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The occurrence of lost and malformed legs in mayfly nymphs as a result of predator attacks

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A high proportion (13 %) of nymphs of the mayfly *Leptophlebia vespertina* in unpolluted streams in Central Sweden have been found to have malformed legs (Nilsson 1981). Gut dissections revealed that predacious caddisfly larvae (*Rhyacophila septentrionis*) had attacked and consumed *Leptophlebia* nymphs (legs recognized). Laboratory experiments showed that *Leptophlebia* nymphs lost legs when together with *Rhyacophila* and *Hydropsyche angustipennis* larvae, but not in the absence of the predators. Lost legs were regenerated to malformed appendages after a couple of days. The survival of nymphs that had lost legs was lower than for intact individuals, but some emerged as adults.

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1. Introduction

In a previous study I observed that about 13 % of the nymphs of species of the ephemerid genera *Leptophlebia* and *Heptagenia* in a Central Swedish stream had malformed legs. It was shown experimentally that malformed legs similar to those found in the wild were generated after legs were removed artificially (Nilsson 1981). Later observations in the wild revealed that nymphs with malformed legs are quite common among many mayfly species. The capability for leg regeneration in hemimetabolous insects is well documented (Newport 1845), but has mostly been discussed in terms of developmental processes (French 1982). Other types of malformations in aquatic insects have previously been either unexplained (Zwick 1976) or ascribed to industrial pollution (Donald 1980).

The high frequencies of malformed nymphs found in many unpolluted Swedish streams prompt some questions: Why do mayfly nymphs lose legs? What factors affect the frequency of leg loss in a mayfly population? If leg losses result from predator attacks, then do the predators consume the legs taken and does the loss influence the survival of the mayfly nymphs? Are mayfly nymphs adapted to discard leg fragments when attacked by a predator? In order to answer these questions a series of experiments were carried out (Table 1).

2. Material and methods

Nymphs (6–8 mm) of *Leptophlebia vespertina* L. and *Heptagenia fuscogrisea* Retz. were chosen as prey. They were obtained from two Central Swedish streams where specimens with malformed legs of both species are common. Larvae of two predatory caddisfly species, *Rhyacophila septentrionis* MacLachland and *Hydropsyche angustipennis* Curtis were collected in streams and used as predators. When the guts of some of the *Rhyacophila* larvae were examined, sclerotized body parts, including legs, from *Leptophlebia* nymphs were identified. The experiments were made in transparent polystyrene aquaria, size 20×30×6 cm. Water depth was 5 cm. When gravel (size 2–5 mm) was used it was about 1 cm deep. To avoid starvation effects the predators were kept together with large numbers of prey prior to the experiments. As food for the prey small amounts of Tetraphyll^R fishfood were given every one or two days. In order to avoid loss of legs caused by handling, a cut pasteur pipette was used to collect the nymphs. Every one or two days, the number of lost and malformed legs per nymph were counted.

3. Results

Prey specimens lost legs in all aquaria which contained predators, but in the absence

Table 1. The effects of two larval caddisfly predators (*Rhyacophila* sp. and *Hydropsyche* sp.) on the survival of individuals and the occurrence of lost (and malformed) legs of two mayfly nymphs (*Heptagenia fuscogrisea* Retz, and *Leptophlebia vespertina* L.). Category "L" indicates that the specimen had lost two legs, "N" that no legs were missing. For explanations regarding test No. 12 and 13 see text.

Test	Prey species	no./cat.	Predator species/no.	Bottom substr.	Duration days	Survivors no.	Survivors % malf.	Killed no./cat.
1	*H. fusc.*	20/N	None	Plastic	12	20	0	–
2	"	"	Rhya./1	"	12	10	80	–
3	"	"	" /3	"	12	8	75	–
4	*L. vesp.*	"	None	"	12	20	0	–
5	"	"	Rhya./1	"	12	9	78	–
6	"	"	" /3	"	12	7	57	–
7	"	"	Hydr./3	"	12	6	67	–
8	"	"	" /5	"	12	4	75	–
9	"	"	None	Gravel	12	20	0	–
10	"	"	Hydr./5	"	12	17	12	–
11	"	40/N	" /20	"	12	35	29	–
12	"	10/N, 10/L	None	Plastic	12	20	–	0/N, 0/L
13	"	10/N, 10/L	Rhya./3	"	8	–	–	4/N, 13/L

of predators no legs were lost. When a predator came in contact with a prey it immediately grasped one of the legs with its jaws and held the nymph tightly. The prey then vigorously undulated its body (as when swimming) until the predator either released the prey, the leg was pulled off or the prey was killed. If the prey got free, it immediately swam away. When a leg was pulled off, the breaking point was always between the femur and the trochanter. A lost leg was replaced by a small malformed leg within a couple of days. The malformed leg is a small, slightly distorted copy of a normal leg (Fig. 1). It was not observed whether the predator ate the pulled-off leg, but no loose legs were found in the aquaria. Nymphs that were killed were entirely or partly consumed.

