OPTIMISING THE SYNCHRONISATION OF THE MAYFLY CLOEON DIPTERUM FOR USE IN CHRONIC, HIGHER-TIER LABORATORY TESTING

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INTRODUCTION

The pre-adult stages of mayflies (Ephemeroptera) reside in freshwater habitats such as ponds, streams, rivers and lakes. The mayfly species, *Cloeon dipterum* is highly abundant throughout the British Isles and across Europe. They are often used as a non-standard test species in acute laboratory studies for plant protection products (PPPs).

Recent studies have found that, in some instances, mayfly larvae are more sensitive to PPPs than other benthic macroinvertebrates. As such, there is a need for the development of methods for the chronic testing of mayfly species, such as *C. dipterum*. During experimental laboratory trials, we have developed methods for measuring effects on survival, growth, physiology and emergence. An integral issue in developing such methods using field caught organisms has been the intrinsic variance when recording the time to emergence. Reducing this variation is key to ensuring the reproducibility of experimental data going forwards.

Here, we present our developmental work with laboratory rearing of mayfly larvae. The main objective of this work is to define the ideal length of a chronic laboratory study alongside the start size of *C. dipterum* larvae to ensure a successful emergence endpoint and to enable the recording of sub lethal, developmental and physiological endpoints.

Table 1: Test conditions

Water:	Natural pond water (filtered at 30 Qm)
Test duration:	35 Days
Test vessels:	300 mL glass crystalizing dishes containing 250 mL pond water
Water Temperature:	18 - 22°C
pH:	6-9
Dissolved oxygen:	>60% ASV (Air Saturation Value)
Photoperiod:	16h light: 8h dark
No. of replicates:	29
Mayfly larvae per replicate:	1
Feeding	Fed 3 times per week using vegetarian fish food
Growth Measurements	Growth (weekly measures of body length). Taking pictures using camera and length measured using image software.

RESULTS

- Ideally, to assess emergence, *C. dipterum* larvae need to emerge between Day 28 and Day 35.
- The data clearly shows that *C. dipterum* larvae larger than 4 mm in size at test initiation will emerge too early (before 28 days; Table 2).
- Similarly, larvae less than 3 mm in size at test initiation may not emerge within the 35 day period.
- This indicates that field caught *C. dipterum* should be 3 to 4 mm in size at test initiation for successful emergence within a chronic study test design.

3 → 4 mm starting size

FURTHER WORK

- Repeat the trial using mayfly at a start size of 2 to 3.5 mm to give more insight into the lower size threshold that still emerge between 28 and 35 days.
- Investigate any difference male and female emergence times.
- Correlate the starting size with wing case development. Does this play a factor in time to emergence?
- Would using *C. dipterum* larvae at the upper end of the start size threshold allow treatment organisms time for recovery and emergence within the test duration?

Table 2: *C. dipterum* Growth Length

Replicate	Day 0	Day 21	Day 28	Day 35
12	2.11	6.34	7.74	8.23
7	2.96	7.48	E	E
1	3.00	8.11	8.44	E
2	3.02	7.32	7.62	E
6	3.14	7.96	E	E
26	3.14	7.83	E	E
19	3.15	5.69	7.18	9.64
17	3.28	6.99	8.33	9.37
5	3.31	8.13	E	E
16	3.36	8.06	E	E
4	3.37	8.91	8.82	E
10	3.41	7.96	8.93	E
9	3.45	7.61	7.73	E
8	3.56	8.14	9.39	E
23	3.59	7.20	8.53	E
22	3.63	7.16	E	E
24	3.80	8.18	9.28	E
28	3.82	8.21	E	E
15	3.85	7.76	8.68	E
25	3.92	9.22	9.69	E
3	3.95	7.65	E	E
13	4.18	7.83	9.41	E
27	4.67	7.91	8.29	E
29	5.57	10.14	E	E
21	5.57	9.67	E	E
18	5.65	E	E	E
11	5.89	10.26	E	E
14	6.24	E	E	E
20	6.75	E	E	E

E = Emerged, replicates sorted according to size on Day 0.