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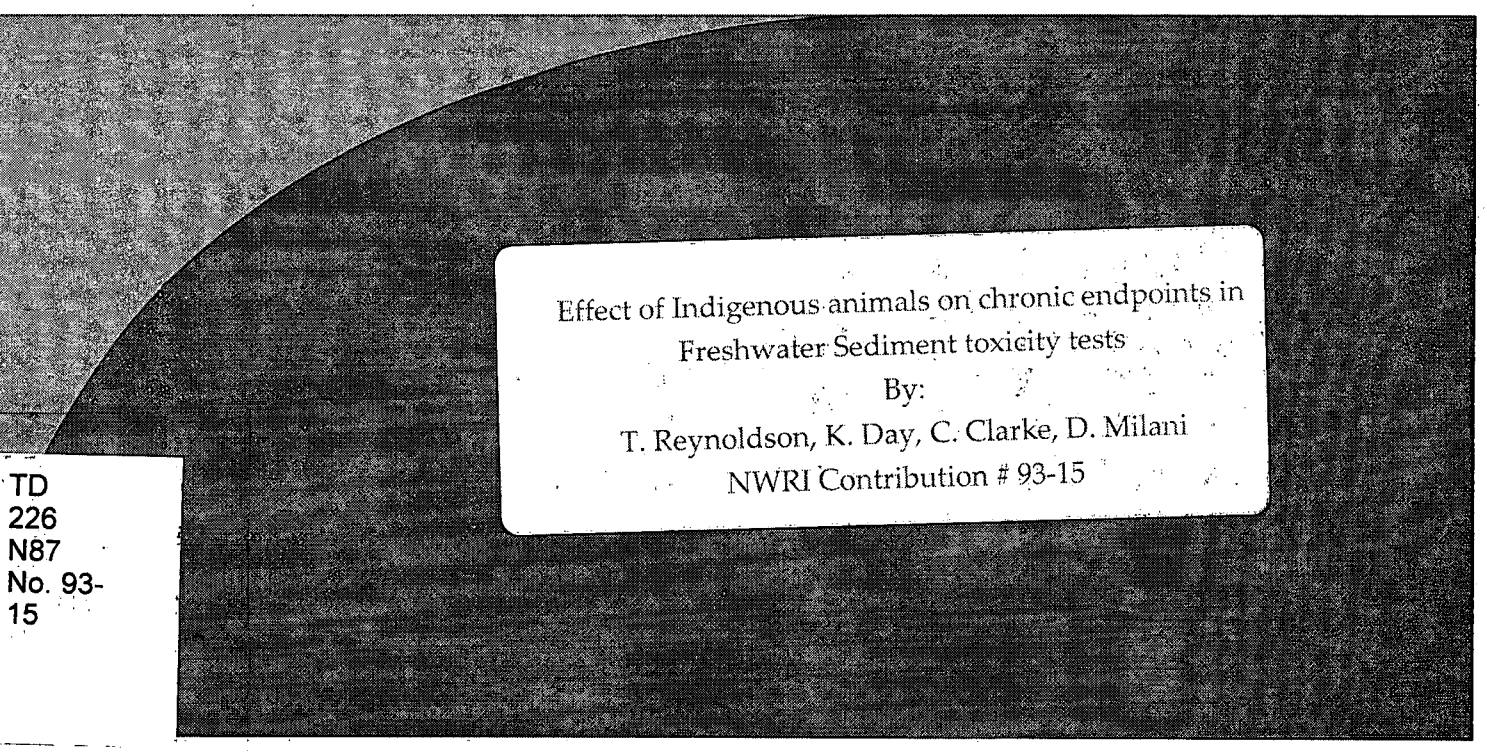

Effect of Indigenous animals on chronic endpoints in Freshwater Sediment toxicity tests

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Effect of indigenous animals on chronic endpoints in freshwater sediment toxicity tests

