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DEFORMITIES IN CHIRONOMID LARVAE AS INDICATORS OF POLLUTION (PESTICIDE) STRESS

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Keywords: Chironomidae; deformities; pesticides; DDT; Australia

ABSTRACT

Chironomid larvae (*Chironomus* spp., *Dicrotendipes conjunctus* and *Procladius paludicola*) collected from Cox Creek and Aldgate Creek, South Australia, showed morphological abnormalities similar to those reported in other studies elsewhere in the world. The sediment of Cox Creek contained high concentrations of pesticides and there was a significant correlation between the occurrence of mouthpart and antennal deformities in larvae and the concentration of DDT and the herbicide, Dacthal®.

Laboratory experiments were conducted using a culture of *Chironomus* sp. to determine whether or not a causal relationship existed between exposure to pesticides and the occurrence of deformities in larvae. Results showed a positive relationship between the concentration of DDT and the percentage of deformed mouthparts (menta). The results for the effect of DDT on antennae and those for the effect of Dacthal® were less clear, but generally showed a higher incidence of deformity for treatments compared with controls.

To compare these results to a natural population (*i.e.* from an unpolluted area) the incidence of deformities was measured for larvae collected from Deep Creek Conservation Park, an area virtually free of pollution. The significance of this work is discussed with regard to its wider application as a useful environmental monitoring technique for freshwater systems.

INTRODUCTION

Many examples of morphological deformities in chironomid larvae have been reported worldwide and it has been suggested that pollutants present at the site of collection of larvae are responsible for such deformities (*e.g.* HAMILTON and SAETHER, 1971; PETTIGROVE, 1989; WARWICK, 1990). This paper describes deformities from polluted locations in South Australia for *Chironomus* spp. and *Dicrotendipes conjunctus* Walker. The frequency of deformities for two unpolluted creeks was also measured so that a basal level of deformities could be compared with polluted locations. Parallel laboratory experiments were designed to test the hypothesis that pesticides are the causal agents of deformities in chironomids and that their frequency is dose-related.

MATERIALS AND METHODS

Chironomids were collected from two unpolluted streams, Deep Creek and Aaron Creek, in July and October 1988 and February 1989. The creeks are situated in Deep Creek Conservation Park, 90 km south of Adelaide in South Australia (Fig. 1). The park receives its prevailing weather from the Southern Ocean and the only potential source of pollutants is in water from the surrounding farmland that drains into Deep Creek, while the catchment area for Aaron Creek is wholly contained within the park.

Samples were collected from Cox Creek and Aldgate Creek (part of the Mount Bold Reservoir catchment in the Mount Lofty Ranges, east of Adelaide, and 90 km north-east of Deep Creek Conservation Park, Fig. 1). Cox Creek receives a

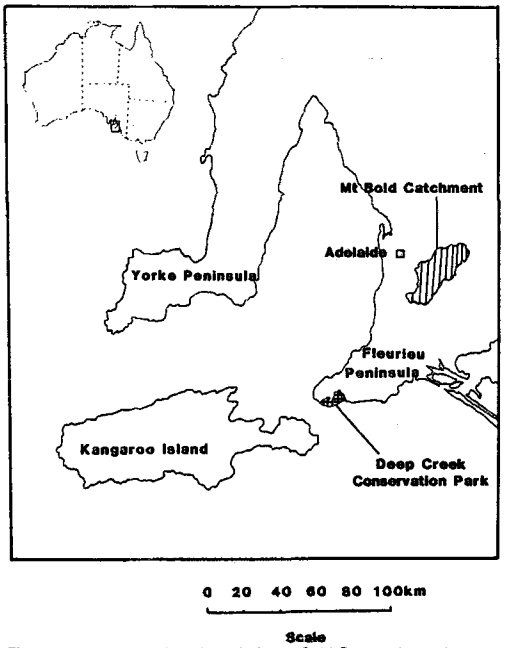

Fig. 1. Map showing location of Mount Bold Reservoir catchment and Deep Creek Conservation Park.

