Effects of chlorpyrifos on macroinvertebrate communities in coastal stream mesocosms

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Running title: Impact of chlorpyrifos on macroinvertebrates in mesocosms

Abstract

This study measured the effects of a single pulse of chlorpyrifos at nominal concentrations of 1 µg/L and 10 µg/L on the macroinvertebrate community structure of a coastal stream mesocosm system. Analysis of data using Principal Response Curves (PRC) and Monte Carlo tests showed significant changes in the treated stream mesocosms relative to that of the controls. These changes in the macroinvertebrate assemblages occurred within 6 h, and persisted for at least 124 days after dosing. Significant community-level effects were detected at the lowest concentration on days 2 and 16 post-dosing, giving a no-observed effect concentration ($NOEC_{community}$) of 1.2 µg/L (measured).

The mayflies *Atalophlebia* sp. and *Koorrnonga* sp., Chironomidae and Acarina were all sensitive to chlorpyrifos and decreased in abundance in treated mesocosms after dosing. The fauna of these coastal stream mesocosms showed similar sensitivity to chlorpyrifos with that of other reported studies, but there was no evidence of recovery after 124 days.

Keywords: Chlorpyrifos, mesocosm, toxicity, organophosphate pesticide, artificial streams, macroinvertebrates

1. Introduction

The organophosphate pesticide, chlorpyrifos, is used extensively in the management of urban and rural agricultural pests because of its high acute toxicity to insects and its relatively low persistence in the environment. It has been used in Australia for over 30 years, yet despite this, there are few toxicological data using local species.

Chlorpyrifos is frequently used for urban and domestic pest control, including turf maintenance, and as a termiticidal barrier in, around or under buildings, and is a common constituent of sewage effluent (NRA, 2000). In Australia, urban development is focused on coastal regions, particularly on the east coast, thus the use of chlorpyrifos in urban areas may pose a risk to coastal streams. These streams along the coastal fringe have fauna and environmental attributes that are different from streams and rivers in inland areas of New South Wales, Australia (Turak et al., 1999).

Chlorpyrifos is considered highly toxic to non-target aquatic organisms, particularly arthropods (Crane et al., 2003). Several pond mesocosm studies have shown significant changes in macroinvertebrate and zooplankton assemblages, and water quality following chlorpyrifos exposure (Leeuwangh 1994; Verdonschot & ter Braak 1994; van den Brink et al., 1996). However, the applicability of these studies to model the impacts of an acute exposure in streams following accidental spills or runoff is questionable. Furthermore, the application of these predominantly northern hemisphere derived data to Australian conditions is questionable. Clarke et al. (1994), Pusey et al. (1994) and Ward et al. (1995) report the impacts of acute and chronic chlorpyrifos exposures on macroinvertebrates in artificial streams in sub-tropical Australia but, with the exception of Olima et al. (1997) who showed that sub-

populations of the freshwater shrimp *Paratya australiensis* were not equally sensitive to chlorpyrifos, no data exists for temperate Australian species or ecosystems.

This study investigates the toxicity of a pulse exposure to chlorpyrifos to macroinvertebrate assemblages in an outdoor stream mesocosm system. Pablo et al. (in review) report the fate and toxicity of chlorpyrifos in our mesocosm system and showed that chlorpyrifos is removed rapidly from the water compartment and more slowly from the gravel/pore water compartment. Despite this, single-species toxicity tests conducted in the mesocosms indicated that even short exposures at realistic environmental concentrations can be acutely toxic to aquatic biota (Pablo et al., in review). Here we report the effects of chlorpyrifos exposure on macroinvertebrate assemblages in these streams, and further, relate the toxicity of chlorpyrifos to nymphs of the mayfly *Atalophlebia* sp. in the laboratory and caged populations (from Pablo et al., in review) to populations in the mesocosm benthos. Our overall prediction is that chlorpyrifos exposure will cause significant changes to the macroinvertebrate assemblages of the streams and that for *Atalophlebia* sp., these changes may be predicted from laboratory toxicity data.

2. Methods

2.1 Description of the stream mesocosm system

A mesocosm system of 12 artificial streams was located at the University of Technology, Sydney, field station near Stroud, approximately 70 km north of Newcastle, New South Wales, Australia (E 151^{o} 57’ 59”, S 32^{o} 26’ 53”) (Fig. 1). The stream mesocosm system was constructed on the banks of Alderley Creek, a tributary of the Karuah River. This system is described in detail in Pablo et al. (in review) and is summarised below. The 12 stream channels were each 4 m long and

constructed from 30-cm diameter PVC pipes, with one third of their walls removed to create a trough to allow light and access to the streams. The substrate was graded 12 mm diameter gravel, with a maximum depth of approximately 80 mm.