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Management Perspective

Sediment toxicity tests are increasingly being recommended and used in the determination and identification of sediment-associated contaminants and their use has been strongly endorsed by the International Joint Commission, particularly for Areas of Concern. While the initial development of sediment bioassay methodologies emphasized acute tests there has been recognition that chronic endpoints, such as growth and reproduction, are a more sensitive and discriminatory measurement of biological effects. Most protocols for conducting whole sediment toxicity tests recommend that manipulation of field-collected sediments .(*i.e*, sieving and mixing) be limited in order to maintain the chemical equilibria of any potential contaminants associated with the sediment. However, there has been some concern expressed as to the potental effects of indigenous animals on confounding the interpretatioon of such tests. The presence of oligochaete worms, which occur commonly in field samples, in sediments used to conduct chronic toxicity tests has been shown to have a marginal effect on survival and a significant ($P < 0.05$) effect on growth of three test species, a chironomid midge, an amphipod and a burrowing mayfly. These results show that the presence of indigenous organisms can greatly affect chronic endpoints in sediment toxicity tests. The obvious concern if the presence of indigenous organisms is not considered is that reduction in growth may be attributed to contaminants in sediments when the effects are in fact due to interactions between the test organism and indigenous animals. We consider these data provide a strong argument for the manipulation of sediments, especially freshwater sediments, to remove indigenous organisms before conducting chronic toxicity tests with benthic invertebrates.

Abstract

Sediment bioassays were conducted using three species of benthic invertebrate, *Chironomus riparius, Hyalella azteca* and *Hexagenia limbata* with various densities of the oligochaete worm *Tubifex tubifex*. It was shown that indigenous animals, simulated by the presence of *Tubifex tubifex*, did not affect survival of the test species ($P > 0.05$) but reduced growth in all three test species. At densities of *T. tubifex* equivalent to 20,000 m^{-2} the growth of, *C. riparius* was reduced by more than 90%, *H. azteca* by more than 60% and *H. limbata* by almost 50%. The densities of oligochaetes used are equivalent to those found in many contaminated sites. Therefore, it is concluded that the presence of indigenous organisms can confound the interpretation of bioassay results, based on chronic endpoints. It is recommended that removal of organisms be considered before toxicity tests are conducted with freshwater sediments from sites with large populations of benthic invertebrates.

Introduction

Sediment toxicity tests are increasingly being recommended and used in the determination and identification of sediment-associated contaminants (Long and Chapman, 1985; International Joint Commission, 1987, 1988; Giesy and Hoke, 1989). While the initial development of sediment bioassay methodologies emphasized acute tests (Burton, 1991) there has been recognition that chronic endpoints, such as growth and reproduction, are a more sensitive and discriminatory measurement of biological effects (Hoke *et al.*, 1990). Most protocols for conducting whole sediment toxicity tests recommend that manipulation of field-collected sediments .(*i.e*, sieving and mixing) be limited in order to maintain the chemical equilibria of any potential contaminants associated with these sediments (ASTM, 1992). Although indigenous species, such as leeches, have been shown to interfere with the results of sediment toxicity tests (Ingersoll and Nelson, 1990), suggestions for the removal of endemic species have been limited to hand-picking of large predators or pressure sieving of sediment through a 1-2 mm mesh. However, cocoons, eggs and young of many freshwater invertebrates, particularly chironomids and oligochaete worms can pass through a 1 or 2 mm mesh. While the presence of such indigenous organisms may not be apparent at the beginning of a chronic sediment toxicity test these organisms may well compete or interfere with the test species ability to aquire food or occupy space over the course of the exposure. Although ecological studies on intraspecific interactions have shown that as densities of aquatic invertebrates reared in containers increase, growth of individual organisms decreases (Fuller and Mackay, 1981; Webb and Merritt, 1987; Rosillon, 1988; Hanes and

Ciborowski, 1992; Day *et al.* , in press), the effects of interspecific interactions on endpoints in chronic sediment toxicity tests have not been investigated.

