovasyn)
Chlorpyrifos (Predator OP)
Profenofos (Curacron)
Date Sampled


Dissolved Oxygen (% Saturation)
120
100
80
60
40
20
0
A
6 Dec 02
19 Dec 02
16 Jan 03
6 Feb 03
Conductivity (µS cm-1)
300
250
200
150
100
B
Temperature (0C)
32
31
30
29
28
27
26
C
Secchi Depth (cm)
20
15
10
5
0
D
pH
8.5
8.0
7.5
7.0
6.5
E
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6

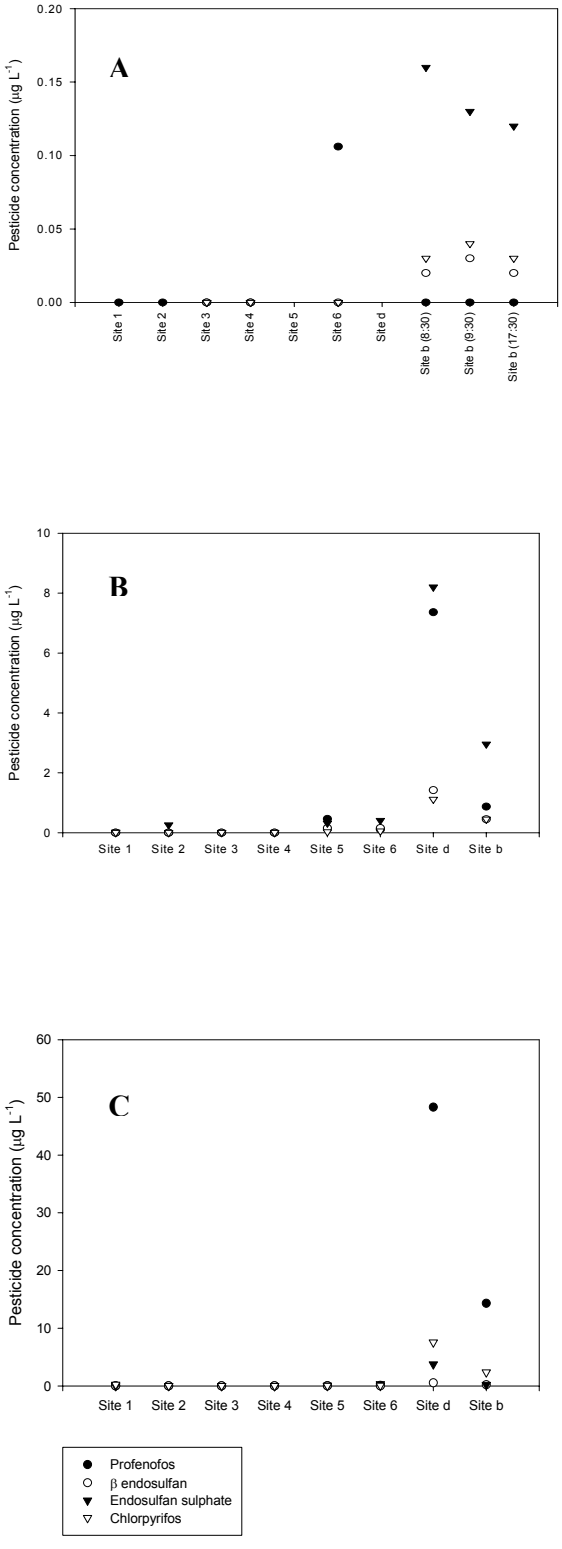
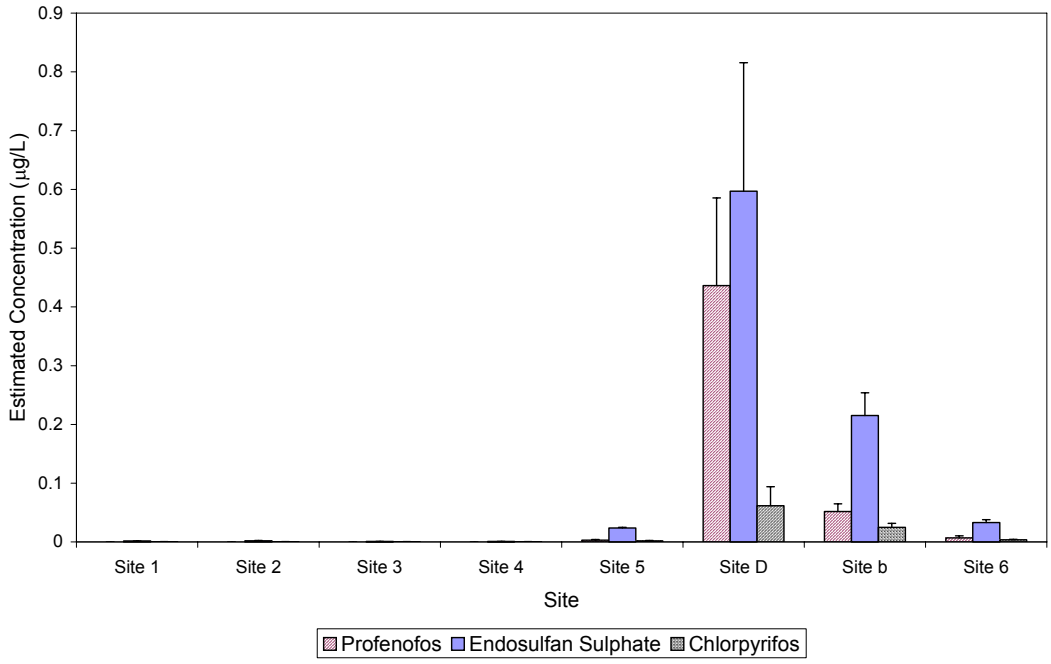