The streams operated as a flow through system, and water was pumped from a pool area of the adjacent creek into a 20 000-L storage tank. Water was gravity fed via polyethylene pipes from the storage tank to the head of each stream. A plastic valve regulated the discharge into each stream at approximately 8 L/min to give a mean flow rate of 0.3 m/min. Fixed drainpipes at the end of each stream maintained water depth at approximately 100 mm above the gravel substrate. The outflow water was passed through a bed of activated charcoal before being returned to Alderley Creek. The streams were set up one year before the dosing experiment to allow maturation of the system (i.e. development of biofilm on the substrate and leaching of chemicals from the PVC stream channels) and macroinvertebrate recruitment to occur naturally via the water inflow, and aerially from the adjacent creek.

2.2 Experimental design

Experiments were conducted in the period April to September 1996, with dosing conducted in May. Application of chlorpyrifos for termite control occurs all year round; so too do rain events, runoff and subsequent chlorpyrifos contamination of nearby rivers and streams (NRA, 2000). This study targets these situations (see Introduction). We chose to conduct the experiment in a cooler season to allow comparison with previous studies (see Pusey et al., 1994), and because we expected that with lower temperatures the half-life of the pesticide would be longer. All 12 streams were used for the experiment and treatments were allocated in a stratified random design. Groups of three adjacent streams were identified *a priori* and one of

each treatment and a control randomly allocated to a stream within each group. This was done to ensure a spread of treatments across the mesocosm system but the groups were not considered as a factor in the analysis of data. The design of the experiment involved solvent control and water-only control streams, each with two replicates, and two chlorpyrifos treatments (nominal 1 and 10 µg/L) each with four replicates. Solvent control streams received solvent (acetone) at the concentration equivalent to that used for the highest chlorpyrifos treatment (0.01% v/v).

2.3 Chlorpyrifos application

The outlets of the streams were blocked and the flow was stopped to maintain static conditions for 6 h after chlorpyrifos application. Technical grade chlorpyrifos (Dursban, 97% purity Dow Elanco) was dissolved in nanograde acetone. Appropriate amounts of chlorpyrifos were made up to approximately 2 L with creek water and were applied to the streams using 2 L glass separating-funnels. The chlorpyrifos solutions were distributed by walking up and down the length of the stream as the funnel emptied. The stream water was then mixed with a polyvinyl chloride (PVC) paddle moving up and down each stream six times.

2.4 Water sampling and chemical analysis

Concentrations of chlorpyrifos were measured in water samples collected at the beginning and end of the 6-h dosing period in each stream. Water samples (500 mL) were collected from each stream using a glass beaker and each sample was then poured into individual 1-L separating funnels for pesticide extraction. Chlorpyrifos was extracted twice from the water samples in the field using 75-mL aliquots of nanograde dichloromethane. After the aqueous and solvent phases had separated,

we combined the dichloromethane aliquots and removed any remaining water by adding anhydrous sodium sulfate. The final organic extracts were poured into 200-mL amber glass bottles and transported to the laboratory for concentration and analysis. Extracts were analysed using a Hewlett-Packard 5890 Series II Plus gas chromatograph (Wilmington, DE, USA) equipped with an HP 7673 automatic injector and a ^{63}Ni electron capture detector. The gas chromatograph was calibrated daily using hexane blank and at least two chlorpyrifos standards bracketing the concentrations analysed. Chlorpyrifos peaks were confirmed by the increase in peak height and area from standard additions and samples from control streams served as field blanks. The practical detection limit was typically 0.02 µg/L. The average extraction efficiency (90.4%) was determined using chlorpyrifos-spiked creek water and the reported chlorpyrifos concentrations have been corrected accordingly.

A detailed description of the fate of chlorpyrifos in the water and gravel/porewater compartments of the streams is given in Pablo et al. (in review). We used the measured concentrations of chlorpyrifos in the water column to explain the effects on the macroinvertebrate assemblages. Dose concentrations for each replicate were determined by calculating the geometric mean of chlorpyrifos concentrations measured in water samples collected at the beginning and end of the 6-h dosing period. Average exposure concentrations (AEC) for each treatment were determined by taking the average of the geometric mean concentrations of the four replicate streams in each treatment.

