1999

Report Cover Sheet for Annual & Final Reports

The following Reporting Requirements MUST BE MET

All Projects
You must submit an **ANNUAL PROGRESS REPORT** by the first Friday in February 1999, detailing the progress of your research. NOTE: IF you are seeking continuation of funding for 2000–2001 for the project, this report will form the basis for CRDC's consideration of ongoing funding. Please complete the budgetary requirements if this is a continuing project.

Terminating Projects
A **FINAL REPORT** must be submitted within three months of completion of the project. This applies in **ALL** cases including research projects, travel, conference attendances, postgraduate, postdoctoral and funded capital items.

Tick Report Purpose

○ **Final Report** (Due 30 September or 3 months after completion of project)

Actual start date: _____ Anticipated completion date: _____

OFFICE USE ONLY:
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Project title (as per original application)

Relationships between populations of selected macroinvertebrates and pesticides in the Namoi River.

CRDC Project Code UTS IC **CRDC Responsible Director (if known)** Dr V. Edge

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Final Report- Project UTS 1C

What was the background of the project?

Of the several pesticides used on the pest management strategy for cotton, endosulfan is ranked as having the greatest impact on the riverine ecosystem. A survey of changes in the density of six abundant macroinvertebrate taxa (ephemeropteran nymphs *Jappa kutera, Atalophlebia australis, Tasmanocoenis* sp., *Baetis* sp. and two trichopteran larvae *Cheumatopsyche* sp. and *Ecnomus* sp.) between upstream and downstream zones of the cotton growing region in the Namoi River, was conducted between November 1995 and February 1996. In November and December 1995, there were few differences in population densities between all sites. In January and February 1996, population densities of the study taxa increased 7-10 fold higher at the two reference sites with low concentrations of endosulfan in sediment and in passive samplers placed in the water column. In contrast, densities of these taxa at sites with exposure to 25 fold higher concentrations of endosulfan remained static and were between one to two orders of magnitude lower than densities at the reference sites in January and February. Population densities of *Baetis* sp., a mobile ephemeropteran, did not show any inverse relationship with endosulfan concentrations. Multivariate redundancy analysis indicated that endosulfan concentrations were the leading environmental predictor of changes in density of the five benthic taxa. Laboratory 48 hour LC50 values of technical endosulfan in river water were 0.6, 1.0 and 0.4 ppb for early instar nymphs of *Atalophlebia australis*, *Jappa kutera* and larvae of *Cheumatopsyche* sp., respectively. Endosulfan sulfate formed a large proportion of the total endosulfan concentrations measured from *in situ* passive samplers, indicating that its main route of entry into the river is through surface runoff during storm events.

What were the project objectives and to what extent were these achieved?

This project evaluated the effect of increased transgenic cotton usage, and the implementation of Best Management Practice, on the concentrations of pesticides entering the riverine environment (as measured by *in situ* passive samplers constructed of solvent filled dialysis bags) and their correlation to densities and recruitment of selected macroinvertebrates in the Namoi River. The study was conducted in the 1997/98 and 1998/99 cotton growing seasons.

The results demonstrated the utility of using the passive samplers as a regulatory tool to audit the impact of Best Management Practice on pesticides entering the riverine environment.

What Methodology was used, and a justification for the use of this methodology?

See attached publications.

Detailed results including statistical analysis of results?

See attached publications for the 1997/98 season. The results for the 1998/99 are still to be analysed.

A discussion of the results, including an analysis of research outcomes compared with the objectives?

See attached publications.

An assessment of the likely impact of the results and conclusions of the Research project for the Cotton industry, and where possible a statement of the costs and potential benefits to the Australian Cotton Industry and future research needs?

Using passive sampling methods, pesticide concentrations measured in solvent-filled polyethylene bags placed in the water column have potential as a tool to audit the impact of Best Management Practices implemented by the Cotton Industry on the riverine environment.

A description of the project technology (eg commercially significant developments, patents applied for or granted, licences, etc)

No commercially significant outcomes.

A technical summary of any other information developed as a part of the Research Project including discoveries in methodology, equipment design, etc.

Use of passive samplers, constructed of solvent-filled polyethylene bags, to give a time-integrated measure of pesticides entering the riverine environment.

Recommendations on the activities or the steps that may be taken to further develop, disseminate, or exploit the project technology

No specific recommendation.

A list of publications arising from the research project

Hyne, R.V., Lim, R.P. and Leonard, A. (1999). Relationship between endosulfan concentrations and macroinvertebrate densities in the Namoi River over two cotton growing seasons. In *Minimising the Impact of Pesticides on the Riverine Environment.* Key Findings from Research with the Cotton Industry. Land and Water Resources Research & Development Corporation. Occasional Paper 23/98, pp. 68-72. Canberra. ACT. Australia.

Leonard, A., Hyne, R.V., Lim, R.P. and Chapman, J.C. (1999). Effect of endosulfan runoff from cotton fields on macroinvertebrates in the Namoi River. **Ecotoxicol. Environ. Saf.** 42: 125-134.

Leonard, A., Hyne, R.V., Lim, R.P., Pablo, F. and Van Den Brink, P. (1999). Correlations between riverine endosulfan concentrations linked to runoff from cotton fields and population densities of macroinvertebrates in the Namoi River, Australia : comparison of two seasons with differing hydrological conditions. **Environ. Toxicol. Chem**. submitted.

A one page plain English summary of the project outcomes must be submitted, and this may be used in CRDC publications and on our proposed web site.

During the 1995/96 and 1997/98 cotton-growing seasons there was an inverse relationship between the population densities of dominant benthic macroinvertebrates and concentrations of endosulfan in the Namoi River. Laboratory toxicity testing with these same species indicated that endosulfan concentrations that have been measured in the Namoi River during storm events would cause decreases in the population densities of mayflies and caddisflies as well as fish. The dominant toxic component of endosulfan in riverine samples was the metabolite endosulfan sulfate indicating that its most likely source is from land run-off during storm events. Throughout both cotton-growing seasons, the mean total endosulfan concentrations in passive samplers constructed of solvent-filled polyethylene bags at the high-exposure sites were 10-25 times higher than those at the reference sites.

Using passive sampling methods, pesticide concentrations measured in solvent-filled polyethylene bags placed in the water column has potential as a tool to audit the impact of Best Management Practices, implemented by the Cotton Industry, on pesticides entering the riverine environment.

CORRELATIONS BETWEEN RIVERINE ENDOSULFAN CONCENTRATIONS LINKED TO RUNOFF FROM COTTON FIELDS AND POPULATION DENSITIES OF MACROINVERTEBRATES IN THE NAMOI RIVER, AUSTRALIA : COMPARISON OF TWO SEASONS WITH DIFFERING HYDROLOGICAL CONDITIONS

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Abstract

Population densities of six dominant macroinvertebrate taxa (mayfly nymphs *Jappa kutera*, *Atalophlebia* sp., *Tasmanocoenis* sp., *Baetis* sp. and the caddisfly larvae *Cheumatopsyche* sp. and *Ecnomus* sp.) were negatively correlated to total endosulfan concentrations in the Namoi River in the 1995/96 and 1997/98 cotton growing seasons (November to March). Total endosulfan concentrations measured in solvent-filled polyethylene bags (passive samplers) at the exposed sites correlated with rainfall, suggesting that endosulfan entered the riverine environment in run-off from land. At the start of both surveys, in November 1995 and November 1997, there was no significant difference ($P<0.05$) between the reference and exposed sites for both total endosulfan concentrations and mean population densities of the combined study taxa. This indicates distance downstream, which was negatively correlated with exposure, was not an important variable in explaining the significantly higher population densities in other months at the reference sites compared to those at sites subsequently exposed to increased (10-25 fold) total endosulfan concentrations. The multivariate analysis of the 1997/98 data with the Principal Response Curves method indicated that endosulfan explained a significant proportion (22 %) of the variation in the total macroinvertebrate community. Principal Components Analysis indicated other covariables were involved, including river discharge. River discharge was positively correlated to increased densities of the mayfly taxa at the reference sites in 1995/96 survey but these correlations were reduced to near zero, except for *Baetis* sp., in the 1997/98 survey.

Key words: endosulfan; hydrology; macroinvertebrates; passive samplers

INTRODUCTION

Storm runoff events in cotton growing areas in Australia are thought to contribute to large fish kills [1]. This may be due to the runoff causing pesticides (particularly the organochlorine pesticide endosulfan) to be mobilised from cotton fields into waterbodies. Fish kills have been putatively attributed to high concentrations of endosulfan in the present study region, including the Namoi River [2]. Although endosulfan is a hydrophobic compound sorbing to soil, during rain storm events a proportion desorbs becoming bioavailable and toxic in run-off from land [3]. The persistence of endosulfan in soil [4] suggests that field run-off during storm events may be a major source of endosulfan causing reductions in populations of riverine macroinvertebrates [5].

Fish populations are often monitored to estimate the actual risk of pesticides in run-off from land. One disadvantage of monitoring fish populations, however, is their relatively high mobility [6], which leads to an unknown environmental exposure. Another difficulty with monitoring fish populations is the high sampling effort required, which may not produce sample sizes sufficient for statistical analysis. Evaluating changes in macroinvertebrate populations, with their high densities and their relative sedentary behaviour [7,8] reduces these difficulties. To evaluate the potential effects of pesticides entering the riverine environment, macroinvertebrates were therefore selected in this study as biomonitors instead of fish. Dominant Namoi River taxa such as mayfly nymphs and caddisfly larvae had 48 h LC50 values for endosulfan [5] similar to native fish [9] and below concentrations of endosulfan

measured in the Namoi River and the nearby Gwydir River following rain storm events [10].

