

The role of parthenogenesis in the biology of two species of mayfly (Ephemeroptera)

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SUMMARY

1. From a single founder virgin female of each species, nine and seven successive parthenogenetic generations of *Cloeon dipterum* and *Cloeon simile*, respectively, were reared: all offspring were female. Eggs were removed from final instar nymphs, subimagos and virgin imagos of each generation and their development assessed. The life cycles of 487 individual *C. dipterum* and 315 *C. simile* were recorded, together with details of growth rate, egg production and adult size of these and other representatives of each generation. In order to distinguish between the effects of parthenogenesis and the effects of culture conditions, fertilized eggs of both species were reared under the same laboratory conditions.
2. Fewer unfertilized than fertilized eggs hatched. There was no consistent trend in the percentage hatch in successive parthenogenetic generations. In both species the time taken for parthenogenetic individuals to complete a generation varied considerably, and no relationship was found between generation length and the number of parthenogenetic generations that had passed, the developmental stage of the donor, or the time taken for the eggs to hatch. After 50 weeks there was, in both species, an overlap of up to six generations. The number of eggs produced by both species was significantly greater in the first parthenogenetic generation than in later generations but the numbers for later generations lie within the recorded range of egg number in imagos captured in the field.
3. To find if parthenogenetic individuals occur in the field, the sex ratios of last instar nymphs and subimagos of *C. simile* were measured in samples collected over 13 successive years from a set of small isolated pools. There was a female bias both in winter and at the start of the emergence season. It is possible that, when mature nymphs and emerging subimagos are damaged by predators, the eggs which are released from them survive and develop.
4. Mature, egg-containing nymphs of both species were present in the field throughout the year, although their numbers were small at the beginning of winter.
5. Oviposition by imagos captured from mating swarms and by virgin imagos, of both species, was recorded for the first time, showing that *C. dipterum* is not always ovoviviparous. It is suggested that early instar nymphs occurring in mid-winter come not only from unfertilized eggs released from injured nymphs and subimagos, but also from fertilized eggs laid in autumn.

Introduction

The females of many species of mayflies produce eggs that may develop parthenogenetically, although little attention has been paid to the effect of tycho-parthenogenesis (accidental or sporadic parthenogenesis) on the

population structure in particular habitats, or to its genetic and evolutionary implications. Hutchison (1993) doubted the general importance of parthenogenesis, considering that it 'could only be functional

in nature in those species in which females can proceed to oviposit without having copulated'. Although some species do at times oviposit without having copulated (Gibbs, 1977, 1979; Bergman & Hilsenhoff, 1978), the relative contributions of parthenogenetic and non-parthenogenetic offspring to populations and the consequences of accidental egg release have not previously been considered.

Butler (1984) claimed that 'when a small proportion of unfertilized eggs hatch, as in some mayflies, it is unlikely the hatchlings would mature and reproduce', although Degrange (1959) had shown that more than 10% of nymphs coming from unfertilized eggs removed surgically from the imagos of various species, mature and produce fertile eggs. Humpesch (1980), studying five species of *Ecdyonurus* and two of *Rhithrogena*, also found unfertilized eggs could develop successfully, although only a small proportion hatched and the embryos took longer to develop than did those from fertilized eggs.

In addition to eggs oviposited by virgin imagos, eggs can be released through the body wall of female imagos, subimagos or final instar nymphs after practically any type of damage to the abdomen (or indeed to the thorax). Such damage could be brought about by movements of the substratum in rivers and streams or by attacks by predators, and we may ask whether eggs produced in this way could develop and produce another generation.

This paper describes the fate of eggs removed from all three developmental stages, and the progress of successive parthenogenetic generations, of *Cloeon dipterum* (Linnaeus) and *Cloeon simile* Eaton, two common species of ponds and streams in Great Britain. The results are related to information obtained from long-term field studies.

Degrange (1959) described the mated female of *C. dipterum* as ovoviviparous, and stated that after copulation it shelters in foliage and retains the eggs for 10–14 days until the embryos have completed development: females then fly to water and, as the abdomen touches the surface, the eggs are ejected and hatch immediately. Successive authors have since reiterated that ovovivipary is the only form of reproduction by the fertilized females of *C. dipterum* (Macan, 1979; Elliott & Humpesch, 1980; Elliott, Humpesch & Macan, 1988; Hutchison, 1993), and that embryonic development must be completed before oviposition. Since such a mode of development would preclude

the possibility of successful development of any eggs released from nymphs and subimagos (or indeed even from newly emerged imagos), a further investigation has been made of the interval between mating and egg laying.

Materials and methods

The eggs were removed from a single, unmated, female imago of *Cloeon dipterum* in May and from a single, unmated, imago of *Cloeon simile* in the following September. Each set was placed in a separate lidded Petri dish containing rainwater and kept at room temperature. The founder *C. dipterum* was taken from Wicken Fen, Cambridgeshire, U.K. (52°18'N, 0°17'E) in the subimago stage, and the founder *C. simile* was taken as a subimago from one of a group of small pools located just above the shore line on the Ardnamurchan Peninsula on the west coast of Scotland (56°43'N, 6°13'W): each founder subimago was reared in isolation to the imago stage, thus ensuring they were unmated.

When the first nymph hatched in each container, unicellular and filamentous algae and detritus were added; additional algae were added 1 week later and every few days thereafter. To compare the progress of early and late hatching nymphs, any nymphs that had hatched by the end of each week were transferred to new labelled containers (not more than ten nymphs per container). After $\approx 90\%$ of the eggs had hatched any further nymphs hatching after a gap of 2 weeks were individually reared. The approximate percentage of eggs hatching in each batch was recorded.

