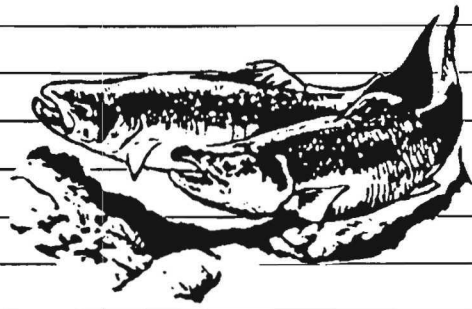


Research

**Information bulletin**

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## Determining the Age and Size of *Hyalella azteca* for Toxicity Testing

Toxicity tests on sediment and sediment pore water are used to assess the quality of aquatic habitats; standard procedures for these tests are presently being developed for certain species. These standards stipulate that animals of known age (e.g., 7–14-day-old *Hyalella azteca*) should be used to reduce variability and increase comparability within and among tests. The variability of concern is generally differences in sensitivity with age and size that some organisms show for some contaminants. Uniform age and size of the test organisms are also particularly important when growth is the test endpoint. Depending upon the culture methods or source of the animals, however, determination of age or size of the test animals may be difficult or time-consuming.

### Animals for Testing Are Sieved From Culture

*Hyalella azteca* has become one of the standard organisms for use in laboratory toxicity testing of freshwater sediments and sediment pore water; these amphipods are easily cultured in the laboratory, sensitive to most contaminants, and are

an all-purpose surrogate representing benthic invertebrates. We culture *H. azteca* in recirculating undergravel filter systems composed of two 37-L aquaria positioned above a third aquarium that contains the undergravel filter. Water pumped from the aquarium with the undergravel filter to the two overlying culture aquaria returns by gravity flow. The animals in culture are fed TetraFin every 2–3 days as needed. For testing, animals of the appropriate size are obtained by sieving them from our culture system. Animals passing through a U.S. Standard #35 sieve (500  $\mu\text{m}$ ) but retained on a #45 sieve (355  $\mu\text{m}$ ) are used for testing. To determine the age of sieved animals used in toxicity testing, the age–size relation for *H. azteca* was determined.

### Age and Size of Animals Were Determined

The size and age of the amphipods were determined by measuring lengths of known-age animals daily for 30 days. At the start of the test, known-age animals were obtained by holding adult *H. azteca* on a partly submerged #45 sieve

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(355  $\mu\text{m}$ ) and retrieving the juveniles less than 24 h old that had been released from the female brood pouch and passed through the sieve. Nine-hundred juveniles less than 24 h old were isolated and transferred into a 37-L aquarium connected to a recirculating undergravel biological filter and cultured for 30 days. The animals were fed 0.1 g TetraFin every 2–3 days during the test and temperature was maintained at 23°C. Twenty animals were removed from the aquarium each day for length measurements. Using a section of clear vinyl tubing that had been calibrated with a stage micrometer, length was determined by measuring the distance from the base of the antennae to the telson of an amphipod image that was projected onto a screen using a slide microprojector (Ken-A-Vision Model X1000-1, Ken-A-Vision Manufacturing Co., Raytown, Missouri).

### Sieved Animals Average Six Days Old

The length ( $\pm\text{SD}$ ) of animals at birth were 0.96 mm ( $\pm 0.03$ ) and increased to 4.64 mm ( $\pm 0.08$ ) for males and 4.37 mm ( $\pm 0.16$ ) for females through the 30-day period (Fig. 1). The stair-step configuration of the curve, which becomes less obvious as the animals get older, delineates the various instars that the animals passed through in their development. Differentiation between males and females was possible after the animals were 17 days old by the presence of the enlarged second gnathopod of the males. After 21 days, males are significantly longer than females. As judged from length, the age of sieved animals from mixed-age cultures averaged 6 days ( $\text{SD} = 1$ ), with a range of 3–9 days (Fig. 2). To eliminate any animals that may have been injured during the sieving process, sieved animals are held for 3 days before testing. Therefore, the age of animals at the start of a test would average 9 days, well within the range of 7–14 days required for the test. The variability in

length of the sieved animals ( $\text{SD} = 0.09$ ) was slightly less than the variability in length of the 7–14-day-old amphipods ( $\text{SD} = 0.10\text{--}0.24$ ) isolated at birth.

