Arch. Environ. Contam. Toxicol. 39, 299–306 (2000)
DOI: 10.1007/s002440010108

ARCHIVES OF
Environmental
Contamination
and Toxicology

A Comparative Study of *Chironomus riparius* Meigen and *Chironomus tentans* Fabricius (Diptera:Chironomidae) in Aquatic Toxicity Tests

M. M. Watts, D. Pascoe

School of Biosciences, Main Building, University of Wales, Cardiff, PO Box 915, Cardiff, CF10 3TL, United Kingdom

Received: 17 December 1999/Accepted: 21 March 2000

Abstract. *Chironomus riparius* Meigen and *Chironomus tentans* Fabricius were examined under controlled conditions and exposed to the reference toxicants cadmium and lindane (γ-hexachlorocyclohexane) to identify any differences that could have implications for their use in aquatic toxicity testing. Preliminary studies showed that both species could be cultured in the laboratory using similar methodology, resulting in the typical bimodal emergence of adult males prior to females. However, adults of *C. riparius* emerged earlier and in greater numbers than *C. tentans*. Comparative measurements of head capsule width and body length for the four larval instars revealed similar dimensions for each species up to and including the third instar. Fourth-instar larvae of *C. tentans* were considerably larger than those of *C. riparius*. Median lethal concentrations (LC50) determined over a 10-day exposure period suggested no difference in species response to cadmium; however, with increasing exposure time, *C. tentans* was significantly more sensitive to lindane. The investigations showed that the pattern of response was similar for the two species, and that both *C. riparius* and *C. tentans* are suitable test organisms for acute exposure assays.

The dipteran family Chironomidae has attracted widespread research interest not only from taxonomists and ecologists but also in recent years from molecular biologists, geneticists, aquaculturists, and particularly toxicologists. Comprehensive studies of these insects date back to those of Miall in 1895, with more recent reviews by Oliver (1971), Pinder (1986), and Armitage *et al*. (1995). This voluminous amount of work stems from the functional importance of chironomids in freshwater ecosystems and a number of characteristics make some species ideal for laboratory testing (Taylor *et al*. 1993), including a short generation time, high fecundity, and the existence of established methods for successful laboratory culture (McCahon and Pascoe 1988; ASTM 1993).

The two species described in this investigation, *Chironomus riparius* and *Chironomus tentans*, are currently recommended as standard toxicity test organisms in Europe and North America (Hill *et al*. 1993; US EPA 1994) and as a consequence, their biology, life cycle characteristics, methods for laboratory maintenance, and response to environmental changes have been well documented. However, most of these investigations have dealt with each species in isolation and very little comparative data exist on basic aspects of their biology (life cycle duration, size of larval instars, etc.) and general performance under laboratory conditions.

There is a similar lack of directly comparable data for species response following either acute or chronic toxicant exposure, although some interlaboratory precision studies have been performed with *C. tentans* (Burton *et al*. 1996). Although the main thrust of research in the field of aquatic toxicology is presently directed at the identification of sensitive chronic endpoints for a wide range of test species, including chironomids, acute toxicity exposures still play a key role in explaining toxic effects. Furthermore, the fundamental means by which new chemicals are screened and effluents assessed for discharge consent limits remains the preserve of acute toxicity data (CEC 1992; APHA 1995). The recent implementation of direct toxicity assessment (DTA) and whole effluent toxicity (WET) schemes based on acute response criteria by the UK Environment Agency and US EPA, respectively, for sewage and trade effluent, lend further relevance to the need for such data.

To provide a direct comparison of the two species an investigation was undertaken with the following objectives: (1) to compare several aspects of biology under controlled conditions (no toxicant) including laboratory culture, adult emergence, and size of larval instars; and (2) to describe the acute effects of the reference toxicants cadmium and lindane, both highly toxic to all forms of aquatic life (Møhlenberg and Jensen 1980; Williams *et al*. 1985; Wren and Stephenson 1991; Maund *et al*. 1992), to second-instar larvae of *C. riparius* and *C. tentans*. This particular life stage was selected because it represents a compromise between sensitivity, as later instars are known to be more tolerant (Williams *et al*. 1986), and ease of handling.

Materials and Methods

Laboratory Culture

Cultures of both *C. riparius* and *C. tentans* were established using 6–8 fertilized egg-ropes and maintained as described by McCahon and

Correspondence to: M. M. Watts

Pascoe (1988). The egg-ropes of *C. riparius* used to seed these cultures were estimated to contain a mean number of 500 individual eggs (SE = 26.7), whereas those used for the *C. tentans* cultures were larger with a mean number of 820 eggs (SE = 33.0). A common 16:8 h light/dark regimen was used for both species. The temperature regimes depended on species; *C. riparius* was cultured at 20°C ± 1°C, and *C. tentans* at 22°C ± 1°C. This difference in temperature was based on the individual species culture requirements described by McCahon and Pascoe (1988) for *C. riparius* and in a standard culture procedure, supplied by Zeneca Agrochemicals (Jealotts Hill, Bracknell, UK), for *C. tentans*. A food ration of 0.5 g ground Tetramin® fish flake (Tetra Werke, Germany) was added to each culture on alternate days. In the case of *C. tentans*, a mesh platform was added to the side of each culture aquarium as an attachment point for the eggs of ovipositing females.