As individuals of the first generation matured, eggs were removed: (i) from last instar nymphs just before emergence (recognized by folding of wing tissues within the wingbud, or by pigmentation of the wingbuds); (ii) from subimagos; or (iii) from unmated imagos. These eggs, and the nymphs which hatched from them, were kept under similar conditions and treated in the same way as those from the founding females. Once the number of nymphs in any generation exceeded 100 some of the replicate dishes of nymphs hatching during each week were discarded. The lineage and life history of all nymphs was recorded. The process was repeated for nine successive generations of *C. dipterum* and seven of *C. simile*.

All containers were kept at room temperature, ranging from 12 to 22 °C, except for 2 weeks at a constant

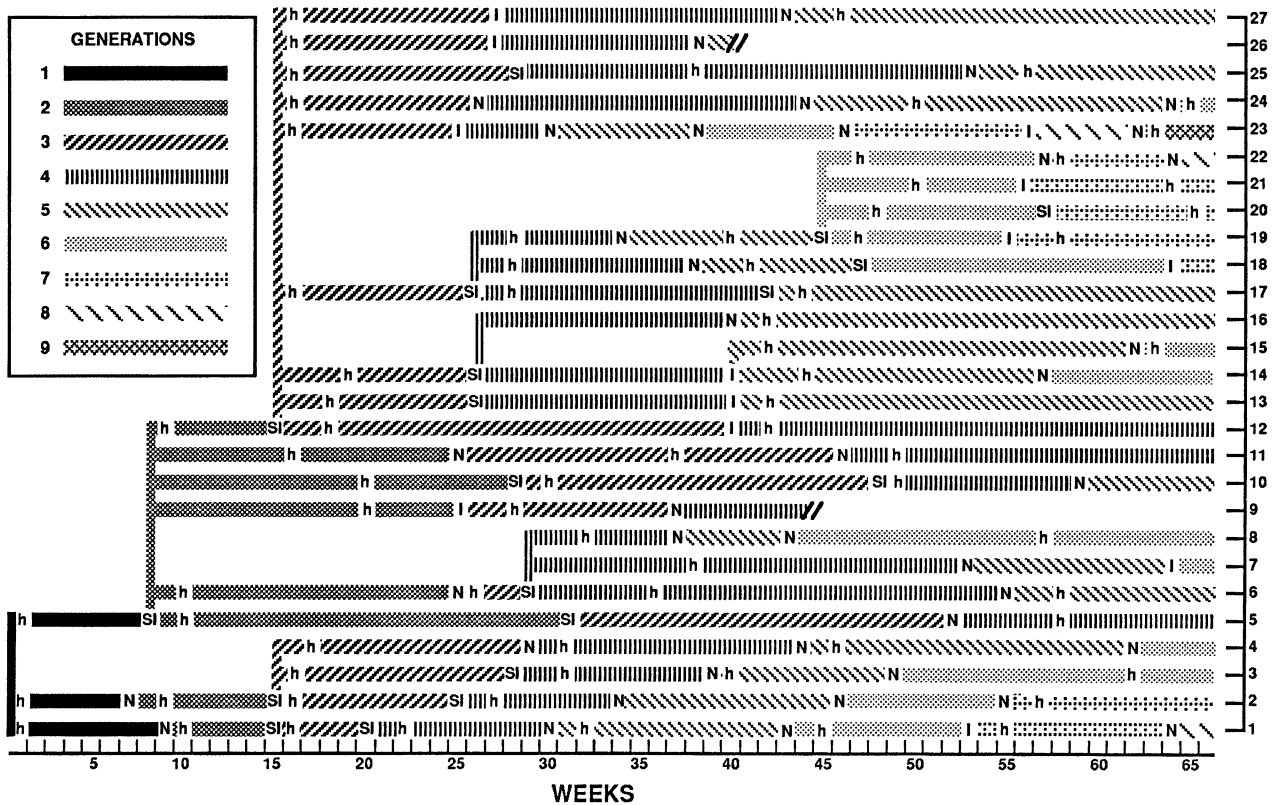


Fig. 1 The life histories of representatives of a succession of parthenogenetic generations (represented by horizontal lines) arising initially from eggs removed from a single virgin imago of *Cloeon dipterum*. Vertical lines connect eggs from the same batch. The developmental stage of the donors of the eggs for each generation is indicated by the letter at the end of the previous generation: h, hatched; N, final instar nymph; SI, subimago; I, imago.

4 °C (for *C. dipterum* representing weeks 33 and 34 in Fig. 1, for *C. simile* representing weeks 17 and 18 in Fig. 2), and 2 weeks at a constant 12 °C (representing weeks 60 and 61 for *C. dipterum* and weeks 44 and 45 for *C. simile* in Figs 1 and 2). These temperatures lie within the day-time ranges recorded during the egg-laying season and in December in the habitats of the founder *C. simile*.

Eggs were removed from ten randomly selected individuals of each generation and counted. Counts were also made of the number of eggs in ten subimagos and ten imagos of *C. dipterum* captured at the same site and time as the founder female, and in twenty subimagos reared from early instar nymphs captured in the field. Counts of the number of eggs in subimagos and imagos of *C. simile* from the source site are given by Harker (1989).

In the great majority of cases the percentage of eggs hatching in each set was recorded. In order to compare the hatching success of eggs from successive generations of parthenogenons with that of eggs from mated

and unmated imagos captured in the field, eggs were extracted from four *C. dipterum* females taken as they flew away from swarms (and which were seen apparently mating) and, on the same day, eggs were extracted from four females imagos captured as subimagos and therefore known to be unmated. These eggs were kept as described above and the number of nymphs hatching from each batch recorded.

