Hydrobiologia **334:** 199–206, 1996.
K. A. Coates, Trefor B. Reynoldson & Thomas B. Reynoldson (eds), Aquatic Oligochaete Biology VI.

A comparison of reproduction, growth and acute toxicity in two populations of *Tubifex tubifex* (Müller, 1774) from the North American Great Lakes and Northern Spain

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Key words: toxicity, cultures, oligochaetes, *T. tubifex*, growth, reproduction

Abstract

Reproduction in *Tubifex tubifex* is being used as part of a suite of indicators of sediment toxicity in Canada and Spain, and reproduction of *T. tubifex* is being considered as a component of sediment objectives for environmental regulation and clean-up in the Canadian Great Lakes. The data being used to set these reproductive targets have been developed from a single culture of *T. tubifex* from Lake Erie. The plasticity of this particular species is well known and before it can be adopted widely as a test organism it is necessary to determine whether a single culture source should be used or if cultures derived from different populations respond similarly. A series of experiments with two cultures, one from Lake Erie the second from a small mountain stream in Northern Spain have shown that the Spanish worms appear to produce fewer cocoons per adult (mean 8.6 S.D. 1.0) than those from Lake Erie (mean 10.4 S.D. 0.3) at 22.5 °C, a standard test temperature. The number of young produced per adult by the Spanish culture is also lower (mean 19.0 S.D. 4.6) than the L. Erie population (mean 30.6 S.D. 2.3), however, the Spanish population has higher reproductions rates at a lower temperature. The Spanish worms also have lower and more variable growth rates than the Canadian population. There also appear to be slight differences in the sensitivities to toxicants, with the Canadian worms having higher LC50s for copper, chromium and cadmium. While there are differences in the responses in the two cultures these are not considered to be sufficient to invalidate the use of either population in a standard bioassay protocol as long as appropriate calibration and validation are undertaken.

Introduction

Benthic macroinvertebrates are frequently used for the classification and monitoring of natural environments and in laboratory toxicity tests. However, the aquatic Oligochaeta often are not considered as useful indicators or as test species because of perceived difficulties in their identification and the perception that they are tolerant to pollution. These perceptions are inaccurate. Identification keys are available, their sensitivity has been demonstrated in laboratory toxicity tests and detailed standard test protocols are available (Naididae: Learner & Edwards, 1963; Chapman & Mitchell, 1986; Tubificidae: Chapman & Mitchell, 1986; Milbrink, 1987; Wiederholm et al., 1987; Casellato & Negrisolo, 1989; Reynoldson et al., 1991; Enchytraeidae: Roembke and Knacker, 1989; Lumbriculidae: Bailey & Liu, 1980; Keilty et al., 1988; Dermott & Munawar, 1992).

The oligochaete *Tubifex tubifex* (Müller, 1774) can adapt to a wide range of environmental conditions and occurs in both oligotrophic and eutrophic conditions and is found in both non-polluted and very polluted waters (Milbrink, 1983; Lauritsen et al., 1985). A 28 day reproductive toxicity test with *T. tubifex* is being used in Canada as part of a remediation program on the Great Lakes (Bailey et al., 1995; Reynoldson et al., 1995). The test also is being used in Spain to determine the toxicity of river sediments in the industrial area of Bilbao. The objective of this study was to determine whether a single culture should be used for future work

to avoid differences in test responses resulting from the source of the culture populations. To address this, separate and paired experiments were conducted examining survival, reproduction and growth in cultures derived from Spanish and Canadian populations of *T. tubifex*.

