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The Freshwater Mussel, *Westralunio carteri* Iredale, as a Biological Monitor of Organochlorine Pesticides

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Abstract

The freshwater mussel *Westralunio carteri* was tested as a biomonitor of the organochlorine pesticides dieldrin, DDE, DDD and DDT. The mussel survived handling, transportation and being caged, and demonstrated significant bioaccumulation of the pesticides. Total DDT analogues at one site reached a maximum of 0·48 mg kg^{-1} wet whole tissue after 112 days and at a second site accumulated to 1·23 mg kg^{-1} wet whole tissue after 66 days.

It is suggested that observed reductions in levels of bioaccumulated residues by aestivating mussels in an intermittent stream were the result of preferential utilization of stored lipids and release of associated pesticides.

Introduction

Mussels as bioaccumulators have many advantages over chemical analysis: they accumulate only biologically available forms of the pollutant; they are continuously present in the environment and therefore may be used to continuously monitor pollutants; fluctuations in concentration are integrated over time; and the magnification afforded by bioaccumulation may be advantageous in respect to the accuracy and expense of analysis of trace pollutants near the limits of analytical detection (Jones and Walker 1979).

Freshwater mussels have frequently been used as biological monitors of both organochlorine and trace metal contamination in Northern Hemisphere systems (Bedford *et al.* 1968; Manly and George 1977; Foster and Bates 1978; Czarnezki 1987). In Australia, only species occurring outside Western Australia have been investigated (Ryan *et al.* 1972; Jones and Walker 1979; Millington and Walker 1983; Jeffree and Simpson 1986). The present study was designed to test the suitability of *Westralunio carteri* Iredale, a species of freshwater mussel endemic to Western Australia, as a bioaccumulator of organochlorine pesticides.

Streams and rivers of the Darling Range, Western Australia, are extensively used for water supply, with the majority impounded by storage reservoirs. The catchments also support an established mixed fruit-growing industry, with many orchards located on head-water streams. Before restrictions on the use of organochlorine pesticides (July 1987), these chemicals were routinely applied to the orchards for pest management. Concern has since arisen over the possible contamination of head-water streams by organochlorine pesticides in surface-water run-off. The pesticides selected for monitoring were dieldrin [85%, 1,2,3,4,10,10-hexachloro-6,7-expoxy-1,4,4a,5,6,7,8,8-octahydro-1,4-*endo*,*exo*-5,8-dimethaonaphthalene(HEOD)], DDE [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene], DDD [1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane] and DDT [1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane]. These pesticides are highly soluble in the lipid or fat tissue of organisms,

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and their low solubility in water facilitates their persistence in the environment (Hellawell 1986). The approximate half-life for DDT and dieldrin in soil is 2·8 and 2·5 years, respectively, and the time taken for 95% of their residues to disappear is 10 and 8 years, respectively (Hellawell 1986). Therefore, organochlorine pesticides persist in a system long after their application has ceased.

Methodology

Study Sites

Three sites in the Canning River catchment, Western Australia, were established for monitoring organochlorine pesticide levels (Fig. 1). Stinton Creek (western branch) and Kangaroo Gully were selected because of the presence of mixed fruit orchards on their head waters, and because organochlorine pesticides had been detected in samples of water and sediment from these sites (Dr R. Rosich, Water Authority of Western Australia, personal communication). A third site, Thompson Road, was selected as a down-stream site on the lower Canning River. This site was not near mixed fruit orchards.

Neither Stinton Creek or Kangaroo Gully had resident populations of *W. carteri* and therefore the experimental design involved the transplantation of specimens to each site.