To distinguish between any effects of the culture conditions on the final adult size and effects related to the parthenogenetic state, both the body length and the length of a single wing was measured for each of 111 subimagos of *C. dipterum* and fifty subimagos of *C. simile*, taken from a range of parthenogenetic generations, and for female subimagos of each species bred from nymphs captured in the field at an early nymphal stage (body length 2 mm or less) and reared under the same conditions as the parthenogenetic nymphs.

The pools on the Ardnamurchan Peninsula from which the founder *C. simile* female had been captured

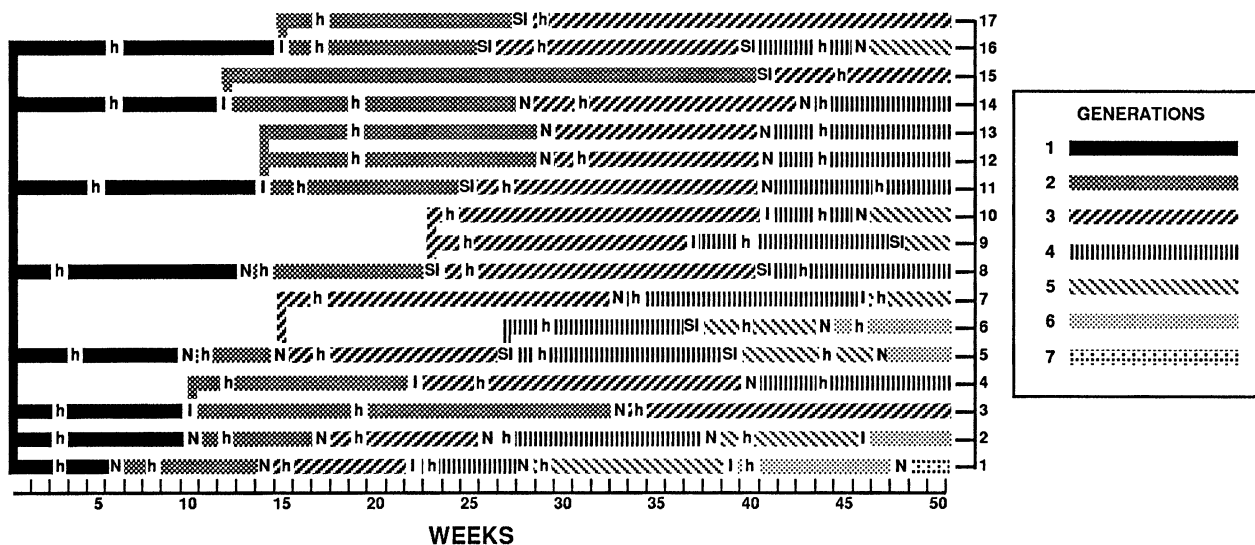


Fig. 2 The life histories of representatives of a succession of parthenogenetic generations (represented by horizontal lines) arising initially from eggs removed from a single virgin imago of *Cloeon simile*. Vertical lines connect eggs from the same batch. The developmental stage of the donor of the eggs from each generation is indicated by the letter at the end of the previous generation: h, hatched; N, final instar nymph; SI, subimago; I, imago.

were sampled in April, June–July and September from 1980 to 1993 inclusive (except for July 1992), and in December in 2 years. These collections were made over a range of times of day. The sex of each mature *C. simile* nymph in these samples, and that of subimagos captured nearby, was recorded.

To investigate whether ovovivipary is the only form of development in *C. dipterum* and *C. simile*, female imagos of both species were, within a few hours of being captured from mating swarms, placed in small individual cages containing water-filled Petri dishes which were regularly examined thereafter. Laboratory-reared females, individually isolated from the time of emergence, were treated similarly.

Results

Eggs of *Cloeon dipterum* that had been removed (a) from four imagos thought to have mated and (b) from four imagos known not to have mated, all hatched within 12–13 days. The percentage hatch from the two sets was, however, very different: 87–92% of set (a) eggs hatched whereas only 31–39% of the unfertilized eggs hatched. Nymphs from set (a) were both male and female, indicating that the observed mating had led to egg fertilization.

Over a period of 66 weeks up to nine generations of *C. dipterum* were reared as parthenogenetic descend-

ants of the founder female, and over a period of 50 weeks up to seven generations of *C. simile* were reared as parthenogenetic descendants of the founder. The details of the life cycles of 487 individual *C. dipterum* and 315 individual *C. simile* were recorded. The lineages of forty-four of these individuals (twenty-seven *C. dipterum*, seventeen *C. simile*) are illustrated in Figs 1 and 2: these representatives were selected to cover the observed range of variability in the length of egg and nymphal stages. All parthenogenetic offspring were female.

The time taken to complete individual generations was very variable, ranging from 4 to 33 weeks in *C. dipterum* and from 5 to 30 weeks in *C. simile*. There were two sources of variation: first, in the time taken for eggs to hatch, and second, in the length of nymphal life. For example, compare the individual lineages of *C. dipterum* featured in lines 9 and 11 of Fig. 1. In the second generation, despite the eggs of the two lines having hatched 4 weeks apart, the nymphs reached maturity at the same time. In the next generation, however, although there was an even bigger difference in time taken to hatch (9 weeks), it was the line which had been slowest to hatch in the previous generation which now hatched first. Indeed, one nymph of this latter batch had reached maturity before any of the eggs of the originally faster line had even hatched.

