Environmental Toxicology and Chemistry, Vol. 18, No. 12, pp. 2921–2925, 1999
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Printed in the USA
0730-7268/99 $9.00 + .00

GROWTH AND FERTILITY OF *CAENORHABDITIS ELEGANS* (NEMATODA) IN UNPOLLUTED FRESHWATER SEDIMENTS: RESPONSE TO PARTICLE SIZE DISTRIBUTION AND ORGANIC CONTENT

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(*Received* 14 *September* 1998; *Accepted* 6 *May* 1999)

Abstract—The nematode *Caenorhabditis elegans* (Maupas) was exposed in a sediment bioassay to 26 different unpolluted freshwater sediments varying in particle size distribution (2.5–18% clay, 25.7–68.2% silt, 18.7–70.9% sand) and organic content (2.5–77.1%). We examined the variation of the test endpoints body length, eggs per worm, and percentage of gravid worms. *Caenorhabditis elegans* tolerated all investigated sediments, with at least 80% (total mean 96.6%) of the worms reaching the stage of reproductive adults. Variation in body length was small (total mean 1,235 ± 97.8 μm), but significant differences among the various sediments were found. We found a weak correlation of body length with particle size distribution, indicating that the nematodes grew better in coarser sediments. The number of eggs per worm showed relatively high variation among treatments (total mean 12.4 ± 4.8) and also within treatments (mean ± 5–95%). *C. elegans* is a suitable test organism for freshwater sediment bioassays, using body length and percentage of gravid worms as test endpoints.

Keywords—Sediment Particle size distribution Organic content *Caenorhabditis elegans* Nematoda

INTRODUCTION

In aquatic systems sediments can accumulate organic and inorganic pollutants, resulting in concentrations that exceed those in the water column by several orders of magnitude [1]. As a consequence, benthic organisms are exposed to especially high concentrations of pollutants. Thus, toxicity tests with different benthic organisms have been developed and have become a valuable tool for sediment hazard assessments [2–4]. Among bioassays with benthic organisms, tests using whole sediment as test matrix are a more realistic approach than tests using pore water or elutriates because they take into account all possible uptake routes for contaminants [5]. However, physicochemical properties of sediments, such as particle size distribution and organic content, can be confounding factors in whole-sediment bioassays [6,7]. Variations in the response of test organisms can occur for two reasons: different degrees of sediment contamination or natural variations of physical and chemical sediment properties that influence the test organisms. To ascertain whether variations in the response of test organisms are due to chemical contamination, the range of response of test organisms in uncontaminated sediments must be determined. This has already been done for a number of freshwater organisms that are established as test organisms for sediment bioassays (e.g., the amphipod *Hyalella azteca*, the midge *Chironomus tentans*, and the oligochaete *Lumbriculus variegatus* [7–10]) as well as for marine and estuarine benthic organisms [11–14].

In terms of both the number of individuals and the number of species present, nematodes represent one of the major groups in aquatic and terrestrial environments [15–18]. They are permanent members of the benthos, have no pelagial stage, and thus are unable to escape from bottom pollution effects. Free living nematodes have several features that make them excellent test organisms for determination of the presence of toxic contaminants in aquatic, marine, and terrestrial environments ([19–22]). Among these features are a life cycle that is strongly connected with the environment in which they are tested and the nearly ubiquitous distribution in nature. *Caenorhabditis elegans* (Maupas) lives mainly in the liquid phase of soils but occurs also in freshwater sediments [23,24], feeding unselectively on bacteria and other small particles [25]. Thus, *C. elegans* is an ecologically relevant organism for bioassays using whole sediment or soils as test substrate. In addition, the broad base of experimental and descriptive studies on the life cycle and genetics [26,27] makes *C. elegans* an ideal species to be used for bioassays. *C. elegans* occurs in two sexes: most frequently as hermaphrodites, which usually reproduce by self-fertilization, and rarely as males [27]. Within 3 d (20°C), *C. elegans* completes a whole life cycle [28,29], allowing the use of reproductive toxicity endpoints. Studies on the sublethal toxicity of cadmium on *C. elegans* in liquid medium have shown first effects on growth and fertility at 141 μg Cd/L [29], which is comparable to chronic cadmium lowest-observed-effect concentration (LOEC) values of other benthic test organisms, such as chironomids [30,31]. *C. elegans* has already been used in bioassays using soils and whole sediments [29,32–34], yet up to now no information on the sensitivity of *C. elegans* to natural sediment properties is available. Thus, the aim of this study was to investigate the tolerance of *C. elegans* to a number of different uncontaminated sediments. To quantify the tolerance of *C. elegans*, we used body length, eggs per worm, and percentage of gravid worms as endpoints for the toxicity test. Differences in sediment composition were quantified by measuring particle size distribution and organic content.