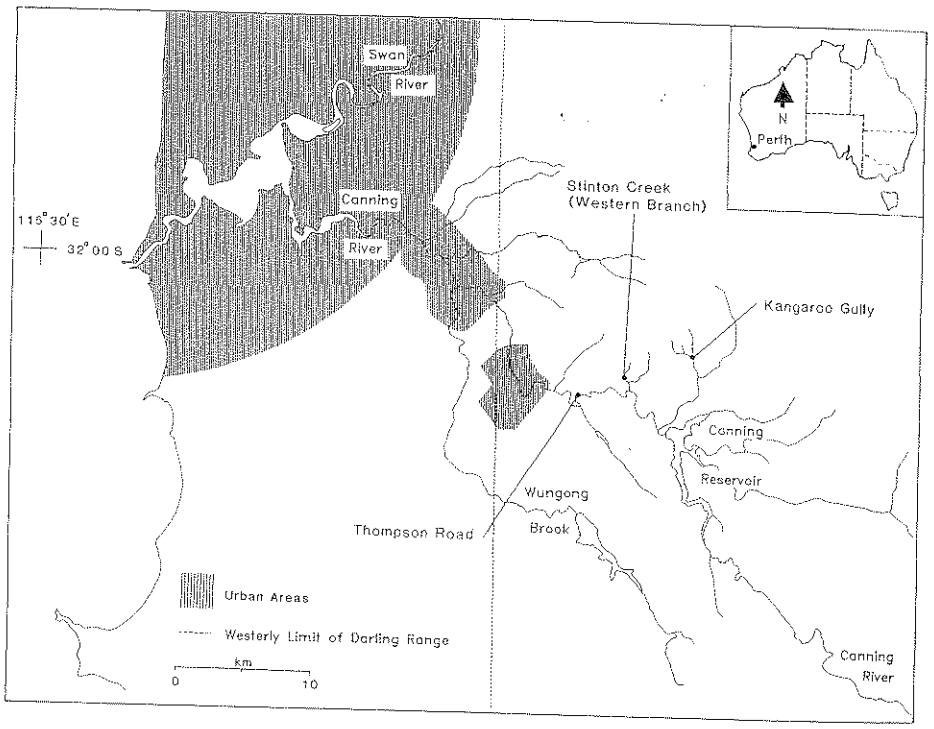


Fig. 1. Location of study sites within the Canning River catchment.

Pesticide Monitoring

Background levels of organochlorine pesticides were determined from an initial sample of 20 mussels taken from Thompson Road on 30 June 1987. Subsequently, 60 mussels were collected from this site on 18 August 1987. Mussels of the same size-class were selected to minimize effects due to the correlation between rates of accumulation and mussel size, i.e. age (Mackay *et al.* 1975; Manly and George 1977; Millington and Walker 1983). Shell height, length and width were measured to the nearest 0·1 mm on each specimen. Ten mussels were then placed in each of six transplant cages constructed of black plastic trellis mesh (2·5-cm aperture) with dimensions 35 × 30 × 25 cm. Two cages were placed at Thompson Road, two transplanted to Stinton Creek and two to Kangaroo Gully. Cages were

buried in the stream sediment to a depth of approximately 15 cm and securely anchored at each corner by 30-cm metal pegs.

One cage was removed from each site after 66 and 112 days (21 October and 9 December 1987, respectively). At the second collection date the cage at Thompson Road was totally inundated by fine sediment, resulting in 100% mortality of the mussels. A random sample of 10 mussels was collected from the stream bed at this site to represent the final sample.

On returning to the laboratory, mussels were placed in containers of fresh river water for 24 h to void the contents of their guts. Shell parameters were remeasured to assess growth rates over the experimental period, and percentage mortality in each cage was determined. Specimens were killed by freezing and the tissue was removed from each shell.

Levels of residue were measured following the method described by Erney (1983). Before analysis, the wet weight of each sample was recorded. Samples were extracted using an anhydrous sodium sulfate and light petroleum solution, passed through a sodium sulfate column, concentrated by reflux condenser and made up to a known volume before determination of residue levels by electron-capture gas chromatography. Results were expressed in mg kg^{-1} wet whole tissue with a detection limit of 0·005 mg kg^{-1}.

The significance of between-sample differences in residue concentrations and changes in shell parameters was determined by one-way analysis of variance. Before analysis, homoscedasticities of sample variances were assessed by Cochran's C and Bartlett's Box tests. Square root and $\log_{10}(x+1)$ transformations were applied to heteroscedastic data. *A posteriori* Student–Newman–Keuls multiple-range tests were calculated to locate significant between-sample differences ($P < 0·05$).