Overall, the time from egg release to the end of

Table 1 Generation time (egg to fully grown nymph; mean and SE) of successive parthenogenetic generations of *Cloeon dipterum* and *C. simile*. *P* values given for two-tailed *t*-test against generation 1: **P* < 0.05; ***P* < 0.01

Generation	Generation time (weeks)			Month of egg release
	Mean	SE	<i>n</i>	
<i>C. dipterum</i>				
1	10.9	0.6	43	May
2	15.1**	1.2	52	June–July
3	13.9	1.3	63	Aug.–Dec.
4	15.4**	1.5	71	Sept.–Jan.
5	17.0**	1.6	82	Nov.–March
6	14.9*	1.3	73	Feb.–June
7	15.4**	1.1	65	March–July
8	14.6**	1.4	34	May.–Aug.
9	14.2	1.3	4	July–Dec.
<i>C. simile</i>				
1	11.3	1.1	34	Sept.
2	14.4*	1.0	56	Oct.–Dec.
3	14.4	1.4	79	Dec.–April
4	11.5	0.8	62	Feb.–July
5	10.6	0.6	58	March–Aug.
6	10.9	0.4	21	June–Nov.
7	11.2	0.3	5	Aug.–Jan.

nymphal life was significantly shorter in the first generation of *C. dipterum* than in all but the third and ninth generations: generation times from the second generation onwards did not differ significantly (Table 1). Generation time of the first generation of *C. simile* differed significantly only from that of the second (Table 1), although there were also significant differences between the fifth and both the second and third generations. The mean generation time of *C. simile* was longer in the winter months, but there were no seasonal differences for *C. dipterum*. By the end of 50 weeks the surviving nymphs of both species included representatives of the third to seventh generations and, by the end of 66 weeks, surviving *C. dipterum* included members of the fourth to ninth generations. There were no significant differences in the length of nymphal life of individuals hatched from eggs removed from sources at different developmental stages (Table 2). (Note that the totals in Table 2 are slightly different from those in Table 1: this is because the developmental stage of the egg donor was not recorded in some of the earlier observations.)

Table 3 gives the summarized observations, for both species, of wing and body lengths of subimagos reared from nymphs captured in the field and of subimagos reared from successive parthenogenetic generations. A one-way analysis of variance was carried out on the values, and the *F* ratios are shown in Table 3; means in different generations were also compared

Table 2 Mean length of nymphal life of offspring from unfertilized eggs which had been removed from donors at different stages of development

Developmental stage of donor	No. of weeks of nymphal life		
	Mean	SE	<i>n</i>
<i>Cloeon dipterum</i>			
Full grown nymph	13.4	1.2	183
Subimago	14.9	1.1	192
Imago	14.6	2.2	104
<i>C. simile</i>			
Full grown nymph	13.0	1.1	123
Subimago	14.1	1.9	53
Imago	12.4	1.1	130

by Tukey tests. The results show that although there were significant differences between generations there was no significant trend with generation in parthenogenetic offspring of either species. In *C. simile* the wings of subimagos reared from nymphs captured in the field were significantly larger than those of subimagos produced by parthenogenesis, but there was no significant trend with generation in the parthenogenetic offspring.

In comparisons of body length there was no significant trend with generation nor was the difference between subimagos reared from nymphs caught in the field and those reared from unfertilized eggs significant.

Generation	Wing length (mm)		Body length (mm)		n
	Mean	SE	Mean	SE	
<i>C. dipterum</i>					
(a) 1	6.1	0.05	5.6	0.06	32
(b) 1	6.2	0.05	5.4	0.03	12
2	5.4	0.02	5.4	0.02	14
3	6.1	0.02	5.8	0.02	10
4	5.6	0.05	5.4	0.03	14
5	5.4	0.06	5.6	0.03	10
6	5.9	0.06	5.6	0.04	13
7	5.4	0.03	5.4	0.03	24
8	6.0	0.04	5.8	0.03	10
9	5.4	0.02	5.5	0.02	4
<i>C. simile</i>					
(a) 1	6.9	0.03	7.0	0.03	18
(b) 1	6.4	0.03	6.6	0.05	10
2	6.2	0.02	6.4	0.03	7
3	5.4	0.02	6.1	0.03	9
4	6.2	0.03	6.9	0.03	7
5	6.4	0.03	7.0	0.03	5
6	6.1	0.06	7.0	0.05	7
7	6.7	0.05	6.1	0.05	5

Table 3 Wing and body lengths (mean and S.E.) of female subimagos of *Cloeon dipterum* and *C. simile* that had emerged from nymphs reared under laboratory conditions: (a) reared from early instar nymphs (body length 2 mm or less) captured in the field; (b) reared from unfertilized eggs. One-way ANOVA: *F* ratios. Wing length: *C. dipterum* $F_{8,102} = 53.0$, *C. simile* $F_{6,43} = 77.6$; body length: *C. dipterum* $F_{8,102} = 33.3$, *C. simile* $F_{6,43} = 40.5$. In each case $P < 0.001$

Egg numbers in successive parthenogenetic generations are shown in Fig. 3. One-way analysis of variance was carried out on these values, giving *F* ratios of $F_{8,81} = 10.78$ for *C. dipterum* and $F_{6,63} = 11.87$ for *C. simile* (both $P < 0.001$). However, although there were significant differences between groups there was no pattern to the variation between generations. Paired comparisons using Tukey tests showed that the number of eggs in the first parthenogenetic generation of *C. simile* was significantly greater ($P < 0.05$) than the number of eggs in later generations, but in neither species was there any significant trend with generation in parthenogenetic offspring.

The mean numbers of eggs in imagos and subimagos of *C. dipterum* captured in the field were significantly greater ($P < 0.05$) than that in any of the parthenogenetic generations, whereas the egg number in subimagos reared in the laboratory from nymphs caught in the field was comparable with that from laboratory-reared parthenogenetic offspring (Table 4).

