LABORATORY AQUATIC INVERTEBRATE SINGLE- SPECIES SCREENING STUDIES

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INTRODUCTION

- Acute aquatic toxicity testing in laboratories provides an efficient means of predicting the short-term effects chemicals may have in the environment. As such, these tests can aid regulatory risk assessments by providing confirmatory or additional data to support regulatory submissions

- Cambridge Environmental Assessment (CEA) has recently used several standard and non-standard aquatic invertebrates to conduct laboratory bioassays in which methods were based on existing OECD and EPA guidelines such as the *Daphnia* sp. acute immobilisation test (OECD 202)

- Here we present methods used for collecting, acclimating, culturing and testing these freshwater invertebrates and make recommendations for standardising test procedures, exposure durations and relevant effect endpoints for each taxa

Figure 1. Collection of invertebrates using sweep nets.

METHOD

Collection

- Eleven freshwater invertebrate taxa (Table 1) were sourced from the CEA mesocosm facility using sweep net sampling (Figure 1) or from external suppliers

Culturing and Acclimation

- A 16hr light: 8hr dark photoperiod and nominal water temp. of 18-24ºC were used to establish cultures
- *Daphnia magna* and Cyclopidae were fed a *Chlorella vulgaris* suspension *ad libitum*
- Chironomidae and *Culex* sp. larvae were fed flaked fish food solution

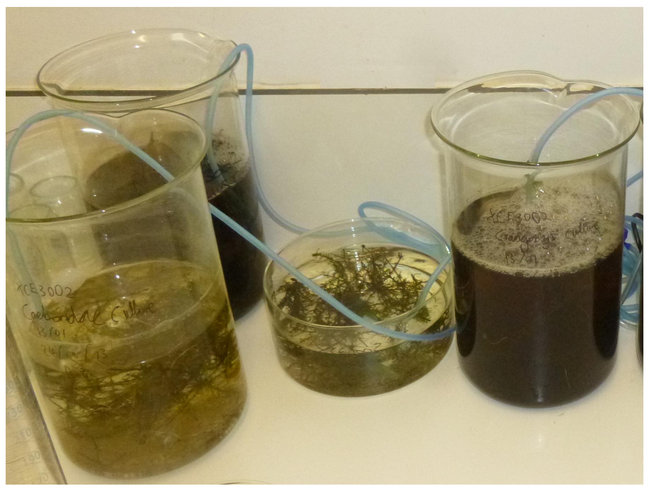

Figure 2. Establishment and acclimation of invertebrate cultures.

- Emerging adult *Culex* sp. were given sucrose solution, liver and defibrinated blood but did not proliferate in the laboratory; culturing outdoors in aged rain water proved most successful
- Other taxa were supplied with *Elodea Canadensis* and dried alder leaves and aerated gently (Figure 2)

Laboratory Bioassay

- Five organisms per replicate were randomly assigned to test vessels; *Asellus aquaticus*, Coenagrionidae and *Crangonyx* sp. were tested individually due to life history characteristics (Figure 3)
- Test media was filtered (45 µm) pond water or Elendt M4 (*D. magna* only)
- Observations were made each day over 48 hrs (96 hrs for *Asellus aquaticus*, Coenagrionidae and *Crangonyx* sp.); Individuals were recorded as dead, immobile or normal
- Immobility was recorded as a loss of free movement following stimuli
- Several stimuli were required for effective assessment: a single sharp tap on the test vessel or gentle swirling of media

RESULTS

- Clear dose related effects were observed for nine taxa
- Cumulative immobility (% initial population) was calculated to estimate the effect concentrations (EC_{50}) for each taxa (Table 1)
- Planariidae and Lymnaeidae showed minimal or no dose response after 48 hrs. However, assessment of Lymnaeidae movement proved challenging
- During 96 hr exposure trials, mortality plateaued after 48 hrs for *Crangonyx* sp. and Coenagrionidae at the highest treatment rate
- *A. aquaticus* immobility continued to increase over time
- Dissolved oxygen decreased by a comparably small amount by the end of 48 hr and 96 hr bioassays
- The pH and temperature remained largely unchanged