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MATERIALS AND METHODS

Sediment samples

Sediment samples were taken in April 1997 (sample from Lake Speichersee was taken in October 1996) from 12 lakes in southern Germany (48°N, 11°–12°30′W). Within each lake, samples were collected from different sites (1–4) to obtain sediments with a wide range of physicochemical properties, such as particle size distribution and organic content. The following lakes were chosen: Lake Starnberg (Sta 1–3), Lake Wörthsee (W 1–4), four lakes from a chain of lakes, Osterseen (O 1–4), Lake Klostersee (K 1–3), Lake Griessee (G 1–3), Lake Seeleitensee (S 1–2), Lake Bannsee (B 1–3), Lake Brunnsee (Br 1–2), and Lake Speichersee (Sp). All lakes, with the exception of Lake Speichersee, have no industrial impact and thus were regarded as unpolluted. In addition, most of the lakes are located in nature reserves. Lake Speichersee is a reservoir basin of the river Isar near the city of Munich, Germany. We took sediment samples from a reference site that had been shown to be uncontaminated [35].

The sediment samples (four subsamples per site) were collected with a gravity corer from the littoral zone (1–2 m water depth) of the lakes. To obtain defined amounts of sediment from all sites, the top 2 cm of each core were used. The four subsamples from each site were pooled. The part of the sediments destined for bioassay and for analysis of organic matter were stored at 4°C and used within 1 week. Samples used for analysis of particle size distribution were fixed with formalin (final concentration 4%) and, before use, sieved through a net with a mesh size of 0.63 mm to remove gravel, larger pieces of wood, and leaves. Less than 5% of the particles were held back by the 0.63-mm sieve. The size ranges for the various fractions were sand, 63 to 630 μm; silt, 4.5 to 63 μm; and clay, <4.5 μm. Particle size distribution was measured with a laser analyzer (Malvern SB.09, Malvern Instruments, Herrenberg, Germany). Organic content was determined as loss on ignition (LOI within 5 h at 550°C) according to German standard methods (DIN 38414-3) [36].

Bioassay

Caenorhabditis elegans var. Bristol, strain N2, was maintained in stocks of dauer larvae on NG agar [26] according to standard procedures [37,38]. Bioassays were carried out according to Traunspurger et al. [29] with few modifications. Briefly, sediments (0.5 g wet wt.) were transferred into 20-ml glass vials and then mixed with 0.5 ml of the test medium. The test medium consisted of a suspension of bacteria (*Escherichia coli,* approx. 10^{10} cells/ml), serving as a food supply, in M9 medium [26] with a pH of 7.2. At the start of the test, 10 juvenile worms of the first stage (270 ± 16 μm mean body length) were transferred to each test vial. Five replicates were set up for each treatment. The samples were incubated on a shaker at 20°C, and after 72 h the test was stopped by heat-killing the worms at approx. 50°C. The samples were mixed with 0.5 ml of an aqueous solution of rose bengal (0.5 g/L) and kept overnight at 4°C to stain the worms for easier recovery. Using a dissecting microscope, the worms were then transferred onto a slide, and body length and number of eggs inside worms were determined under a microscope (100× magnification). Worms having eggs inside the body were regarded as gravid. One-way analysis of variance (ANOVA) and Pearson linear correlation analysis [39] were carried out using SPSS® microcomputer software [40].

Table 1. Loss on ignition (mean ± standard deviation), percentage of clay, silt, and sand, and mean particle size of sediments from Lake Starnberg (Sta1–Sta3), Lake Wörthsee (W1–W4), Lakes Osterseen (O1–O4), Lake Klostersee (K1–K3), Lake Griessee (G1–G3), Lake Seeleitensee (S1–S2), Lake Bannsee (B1–B3), Lake Brunnsee (Br1–Br3), and Lake Speichersee (Sp)