Table 1. Analysis of variance and multiple range tests for organochlorine residue levels in mussel tissue

F-values and associated levels of significance (***$P < 0·001$; **$P < 0·01$; *$P < 0·05$; n.s., not significant). *A posteriori* Student–Newman–Keuls multiple range tests indicating between-sample differences ($P < 0·05$). Transformations in parentheses

Pesticide	*F*-ratio	*F*-prob	Student–Newman–Keuls
Thompson Road			
Dieldrin	2·81	n.s.	
DDD	0·00	n.s.	
DDE (sq. rt)	0·99	n.s.	
Total DDT analogues (sq. rt)	2·79	n.s.	
Stinton Creek			
Dieldrin (sq. rt)	27·53	***	112 day > 66 day > Initial
DDD (sq. rt)	138·09	***	112 day > 66 day > Initial
DDE (sq. rt)	101·51	***	112 day > 66 day > Initial
Total DDT analogues (sq. rt)	106·40	***	66 day; 112 day > Initial
Kangaroo Gully			
Dieldrin	17·13	***	66 day > 112 day; Initial
DDD (sq. rt)	93·78	***	66 day > 112 day > Initial
DDE (sq. rt)	82·67	***	66 day; 112 day > Initial
Total DDT analogues [$\log_{10}(x+1)$]	33·53	***	66 day > 112 day > Initial

Results

Percentage survival of the 60 caged specimens across all sites was 75%; specimens transplanted to Stinton Creek and Kangaroo Gully had percentage survivals of 90% and 95%, respectively, but survival was 40% at Thompson Road.

After 66 and 112 days, there were no significant increases in shell height, length or width of caged specimens at any site (ANOVA, $P > 0·05$).

Mean residue levels in samples of mussels from Thompson Road, Stinton Creek and Kangaroo Gully are shown in Fig. 2. At Thompson Road, there was no significant change in levels of dieldrin, DDD, DDE and total DDT analogues between samples (ANOVA, $P > 0·05$; Table 1).