Adult Emergence Under Culture Conditions

Four egg-ropes ≤24 h old were removed from established cultures of *C. riparius* and *C. tentans*, and transferred to separate 5-L aquaria that were subject to the same temperature, photoperiod, and feeding regimens appropriate to the maintenance of each species. Estimates of the mean number of individual eggs contained in the egg-ropes used for this experiment were 750 eggs/rope (SE = 29.6) for *C. tentans* and 400 eggs/rope (SE = 11.3) for *C. riparius*. Each of the two test systems was maintained to the point of adult emergence, following which no further food was added and the number and sex of emerged adults recorded daily. The study was ended when emergence was not observed for 3 consecutive days.

Size of Larval Instars

Four egg-ropes ≤24 h post-oviposition were removed from cultures of *C. riparius* and *C. tentans*, transferred to separate 5-L culture aquaria and maintained as a separate culture system. Larvae of both species were sampled on the first day of hatching from the egg-rope and subsequently at 2-day intervals from the start of the test, increasing to 3 days in the later stages. On each sample date, four random substrate samples were taken from each test aquarium using a 2.5-cm diameter coring device (Taylor *et al*. 1993). These substrate cores, typically containing 20–50 individual larvae, were transferred to sorting trays filled with dechlorinated water so that larvae could be easily located and removed.

Measurement of each larva was performed by transference onto a microscope slide in a small volume of water. A coverslip was loosely placed over the larva as an aid to manipulating it into the desired position for observation. The coverslip was only loosely positioned to avoid distortion of the head capsule and to prevent damaging the larvae. With the exception of the first instars (which were killed) this technique allowed measured larvae to be returned to the test system, thereby avoiding a substantial alteration in the population size during the course of the study. Head capsule width and body length of 40 randomly selected individual larvae of both species were recorded on each sample date, using a binocular microscope fitted with a calibrated eye-piece micrometer. The number of larvae measured was reduced to 20 in the later stages of the investigation as it became apparent that all larvae were at the same developmental stage (fourth instar). This observation also serves to explain why the frequency of sampling was reduced (from every 2 to every 3 days) toward the end of the study.

Data Analysis

Median emergence times (EmT50) of *C. riparius* and *C. tentans* for males, females, and for all adults collectively were calculated and compared using a FORTRAN program written in this laboratory and based on the time-response analysis methods of Litchfield (1949). Percentage emergence data were calculated based on an estimation (Sibley *et al*. 1997) of the total number of individual eggs contained within the four egg-ropes used in each aquarium.

Plots of larval head capsule width versus body length for both *C. riparius* and *C. tentans* were produced using Microsoft Excel (version 5). In addition, hierarchical cluster analysis (between-group linkage method and squared Euclidean distance coefficient) was performed solely on head capsule width measurements using SPSS for Windows (version 6.1). Head capsule width, not body length, was used in the cluster analysis in recognition of the fact that this parameter represents the best quantitative descriptor of larval instar (Dyar 1890). Furthermore, Holloway (1983) found that considerable overlap in body length occurs between instar stages, which could significantly affect the cluster membership assigned to a particular individual. Subsequent to this analysis, descriptive statistical measures for head capsule width and body length were assigned to each instar using Minitab for Windows (version 9.1).

Acute Toxicity

Four egg-ropes of *C. riparius* and *C. tentans* ≥48 h post-oviposition were removed from cultures and transferred to separate 1-L beakers, which were maintained under appropriate conditions until the larvae had reached the second instar stage (about 5 days).

Following initial range finding studies, the mortality of *C. riparius* and *C. tentans* larvae at five concentrations of cadmium (0.42–4.78 mg/L) and eight of lindane (1.2–32.9 μg/L) was recorded over a 10-day period at a temperature of 22°C ± 1°C with a 16:8 h light/dark regime. Each test concentration was assigned 20 5 cm × 2 cm soda glass vials that served as the exposure chambers for the study. Of these, 10 vials were allocated to house *C. riparius* larvae and the remaining 10, *C. tentans* larvae. Prior to larval introduction, each vial received 10 ml of the appropriate toxicant solution (or dechlorinated tap water as control) and a small amount (0.1 g wet weight) of shredded Whatman No. 1 filter paper as substrate. Vials were left to stand at exposure conditions for 24 h in order to allow chemical equilibration between the aqueous and solid phases. Test solutions were replaced following this period and a second instar larva of *C. tentans* or *C. riparius* selected at random and assigned to a vial.