Temperature, pH, conductivity, turbidity and dissolved oxygen (DO) concentration of the water in each stream were measured daily between 0900 and 1000 h. These measurements were made half-way along each stream using a Horiba V-10 Water Quality Checker.

2.5 Macroinvertebrates sampling

Benthic macroinvertebrates were collected from the streams using gravel-filled perforated baskets (15 cm length x 10 cm width x 6 cm depth) that were set into the substrate of the streams 4 weeks before the first sampling event. The baskets served as quantitative sampling units and the perforated design of the baskets allowed unimpeded movement of macroinvertebrates. The streams were sampled for macroinvertebrates on three times before dosing (-40 d, -12 d and -1 d), immediately after the dosed water in the streams was released (on day 0) and thereafter on days 2, 4, 16, 31, 58 and 124.

For each sample, two baskets were collected from each stream in a stratified random design; one basket was chosen randomly and removed from each of the upper and lower halves of the stream. The gravel-filled baskets were gently removed from the streambed and placed in a small net of 200-µm mesh while still underwater. The contents of the sampling baskets were washed through a 10 mm-mesh sieve for two minutes to separate the gravel from the smaller particles and biota, with the latter collected in a 200-µm mesh net. The contents of the net were emptied onto a white tray and all live macroinvertebrates in the sample were picked in the field under low magnification. All macroinvertebrates were preserved in 70% ethanol and later identified to genus or family level, using keys listed in Hawking (2000). The two baskets from each stream were processed separately and the data for each later pooled to make one sample for analysis.

2.6 Data analysis

Macroinvertebrate community data were analysed using Principal Response Curves (PRC) (van den Brink and Ter Braak, 1999) using CANOCO version 4 (Ter Braak and Smilauer, 1998). This method of multivariate data analysis has been developed for use with community response data from experiments designed with repeated temporal sampling, such as mesocosm experiments (van den Brink and Ter Braak, 1999). The PRC method is based on redundancy analysis but is extended to adjust for changes in the controls over time (van den Brink and Ter Braak, 1999). Macroinvertebrate data were ln(2x+1) transformed before analysis and all other recommended settings were used (van den Brink and Ter Braak, 1999).

The PRC analysis incorporates an analysis of the responses of each species in the dataset by providing a weighting that reflects how closely the response pattern of that species follows the pattern in the PRC. Species or taxa with negative species weights are expected to increase in abundance in the treatments relative to the controls, and taxa with positive species weights are expected to decrease in abundance in the treatments relative to the controls. Taxa with near zero weights either show no response or a response that is unrelated to the PRC (van den Brink and Ter Braak, 1999).

To determine the significance of the treatments, Monte Carlo permutation tests were performed per sampling date. AECs were ln(2x+1) transformed and used as the explanatory variable (van den Brink et al*.,* 1996). Each sampling point in which a significant effect was detected by the Monte Carlo permutation tests was then analysed to determine a no observed effect concentration (NOEC) value at the community level. The community data were reduced to a single variable by taking the sample scores of the first principal component of a principal components analysis (van den Brink et al*.,* 1996; van den Brink and Ter Braak, 1999). The significance of

each treatment was tested by comparing the sample scores of each treatment with those of the controls for each time point in turn using Williams test (Williams, 1972) in the TOXSTAT 3.5 program (Gulley and West, Inc., 1996).

The response of some univariate community parameters, the abundances of important taxa (identified by species weights from PRC) and physico-chemical water quality variables were tested using repeated-measures analysis of variance (ANOVA) using SPSS (Release 10.0.5, SPSS Inc, Chicago, USA). Treatments and times were considered as fixed factors. All data were tested for homogeneity of variance using Cochran's test (Winer, 1971) and if required, data were ln(x+1) transformed. All control samples were pooled for these analyses. For all univariate and multivariate statistical comparisons the significance level (α) was 0.05.

3. Results

3.1 Water quality

The fate of chlorpyrifos in the mesocosms is described in detail in Pablo et al. (in review). Chlorpyrifos concentrations measured in the water compartment were close to nominal values and the fate of chlorpyrifos in the high and low-dose streams of the mesocosm showed similar patterns. The concentrations in water decreased quickly and were undetectable after 48 h in the low dose and after 96 h in the high dose. The average exposure concentrations (±SE) were 1.2 ± 0.1 and 10.0 ± 0.6 µg/L for the low and high treatments respectively.