A
B
C
Pesticide concentration (µg L-1)
0.20
0.15
0.10
0.05
0.00
10
8
6
4
2
0
60
50
40
30
20
10
Site 1
Site 2
Site 3
Site 4
Site 5
Site 6
Site d
Site b (8:30)
Site b (9:30)
Site b (17:30)
Site b
Profenofos
β endosulfan
Endosulfan sulphate
Chlorpyrifos

Figure 6.


0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Estimated Concentration (µg/L)
Site 1
Site 2
Site 3
Site 4
Site 5
Site D
Site b
Site 6
Site
Profenofos
Endosulfan Sulphate
Chlorpyrifos

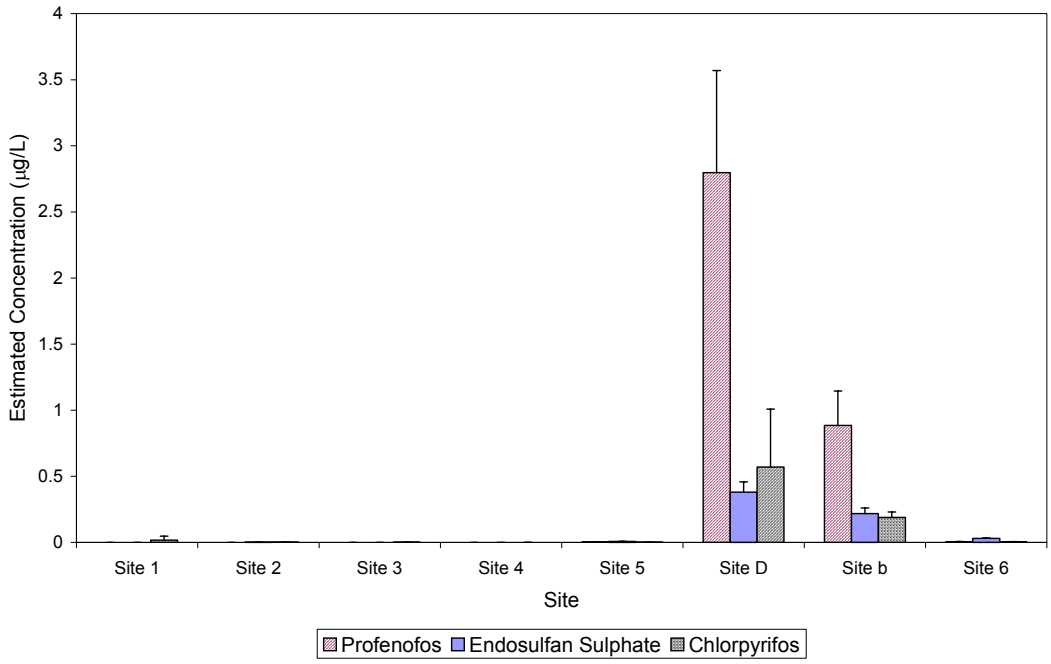

4
3.5
3
2.5
2
1.5
1
0.5
0
Estimated Concentration (µg/L)
Site 1
Site 2
Site 3
Site 4
Site 5
Site D
Site b
Site 6
Site
Profenofos
Endosulfan Sulphate
Chlorpyrifos

Figure 7.

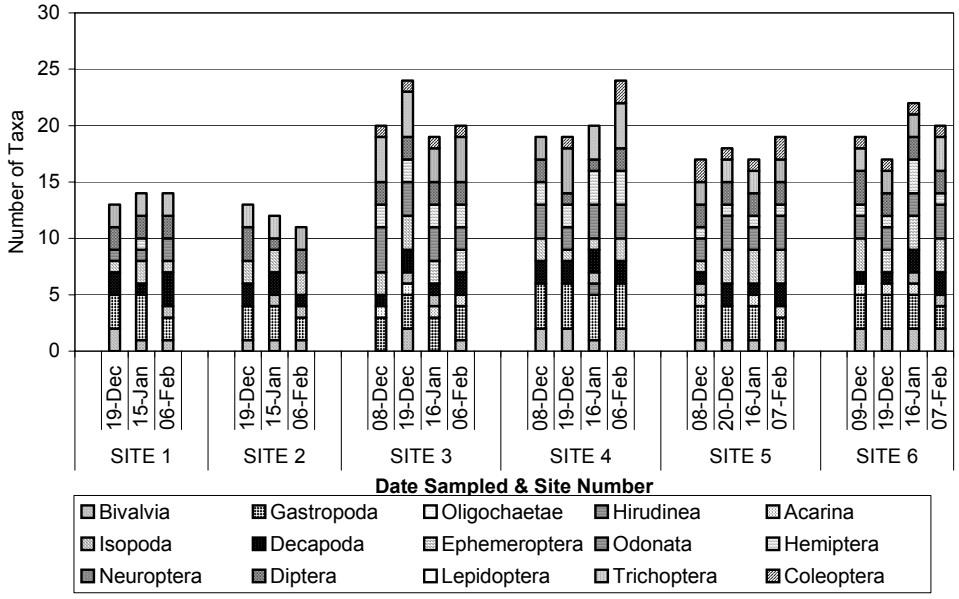


Figure 8.