Toxicant levels and water quality were maintained by daily formulation and replacement of test solutions which ensured that dissolved oxygen levels remained ≥80% of the air saturation value (Taylor *et al*. 1991). Food was administered daily to each vial in the form of a 500-μl measure of a 1 g/L suspension of ground Tetramin® fish flake (equivalent to 0.5 mg food per larva), which is slightly in excess of larval requirements for both species (Holloway 1983; Ankley *et al*. 1994; Sibley *et al*. 1997). Mortality of larvae, defined as failure to respond to mechanical stimulation was recorded daily throughout the 10-day test period.

Water Quality

Measures of conductivity (225 μS/cm) and pH (7.2) of the dilution water were taken using hand-held meters and hardness (114 mg as $CaCO_3$/L) was determined by flame atomic absorption spectrophotometry on an Instrumentation Laboratory Model 457.

Toxicant Analysis

Samples of each toxicant solution were collected and measured daily. Aliquots of 1 ml were removed from each of the 10 replicates per species assigned to a particular concentration and pooled to provide a volume sufficient for analysis. Cadmium samples were fixed at 1% with Aristar nitric acid and analyzed against suitable standards (0.1–2.0 mg/L) by flame atomic absorption spectrophotometry using standard operating procedures. Lindane samples were extracted from aqueous solution into n-hexane (by shaking for 1–2 min) and measured against a 10 μg/L standard by gas liquid chromatography on a Pye Unicam 4500 with a 5% SE-30 packed column and an electron capture detector.

Data Analysis—Acute Toxicity

FORTRAN programs developed in this laboratory and based on the concentration-response analysis methods of Litchfield and Wilcoxon (1949) were used to compute and compare median lethal concentrations (LC50). Calculations were based on measured cadmium and lindane concentrations. Data were considered suitable for LC50 analysis only at those observation times when mortality of $>0\%$ and $<100\%$ (though 0 and 100 may also be included) was recorded in at least three concentrations.

Results and Discussion

Laboratory Culture

The method of laboratory maintenance of *C. riparius* and *C. tentans* used in this study was effective because it represented a self-replenishing system, capable of providing a regular supply of test organisms in large numbers at various stages of development. Although the total number of egg-ropes produced was greater in cultures of *C. riparius*, the number produced by both species was sufficient to reseed the culture aquaria and provide organisms for use in tests. Within the culture system, no difference in the longevity of adults was noted between males and females of the same species, although adults of *C. riparius* survived longer with a median survival time of 112 h (ranging from 105 h to 119 h) for males and 101 h (ranging from 90 h to 113 h) for females. Median survival times for male and female *C. tentans* were 54 h (ranging from 49 h–59 h) and 53 h (ranging from 47 h–60 h), respectively. However, the work of several authors describes a different situation, for example Downe and Caspary (1973) found that *C. riparius* adults only survived for 2–3 days, and Sadler (1935) noted that male and female adults of *C. tentans* reared outdoors survived for 5 days and 3 days 12 h, respectively. To explain the differences between survival times noted in the present investigation and those reported by other authors, further study would be required to establish firm conclusions. However, differences in the exposure conditions in relation to both adults (temperature, photoperiod) and larvae (competition for food, space), would presumably have a considerable influence on adult longevity.

Adult Emergence Under Culture Conditions

The adults of *C. riparius* and *C. tentans* shared some features relating to the pattern and timing of emergence and also the number of adults emerging daily from the two test aquaria (Figure 1). Both species displayed a bimodal emergence with a peak in male numbers followed by the corresponding female peak. This phenomenon of protandry, a reproductive strategy adopted by males to increase their chances of mating success (Wicklund and Fagerström 1977), is well established in chironomids (and other insects) and has been recorded under laboratory (Holloway 1983) and field (Learner and Potter 1974) conditions. The two test species were also comparable in terms of the duration of the emergence period, which began 17 days following the start of the study and continued for about 30 days. Although the total number of adults that emerged was similar between species, these numbers represent only 41% of the total number of *C. tentans* originally introduced, (estimate of 750 eggs/egg-rope), whereas the number of *C. riparius* that emerged represents 74% of the original total, (estimate of 400 eggs/egg-rope) (Table 1). These estimates of egg numbers differ from those estimated for the egg-ropes used to seed the cultures (see previous section), suggesting some variation in egg number between the egg-ropes produced by individual females. Similar variation has been reported by several workers, for example *C. tentans* egg-ropes have been reported to contain anything from 630 to 2,300 eggs (Sadler 1935; Sibley *et al*. 1997), with *C. riparius* egg-ropes typically holding 400–500 eggs (Downe and Caspary 1973). The sex ratio of adults shows some deviation from 1:1, with an approximate 10% bias toward males in both cases.

