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A comparative study of the costs of alternative mayfly oviposition behaviors

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Abstract Oviposition behavior of insects has associated fitness costs related to the probability that females survive to oviposit. During summer 2003, we observed the oviposition behavior and compared the mortality rates of females of 17 mayfly species in one western Colorado watershed. We dissected adult females collected on terrestrial sticky traps, in drift nets submerged in streams, and in stomachs of brook trout to determine whether the mayflies had oviposited before capture, drowning, or consumption. Females oviposited by either splashing on the water surface releasing all their eggs (splashers), dropping their eggs from the air (bombers), dipping their abdomens multiple times releasing a few eggs at a time (dippers), landing on rocks and ovipositing on the undersides (landers), or floating downstream while releasing their eggs (floaters). Almost 100% of lander and 50% of dipper females had not oviposited when captured on sticky traps, increasing their vulnerability to preoviposition mortality by aerial predators compared to mayflies with other behaviors. In contrast, most females had laid their eggs before drowning or being eaten by a fish (50–90%). However, groups with oviposition behaviors most exposed to the water surface (floaters, then splashers, dippers, and landers) were more vulnerable to drowning before completing oviposition. In addition, splashers and floaters were most vulnerable to predation by brook trout before ovipositing. These data suggest that fitness costs associated with preoviposition mortality may be considerable depending on mayfly oviposition behavior. Furthermore, previously demonstrated benefits of low predation rates on eggs of lander species may be offset in part by costs to female survival.

Keywords Ephemeroptera · Oviposition behavior · Fitness costs · Mortality · Fish predation

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Introduction

Oviposition behaviors in insects are extremely diverse and range from relatively unselective to highly specialized (Hinton 1981). Females with alternative oviposition behaviors experience variable risks and consequently incur different relative costs and benefits (Alcock 1998). Female insects can increase their reproductive success by ovipositing at locations that maximize the survival of their offspring or minimize egg mortality (Kouki 1991; Thompson and Pellmyr 1991; Price et al. 1998). Females can also increase their fitness by minimizing preoviposition mortality (Michiels and Dhondt 1990; McMillan 2000).

In general, aquatic insects have been considered to have nonselective oviposition behaviors (Hinton 1981). However, some Odonata (Wildermuth and Spinner 1991), Diptera (Blaustein and Kotler 1993; Canyon et al. 1999), Hemiptera (Smith 1976), Trichoptera (Reich and Downes 2003), and Ephemeroptera (Elliott and Humpesch 1983; Peckarsky et al. 2000) have highly specialized oviposition behaviors with the potential to increase egg or offspring survival. Nonetheless, adaptive values of different oviposition behaviors in aquatic insects have rarely been evaluated either from the perspective of postoviposition (Fincke 1986) or preoviposition costs (but see Michiels and Dhondt 1990).

Adult females of all species of mayflies (Ephemeroptera) are terrestrial, but return to the water to lay their eggs and have no parental care or egg-guarding behaviors (Needham et al. 1905). Oviposition behaviors of mayflies are highly diverse (Edmunds et al. 1976; Elliott and Humpesch 1983), which is likely to result in variable rates of preoviposition mortality. For example, depending on their oviposition behavior, females may be more or less vulnerable to consumption by aquatic predators (principally fish), aerial predators (e.g., predatory insects or birds), or drowning before releasing their eggs. Jackson and Fisher (1986) estimated that only 3% of the biomass of emerged aquatic insects returned to the stream to oviposit, which suggests high preoviposition mortality rates of adult females.

Little is known about the mortality rates of female mayflies during oviposition, or the relative cost or benefits of alternative oviposition behaviors. The goals of this study were to (1) describe the oviposition behaviors of different mayfly species in the East River drainage basin near the Rocky Mountain Biological Laboratory (RMBL) in western Colorado, and (2) compare the levels of female preoviposition mortality among mayflies with different oviposition behaviors. Using this comparative approach we developed hypotheses about the costs associated with different oviposition behaviors in mayflies and determined that certain types of oviposition behaviors are associated with a significantly higher probability of egg mortality before oviposition.

Materials and methods

Study area

We studied mayfly populations ovipositing in the East River near the RMBL at ~2,900 m elevation in the West Elk Mountains, Gunnison County, CO, USA (38°57′30″N, 106°59′15″W). At RMBL the East River is a third-order trout stream, 10–15 m wide, with mean discharge decreasing from ~4 to 0.50 m^3/s throughout the flight season of these mayflies (June to September). The riparian vegetation of the study site is dominated by Engleman spruce (*Picea englemanii*), Douglas Fir (*Pseudotsuga menziesii*), aspen (*Populus tremuloides*), and many species of willows (*Salix* spp.). However, the canopy above these streams is generally open, providing a high sunlight environment for observation of mayfly flight behavior during swarming, mating, and oviposition.

