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Developing Standardized Guidance for Conducting Toxicity Tests With Glochidia of Freshwater Mussels

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Abstract

A joint research project is ongoing to develop standardized guidance for conducting toxicity tests with various life stages of freshwater mussels. Multiple tests were conducted to assess the survival times of glochidia of 13 mussel species. Copper, ammonia, or chlorine toxicity tests with glochidia also were conducted to examine the influences of exposure time, test chambers, and ages of glochidia. No differences were found in the survival time of glochidia held in static or flow-through conditions. Over 90% survival of glochidia was observed for ≥ 2 d with 62% of the species tested and for ≥ 1 d with 77% of the species tested. EC50s of the three chemicals decreased over the exposure periods of 6 to 48 h. Copper concentrations declined substantially in polystyrene plates but not in glass dishes during 48-h exposure periods. Similar EC50s were found when using 2- or 24-h-old glochidia or when using glochidia sampled over time during the spawning season. These results indicate that the toxicity tests with glochidia could be conducted for 48 h with a control survival of over 90% although shorter or longer tests might be appropriate for a particular species depending on the life history characteristics of the species and glochidia survival time.

Background

70% of freshwater mussel species in USA are considered as endangered, threatened, or of special concern (Figure 1). Glochidia, a parasitic larval stage of the complex life cycle of freshwater mussels (Figure 2), could be sensitive to many chemicals compared to other test organisms (Keller and Ruessler 1997; Augspurger et al. 2003). However, concerns have been expressed about the lack of standardization of the approaches and therefore the use of toxicity data generated with glochidia (Charles Stephan, USEPA, Duluth, MN, personal communication). The present study was performed to evaluate the methods for conducting toxicity tests with glochidia of freshwater mussels.

Study Questions

- How long should a toxicity test with glochidia be conducted?
- What criteria should be used to judge acceptability of tests with glochidia?
- Is there any different result when using various test chambers?
- Is there any different result when using glochidia of different ages or when testing glochidia sampled over time from the same females during a spawning season?

Materials and Methods

Survival tests:

- Glochidia collection: Flushed from the gills of a female (Photo 1).
- Test water: ASTM reconstituted water, hardness 170 mg/L as $CaCO_3$, 20°C.
- Glochidia viability: Confirmed with about 300 glochidia using saturated NaCl solution; tests started if viability >90%.
- Test chambers: 24-well polystyrene tissue-culture plates, 200-ml crystallizing dishes, or 250-ml beakers in an intermittent flow diluter system (Photo 2 and 3).
- Number of glochidia per replicate: 20 to 30 in a well, 1000 to >5000 in a dish or beaker.
- Number of replicates: 3 wells or 3 subsamples of about 100 individuals from 3 dishes or beakers.
- Water addition: every other day for wells and dishes; 7 times/d for beakers in the diluter system.
- Survival duration: percentage survival (viability) determined daily; survival indicated by glochidia valve closure upon addition of NaCl solution.

Toxicity tests:

- Test chemicals: Copper, ammonia, chlorine
- Test type: Static (copper and ammonia tests) or flow-through (chlorine test)
- Test duration: 6, 24, 48, or 72 h
- Temperature: 20°C
- Photoperiod: 16L:8D
- Test chamber size: 24-well plate, 200-mL dish, or 300mL beaker
- Test solution volume: 4 mL in well, 100 ml in dish or beaker
- Renewal of solution: None for static, every 15 min for flow-through tests

- Age of test organism: <2-h old (or depending on the study objective)
- Number of organisms/chamber: 20 to 30/well, 1000 to 2000/dish or beaker
- Number of replicates/concentration: 3 wells or 3 subsamples of about 100 individuals from 3 dishes or beakers
- Feeding: None
- Dilution water: ASTM Reconstituted water (170 mg/L as $CaCO_3$, pH 8.3)
- Dilution factor: 0.5
- Endpoint: Survival based on glochidia valve closure with NaCl (EC50 calculated based on nominal concentration; TOXSTAT 3.5, West, Inc. 1996)
- Test acceptability: >90% survival in control

Results and Discussion

Survival tests

- Survival times of glochidia of 13 species ranged from <2 to >10 d (Figure 3); over 90% survival of glochidia was observed for • 2 d with 62% of the species tested and for • 1 d with 77% of the species tested.
- There was no substantial difference in the survival of glochidia of fatmucket held in various test chambers under static-renewal or flow-through conditions during the first three days (Figure 4).
- These results suggest that the toxicity tests with glochidia of most species could be conducted for 48 h with a control survival of over 90% using various test chambers, although shorter test periods might be needed for some species to maintain a control survival of >90%.

Toxicity tests

- EC50s of the three chemicals for glochidia of two mussel species typically decreased over the exposure periods (Figure 5), suggesting that a 48-h test should be considered if glochidia are able to survive for >2 d.
- Similar EC50s were generally observed when using glochidia isolated from the same females over time during the spawning season (Figure 5), indicating that mature lochidia of a long-term brooding species could be held in female mussels for a long time before the start of a toxicity test.
- 48-h EC50s for chlorine were above the USEPA acute water quality criterion (WQC), whereas 48-h EC50s for copper and ammonia were typically at or below the WQC, suggesting that the acute WQC adequately protect glochidia from chlorine exposure but not from copper or ammonia exposure.
- Copper EC50s were consistently lower for glochidia held in glass dishes compared to those in polystyrene plates (Figure 6). This was consistent with the measured copper concentrations, which decreased during 48-h exposures in plates but did not change in dishes (Figure 7). The decrease of copper concentration in plates presumably resulted from adsorption losses of copper on the polystyrene surface.
- Copper EC50s were similar when using 2- or 24-h-old glochidia of fatmucket (Figure 8), indicating that sensitivity of glochidia held for 24 h after removal from a female was similar to newly-released glochidia.

Conclusions

- 48 h is a reasonable time period to conduct toxicity tests with glochidia of most species evaluated, although shorter or longer tests might be needed for a particular species depending on glochidia survival time and the life history characteristics of the species.
- >90% control survival could be considered as an acceptability criterion for 24- or 48-h toxicity tests conducted with glochidia.
- Using large glass dishes for toxicity tests has more volume of water available for conducting water quality and chemical analyses and for subsamples to estimate toxicity, and might also maintain more stable chemical concentration during 48-h exposure periods than using polystyrene multi-well plates.
- 24 h-old glochidia isolated from a long-term brooding species could be used for testing, indicating the possibility of overnight shipping of glochidia for testing.
- Mature glochidia of a long-term brooding species could be held in a female mussel for months before isolating them for testing.

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