The aim of this study was to determine if these laboratory toxicity data were supported by field data over two seasons, i.e. if the potential risk of pesticides as indicated by results of laboratory tests is also an actual one. This consisted of evaluating correlations between riverine endosulfan concentrations and changes in the population densities of the dominant macroinvertebrate taxa in the Namoi River, with special reference to mayfly nymphs and caddisfly larvae. The study consisted of two surveys conducted in the 1995/96 and in the 1997/98 cotton growing seasons when different hydrological conditions occurred. This allowed the influence of river discharge, rainfall, distance downstream and other environmental variables on the population densities of the study taxa to be investigated in reference to the influence of endosulfan concentrations.

MATERIALS AND METHODS

Field study design

Changes in the population densities of the study taxa and concentrations of endosulfan in the Namoi River were examined with a modified BACI design [11] in 1995/96 and 1997/98. Sampling occurred before, during and after the endosulfan

spraying season for cotton from November to March. The sites in both surveys were sampled every four weeks, which is less than the minimum emergence time of the study taxa [12,13,14]. All sites in both surveys had similar riffle-pool habitats and were at least 5 km apart [Fig. 1]. Previous studies have shown that this distance made it likely the sites were independent of drifting macroinvertebrates [7,8]. Each site was the replicate unit in this study with the treatment consisting of reference and pesticide-exposed sites. Sites were categorised as reference or exposed using measured concentrations of pesticides in the solvent of the solvent-filled polyethylene bags (passive samplers). Sampling of the passive samplers was coincident with the macroinvertebrate samples every four weeks. The passive samplers were filled with trimethylpentane [15] and placed in the water column of the river [5].

In the 1995/96 survey [5], eight sites were selected along the Namoi River (Fig. 1). The total endosulfan concentrations (sum of α-endosulfan, β- endosulfan and endosulfan sulfate) in the samplers indicated the eight sites consisted of two reference sites (sites 1 and 2 upstream of the cotton growing areas), a site with low pesticide exposure (site 3), and five sites with high pesticide exposure (sites 4 to 8). Four macroinvertebrate sampling units at each of the eight sites were taken with a 500-μm mesh Surber sampler.

The design of the 1997/98 survey was expanded to six macroinvertebrate sampling units at each of the 17 sites. Except for the site furthermost downstream

(site 8), the sites sampled in 1995/96 were re-sampled in 1997/98 and supplemented with nine additional sites with similar riffle-pool habitat (represented by letters in Fig. 1). The selection of these sites was based on the 1995/96 pesticide concentrations. These 17 sites potentially consisted of six reference sites (A,B,1,C,2,D), six low-exposure sites (E,F,G,H,3,I) and five high-exposure sites (4,5,J,6,7).

The main differences to the methodology of the 1995/96-survey [5] in 1997/98 was the inclusion of the whole macroinvertebrate community and the method of enumeration. The efficiency of the enumeration in 1997/98 was improved by sub-sampling a composite sample on a wet weight basis [16]. A composite macroinvertebrate sample was made by pouring the six sampling units (collected from each site on each occasion) into a bucket containing eight litres of water. A suspension of the organic particles was obtained by mixing the sample and then allowing the sand and pebbles to settle for ten seconds. The organic particles containing macroinvertebrates were removed from the suspension by filtering through a 150-µm mesh sieve. This was repeated six times leaving the gravel fraction with very little organic matter. From the combined organic fractions retained by the sieve, all large (> 1 g) organic debris including sticks, large crustaceans and filamentous macrophytes were removed after inspection for adhering macroinvertebrates. To increase the dispersion of the macroinvertebrates within the remaining organic fraction, the suspension and filtering procedure was repeated. To ascertain that the individuals were randomly dispersed in the sub-samples of the organic matrix, an index of dispersion was calculated [16,17]. This involved counting

all 10 % aliquots (by wet weight) for sites A, 2 and 4 in November 1997. The accuracy was set by counting 20 % of the total wet sample weight as two separate 10 % aliquots. If more than 100 individuals of a taxon were counted in the first aliquot then, this taxon was omitted in the second aliquot [17]. For the gravel fraction, animals were counted in a 25 % by wet weight sub-sample in all cases.

For both surveys, abiotic parameters measured at each site included river discharge, total rainfall, river channel width, substrate size and concentrations of pesticides in solvent-filled polyethylene bags. For all these parameters, mean values were calculated for each monthly sampling period. The mean monthly values of river discharge were obtained from hourly measurements by three hydrological gauging stations located within the study section of the Namoi River [New South Wales Department of Land and Water Conservation]. Daily discharge means for each gauge station were used to calculate integrated values for each monthly sampling period and these were adjusted by interpolation for the distance of each site from the nearest upstream and downstream gauging stations. Mean total monthly rainfall data were interpolated in the same way by using daily values from the three nearest gauging stations (twelve stations were within the study area) obtained from the Bureau of Meteorology. Besides the monthly mean measurements, spot measurements of water quality variables (temperature, pH, conductivity, turbidity and DO) and distance downstream were carried out in both surveys on each sampling occasion, using methods described in detail previously [5]. Subjective measurements of cow trampling pressure were also scored on a scale of one to ten.

Pesticide sampling

In situ passive samplers containing trimethylpentane were used to quantify the bioavailable fraction of endosulfan [15]. At each site, three passive sampler was placed inside each of three large rock filled nylon mesh bags (0.8 mm mesh). The mesh bags were secured by cable ties in the water column to one metre high metal fencing posts hammered into the substrate. The passive samplers were replaced monthly and the recovery of trimethylpentane was over 90%. The solvent containing the pesticides was analysed directly by gas chromatography-electron capture detector (GC-ECD) for organochlorines (USEPA method G19) and GC-nitrogen phosphorous detector for organophosphates (USEPA method 507) and they were confirmed using GC-mass spectrometry as described previously [5]. For quality assurance an interlaboratory program was instituted between the Chemical Laboratories of the NSW EPA and our laboratory at the Centre for Ecotoxicology, using randomly selected subsets of the solvent from the passive samplers. A subset of samples collected each month was mixed and split into two sets of pooled samples and given to each laboratory for endosulfan analysis. Variations between the two laboratories were within 10-15%.

Statistical analysis

Univariate analysis

Statistical analyses were conducted, to test if the individuals from each taxon in a sub-sample of the composite macroinvertebrate samples were randomly dispersed, and the accuracy of the sub-samples, in order to estimate the total number of organisms in the original composite samples. Determining the 95 % confidence intervals and the 95 % coefficient of variation indicated accuracy of the sub-sample counts. The statistical test for random dispersion of individuals in each sub-sample was based on the conformity of the variance to mean ratio in the Poisson distribution [16,17]. An index of dispersion was calculated which approximates the Chi-square distribution.

Unless stated otherwise, the probability levels of rejecting the null hypothesis and all confidence intervals was set at 95 %. All marginal cases in simple comparisons of mean values were verified using a one-way ANOVA followed by a Tukey's HSD test for equal sample sizes (or if necessary unequal sample sizes) using Statistica for windows, version 5.1, [18]. The assumptions of homogeneity of variance were tested using a combination of Cochran's, Hartley's and Bartlett's tests and the normality of the distribution was assessed using a normal probability plot of the residuals. If the assumptions were violated, the non-parametric Mann-Whitney U test was used. Due to the small sample sizes the exact probabilities were determined using a cumulative one-sided probability of the Mann-Whitney U statistic.

Multivariate analysis

One of the aims of the present paper was to study the relation of several environmental variables (e.g. river discharge) with the effects of endosulfan on the macroinvertebrate community. We therefore performed a multivariate analysis using the indirect ordination method, Principal Components Analysis (PCA). In this way an overall representation of species-based correlations of measured environmental variables with endosulfan exposure was obtained. The PCA was performed with the DOS statistics software package CANOCO, version 3.1.4. [19]. PCA assumes a linear response curve in the species data. This assumption was confirmed by determining the species gradients using detrended (by segments) correspondence analysis [19]. This indicated the species response curves were monotonic or linear (gradients less than 1.5 in both surveys). For more information on the use of multivariate techniques in community ecotoxicology, refer to Van Wijngaarden et al., 1995 [20] and Van den Brink et al., 1996 [21].

The Principal Response Curve method is a multivariate technique especially designed for the analysis of data from microcosm and mesocosm experiments. The advantage of this method over other methods is that it is able to focus on the part of the variance explained by the treatment. This is done by excluding the variance explained by differences between replicated and sampling data. The data collected in this study were not of an experimental nature but are a result of a biomonitoring program. The PRC method is a modified version of Redundancy Analysis [22,23].

This is the first paper that uses the Principal Response Curves for the analysis of biomonitoring data. Monte Carlo permutation tests were performed to indicate possible significant treatment effects of endosulfan exposure on the macroinvertebrate community.

RESULTS

Hydrological conditions of the Namoi River and rainfall during the two field surveys

Differences in the hydrological conditions of the Namoi River during the field surveys of 1995/96 and 1997/98 were evaluated using the mean river discharge recorded by three hydrological gauging stations within the study section. These three stations were situated within 500 m of sites E, 5 and 7 (Fig. 1). A gauging station located at site A on the Peel River was excluded due to the river discharge being atypical of that of the Namoi River where the other 16 sites were located. Data from a hydrological gauging station near site 3 were also omitted, as the data set was incomplete. There were no significant ($P>0.05$, ANOVA) differences in the mean discharge data between the three stations for each survey [data not shown].

The volume of water in Keepit Dam was only 9 % of its capacity in October 1995 due to the prolonged drought over the previous Winter and Spring. Consequently, river discharge in the 1995/96 survey was unregulated and very variable (Fig. 2). A large storm event in January 1996, led to flooding in the

catchment. Despite the high peak in river discharge, macroinvertebrates were sampled that month immediately after the floodwater had subsided.

Above average winter/spring rainfall in 1997 resulted in the Keepit Dam water level reaching 65 % of its capacity in October 1997. This facilitated the regulation of discharge in the Namoi River during the growing phase of the cotton crop between mid-December 1997 and mid-February 1998. The persistence of high water levels in the river during this period restricted the sampling of macroinvertebrates at all sites until the discharge from Keepit Dam had ceased in mid-February 1998. This coincided with significantly lower rainfall in the locality of the sites exposed to endosulfan (sites 5, 6 and 7) in January / February 1998 compared to the corresponding sites and times in 1996 (Fig. 3).