The percentage hatch from different egg batches (taken on the same day from donors at the same stage of development and themselves originating from a single batch of eggs and kept in the same container) differed by up to 2-fold (Fig. 4). However, there was no consistent trend in the percentage hatch with successive parthenogenetic generation.

The sex ratios of last instar nymphs and subimagos of *C. simile* collected from the source of the founder female are shown in Table 5. In the summer months the sex ratio was about 1, while there were nearly twice as many females as males in the April and December samples (there were no subimagos in December).

Despite the widely held view that these species are ovoviviparous, of the female imagos taken from mating swarms and placed over water four *C. simile* and eleven *C. dipterum* oviposited within 12 h, well before embryonic development could be completed. Five laboratory-reared unmated *C. simile* imagos were observed ovipositing within 5 days of emergence, and three laboratory-reared unmated *C. dipterum* imagos oviposited within 2 days of emergence (one only 2 h after emergence). When the remaining laboratory-reared imagos (thirty-eight *C. dipterum*, twenty-four *C. simile*) became moribund their eggs were removed: none appeared to have begun to develop, although the majority began to do so after being placed in water.

Discussion

The possibility that virgin females of either *C. simile* or *C. dipterum* might oviposit has long been discounted (Elliott & Humpesch, 1980). In the case of *C. dipterum*

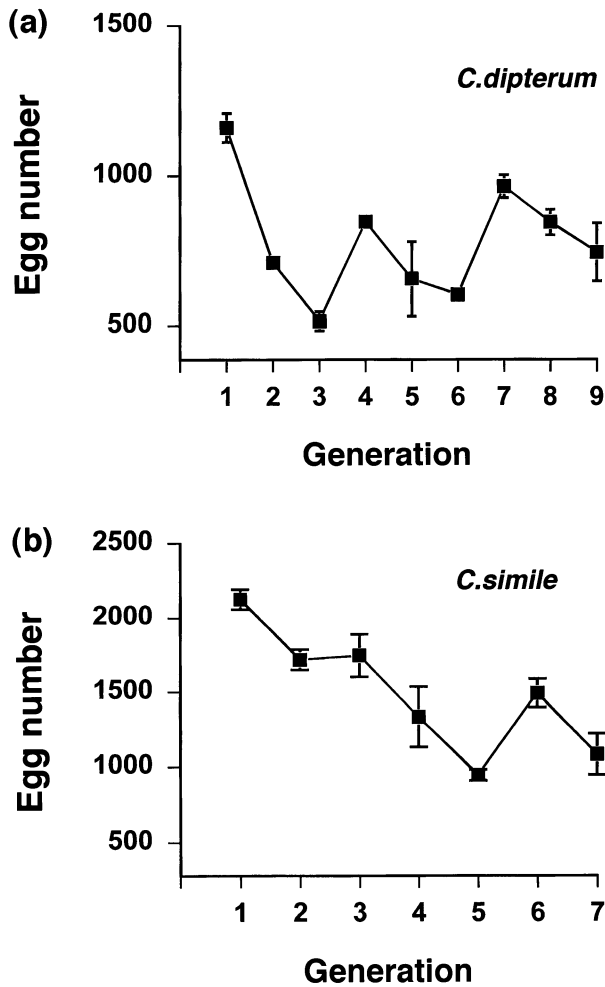


Fig. 3 Number of eggs (mean ± SE) produced by ten individuals in each successive parthenogenetic generation of (a) *Cloeon dipterum* and (b) *C. simile*.

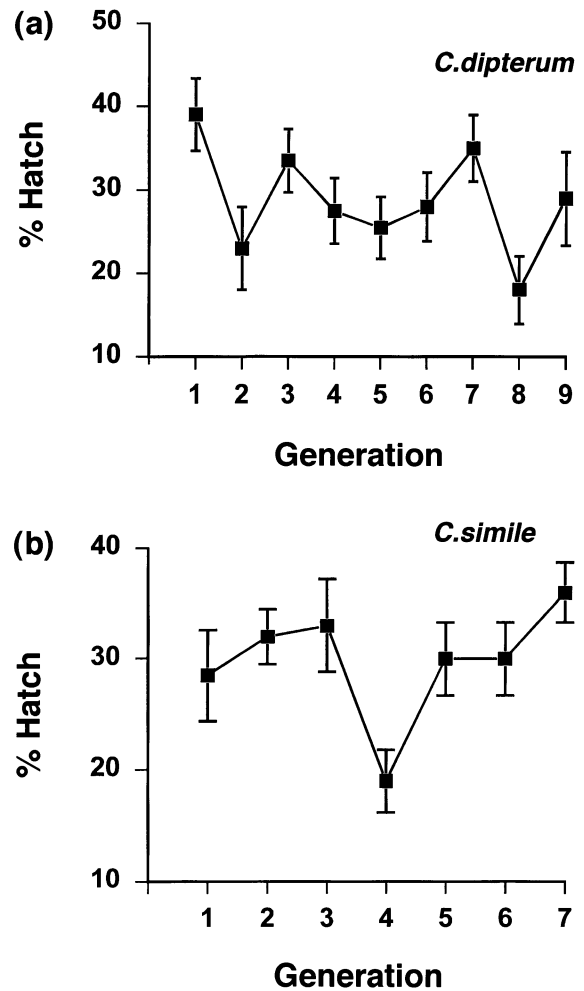


Fig. 4 Percentage of eggs (mean ± SE for each batch) which hatched in the egg samples of each successive generation of (a) *Cloeon dipterum* and (b) *C. simile*.

it has been believed that after copulation the embryos develop within the imago for 8–14 days, and that imagos avoid water until the eggs are ready to hatch (Degrange, 1959). Degrange concluded that a mating flight, as well as copulation, is a prerequisite for oviposition, believing that the muscular activity involved in flight drives the eggs down the oviduct where they develop. However, in this study eggs were found in both oviduct and bursa copulatrix at the time of emergence from the subimago, and oviposition by unmated imagos of both species was observed. It is clear that neither vigorous flight nor copulation are essential for oviposition. What is not known, nor has it previously been questioned, is how frequently contact with water in itself triggers oviposition by virgin females in nature.

