'Echo' Moulting Used to Estimate Moulting Periodicity of Mayflies (Ephemeroptera) and Stoneflies (Plecoptera), in Nature

by

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Larvae of mayflies (Insecta, Ephemeroptera) and Stoneflies (Insecta, Plecoptera), removed from nature, continue with their moulting cycle ('echo'), even if they are unfed. This makes it possible to estimate their intermoult period in nature, at the time of their collection. Three equations were developed to make the estimations. Using animals in the length class of 3 to 4 mm, and at 9°C, a field intermoult period of 11.7 days was obtained for *Baetis muticus* (Ephemeroptera) and a field intermoult period of 26.8 days was obtained for *Isoperla grammatica* (Plecoptera). The intermoult period estimation for *Baetis rhodani* (Ephemeroptera), 10.0 days, was less satisfactory, for reasons which are discussed. These intermoult period estimations, which can be made without any knowledge of the feeding regime of the insect, are of value in assessing the toxic effects of different waters; these effects may be most severe when the insect moults.

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INTRODUCTION

Mayflies (Ephemeroptera) and stoneflies (Plecoptera) make numerous moults as larvae. Moulting is a consequence of growth and it is reasonable to suppose that if a mayfly is grown in the laboratory, on its preferred diet, then both its growth rate and its moulting frequency will reflect these in nature. In this current investigation, the preferred diet for *Baetis muticus* (L.), in its local native streams, was found to be decaying tree leaves (not algae). Accordingly decaying tree leaves were presented to the animals when they were grown experimentally (see below). This expected connection between laboratory and field performance of mayflies, if the diets are matched in the two situations, was confirmed by Humpesch (1981) for *Ecdyonurus dispar* (Curt.). He found that the growth rate was similar; by implication the moulting frequency was believed to be similar also. However moulting frequency of mayflies in nature, determined directly, has never been an attempted study, by any previous investigator.

If small larvae of mayflies and stoneflies are collected and held, unfed, as separate individuals, at constant temperature, they moult successfully, but at various days following the starting day (day 0). In results reported in this current

investigation some individuals moulted on day 1 and others much later, one as late as day 12. This suggests that the animals are completing intermoult periods which began in nature, before they were collected. The moulting cycle therefore appears, at first sight, to be independent of feeding and growth. It was seen that data derived from laboratory experiments could make it possible to estimate moulting frequency, at a particular stage in the life cycle, in nature.

MATERIALS AND METHODS

Cianciara (1980) has reported that in the mayfly *Cloeon dipterum* (L.), moulting frequency decreases during the life of the animal, if it is held at constant temperature. This was borne in mind in the present investigation; larvae in the 3-4 mm length class were used throughout.

Larvae were maintained, unfed, each in a separate, flat, tissue culture flask, capacity 200 ml. In experiments 40 ml of water was used, surface about 10 x 7 cm, depth 6 mm. To prevent evaporation the flask opening was covered with a cap of aluminium foil. Water was from Windermere, paper-filtered and autoclave-sterilized before use. This water has a pH of 7.0 and it is well endowed with background ions, making it amenable for aquatic insects. The larva were given a 2 cm square of nylon mesh to rest on. Larvae of the selected length class were washed and placed in their flasks within 2 h of their collection. The collections were made in the spring season and stream water temperature (day) was 6°C to 7°C, a fairly close approximation to the laboratory experimental temperature, 9°C with alternate 12 h light and dark cycles. Daily inspections were made for moulting and survivorship, keeping separate records for each individual.

Three basic assumptions were made: – (a) that time to the next moult, of animals collected from nature, is under the control of a 'clock' (see Saunders, 1976) or some other mechanism; (b) when animals are collected from nature the times since their last moult are uniformly distributed over the intermoult period, i.e. they are completely asynchronous as to moulting periodicity; (c) death in the experiment (due to starvation) and time at which an animal is due to moult are unrelated, i.e. death is not more likely when an animal is about to moult. Assumption (b) leads to the statistical assumption that time to moult from day 0 of the experiment is uniformly distributed on the range 0 to M, where M is the intermoult period.