Species emergence differs in that adults of *C. riparius* reach a peak in numbers earlier and over a shorter period of time than *C. tentans*. Analysis of the median emergence times for each species confirms the trends described (Table 1). The EmT50 of males and females of both species are significantly different ($p < 0.05$), showing that the males emerge earlier than the females. In addition, analysis also confirms that *C. riparius* males, females and adults collectively reared at 20°C reach their median emergence significantly earlier ($p < 0.05$) than *C. tentans* reared at 22°C. Although temperature is one of the primary influences governing growth and development in aquatic insects, with increases in temperature known to hasten the development of chironomid larvae (Nebeker 1973; Mackey 1977), the higher culture temperature used for *C. tentans* did not result in an earlier emergence compared to *C. riparius*. Though culturing both species at the same temperature would have provided directly comparable data, the fact that *C. riparius* emerged earlier at a lower temperature suggests that this trend would have occurred even if both species had been cultured at either 20°C or 22°C.

The differences noted in emergence characteristics may simply mirror the natural course of events as they apply to each species, or alternatively stem from the laboratory conditions used. Because the aim of the exercise was a laboratory comparison, factors peculiar to this environment should be considered in explaining the differences seen. Competition for space and food could provide an explanation for the earlier emergence and greater percentage of *C. riparius* adults. For example, fourth instar larvae of *C. tentans* are considerably larger

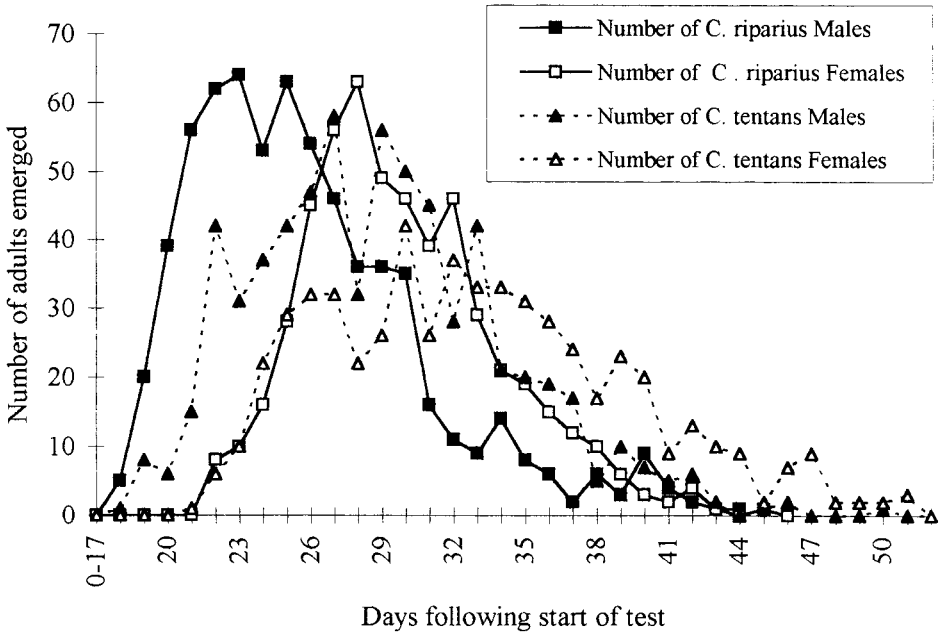

Fig. 1. Number of adults of *C. riparius* and *C. tentans* emerging daily from laboratory cultures

Table 1. Summary of emergence data for *C. riparius* and *C. tentans* adults under culture conditions

Test Statistic	Species	
	C. riparius	*C. tentans*
Male EmT50 (Upper–Lower 95% CI)	25.4days (26.3–24.4)	28.1days (29.1–27.2)
Female EmT50 (Upper–Lower 95% CI)	29.3days (30.1–28.5)	32.0days (33.1–30.9)
Collective adult EmT50 (Upper–Lower 95% CI)	26.9days (27.8–26.0)	30.0days (31.1–28.9)
No. of adults emerged	1,190 individuals	1,218 individuals
% of adults emerged	74%	41%
% of males:females	55.5%:44.5%	53.9%:46.1%

than those of *C. riparius* and would therefore require more space and food to develop successfully. Since the number of individual eggs per rope is also higher for *C. tentans*, it would seem logical (assuming most of the eggs hatch) that competition would be further increased with the result that only the "fittest" larvae develop successfully to adults. In this respect *C. tentans* may have been disadvantaged by the design of the experiment, since the original stocking density (in terms of egg number) was greater than *C. riparius* and may have contributed to the comparatively low level of adult emergence. However, the intention of the study was to establish if both species could be cultured using the same method, in terms of substrate type, feeding level, egg-rope number, etc. In the light of the low emergence of *C. tentans*, such factors as stocking density and larval competition within the culture system would need to be considered, and the methodology altered accordingly for future studies.

