DEVELOPING METHODS TO DETERMINE AQUATIC INVERTEBRATE BEHAVIOURAL ENDPOINTS FOR REGULATORY ECOTOXICOLOGY STUDIES

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Background

Under current plant protection product regulation, Tier I aquatic invertebrate studies measure mortality (acute) or reproduction (chronic) to address the current protection goal of maintaining populations. Agreed guidelines are available for reproduction studies for standard test species, but other taxa/species are sometimes more sensitive e.g. Ephemeroptera, Plectoptera, Trichoptera (EPT) species. In the absence of agreed methods for measuring reproduction endpoints for EPT species (due to the challenges of recreating their life cycle in laboratory conditions), a proxy measure for reproductive effects could be useful. Although behavioural endpoints do not directly relate to the protection goal, they may still be useful in regulatory risk assessment. For example, for substances with steep dose-response curves, and thus narrow exposure windows between acute and chronic effects, significant differences in reproduction can be due to mortality of adults rather than true reproductive effects; therefore, designing specific reproduction studies may not be necessary. Instead, a risk assessment could be undertaken using acute and behavioural endpoints and if acceptable risks were concluded, then infer that it would be unlikely for effects at the population level to arise. Here we share our experiences of developing methods to measure behavioural endpoints in two non-standard (EPT: mayfly, caddis) test species that are suitable for use in regulatory toxicity tests, integrating regulatory needs with practicalities of ecotoxicology testing.

Methods used

- Animals collected from mesocosm facility 3 days before trials started; kept in 30 µm filtered aerated pond water at 18°C; fed *ad libitum*
- **Feeding trials** undertaken to determine type/amount of food and frequency of feeding that resulted in highest survival
    - Animals housed individually in glass vessels, including gravel for caddis to aid movement
    - Mayfly diets tested: algae (broken/intact), periphyton, crushed alder leaves, vegetarian fish food
    - Caddis diets tested: bloodworm, fish food
- **Sublethal parameters** investigated in parallel during feeding trials:

Parameter investigated	Method used for	
	Mayfly	Caddis
Growth	Length (photo taken over graph paper)	Case and head capsule measured once a week (photo taken over graph paper)
Food consumption	• Algal absorbance using spectrophotometer • Qualitative assessment of phytoplankton remaining on slide • Qualitative assessment of crushed alder leaf eaten • Mass of fish flake eaten	• Qualitative assessment of amount of fish flake eaten • Number of bloodworm eaten
Excretion	Count faecal pellets	Count faecal pellets
Behaviour	• Movement towards food source (time taken over known distance) • Response to external stimulus (video used to determine duration of movement following pipette stimulus)	• Movement towards food source (time taken over known distance) • Time to taken to return to case (larvae flushed out of case using pipette) • Larvae behaviour when active (reaction of larvae to pipette moved towards head) • Larvae behaviour when inactive (response to external stimulus; attachment to gravel)

Results

Laboratory conditions resulting from feeding trials that allow 4-5 week survival in controls:

- 250 mL of 30 µm filtered pond water in glass vessels
- 1-3 water changes per week
- Temperature: 18°C
- Feeding regime: 2 vegetarian fish food flakes 3x per week (mayfly); 6 bloodworm 3x per week (caddis)
- Duration of study: 4-5 weeks

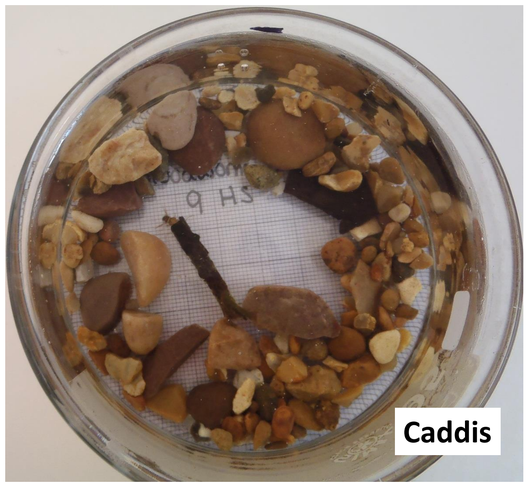
Sublethal parameters identified for <u>mayfly</u>:

- Growth – length of larva (mm); moulting (frequency)
- Response to external stimulus (simulating predator response; seconds)
- Food consumption (mg/week)

Potential sublethal parameters identified for <u>caddis</u>:

- Growth – size of capsule (mm); size of case (mm)
- Response to presence of food (minutes)
- Response to case removal (simulating predator response; seconds)
- Food consumption (% eaten/feeding occasion)


Mayfly


Caddis

NEXT STEPS

- Currently assessing the suitability of these behavioural endpoints for assessing the toxicity of a particular test item
- Currently assessing the impact of exposure profile on the effects observed i.e. short term exposures, pulsed exposures
- Develop comparable behavioural endpoints for standard test species: *Daphnia*, *Chironomus*