The following notation was used:

d = number of days of the experiment so far.

n_1 = number of animals which moult during the d days of the experiment so far (irrespective of whether they live or die subsequently).

n_2 = number of animals which do not moult during the d days of the experiment but which survive to day d.

n_3 = number of animals which die before day d of the experiment and have not moulted. Their dates of death are $e_1, e_2, e_3 \ldots e_{n_3}$.

n   = $n_1 + n_2 + n_3$ = total number of animals which started an experiment.

M   = Intermoult period. $M \geqslant d$ is a necessary condition for the experiment, otherwise an individual animal could, if it was capable of doing so, enter a third intermoult period during the experiment.

The statistical method used to estimate the intermoult period is the method of maximum likelihood.

It follows from assumptions (b) and (c) that the likelihood (L) of the experimental observations made is:

$$L = \left(\frac{1}{M}\right)^{n_1} \left(\frac{M-d}{M}\right)^{n_2} \prod_{i=1}^{n_3} \left(\frac{M-e_i}{M}\right)$$

The problem becomes one of choosing M to maximize L.

Proceeding on the assumption that $n_1 > 0$,

$$\log_e L = -n \log_e M + n_2 \log_e (M-d) + \sum_{i=1}^{n_3} \log_e (M-e_i)$$

$$\frac{\partial \log_e L}{\partial M} = \frac{-n}{M} + \frac{n_2}{M-d} + \sum_{i=1}^{n_3} \frac{1}{M-e_i}$$

The likelihood is a maximum where $\partial \log_e L/\partial M = 0$, and so to obtain the maximum likelihood estimate ($\hat{M}$) of the intermoult period must solve:

$$\frac{n}{\hat{M}} = \frac{n_2}{\hat{M}-d} + \sum_{i=1}^{n_3} \frac{1}{\hat{M}-e_i} \qquad \text{(Equation 1)}$$

If $n_2 > 0$, $n_3 > 0$ then the solution to Equation 1 must be found by numerical iteration.

If $n_2 = 0$, $n_3 > 0$ then Equation 1 simplifies:

$$\frac{n}{\hat{M}} = \sum_{i=1}^{n_3} \frac{1}{\hat{M}-e_i} \qquad \text{(Equation 2)}$$

and again must be solved by numerical iteration.

If $n_3 = 0$ then the summation part of Equation 1 can be eliminated:

$$\frac{n}{\hat{M}} = \frac{n_2}{\hat{M}-d}$$

This can be transformed as follows: $-n\hat{M}-nd = n_2\hat{M}$; $\hat{M}(n-n_2) = nd$; $\hat{M} n_1 = nd$ (since $n = n_1 + n_2$ and $n-n_2 = n_1$);

$$\hat{M} = \frac{nd}{n_1} \qquad \text{(Equation 3)}$$

The approximate standard error (S.E.) of the estimate $\hat{M}$ is obtained from the second derivative of $\log_e L$

$$\text{S.E. } (\hat{M}) = \left(\frac{-n}{\hat{M}^2} + \frac{n_2}{(\hat{M}-d)^2} + \sum_{i=1}^{n_3} \frac{1}{(\hat{M}-e_i)^2} \right)^{-\frac{1}{2}}$$

The simplicity of Equation 3 will make it very attractive to an investigator. Theoretically the most acceptable estimate for M is obtained when d is at its maximum, because the estimate will then make use of all the data obtained.

RESULTS

Baetis muticus L. (Ephemeroptera)
The larvae showed good survivorship (Fig. 1) and moulting (Table 1) and Equation 3 was used up to day 10. The best value for $\hat{M}$ is taken to be 11.7 days.

Isoperla grammatica Poda (Plecoptera)
The larvae again showed good survivorship (Fig. 1), but with a lower incidence of moulting (Table 2). Day 8 was the last day on which Equation 3 could be
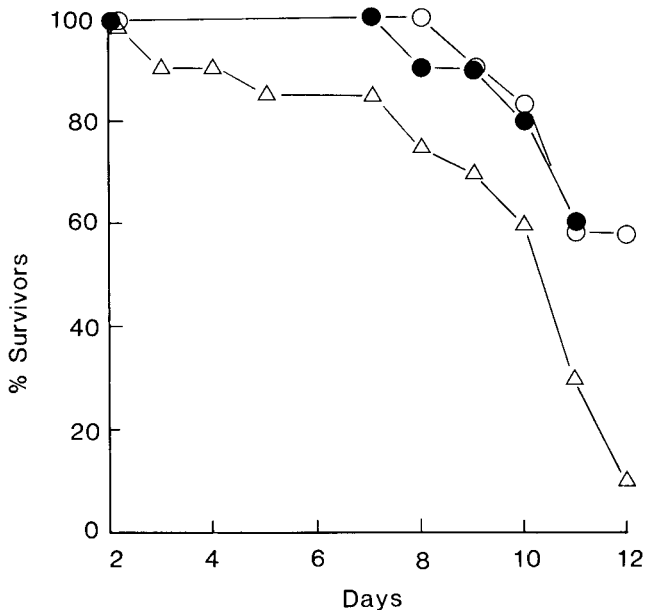