Methods

Characteristics of culture animals and sediment

The Spanish population of worms and culture sediment were obtained from Barazar in Gorbea Natural Park, Bizkaia, Spain. This is a mountain stream at an altitude of 570 m, the stream is approximately 2 m wide and less than 50 cm deep (43°03′ N, 2°43′ W). The Canadian culture sediment came from Big Creek Marsh, Long Point, Lake Erie (42°36′ N, 80°27′ W), a United Nations biosphere preserve, which is a shallow embayment of 850 ha with a water depth of 0.75–1 m. Canadian cultures of *T. tubifex* were derived from populations from western Lake Erie and Hamilton Harbour, Lake Ontario. Sediments from Barazar used for cultures and experiments were collected in December 1993 (BAR) and two separate batches of sediment were collected from Long Point in Lake Erie in the spring (LP7) and the fall (LP8) of 1992. Sediment from both sources (Table 1) are predominantly fine grained silts, the Long Point sediment is higher in organic material and generally higher in metal concentrations (Table 1).

Table 1. Characteristics of culture sediments from Spain and Canada.

	Barazar - Spain	Long Point - Canada
Sediment Characteristics (% composition)		
Sand	5.6	14.5
Silt	73.4	76.8
Clay	20.9	8.7
LOI	8.8	17.0
Metals & Major Elements ($g\ g^{-1}$ dry weight)		
Cr	41.5	41.5
Zn	213	96.6
Cd	9.5	0.4
Pb	89.6	25.8
Ni	45.9	16.9
Mn	217	1200
Fe	30992	28300
Cu	20.7	12.1
S	646.2	9100
P	367.5	2100
Ca	2269	172500

Laboratory culture

Canadian and Spanish populations of *T. tubifex* were maintained at the National Water Research Institute (NWRI), Burlington, Ontario, on sieved (250 μm pore size), previously frozen sediment from Long Point (LP) (Table 1). The culture vessels were covered 20 × 20 × 20 cm aquaria with a 5 cm layer of sediment, and 10 cm of overlying dechlorinated, City of Burlington, Lake Ontario, tap water (pH 7.8–8.3, conductivity 439–578 μS, hardness 119–137 mg l^{-1}). The aquaria were gently aerated, maintained on an 8:16 h light dark cycle and at ambient room temperature (20 ± 2 °C). The cultures were checked regularly, and when the proportion of sexually mature individuals declined the sediment was replaced, approximately every three months.

Spanish cultures were maintained at the Universidad del Pais Vasco (UdVP), in Bilbao, Spain. The sediment from Barazar (BAR) (Table 1) was sieved through a 250 μm pore-size sieve and kept in the dark at 4 °C prior to use. The cultures were maintained in aquaria (h 25 cm, w 40 cm, d 20 cm) with a 7 cm layer of sediment and 10 cm of overlying dechlorinated tap water (pH 7.1, conductivity 180.5 μS, $CaCO_3$ alkalinity 50 mg l^{-1}). The aquaria were kept in an incubator at 22.5 ± 1 °C with gentle aeration in the dark.

Reproduction tests

Differences in reproduction in the two populations of *T. tubifex* were investigated in two series of experiments. First, Spanish and Canadian populations were tested separately at UdVP and at the NWRI with the different, respective, culture sediments (BAR and LP, Table 1) and overlying tap water. Second, paired experiments with Spanish and Canadian worms, both in culture at NWRI, were conducted with LP sediment.

In both experiments tests were performed using the protocol of Reynoldson et al. (1991). For each test five replicates were established. Each replicate contained 100 ml of sediment and 100 ml of overlying tap water in a 250 ml glass beaker. The sediment was sieved through a 250 μ m pore-size sieve to eliminate the indigenous invertebrates (Reynoldson et al., 1994). Four sexually mature worms were added to each replicate container. The tests were maintained in the dark at the test temperature (20 or 22.5 ± 0.5 °C) and gently aerated; pH, dissolved oxygen and temperature were

checked weekly in each beaker. At the start of each test, each replicate beaker received 80 mg of Tetramin® as a food supplement. Reproduction was measured by counting the number of cocoons and young produced in each replicate beaker and expressed as the number produced per adult.

