CONTINUOUS CULTURE ESTABLISHMENT AND GESTATION SYNCHRONISATION OF *CLOEON DIPTERUM* (MAYFLY)

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INTRODUCTION

Cloeon dipterum are often used as a non-standard test species for plant protection products (PPPs). With their aquatic larval phase and emergence into a terrestrial environment they present as a vulnerable species to a range of products. Laboratory testing requires wild caught larvae, introducing variability into the starting populations for controlled testing studies. The availability of the animals is restricted by their lifecycle, which limits when these studies can be performed. In conjunction with unknown optimal rearing and reproductive conditions, these factors have made inter-laboratory validation of study protocols for this species unsuccessful to date.

Establishing a laboratory culture of *C. dipterum*, capable of completing its lifecycle within the laboratory, would overcome many of these limitations. Here we present our development work into the establishment and synchronisation of a laboratory mayfly culture. This includes establishing and developing culturing techniques and experimental forced copulations with histological assessment throughout the mating and reproductive stages to attempt to identify the indicators of success or failure.

The results of trials are intended to progress the reliability of these studies for use in a regulatory context and provide further research opportunities (such as future studies into the energy-budgets of these species in order to parameterise population models).

CULTURING

Wild mayfly larvae were collected from the Cambridge Environmental Assessments (CEA) mesocosm facility. Algae was cultured on acrylic tiles in order to supply consistent feed without decreasing the water quality for a large culture, allowing for optimal conditions for mayfly growth and development.

REPRODUCTION

Forced copulation to produce subsequent generations was trialled (methods adapted from published literature[1]) . An imago male and imago female (Fig.2) held gently with tweezers were brought together and the tip of the male abdomen placed between the females' 7th and 8th segments (Fig. 1). The male grasps the female with his claspers and inseminates. Based on literature findings, "egg layings" were initiated from Day 9 post-copulation, until the natural death of the female. Initial copulation attempts failed to produce offspring. Further investigations were made to assess whether copulation or embryonic development failure was the cause of the lack of reproductive success. Mating, with pre-determined sacrificial days, was run alongside regular laying attempts. The eggs from the sacrificed female were collected and stained and developmental stage was assessed[2].

Figure 1: Forced copulation of male imago bottom and female imago (top). Held by wings with forceps

Figure 2: Female imago, ~24 hours old

Figure 3: Virgin egg, 40X magnification

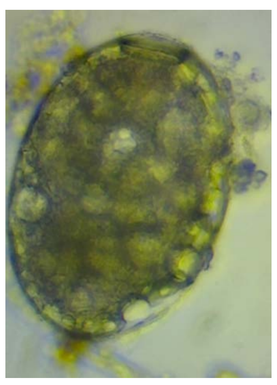

Figure 4: 7 day post-mating egg, 40X magnification

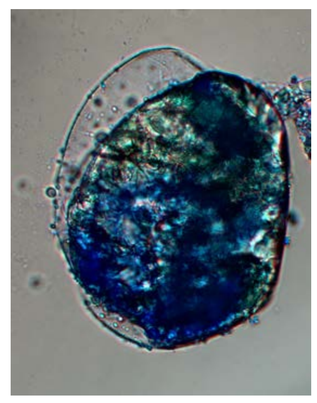

Figure 5: 22 day post-mating egg, 40X magnification

Culture Type and Order	Ad Hoc Fish Food (22/02)	Consistent Periphyton (22/03)	Overwintering (All) (22/04)	Overwintering (After wintering) (22/04)	Reproduction Experimental (23/01)
Emergence (%)	42.5	87.83	3.57	20.45	Ongoing