As part of a major programme to develop biological sediment guidelines for the Laurentian Great Lakes we have been testing reference sediments from sites with varying densities of oligochaete worms. We have observed that in sediments where oligochaetes were abundant that growth of the test species was reduced. In this paper, we investigate the potential effects of a common indigenous species, the oligochaete worm *Tubifex tubifex* (Müller, 1774), on the responses of three frequently used test species *Chironomus riparius* Meigen, *Hyalella azteca* (Saussure) and *Hexagenia limbata* (Serville) in sediment toxicity tests..

Methods

Cultures of *H. azteca, C. riparius* and *T. tubifex* are maintained at the National Water Research Institute under constant temperature (23°C±1°C) and light conditions (16L:8D) according to the methods outlined in Borgmann *et al.* (1989), Day *et al.* (1993) and Reynoldson *et al.* (1991), respectively. Nymphs of the mayfly, *H. limbata*, were obtained from eggs collected in July 1991 and stored in plastic bags at 8°C according to the procedure of Hanes and Ciborowski (1992). The substrate used for all experiments was collected from a reference ('clean') site in Severn Sound, Georgian Bay, Lake Huron. The physical characteristics of this sediment are 5.6% sand, 73.6% silt and 20.7% clay, the total organic content was 2%. The resident benthic fauna includes oligochaetes,

chironomids, ostracodes and nematodes (Reynoldson, unpublished data).

Tests with *C. riparius* were initiated with first instar larvae (72 h old) and were of 10 day duration. Each replicate consisted of fifteen larvae placed in a 250 ml beaker containing 60 mg (wet weight) of sediment and 150 ml of overlying water (carbon-filtered, dechlorinated and aerated City of Burlington tap water); pH 7.8 - 8.3, conductivity 439 - 578 μohms. cm^{-1}, hardness 119 - 137 $mg.l^{-1}$). Each beaker received 8 mg of Nutrafin® at the beginning of the test and then biweekly. At the conclusion of the test, the contents of the beaker were sieved through a 500μ sieve and the surviving animals counted. Survivors were then dried for 24 h at 60°C and weighed as a group. Test endpoints were survival (%) and growth, calculated as mg dry weight/individual after 10 days. Conditions of the tests conducted with *H. azteca* were similar to those of *C. riparius* with the exception that the initiation of the test was with juvenile animals 3 to 14 d old, and the duration of the test was 28 d. Tests with *H. limbata* were begun with 1½ - 2 month old nymphs, each weighing 5 to 10 mg (wet weight). Ten nymphs were added to each 1 L widemouth glass container, with 150 mg of sediment and 10 cm of overlying water. Animals in each replicate were fed with 500μl of a mixture of Brewer's yeast, Cerophyll® and Nutrafin® at the beginning of the test and then weekly. The duration of the test was 21 d after which all surviving animals were gently sieved from the sediment, counted and weighed as described above.

The effects of indigenous organisms on the survival and growth of the test species were

simulated by the addition of varying densities of both juvenile and adult tubificid worms (*Tubifex tubifex* : Oligochaeta, Tubificidae) to the individual replicate beakers. Five treatment densities were used equivalent to field densities of between 1,200 to 20,000 m^{-2}. These densities are typical of those found in various sites in the Laurentian Great Lakes (Barton, 1989). Controls containing no worms were also included for comparison (Table 1). Five replicates were used for each density tested for *C. riparius* and *H. azteca* and four replicates were used for *H. limbata*.

Data were analyzed using the SYSTAT® statistical software package. To determine whether oligochaete density was a potentially important effect one way ANOVA was used. If a significant difference ($P < 0.05$) was observed between the treatments a multiple comparison procedure was used to identify which treatments were significantly different from the control. Differences between treatments and control were tested for each species using Tukey's HSD multiple comparison procedure which is more conservative in identifying differences than other multiple comparison procedures, with less likelihood of making Type I errors.