Growth experiments

Differences in growth rates of the two populations were examined. First, the effect of individual size on growth was examined at a single temperature (25 ± 0.5 °C) and second, the effect of temperature on growth rate was examined. In the first experiment growth of the two populations was measured using individuals ranging from 0.3–6.0 mg (wet weight), this range represents individuals that are a few days old (hatched from the cocoon) to those approaching their first reproduction. In the second experiment differences in growth response to temperature (5, 10, 15, 20, 25 and 30 ± 0.5 °C) were examined in eight week old animals that were sexually immature and had not reproduced. Animals in the 1.0–4.0 mg (wet weight) size range were used and all experiments lasted for 10 days. Animals were maintained separately in 100 ml glass containers containing 50 ml of LP sediment and 50 ml of Lake Ontario tap water.

All worms were sexually immature at the start of the experiment. Wet weights were measured for each individual at the beginning and the end of the experiment. It was necessary to use wet weight rather than dry weight as growth rates were calculated for individual worms. There is a high correlation between wet and dry weight ($r^2 = 0.997$) in *T. tubifex*, and therefore wet weight measurements were considered acceptable. All data are expressed as wet weights. Growth was calculated as % growth per day ($G_w\%$):

$$G_w\% = (\ln FW - \ln SW) \times 100d^{-1},$$

where FW = final weight (mg), SW = start weight (mg) and d = number of days.

Sensitivity to reference toxicants

Paired experiments were conducted with the two populations to study their relative sensitivity to selected toxicants. Acute toxicity was examined in dechlorinated, Lake Ontario tap water over a 96 h period using methods similar to those reported by Chapman et al. (1982). The animals were examined every 24 hours and were considered dead if they did not respond to tactile stimulation. At each dose, five replicate 250 ml beakers containing 100 ml of water were established to which five individual worms were added (wet weight = 3.1–10.0 mg). The experiments were conducted at 22 ± 0.5 °C and maintained in the dark, the beakers were not aerated. Oxygen, pH and conductivity were measured daily. Dead animals were counted and removed daily.

Three metals, cadmium ($CdCl_2$), chromium ($K_2Cr_2O_7$) and copper ($CuSO_4$) and an organic compound, (hexachlorocyclohexane – lindane) were tested in acute toxicity experiments using a series of concentrations (Table 2) with dechlorinated Lake Ontario tap water ($CaCO_3$ alkalinity 90–120 mg l^{-1}, hardness 119–137 mg l^{-1}). Nominal concentrations (Table 2) of trace metals were confirmed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) on a multichannel Jarrell-Ash (Franklin, Ma) Atom Comp® 1100. Lindane analysis was performed by dual capillary column gas/liquid chromatography with dual and single electron capture detection using a Hewlett Packard GC/ECD (model 5890). Confirmation analyses were done by GC/MS. The margin of error is estimated to be 10% from extraction, isolation and instrumentation.

Calculation of LC50s was conducted by the least squares probit method using a programme developed by the University of Guelph (T. James).

Results

Reproduction

Reproduction was first compared from the results of a number of repeated bioassays using the Spanish (BAR) and Canadian culture sediments (LP7 and LP8). These data (Table 3) provide an indication of the range in reproductive performance of *T. tubifex* from several separate runs on each culture sediment. A comparison of reproduction in the two cultures, in their respective sediment using the Mann Whitney test with mean values for each run showed that the numbers of cocoons produced by the Spanish worms on BAR sediment was significantly less ($P<0.05$) than the Canadian worms in both LP7 and LP8 sediments. There was no significant difference ($P>0.05$) between the number of young produced at BAR and LP7 but the difference was significant ($P<0.05$) between the Spanish worms and the Canadian worms in LP8. Because these tests

Table 2. Dose ranges for four toxicants in 96 h acute toxicity tests with *T. tubifex*, showing nominal and measured concentrations (mg l^{-1}).