Despite the differences in hydrological conditions during the two surveys, trends in water temperatures were similar for both surveys (Fig. 2). The temperature increased from about 11°C in August to about 25°C in November. These temperatures peaked at just below 30°C in January before decreasing to about 25°C in March.

Pesticide concentrations

The mean recoveries of the trimethylpentane solvent in the passive samplers were greater than 90% after exposure in the river for four weeks. This was similar to their recovery rates from estuarine waters after six weeks exposure [15]. Data were available for only four sites in both November and December 1995 due to initial difficulties with deployment of the passive samplers. Passive samplers had to be exposed for eight weeks over January and February 1998 at nine of the 17 sites (eight reference sites and one site exposed to high endosulfan concentrations) due to the continuously high river discharge in January 1998, which prevented their retrieval from the river. However, at sites A, 1, 2, 3, 5, J, 6 and 7 the samplers were retrieved and replaced in January 1998.

Pesticides measured in the solvent of the passive samplers in both studies included high concentrations of the endosulfan isomers and their sulfate metabolite, plus lower concentrations of the organophosphates chlorpyrifos, sulprofos and profenofos, and the herbicide trifluralin [5]. In a laboratory flow-through experiment with water concentrations of a chlordane/ dieldrin mixture of 10 µg/L, similarly constructed passive samplers accumulated the pesticides linearly and did not reach equilibrium when exposed continuously for six weeks [15]. In a similar experiment, we confirmed that the endosulfan isomers and endosulfan sulfate were absorbed linearly by the trimethylpentane containing passive samplers over the measured aqueous concentration range of 0.01 to 1.0 µg/L, with an accumulation factor of approximately 1600 for the compounds after 28 days. Other studies have show that endosulfan will accumulate in passive samplers to a similar magnitude as chlordane [24].

In November 1995 and November 1997, before pesticide spraying commenced, differences in total endosulfan concentrations between the reference and the sites subsequently exposed to endosulfan were negligible (Fig. 4). A one-way ANOVA indicated these differences were not significant ($P>0.05$) in November 1997. Statistical differences in total endosulfan concentrations between the reference and exposed sites could not be determined in November 1995 as data was available for only two reference and two exposed sites.

Throughout both surveys, the mean total endosulfan concentrations in the passive samplers remained low (less than 50 µg/l) at the reference sites and increased 10 to 25 fold at the exposed sites between December and February (Fig. 4). Between December 1997 and March 1998, 10 to 12 fold higher (significant at $P<0.05$) endosulfan concentrations were measured in passive samplers at the five most downstream sites (sites 4 to 7) compared to the twelve most upstream sites (sites A to I) (Fig. 4). During the 1997/98 survey, the concentrations of endosulfan were lower than expected in the middle reaches of the study section of the river (sites E to I) based on concentrations at site 3 in January and February 1996 (Fig. 4). Based on these results the experimental design for the 1997/98 survey was improved with 12 reference sites and five sites exposed to high endosulfan concentrations.

Evidence of run-off from land being the main route of entry of endosulfan into the river is provided by the correlation between rainfall in the locality of exposed sites (sites 5, 6 and 7) and total endosulfan concentrations in the solvent of passive samplers positioned in the water column of the Namoi River (Fig. 3). This correlation shows that the significantly lower rainfall in January and February 1998 led to significantly lower riverine endosulfan concentrations, compared to the corresponding periods and sites in 1996. In addition, in February of both 1996 and 1998 when the most of the endosulfan spraying had ceased, over 80% of the endosulfan was in the form of endosulfan sulfate [data not shown]. This is the biological metabolite of the parent alpha and beta isomers and is the dominant degradation product in aerobic soils [25].

Population densities of selected macroinvertebrate taxa

The number of individuals represented by the six taxa selected for study in the 1995/96 survey [5] represented more than 88 % of the macroinvertebrate community at all the study sites in the 1997/98 survey (Table 1). Similar riffle-pool habitat at all study sites was sampled in both surveys.

The change in the method of enumeration of macroinvertebrates collected in the 1997/98 survey from that of the 1995/96 survey did not alter the validity of comparisons to the data between the two surveys when all individuals collected

were enumerated. This was due to the fact that sub-sample counts in 1997/98 represented a random estimate of the mean (in a Poisson distribution). In only two cases out of a total of 21 examined, the data for the most dominant taxa (Table 1) did not fit a Poisson distribution. If the mean number of individuals was greater than five for two separate 10 % sub-samples the 95 % coefficient of variation indicated the counts were within 35 % of the mean. For rarer taxa with less than five individuals in each sub-sample the coefficient of variation was between 35 % and 85 % of the mean count. In the process of separating the organic fraction from the gravel fraction, virtually all individuals (99.9 %) were removed with the organic fraction of the sample. The exception was the molluscs of which 6.5 % remained in the gravel fraction.

From December 1995 to February 1996 there was a continuous increase in the mean population density of the combined mayfly and caddisfly taxa at reference sites 1 and 2, but no increase at the exposed sites 3 to 8 (Fig. 5). In contrast, during the 1997/98 survey the mean population density of the six study taxa did not increase significantly at the twelve reference sites (A, B, 1, C, 2, D, E, F, G, H, 3, I). However by March 1998, a significant decrease in the mean population density occurred at the five highly exposure sites (4, 5, J, 6, 7) (Fig. 5). The differences in the mean population density of the combined study taxa between the reference and exposed sites were not significant ($P=0.08$) in December 1997, but were significant ($P<0.01$) by February and March 1998 (Fig. 5) following exposure to peak endosulfan concentrations in December 1997.

Time dependent treatment effects can be examined in the multivariate ordination method of Principal Components Analysis (PCA). Data values are replaced by treatment means that allow the variances in the macroinvertebrate dataset to be attributed to time, treatment and their interaction [23]. The delay in response of the study populations was examined by the inclusion of a time interaction with endosulfan exposure (expressed as nominal binary values of 0 or 1) at the reference sites (Ref*T) and the exposed sites (Exp*T), respectively. This avoids the assumption that response of the study taxa coincides with exposure to maximum endosulfan concentration (Fig. 6 and 7). The relationship of Ref*T and Exp*T to the study taxa populations at each time point is shown by the positions of the respective centroids in ordination diagrams of Principal Components Analysis (PCA) (for interpretation consult Jongman et al., [26]) (Fig. 6 and 7). The dominant trend in these plots is the progressive separation of Ref*T and Exp*T at each time period, during the course of both studies. This agrees with the changes in the mean population densities of the study taxa between reference and exposed sites shown by the univariate plots (Fig. 5).

In addition to endosulfan concentrations, the PCA also indicated other variables such as rain, turbidity and distance downstream were negatively correlated with the taxa centroids (Fig. 6 and 7). Variables with weaker correlations which were excluded from the analysis included cow trampling pressure, pH, DO, temperature and other pesticides measured in the solvent of the passive samplers including profenofos, chlorpyrifos, sulprofos and trifluralin. The risk of profenofos toxicity to

the macroinvertebrates was highest in February 1998 as indicated by its concentrations in the passive samplers. However, profenofos is generally less toxic than endosulfan [24] and in February 1998 its mean concentration at the exposed sites was seven fold less than the mean endosulfan concentration (239 µg/l) in the solvent of the passive samplers. Conductivity values were also correlated with distance downstream but the variable was excluded in the PCA as high population densities have been observed within all the recorded values and mayfly nymphs, particularly, are able to osmoregulate [27].

The variables, river discharge and width of the river channel, were both positively correlated to the taxon centroids in both surveys (Fig. 6 and 7). Their correlations were stronger during the 1995/96 survey (Fig. 6) than those in the 1997/98 survey (Fig. 7). However, the similar temporal variability, except for January 1996, of discharge across the reference and exposed sites (Fig. 2) suggests it does not explain the low taxon densities at the exposed sites in both surveys.

Of the measured variables, distance downstream was most highly correlated to the time interactions with endosulfan exposure (Exp*T) (Fig. 6 and 7), but it does not account for the increasing differences between Ref*T and Exp*T in both surveys. The first axis of the PCA biplots identified most of the variation which separated the nominal variables of Ref*T and Exp*T (Fig. 6 and 7). This axis accounted for 54 % and 33 % of the total variation in the species data in the

1995/96 and 1997/98 surveys, respectively. The divergence of Ref*T and Exp*T during each study is clearly shown in both ordination diagrams (Fig. 6 and 7). The univariate analyses support this trend with differences between the exposed and reference groups not being significant in November 1995 and November 1997, and only becoming significant following increased endosulfan exposure (Fig. 5). This suggests that either endosulfan, turbidity or another unmeasured variable with similar temporal and spatial variability explains the differences in population densities between the reference and exposed sites (Fig. 6 and 7).

The differences between the reference and exposed sites based on densities of taxa in the macroinvertebrate community were evaluated in the 1997/98 survey. In this survey, the PRC analysis indicated differences in the taxa densities between the reference and exposed sites were not significant ($P=0.17$) in November 1997 but became significant following high endosulfan exposure in December 1997 ($P=0.01$), February 1998 ($P<0.01$) and March 1998 ($P<0.01$) (Fig. 8). This analysis focuses on and tests the significance of Ref*T and Exp*T [19, 23]. The treatment contributed to 22 % of the total variation in the species data, 17 % of this is shown on the first axis (Fig. 8). The taxa contributing most to this pattern were the mayflies and caddisflies, although the trend was consistent across all taxa (inset Fig. 8). Time was used as a covariable to subtract the variation due to time alone from the explained variation [23].