Size of Larval Instars

The plots of head capsule width (HCW) versus body length (BL) show that larval measurements fall into one of four groups (Figures 2 and 3), corresponding to the four larval instars of *C. riparius* and *C. tentans*. Larval dimensions were similar up to and including the third instar stage, but the fourth instars of *C. tentans* were larger both in terms of HCW and BL. Cluster analysis showed that larvae of both species could be assigned to one of four clusters, thereby allowing the calculation of descriptive statistical measures for each instar (Table 2). These data show that there is some overlap in body length measurements between instars of the same species, as noted by Holloway (1983); however, no overlap in HCW was evident between instars. The HCWs and BLs of both *C. riparius* and *C. tentans* recorded in this investigation compare favorably with those reported in recent literature (Environment Canada 1997). For example, HCWs of fourth instar *C. riparius* and *C. tentans* larvae have been reported to range from 0.43–0.60 mm and 0.63–0.71 mm, respectively (Townsend *et al*. 1981; Day *et al*. 1994), values that encompass the mean HCW recorded in this study. This suggests that the test conditions were suitable for normal growth and development of both test species. Although competition between *C. tentans* larvae may have resulted in fewer animals surviving to eclosion in the previous experiment, this study, which incorporated a similar stocking density, suggests that the development of the surviving larvae was not affected.

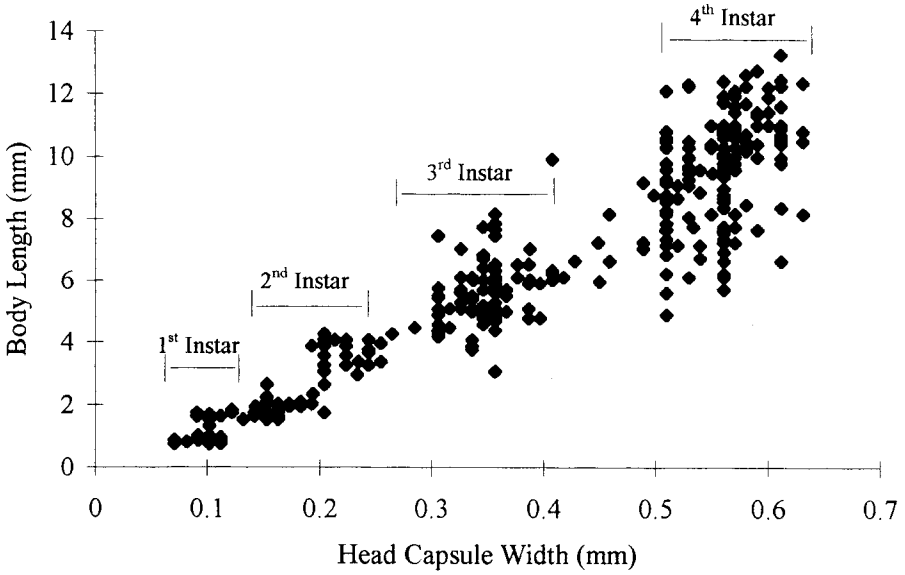

Fig. 2. The relationship between head capsule width and body length of *C. riparius* larvae

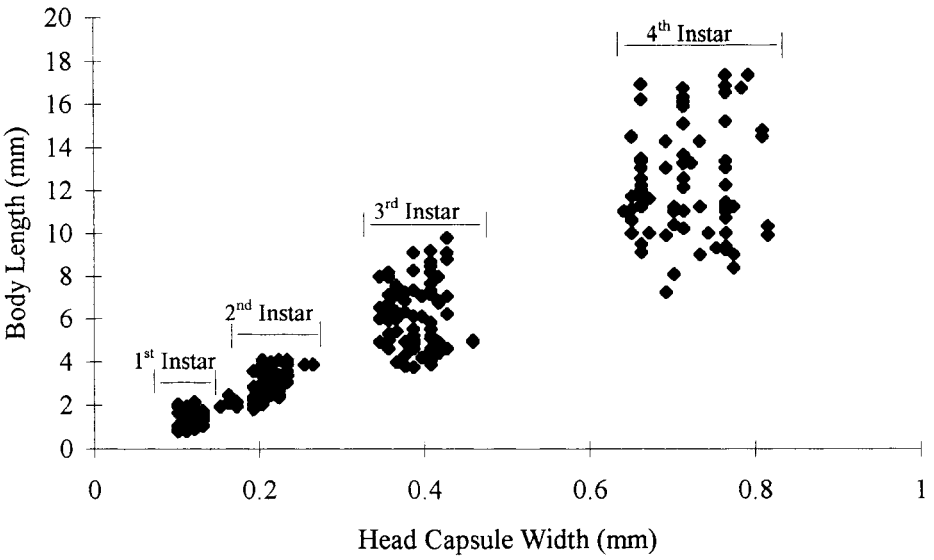

Fig. 3. The relationship between head capsule width and body length of *C. tentans* larvae