The physico-chemical water quality variables in the mesocosms varied among sampling periods as conditions changed in the water supply from Alderley Creek, but there were no significant differences among the treatments ($p>0.05$) nor were there significant time x treatment interactions that would indicate treatment related

differences among the streams. Water temperatures (mean reading 14.7°C, SE = 1.0) were normal for autumn/winter water temperatures in this area (Hose and Turak, 2004). The mean dissolved oxygen concentrations (mean 9.1 mg/L, SE = 0.3) indicated the streams were well aerated throughout the experiment. pH (mean 7.07, SE = 0.11) and conductivity (mean 219 µS/cm, SE = 25) were consistent over time and among streams, and were within ranges expected for rivers in the region (Hose and Turak, 2004). Turbidity (mean 13.4 NTU, SE = 1.8) was high immediately following dosing due to the disturbance of the pesticide addition and mixing, and declined sharply after the dosing period. The range of turbidity readings was within range expected for rivers in the region (Hose and Turak, 2004).

3.2 Community analyses

Fifty taxa were recorded in the benthic assemblages during the study. Mayflies accounted for 19% of the total macroinvertebrate abundance, of which *Atalophlebia* sp. and *Koorrnonga* sp. were the most abundant taxa. Oligochaeta, Turbellaria and Diptera were also numerically dominant. For Diptera, this was due to the high abundance of Chironomidae in most samples.

Macroinvertebrate assemblages from solvent control and water-only control streams were not significantly different ($p = 0.37$). Although the replication used for the test was small, results of previous artificial stream studies have indicated that the volumes of the acetone solvent used would have no detectable effect (Hose et al., 2003). The two control groups were pooled for further analysis giving a total of four control streams.

A significant pattern ($p = 0.02$) was detected by the PRC analysis. Differences among sampling times accounted for 20.5% of all variance, while differences among

treatments accounted for 19.7% of all variance. The remainder was attributed to variability among replicates. The response pattern in the first PRC axis was significant ($p = 0.01$), and this axis captured a much greater proportion of the total variance explained by the treatment regime (46.7%) than the second axis (12.9%), which was not significant ($p = 0.91$). For this reason, only the first axis of the PRC analysis is presented.

Before exposure, the macroinvertebrate assemblages in the streams were similar, as indicated by the closeness of response curves (Fig. 2). After exposure, the macroinvertebrate assemblages in both treatments were significantly different ($p<0.05$) from those in the controls and remained so for the course of the study (Fig. 2). The deviation of the treatments from the controls was consistent with the results of Monte Carlo tests, which detected significant ($p<0.05$) treatment effects for all post-dose sampling times.

Further analysis of the assemblage data indicated that significant differences detected by the Monte Carlo tests were due to samples from treatments being significantly different from those in the controls. The $NOEC_{community}$ values fluctuated during the experiment. There was considerable within-treatment variability for the sample scores from the PCA (on which the Williams test is based), which is likely to be the cause of the fluctuating NOEC values that range from <1.2 to 10.0 µg/L. On days 2 and 16, effects were detected at the lowest concentration (Fig. 2), thus the $NOEC_{community}$ value was undefined but less than 1.2 µg/L.

The dose response pattern among treatment and control streams was strongly influenced by differences in the abundances of several taxa. *Atalophlebia* sp., *Koorrnonga* sp., Chironomidae and Acarina all had strongly positive species weights (Fig. 2), indicating their abundances were greater in samples from the control and

lower dose treatments than those from the highest dose treatment. In contrast, capilloventrid oligochaetes and *Ferrissia* sp. (Ancylidae: Gastropoda) had negative species weights, indicating that they were more abundant in treatments than the controls. Repeated measures ANOVAs on the abundances of these taxa indicated significant differences ($p<0.0.5$) among treatments and controls, but only a significant time x treatment interaction for *Atalophlebia* sp. The abundance of *Atalophlebia* sp. was reduced in the treated streams compared to the control streams after dosing (Fig 3a).

Taxon richness of the streams was not significantly affected by chlorpyrifos application, as evidenced by a non-significant ($p>0.05$) time x treatment interaction, although there appears to be a slight decline in richness in the highest treatment following chlorpyrifos exposure (Fig. 3b).

4. Discussion

The addition of chlorpyrifos to the artificial streams resulted in a rapid (6-h) change in the macroinvertebrate assemblages of the streams, which persisted for at least 124 days after dosing. Although the chlorpyrifos was removed relatively quickly from the system (Pablo et al., in review), the macroinvertebrate assemblage did not recover. The changes observed were mostly due to a reduction in the abundance of dominant taxa, rather than a loss or change in taxon richness among treatments.