Copper		Cadmium		Chromium		Lindane	
nominal	actual	nominal	actual	nominal	actual	nominal	actual
0	<0.002*	0	<0.003*	0	<0.005*	0	0.008
0.025	<0.004*	0.1	0.128	1	0.724	0.5	0.346
0.050	0.056	0.2	0.248	2	2.456	1.0	0.593
0.100	0.114	0.4	0.564	4	5.360	1.5	1.246
0.200	0.198	0.8	1.028	8	9.960	2.0	3.258
0.400	0.368	1.6	2.004	16	17.560	3.0	1.373
0.800	0.772	3.2	4.040	32	35.960	4.0	3.113
1.600	1.882	6.4	7.840	64	74.400	8.0	4.426
3.200	3.660	12.8	15.000	128	142.000	16.0	5.972
6.400	8.160			256	370.400		

* indicates below detection.

Table 3. Results of repeated bioassays with Spanish and Canadian cultures on different culture sediments, showing numbers of cocoons (CCAD) and young (YGAD) produced per adult *T. tubifex* (standard deviation in parentheses).

BARAZAR		LONG POINT 7		LONG POINT 8	
CCAD	YGAD	CCAD	YGAD	CCAD	YGAD
6.1 (0.6)	5.1 (7.0)	8.3 (0.9)	15.8 (2.2)	6.6 (0.6)	9.6 (1.0)
8.4 (0.4)	21.0 (4.0)	8.7 (0.8)	9.1 (0.6)	10.9 (0.5)	28.8 (3.3)
6.3 (1.9)	12.5 (11.1)	7.3 (1.4)	15.1 (1.6)	10.8 (0.5)	38.3 (3.5)
6.9 (0.3)	13.9 (4.3)	9.9 (0.8)	18.2 (3.2)	9.4 (1.3)	22.8 (2.0)
8.7 (0.5)	27.4 (4.9)	8.8 (0.4)	15.5 (0.9)	9.5 (1.2)	25.7 (4.7)
9.0 (0.1)	26.6 (8.6)	10.1 (1.1)	20.5 (3.0)	6.6 (0.6)	9.6 (1.0)
6.9 (0.8)	8.0 (3.9)	8.3 (0.9)	18.4 (2.1)	10.7 (0.5)	32.8 (3.6)
		7.9 (0.4)	14.0 (2.1)	10.3 (0.8)	19.1 (3.3)
		10.8 (0.7)	31.1 (3.8)	9.0 (0.7)	26.7 (2.0)
		10.5 (0.5)	18.4 (3.2)	10.4 (1.6)	45.4 (1.7)
				10.6 (1.3)	36.2 (9.1)
				9.6 (1.4)	29.1 (5.5)

were performed at different laboratories using different culture sediment the variability in response could be attributed to differences between the populations, the laboratories or the sediment.

To exclude methodological differences or effects due to differences in sediment quality, reproduction was compared in four paired tests on LP sediment using individuals from Canadian and Spanish populations. The Spanish worms were maintained in culture at NWRI for several months prior to the experiment, using LP sediment. These experiments were conducted at two temperatures: 20 ° and 22.5 ± 0.5 °C.

The results from two experiments at each temperature are presented in Table 4. At the standard test temperature (22.5 °C) the results are in the range observed in the different culture sediments (BAR, LP7 and LP8 in Table 3), with the exception of Spanish cocoon production in the first experiment. The two populations were compared using the Mann Whitney test. In both experiments at 22.5 °C cocoon production was significantly ($P<0.05$) lower for the Spanish worms, and in the second experiment the number of young produced was significantly ($P<0.05$) lower. At 20 °C the opposite occurred; cocoon production was significantly ($P<0.05$) lower in the Canadian population in both experiments and young production lower ($P<0.05$) in the first experiment.

Spanish *T. tubifex* are smaller than those from the Canadian culture population. For 11 sexually mature animals randomly selected from the two populations

Table 4. Comparison in number of cocoons (CCAD) and young (YGAD) produced by Spanish and Canadian cultures of *T. tubifex* in sediment from Long Point at 20 °C and 22.5 °C (standard deviation in parentheses).