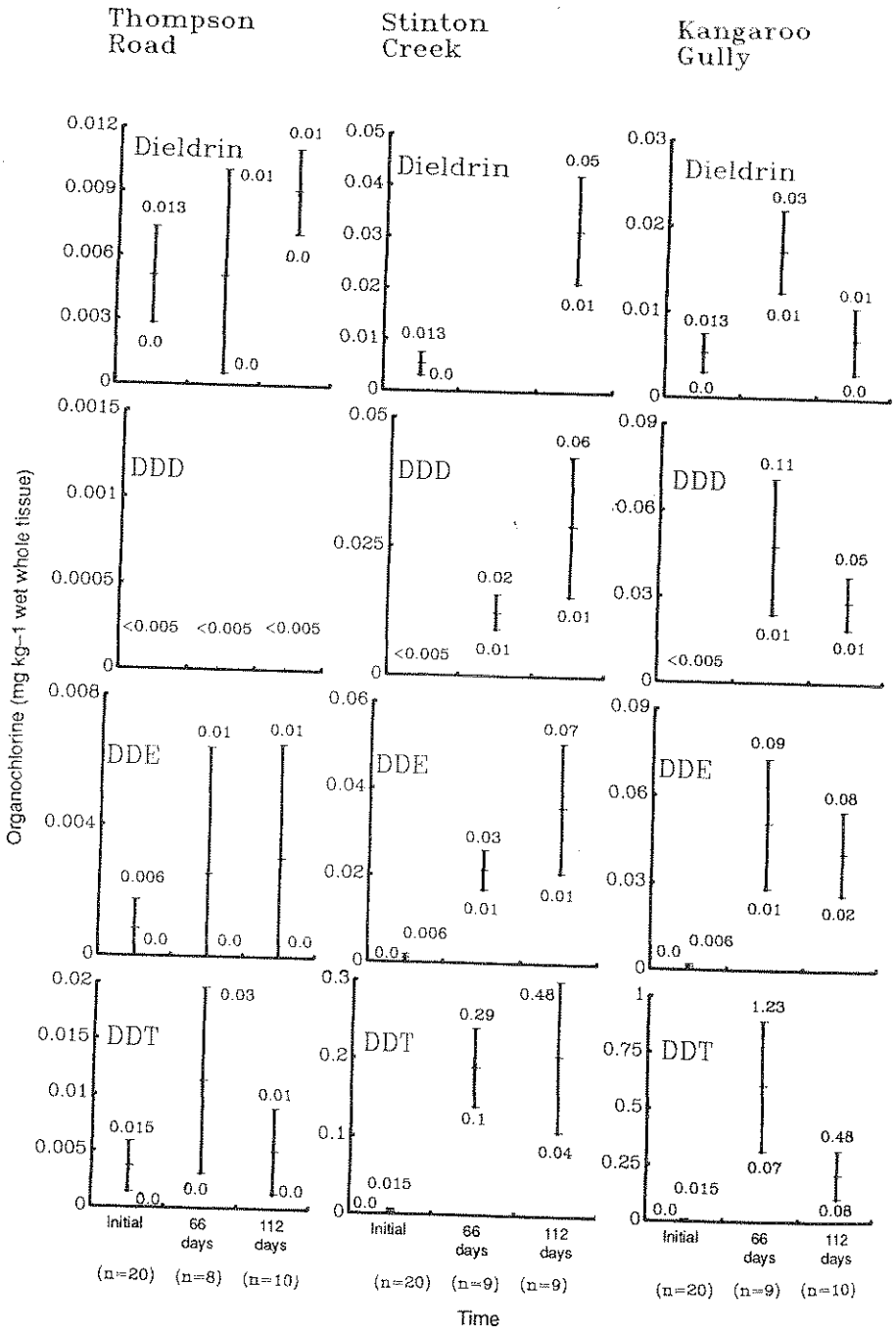
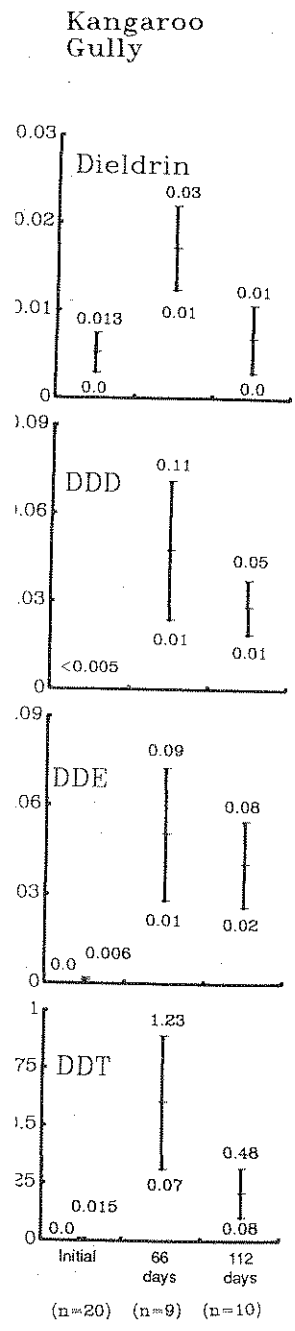


Fig. 2. Changes in mean concentration of organochlorine residues (mg kg^{-1} wet whole tissue) over time at Thompson Road, Stinton Creek and Kangaroo Gully, with 95% confidence intervals. Values for maximum and minimum residue levels, and number of replicates (n) in each sample are presented.



(mg kg^{-1} wet whole tissue) with 95% confidence intervals. plicates (n) in each sample are

In all instances, samples from Stinton Creek and Kangaroo Gully showed increases in levels of residue with time. The greatest accumulation at both sites was in total DDT analogues; these reached maxima of 0·48 and 1·23 mg kg^{-1} wet whole tissue at Stinton Creek and Kangaroo Gully, respectively (Fig. 2).

The results of one-way analysis of variance for mussels from Stinton Creek are presented in Table 1. In all cases there were highly significant between-sample differences in residue levels ($P < 0·001$), with levels in the initial sample significantly lower than in transplanted samples. There was significant accumulation of dieldrin, DDD and DDE at 66 days and further accumulation at 112 days, whereas there was no significant difference in total DDT analogues between the 66 and 112 day samples.