Results

There was no statistical difference observed in the survivorship of any of the species between the control and treatments with oligochaetes at any density. Percent survival of *H. limbata* was above the acceptability criterion of 80% (Bedard *et al.*, 1992) in all treatments (Table 2). However, while there were no statistical differences between

control and treatment survival in *C. riparius* and *H. azteca*, there was a trend of declining survival with increasing oligochaete density in both species. The survival of *C. riparius* was below the acceptability criteria (ASTM, 1992) of 70% in the three higher densities of worms and *H. azteca* did not meet the 80% acceptability criteria (ASTM, 1992) in the two higher densities.

Growth of all three species showed significant ($P < 0.05$) between treatment differences in a one way ANOVA. Of the three species, *C. riparius* was most sensitive to the presence of oligochaetes, based on growth effects (Fig. 1a). At the highest worm densities growth was reduced by 92% (Table 3) and even at the lowest treatment density growth was significantly ($P < 0.001$) lower than the control showing the greatest incremental reduction in growth (Table 3). While the growth response appears to follow a logarithmic relationship ($r^2 = 0.98$), there may be two parts to the response, linear at low density and no effect at high worm densities.

Growth of *H. azteca* was also affected by increasing densities of *T. tubifex*. The growth response shown in Figure 1b suggests a two-step response with no effect occurring until a threshold worm density of between 10-20 individuals per beaker is achieved. This was confirmed using ANOVA and Tukey's HSD test. There was no significant difference in the growth response among the three lower densities ($P > 0.05$) nor among the three higher densities ($P > 0.05$).

Growth of *H. limbata* also decreased with increasing worm density (Fig. 1c, Table 3). The distribution of the data around the fitted line and the high correlation ($r^2 = 0.91$) suggests that in the density range used the response is directly density dependent.

Discussion

The presence of oligochaete worms in sediments used to conduct chronic toxicity tests has been shown to have a marginal effect on survival and a significant ($P < 0.05$) effect on growth of the test species. The pattern of the growth response varies depending on both the density of oligochaetes present and the test species. This suggests that the nature of the inter-specific reactions may be different.

Hexagenia shows the simplest growth response to increasing worm density and has a negative linear relationship in the density range used. Direct competition for food or interference in the mayflies burrowing or feeding activity due to the presence of the oligochaetes are the most likely explantions for the reduced growth.

Chironomus shows a logarithmic response to increasing worm density, however, the difference between this response and that of *Hexagenia* could simply be due to the range of worm densities over which the response is observed. The growth observed in *C. riparius* suggests that at the higher oligochaete densities the effect is density independent. *Chironomus riparius* was also the most sensitive of the test species to oligochaete density. The addition of five individual worms caused a 38% reduction in

growth (Table 3) and growth was reduced by 92% at the highest density. This compares with reductions of 48% and 67% for *Hexagenia* and *Hyalella* respectively at the highest oligochaete densities.

The response of *Hyalella* to increasing oligochaete density was different. There was no significant ($P > 0.05$) effect on growth with the addition of five and ten oligochaetes (Table 3), this contrasts with *C. riparius* whose growth was reduced almost 60% at the same density. However, at the next density (25 worms) there was a 50% reduction in growth of the amphipod. Further increases in worm density, to 75 worms per container (>20,000 m-2), had no further effect on growth ($P > 0.05$). This suggests a stepwise response with a threshold density between 10 and 25 worms. As *H. azteca* is an epibenthic species, we speculate that its interaction with *T. tubifex* does not result in competition for food or space. Rather that once a critical density of worms is present then *Hyalella* is either prevented from feeding or is required to expend more energy in an activity other than somatic growth. For example the burrowing and surficial disturbance by the oligochaetes may deter the amphipod from surface grazing or require it to spend more time in the water column.