Changes to the macroinvertebrate assemblages were caused primarily by reductions in the abundance of the mayfly taxa *Koorrnonga* sp. and *Atalophlebia* sp. The PRC analysis suggests that of these two taxa, *Atalophlebia* sp. was more sensitive. The almost complete loss of *Atalophlebia* sp. from the benthos of the 10 µg/L treatment after dosing could be predicted from the laboratory and caged

mesocosm tests (Pablo et al., in review) that both had 100% mortality at or below 10.0 µg/L. However, both laboratory and cage tests also predicted 100% mortality to occur at 1.2 µg/L (after 6-24 h exposure). If we assume that the reduction in abundance of *Atalophlebia* sp. in the benthos is due to mortality, then the observed reduction in benthic abundance at 1.2 µg/L suggests only 50% mortality (relative to the control abundance). We consider that the lesser effect of chlorpyrifos on the benthic populations compared to laboratory or caged populations is most likely due to the ability of benthic animals to reduce their exposure by burrowing into the substratum where chlorpyrifos concentrations were less (Pablo et al., in review) and bioavailability potentially lower due to sediment binding (Ankley et al., 1994). Such evidence highlights the importance of environmental conditions (such as the presence of substrate) in mitigating pesticide impacts on biota and consequently, the need for higher tiered tests in ecological risk assessment of pesticides.

Together with the mayflies, Chironomidae and Acarina shared large positive species weights, indicating very strong responses to chlorpyrifos exposure. Pusey et al. (1994) reported chironomid species were acutely sensitive to a 6-h pulse exposure to chlorpyrifos in stream mesocosms, and Eaton et al. (1985) reported a 95% reduction in chironomid abundance in a stream receiving 0.13 µg/L. Chironomids were also the most sensitive (10-d LC50 0.07 µg/L) of a range of species tested by Phipps et al. (1995). Unfortunately, further analysis of the chironomids collected by Pusey et al. (1994) was inconclusive in establishing a relationship between mouthpart asymmetry and chlorpyrifos exposure (Clarke et al., 1994).

The weakly negative species score for capilloventrid oligochaetes, suggests they were more abundant in treatment than in control streams. Although van den

Brink et al. (1996) detected an increase in oligochaetes following application of chlorpyrifos to pond mesocosms, we believe our result is due to patchy distribution of oligochaetes among the streams. Indeed, the absence of significant time x treatment interactions (indicating a dose response) for both Capilloventridae and all oligochaetes combined suggests a tolerance to chlorpyrifos at up to 10.0 µg/L. This is consistent with previous studies that suggest that chlorpyrifos is not acutely toxic to oligochaetes in the range of 0.1-44 µg/L (Verdonschot and ter Braak, 1994; van den Brink et al., 1996).

After dosing, no significant change occurred in taxonomic richness of macroinvertebrates in the streams, but mean richness declined slightly in the streams dosed with 10.0 µg/L chlorpyrifos (Fig. 3). This general finding is consistent with that of Pusey et al. (1994) who found no significant reductions in taxon richness, indicating no localized extinctions, but recovery was rapid. Eaton et al. (1985) reported significant reductions in the taxonomic richness of streams dosed with chlorpyrifos, but their experiment involved repeat and chronic exposures, after which invertebrate species richness might be expected to change.

Interestingly, both Pusey et al. (1994) and van den Brink et al. (1996) report recovery of macroinvertebrate assemblages in mesocosms following chlorpyrifos exposure. Macroinvertebrates in the streams of Pusey et al. (1994) took up to 104 days to recover and the ponds of van den Brink et al. (1996) took 168 days. It is surprising, then, that recovery was not evident in the mesocosms of our study. A natural stream receiving the same dose may have had at least some repopulation via downstream drift from upstream intact populations. Such repopulation of the mesocosm was potentially limited by the mechanical impact of the pump on invertebrates, and the limited catch of drifting invertebrates by drawing water from a

still area of the stream. Hence, we were largely reliant on aerial recruitment to the streams. The lack of recovery and repopulation of the streams may be exacerbated by conducting the experiment over autumn and winter months when recruitment is likely to be at its lowest. Continuation of our study further into spring may have detected some recovery by the macroinvertebrate assemblages.