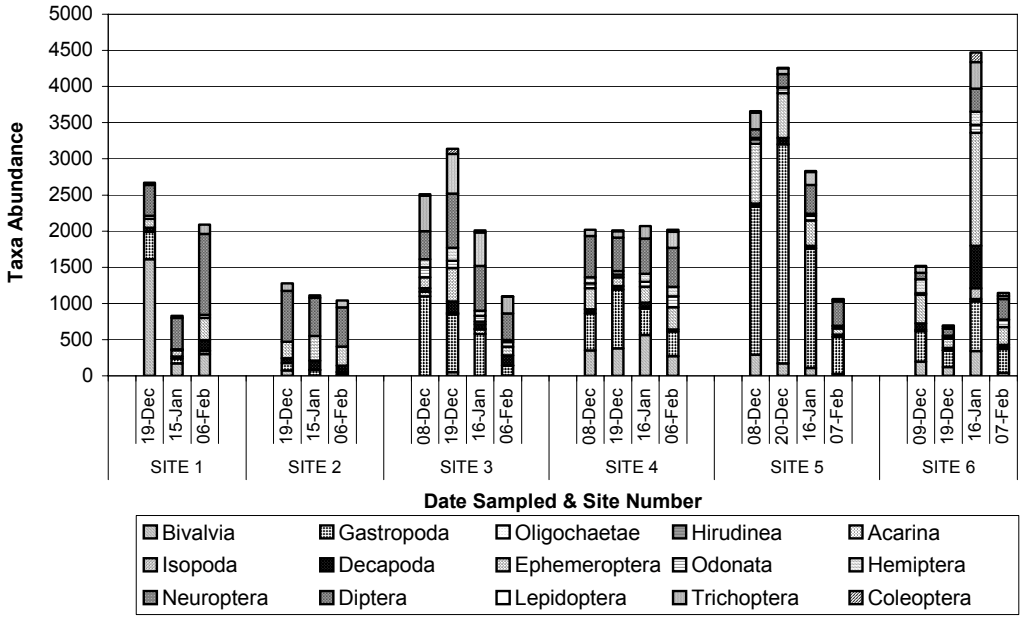


Figure 9.

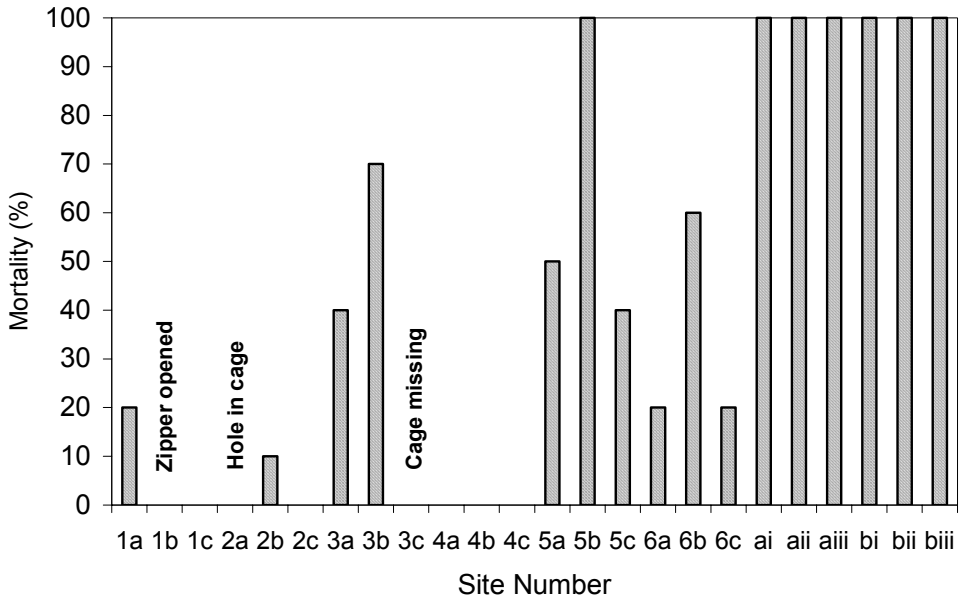

100
90
80
70
60
50
40
30
20
10
0
Mortality (%)
Zipper opened
Hole in cage
Cage missing
1a 1b 1c 2a 2b 2c 3a 3b 3c 4a 4b 4c 5a 5b 5c 6a 6b 6c ai aii aiii bi bii biii
Site Number

Figure 10