Studies of the stream insects of this area have reported the larval biology of mayflies in the East River near RMBL, mostly in the families Baetidae, Heptageniidae, Ephemerellidae, and Ameletidae (Peckarsky 1985, 1991). Previous studies have not considered the adult biology of mayfly species other than *Baetis bicaudatus* (Peckarsky et al. 2002).

Observations of mayfly oviposition and preoviposition mortality

During the flight period in summer 2003 we recorded the oviposition behaviors of 17 different mayfly species (Fig. 1) and compared potential causes of female mortality and effects of oviposition behavior on preoviposition mortality. We obtained comparative data using three general approaches.

1. *Traps to estimate relative abundance of ovipositing females* (*potential risk to females of aerial predation before ovipositing*)

Because direct measures of aerial predation by birds, bats, or insects were not feasible, we estimated the number of females caught during flight with or without eggs as a surrogate of preoviposition risk of aerial predation. We estimated the relative abundance of flying females over daily and seasonal cycles from collections of female mayflies on two transects of sticky traps, evenly spaced (50 cm apart) and suspended vertically 3–8 cm above the East River (to catch females flying near the stream surface). Sticky traps (11 total) consisted of transparent acetate sheets (27.9×21.6 cm) with tree pest adhesive (Tanglefoot®) applied to upstream and downstream sides. Sticky traps were deployed on 7, 14, 21, and 28 July and 4 August, and changed two or three times daily.

We also placed 13 horizontal "platform traps" 3–8 cm above the stream surface to catch females ovipositing in a 150-m reach of the East River. Platforms were made of plywood (27.9×21.6 cm) and anchored to the streambed using rebar. Platforms were distributed at random (>3 m apart) and remained in place for 24 h on the same dates as the vertical traps. We applied tree Tanglefoot® to three transparent acetate sheets stacked on top of each platform. One of the three sheets was removed at 0800, 1400 and 2000 hours.