Toxicity tests using benthic invertebrates are an essential component of any current assessment of potentially contaminated sediments. These results show that the presence of indigenous organisms can greatly affect chronic endpoints in sediment toxicity tests. The obvious concern if the presence of indigenous organisms is not considered is that

reduction in growth may be attributed to contaminants in sediments when the effects are in fact due to interactions between the test organism and indigenous animals (Burton *et al.* 1992).

Several laboratory studies have examined intraspecific interactions, but their are few studies on interspecific effects in sediment toxicity tests. Hanes and Ciborowski (1992) showed that increased density, in a density range equivalent to between 159 to 7950 larvae m^{-2}, was considerably more important than reduced food supply in reducing growth in *Hexagenia rigida*. In an examination of the effects of intraspecific density on survival and growth of *Nereis arenaceodentata*, a marine polychaete, Moore and Dillon (1992) showed little effect of density on survival over a six-week exposure. They did demonstrate that growth was significantly reduced ($P < 0.05$) after six weeks. In earlier experiments Reynoldson *et al.* (1991) described the effects of density on a reproduction endpoint in a bioassay with *Tubifex tubifex* and again showed that increased density reduced reproduction. Rasmussen (1985) manipulated densities of *C. riparius* in enclosures in a field study and found a highly significant negative correlation between density and growth in the range used (10,000 - 42,000 larvae m^{-2}) .

The use of *T. tubifex* to simulate the potential effect of indigenous organisms is considered realistic. In many contaminated sites *e.g.*, Hamilton Harbour, Toronto Harbour, the Detroit River, oligochaetes are often the only species found. In the Detroit River densities of oligochaetes greater than one million m^{-2} have been observed

(Thornley and Hamdy, 1984). In Hamilton Harbour in 1987 and 1988 we have recorded densities of oligochaetes ranging between 5,000 - 35,000 m^{-2}. The equivalent densities used in these experiments of 1,200 - 20,000 m^{-2} are certainly within the range found in sites with contaminated sediments.

We consider these data provide a strong argument for the manipulation of sediments, especially freshwater sediments, to remove indigenous organisms before conducting chronic toxicity tests with benthic invertebrates. We are currently considering an number of manipulation options such as freezing, sieving, heating and gamma irradiation (Day *et al.*, in press).

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Table 1. Treatment densities of *Tubifex tubifex* used in sediment toxicity tests with *C. riparius, H. azteca* and *H. limbata.*

C. riparius and H. azteca		*H. limbata*	
No. worms/beaker	Equivalent Density (no.m-2)	No. worms/beaker	Equivalent Density (no.m-2)
0 (control)	0	0 (control)	0
5	1460	10	1280
10	2920	25	3180
25	7310	50	6360
50	14620	100	12730
75	21920	150	19100

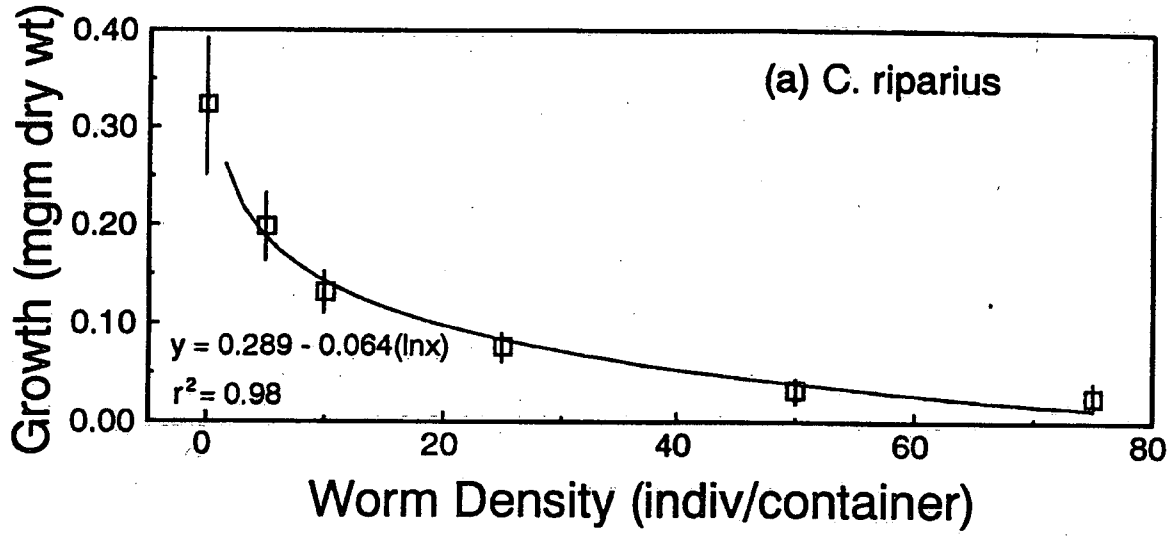
Table 2. Effect of *Tubifex tubifex* density on survival of three benthic invertebrate species in sediment toxicity tests.