Sediment	Loss on ignition (%)	%Clay	%Silt	%Sand	Mean particle size (μm)
Sta1	4.6 ± 0.9	3.4	25.7	70.9	157.8
Sta2	4.8 ± 0.1	13.1	68.2	18.7	24.7
Sta3	2.5 ± 0.1	12.5	38.1	49.4	60.8
W1	5.4 ± 0.2	3.5	41.1	55.4	72.3
W2	4.5 ± 0.2	3.1	37.6	59.3	81.3
W3	4.0 ± 0.3	4.8	36.2	59.0	83.9
W4	4.6 ± 0.8	4.5	38.5	57.0	78.7
O1	14.7 ± 2.1	6.7	53.3	40.0	45.3
O2	6.8 ± 0.1	6.8	57.6	35.6	43.2
O3	9.8 ± 0.2	3.9	37.6	58.5	78.5
O4	10.9 ± 0.1	3.1	32.6	64.3	97.6
K1	9.9 ± 0.5	2.6	30.2	67.2	102.6
K2	4.8 ± 0.5	2.9	28.1	69.0	117.7
K3	12.5 ± 0.2	3.1	30.0	66.9	107.6
G1	77.1 ± 0.9	3.5	40.3	56.2	74.6
G2	73.1 ± 1.1	2.5	32.9	64.6	89.9
G3	69.3 ± 2.3	3.2	36.7	60.1	84.0
S1	42.2 ± 4.3	4.6	48.1	47.3	64.8
S2	47.5 ± 1.4	2.9	36.8	60.3	82.1
B1	74.3 ± 2.8	4.1	42.6	53.3	70.3
B2	25.6 ± 5.7	5.8	49.8	44.4	51.8
B3	62.3 ± 1.8	4.2	46.1	49.7	61.9
Br1	24.2 ± 5.2	3.3	35.8	60.9	89.4
Br2	7.3 ± 0.3	3.7	45.2	51.1	64.2
Br3	7.2 ± 0.2	3.5	45.7	50.8	63.8
Sp	8.0	18.0	55.0	27.0	29.0

RESULTS AND DISCUSSION

The investigated sediments showed a wide range of organic content (2.5–77.1% LOI; Table 1) and mean particle size (24.7–157.8 μm; Table 1). Overall, sediments exhibited relatively low proportions of clay (2.5–18%; Table 1 and Fig. 1), whereas silt and sand were more abundant (25.7–68.2% and 18.7–70.9%, respectively; Table 1 and Fig. 1).

Sediment bioassays with *C. elegans* showed that the nematodes tolerated all investigated sediments. All test organisms survived, and 97% of the nematodes reached the stage of a

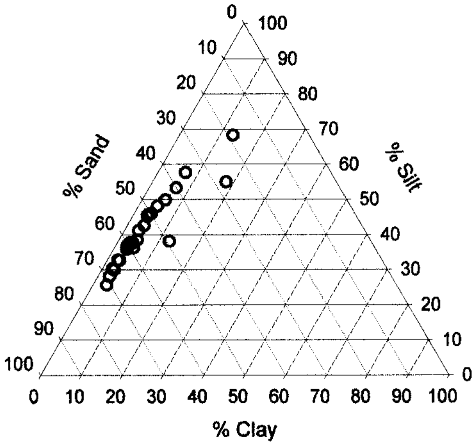

Fig. 1. Particle size distribution of 26 sediment samples from Lakes Starnberg, Wörthsee, Osterseen, Klostersee, Griessee, Seeleitensee, Bannsee, Brunnsee, and Speichersee; — = percentage silt, ---- = percentage clay, and ⋯⋯ = percentage sand.

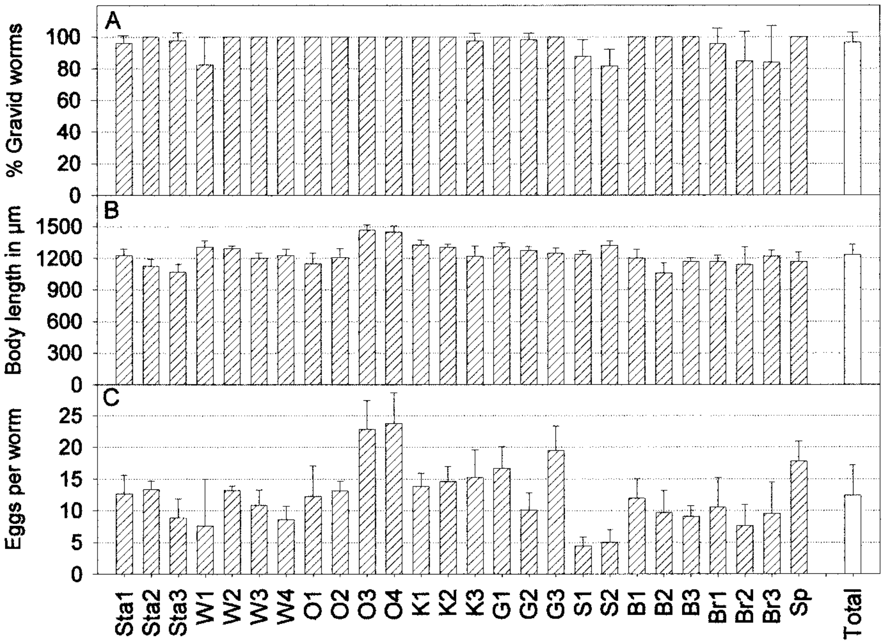

Fig. 2. Percentage of gravid worms (**A**), body length (**B**), and eggs per worm (**C**) (mean ± standard deviation) of *C. elegans* after 72-h exposure to various sediments taken from different lakes: Sta = Lake Starnberger See, W = Lake Wörthsee, O = Lake Osterseen, K = Lake Klostersee, G = Lake Griessee, S = Lake Seeleitensee, B = Lake Bannsee, Br = Lake Brunnsee, Sp = Lake Speichersee. Numbers represent different sites of the lakes; white bar = total mean ± standard deviation of sediment samples.

reproductive adult. The percentage of gravid worms was <90% in only 4 of 26 sediments and ≥80 % in all treatments (Fig. 2A). This indicates that sediments with organic contents and particle size distributions within the range investigated here should be suitable substrates for *C. elegans.* As the investigated sediments varied only slightly in the proportion of clay (2.5–18%), we cannot generalize these findings to all types of freshwater sediments. However, investigations with sediments of higher clay content indicate that *C. elegans* is tolerant up to 28% clay (S. Höss, unpublished data). The latter data were not included in the present study because we could not rule out contamination in those sediment samples. Acute bioassays with clean control sediment using the amphipod *Hyalella azteca* and the midge *Chironomus tentans* have been regarded as successful if survival was ≥80% [9,41]. Applying the limit of 80% to the chronic nematode bioassay, *C. elegans* seems to be a suitable test organism for freshwater sediments, within the range of the investigated characteristics.

Whereas the reproductive endpoint percentage of gravid worms was tolerant to a broad range of particle size distribution and organic content, it appeared to be relatively sensitive to sediment-associated contaminants. Recalculating the toxicity data of cadmium from a former study, percentage of gravid worms was found to be in the same range of sensitivity as the endpoints body length, eggs per worm, and offspring per worm (LOEC 142, 142, and 282 µg Cd/L, respectively; [29]), which is comparable to chronic cadmium LOEC values for chironomids (100–500 µg Cd/L; [30,31]) but less sensitive than the amphipod *Hyalella azteca* (LC50 0.6–85 µg Cd/L; [31,42–44]). In a sediment bioassay using *C. elegans,* percentage of gravid worms turned out to be the most sensitive toxicity endpoint [29].

Regarding body length of *C. elegans,* we found significant differences between the various treatments (Fig. 2B; one-way ANOVA, Kruskal–Wallis *H* test, $p < 0.001$). To test whether growth of the nematodes was influenced by particle size distribution or organic content, we plotted body length against the different sediment properties (Figs. 3 and 4). Body length was not correlated with organic content (Fig. 3; $r^2 < 0.01$, $p = 0.77$). Thus, the content of organic matter in sediments apparently does not influence the outcome of the nematode bioassay, which is consistent with results of an earlier study [34] that showed that *C. elegans* could even grow normally in sediments without organic matter. This may be related to the fact that the nematodes were fed throughout the bioassay and thus were not limited by food. In addition, studies with *H. azteca* or *C. tentans* showed that sufficient food supply prevented false-positive results [7].

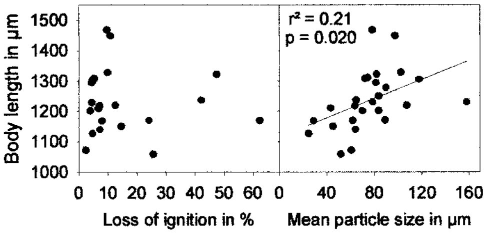

Fig. 3. Correlation of organic content and mean particle size with body length of *C. elegans*; r^2 = linear regression coefficient; $p < 0.05$ = significant.

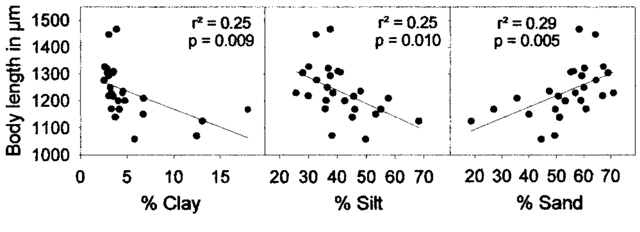

Fig. 4. Correlation of clay, silt, and sand content with body length of *C. elegans*; r^2 = linear regression coefficient; $p < 0.05$ = significant.