When a nymph was attacked by a predator and lost one or more legs, its subsequent behaviour changed. Few swimming nymphs when caught and examined had lost legs indicative of failed predator attacks but nymphs taken when immobile on the bottom of the aquarium often had lost one or more legs, suggesting either that the mobility of these nymphs was reduced or that their response to injury was to remain immobile. At the end of an experiment the nymphs had often lost three, four or even more legs. This, however, is rarely the case in the wild, where the great majority only have one leg missing (Nilsson unpubl.). Of those nymphs attacked by a predator early in the experiments, a high proportion (81 %) lost only one leg. It seems that few nymphs lose more than one leg per attack.

The survival of nymphs after 12 days varied between 20 and 85 % (Table 1). Somewhat surprisingly, prey exposed to one, as compared to three, *Rhyacophila* individuals initially suffered heavier losses. The aquaria with higher densities of predators did not show either higher mortality or leg loss than the aquaria with fewer predators (Table 2). The final frequency of lost legs among the two prey species did not differ between the experiments except

Table 2. Mayfly mortality and leg loss (%) in the test aquaria with predators present. For test specifications see Table 1.

Test no.		Day no. 1	2	5	7	9	12
2	Mortality	15	40	45	50	50	50
	Leg loss	9	14	17	18	22	22
3	Mortality	0	5	30	45	60	60
	Leg loss	4	5	6	15	15	17
5	Mortality	40	50	55	55	55	55
	Leg loss	18	17	22	24	24	26
6	Mortality	10	15	35	40	50	65
	Leg loss	3	3	7	8	15	26
7	Mortality	5	10	20	30	50	70
	Leg loss	0	3	7	13	13	19
8	Mortality	0	5	45	55	70	80
	Leg loss	0	2	23	22	31	29

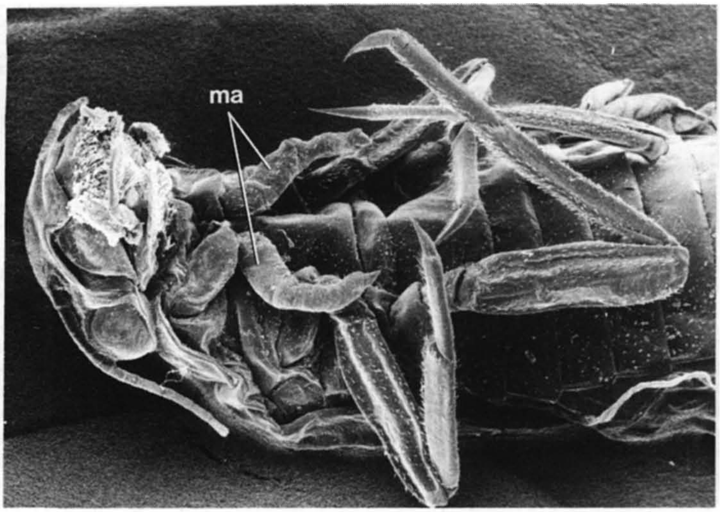

Fig. 1. Scanning electron micrograph of the ventral view of the anterior two thirds of a *Leptophlebia vespertina* L. nymph used in one of the experimental tests. Both fore legs were taken by a predator and subsequently regenerated. ma = malformed. 40 ×.

for when the bottom substrate was gravel. The presence of bottom substrate also greatly reduced the capture success of the *Hydropsyche* larvae (Table 1). When nymphs lacking two legs were kept with intact nymphs and predators, they were significantly more often preyed upon than normal nymphs (four normal and 13 with lost legs were killed) (χ^2-test, $P<0.05$) (Table 1).

A few nymphs from the experiments emerged. It was found that legs which were malformed in the last instar nymph did not reach full size in the adult.

4. Discussion

Changed locomotory behaviour and lower survival rates among injured nymphs are not unexpected. Under natural conditions the effects of leg loss probably would have been still more severe. On the other hand, some injured nymphs in the experiments did survive and emerged as adults. A leg loss thus does not necessary imply a lowered chance of emerging as adult. To test if leg losses affect individual fitness, one would have to compare the reproductive success of intact and handicapped individuals. In many mayfly species copulating males grasp females with the fore legs. Loss of these legs therefore may well impair copulation.

An escape mechanism has been recorded among many lizards and harvestmen (Opilionidae) which involves the release of a tail or leg when encountering a predator (autotomy) (Miller 1977, Vitt et al. 1977). Contrary to the mechanism of the mayfly leg loss, autotomy involves highly developed physiological and morphological adaptations (Dial & Fitzpatrick 1983). However, as the breaking site of a mayfly leg is always between the femur and the trochanter, some kind of morphological adaptation seems to exist.

The occurrence of deformities in aquatic insects has previously been ascribed to the action of industrial pollution (Donald 1980), but this study shows that it can have an entirely different explanation.

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