Culturing and Testing Early Life History Stage Larvae of *Asellus aquaticus, Crangonyx pseudogracilis* and *Lymnaea stagnalis*

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1. INTRODUCTION

- Lower-tier toxicity testing provides an efficient means of predicting the effects of chemicals in the environment. In standard tests such as *Daphnia magna* and *Chironomus riparius* (OECD 202 and OECD 235) age specific organisms are routinely used.
- Cambridge Environmental Assessments (CEA) have developed culturing methods to make age specific testing of non-standard freshwater invertebrates possible.
- Here, we present our experiences of collecting, culturing and testing early life history stage *Asellus aquaticus, Crangonyx pseudogracilis* and *Lymnaea stagnalis* in laboratory conditions.

OBJECTIVE
To produce age-standardised juveniles for non-standard acute invertebrate testing

Species	Collection	Feeding	Juvenile isolation
***Asellus aquaticus* (Water Hoglouse)**	Copulating pairs from CEA mesocosm facility	Alder leaves and elodea	Twice weekly into cultures of less than 28 days old
***Crangonyx pseudogracilis* (Freshwater shrimp)**	Copulating pairs/ gravid females from CEA mesocosm facility	Alder leaves and elodea	Twice weekly into cultures of less than 28 days old
***Lymnaea stagnalis* (great pond snail)**	Adults collected from CEA mesocosm facility	Cucumber and fish food	Eggs were transferred to a separate hatching culture, once hatched they were transferred to juvenile cultures (<21 days old)

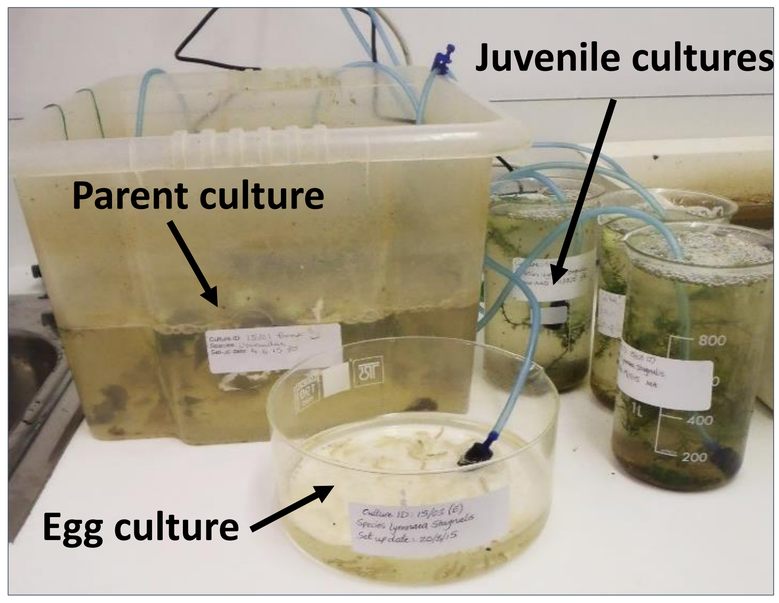

FIGURE 1. *Lymnaea stagnalis* laboratory cultures separated into adults, eggs and juveniles.

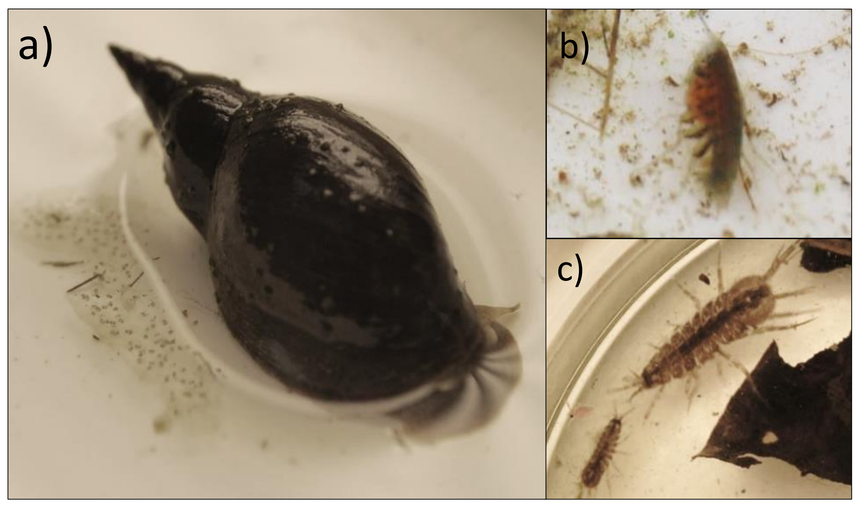

FIGURE 2. a) *Lymnaea stagnalis* b) *Crangonyx pseudogracilis* c) *Asellus aquaticus*

3. KEY FINDINGS

- All three species were successfully cultured and bred to produce age specific juveniles for acute testing
- Laboratory cultures were robust and rapidly produced many offspring, however, removing juveniles from parent culture can be time consuming
- Using positively identified adult *Lymnaea* to produce eggs was more preferable to egg collection from the wild, as juvenile snails are difficult to accurately identify
- Established adult cultures of *Lymnaea* were low maintenance with low adult mortality; however abundant vegetation was required to induce egg laying
- During acute testing, juvenile *Lymnaea* were too small to detect movement in the designated age range therefore immobility had to be determined using a method of re-attachment.
- Using these methods robust endpoints (NOEC and ECx values) were derived for all three species

4. CONCLUSIONS AND RECOMENDATIONS

- All three species were successfully collected cultured and bred to age specific requirements
- To reduce variability the recommended age of Asellus and Crangonyx to be used in tests is a maximum of 14 days
- Further refinement of methods is required to improve the efficiency of the collection of juveniles from parent cultures
- For *Lymnaea stagnalis,* as mobility was difficult to confirm, older and larger organisms could be used and other non lethal parameters such as egg production could be measured

REFERENCES

- OECD Guidelines for Testing of Chemicals, No. 202: “Daphnia sp. Acute Immobilisation Test”, adopted, 2004.
- OECD Guidelines for Testing of Chemicals, No. 202: “Chironomus sp., Acute Immobilisation Test ”, adopted, 2011.