Acute Toxicity

In all experiments, 100% of the control animals survived. The median lethal concentrations (LC50s) for the larvae of both species (Table 3) revealed a high tolerance of cadmium especially over an acute exposure period (up to 96 h). Mortality was only recorded at the highest exposure concentration (4.78 mg/L) for both species in the first 24 h of exposure. At 48 h, dead *C. riparius* larvae were noted in four separate concentrations, whereas for *C. tentans*, mortality only occurred at three concentrations, which may account for the high value recorded at this observation time. As exposure time increases, the LC50 for both species show a similar gradual reduction, resulting in a virtually identical final 10-day value. Comparison of these data using Litchfield and Wilcoxon's (1949) method, revealed that with the exception of the 48-h observation time, no significant difference ($p > 0.05$) in larval response to cadmium was recorded between the two species.

The LC50 values for lindane (Table 3) suggest that it is more toxic to *C. riparius* and *C. tentans* larvae than cadmium by one to two orders of magnitude. Larval response to this toxicant differs from that to cadmium with large reductions in LC50 (compared to the preceding value) occurring in the first 96 h for both species. Following this initial period, the difference between each value becomes less marked. In further contrast to the results with cadmium, the LC50 was found to be significantly different ($p < 0.05$) between the two species at each time point following 144-h exposure, with *C. riparius* larvae less sensitive than *C. tentans*.

The results of this study suggest that toxicity can vary depending on the choice of test species and the nature of the contaminant. Both *C. riparius* and *C. tentans* larvae were found to be tolerant of cadmium with concentrations in mg/L necessary to cause mortality, which is in agreement with previously reported chironomid LC50s (Rehwoldt *et al*. 1973; Williams *et al*. 1986). Lindane was markedly more toxic and in addition

Table 2. Descriptive statistical measures in each of the four larval instars of *C. riparius* and *C. tentans* following hierarchical cluster analysis based on head capsule width

Species/Instar	Mean HCW[a] (mm) (SE)	Mean BL[b] (mm) (SE)	Max. HCW (mm)	Min. HCW (mm)	Max. BL (mm)	Min. BL (mm)
C. riparius						
first	0.12 (0.003)	1.34 (0.057)	0.18	0.07	2.65	0.76
second	0.22 (0.003)	3.53 (0.118)	0.19	0.26	4.28	1.73
third	0.36 (0.003)	5.69 (0.112)	0.45	0.29	9.91	3.06
fourth	0.56 (0.002)	9.55 (0.142)	0.63	0.49	13.3	4.89
C. tentans						
first	0.11 (0.001)	1.25 (0.043)	0.13	0.10	2.14	0.82
second	0.20 (0.002)	2.98 (0.085)	0.26	0.15	4.08	1.83
third	0.39 (0.003)	6.19 (0.172)	0.46	0.35	9.79	3.73
fourth	0.71 (0.005)	12.29 (0.298)	0.82	0.64	17.34	7.24

[a] Head capsule width.
[b] Body length.

Table 3. Median lethal concentrations (LC50) at 24-h intervals for second instar larvae of *C. riparius* and *C. tentans* exposed to cadmium and lindane for 10 days

	Cadmium LC50 (mg/L) (Upper–Lower 95% CI)		Lindane LC50 (μg/L) (Upper–Lower 95% CI)	
Time (h)	*C. riparius*	*C. tentans*	*C. riparius*	*C. tentans*
24	x[a]	x[a]	68.06 (112.9–41.0)	82.74 (129.9–52.7)
48	2.62 (3.8–1.8)	9.34 (25.3–3.4)	43.52 (52.6–7.5)	39.77 (64.0–24.7)
72	2.27 (3.4–1.5)	x[a]	20.28 (47.8–5.6)	12.56 (23.3–6.7)
96	1.76 (3.1–1.0)	1.68 (3.4–0.8)	6.22 (11.8–3.3)	5.45 (10.3–2.9)
120	1.24 (1.9–0.8)	1.49 (2.5–0.9)	x[a]	3.76 (6.22–2.3)
144	x[a]	x[a]	5.06 (8.1–3.2)	2.07 (3.2–1.3)
168	1.00 (1.5–0.7)	1.16 (1.8–0.7)	3.98 (6.1–2.6)	1.88 (3.1–1.2)
192	0.79 (1.1–0.6)	1.01 (1.8–0.6)	3.80 (5.9–2.4)	1.25 (2.0–0.8)
216	0.75 (1.1–0.5)	0.75 (1.1–0.5)	3.41 (5.5–2.1)	1.24 (1.9–0.7)
240	0.70 (1.0–0.5)	0.74 (1.1–0.5)	2.60 (4.4–1.5)	1.23 (1.9–0.8)