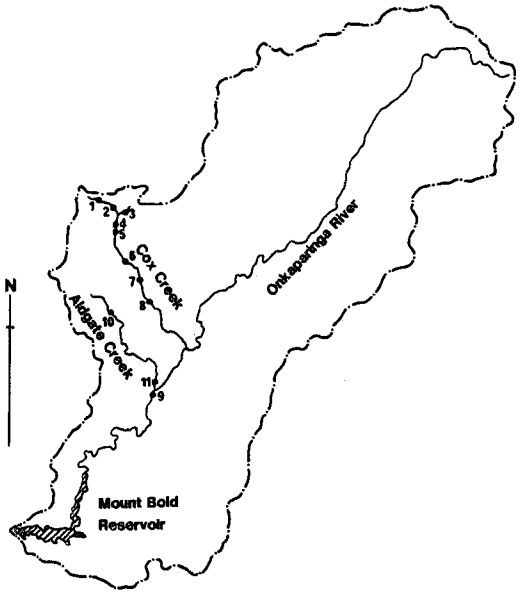

Fig. 2. Map of Mount Bold Reservoir Catchment showing collection sites.

heavy loading of soil and pesticides from Piccadilly Valley, which is an intensive vegetable growing area. The high rainfall, steep slopes and farming techniques (*i.e.* frequent cultivation) predisposes the valley to severe erosion. In March 1987 11 sites were sampled, eight on Cox Creek, two on Aldgate Creek (an urban stream with no pesticide input) and one on the Onkaparinga River downstream of the confluence with Cox Creek (Fig. 2). Four of the sites on Cox Creek were sampled again in June and September 1988 and February 1989, as was a new site on Aldgate Creek.

Pools, runs and emergent vegetation were sampled at each site using a kick technique and a sweep net of mesh size 200 µm. Samples were preserved in the field in 4% formalin or 70% ethanol. Some live specimens and egg masses were retained to enable laboratory rearing and identification of the chironomid fauna.

During the 1987 sampling of Cox Creek and Aldgate Creek approximately 100g of fine surficial sediment was collected from the stream bottom in depositional zones at each site. A single sediment sample was taken from Aaron Creek in October 1988. These samples were tested for pesticides using the method of THOMA and NICHOLSON, 1989. Heavy metal analyses were carried out on the 1987 samples from sites 6, 10 and 11 using CCP-AES following digestion with 1:1 nitric acid/ hydrochloric acid.

The head capsules of larvae to be examined for deformities were mounted in polyvinyl lactophenol or Hoyer's medium using the method of WARWICK, 1985. Each specimen was examined at 400X magnification for abnormalities of the mentum, mandibles and antennae.

A species of *Chironomus* (new species, J. Martin pers. comm.) was reared in the laboratory using the method of MARTIN *et al.*, 1980 with the following modifications. A basic 2X Martins solution (MARTIN *et al.*, 1980) was used and supplemented with 0.2 ml l^{-1} of $FeSO_4$ (as a 1.0 g l^{-1} solution) and 1 ml l^{-1} of a solution containing 200 ml of 60% ammonium lactate, 1 g $MnCl_2.4H_2O$ and 1 g KI per litre. At the time of establishing the breeding tank 1 ml of a solution containing 6 g l^{-1} of thiamine hydrochloride was also added. The larvae were fed with raw ground flour at the rate of 0.2 g initially and approximately 0.5 g per week after that. The lighting regime was identical to that of MARTIN *et al.*, 1980 and temperature was maintained as close as possible to 21°C.

DDT and Dacthal®, a pre-emergence herbicide, were used as test compounds for laboratory experiments. These pesticides were the two present in highest concentrations in Cox Creek during the sampling in March, 1987. Exposure of larvae to each compound was carried out in tanks with a bottom area of approximately 150 cm^2 (11 cm x 14

cm, and 20 cm high). One litre of 2X Martin's solution was added to each tank, along with $FeSO_4$ and the ammonium lactate solution at the appropriate dose. One ml of the thiamine hydrochloride solution was added at the time an egg mass was placed in each tank. A Kleenex facial tissue washed in ethanol (to remove contaminants from paper processing) was shredded and used as a substrate. The pesticide to be tested was added in the appropriate amount, as described below. The tanks were continuously aerated and lighting, temperature and feeding regimes were identical to those described above. Most larvae were sacrificed as they reached the 4th instar (or 3rd instar if numbers were low) for mounting and examination for deformities.

The concentrations of DDT chosen were 1, 5 and 10 μg l^{-1} based on the concentrations used by HAMILTON and SAETHER (1971) in their laboratory study. Five replicates were run for the 1 and 10 μg concentrations and four for the 5 μg concentration. Only two control tanks were set up because of a shortage of tanks and egg masses. The controls contained 1 ml of acetone instead of the DDT solution in acetone.