Table 4 Number of eggs (mean and SE) in (a) ten subimagos and ten imagos of *Cloeon dipterum* captured in the field, and (b) twenty subimagos of *C. dipterum* reared from early instar nymphs (body length 2 mm or less) captured in the field

		Imagos	Subimagos
(a)	Mean	1245	1285
	SE	80	85
	Range	990–1620	998–1630
(b)	Mean		693
	SE		77
	Range		90–1110

Upon emergence a subimago usually flies away from water, and it is thought that the moult to the imago takes place at some distance away (Elliott & Humpesch, 1983). Nevertheless, subimagos are often caught on waterside vegetation and, although some

Table 5 Ratio of males : females of last instar nymphs of *Cloeon simile* in standard samples taken every year from 1981 to 1993 inclusive (except July 1992), and the sex ratio of subimagos collected at the same time from the surroundings of the collecting sites at Ardnamurchan Point. December readings in 2 years only. χ^2 test for inequality: ** $P < 0.01$; NS, $P > 0.05$

Month	Nymphs		Subimagos		Combined ratio
	Male : female	No.	Male : female	No.	
April	1 : 1.9**	242	1 : 2.2**	68	1 : 2.0**
July	1 : 0.8**	740	1 : 0.9 NS	146	1 : 0.8**
Sept.	1 : 0.9 NS	853	1 : 1.2 NS	126	1 : 1.0 NS
Dec.	1 : 2.0**	86			

leking sites are well away from water, swarms of both *C. dipterum* and *C. simile* are common near water and individuals are frequently trapped in the water surface (J.E. Harker, unpublished). We do not know what proportion of the *Cloeon* imagos floating on a particular stretch of water (and perhaps ovipositing) have never entered a mating swarm and/or have not mated. It is possible that parthenogenetic reproduction in these species is much more widespread than has been suspected hitherto. It is also possible that parthenogenesis could be more common in some regions than others (as has been shown to be the case for *C. triangulifer* McDunnough, a species in which males are absent in some places (Gibbs, 1977)). Observations of populations of *Cloeon* from other regions of Great Britain are needed therefore before any generalizations can be made.

A further possible source of unfertilized eggs may be mature nymphs or emerging subimagos whose body walls have been damaged physically or by predators: the release of eggs from these two stages of both *C. dipterum* and *C. simile* has frequently been observed (J. E. Harker, unpublished). Evidence presented here suggests that at least some eggs so released could develop and produce viable nymphs, and that the success rate would be independent of whether the eggs came from nymphs, subimagos or imagos.

If parthenogenetic eggs do make a significant contribution to a population then the sex ratio of that population should show a female bias (at least in the case of species in which all the parthenogenons are female). Such a female bias was found in *C. simile* nymphs collected from the Ardnamurchan pools in December, and in both nymphs and adults collected there at the beginning of the emergence season. Furthermore, there were more mature females than males in *each* of the samples taken at the beginning of the emergence season in *each* of the 13 years. Although

such an apparent imbalance could also arise by females maturing earlier than males, no sex difference in maturation time has been found, and synchrony of emergence by the short-lived males and females would be expected in order to ensure sexual reproduction.

Why should there be a female bias at these times of year? In May and June eggs have been seen issuing from the damaged bodies of many nymphs and emerging subimagos as these are attacked by gyrinids (virtually the only predators in the Ardnamurchan pools). Macan (1979) found that eggs of *C. simile* laid in May–June gave rise to adults in August–September, which suggests that parthenogenetic progeny from eggs released in May–June would bias the sex ratio of adults emerging in August–September: yet the present results provide no evidence of an imbalance of the sexes in August–September. The author has found, however, that unfertilized eggs generally take longer to develop than fertilized ones, so that any nymphs arising from unfertilized eggs released in May or June may not be sufficiently mature to sex until after the emergence season ends in late September. Consequently, such nymphs would still be present in December and, indeed, the December samples contained twice as many females as males. This bias in December could, however, equally arise by females maturing rapidly and reaching the stage at which they can be sexed earlier than males: therefore, the later samples collected in April, at the beginning of the emergence season, need also to be considered. The April samples also showed a female bias, thus strongly supporting the notion of a genuine inequality in the sex ratio of overwintering nymphs in these pools.

Since parthenogenetic adults of *C. dipterum* reared in the laboratory contained fewer eggs than the majority of adults captured in the field, we might suspect a rather low overall success rate for parthenogenetic offspring. The low egg production observed

in the present experiments could, however, also be due to the conditions under which the nymphs were reared. Some support for this second alternative comes from the fact that tiny nymphs captured in the field and reared under the same laboratory conditions as the parthenogenetic offspring, similarly produced few eggs. Further, the mean number of eggs in the first parthenogenetic generation of *C. dipterum* in the present experiments, and even the number found in some of the later generations, is actually higher than the figure of 600–700 quoted by Nagell (1981), or the 898–910 cited by Illies (1968) for that species in the field. In the case of *C. simile*, the mean number of eggs in the first parthenogenetic generation is within the range cited for imagos in the field by Degrange (1960) and Soldan (1979), although less than the 2378–3415 quoted by Illies (1968). *Cloeon simile* adults captured at the source site of the founder female of the present experiments show large variations in egg number seasonally as well as between individuals at any one time, ranging from 60–100 in April to 3–4000 in July (Harker, 1989). By comparison, the number of eggs produced, even by the sixth parthenogenetic generation of the present series, is well above that found in the field at some times of year.