[a] Data were unsuitable for LC50 analysis, mortality was not recorded on three separate occasions.

was found to cause a significant difference in larval response between species, with *C. tentans* larvae more sensitive. The observation that lindane was the more toxic of the chemicals tested is predictable, because it is specifically targeted against arthropod species, whereas cadmium exerts its toxic affects across a much wider taxonomic spectrum. The high sensitivity of chironomids to lindane has previously been reported by other authors (Fisher and Wadleigh 1985; Taylor *et al*. 1991; Maund *et al*. 1992). Although the absorption of cadmium and lindane by *C. riparius* and *C. tentans* is likely to follow a similar pathway involving transfer into the circulatory system across the epithelial barrier of larval gills, digestive system, and integument, the mode of action of these toxicants and the systems affected are fundamentally different, which may also be important in explaining the differing toxicity.

While there can be little dispute that cadmium and lindane are toxic to *C. riparius* and *C. tentans* larvae, the rate of change of the LC50s suggest that toxicity proceeds at a much slower rate in the latter stages of the test compared to the initial (up to 96 h) exposure period. This may be attributed to the induction of separate detoxification systems (metallothioneins, monooxygenase, and glutathione-s-transferease enzymes) in the larvae following the initial exposure. However, to detoxify the test chemicals at a rate that would manifest as a slowing down of toxicity, the larvae would need to synthesize a sufficient quantity of protective enzymes to account for all the toxicant, and in this respect the total capacity for induction represents the capacity to counteract toxicity (Roesijadi 1992). The mortality data obtained for both species suggests that, at least at the lower exposure concentrations, sufficient protection is inferred on the larvae to offset the toxic effects of cadmium and lindane.

Conclusion

These separate investigations have shown that under controlled conditions and following acute toxicant exposure, the pattern of response for *C. riparius* and *C. tentans* was similar. Although differences in EmT50 were noted, this does not represent an obstacle to the use of either species in toxicity tests, provided that this and any other possible differences have been identified and are considered when interpreting the data. The results support the current practice of using either *C. riparius* or *C. tentans* in research and regulatory tests since, at least under acute exposure conditions, the only determinant of spe-

cies sensitivity was the toxicant. However, the difference in sensitivity to lindane highlights the fact that in regulatory testing, reliance on one species is not sufficient to ensure maximum protection, necessitating an approach involving a suite of species to comprehensively assess the impact of a particular chemical. In addition, standardization relating to all aspects of organism exposure should be adopted and applied to facilitate the direct comparison of results from different sources.

Acknowledgment. The authors thank Zeneca Agrochemicals, Mr. M. O'Reilly, Mrs. O. James, and the two reviewers for their constructive comments on the manuscript.

References

Ankley GT, Hoke RA, Benoit DA, Leonard EM, West CW, Phipps GL, Mattson VR, Anderson LA (1994) Development and evaluation of test methods for benthic invertebrates and sediments: effects of flow rate and feeding on water quality and exposure conditions. Arch Environ Contam Toxicol 25:12–19

APHA (American Public Health Association) (1995) Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, DC

Armitage PD, Cranston PS, Pinder LCV (1995) The Chironomidae—biology and ecology of non-biting midges. Chapman and Hall, London

ASTM (American Society for Testing and Materials) (1993) Standard guide for conducting sediment toxicity tests with freshwater invertebrates. E1383-93, Annual Book of Standards, 11.04, Philadelphia, PA, pp 1173–1199

Burton GA Jr, Norberg-King TJ, Ingersoll CG, Benoit DA, Ankley GT, Winger PV, Kubitz J, Lazorchak JM, Smith ME, Greer E, Dwyer FJ, Call DJ, Day KE, Kennedy P, Stinson M (1996) Interlaboratory study of precision: *Hyalella azteca* and *Chironomus tentans* freshwater sediment toxicity assays. Environ Toxicol Chem 15:1335–1343

CEC (Council of the European Communities) (1992) Council directive 92/32/EEC of 30th April 1992 amending for the seventh time directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Off J Eur Commun 35:1–29

Day KE, Scott-Kirby R, Reynoldson TB (1994) Sexual dimorphism in *Chironomus riparius* (Meigen): impact on interpretation of growth in whole-sediment toxicity tests. Environ Toxicol Chem 13:35–39

Downe AER, Caspary VG (1973) The swarming behaviour of *Chironomus riparius* (Diptera: Chironomidae) in the laboratory. Can Entomol 105:165–171

Dyar HG (1890) The number of moults of Lepidopterous larvae. Psyche Camb Mass 5:420–422

Environment Canada (1997) Biological test method: test for survival and growth in sediment using the larvae of freshwater midges (*Chironomus tentans* or *Chironomus riparius*). Report EPS 1/RM/32, 131 pp