Dacthal® was used at concentrations of 0.2, 2, 10 and 100 μg l^{-1} which cover the range of concentrations of this compound found in Cox Creek in 1987. Commercial Dacthal® contains 750 g kg^{-1} of the active ingredient, chlorthal-dimethyl, a dimethyl ester of tetrachloroterephthalic acid. Larvae removed from the breeding tanks at the time of establishing the Dacthal® experimental tanks were mounted and examined as controls. The concentrations given refer to the dosage of the chemical at the start of the experiment. Pesticide levels were not monitored over the period of larval development because both chemicals are persistent and do not decompose rapidly in the environment.

Specimens of the chironomid species used in the laboratory experiments and those collected from the field are lodged in the Department of Crop Protection insect collection (Waite Campus, University of Adelaide).

RESULTS

Field Studies

The concentrations of pesticides in sediments of Cox Creek, Onkaparinga River and Aldgate Creek in 1987 are given in Table 1. Eight compounds were quantified, Dacthal®, DDT, DDE, TDE, chloropyrifos, hexachlorobenzene, dieldrin and endosulfan. The sediment sample from Aaron Creek returned a negative result for the presence of pesticides.

Table 1. Pesticide residues in sediments (concentration in μg kg^{-1} dry weight) collected 18.3.87 from Cox Creek, Onkaparinga River and Aldgate Creek. (– = not detected; Chlor. = Chlorpyrifos; HCB = Hexachlorobenzene; Dield. = Dieldrin; Endo. = Endosulfan.)

Site	Dacthal	ΣDDT	DDE	TDE	DDT	Chlor.	HCB	Dield.	Endo.
1	–	220	130	62	30	–	–	–	–
2	40	190	46	38	110	14	2	–	–
3	1.3	120	34	64	21	–	1	1	–
4	278	830	340	190	300	88	12	31	63
5	379	1800	380	130	1330	15	8	–	8
6	92	130	51	32	46	–	4	–	5
7	56	170	69	56	45	–	6	–	5
8	25	60	24	23	13	–	7	6	–
9	–	22	10	12	–	2	–	–	–
10	–	–	–	–	–	–	–	–	–
11	–	–	–	–	–	–	–	–	–

Table 2. Frequency of deformities (mentum, antennae and mandibles, combined) of *Dicrotendipes conjunctus* from Cox Creek, Onkaparinga River and Aldgate Creek on 18.3.87.

Site	Total no. examined	% deformities
1	45	8.9
2	26	19.2
3	30	33.3
4	1	0
5	0	0
6	12	41.7
7	17	35.3
8	5	0
9	3	0
10	3	33.3
11	11	9.1

The frequency of deformities in *Dicrotendipes conjunctus* found in 1987 is summarized in Table 2. Larvae of *Chironomus* spp. were collected in small numbers with only sites 8 and 9 producing more than 10 specimens. At these two sites 13% showed deformities. Thirty-four larvae of *Chironomus cloacalis* Atchley & Martin were reared from an egg mass collected from site 3 and seven (21%) showed deformities. Although pesticides were not detected in Aldgate Creek, the frequency of deformities was high, and so the sediments at sites 6, 10 and 11 were tested for heavy metal concentrations. The results are presented in Table 3. The frequency of deformities in three taxa, *D. conjunctus*, *Chironomus* spp. and *Procladius paludicola* Skuse, from the creeks sampled in 1988 and 1989 are presented in Table 4. There was little variation between sites and seasons so the data

Table 3. Heavy metal analyses of sediments collected 18.3.87 (concentration μg g^{-1} dry weight).

Site	Cr	Cu	Pb	Cd	Ni	Zn
6	7.0	5.9	19.2	0.2	1.5	44.0
10	2.4	2.1	10.0	<0.1	–	19.2
11	20.0	18.6	27.0	0.3	7.4	67.1

Table 4. Frequency of deformities (mentum, antennae and mandibles, combined) of chironomid larvae collected from polluted and unpolluted sites from June 1988 to February 1989. (*includes egg masses collected and raised to fourth instar larvae in the laboratory)

		number collected	% deformed
Cox Creek*	– *D. conjunctus*	146	3.4
	Chironomus spp.	172	12.2
Aldgate Creek	– *D. conjunctus*	7	14.2
	Chironomus spp.	9	0
Aaron Creek	– *D. conjunctus*	108	1.9
	Chironomus spp.	17	0
	P. paludicola	105	1.9
Deep Creek	– *D. conjunctus*	1	0
	Chironomus spp.	24	0
	P. paludicola	80	1.3