The macroinvertebrate assemblage NOEC varied over the course of the study and NOEC values of 10 µg/L after 6 h and 28 days are at odds with the Monte Carlo tests which identified significant differences among treatments at all post dose sampling events. van Wijngaarden et al. (2005) reported similar temporal variability in community level NOEC values for zooplankton exposed to chlorpyrifos in laboratory microcosms. In our study, the variability in NOEC values is a likely artefact of the relatively lower power of the Williams test compared to the Monte Carlo test. Monte Carlo tests have greater power to detect differences among treatments because they test the significance of the whole ordination space while the Williams test is performed on a reduced space (i.e. only the variance explained by the first PCA axis). Assuming a monotonic dose response (as suggested by the PRC, Fig. 2), all post dose sampling events (that had significant Monte Carlo tests) should have a NOEC $\leq$1.2 µg/L.

Although coastal streams represent a unique and little studied component of Australia's aquatic ecosystems, the fauna appear to respond similarly to that of lentic and lotic ecosystems elsewhere in Australia and abroad. Pusey et al. (1994) report a NOEC value of 0.06 µg/L for macroinvertebrates in stream mesocosms and, van den Brink and Ter Braak (1999) and van Wijngaarden et al. (2005) both report community level NOEC values for macroinvertebrate assemblages at 0.1 µg/L after a single pulse application to pond mesocosms and indoor microcosms, respectively.

However, van Wijngaarden et al. (1996) expressed concern that 0.1 µg/L may yet cause long-term indirect effects on populations or the community since it corresponds to acute EC10 values for several taxa. This concern is justified given that several studies have shown significant effects on macroinvertebrate assemblages or populations subject to chronic application of chlorpyrifos at 0.1 µg/L (Ward et al., 1995; Rakotondravelo et al., 2006).

In this study we have considered the response of biota to concentrations of chlorpyrifos in the overlying water compartment and have ignored the considerable concentrations of chlorpyrifos in the gravel/pore water compartment (Pablo et al., in review). The reason for adopting this approach is because macroinvertebrates tend to occur in the upper layers of the substrate and are likely to have greater exposure to the surface water than to the pore water (Chapman et al., 2002). Furthermore, past studies have all reported surface water concentrations; doing so here allows simple and direct comparison with previous research.

The literature on chlorpyrifos impacts on aquatic biota is extensive, more so than many other pesticides. The similarity of responses among multispecies studies suggests there is a reasonable consistency in the response of macroinvertebrate assemblages irrespective of ecosystem type and geographic region (see van Wijngaarden et al. (2005)). This is consistent with evidence from laboratory-based studies (Maltby et al., 2005). It is a limitation of comparison that the numerous multi-species studies have been analysed by different processes (e.g. PRC, our study, van den Brink and Ter Braak 1999, van Wijngaarden et al., 2005; RDA, van den Brink et al., 1995; ANOVA, Pusey et al., 1994). For direct comparison of findings and toxic concentrations, consistent analysis among studies is desirable and should be a focus of future work on this pesticide. Given the extent and consistency of data now

available for chlorpyrifos, and the lack of data for so many other agricultural chemicals, it may be time to focus pesticide research on new chemicals, or the impact of chlorpyrifos in ecosystems other than fresh surface waters. In particular, there are relatively few data available for marine ecosystems, and Hose (2005) suggests that groundwater ecosystems may be particularly at risk from chlorpyrifos, more so than the stream fauna studied here.

5. Conclusion

Significant changes to the macroinvertebrate assemblages of the artificial streams were detected after a 6-h exposure to chlorpyrifos at 1.2 µg/L. This concentration is similar to those causing effects in mesocosm studies elsewhere, suggesting that the fauna of this coastal stream mesocosm respond similarly to the fauna of mesocosms representing other habitats. Population-level effects on the mayfly *Atalophlebia* sp. in the mesocosms were less than those predicted from laboratory studies or caged mesocosm tests, highlighting the need for realistic test conditions to adequately assess the toxicity of pesticides to aquatic biota.