The lack of replicate measurements of pesticides in the 1995/96 survey (Fig. 4) prevented significance testing using the Principal Response Curve (PRC) analysis [19]. However, a partial Redundancy analysis of the 1995/96 survey data that analysed the taxa densities at all sites across time, but did not examine time as a separate variable, showed that the population densities of mayflies and caddisflies were significantly negatively correlated to total endosulfan [5].

DISCUSSION

Population densities of mayfly nymphs and caddisfly larvae in the Namoi River were significantly lower at downstream sites compared to upstream reference sites, after exposure to 10 to 25 fold higher concentrations of endosulfan in both the 1995/96 [5] and the 1997/98 surveys. However, in November 1995 and November 1997, before endosulfan concentrations in the river peaked, there was no significant differences in taxon densities between the reference and exposed sites. These patterns were indicated by univariate analyses and supported in the macroinvertebrate community data in 1997/98 using the ordination method of Principal Response Curve (PRC) analysis [19]. This analysis indicated that exposure to endosulfan explained 22 % of the total variability in the population densities of the community. The analysis also provided greater insight to the relationship between macroinvertebrate populations to endosulfan exposure compared with the univariate analyses, such as the delay in response of the populations to peak endosulfan concentrations in December 1997. At this time the

PRC, unlike the univariate analyses, indicated significant differences (P=0.01) between the taxon population densities at the reference and exposed sites. The differences increased further in February and March 1998, indicated in the PRC diagram by the divergence of the exposed and reference sites. The results of the PRC analysis indicated that, in combination with Monte Carlo permutation tests, PCA is a powerful tool to indicate treatment effects even in the case of a field-based experimental design as in biomonitoring programs.

The delay in response may be due to the fact that only the smaller instars being affected by prevailing endosulfan concentrations from run-off events. This is supported by laboratory toxicity data in which smaller instars of these taxa were more sensitive to endosulfan than larger instars [5]. The smallest instars (0.3-mm body length after 24 hours) have never been tested due to the difficulty of culturing them in the laboratory or collecting them in the field. The effects on the smallest instars would not have been immediately detected in the field samples due to only relatively large individuals being collected in the aquatic nets of 500 µm mesh [14]. The probability of small individuals influencing the overall population densities is increased by local recruitment of these taxa [7] and the survival of small individuals being highly dependent on their environment (e.g. river discharge) and less on biological factors such as competition [28]. A greater effect of endosulfan on smaller individuals is also consistent with the static population densities measured at the exposed sites during the 1995/96 survey [5].

Changes in population densities of the dominant mayfly nymphs and caddisfly larvae recorded in the 1995/96 survey [5] were indicative of changes in the whole riffle-pool macroinvertebrate community based on the 1997/98 data. The six study taxa represented 88 % of the community in the 1997/98 survey. The differences in population densities between the reference and exposed sites in the PRC analysis were consistent across all taxa. This is despite the taxa having potentially dissimilar exposure to environmental stressors because of their differing micro-habitat preferences and feeding ecology [29,30,31]. The consistent difference between the taxon densities at the reference and exposed sites across the macroinvertebrate community suggested that the stressor(s) was ubiquitous to all taxa, probably due to it being carried in the water.

This strongly suggests that the main stressor may be the bioavailable fraction of endosulfan [32] in the water column, measured at each site by the passive samplers. A link between rainfall and river endosulfan concentrations was indicated by the significant reductions in both rainfall and total endosulfan concentrations in the passive samplers located at exposed sites in January and February 1998 compared to the corresponding sites and times in 1996. We also showed previously that there was a significant regression between total endosulfan concentrations in the bottom sediment and those in the solvent of passive samplers positioned in the water column of the river [5]. This suggests that endosulfan is entering the riverine environment during storm events in both runoff water and runoff sediment, as found in other studies [33]. The main advantage of using passive samplers in a monitoring program is that they give a time-integrated measure of the pesticide exposure

between sites that can be related to any changes in the benthic macroinvertebrate population densities at those sites between the sampling periods. The utility of passive samplers in field monitoring studies would be improved if a model could be developed that predicts actual river loads and extends the model proposed by Huckins et al., [34].

The absence of significant differences between the mean population densities of the mayfly nymphs and caddisfly larvae at the reference and exposed sites when endosulfan exposure was low in November 1995 and November 1997, indicates distance downstream itself was not the predictor of these differences between December and March. This is consistent with the studies of Marchant et al. [28] and Lake et al. [35] who found that functional feeding groups of macroinvertebrates may not be ordered in a downstream gradient in many Australian riverine systems as predicted by the river continuum concept [36]. This is particularly true for riverine systems such as the Namoi River which are more tropical than the northern temperate systems on which the river continuum theory was based [36]. Further support for this argument is the disruption of upstream-downstream linkages (vital in the assumptions of the river continuum concept) by the Keepit Dam [37]. The most influential variable to Australian macroinvertebrate communities is the great temporal variability in river discharge [35].

The hydrological conditions differed during the two surveys and this may explain the different patterns at the reference sites in the PCA ordination diagrams between

the two surveys. During the 1995/96 survey, increases in the population densities of the study taxa at the two reference sites were positively correlated to river discharge. In the 1997/98 survey this correlation was reduced to nearly zero for the mayfly taxa, except for *Baetis* sp. The increase in population densities during the 1995/96-survey was probably due to the cessation of drought conditions following rain storm events. This led to a high temporal variability in the river discharge but population stability of the study taxa was ensured given their ability to adapt to natural change in discharge [38,39,40] and their high recruitment frequency [12,13,14]. Cohort analyses (between October 1995 and February 1996) of body length data indicated at least two generations would have recruited to each site over the five month survey period in 1995/96 [14]. In the 1997/98 survey, the reduced correlations of population densities to river discharge was probably due to continuous high river discharge between mid-December 1997 and mid-February 1998 from the regulated release of water from Keepit Dam. This may have prevented the macroinvertebrates from leaving refugia and feeding.

Although the differences in river discharge at the reference sites may explain changes in population densities of the study taxa when comparing the two surveys, this was not the case at the endosulfan-exposed sites. At these sites the multivariate correlations indicated endosulfan, possibly in association with turbidity or other unmeasured variables, contributed to the reduction of population densities of the study taxa during surface run-off events. The interaction of other stressors with the toxicity of endosulfan is likely to be important during these events. This includes toxicity from other pesticides sprayed on cotton, and increased turbidity

and flow rate that are likely to compound the effect of endosulfan on populations of the study taxa. Turbidity was only measured as spot measurements in this study and so the correlative strength of this variable using time integrated data remains unknown. However, increased suspended solids which is measured as turbidity is detrimental to macroinvertebrates causing gill damage, clogging of filter-feeding organs and smothering of habitat [41]. It is unlikely that turbidity has an ameliorative effect on the toxicity of endosulfan to the biota in the Namoi River [42]. Field turbidity measurements during surface run-off events [data not shown] were two orders of magnitude less than those which reduced the toxicity of endosulfan to eastern rainbow fish in a laboratory study [42]. The positive correlation of river channel width with population densities indicates increased flow rate during surface run-off events may contribute to stress in the populations of macroinvertebrates. This is due to raised metabolic rates from maintenance of position in high currents [43], avoiding current forces by migration down to the hyporheos [39], or by drifting downstream [8]. Other factors possibly contributing to the overall stress to the macroinvertebrates during surface run-off events include organophosphate pesticides that were present in the solvent of the passive samplers. The interaction of these pesticides, flow and suspended sediment may lead to an underestimation of endosulfan toxicity in the field compared with laboratory toxicity results with endosulfan as the single toxicant [44].

Despite possible interactions with other stressors, this study when combined with evidence from laboratory toxicity studies [5] and concentrations of endosulfan in riverwater [10] provides a "weight of evidence" strongly suggesting endosulfan as

the main contributor reducing populations of the dominant macroinvertebrates in the riffle-pool habitats. In these habitats, we visually observed the macroinvertebrate populations to be many orders higher in density than in mud/sand substrates and in edge habitats. This may be due to the many stable micro-habitats provided by the "reef-like" rocky substrate. So although these riffle-pool habitats constitute only a small area of the Namoi River, they may generate a large portion of the secondary production of the river. Consequently, they are important to the ecology of the river, in the decomposition cycle and as a component of the food chain [32]. An independent study examining the macroinvertebrate community in edge habitats throughout the Namoi River catchment also found downstream sites exhibited signs of impact compared to upstream sites [45].

The coincidence of fish kills with high endosulfan exposure [1,2], the extreme sensitivity of fish to endosulfan [9], and the longer generation times of fish relative to macroinvertebrates, would suggest that native fish populations will be reduced and not likely to recover between spraying seasons. This is in contrast to the populations of mayfly nymphs and caddisfly larvae that were observed to partially recover before the spraying seasons of 1995/96 and 1997/98. This is likely due to their high fecundity [46] and short generation times [12,13,14].

CONCLUSIONS

1. During the 1995/96 and 1997/98 cotton-growing seasons in the Namoi River the population densities of the dominant benthic macroinvertebrates were significantly lower at downstream sites exposed to high concentrations of endosulfan, compared to upstream reference sites.
2. Throughout both cotton-growing seasons, the monthly mean total endosulfan concentrations in solvent-filled polyethylene bags at the highly exposure sites were 10-25 times those at the reference sites.
3. The correlation between river discharge and mayfly population densities at the reference sites was lower in the 1995/96 survey than in the 1997/98 survey. River flow was unregulated and variable in the 1995/96 season, but was tightly regulated in the 1997/98 season.

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Table 1. Percentage abundance of mayfly nymphs and caddisfly larvae in the riffle-pool community of the Namoi River, November 1997 to March 1998.

Mayflies				Caddisflies		Total
Jap kut	*Ata* sp.	*Bae* sp.	*Tas* sp.	*Che* sp.	*Ecn* sp.	
5.2	5.1	11.3	35.2	21.1	10.5	88.5

Note: the mayfly nymphs are denoted by *Jap kut* (*Jappa kutera*), *Ata* sp. (*Atalophlebia* sp.), *Bae* sp. (*Baetis* sp.), *Tas* sp. (*Tasmanocoenis* sp.) and the caddisfly larvae by *Che* sp. (*Cheumatopsyche* sp.) and *Ecn* sp. (*Ecnomus* sp.). The remaining 11.5 % of the macroinvertebrate community were in the following order of dominance, chironomids, molluscs, shrimps and oligochaetes.

