

Flight Direction in Some Rocky Mountain Mayflies (Ephemeroptera), with Observations of Parasitism

by

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Alexander S. FLECKER & J. David ALLAN: Flight Direction in Some Rocky Mountain Mayflies (Ephemeroptera), With Observations of Parasitism. Aquatic Insects, Vol. 10 (1988). No. 1, pp. 33-42.

Adult mayflies (Ephemeroptera) were sampled using sticky traps at several locations along a Rocky Mountain stream. Eight genera were collected, of which imagos of *Baetis* spp and *Rhithrogena hageni* were most common. *Baetis* was represented almost exclusively by females and flight direction was significantly in the upstream direction. In *R. hageni*, both males and females were included. Females appeared to fly into clearings or downstream, whereas males showed no directionality, or a downstream bias. This difference in adult flight direction may be partly explainable by the greater amount of downstream drift in *Baetis* relative to *R. hageni*. Finally, mermithid nematodes were found to be frequent parasites of *Baetis* but not of *R. hageni*, causing total absence of eggs in infected females.

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INTRODUCTION

The downstream transport of the immature stages of stream-dwelling insects, termed drift, is of widespread occurrence in running waters. This apparent unidirectional movement during the larval portion of the life cycle led Müller (1954) to posit that adults should fly upstream to compensate for gradual displacement downstream (first described by Mottram, 1932, citation in Müller, 1982). Although Müller's intriguing hypothesis, which he termed the colonization cycle, has been well studied in Scandinavia (see Müller, 1982 for review) there are relatively few studies from other regions of the world (see Pearson and Kramer, 1972; Bird and Hynes, 1981).

We report here on the movement patterns of two genera of mayfly (Ephemeroptera) from a Rocky Mtn stream. Our objectives were to 1) determine the directionality of adult flight for several common mayfly species, 2) examine whether flight patterns differ according to the position within sites commonly used for swarming and ovipositioning, 3) determine the proportion of females carrying eggs, as well as rates of parasitism by mermithid nematodes.

METHODS

The study was conducted at Cement Creek, Gunnison Co., Colorado during summer 1985. Cement Creek is a third order, clear, stony bottom stream that is bordered by willow, cottonwood, spruce, alder and aspen (a detailed description is found in Allan, 1975; Allan and Flecker, 1987). Reaches of the stream vary from being covered by a thick canopy to open and unshaded. Mayfly swarming and ovipositioning were frequently observed in open reaches, and rarely seen in heavily canopied sections of stream (Allan and Flecker, 1987); thus clearings appeared to represent preferred locations for mating activities. Mayflies were collected using 1 x 1 m sticky traps that were placed at the upstream, center, and downstream edges of several clearings where a number of mayfly species were commonly observed swarming and ovipositioning (unpubl). Each location was chosen because a distinct clearing occurred in an otherwise unbroken section of heavily canopied stream.

Sticky traps were made of clear plastic attached to a wooden frame and coated with a layer of transparent tanglefoot on both sides. Two traps were placed one above the other at each of the different sections of the clearing. Traps were roughly in the center of the stream and the lower edge was 10-20 cm above the water. Collections were made over three sampling periods (26-28 July, 31 July-4 August, 5-7 August) at location A (60 m downstream of the main swarm location used by Allan and Flecker (1987)). In addition we sampled a site located ca. 2 km upstream on 5-7 September (loc. C) and a third site ca. halfway between the other localities on 9-13 August (loc. B). The duration of the sampling period varied from 2-4 days in order to collect a sufficient number of adults for quantitative analysis. At the end of each period, mayflies were removed from the separate sides of the trap using paint solvent and preserved in 70% ethanol. Mayflies were sorted according to sex and subimago vs. imago for each species present. Flight directionality was analyzed using a G-test for goodness of fit (Sokal and Rohlf, 1981).

Finally, we examined a subsample of adult females from each sampling period to determine the proportion of females that were carrying eggs as well as rates of parasitism by nematodes. G-tests of independence (Sokal and Rohlf, 1981) were used to assess whether the proportion of females with eggs or parasites differed according to flight direction on each of the dates.