It seems likely that the rearing conditions of the nymphs, rather than whether they had come from fertilized or unfertilized eggs, had the greater influence on the number of eggs produced in the present experiments. Overall, the results suggest an egg production sufficient to allow successive generations of parthenogenetic nymphs to survive in the field.

More important than the number of eggs produced, however, is the number that hatch. There was great variation in the hatching success. There was no consistent relationship between hatching success and the number of parthenogenetic generations that had passed, and the variability in percentage hatching remains unexplained. Overall, however, only about a third as many unfertilized as fertilized eggs hatched, a finding in keeping with Degrange's (1960) records of low hatching success by the unfertilized eggs of twenty-four facultatively parthenogenetic species. Yet there are also records of low hatching success of fertilized eggs of some widespread and commonly occurring species: for example Humpesch & Elliott (1980) and Humpesch (1982) found that less than 48% of apparently fertilized eggs of two species of *Ecdyonurus* and four species of *Rhithrogena* hatched.

Even if some parthenogenetic eggs have a low hatching success, they could still make a significant contribution to mayfly populations.

The time taken for hatching of the parthenogenetic eggs of both species of *Cloeon* to begin was, from the second generation onwards, very variable. Variation may be related to temperature fluctuations but, since Elliott & Humpesch (1980) found that small temperature fluctuations within the range of 10–20 °C had rather little effect on the hatching times of eight British and three other European species, it seems more likely that variability in hatching time is a feature of parthenogenetic development. Yet the interval between the extraction of eggs from nymphs of *C. dipterum* and the beginning of hatching was sometimes shorter than the period for which imagos of this species are thought to retain their eggs after mating (Degrange, 1959; Elliott *et al.*, 1988), although the period over which hatching continued was significantly greater for unfertilized than fertilized eggs.

As a consequence of the range of hatching times both within and between individual batches of fertilized eggs of *Cloeon* in the field, we could expect newly hatched nymphs to continue entering the population for several weeks after the end of the mating season (late September in Britain). Any newly hatched nymphs entering a British population after the end of October seem unlikely, however, to have come from fertilized eggs. Yet newly hatched *C. dipterum* nymphs have been taken in January in catches from East Anglia and Norfolk, although none have been found in the previous 3 months (J.E. Harker, unpublished). Elliott *et al.* (1988) likewise found very small nymphs late in winter but, since they believed that eggs of *C. dipterum* are laid only as they are about to hatch, they concluded that these nymphs must have hatched in autumn but not grown thereafter. In view of the present evidence there is, however, the possibility both of eggs overwintering and of eggs hatching in winter.

It is important to establish whether nymphs containing mature eggs are present once the imaginal season has ended. Nagell (1981) recorded *C. dipterum* nymphs feeding and growing (albeit slowly) when kept at 1 °C. Similarly, Brown (1961) recorded *C. dipterum* nymphs with fully developed wingbuds in January in England. Nymphs collected over the winter in East Anglia and Norfolk have, in each of a number of years, shown a monthly increase in mean length, and a few mature egg-containing nymphs have

been present in these samples in every winter month (J.E. Harker, unpublished). Nymphs of *C. simile* with fully developed wingbuds, and containing eggs, have been collected in Ardnamurchan in December. Since Elliott & Humpesch (1980) found that the lowest temperature at which embryos would develop is 3 °C, the development of any eggs released would depend on water temperature, but could occur in some areas.

Ephemeroptera are unique insects in two ways. The first is that females lack spermathecae—the pouches in which other insects store sperm that is later released as eggs pass the spermathecal opening. In mayflies the bursa copulatrix, into which the sperm are ejaculated, may already be packed with eggs at the time of copulation (Needham, Traver & Hsu, 1935). These eggs, and those still in the oviduct, may all be laid within minutes of copulation, giving the possibility of a significant number of unfertilized eggs. In such circumstances an ability to undergo parthenogenetic development may be of significant advantage.

The second unique feature of Ephemeroptera is the moulting of a winged stage. It is thought that the Palaeozoic ancestors of modern mayflies had more than two winged stages, the rest having now been eliminated (Kukalova-Peck, 1978), but it is unclear why *two* stages have been retained. Indeed, the retention of two winged stages is particularly unexpected in view of the fact that it is metabolically very expensive to produce a new cuticle, and in mayflies metabolites cannot be replaced since the mouthparts of both winged stages are vestigial and neither can feed. Furthermore, during eclosion the emerging insect is extremely vulnerable both to predators and water loss.

It has been argued (Schaefer, 1975; Edmunds & McCafferty, 1988) that the overriding advantage of the subimaginal cuticle covered with fine setae is that it prevents the insect from being trapped in the surface film while emerging. By undergoing another moult this cuticle can be replaced by a smooth cuticle that is aerodynamically advantageous during the mating dance (Marden & Kramer, 1994). While the latter may advantage males, the females of many species fly little (Harker, 1992): indeed, the females of some species (particularly those of *Caenis*) seldom escape completely from the subimaginal cuticle, and some parthenogenetic *Caenis* species oviposit before the eclosion of the subimaginal cuticle has begun (Froehlich, 1969). Perhaps these latter instances foreshadow the loss of

a subimaginal stage, at least in females, and an early maturation of the eggs.