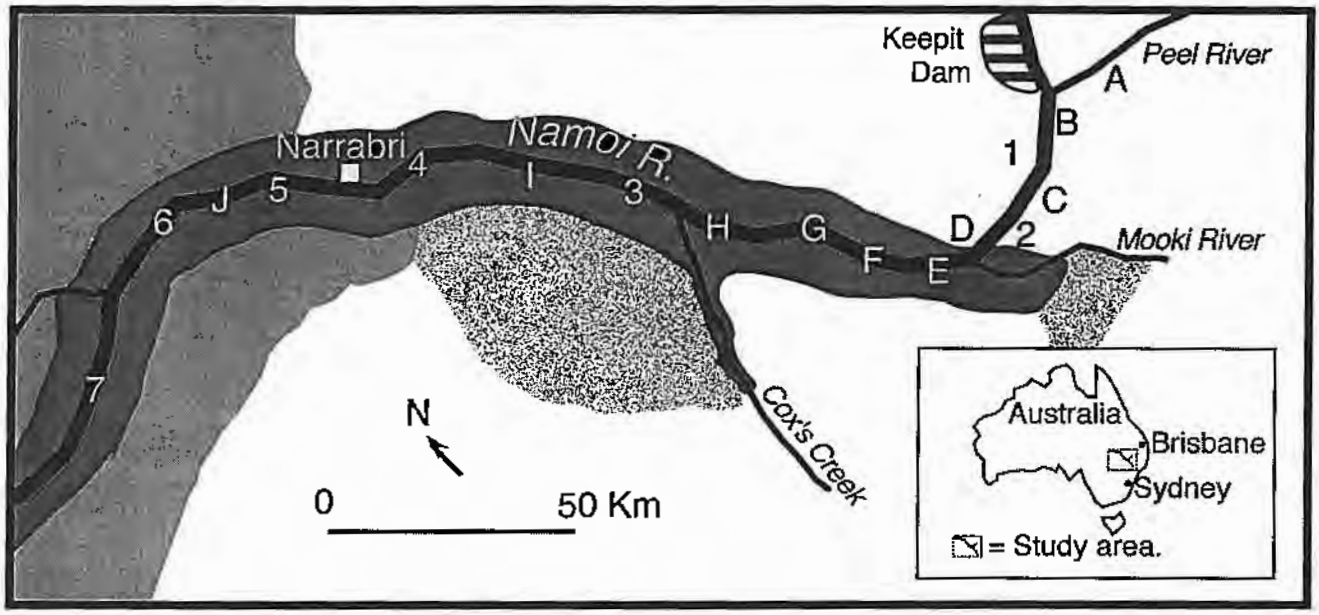


Fig.1. The Namoi River and locations of the sampling sites (1 - 7, A - J), the major town (□) and cotton growing areas. These areas are categorised by non-irrigated (▩), river-irrigated (■) and groundwater-irrigated cotton (■). Note that site 8 included only in the 1995/96 study was 110 Km downstream of site 7.

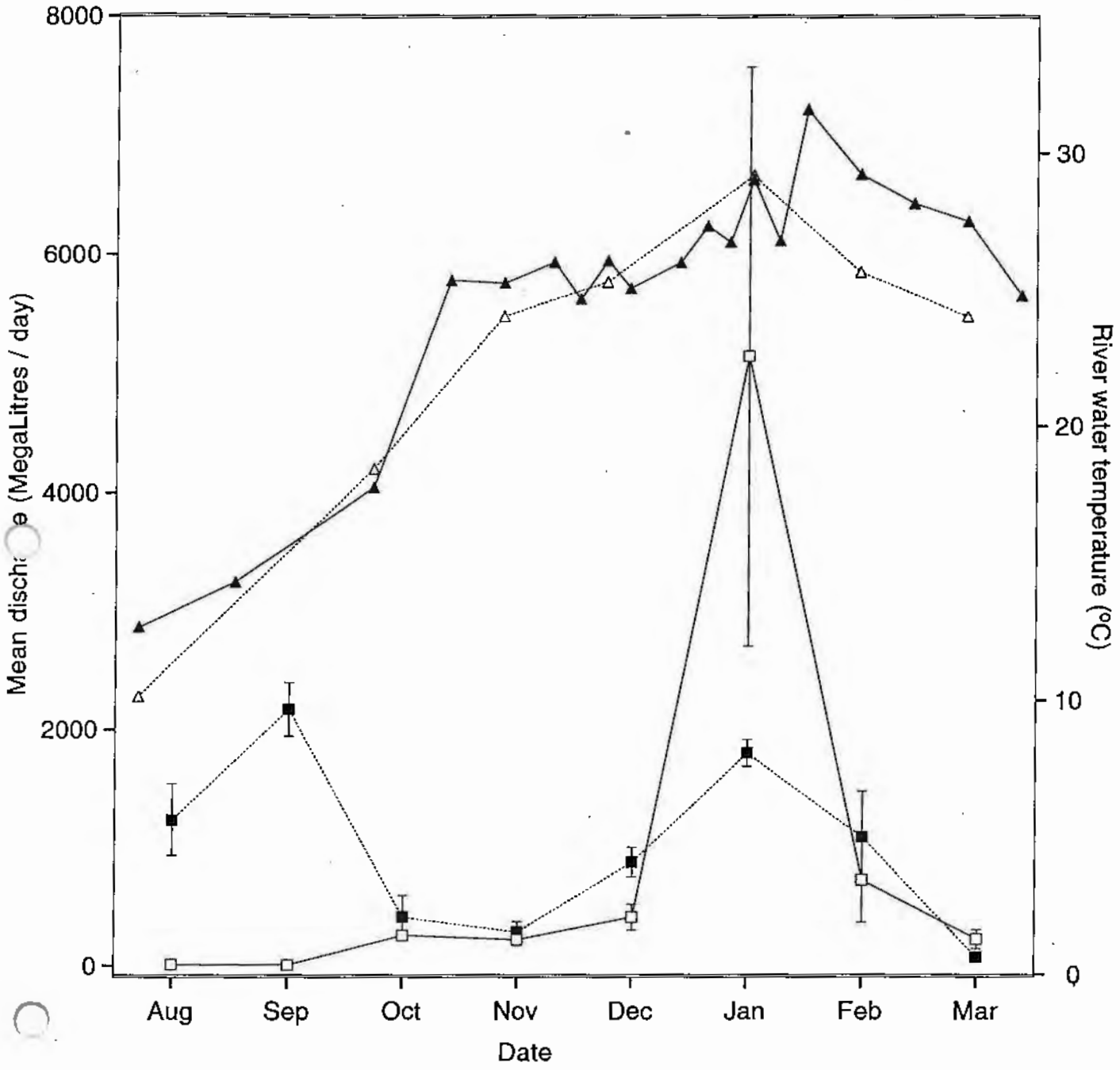
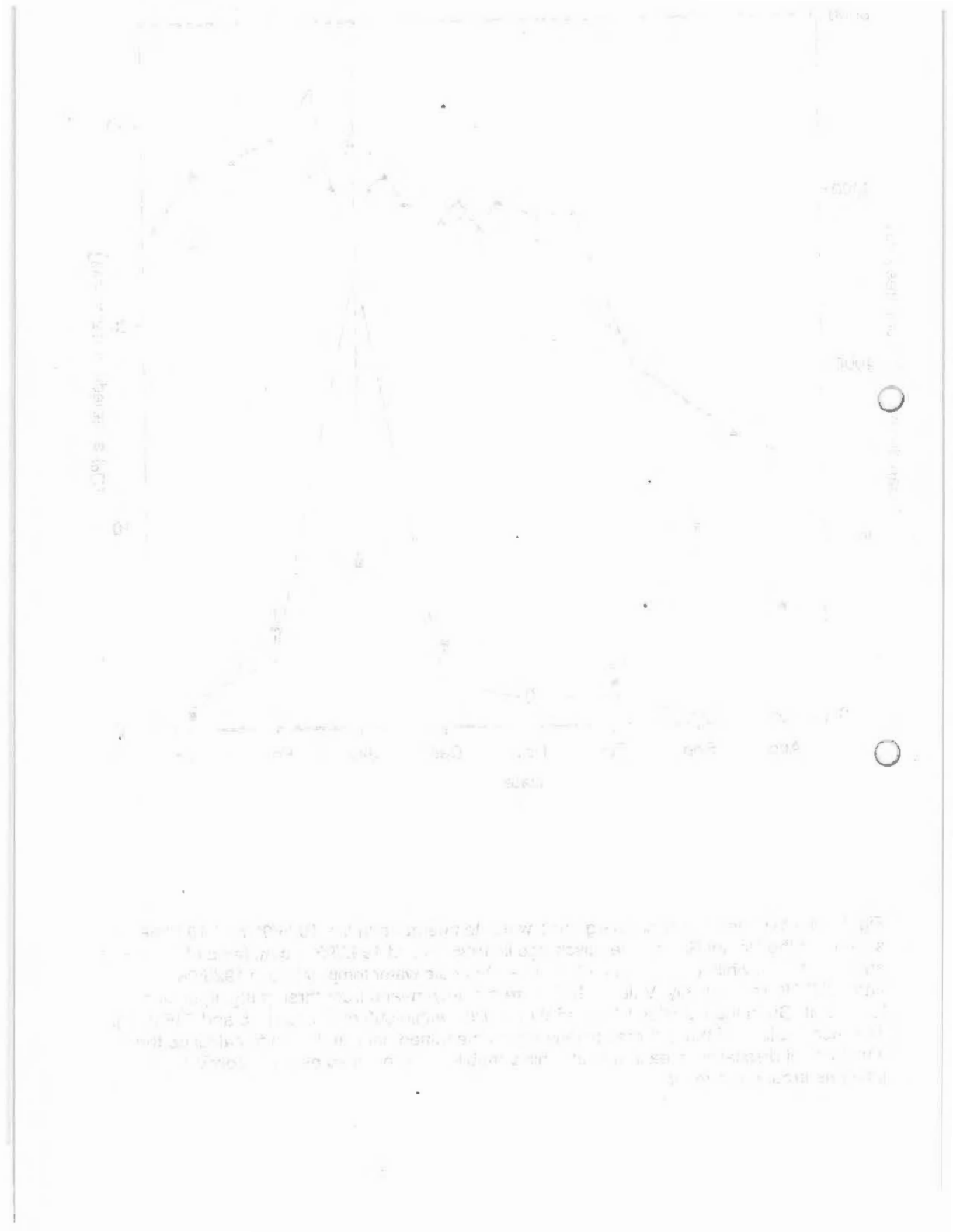