Table 1: Emergence success of each culture

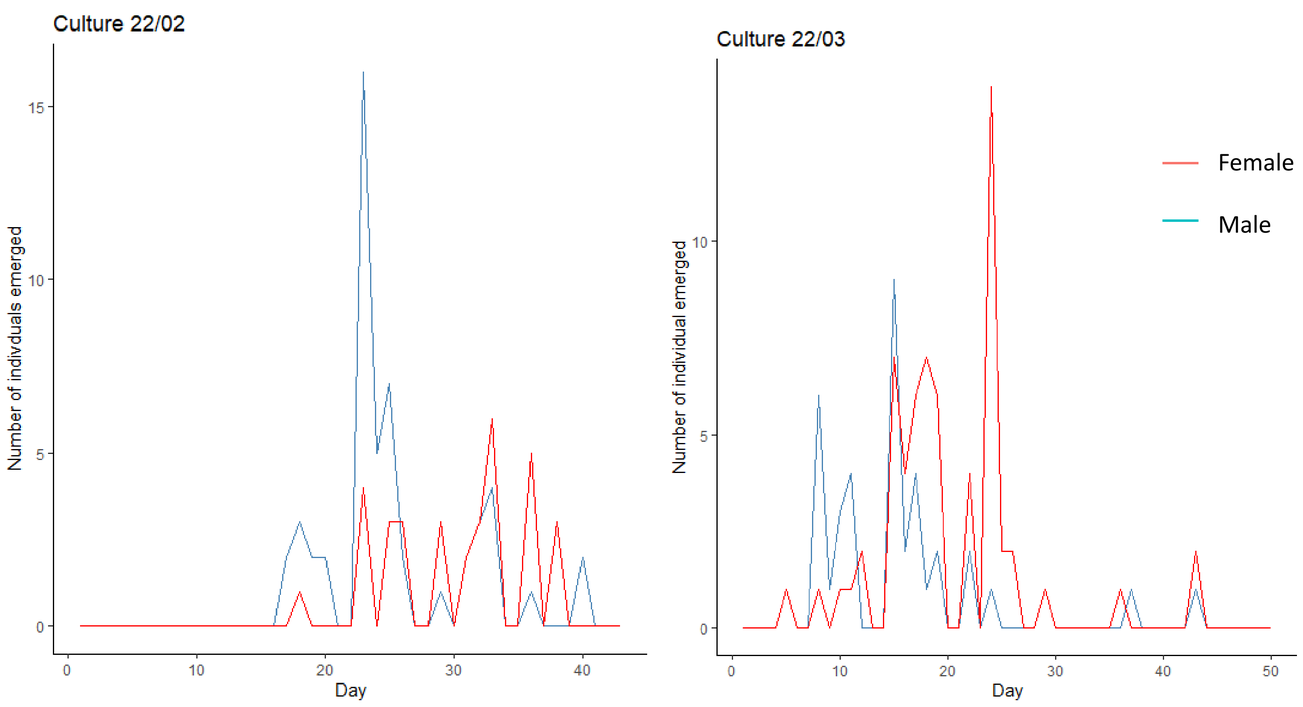

Figure 7: Total emergence per day of 22/02 and 22/03 cultures, split by sex

KEY FINDINGS

EMERGENCE SUCCESS

Optimising culture maintenance at the larval stage to enable a higher successful emergence rate and longer-lived female imagoes.

- Emergence success rate increased from 42.5% to 88% with modifications to feeding procedures.

Natural ambient conditions influence emergence success in the laboratory

- Artificially induced over-wintering enabled larvae to complete their growth lifecycle
- Artificially induced over-wintering increased mortality and reduced emergence success rates in comparison to optimal Spring collected larvae.

Emergence trends identified

- From repeated culturing attempts, patterns of emergence were observed.
- Typical emergence of mayflies followed a trend of initially a few individuals emerging over the first 1 to 2 weeks of optimum conditions before having an explosion of many individuals over the next 2 weeks, and a subsequent decline.
- When investigated according to sex, males often emerge first, and in higher proportions, followed by females. A significant cross-over occurs during the population explosion, when male emergences decline prior to female emergences (Fig. 6).
- Developments allowed for a planned mating schedule within the laboratory trials.

REPRODUCTION

The reproduction trials have so far produced no evidence of egg development when compared to virgin eggs (Figs 3, 4 and 5), indicating that mating has not taken place. We intend to re-evaluate our technique and amend the procedure for future attempts.

FUTURE STUDIES

Future work pertaining to the differing environmental factors is scheduled to take place this summer. Mayflies will be reared in different environmental conditions; growth rate (body length and instar) and emergence success will be recorded. The factors include photoperiod, water temperature and food ration. The results of which will inform a Dynamic Energy Budget (DEB) model to further understand population level effects of chemical exposure with differing environmental scenarios e.g. changes to food availability and increasing water temperatures.

[1] Almudi, I., Martín-Blanco, C. A., García-Fernandez, I. M., López-Catalina, A., Davie, K., Aerts, S., & Casares, F. (2019). Establishment of the mayfly Cloeon dipterum as a new model system to investigate insect evolution. *EvoDevo*, *10*(1), 1-10.
[2] North Carolina State University. (2015) "Embryogenesis" https://genent.cals.ncsu.edu/bug-bytes/embryogenesis/

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