Fisher WS, Wadleigh RW (1985) Effects of temperature on the acute toxicity and uptake of lindane by *Chironomus riparius* Meigen (Diptera: Chironomidae). J Econ Entomol 78:1222–1226

Hill IR, Matthiesen P, Heimbach F (eds) (1993) Guidance document on sediment toxicity tests and bioassays for freshwater and marine environments. Society of Environmental Toxicology and Chemistry—Europe, Workshop on sediment toxicity assessment. Renesse, The Netherlands

Holloway MTP (1983) Factors controlling the productivity of a benthic detritovore (*Chironomus riparius*). Ph.D. thesis, UWIST, Cardiff, UK

Learner MA, Potter DWB (1974) The seasonal periodicity of emergence of insects from two ponds in Hertfordshire, England, with special reference to the Chironomidae (Diptera: Nematocera). Hydrobiologia 44:495–510

Litchfield JT (1949) A method for the rapid graphic solution of time-percentage effect curves. J Pharmacol Exp Ther 97:399–408

Litchfield JT, Wilcoxon F (1949) A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 96:99–113

Mackey AP (1977) Growth and development of larval Chironomidae. Oikos 28:270–275

Maund SJ, Peither A, Taylor EJ, Jüttner I, Beyerle-Pfnür R, Lay JP, Pascoe D (1992) Toxicity of lindane to freshwater insect larvae in compartments of an experimental pond. Ecotoxicol Environ Saf 23:76–88

McCahon CP, Pascoe D (1988) Culture techniques for three freshwater macroinvertebrate species and their use in toxicity tests. Chemosphere 17:2471–2480

Miall LC (1895) The natural history of aquatic insects. Macmillan and Co., New York, pp 122–152

Møhlenberg F, Jensen A (1980) The ecotoxicology of cadmium in fresh and sea water and water pollution with cadmium in Denmark. The National Agency of Environmental Protection, Denmark

Nebeker AV (1973) Temperature requirements and life cycle of the midge *Tanytarsus dissimilis* (Diptera: Chironomidae). J Kansas Entomol Soc 46(2):160–164

Oliver DR (1971) Life history of the Chironomidae. Ann Rev Entomol 16:211–230

Pinder LCV (1986) Biology of freshwater chironomidae. Ann Rev Entomol 31:1–23

Rehwoldt R, Lasko L, Shaw C, Wirhowski E (1973) The acute toxicity of some heavy metal ions toward benthic organisms. Bull Environ Contam Toxicol 10:291–294

Roesijadi G (1992) Metallothioneins in metal regulation and toxicity in aquatic animals. Aquat Toxicol 22:81–114

Sadler WO (1935) The biology of the midge *Chironomus tentans* Fabricius, and methods for its propagation. Mem Cornell Univ Agric Exp Stn 173:1–25

Sibley PK, Benoit DA, Ankley GT (1997) The significance of growth in *Chironomus tentans* sediment toxicity tests. Relationship to reproduction and demographic end points. Environ Toxicol Chem 16:336–345

Taylor EJ, Maund SJ, Pascoe D (1991) Toxicity of four common pollutants to the freshwater macroinvertebrates *Chironomus riparius* Meigen (Insecta: Diptera) and *Gammarus pulex* (L.) (Crustacea: Amphipoda). Arch Environ Contam Toxicol 21:371–376

Taylor EJ, Blockwell SJ, Maund SJ, Pascoe D (1993) Effects of lindane on the life cycle of a freshwater macroinvertebrate *Chironomus riparius* Meigen (Insecta: Diptera). Arch Environ Contam Toxicol 24:145–150

Townsend BE, Lawrence SG, Flannagan JF (1981) *Chironomus tentans* Fabricius. *In Manual for the culture of selected freshwater invertebrates*. Canad Spec Publ Fish Aquat Sci 54:109–126

US EPA (United States Environmental Protection Agency) (1994) Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA/600/R-94/024, Technical report, Washington, DC, 133 pp

Wicklund C, Fagerström T (1977) Why do males emerge before

females? A hypothesis to explain the incidence of protandry in butterflies. Oecologia 31:153–158
Williams KA, Green DWJ, Pascoe D (1985) Studies on the acute toxicity of pollutants to freshwater macroinvertebrates. 1. Cadmium. Arch Hydrobiol 102:461–471
Williams KA, Green DWJ, Pascoe D, Gower DE (1986) The acute toxicity of cadmium to different larval stages of *Chironomus riparius* (Diptera: Chironomidae) and its ecological significance for pollution regulation. Oecologia (Berlin) 70:362–366
Wren CD, Stephenson GL (1991) The effect of acidification on the accumulation and toxicity of metals to freshwater invertebrates. Environ Pollut 71:205–241