RESULTS

A total of 9 mayfly genera was captured on sticky traps, including *Baetis* (*B. bicaudatus* Dodds and *B. intermedius* Dodds), *Rhithrogena hageni* Eaton, *Cinygmula mimus* Eaton, *Epeorus longimanus* Eaton, *Ameletus*, *Siphonurus*, *Paraleptophlebia*, *Drunella* spp. and *Ephemerella* spp. However, few were sufficiently abundant to determine patterns of flight directionality, indicating

that the technique used was effective in capturing only some mayfly species. For example, *E. longimanus* was the most conspicuous mayfly present at Cement Creek (up to thousands of individuals were observed swarming on many evenings), but this species rarely appeared on sticky traps. The common species in sticky trap collections were *Baetis* spp (Family Baetidae) and *R. hageni* (Family Heptageniidae). Both *Baetis bicaudatus* and *B. intermedius* were present, but as adult females cannot be reliably identified (Jensen 1966), we grouped them together as *Baetis* spp.

Baetis was observed on all sampling dates, with adult females representing > 95% of the total number of *Baetis* captured. Since subimagos and adult males were infrequently captured we report flight activity for adult females only. This female-skewed sex ratio was due in part to differences in the flight patterns or ability to detect the trap by the sexes, as the female : male sex ratio on emergence was ca. 1.75:1 (unpubl.), whereas traps exhibited > 95:1 female bias. Flight activity of female *Baetis* was significantly biased upstream on most trap dates (Table 1). The proportion of total females flying upstream on a given date ranged from ca. 62% to 80%, and was always greater at the downstream edge of the clearing (Table 1).

Both male and female *Rhithrogena* were relatively common on sticky traps, although on all dates more females than males were collected. Direction of female flight appeared to depend on trap position within the clearing at loc. A, where we collected on three dates. Females at the downstream end of the clearing showed an upstream bias, whereas female flight in the upstream and center regions of the clearing was disproportionately downstream. This was not true for collections at loc. B and C, which also were later in the season. These two collections showed females to be common in the center of the clearing only, and flight was biased downstream (9-13 August) or was not significantly different between directions (7-9 September) (Table 2). When all collections from a clearing were grouped for a given date, flight directionality was generally downstream (Table 2). Males always displayed either a downstream bias in flight direction or no significant directional flight, regardless of trap position (Table 3).

Most adult female *Baetis* collected from sticky traps were carrying eggs. The proportion of gravid females ranged from ca. 65 to > 95% (Table 4). On two sampling dates, the proportion of gravid females differed significantly between upstream vs. downstream flight direction. A greater proportion of gravid females was observed flying upstream on 31 July-4 August, whereas the opposite was found on 29 August-2 September. Those females without eggs were usually infested with mermithid nematodes (Table 4). A single nematode would completely fill the abdomen in an infected *Baetis*. The rate of parasitism by nematodes increased as the summer progressed, and > 20% of adult females were infested by the final sampling date. When parasitized females were analyzed separately, a significantly greater proportion of these were found to be flying upstream as opposed to downstream on the early September sampling

Table 1. Numbers of adult female *Baetis* flying in an upstream vs. downstream direction. Data analyzed by G-test for goodness of fit test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Trap	Direction of Flight				net flight direction
	Downstream No.	(%)	Upstream No.	(%)	
26 - 28 July 1985, loc. A					
Up	32	30.8	72	69.2	up***
Center	23	33.8	45	66.2	up**
Down	6	5.0	114	95.0	up***
Total	61	20.9	231	79.1	up***
31 July - 4 August 1985, loc. A					
Up	61	22.3	212	77.7	up***
Center	60	33.0	122	67.0	up***
Down	5	3.1	154	96.9	up***
Total	126	20.5	488	79.5	up***
5 - 7 August 1985, loc. A					
Up	66	54.1	56	45.9	ns
Center	133	44.3	167	55.7	up*
Down	15	10.1	133	89.9	up***
Total	214	37.5	356	62.5	up***
9 - 13 August 1985, loc. B					
Up	65	94.2	4	5.8	down***
Center	78	60.0	52	40.0	down*
Down	159	18.8	708	81.7	up***
Total	302	28.3	764	71.7	up***
7 - 9 September 1985, loc. C					
Up	15	23.8	48	76.2	up***
Center	11	57.9	8	42.1	ns
Down	15	18.1	68	81.9	up***
Total	41	24.8	124	75.2	up***

