Bull. Environ. Contam. Toxicol. (2005) 75:617–622

DOI: 10.1007/s00128-005-0797-6

Bulletin of Environmental Contamination and Toxicology

Preparatory Work to Propose Water-Only Tests with the Amphipod *Hyalella azteca:* Comparison with Sediment Toxicity Tests

A. R. R. Péry,[1] S. Dargelos,[1] H. Quéau,[1] J. Garric[1]

[1] Laboratoire d'Écotoxicologie, Cemagref, 3b quai Chauveau, 69009 Lyon, France

Received: 30 May 2005/Accepted: 15 August 2005

Hyalella azteca growth and survival tests have now been performed for more than ten years. The tests have low variance and acceptable interlaboratory differences (Burton et al. 1996). These amphipods are sensitive not only to overlying waters but also to whole sediment (Ingersoll et al. 2000). It has been shown that sediment organic matter content is a potential confounding factor in sediment toxicity tests, for the amphipods can obtain additional food from the sediment (Orr et al. 2004). This implies that, in the laboratory tests, sediment is a significant source of feeding.

However, the use of *Hyalella azteca* for sediment toxicity assessment has been recently criticized for ecological reasons (Wang et al. 2004). In natural habitats, no food is taken from the sediment, and other covers than sediments are available to protect amphipods from light. In contrast, in the laboratory, amphipods are forced to be in contact with the sediments to find shelter and feeding. This means that *Hyalella azteca* is probably more appropriate for testing surface water than sediments.

Here we aim to show supporting evidence for this statement and to propose a protocol for surface water toxicity tests with *Hyalella azteca*. We first plan to assess the importance of providing a shelter to the amphipods for water only experiments and propose a protocol to prepare shelters for water-only tests. We then perform 104d-experiments with growth, survival and reproduction measurements for six different feeding conditions to determine optimal feeding for these tests. We finally compare the growth and survival pattern for amphipods using our protocol and for amphipods exposed to natural clean sediments with comparable feeding to assess the importance of sediment as a shelter and feeding source.

MATERIALS AND METHODS

Hyalella azteca organisms came from our laboratory culture. The test beakers containing no sediment were filled with 0.4 L water (taken from an uncontaminated nearby spring with pH 7.7, conductivity 400µS/cm, only traces of chemicals), three days before the beginning of the tests. A shelter was

Correspondence to: A. R. R. Péry

provided for the amphipods through the use of 4cm² of 200µm nylon sieve put down at the bottom of the beakers. The test beakers containing sediment were filled with 0.3 L of water and 0.1 L of sediment. During the tests, the beakers were set in a water bath at 21°C with a 16:8h light:dark photoperiod. Test water was gently aerated. Specific conductivity, temperature, pH, dissolved oxygen, nitrates and ammonium concentrations were measured daily. Water was renewed every week. Newly produced amphipods were isolated from the culture and exposed one week after as in Orr et al. (2004). At the beginning of tests, each beaker contained 10 organisms. During the tests growth was monitored through length measurements on the dorsal side from the base of the first antenna to the end of the next to last segment. Using an image analysis method (Sigma Scan Pro 5), we found it easier than ending measurements at the third uropod, as proposed by Orr et al. (2004).

In the first experiment, we tested the importance of the shelter and the necessity of preparation. This preparation consisted in leaving the sieves for 14 days with food (0.1g per sieve) and aeration to ensure bacterial development. Three conditions were compared, with seven beakers per condition : no sieve, unprepared sieve, prepared sieve. Survival and growth measurements were performed at day 14 and 28.

In the second experiment, we tested the effects of food on the survival, growth and reproduction of the amphipods. Six feeding levels were tested : 0.04, 0.06, 0.1, 0.2, 0.3 and 0.4 mg Tetramin® fish food (Tetrawerke, Melle, Germany) per individual per working day, with five replicates per feeding condition at the beginning of the test. Survival and growth were monitored each week, on Friday, during the first 28 days, then every two Fridays. Reproduction was monitored each Friday. The test lasted 140 days. To avoid effects on density due to mortality, the number of beakers per feeding level was adapted to the total number of survivors after each measurement day, so that density per beaker was always between 8 and 11 individuals.

In the third experiment, we compared organisms exposed, with feeding levels determined in the previous experiments, to either a nylon sieve or one of two clean natural sediments, with 5 replicates per condition. All the selected sediments came from unpolluted sites regularly analysed by our institute (Cemagref, Lyon, France) for contaminants to confirm their potential use as reference sediment. We collected them with a one-litre grab sampler, the first at Port-Galland (France), in a tributary of the river Ain and the second in Beaujeu (France), near the source of the river Ardières. Sediments were 2 mm sieved and homogenised before use. Particle size distribution as well as percentage of dry matter, loss on ignition, nitrogen and organic content is well known and currently monitored in our institute using standard methods of measurement (Péry et al. 2004). Sediment from Port-Galland is fine (93 % below 50 µm) and contains a small amount of organic carbon (about 3% with a C/N ratio of 8). Sediment from Beaujeu is a little more coarse (70 % below 50 µm, and 28 % between 50 and 200 µm) and contains

high amount of organic carbon (about 9% with a C/N ratio of 10). Survival and growth measurements were performed at day 14 and 28.

Mean length and survival values obtained during the tests were compared, day by day, using Student-t-tests with the mean values of each replicate. Mean reproduction values were compared using Student-t-tests with the mean values of each day.

RESULTS AND DISCUSSION

During all the experiments, temperature was constant (21+/-0.5°C) as was pH (between 7.9 and 8.3). Specific conductivity was between 300 and 450 µS/cm, and the percentage of dissolved oxygen was always above 80 %. Nitrate and ammonia levels were always below 2 mg/L, except for feeding level 0.4 mg/individual/day, for which values above 10 mg/L were observed.

In the first experiment, no growth was observed for beakers with no or unprepared sieves (less than 0.2 mm increase after 28 days) and survival was extremely low (mean values 13 and 7 % respectively, for unprepared and no sieve after 28 days of exposure). On the contrary length increase with prepared sieve was 1+/-0.15 mm after 14 days and 2.7+/-0.37 mm after 28 days. We consequently conclude that sieves not only act as shelters to protect from light, but also as a source of food, at least for young amphipods.

Figure 1 presents the results of the growth test. No data are presented for feeding level 0.4 mg/individual/day. Indeed, no organism had survived up to 28 days. This could be due to the high nitrate and ammonia levels (Ankley et al. 1995). For the other feeding conditions, survival always remained above 55 % during the whole experiment. Significant differences ($p<0.05$) between feeding conditions only appear at day 14. Conditions 0.1, 0.2 and 0.3 mg/individual/day provides no length difference during the whole test with common ultimate length of 5.6 mm. On the contrary from day 14 till the end of the test, lengths for feeding conditions 0.04 and 0.06 mg/individual/day are significantly lower than for other conditions. After day 70, these two conditions also lead to significant differences between each other leading to ultimate lengths of respectively 4.3 and 5.1 mm.

The results for reproduction are presented in Table 1. We present the mean number of newly born per individual (the number of individuals being the mean number of individuals during the experiment). The values obtained for feeding condition 0.1 mg/individual/day are consistent with data from other authors (Othman and Pascoe 2001) who found that the number of neonates produced per female ranged from 3-17 (mean 9) in a laboratory culture. Surprisingly, the reproduction for levels 0.2 and 0.3 mg/individual/day is much less than what is observed for 0.1 mg/individual/day. Over feeding might be responsible for some deterioration of the water quality.

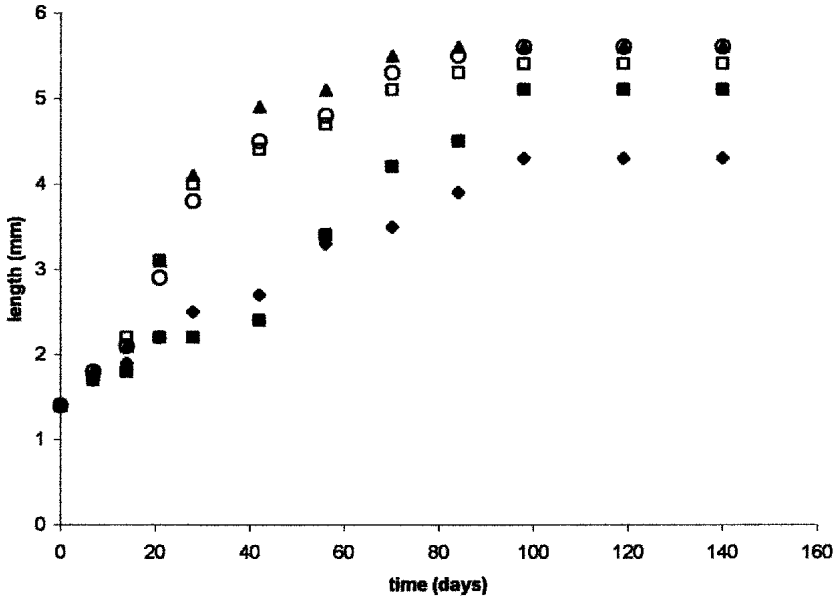

Figure 1. Growth pattern for Hyalella azteca exposed to different feeding levels (black squares : 0.04 mg/individual/day ; diamonds: 0.06 mg/individual/day; triangles: 0.1 mg/individual/day; white squares: 0.2 mg/individual/day; white circles: 0.3 mg/individual/day).