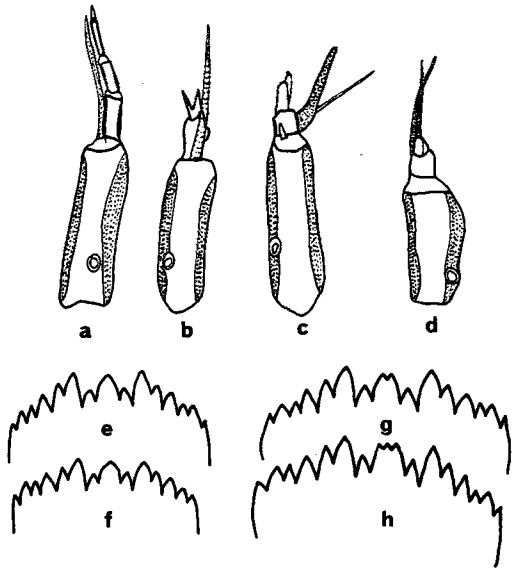

Fig. 3. Deformities of antennae of *Dicrotendipes conjunctus:* a) normal antenna; b-d) abnormal antennae; and deformities of the mentum in *Chironomus* sp.; e) normal mentum; f-h) abnormal menta showing missing lateral tooth (f) and deformed median tooth (g,h).

were pooled for each creek and each species. Although the sample sizes are small, the frequency of deformities are generally seen to be higher in all polluted sites compared with Deep Creek Conservation Park. Examples of deformities are shown in Fig. 3.

Laboratory Studies

To test the hypothesis that DDT causes deformed menta and antennae in *Chironomus* sp., larvae were exposed to a graded series of concentrations of this compound as described previously and larvae were allowed to develop. The results (Fig. 4) show that the percentage of deformed menta increases as the concentration of DDT is increased but the same relationship is not evident for the effect of DDT concentration on the antennae (Fig. 5). Unfortunately no data were forthcoming for one of the two control tanks because the egg mass was inviable and did not develop. Therefore only a single value for zero DDT concentration is plotted in Figs 4 and 5. Comparison of the residuals for percentage deformities showed that the variances for these data were heterogeneous and so they were subjected to square-root transformation.

The correlation of DDT concentration and the resultant incidence of mentum deformities was significant at the 10% level but not at 5% (r = 0.47;

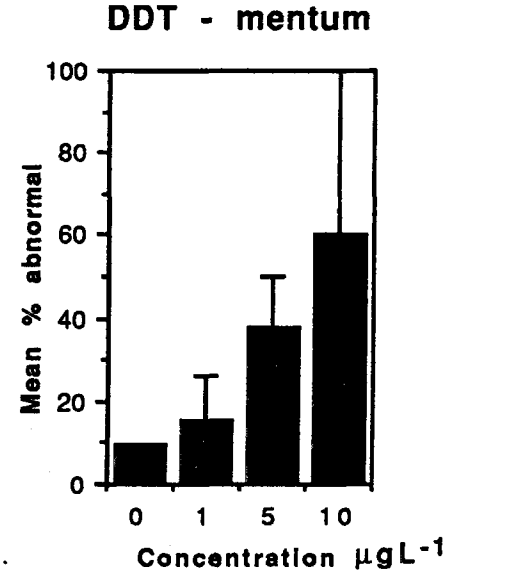

Fig. 4. Mean percentage of larvae (±1 S.D.) of *Chironomus* sp. which developed mentum deformities in response to different concentrations of DDT.

d.f. = 12). The range in the proportion of deformed larvae between replicates at the 10 μg l^{-1} DDT concentration (0 to 100%) was much larger than for other treatments. At this concentration only seven

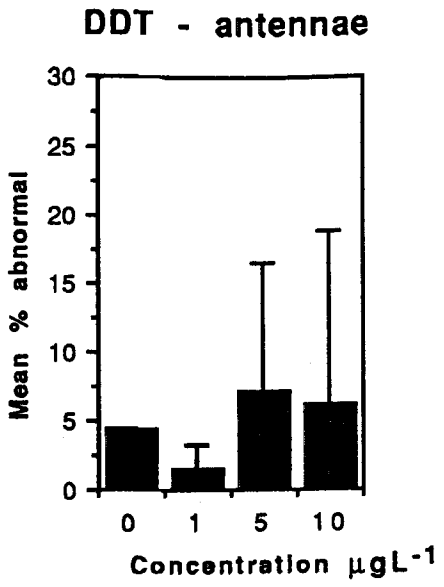

Fig. 5. Mean percentage of larvae (±1 S.D.) of *Chironomus* sp. which developed antennal deformities in response to different concentrations of DDT.