Mean particle size weakly correlated with body length ($r^2 = 0.21$, $p = 0.020$; Fig. 3). Plotting body length against the relative content of clay, silt, and sand gave slightly better correlations (Fig. 4). We found negative correlations for the clay fraction ($r^2 = 0.25$, $p = 0.009$) and for the silt fraction ($r^2 = 0.25$, $p = 0.010$) and a positive correlation for the sand fraction ($r^2 = 0.29$, $p = 0.005$). This means that *C. elegans* grew slightly better in coarse than in fine sediments, which was already indicated by an earlier study [34]. Size and species composition of nematode populations in sediments can be influenced by the sediment composition, either directly or indirectly via food availability [45]. For soils, nematode growth was found to be lower in fine-textured than in coarse-textured soil [46,47]. Other studies with benthic organisms, which are commonly used for sediment bioassays, showed that particle size distribution can be an important factor for the interpretation of a sediment bioassay. A study using the amphipod *Rhepoxynius abronius* showed that survival decreased with increasing content of fine sediment (silt–clay fraction) and that particle size turned out to be the best single predictor of amphipod survival in uncontaminated sediment [12]. In addition, the growth of the amphipod *Grandidierella japonica* was influenced by sediment particle size [48]. Although *Chironomus tentans* is relatively tolerant to sediment composition [8,10], Ankley et al. [7] pointed out that further attention should be given to the possible effect of particle size distribution on growth in the *C. tentans* assay. Also in this study, the negative correlations with clay content suggest that results from bioassays with *C. elegans* in sediments with high contents of clay should be interpreted with care, and, if possible, that reference sediments with similar particle size distribution should be used. However, an important result of this study was that, despite the wide range of particle size distribution and organic content, body length of *C. elegans* showed only a small variation between the different treatments (total mean 1,238 ± 99 μm; Fig. 2B). This means that growth is a useful test endpoint, which was already shown in previous studies using *C. elegans* as test organisms [29,34,49].

Eggs per worm showed a relatively high variation among the different treatments (total mean 12.4 ± 4.8; Fig. 2C) and correlated neither with particle size distribution (correlation with mean particle size: $r^2 = 0.02$, $p > 0.05$) nor with organic content ($r^2 < 0.01$, $p > 0.05$). This parameter had a high variation within the treatments (5–98% standard deviation). In comparison, Traunspurger et al. [29] found a standard deviation of 40% for eggs per worm in the control sediment, although this endpoint was as sensitive to cadmium as was body length and percentage of gravid worms. The high variation of eggs per worm may be caused by difficulties in determining the exact number of eggs inside the body of the worms, especially if high numbers of eggs are present. Although the worms are transparent, eggs are sometimes hidden behind other eggs, so that the number is underestimated. A high variation among the replicates within a treatment generally weakens the significance of a toxicity endpoint.

According to the results of this study, we recommend using percentage of gravid worms and body length as toxicity endpoints. With respect to these two parameters, *C. elegans* has been shown to be relatively tolerant also to water-quality criteria, such as ammonia and water hardness. Body length and percentage of gravid worms were not influenced by total ammonia concentrations up to 567 mg/L NH_4-N (Höss et al., unpublished data). In addition, water hardness in the range from 0.5 to 2.5 mmol Ca+Mg/L was tolerated by *C. elegans* (S. Höss et al., unpublished data). In a study testing the pH tolerance of *C. elegans*, the worms were able to survive from pH 3.1 up to 11.9 [50].

CONCLUSIONS

Caenorhabditis elegans tolerates sediments with a wide range of particle size distribution and organic content and thus is a suitable test organism for the ecotoxicological assessment of sediments. In all the 26 sediments we studied, at least 80% of the worms reached the stage of a reproductive adult. The sublethal test endpoint body length showed only a small variation between the different sediment treatments and was weakly correlated with particle size. It has to be considered in sediment assessments using *C. elegans* that the nematodes grow slightly better in coarser sediments. As we found large variations of eggs per worm within the treatments, we doubt that this parameter is a valuable test endpoint for sediment hazard assessments. However, the ecologically relevant parameters of body length and percentage of gravid worms were found to be suitable endpoints for the sediment bioassay with *C. elegans.*

Acknowledgement—We want to thank Sarah Gräslund and Melanie Kolz for technical assistance. We are also grateful to two anonymous reviewers as well as to Matthias Höss and Sebastian Diehl for critically reading the manuscript. We thank the *Caenorhabditis* Genetic Center (Theresa Stiernagle) for providing the nematodes. This study was supported by the German Research Council Deutsche Forschungsgemeinschaft, Schwerpunkt ROSIG, grants STE 673/1 and 673/2, and by the Bundesanstalt für Gewässerkunde.

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