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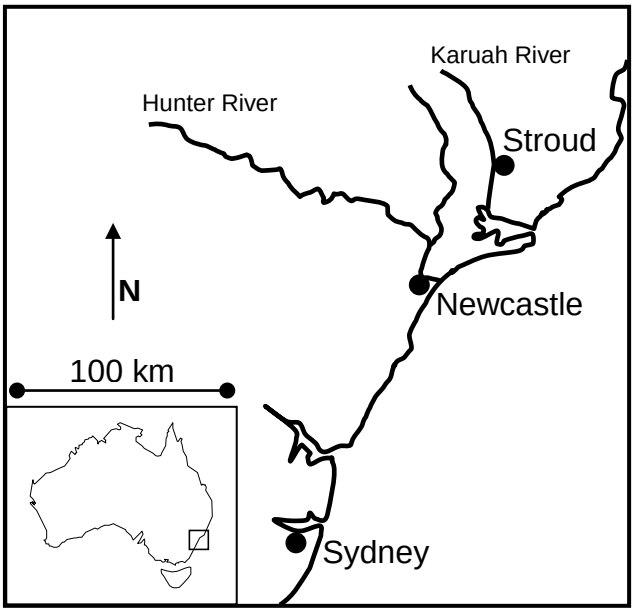
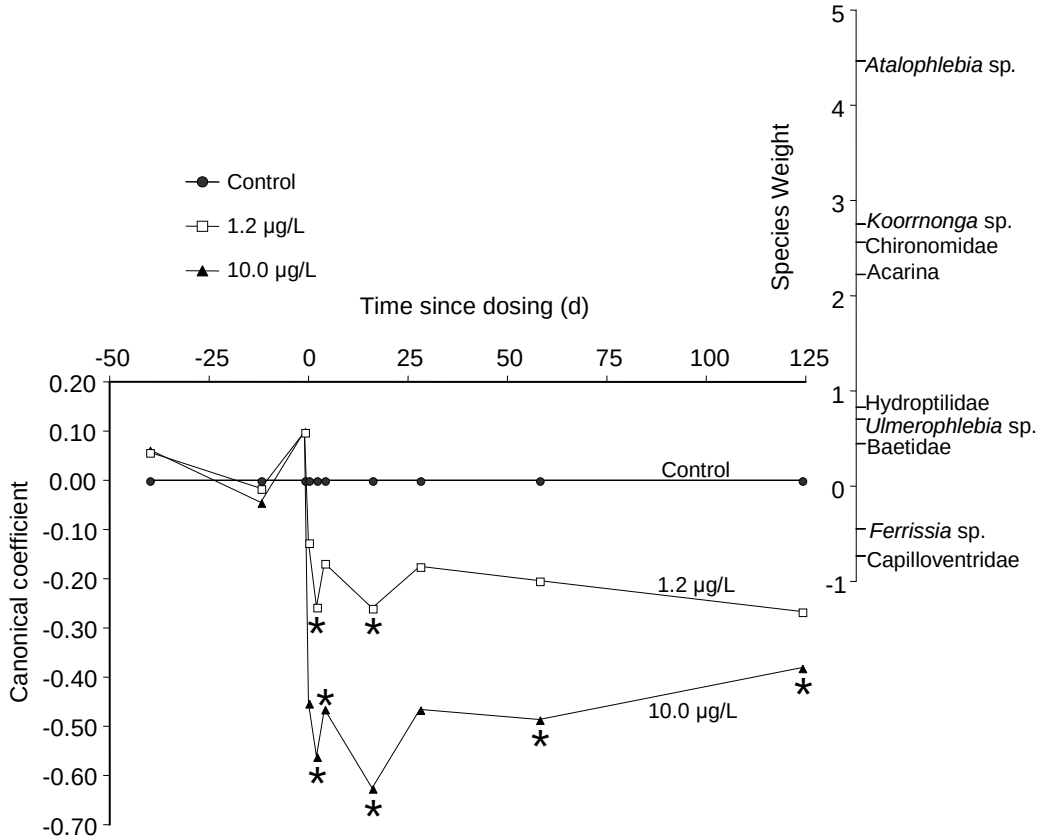
Fig. 1. Location of the artificial stream system at Stroud, NSW, Australia.

Fig. 2. Principal Response Curves with species weights for benthic macroinvertebrate community data indicating the effects of a single 6-h pulse of chlorpyrifos (commencing at time 0). ● = Control, ☐ = 1.2 µg/L, ▲= 10.0 µg/L. Species with weights between 0.4 and -0.4 have been omitted for clarity. Asterisk indicates data point is significantly different ($P<0.05$) from control using PCA/Williams test approach (see text for explanation).

Fig. 3. The mean (± SE) number of a) *Atalophlebia* sp. nymphs and b) taxa in live-picked benthic samples collected before and after chlorpyrifos exposure at time = 0. ● = Control, ☐ = 1.2 µg/L, ▲= 10.0 µg/L.