date (Table 4). Thus parasitized and unparasitized females did not differ in their tendency to fly upstream.

In contrast, a lower proportion of female *Rhithrogena* contained eggs than was observed for *Baetis*. Proportions varied from ca. 8 to 50% depending on flight direction and date (Table 4). There were no significant differences in the proportions of gravid females flying up vs. downstream on any of the dates. In addition, mermithids were never observed in any of the *Rhithrogena* examined (Table 4).

Table 2. Numbers of adult female *Rhithrogena* flying in an upstream vs. downstream direction. Data analyzed by G-test for goodness of fit test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Trap	Direction of Flight				net flight direction
	Downstream No.	(%)	Upstream No.	(%)	
26 - 28 July 1985, loc. A					
Up	56	76.7	17	23.3	down***
Center	32	71.1	13	28.9	down**
Down	3	14.3	18	85.7	up***
Total	91	65.5	48	34.5	down***
31 July - 4 August 1985, loc. A					
Up	100	89.3	12	10.7	down***
Center	107	87.0	16	13.0	down***
Down	6	28.6	15	71.4	up*
Total	213	83.2	43	16.8	down***
5 - 7 August 1985, loc. A					
Up	14	73.7	5	26.3	down*
Center	34	70.8	14	29.2	down***
Down	0	0.0	11	100.0	up***
Total	48	61.5	30	38.5	down*
9 - 13 August 1985, loc. B					
Up	1	100.0	0	0.0	—
Center	85	72.6	32	27.4	down***
Down	9	40.9	13	59.1	ns
Total	95	67.9	45	32.1	down***
7 - 9 September 1985, loc. C					
Up	0	0.0	0	0.0	—
Center	23	53.5	21	46.5	ns
Down	0	0.0	0	0.0	—
Total	23	53.5	21	46.5	ns

DISCUSSION

Flight Direction

The tendency to fly in an upstream direction has been reported to vary with sex, stage (imago vs. subimago in Ephemeroptera, stage of egg maturation in Trichoptera) and species (Roos, 1957; Svensson, 1974; Bird and Hynes, 1981). For some species, strong upstream directionality apparently occurs only in mature females (Roos, 1957; Madsen et al., 1973, 1977). Mayfly subimagos and the males of various taxa may in fact show a weak bias toward downstream flight (Bird and Hynes, 1981). Thus upstream flight when it occurs apparently is post-mating, ovipositioning behavior. Differences in movement patterns

among species are sufficiently pronounced that Svensson (1974) recognized five types based on extensive trapping of Trichoptera, including a strong (his type 1) and a weak (his type 2) tendency to fly upstream. Both Roos (1957) and Svensson (1974) recognized that strongly lotic species show the greatest upstream flight behavior, while lentic species do not exhibit any directionality and rarely are captured on non-attracting traps.

Baetis females displayed a consistent bias in flight direction. Regardless of trap position in the clearing, females flew predominantly upstream. These results are in accord with the colonization cycle, and accord with most other reports for *Baetis* (Roos, 1957; Bird and Hynes, 1981; Müller, 1982). In contrast, Pearson and Kramer (1972) reported *B. bicaudatus* to be equally common flying both upstream and downstream. However, Pearson and Kramer based their