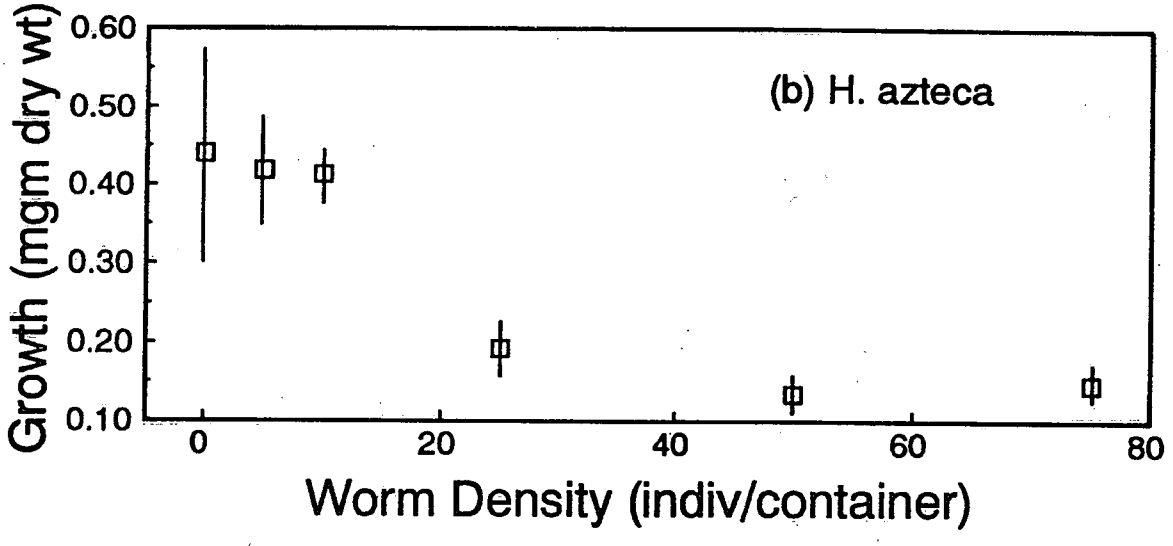
	C. riparius			*H. azteca*			*H. limbata*		
Density Expressed as No. m-2	No. individuals remaining x	(SD)	Survivorship (%)	No. individuals remaining x	(SD)	Survivorship (%)	No. individuals remaining x	(SD)	Survivorship (%)
0 (control)	12.8	(3.0)	85.3	13.6	(1.8)	89.3	9.8	(0.5)	97.5
1250-1500	11.8	(1.6)	78.8	12.2	(4.6)	81.4	10.0	(0.0)	100
3000	11.4	(1.1)	76.6	14.6	(0.5)	97.2	10.0	(0.0)	100
6000-7500	10.2	(2.2)	68.0	14.0	(0.8)	93.3	9.8	(0.5)	97.5
12500-15000	9.8	(3.3)	65.2	11.2	(2.8)	74.4	9.8	(0.5)	97.5
20000-22000	9.2	(2.4)	61.2	8.5	(3.3)	47.8	9.8	(0.5)	97.5