Amphipods sieved from culture as described would be about 19 days old at the end of a 10-day test. Nineteen-day-old animals averaged 3.40 mm ( $\text{SD} = 0.24$ ) long under culture conditions. However, length of animals isolated from the culture and held for 10 days under simulated-test conditions in culture water (no sediment, netting for substrate, static renewal with 95% replacement of the water every 3 days, 0.05 g TetraFin/L test solution added after water renewal) averaged only 2.60 mm ( $\text{SD} = 0.13$ ; Fig. 1). These differences in size suggest that animals isolated from culture and subjected to simulated test conditions may not grow as fast or as large as those in culture. The reduced growth was attributed to the restricted diet stipulated by the testing method; the amount of food is minimized to reduce the effects on potential toxicity.

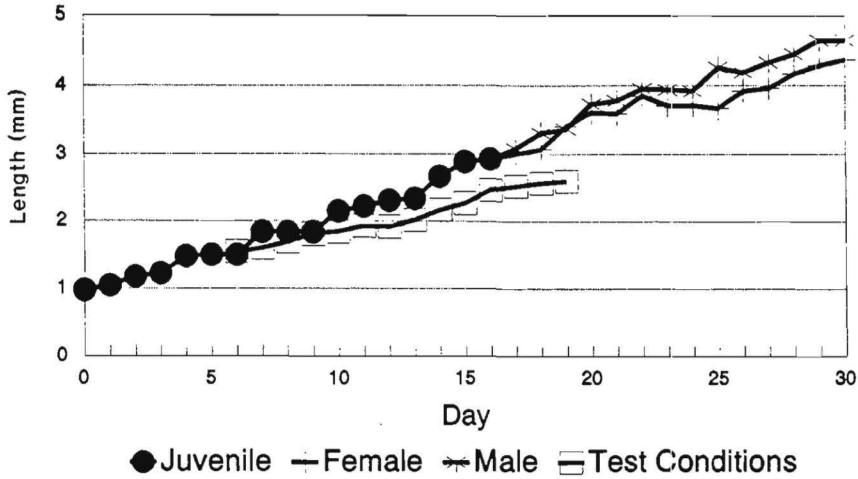
*Hyalella azteca* from our culture system that passed through a #35 sieve and were retained on a #45 sieve and held for 3 days before testing were within the 7–14-day range required for sediment testing. This demonstrates that sieving animals from culture is a viable means of obtaining known-age animals within a specific age and size range required for testing.

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# Hyalella azteca

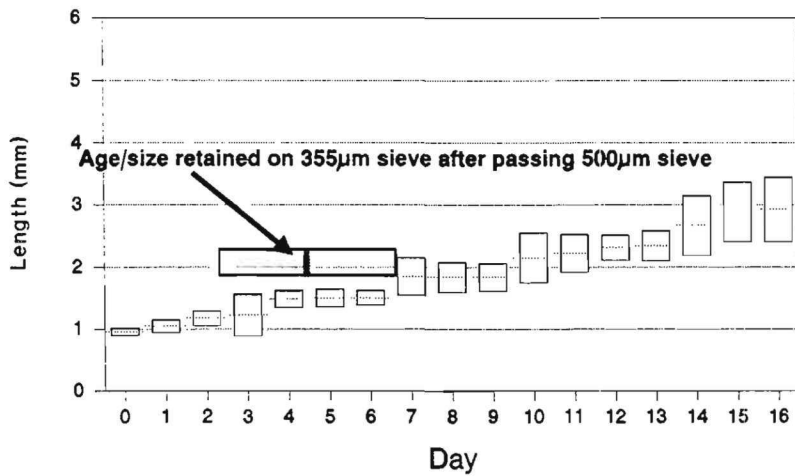
## Total Length



**Fig. 1.** Age and length of *Hyalella azteca* through 30 days, and comparison of age and length with animals sieved from culture and held under test conditions for 12 days.

# Hyalella azteca - Age/Size

## Sieved animals



Mean (+/- 2SD)

**Fig. 2.** Comparison of age and size of known age *Hyalella azteca* and those animals obtained by sieve from a culture system.