Table 3. Numbers of adult male *Rhithrogena* flying in an upstream vs. downstream direction. Data analyzed by G-test for goodness of fit test: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Trap	Direction of Flight				net flight direction
	Downstream		Upstream		
	No.	(%)	No.	(%)	
26 - 28 July 1985, loc. A					
Up	28	87.5	4	12.5	down***
Center	26	61.9	16	38.1	ns
Down	7	63.6	4	36.4	ns
Total	61	71.8	24	28.2	down***
31 July - 4 August 1985, loc. A					
Up	82	94.3	5	5.7	down***
Center	46	83.6	9	16.4	down***
Down	19	76.0	6	24.0	down**
Total	147	88.0	20	12.0	down***
5 - 7 August 1985, loc. A					
Up	19	100.0	0	0.0	down***
Center	24	88.9	3	11.1	down***
Down	0	0.0	1	100.0	ns
Total	43	91.5	4	8.5	down***
9 - 13 August 1985, loc. B					
Up	2	100.0	0	0.0	ns
Center	44	51.8	41	48.2	ns
Down	23	69.7	10	30.3	up*
Total	69	57.5	51	42.5	ns
7 - 9 September 1985, loc. C					
Up	0	0.0	0	0.0	—
Center	0	0.0	4	100.0	down*
Down	0	0.0	0	0.0	—
Total	0	0.0	4	100.0	down*

Table 4. Percent females carrying eggs and parasitized by mermithid nematodes. Significant differences in proportion of gravid or infested females, as analyzed by G-test of independence, are indicated.

Genus	Date	Flight Direction	n	% Females	
				w/Eggs	w/Nematodes
<i>Baetis</i>	26 - 28 VII	Upstream	70	91.4	7.1
		Downstream	35	88.6 ^{ns}	5.7 ^{ns}
<i>Baetis</i>	31 VII - 4 VIII	Upstream	61	93.4*	3.3
		Downstream	66	81.8	3.0 ^{ns}
<i>Baetis</i>	9 - 13 VIII	Upstream	100	96.0	3.0
		Downstream	100	98.0 ^{ns}	2.0 ^{ns}
<i>Baetis</i>	29 VIII - 2 IX	Upstream	365	73.2**	23.0**
		Downstream	145	90.0	9.7
<i>Baetis</i>	7 - 9 IX	Upstream	65	72.3	23.1
		Downstream	23	65.2 ^{ns}	21.7 ^{ns}
<i>Rhithrogena</i>	26 - 28 VII	Upstream	13	15.4	0.0
		Downstream	49	36.7 ^{ns}	0.0
<i>Rhithrogena</i>	31 VII - 4 VIII	Upstream	6	33.3	0.0
		Downstream	47	8.5 ^{ns}	0.0
<i>Rhithrogena</i>	9 - 13 VIII	Upstream	48	52.1	0.0
		Downstream	22	36.4 ^{ns}	0.0
<i>Rhithrogena</i>	29 VIII - 2 IX	Upstream	21	33.3	0.0
		Downstream	18	27.8 ^{ns}	0.0
<i>Rhithrogena</i>	7 - 9 IX	Upstream	23	13.0	0.0
		Downstream	24	25.0 ^{ns}	0.0

findings on visual observations that may have confounded any potential differences between sexes. The weight of evidence for *Baetis* suggests that compensatory (upstream) flight occurs as females are searching for suitable sites to oviposit after mating.

We found no evidence that *Rhithrogena* makes compensatory upstream flight. Although on some dates female *Rhithrogena* displayed a bias towards upstream flight at the traps on the downstream side of the clearing, flight direction of females at the upstream and center regions was skewed downstream. This suggests that females fly from all directions into clearings and that a greater number of females enter than exit. Male *Rhithrogena* generally showed either no significant flight directionality or downstream directional bias. These data clearly do not support the colonization cycle for the mayfly *Rhithrogena*.

Both *Baetis* and *Rhithrogena* are rheophilic mayflies that occupy fast-flowing regions of the study stream (Allan, 1975). Thus differences in their flight

Table 5. Ratio of drift density (no. m^{-3}) / benthic density (no. m^{-2}) for *Baetis* and *Rhithrogena* at Cement Creek during several sampling dates from 1977 (from Allan unpubl.). Sites are those described in Allan (1982).