Fig. 1. Mortality slopes, starting from day 2, for *Baetis muticus* (•), *Baetis rhodani* (Δ) and *Isoperla grammatica* (o), made from the same data used in Tables 1-3.

Table 1. *Baetis muticus*, 3-4 mm in length, unfed in Windermere water at 9°C, daily records of moulting and mortality. The date of death of an individual which died between day 10 and day 11 was taken as 10.5 days.

Days	n_1	n_2	n_3	n_3 constituent	$\hat{M}$	(S.E.)
0	0	10	0		–	
1		Not done			–	
2	1	9	0		20.0	(19.0)
3	1	9	0		30.0	(28.5)
4	2	8	0		20.0	(12.6)
5		Not done			–	
6	5	5	0		12.0	(3.8)
7	7	3	0		10.0	(2.1)
8	8	2	0		10.0	(1.6)
9	8	2	0		11.2	(1.7)
10	9	1	0		11.1	(1.2)
11	9	0	1	10.5	11.7	(1.3)

applied, $\hat{M} = 24$ days; but subsequently, using Equation 1, there was no marked deviation, $\hat{M} = 26.8$ days on day 16.

Baetis rhodani Pict. (Ephemeroptera)
The larvae showed a much poorer survivorship (Fig. 1), although with a high incidence of moulting; day 2 was the last day on which Equation 3 could be applied. Using Equation 1 from day 3 to day 14 there was a marked upward

Table 2 *Isoperla grammatica*, 3-4 mm in length, unfed in Windermere water at 9°C, daily records of moulting and mortality. The date of death of an individual which died between day 8 and day 9 was taken as 8.5 days, etc.

Days	n_1	n_2	n_3	n_3 constituents	$\hat{M}$	(S.E.)
0	0	12	0		–	
1	0	12	0		–	
2	0	12	0		–	
3	0	12	0		–	
4	1	11	0		48.0	(46.0)
5	2	10	0		30.0	(19.4)
6	3	9	0		24.0	(12.0)
7	3	9	0		28.0	(14.0)
8	4	8	0		24.0	(9.8)
9	4	7	1	8.5	26.8	(10.9)
10	4	6	2	8.5, 9.5	29.3	(12.0)
11	4	3	5	8.5, 9.5, 10.5, 10.5, 10.5	31.0	(12.6)
12	5	2	5	8.5, 9.5, 10.5, 10.5, 10.5	25.4	(8.6)
13	5	1	6	8.5, 9.5, 10.5, 10.5, 10.5, 12.5	26.0	(8.7)
14	5	1	6	8.5, 9.5, 10.5, 10.5, 10.5, 12.5	26.5	(8.9)
15	5	0	7	8.5, 9.5, 10.5, 10.5, 10.5, 12.5, 14.5	26.8	(9.0)
16	5	0	7	8.5, 9.5, 10.5, 10.5, 10.5, 12.5, 14.5	26.8	(9.0)

Table 3. *Baetis rhodani* 3-4 mm in length, unfed in Windermere water at 9°C, daily records of moulting and mortality. The date of death of individuals which died between day 2 and day 3 was taken as 2.5 days, etc.