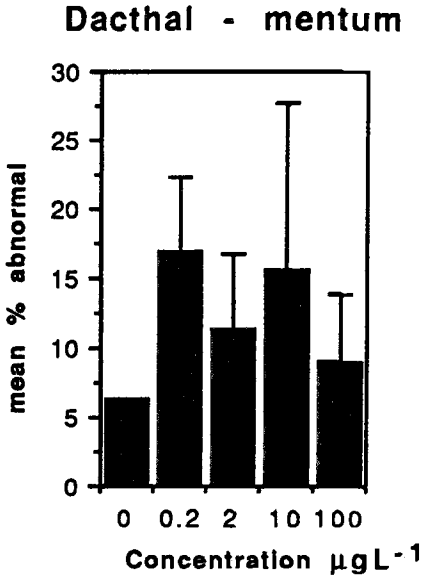

Fig. 6. Mean percentage of larvae (±1 S.D.) of *Chironomus* sp. which developed mentum deformities in response to different concentrations of Dacthal®.

larvae, in total, survived to the fourth instar in four of the five replicates and this confounded the analysis. These data indicate that 10 µg l^{-1} is approaching the lethal level for chironomid larvae, as was found by HAMILTON and SAETHER (1971) in their experiments with DDE.

The correlation of DDT concentration and the incidence of antennal deformities was not significant ($r = 0.03$; d.f. = 12; $P > 0.5$) and the hypothesis is therefore not supported. As for the previous experiment the range in deformity levels was high. However, the data still shows some trend in that the mean proportion of abnormalities at 5 and 10 µg l^{-1} are higher than the controls.

To test the hypothesis that Dacthal® causes deformities of the mentum and antennae in *Chironomus* sp., larvae were exposed to a graded series of the compound. Fig. 6 shows the percentage of deformed menta and, after the data were subject to square-root transformation, the correlation of Dacthal® concentration and the resultant incidence of deformities was not significant ($r = 0.04$; d.f. = 12; $P > 0.5$). However, the percentage of deformities in all treatments is higher than the control, thus indicating that this compound may induce deformities in *Chironomus* sp. Fig. 7 shows the frequency of antennal deformities and appears to indicate an inverse relationship over the concen-

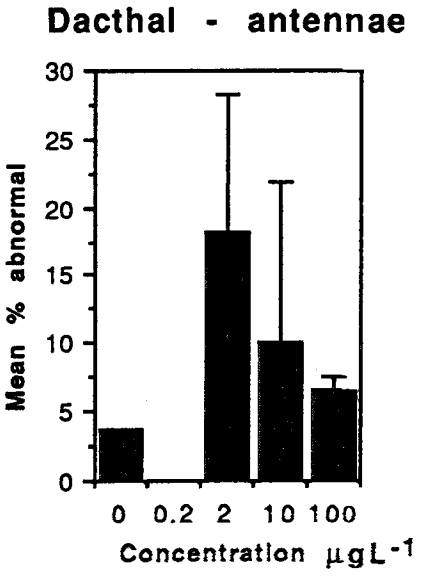

Fig. 7. Mean percentage of larvae (±1 S.D.) of *Chironomus* sp. which developed antennal deformities in response to different concentrations of Dacthal®.

tration range from 2 to 100 μg l^{-1}. Over all treatments the correlation of Dacthal® concentration and incidence of antennal deformities (after the data were subject to square-root transformation) was not significant ($r = 0.02$; d.f. = 12; $P > 0.5$).

DISCUSSION

Sediment collected from Aaron Creek revealed no pesticide contamination and no pollution enters Deep Creek other than some nutrients from fertilizer treatment of surrounding farmland. Thus, the values for the frequency of deformity in *D. conjunctus, Chironomus* spp. and *P. paludicola* from Deep Creek Conservation Park are probably representative of naturally occurring (or background) levels of deformity for these species in the Adelaide region.