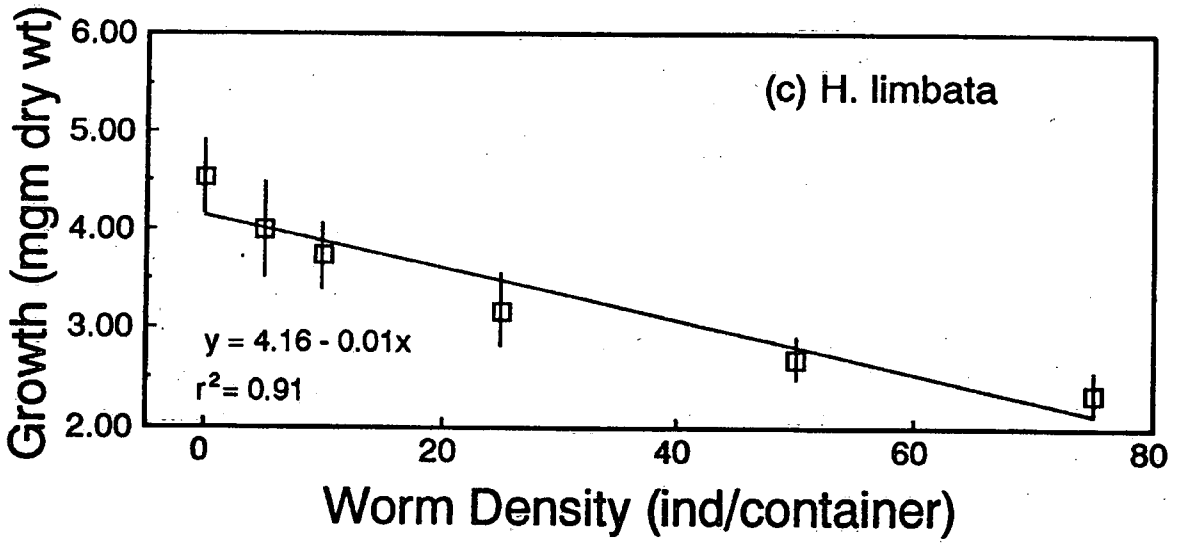
Table 3. Relative and incremental change in growth in three invertebrate species in reponse to density of the oligochaete worm Tubifex tubifex.

Oligochaete Density (No. m^{-2})	C. riparius % reduction		H. limbata % reduction		H. azteca % reduction	
	Total	Incremental	Total	Incremental	Total	Incremental
1250-1500	38.7*	38.7	11.8	11.8	4.9	4.9
3000	59.5*	20.8	17.4	5.6	6.0*	1.1
6000-7500	76.4*	16.9	30.3*	12.9	56.3*	50.3
12500-15000	90.0*	13.6	40.8*	10.5	69.5*	13.2
20000-22000	92.2*	2.2	48.3*	7.5	66.6*	-2.9

* Growth is significantly ($P < 0.05$) less than control


(a) C. riparius
Growth (mgm dry wt)
0.40
0.30
0.20
0.10
0.00
y = 0.289 - 0.064(lnx)
r² = 0.98
0
20
40
60
80
Worm Density (indiv/container)


(b) H. azteca
Growth (mgm dry wt)
0.60
0.50
0.40
0.30
0.20
0.10
0
20
40
60
80
Worm Density (indiv/container)


(c) H. limbata
Growth (mgm dry wt)
6.00
5.00
4.00
3.00
2.00
y = 4.16 - 0.01x
r² = 0.91
0
20
40
60
80
Worm Density (ind/container)

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