Fig. 2. Monthly mean river discharge and water temperature in the 1995/96 and 1997/98 surveys of the Namoi River. River discharge in 1995/96 and 1997/98 is denoted by (—□—) and (·····■·····), while (·····△·····) and (—▲—) denote water temperature in 1995/96 and 1997/98, respectively. Values shown are monthly means from three gauging stations located at Gunnedah, Mollee Weir and Weeta Weir within 500 m of sites E, 5 and 7 [Fig. 1.]. The mean values of these three stations were determined daily and used to calculate the variability of discharge in each month. This variability is expressed as 95 % confidence intervals around the mean.



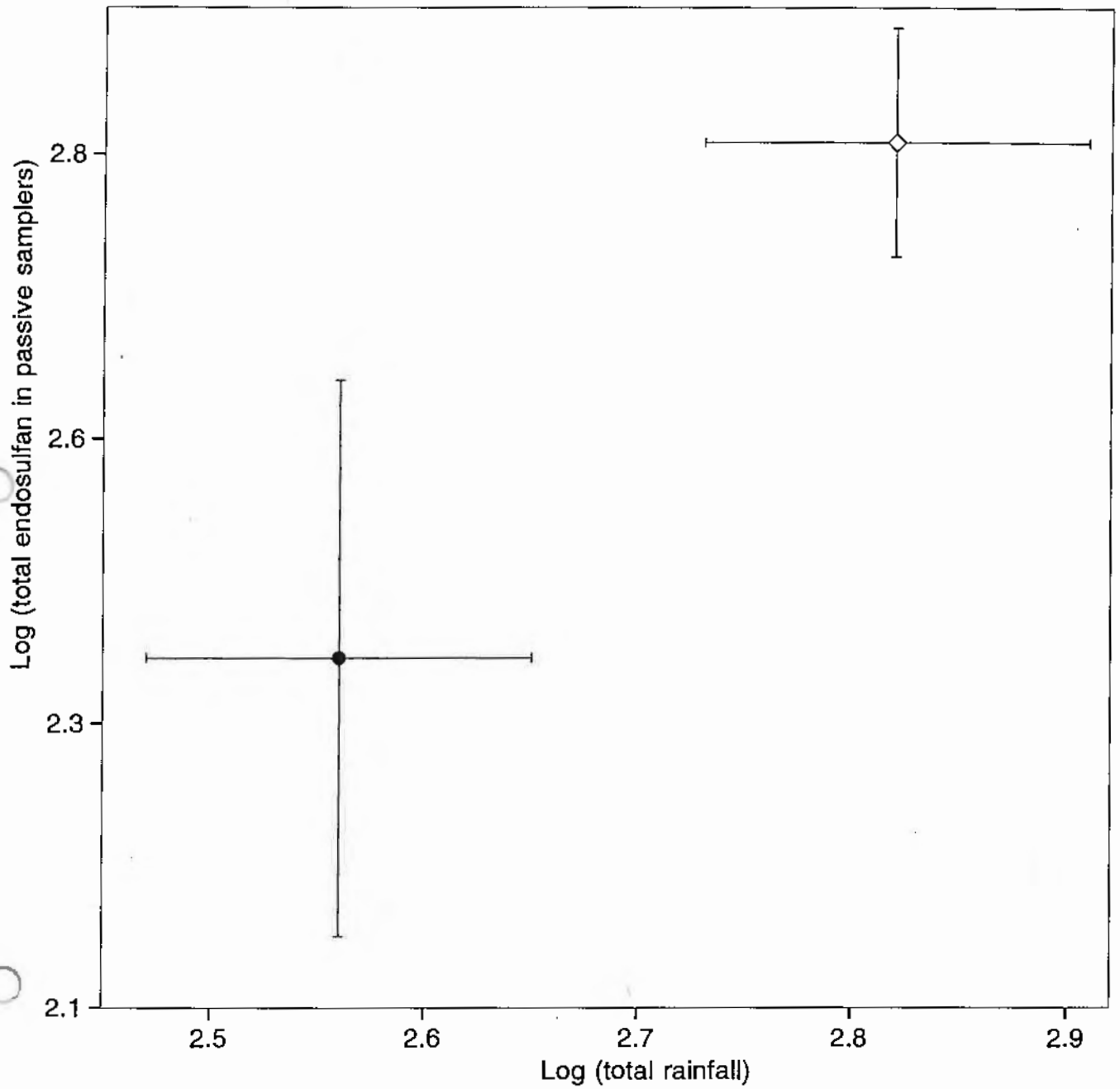


Fig. 3. Variability in total rainfall (mm) and total endosulfan at exposed sites in the Namoi River in January and February in 1996 (◇) and 1998 (●), repectively. The endosulfan values shown are the mean of total endosulfan concentrations (µg/l) measured in the solvent of passive samplers at three exposed sites (sites 5, 6 and 7) common to both surveys. The total rainfall values are the mean of the three nearest rainfall gauging stations [Bureau of Meteorology]. The error bars are 95 % CI values.

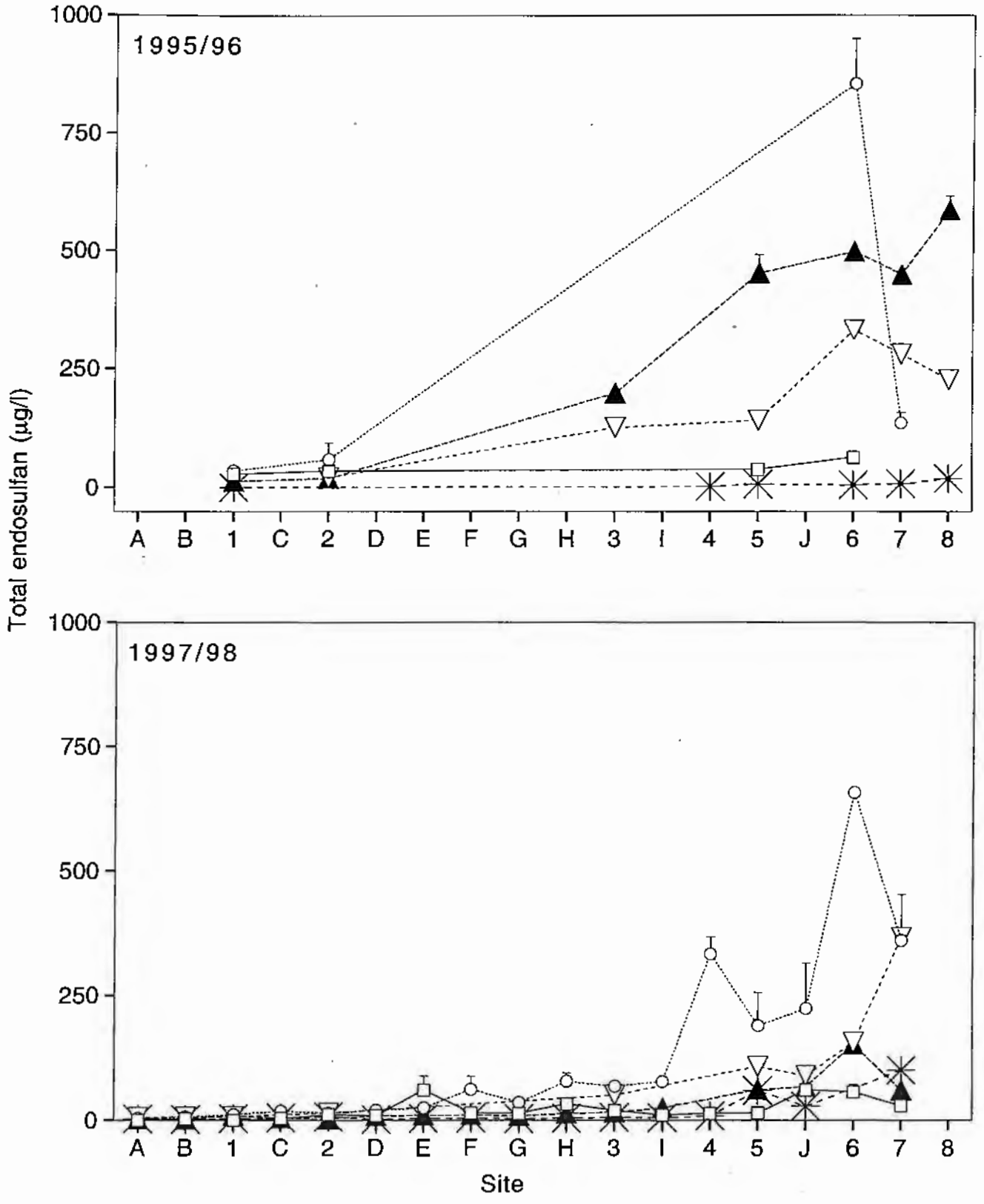


Fig. 4. Concentrations of total endosulfan in the solvent of passive samplers positioned in the water column of the Namoi River in the endosulfan spraying season in 1995/96 and 1997/98. These sampling times are indicated as follows: November (—□—), December (·····○·····), January (----▽----), February (----▲----) and March (- -✳- -). Initial technical problems resulted in data loss particularly in November and December 1995. Only nine sites were sampled in January 1998 due to inaccessibility to the remaining sites. Data from sites 4 and 6 were missing in February and March 1998 due to loss of the samplers. All axes have the same units for each graph. Values shown in µg/l are the mean of two or three samplers