Days	n_1	n_2	n_3	n_3 constituents	$\hat{M}$	(S.E.)
0	0	20	0		–	
1	2	18	0		10.0	(6.7)
2	4	16	0		10.0	(4.5)
3	5	13	2	2.5, 2.5	11.7	(4.5)
4	5	13	2	2.5, 2.5	15.3	(5.9)
5	8	9	3	2.5, 2.5, 4.5	11.6	(3.1)
6	8	9	3	2.5, 2.5, 4.5	13.6	(3.6)
7	9	8	3	2.5, 2.5, 4.5,	13.9	(3.3)
8	10	5	5	2.5, 2.5, 4.5, 7.5, 7.5	13.9	(2.9)
9	10	4	6	2.5, 2.5, 4.5, 7.5, 7.5, 8.5	15.1	(3.2)
10	10	3	7	2.5, 2.5, 4.5, 7.5, 7.5, 8.5, 9.5	16.1	(3.4)
11	10	0	10	2.5, 2.5, 4.5, 7.5, 7.5 8.5, 9.5, 10.5, 10.5, 10.5	16.5	(3.4)

trend in the value for $\hat{M}$. For reasons which are discussed below, the day 2 figure, $\hat{M} = 10$ days is taken to be the best estimate (Table 3).

DISCUSSION

The intermoult period calculations made for *B. muticus* and *I. grammatica* appear to be satisfactory. In other experiments, where individuals of *B. muticus,* of the same size range as that used here, were fed on decaying oak leaves at 9°C, high exponential growth rates, made from length measurements, $G = 0.49 - 0.67\%$ day[-1] were obtained.

Using combined data from these animals, there were 20 intermoult periods, ranging from 8 to 13 days, mean $= 10.35$ days (S.E. $= 0.35$). This mean figure is in good agreement with $\hat{M}$ obtained from this current work. However, its was of great interest that some fed individuals then went on to give much longer intermoult periods, of up to 22 days. During this time their growth rates were much lower, $G = 0.05 - 0.11\%$ day^{-1}. This falling off in performance was attributed to intermittent feeding rather than to increasing maturity of the animals. This suggests then that there is a connection between feeding and growth and moulting periodicity, putting assumption (a) in doubt.

Certainly there is little evidence that a 'clock' mechanism is operating, because it has been reported that moulting periodicity may be altered by temperature (Humpesch, 1981).

The most likely explanation for the results obtained is that, in nature, animals of a particular size class establish a rhythm of regular moulting, at a fairly constant interval. If their feeding is interrupted, or stopped completely, then their established moulting cycle will continue for a while, as an 'echo'. This

allows an investigator sufficient time to establish their field moulting periodicity. Evidently however, 'echo' does not continue indefinitely; the results for *B. muticus* suggest that a new moulting periodicity can be established, reflecting a lowered growth rate, and demonstrating some degree of flexibility.

There are two possible explanations for the results obtained here for *B. rhodani*. They may be valid, i.e. giving indication that the animal adjusts, almost immediately, to the unfed condition, by prolonging its intermoult period. On the other hand these results may not be valid, because assumption (c) did not apply. Here we note again the steep mortality slope for *B. rhodani* (Fig. 1) and it is conceivable that the animal was so weakened on a particular day, especially a later day in the experiment, that it died when it attempted to moult. This would have had the effect of progressively increasing $\hat{M}$, using Equation 1. It is of interest that Fox & Simmonds (1933) have made a case that *B. rhodani* appears to have an outstandingly high metabolic rate (based on oxygen consumption) as compared with other mayflies. The $\hat{M}$ value for *B. rhodani,* 10.0 days using Equation 3 at 2 days, is considered to be the best estimate for the time being, although it is clearly much less satisfactory than the $\hat{M}$ values for *B. muticus* and *I. grammatica* because it does not make use of all the data obtained. Intermoult period estimations for *B. rhodani* and other similar species, if they exist, might better be made using an even larger number of replicate animals, held under observation for 2 days only. It would then be expected that Equation 3 could be used more convincingly; if n_3 was represented then those (presumably few) individuals to which it applied could be ignored.

The calculation of moulting periodicity of insect larvae in nature, accomplished in this contribution, has two areas of usefulness. If a feeding regime is set up in the laboratory, and moulting periodicity is recorded, then reference to the natural periodicity will give good indication as to whether that feeding regime is satisfactory. This seems to have been so for *B. muticus*, fed on decaying oak leaves. Again, in testing for sensitivity to toxic, especially acid, water there is evidence that aquatic insects may be particularly vulnerable when they moult. Moulting periodicity data, which can be obtained as described here, without any knowledge of the feeding preference of the animal in nature, is an essential background to such work.

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