1 Fig. 1




Control
1.2 µg/L
10.0 µg/L
Time since dosing (d)
Canonical coefficient
Species Weight
-50
-25
0
25
50
75
100
125
0.20
0.10
0.00
-0.10
-0.20
-0.30
-0.40
-0.50
-0.60
-0.70
5
4
3
2
1
0
-1
Atalophlebia sp.
Koorrnonga sp.
Chironomidae
Acarina
Hydroptilidae
Ulmerophlebia sp.
Baetidae
Ferrissia sp.
Capilloventridae
Control
1.2 µg/L
10.0 µg/L
*

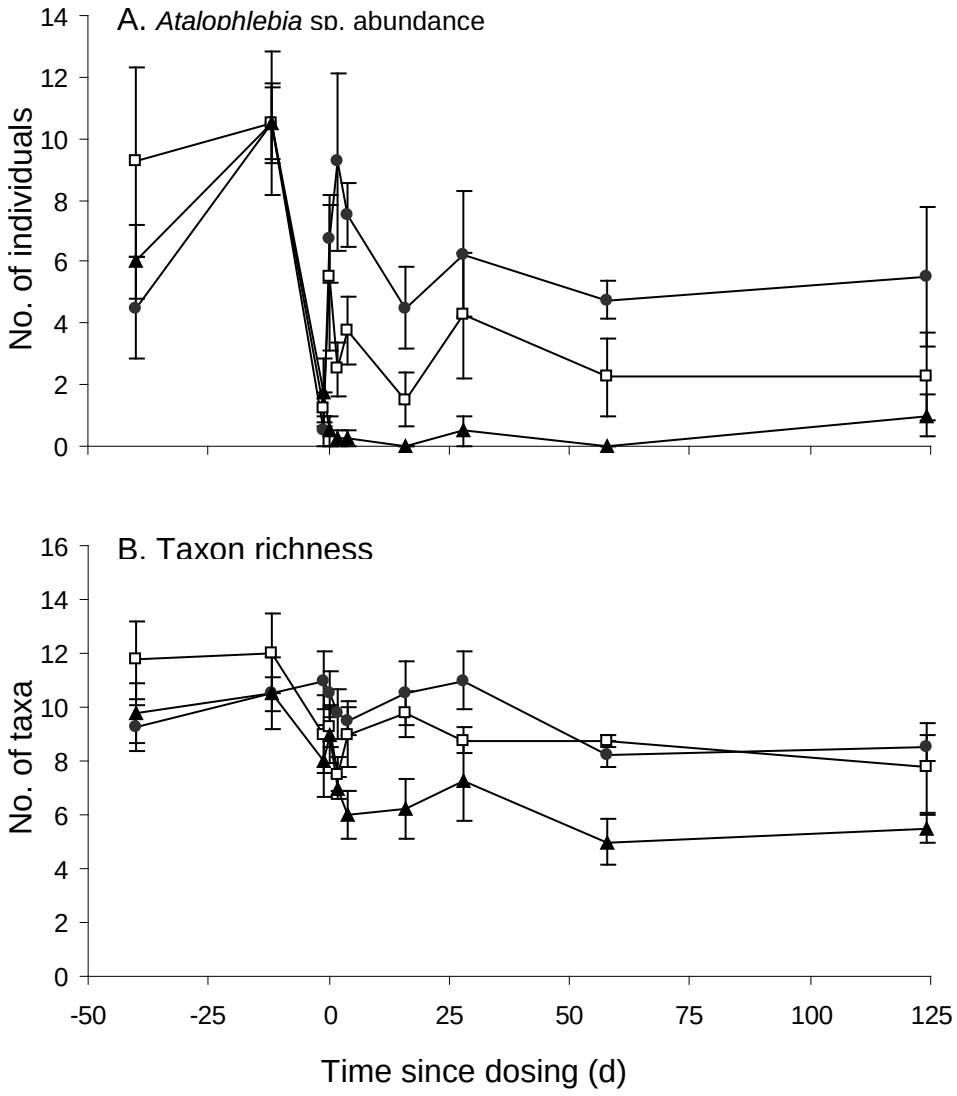

A. *Atalophlebia* sp. abundance
No. of individuals
14
12
10
8
6
4
2
0
B. Taxon richness
No. of taxa
16
14
12
10
8
6
4
2
0
-50
-25
0
25
50
75
100
125
Time since dosing (d)

Fig. 3