	20 °C CCAD	20 °C YGAD	22.5 °C CCAD	22.5 °C YGAD
Exp 1				
Spain	7.0 (0.6)	22.3 (3.4)	4.3 (2.2)	18.8 (9.4)
Canada	5.0 (0.4)	4.6 (2.2)	10.3 (1.3)	27.4 (5.6)
Exp 2				
Spain	6.7 (0.8)	17.2 (8.6)	8.6 (1.0)	19.0 (4.6)
Canada	4.9 (0.2)	7.0 (1.2)	10.4 (0.3)	30.6 (2.3)

Table 5. Summary of reproduction in Spanish and Great Lakes reference sites, with some sediment characteristics.

	Mean	Range
Great Lakes (n = 163)		
Cocoons per adult	9.1	3.3–11.8
Young per adult	24.6	1.1–48.9
LOI %	11.4	1.0–38.7
Sand%	32.8	0.0–99.8
Silt%	32.0	0.0–86.3
Spain (n = 4)		
Cocoons per adult	8.6	6.9–10.5
Young per adult	12.9	1.2–35.5
LOI %	5.2	2.4–8.8
Sand%	46.1	5.8–91.6
Silt%	46.5	5.8–73.4

the mean wet weights were respectively, 5.6 mg (S.D. 2.6) and 8.1 mg (S.D. 1.4) for Spanish and Canadian worms. However, the biomass of mature worms does not appear to influence number of cocoons ($r^2 = 0.03, n = 56$) or young ($r^2 = 0.01, n = 56$) produced per adult in Canadian worms. In Spanish worms a similar relationship between initial biomass and number of cocoons ($r^2 = 0.01$, $n = 35$) and young ($r^2 = 0.001$, $n = 35$) was observed.

As part of a programme to develop biological sediment objectives, a large number of reference sites have been sampled in the North American Great Lakes (Reynoldson et al., 1995). From these data we have provided information on the range of cocoon and young production in *T. tubifex* in a variety of sediments from 163 Great Lakes sites as well as four Spanish sites (Table 5). The results from the few unpolluted Spanish sites so far examined show the average number of cocoons produced per adult again to be lower than the average for the Great Lakes but well within the range observed in the much larger Great Lakes data set. Average young production at Spanish reference sites is about half that observed in the Great Lakes. The organic content (percent LOI) at the Spanish reference sites is also generally lower, the average value being half that of the Great Lakes sites.

Growth rates

We have also investigated the growth behaviour of the two populations of *T. tubifex*. Figure 1 shows the general pattern in somatic growth of worms at 25 °C. There is a strong relationship between initial size and somatic growth and smaller individuals have much higher rates of growth. In the size range of individuals tested (Spain 0.30–5.91 mg; Canada 0.37–6.24 mg), the Spanish population appears to show a logarithmic relationship with initial size ($r^2 = 0.67$), while a linear relationship ($r^2 = 0.46$) provided the best fit for the Canadian culture, although we would expect the logarithmic growth phase to occur if smaller Canadian worms had been included. The wet weights of newly hatched (1 day old) individuals are, for Spanish and Canadian worms respectively, 0.07 (S.D. 0.01) mg and 0.08 (S.D. 0.00) mg.

To compare growth differences between the Spanish and Canadian populations over a range of temperatures we used animals that were larger than 1.0 mg initial weight and excluded those individuals that became sexually mature over the period of the experiment. The Spanish population has a consistently lower growth than the Canadian, and at lower temperatures (10–15 °C) the Canadian worms have growth rates that are 2–4 times greater (Figure 2), however, these differences are not statistically significant (t-test; $P > 0.05$) except at 30 °C.