Comparison of the field data between 1987 and 1988/89 is difficult because of the reduced distribution of *D. conjunctus* found during 1988/89. The frequency of deformity is lower for *D. conjunctus* in 1988/89 but the reasons for this are unclear without corresponding data for pesticide concentration in sediment for the same period. The low number of surviving larvae at sites 4 and 5 in 1987 (Table 2) corresponds with the highest concentration of pesticides (Table 1). This indicates that the concentrations in the sediments at these sites is high enough to be lethal.

As the frequency of deformities in chironomid larvae from Cox Creek and Aldgate Creek is generally higher than that measured for the relatively unpolluted streams of Deep Creek Conservation Park, it was hypothesised that a contaminant or contaminants in the former water bodies were the causative agents involved. The number of larvae collected in Aldgate Creek during this work is too low to be conclusive, but continuing field work has shown a steady and relatively high frequency of deformity at the Aldgate Creek site. The fact that no pesticides were found in the sediment suggests that the metals present may be the cause of deformities. KÖHN and FRANK (1980) suggest that heavy metals caused deformities in chironomids in the Teltowkanal in Berlin.

Although inconclusive in some respects, the results of the laboratory experiments conducted show that DDT and Dacthal® cause deformities in *Chironomus* sp. The frequency of deformity in dosed tanks was generally higher than those of controls, and for mentum deformities caused by DDT, the response is dose related. This is the first time that such compounds have been shown to induce the deformities observed in chironomid larvae. The frequency of deformities for both the DDT and Dacthal® treatments are much lower for the antennae compared with the mentum. This may be due to the fact, as sensory structures, the antennae may not be able to withstand much damage or deformation before ceasing to operate normally. The mentum probably still functions satisfactorally even when significantly distorted. The data for the antennae from the Dacthal® experiments show an inverse correlation between the incidence of deformed antennae and the Dacthal® concentration (above 0.2 μg l^{-1}). WARWICK (1985) re-examined the larvae exposed to DDE by HAMILTON and SAETHER (1971) and found a similar relationship. Field data cited by WARWICK (1988) indicate that antennal deformities do not increase at very high levels of contamination whereas the incidence of mentum deformities does increase. Warwick suggested that deformation in the antennae is replaced at higher concentrations by deformation in other more sclerotized and less sensitive structures, such as the teeth of the mentum. The data from the Dacthal® experiments seem to confirm this trend, but the data from the DDT experiments do not, even though the concentration range is similar to that used by HAMILTON and SAETHER (1971).

Examination of larvae from the experimental tanks was often confounded by broken teeth on the mentum and/or mandibles. Larvae from the field usually showed more wear than breakage, with 10-15% of menta showing breaks. However, in many experimental tanks the frequency of broken teeth was often over 50% and reached 87% in one case. Furthermore, laboratory bred larvae often had several teeth broken whereas field collected larvae usually had only one or two teeth missing. The high level of broken teeth in experimental treatments may be attributable to the effect of the test compounds, in that such breaks occur because of thinning and weakening of the cuticle. However, high rates of broken teeth also occurred in larvae derived from field-collected egg masses raised in the laboratory, and in larvae from breeding tanks that were used as controls. This suggests that broken teeth in laboratory reared larvae may be caused by a deficiency in the artificial diet. The occurrence of broken teeth needs to be examined in detail, both to determine the possible causes and to assess the difficulty created in examining the mentum for other types of deformities.

Chironomids are present in a wide variety of habitats and environmental conditions (PINDER, 1986) and, as such, are highly suited for use as biological monitoring agents. SAETHER (1979) sug-

gests that the distribution patterns of individual species may be helpful in pin-pointing localized areas of pollution. With more detailed testing of a range of species it may be possible to select very sensitive species that show deformities at relatively low levels of pollution, or, resistant species that respond only to high levels of pollution. WARWICK (1989) found that *Procladius* was more resistant to contaminant stress than *Chironomus*, though no such trend is obvious for the Australian species examined in this study. WARWICK (1990) has recorded deformities in several other genera of Chironomidae which further widens their scope for use as a potential monitoring system. Opportunity exists for far more detailed work on a scheme using chironomids for the assessment of water quality, if the suggestion of WARWICK and TISDALE (1988) is true, *i.e.* that there are different 'limits of detection' for each structure on the head capsule of a larva (*e.g.* sensitivity of the mentum versus the antennae).

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