Table 1. Mean reproduction per individual as a function of feeding.

Feeding level (mg/individual/day)	Mean reproduction per individual
0.3	8.2
0.2	8.9
0.1	21.6
0.06	9.8
0.04	7.4

Figure 2 presents the results obtained for the growth comparisons between natural sediments and sieve only in *ad libitum* conditions (we chose 0.1 mg/individual/day according to the previous experiment). There is no significant difference ($p>0.5$) between sediment conditions at day 14 and 28. This suggests that prepared sieves are sufficient to ensure correct growth for *Hyalella azteca* and that sediment organic matter only benefits these amphipods in cases of feeding limitation. We obtained similar observations for the midge *Chironomus riparius* (Péry et al. 2004).

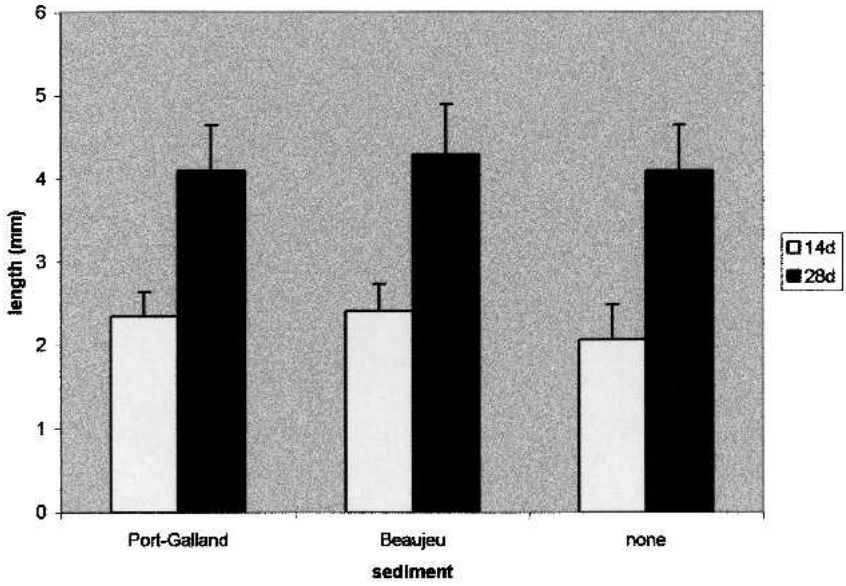

Figure 2. Length growth as a function of time (14d and 28d) and sediment with feeding *ad libitum* (0.1 mg/individual/working day).

To conclude, when performing water-only tests in semi-static conditions with the amphipod *Hyalella azteca*, it is necessary to use sieves prepared as described in this paper. Feeding condition 0.1 mg/individual/working day appeared to be relevant to ensure optimal growth and reproduction. Finally, it seems that sediments do not provide extra benefit compared to prepared sieves, provided there is sufficient cover and some bacterial development.

Acknowledgments. This study is part of the ERAPharm Project with financial support granted by the European Commission (Contract n° 511135). We also thank Gavin Eaton for its contribution to the writing of the manuscript.

REFERENCES

Ankley, GT, Schubauer-Berigan MK, Monson PD (1995) Influence of pH and hardness on toxicity of ammonia to the amphipod *Hyalella azteca*. Can J Fish Aquat Sci 52:2078-2083

Burton GAJr, 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

Ingersoll CG, Ivey CD, Brunson EL, Hardesty DK, Kemble NE (2000) Evaluation of toxicity: whole sediment versus overlying water exposures with amphipod *Hyalella azteca*. Environ Toxicol Chem 19:2906-2910

Othman MS, Pascoe D (2001) Growth, develoment and reproduction of *Hyalella azteca* (Saussure, 1858) in laboratory culture. Crustaceana 74:171-181

Orr TB, Meister SM, Halbrook RS (2004) Density and sediment organic matter content as potential confounding factors in sediment toxicity tests with *Hyalella azteca*. Bull Environ Contam Toxicol 73:371-378

Péry ARR, Sulmon V, Mons R, Flammarion P, Lagadic L, Garric J (2004) A model to understand the confounding effects of natural sediments in toxicity tests with *Chironomus riparius*. Environ Toxicol Chem 22 :2476-2481

Wang FY, Goulet RR, Chapman PM (2004) Testing sediment biological effects with the freshwater amphipod *Hyalella azteca*: the gap between laboratory and nature. Chemosphere 57:1713-1724