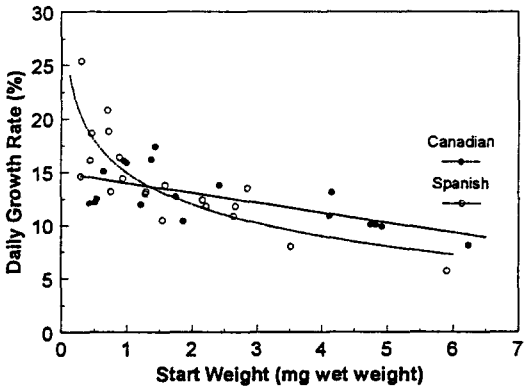

Figure 1. Effect of size on growth rates of two populations of *T. tubifex*.

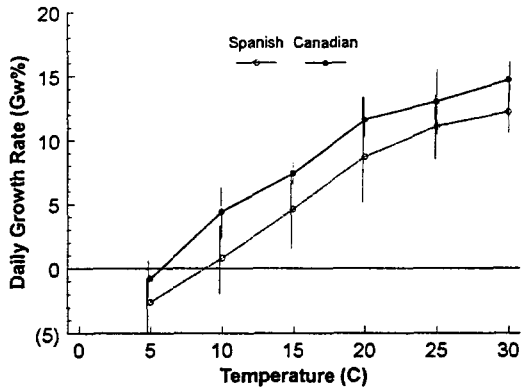

Figure 2. Relationship between temperature and growth rate (mean with S.D.) in two populations of *T. tubifex*.

Sensitivity to reference toxicants

To compare relative sensitivities to toxicants we examined the acute toxicity of the two populations to four selected chemicals. LC50 values were calculated for 24, 48, 72 and 96 h and are presented in Table 6 for each time period and toxicant. In addition, calculated LC50 values for cadmium and copper for worms from the Spanish population estimated at the Universidad del Pais Vasco are also presented together with selected relevant literature values (Table 6).

Based on LC50 data, the Canadian *Tubifex* are slightly more tolerant to all four contaminants. The response of the two populations in lindane shows both to be relatively insensitive up to 72 h.

Discussion

There are only a few published examples (Maltby, 1984; Mearns et al., 1986; Munzinger & Monicelli, 1991) of intercalibration tests for bioassays performed by different laboratories using different cultures of the same species. The performance of *Daphnia magna* cultures is examined periodically in a number of laboratories of the European Community (EC) in 'ring tests', using a reference chemical and standard protocols (Cabricenc, 1986).

Differences in growth rates and reproduction in lentic and lotic populations of *T. tubifex* have been described by Poddubnaya (1980). Lotic forms (Spanish worms) would be expected to be more eurybiotic, with more rapid growth and higher reproduction than lentic forms (Canadian worms) which should be adapted to the relatively stable conditions in the deep and colder waters of lakes. These experiments have shown that there are differences in growth and reproduction in the two populations. The growth rates showed the same general pattern (Figure 1). However, the Spanish worms had lower growth rates at all temperatures (Figure 2). This difference may be important if an endpoint based on growth is used in bioassay studies. If such an endpoint is considered then standard size individuals should be used as small animals (<1.0 mg) have higher growth rates than large animals. Two points are also of ecological interest. First, the different growth rates (Figure 2) suggest that these two populations are adapted to different environmental conditions. Second, the greater variability of growth rates of the Spanish worms (Figure 2) may indicate an adaptation of the Spanish population to the fluctuating conditions that occur in stream environments.

Reproduction is lower in the Spanish worms at 22.5 °C but higher at 20 °C. The ability to reproduce and grow over a broader range of temperature is an adaptation one would expect in a population exposed to the greater temporal variability in temperature observed in stream compared to lake environments. Despite the lower number of young produced by the Spanish worms it is within the range of values obtained from extensive work on reference sites in the Laurentian Great Lakes. Whether the differences between the two populations of *T. tubifex* have been affected by several generations in standarized laboratory conditions is unknown.

There are practical concerns arising from variability in sensitivity to toxicants in different populations. First, the reliability of chemical criteria derived from standard LC50 data in dose response tests; second, accurately establishing a difference between a test response and literature values for the same response. These two populations appear to be more tolerant to

Table 6. 24–96 h LC50 ($mg\ l^{-1}$) values calculated for cultures of *T. tubifex* from Spain and Canada with four chemicals.