Although there are no records of *C. dipterum* ovipositing before shedding the subimaginal cuticle, there are records of male imagos copulating with female subimagos (Degrange, 1960). Since both the subimaginal and imaginal cuticles of *C. dipterum* are laid down beneath the nymphal cuticle some days before the subimago emerges (J.E. Harker, unpublished), the imaginal stage, both anatomically and physiologically, begins during the last larval instar. It is not surprising therefore that eggs from the last instar nymph are as competent to produce parthenogenetic embryos as are those from an adult. Although there are no records of the complete absence of a winged stage in any mayfly species, and a loss of both winged stages would obviously affect the powers of dispersal, there does not appear to be any physical barrier to oviposition by nymphs.

Overall, the ability to reproduce sexually or asexually, depending on circumstances, may be a major factor in the success of this very primitive group of insects.

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References

- Bergman E.A. & Hilsenhoff W.L. (1978) Parthenogenesis in the mayfly genus *Baëtis* (Ephemeroptera: Baetidae). *Annals of the Entomological Society of America*, **71**, 167–168.
- Brown D.S. (1961) The life-cycle of *Cloëon dipterum* (L.) (Ephemeroptera: Baetidae). *Entomologist*, **94**, 114–120.
- Butler M.G. (1984) Life histories of aquatic insects. *The Ecology of Aquatic Insects* (eds V.H. Resh and D.M. Rosenberg), pp. 24–55. Praeger Press, New York.
- Degrange C. (1959) L'ovolarviparité de *Cloëon dipterum* (L.) (Ephemeroptera-Baëtidae). *Bulletin de la Société Entomologique de France*, **64**, 94–100.
- Degrange C. (1960) Recherches sur la reproduction des Éphéméroptères. *Travaux du Laboratoire de Pisciculture de l'Université de Grenoble*, **50/51**, 7–193.
- Edmunds G.F. & McCafferty W.P. (1988) The mayfly subimago. *Annual Review of Entomology*, **33**, 509–529.
- Elliott J.M. & Humpesch U.H. (1980) Eggs of

- Ephemeroptera. *Reports of the Freshwater Biological Association*, **48**, 41–52.
- Elliott J.M. & Humpesch U.H. (1983) *A key to the adults of the British Ephemeroptera*. Freshwater Biological Association Scientific Publication 47.
- Elliott J.M., Humpesch U.H. & Macan T.T. (1988) *Larvae of the British Ephemeroptera: a key with ecological notes*. Freshwater Biological Association Scientific Publication 49, Ambleside.
- Froehlich C.G. (1969) *Caenis cuniana* sp. n. a parthenogenetic mayfly. *Beiträge zur Neotropischen Fauna*, **6**, 103–108.
- Gibbs K.E. (1977) Evidence for obligatory parthenogenesis and its possible effect on the emergence period of *Cloeon triangulifer* (Ephemeroptera: Baetidae). *Canadian Entomologist*, **109**, 337–340.
- Gibbs K.E. (1979) Ovoviviparity and nymphal seasonal movements of *Callibaetis* (Ephemeroptera: Baetidae) in a pond in southwestern Quebec. *Canadian Entomologist*, **111**, 927–932.
- Harker J.E. (1989) Mayflies. *Naturalists' Handbook*, Vol. 13, pp. 1–56. Richmond Publishing Co, Slough, UK.
- Harker J.E. (1992) Swarm behaviour and mate competition in mayflies (Ephemeroptera). *Journal of the Zoological Society of London*, **228**, 571–587.
- Humpesch U.H. (1980) Effect of temperature on the hatching time of parthenogenetic eggs of five *Ecdyonurus* spp. and two *Rhithrogena* spp. (Ephemeroptera) from Austrian streams and English rivers and lakes. *Journal of Animal Ecology*, **49**, 927–937.
- Humpesch U.H. (1982) Effect of fluctuating temperature on the duration of embryonic development in two *Ecdyonurus* spp. and *Rhithrogena* cf. *hybrida* (Ephemeroptera) from Austrian streams. *Oecologia*, **55**, 285–288.
- Humpesch U.H. & Elliott J.M. (1980) Effect of temperature on the hatching time of eggs of three *Rhithrogena* spp. (Ephemeroptera) from Austrian streams and an English stream and river. *Journal of Animal Ecology*, **49**, 643–661.
- Hutchinson G.E. (1993) *A Treatise on Limnology, Vol. IV. The Zoobenthos* (ed. Y.H. Edmonson). John Wiley & Sons, Chichester.
- Illies J. (1968) Ephemeroptera (Eintagsfliegen). *Handbuch der Zoologie IV. Arthropoda Pt 2. Insecta* (ed. W. Kükenthal), pp. 1–63. W. de Gruyter, Berlin.
- Kukalova-Peck J. (1978) Origin and evolution of insect wings and their relation to metamorphosis, as documented by the fossil records. *Journal of Morphology*, **156**, 53–126.
- Macan T.T. (1979) *A key to the nymphs of the British species of Ephemeroptera with notes on their ecology*. Freshwater Biological Association Scientific Publication 20, Ambleside, UK.
- Marden J.H. & Kramer M.G. (1994) Surface-skimming stoneflies: a possible intermediate stage in insect flight evolution. *Science*, **266**, 427–430.
- Nagell B. (1981) Overwintering strategy of two closely related forms of *Cloeon* (*dipterum?*) (Ephemeroptera) from Sweden and England. *Freshwater Biology*, **11**, 237–244.
- Needham J.G., Traver J.R. & Hsu Y. (1935) *The Biology of Mayflies*. Comstock Publishing Co., Ithaca.
- Schaefer C.W. (1975) The mayfly subimago: a possible explanation. *Annals of the Entomological Society of America*, **68**, 183.
- Soldan T. (1979) The structure and development of the female internal reproductive system in six European species of Ephemeroptera. *Acta Entomologica Bohemoslovaca*, **76**, 353–365.

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