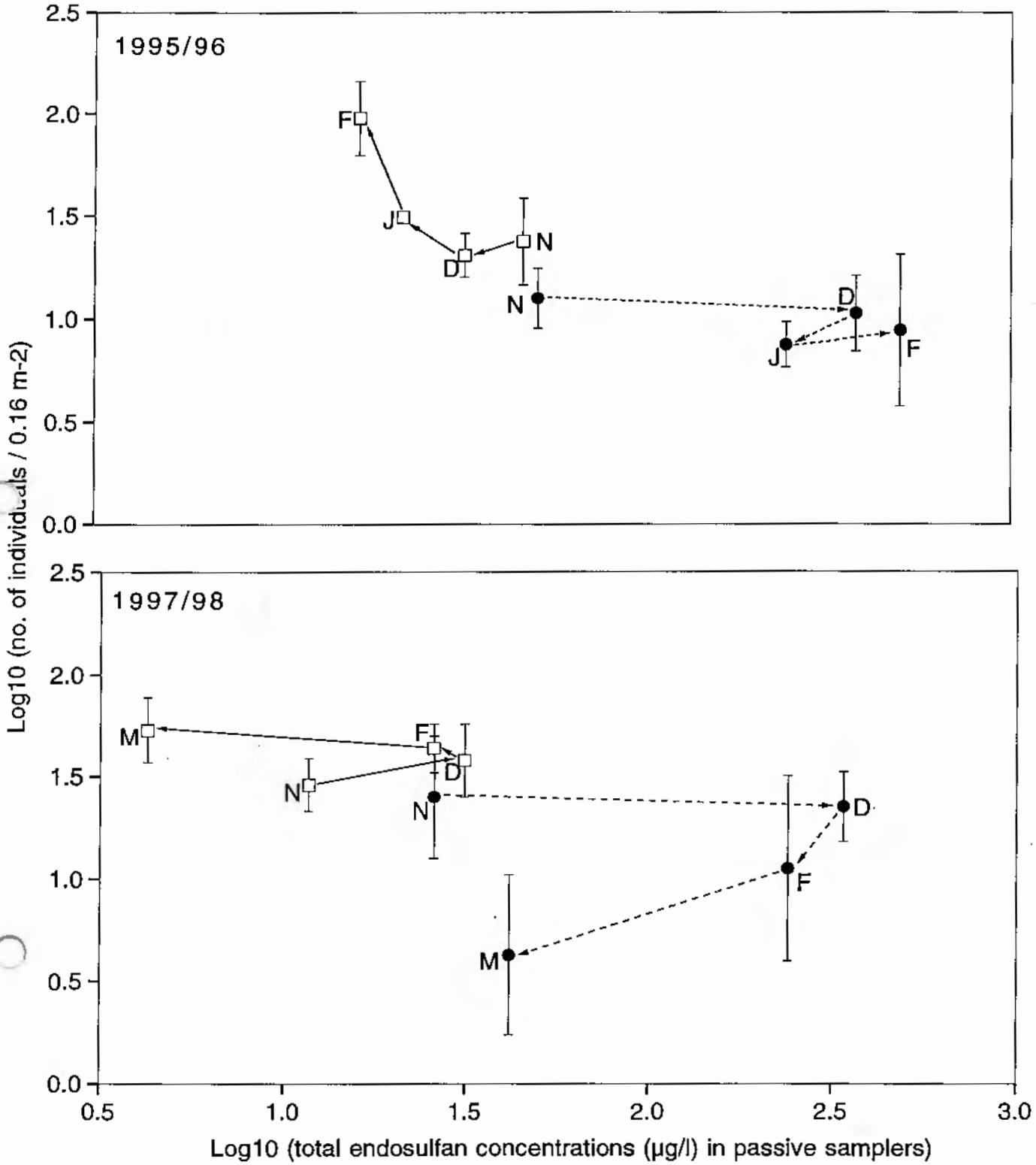


Fig. 5. Densities of dominant mayfly nymphs and caddisfly larvae in the Namoi River at reference (□) and exposed sites (●) in the 1995/96 and 1997/98 spraying seasons for endosulfan. Scales are the same on the axes of the two plots. The x-axis indicates changes in total endosulfan concentrations in the solvent of passive samplers positioned in the water column. These concentrations were used to categorise sites into two reference and six exposed in 1995/96 and five exposed and twelve reference sites in 1997/98. The different sampling times are indicated by: N =November; D =December; J =January; F =February and M =March. The error bars are 95 % confidence intervals except for the two reference sites in 1995/96. These were based on minimum and maximum values, except in January 1996 when there was only data from one

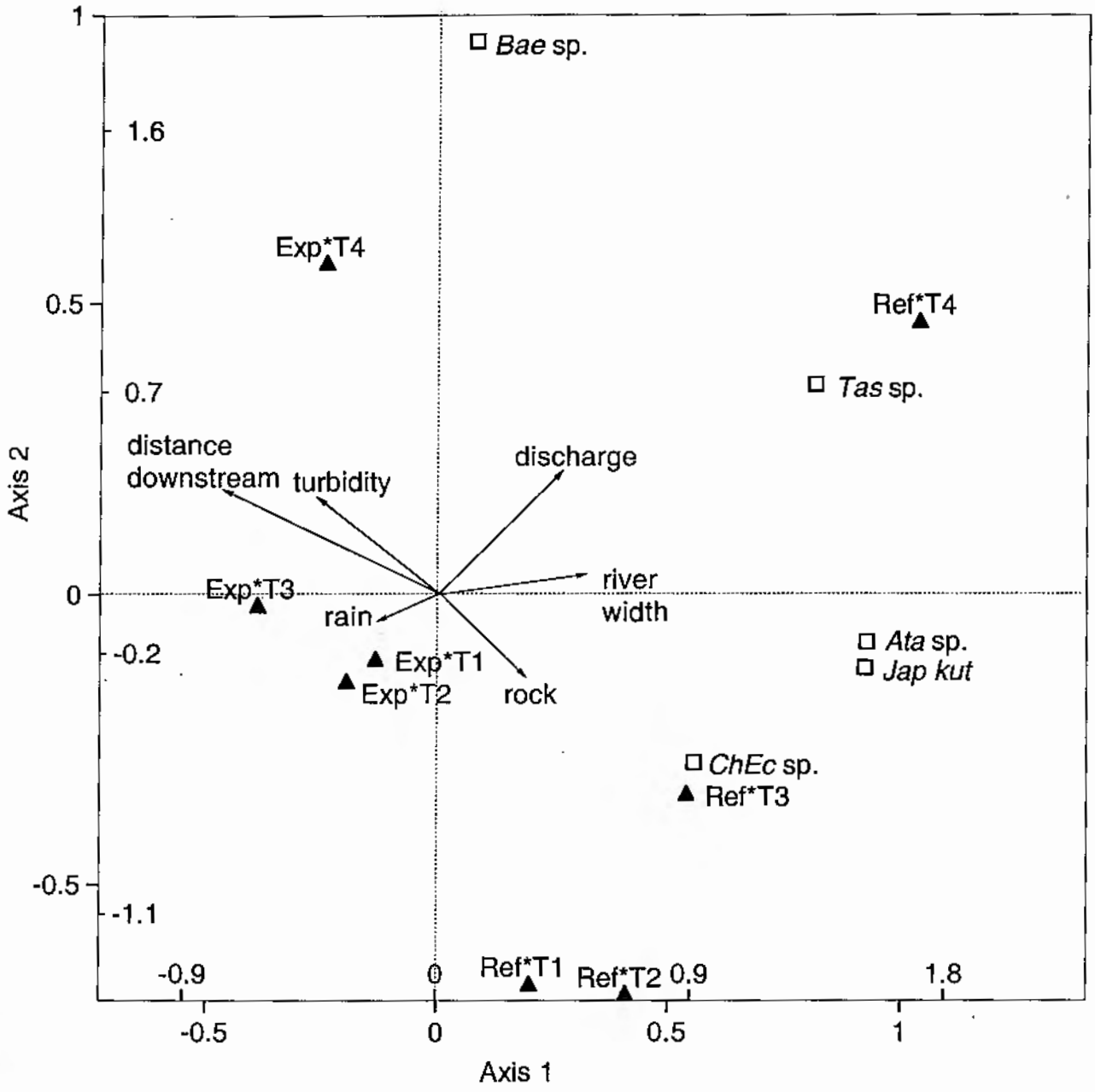


Fig.6. Principal Components Analysis of species data and measured environmental variables in 1995/96. The sites were defined as reference or exposed sites by endosulfan concentrations measured in solvent-filled passive samplers located at each site. The interaction of time (T1 = November, T2 = December, T3 = January and T4 = February) with the reference (Ref) and the endosulfan-exposed (Exp) sites is plotted as a nominal variable (▲). The scales on the inside of the axes are the environmental variables (river discharge, rock size, river width, rainfall, turbidity and distance downstream) and those on the outside of the axes refer to species scores. The percentage of variation explained in the species data was 54 % on axis 1 and 23 % on axis 2. The taxon centroids (□) included the mayfly nymphs of *Jappa kutera* (*Jap kut*), *Atalophlebia* sp. (*Ata* sp.), *Baetis* sp. (*Bae* sp.), *Tasmanoceonis* sp. (*Tas* sp.) and the combined caddisfly larvae of *Cheumatopsyche* sp. and *Ecnomus* sp. (*ChEc* sp.)