Date	Site	<i>Baetis</i>	<i>Rhithrogena</i>
13 - 16 VI	3	31.0	0.54
5 - 7 VII	1	3.55	0.0
	2	6.22	1.78
25 - 26 VII	1	13.76	0.52
	2	3.61	2.16
	3	2.28	0.10
12 - 14 X	1	0.18	0.0

behavior cannot be ascribed to the lotic-lentic dichotomy as in Trichoptera (e.g., Svensson, 1974). However, these two species do differ markedly in their tendency to enter the drift. *Baetis* is the most common invertebrate in the drift of Cement Creek and generally exhibited much greater drift densities than did *Rhithrogena* (Allan, 1987; and unpubl.). Drift propensity, measured by the ratio of drift:benthic densities, always was much greater for *Baetis* than for *Rhithrogena* (Table 5), and this would suggest a greater need for upstream flight. In a similar vein, Svensson (1974) suggested that upstream flight was less common in his study than that of Roos because his collections came from a small stream where downstream displacement may have been minor, whereas Roos studied a large river where upstream flight might be a greater imperative. Our contrasting results for *Baetis* and *Rhithrogena* suggest that the likelihood of drifting long distances downstream as nymphs may enhance the frequency of upstream compensatory flight.

Many of the same species that show upstream flight behavior also exhibit distinctive mating systems whereby males form aggregations or swarms at landmarks, to which females approach and where mating occurs in flight (Downes, 1969; Savolainen, 1978; Allan and Flecker, 1987). Our observation that a number of species (especially *E. longimanus* and *R. hageni*) swarmed in clearings led us to predict that both males and females might fly into clearings either as subimagos or as imagos not yet mated. However, *E. longimanus* rarely flew along the stream. Collections of *R. hageni* did support the expectation that both sexes tend to fly into clear areas and not exit clearings for shaded stream sections. While some such mechanism presumably plays a role in bringing together males and females at swarm locations, our evidence on this admittedly is weak. At present, rather more is known about flight behavior of mated females than of not yet mated individuals of both sexes.

Furthermore, our data also suggest that trap location may influence the outcome and interpretation of flight directionality results. For example, if traps

were placed on the downstream end of a clearing we might arrive at a different conclusion for *Rhithrogena* than if they were placed at the upstream end.

Mermithid Parasites

Larval mermithids (Nematoda: Mermithoidea) have been observed in a number of mayfly nymphs at this site (Haley and Allan unpubl.) including *Baetis* and *Epeorus*. Hominick and Welch (1980) review the scant literature on mermithid-mayfly associations; only some mayfly species from a given site may be parasitized, and the frequency usually is quite low (< 5%). Typically a single worm is present, but virtually the entire abdomen and part of the thorax is occupied. Mermithids exit the host by penetrating its body wall, killing the host. Although larval infection rates rarely approached 5% (Haley and Allan, unpubl.) up to 20% of adult *Baetis* females were parasitized by late summer. These females were sufficiently capable of flight to encounter sticky traps and showed the same upstream flight behavior of mated females. Quite possibly, parasitized females experience slowed maturation (Hominick and Welch, 1980), and this explains their increased representation in the population later in the season.

Mermithids typically render their hosts sterile (if the host survives) due to depletion of nutrients (Needham et al., 1935; Hominick and Welch, 1980), consistent with our observations. Hominick and Welch suggest that adult mayflies may aid the dissemination of their parasites, presumably when the host alights on water. If one assumes that downstream displacement during larval life is as much a problem for the parasite as for the host, our finding that parasitized females of *Baetis* exhibit a pronounced bias toward upstream flight suggests the possibility that the host also may provide to the parasite a mechanism for compensatory upstream movement.

ACKNOWLEDGEMENTS

We thank B. Feifarek, A. Hicks, J. Lovell, N.L. McClintock, S. Travers and numerous friends for help with field work. The Rocky Mountain Biological Laboratory provided space and facilities. Support by NSF grant BSR 8500738 is gratefully acknowledged.

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