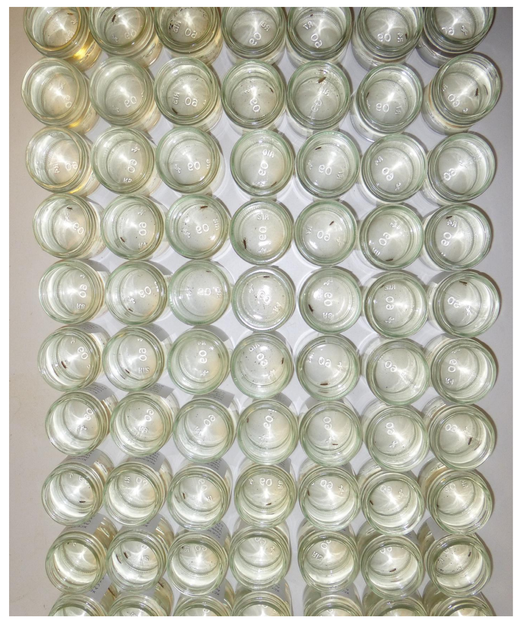

Figure 3. Asellus aquaticus laboratory bioassay.

Table 1. Effect concentrations for 48 hr and 96 hr invertebrate bioassays

Taxon	Taxonomic Class	Common name	48-h EC_{50} (µg/L)	96-h EC_{50} (µg/L)
Asellus sp.	Malocostraca	Hog louse	0.1 - 1.0	~0.1
Baetidae	Insecta	Mayfly larvae	0.01 - 0.1	N/A
Chaoboridae	Insecta	Phantom midge larvae	0.1 - 1.0	N/A
Chironomidae	Insecta	Non-biting midges	1.0 - 10	N/A
Coenagrionidae	Insecta	Damselfly larvae	1.0 - 10	1.0 - 10
Crangonyx sp.	Malocostraca	Freshwater shrimps	0.01 - 0.1	0.01 - 0.1
Culex sp.	Insecta	Mosquito larvae	0.1 - 1.0	N/A
Cyclopidae	Maxillopoda	Cyclops	0.01 - 0.1	N/A
Daphnia magna	Branchipoda	Water flea	1.0 - 100	N/A
Lymnaeidae	Gastropoda	Pond snail	>100	N/A
Planariidae	Turbellaria	Flatworm	>100	N/A

N/A = not applicable

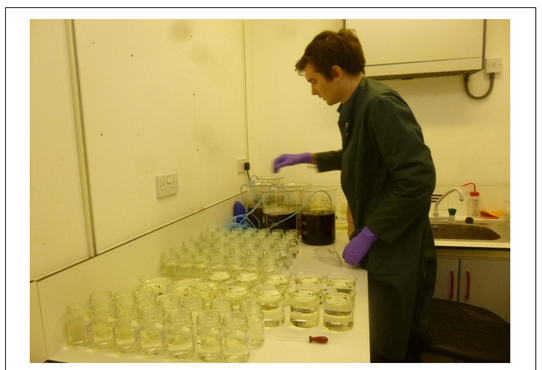

Figure 4. Daily observation of organisms during study.

CONCLUSIONS & RECOMMENDATIONS

- Use of non-standard invertebrate taxa yielded distinct results and endpoints after 48 hrs in most cases
- Further methods for assessing immobility may need to be refined for certain taxa e.g. Lymnaeidae
- Feeding *ad lib.* proved convenient, however excess food can stagnate in some cultures
- Periodic water changes over prolonged periods, or further research into optimal feeding rates for long term culturing is required for some taxa
- *Culex* sp. laboratory culturing was problematic; we recommend revisiting the method and potentially warming food supplies for long-term culturing