Chemical	24 h LC50	48 h LC50	72 h LC50	96 h LC50
Copper				
Spain (NWRI)	0.49	0.18	0.11	0.07
Canada (NWRI)	0.86	0.26	0.16	0.09
Brkovic-Popovic 1977	1.38	0.89		
Cadmium				
Spain (UdPV)	1.2	0.9	0.6	0.4
Spain (NWRI)	6.5	3.6	2.4	1.7
Canada (NWRI)	9.8	6.5	5.4	3.2
Brkovic-Popovic 1977	1.2	0.7		
Chapman et al. 1982				0.32
Chromium				
Spain (UdPV)	57.4	33.2	16.5	9.8
Spain (NWRI)	122.1	54.5	28.7	15.5
Canada (NWRI)	137.8	95.5	49.5	38.1
Brkovic-Popovic 1977	86.0	4.6		
Lindane				
Spain (NWRI)	none	none	5.0	3.5
Canada (NWRI)	none	none	4.4	3.9

UdPV - tests conducted in Bilbao, Spain.
NWRI - tests conducted in Burlington, Canada.

cadmium and chromium than reported by Brkovic-Popovic & Popovic (1977) and Chapman et al. (1982) but more sensitive to copper (Table 6). Unpublished data of similar acute tests performed in Spain with immature worms of several sizes show lower but very similar LC50 values for cadmium and chromium to the values obtained at NWRI with Spanish worms (Table 6). These data suggest that the implications for developing criteria are small given the safety margins incorporated into most procedures for setting criteria from toxicity data. Similarly the implications for other comparisons may be relatively minor given the amount of variability which presently exists in such tests. Literature data show considerable variation usually because tests differ from each other in the temperature used, the alkalinity of water, the age of organisms or the conditions of the test.

These data show differences in the responses in the measured endpoints in the two culture populations. Applying the responses from data derived from one population to results from a second population should be done cautiously. For example, the use of reference data on *T. tubifex* for the Great Lakes would be inappropriate for determining whether or not reproduction has been impaired in sediments from Spanish rivers. By rearing separate cultures from two different populations in the same laboratory we have eliminated differences in handling and maintenance. Therefore, the differences observed are attributes of the different populations. One reason given for the variability in responses observed in different laboratories is the genetic variability of the cultures derived from different natural populations (Baird et al., 1989; Soares et al., 1992). Anlauf (1994) has described genetic differences in *T. tubifex* populations from lacustrine habitats in Germany. These variants showed differences in growth and reproduction, indicating that physiological differences should be established in a test population before it is used in toxicity testing. The Canadian and Spanish populations do show differences in the genome that could be responsible for the difference in relative variability in the two populations. Starch gel electrophoresis data (Coates, pers. comm.) showed the Canadian population to be homozygotic at 19 loci for 13 allozymes, and the Spanish population to be homozygotic at 13 loci for 10 allozymes.

In order to avoid confounding effects due to the source population of test organisms, quality criteria for

survival, reproduction and reference toxicants should be established permitting comparison of results from different laboratories. In aquatic oligochaete toxicity tests, as far as we know, only Roembke (1989) and ASTM (1994) have proposed quality criteria for validating such tests. In order to produce consistent data in toxicity testing with *T. tubifex* at a minimum: animals of a standard age or size range should be used; reproductive tests should measure their first reproduction; a standard temperature should be used in all laboratories; and, a standard sediment should be used for culturing and as a positive control. Finally, we suggest that limits be established for selected reference toxicants to allow intercalibration of different cultures of *T. tubifex* being used in toxicity tests, as is done for other indicator species. This would provide a basis for establishing the condition and sensitivity of the worms used in sediment toxicity testing.

Acknowledgements

The authors wish to thank Ms Sherri Thompson for her assistance in the laboratory, and the Basque Government for financial support (project AMB 93 0460) to the junior authors.

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