Culturing and Testing Early Life-History Stage Larvae of *Chaoborus crystallinus* and *Cloeon dipterum*

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INTRODUCTION

- Lower-tier acute toxicity tests involve age-specific standard organisms e.g. <24 hour old *Daphnia magna* neonates and *Chironomus riparius* larvae (OECD 202 and OECD 235). However, higher-tier toxicity tests include non-standard species organisms of any age, making the results variable.
- Cambridge Environmental Assessments (CEA) are developing methods to culture non-standard freshwater invertebrates for use in age-specific tests.
- Our aim was to develop culturing methods to ensure freshwater invertebrates that we were testing on were all standardised to a certain larval age range. Here we present two examples (*Chaoborus crystallinus* and *Cloeon dipterum*).

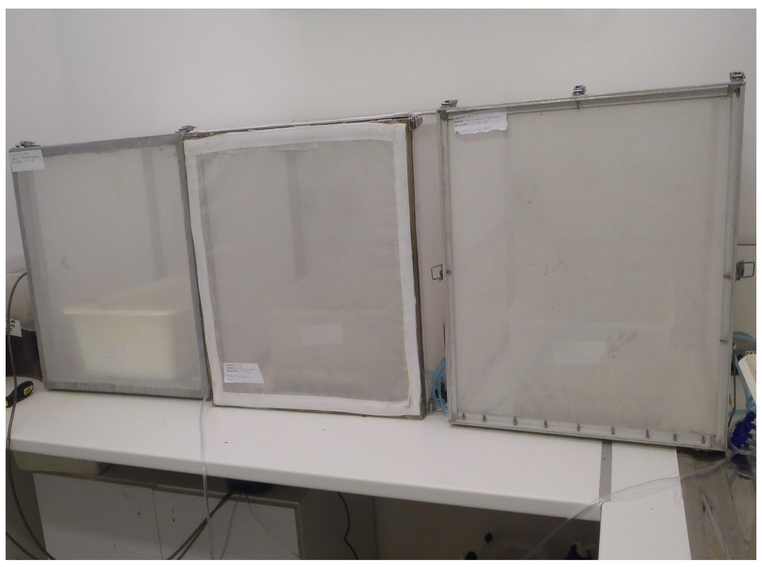


FIGURE 1. *Cloeon dipterum* and *Chaoborus crystallinus* laboratory cultures in mesh cages

Cloeon dipterum

Method 1) Late instar nymphs collected from the CEA mesocosm facility were transferred to laboratory cultures enclosed in fine net cages (Figure 1). Low light at dawn and dusk was introduced to encourage oviposition.

Method 2) An outdoor culturing system was established using mesocosms and several surrounding containers of filtered water and *Elodea sp*. enclosed in mosquito netting to encourage oviposition under natural conditions.

Method 3) Larval stages 1 and 2 (Figure 2) were collected from CEA mesocosms and acclimatised in the laboratory.

FIGURE 3. Early-life history stage of *Cloeon dipterum* at 5x magnification.

METHODS DEVELOPMENT

Chaoborus crystallinus

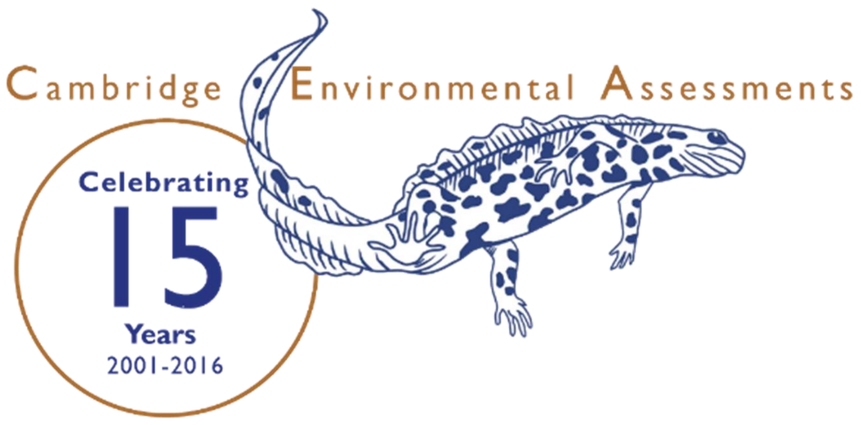
Method 1) Late instar larvae collected from mesocosms were placed in laboratory cultures at 20°C, enclosed in fine net cages (Figure 1). Low light at dawn and dusk and beakers of freshly collected mesocosm water were introduced to encourage oviposition.

Egg rafts were laid on the surface of the satellite cultures and removed into individual crystalizing dish cultures (Figure 3).

FIGURE 2. *Chaoborus crystallinus* fertilised eggs in crystallising dish

After approximately two days at 20°C 100 – 200 first instar *C. crystallinus* hatch.

Few days after hatching they were transferred into a 3L beaker of coarse (2mm) filtered pond water, to reduce intraspecific competition for space and resources.



OBJECTIVE

- To age-standardise (<21 days) cultures of *Cloeon dipterum* nymph and (<24 hours) cultures of *Chaoborus crystallinus* larvae.

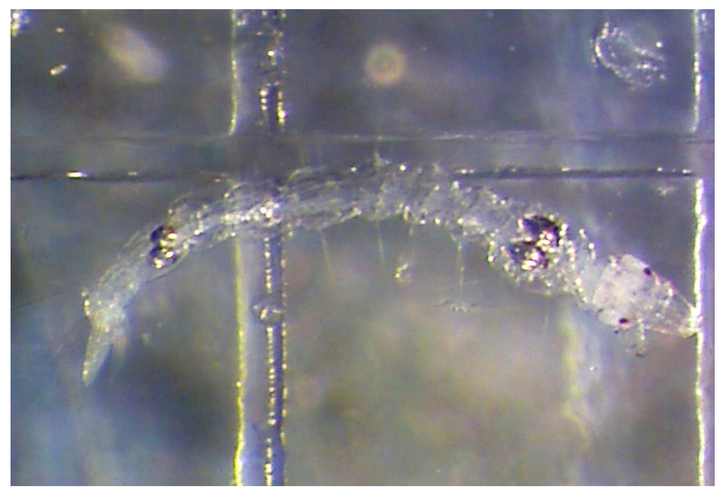


FIGURE 4. 1st Instar *Chaoborus crystallinus* larvae on a Sedgewick Rafter slide at 30x magnification. Cell = 1mm x 1mm.

FINDINGS

Chaoborus crystallinus

- Oviposition was seen in laboratory cultures, resulting in a large production of egg rafts.
- Egg rafts from laboratory cultures hatched within five days and produced between 100 – 200 1st instar larvae.

Cloeon dipterum

- Adults emerged from the laboratory cultures but no evidence of oviposition was observed.
- Eggs and nymphs were not obtained from outdoor cultures as they were difficult to detect.
- Larvae from mesocosms were easily identified to larval stage 1 and 2 without the use of a microscope.

CONCLUSIONS AND RECOMMENDATIONS

- Isolating organisms of specific stage or size was a simple method of standardising, however this is not necessarily the most sensitive stage.
- More development of culturing methods is required in order to introduce a standardised testing procedure of young larvae across the industry.
- This will provide higher-tier toxicity testing with more robust and reliable endpoints, to be used in risk assessments.

REFERENCES

- OECD (2004), *Test No. 202: Daphnia sp.* Acute Immobilisation Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.
- OECD (2011), *Test No. 235: Chironomus sp.,* Acute Immobilisation Test, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris.