MAYFLIES: THE INFLUENCE OF DIET ON GROWTH TO EMERGENCE

CAMBRIDGE ENVIRONMENTAL ASSESSMENTS

Optimising the feeding of the Mayfly *Cloeon dipterum* under laboratory conditions; the influence of diet on growth and emergence.

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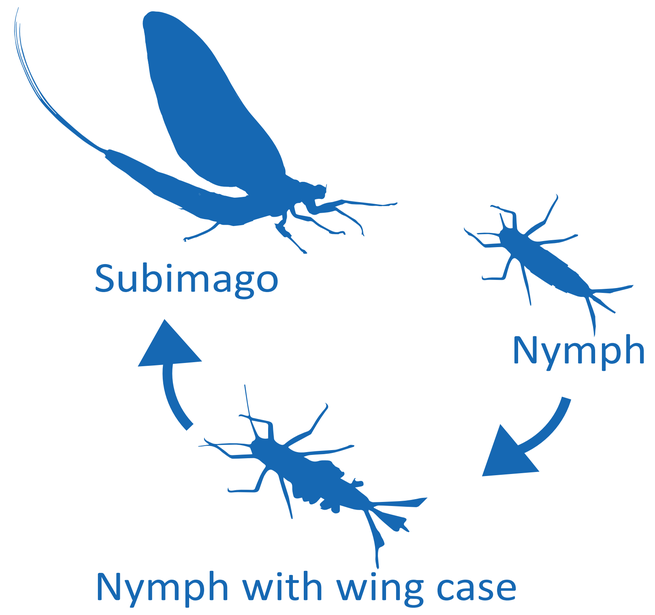
INTRODUCTION

The mayfly species, *Cloeon dipterum* is highly abundant throughout the British Isles and across Europe. The pre-adult stages of mayflies (Ephemeroptera) reside in freshwater habitats (ponds, streams, rivers and lakes) and have often been shown to be highly sensitive to Plant Protection Products (PPPs). This makes them a key species in ecotoxicology studies and aquatic risk assessments.

The use of wild-caught mayfly to maintain a culture in a laboratory environment can be problematic. In their natural environment they would feed upon available detritus and algae. A major challenge in using mayfly as a laboratory test organism is in the generation of a sustainable and quantifiable diet in order to maintain effective growth and survival.

In this poster we present our development work: a comparison of the survival, growth and emergence of *C. dipterum* larvae fed on a variety of diets, including green algae (*Desmodesmus or Nannochloropsis*), mixed periphyton, crushed alder leaves, and vegetarian fish food.



METHODS

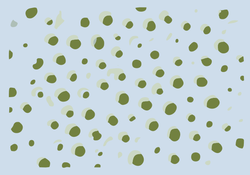
Wild-caught mayfly larvae; acclimatised to laboratory conditions.

Acclimation conditions: All organisms were retained in a single glass beaker in approx. 3 L of 30 µm filtered natural pond water with *Elodea,* provided as a refuge. Organisms were acclimated for a minimum of 36 h (18 ± 2 °C, n = 5 per treatment) and fed in excess with algae and vegetarian fish food.

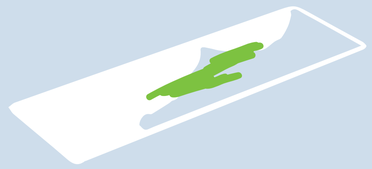
Test conditions: Individual organisms in 250 mL of 30 µm filtered pond water. Fed on one of five different diets for a total of 5 weeks.

Vegetarian fish food flakes

Desmodesmus subspicatus



Nannochloropsis



Glass slides with naturally occurring periphyton

Crushed inoculated alder (*Alnus*) leaves

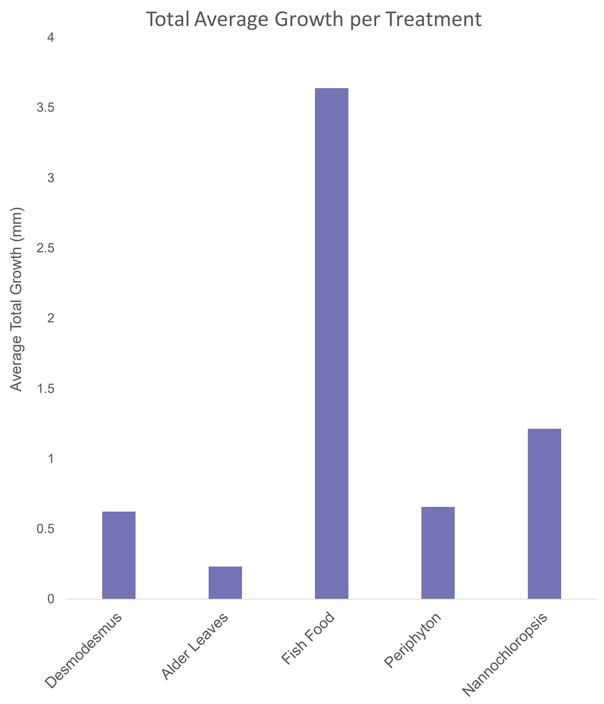
Measurements: survival, moulting, growth (weekly measures of body length) and (successful) emergence.

SURVIVAL and EMERGENCE

The assessment of survival and emergence are critical endpoints that need to be assessed in any non-standard higher tier tests. The Table below shows the percentage of individuals that survived and emerged by the end of the study per treatment.

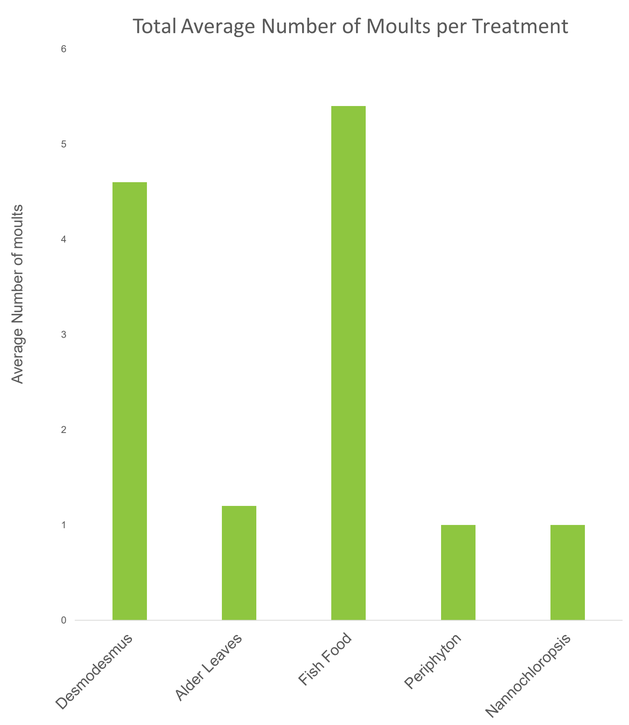
	Treatment				
Endpoint	*Desmodesmus*	Alder leaves	Veg Fish Food	Periphyton	*Nannochloropsis*
Survival (%)	**80**	**40**	**100**	**80**	**80**
Emerged (%)	0	0	80	0	40

Vegetarian fish food was determined as the optimal food source for both survival and emergence. *Nannochloropsis* was the only other food source that enabled any successful emergence. All other treatments failed to emerge.



GROWTH

The largest growth measures were seen in the vegetarian fish food diet group, followed by the *Nannochloropsis* diet group.



MOULTS

The highest average number of moults during the test were observed in the vegetarian fish food diet and the *Desmodesmus* diet groups.

KEY FINDINGS

- The vegetarian fish food diet was the most successful for high survival and emergence percentages.
- Little or no emergence was observed in all other diet treatments.
- The vegetarian fish food diet also promoted the largest organism growth; growth measures are a valuable endpoint when considering the standardization of testing in mayflies.
- *Nannochloropsis* was partially successful as a diet treatment; since this is a natural food source further optimisation could be of interest (e.g. breaking down the cells to aid ingestion).
- Alder leaves, *Desmodesmus* and periphyton were the least successful in this test; in these cases the food source may be nutritionally deficient, too large for ingestion or non-digestible.
- Moulting in mayfly isn't related to the change in instars. The fish food treatment had the most moults and grew the most. *Desmodesmus* diet caused multiple moults but no emergence and very small amount of growth.

CONCLUSION

- The Mayfly larvae survived, grew and emerged successfully on vegetarian fish food.
- Vegetarian fish food can be replicated and quantified easily.
- There is no need for an extra culture of food in the lab as fish food is readily accessible.
- The use of this diet will enable greater standardisation of a laboratory culturing method for mayflies.

References:
1 EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues) (2013). Scientific Opinion - Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal 2013; 11(7): 3290.