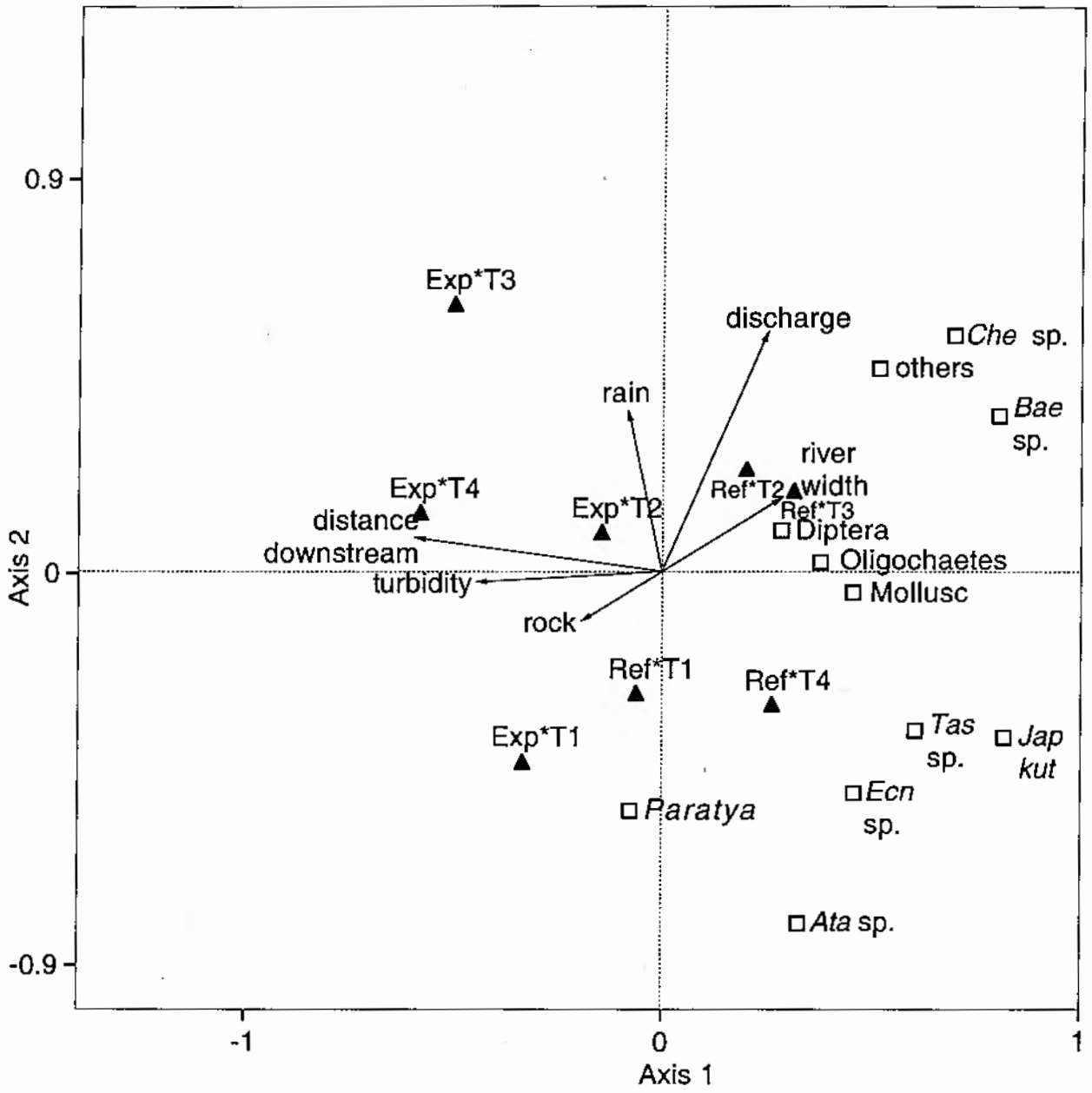


Fig. 7. Principal Components Analysis of species data and measured environmental variables in 1997/98. The sites were defined as reference or exposed sites by endosulfan concentrations measured in solvent-filled passive samplers located at each site. The interaction of time (T1 = November, T2= December, T3= February, T4= March) with the reference (Ref) and the endosulfan-exposed (Exp) sites is plotted as a nominal variable (▲). The scales on the outside of the axes are for the environmental variables (river discharge, rock size, river width, rainfall, turbidity and distance downstream) which in this case were equal to the scale of the species scores. The first axis explains 33 % of the variation in the species data, while the second axis explains 21 %. The taxon centroids (□) included the mayfly nymphs of *Jappa kutera* (*Jap kut*), *Atalophlebia* sp. (*Ata* sp.), *Baetis* sp. (*Bae* sp.), *Tasmanoceonis* sp. (*Tas* sp.), oligochaete worms (oligoch), diptera, the shrimp *Paratya australiensis (Paratya)*, molluscs, and unidentified taxa (others). The "others" centroid was not identified as it consisted of less than 0.1 % of the community

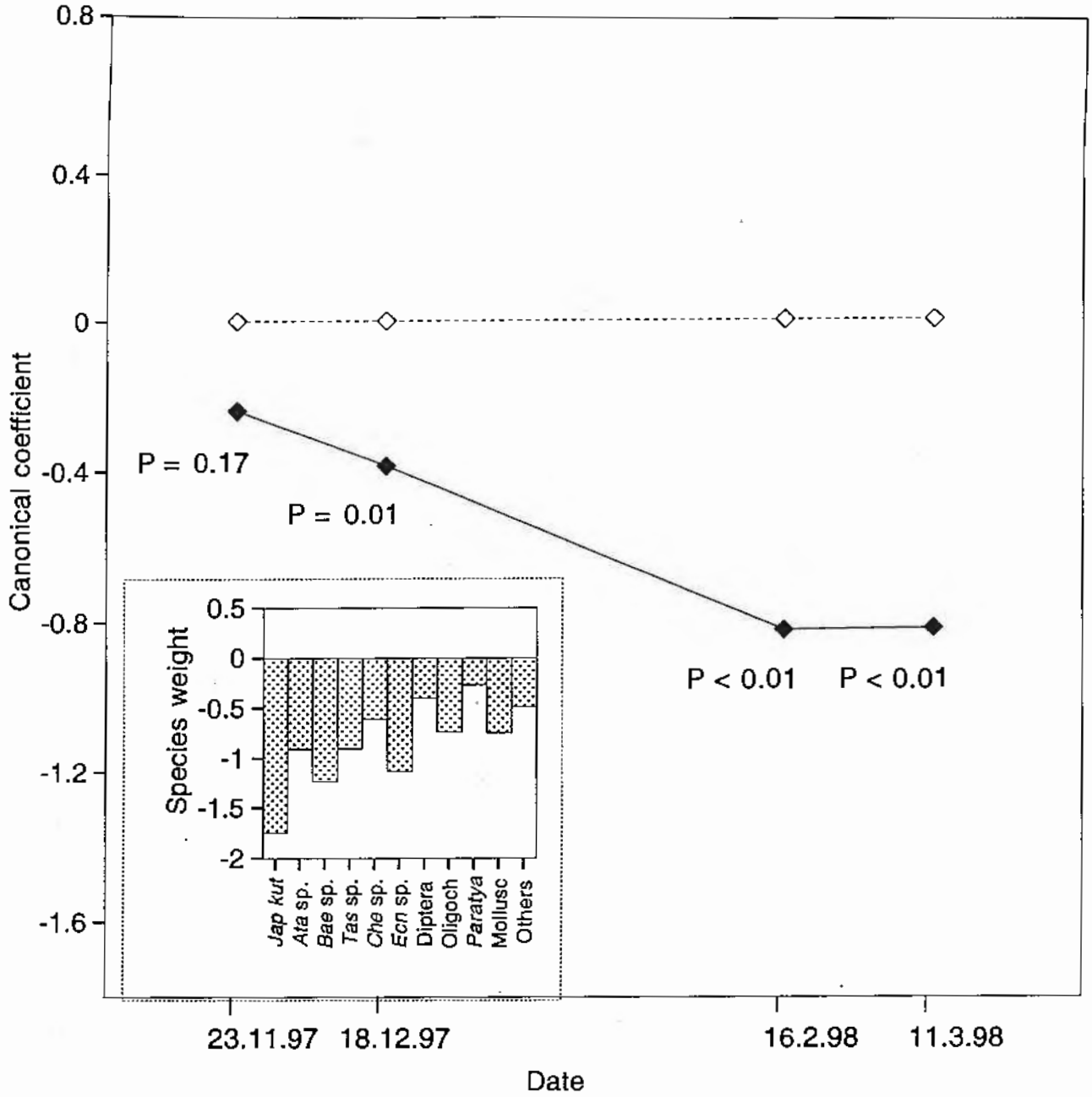


Fig. 8. Principal Response Curves of taxa densities for the macroinvertebrate community of the Namoi River at reference sites (----◇----) and endosulfan-exposed sites (—◆—) between November 1997 and March 1998. The relationship between total endosulfan concentrations in the passive samplers and time was collinear with other variables. However, this relationship accounted for 21.9 % of the total variation, 17.2 % is shown in this figure. Study taxa are indicated in the inset bar chart of species weights, include the mayfly nymphs of *Jappa kutera* (*Jap kut*), *Atalophlebia* sp. (*Ata* sp.), *Baetis* sp. (*Bae* sp.), *Tasmanoceonis* sp. (*Tas* sp.)and the caddisfly larvae of *Cheumatopsyche* sp. (*Che* sp.) and *Ecnomus* sp. (*Ecn* sp.), oligochaete worms (oligoch), diptera, the shrimp *Paratya australiensis (Paratya),* molluscs, and unidentified taxa (others). The significance of each timepoint is indicated by the P values generated from unrestricted Monte-Carlo permutations [20].

1999/UTS1C
Final Report: Relationships between populations of selected macroinvertebrates and pesticides in the Namoi River
Alex Leonard, University of Technology – Sydney