Significant between-sample differences in residue levels were also detected from Kangaroo Gully ($P < 0·001$) (Table 1). Multiple range tests demonstrated that in all cases residue levels in the initial sample were significantly lower than in transplanted samples, with the exception of dieldrin after 112 days. Levels of dieldrin, DDD and total DDT analogues were significantly lower in the 112-day than in the 66-day sample, with no significant difference in DDE between these samples.

Discussion

This study demonstrated bioaccumulation of organochlorine pesticides by *W. carteri*. Levels of pesticides accumulated in mussels at Stinton Creek and Kangaroo Gully, immediately below orchards, related to land-use practices and were comparable to those reported in the literature for bioaccumulation by other species of freshwater mussel. Bedford *et al.* (1968) observed mean levels of DDT and its metabolites of 0·19, 0·41 and 0·51 p.p.m. accumulated after 2, 10 and 6 weeks, respectively, and Ryan *et al.* (1972) reported tissue concentrations for endrin of 0·38 and 3·44 p.p.m. in field and laboratory trials, respectively.

W. carteri survived handling, transportation and being caged. When cages were collected from sites that were still flowing, specimens of *W. carteri* were actively filtering and appeared to be in good condition, although no significant growth was detected. Generally, mortality was low among caged specimens of *W. carteri*. The only exception occurred at Thompson Road, where one cage placed in a depositional zone was totally inundated by fine sediment, resulting in 100% mortality.

Body size was used as an index of mussel age (*sensu* Jones and Walker 1979) as there was no other directly applicable method. The commonly used relationship between growth checks and seasonal climatic change could not be used as no such relationship could be established for specimens from the study area.

High between-specimen variance in residue levels was detected. Organochlorine pesticides are known to accumulate preferentially in tissue lipids (Hellawell 1986). For instance, Phillips (1978) demonstrated that high seasonal variability observed in DDT residue in coho salmon was due to variations in the lipid content of the tissue, and seasonal fluctuations were smoothed when concentrations were based on lipid weight. A similar approach in these types of studies may reduce the observed variance in residues in mussel tissues.

Mussels at Thompson Road demonstrated no significant bioaccumulation of pesticide residues over time. Thompson Road is approximately 10 km and 15 km, respectively, down-stream of the sites on Stinton Creek and Kangaroo Gully. It seems likely that the discharge from these latter sites is diluted by tributaries, particularly during periods of increased winter discharge, thereby reducing the residue concentrations at Thompson Road.

Significant losses of dieldrin, DDD and total DDT analogue levels were observed in mussels at Kangaroo Gully. This is in contrast to investigations by Millington and Walker (1983), who reported no significant loss of accumulated Zn in *Velesunio ambiguus* (Philippi) and Jeffree and Simpson (1986), who similarly found no significant loss of accumulated Ra-226 in *V. angasi* (Sowerby). In the latter case, accumulated Ra-226 was stored in an inert form, in granular deposits dispersed throughout the tissue, preventing its loss. In the present

study, the losses may relate to the association of pesticide residues with lipids (Hellawell 1986) and the aestivation of mussels transplanted to Kangaroo Gully, an intermittent stream which had ceased flowing before 112 days. Levels of these residues in specimens at Stinton Creek, which was still flowing, demonstrated no significant decreases.

Although *W. carteri* has not been recorded from Kangaroo Gully, the species does occur in intermittent streams (Storey, unpublished data) and is adapted to intermittent flow. When dry, it resists dehydration by sealing the shell. The mussel will open and begin to feed as soon as stream flow is restored. Mussels taken from the dry stream bed at Kangaroo Gully in December opened and commenced feeding within hours of being placed in water. Evidence for preferential lipid metabolism from studies on aestivating lung fish (Hochachka and Guppy 1987) and frogs (Van Beurden 1980) suggests that mussels, too, may reduce their metabolic rate and survive on stored lipids when sealed. The waste products of this metabolism, including released organochlorine residues, would presumably be stored during aestivation then expelled when the mussel reopens. As a result, residue levels as a proportion of whole mussel tissue would be reduced. Until more is understood of this relationship, bioaccumulation trials in intermittent or highly regulated rivers, both of which are common in Western Australia, should be restricted to periods of continuous flow.

Acknowledgments

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