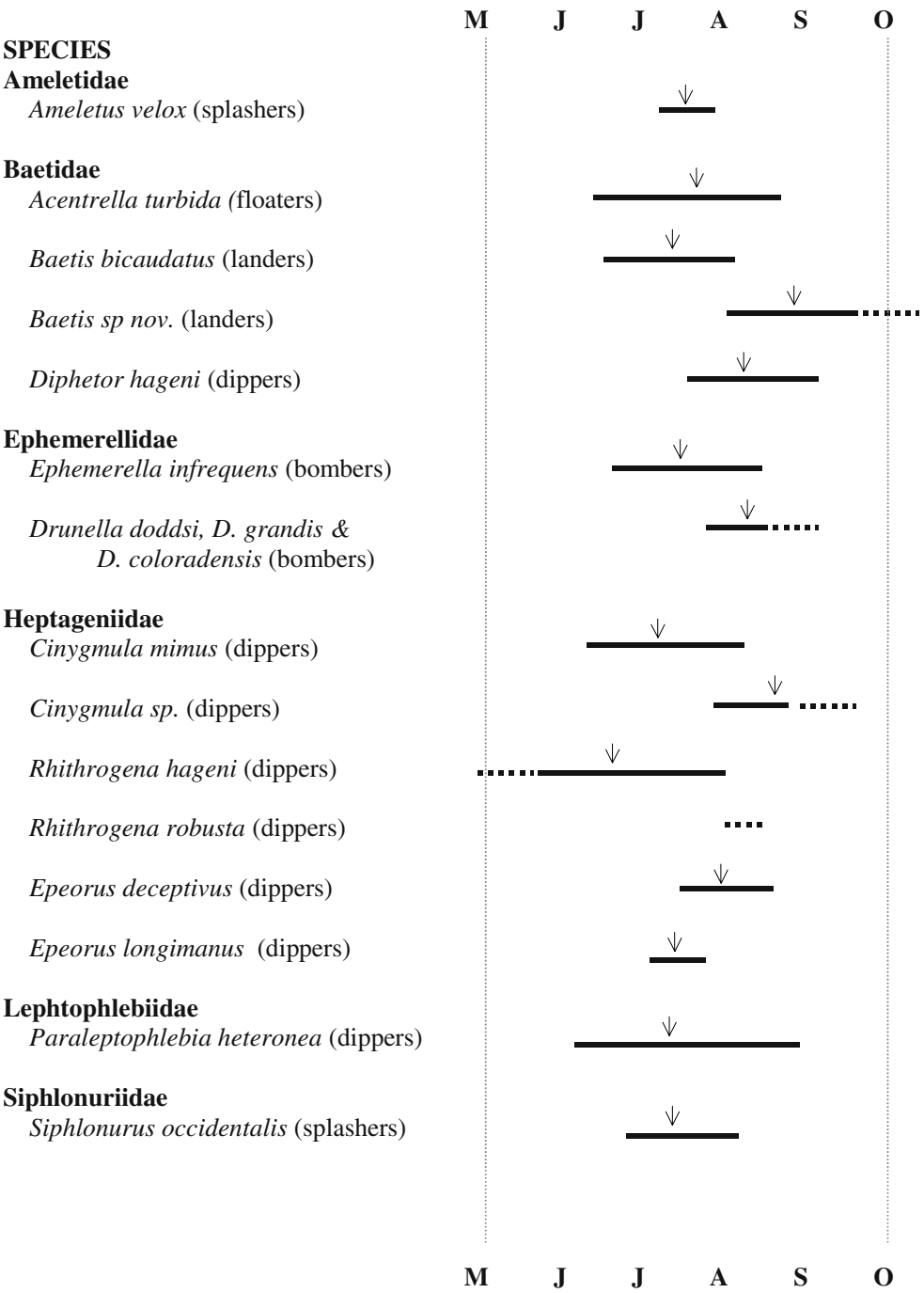


Fig. 1 Flight periods of 17 species of Ephemeroptera in the East River watershed near RMBL. *M* May, *J* June and July, *A* August, *S* September, *O* October. *Black lines* show duration, *dotted lines* show flight periods when less than 20 individuals were observed, and *arrows* approximate peak flight period. Information compiled from horizontal and vertical sticky traps, malaise traps, and weekly observations in 2003. *Vertical dashed lines* indicate approximate dates of ice off and ice on.

In the laboratory we removed mayflies from the sticky traps by placing each sheet in a pan of solvent (Googone®). We subsequently identified mayflies according to species, sex, and subimago vs imago. We also measured total body length excluding cerci and antennae and dissected females to estimate the percentage of eggs remaining in the abdomen.

To supplement the trap data, every week from 3–31 July, we placed a ~15-m-long by 1-m-tall mesh net across a 13-m-wide transect of the East River for 5 min at 1300 and 1600 hours. We collected all mayfly subimagoes, imagoes, and exuvia in 70% ethanol for further analysis in the laboratory. We also made daily collections of flying mayflies using a Malaise trap above one location in the East River from 15 July to 15 October. Finally, once per week for 9 weeks (June to August) during the flight period (0730–2130 hours) we made continuous observations of mayfly oviposition behavior at three locations along the

East River. After observations, individuals were collected with sweep nets to identify the mayfly species exhibiting each type of oviposition behavior.

2. *Drift nets to estimate relative mortality of females that drown before ovipositing*

To estimate the relative proportions of females with and without eggs that got carried away in the water column during oviposition, we placed at random locations in the East River six drift nets (opening 45×29 cm) on 7 July and four drift nets on 14, 21, and 28 July and 4 August 2003. Nets were deployed two times daily at 0900 and 1300 hours and were left in the stream for 3 h. The nets were fastened to the stream bottom with rebar and completely submerged to avoid collecting females touching the water surface during oviposition. The discharge passing through each net averaged overall dates was ~0.12 m^3/s. Imagoes and subimagoes that were captured in the drift nets were preserved in 70% ethanol. In the lab we sorted mayflies according to species and sex and dissected females to estimate the percentage of eggs remaining in each female, as described above.

Finally, to determine whether ovipositing females could reattach onto rocks once entrained in the current during July 2003, we collected 20 ovipositing female *B. bicaudatus* and released them into a 5-m-long wooden trough in the East River with eight rocks (200–450 cm^2) protruding from the water surface and a drift net at the downstream end. The current velocity in the trough was 0.43±0.12 m/s, which was comparable to the mean velocity in the stream at that time (0.52±0.23 m/s). Ten minutes after the release we checked every rock and counted the females remaining and females that drifted into the net.

3. *Relative mortality of females consumed by fish before ovipositing*

Possible aquatic predators of female mayflies in this system include brook trout (*Salvelinus fontinalis* Mitchell), which feed on aquatic and terrestrial stages of aquatic insects and swallow their prey whole (Allan 1978, 1981). Although brook trout are not native to this system (Pister 2001), they were the only species of predatory fish at the East River study site. To estimate the relative abundance of females with and without eggs that were consumed by brook trout, we electroshocked the East River on 7, 14, 21, and 28 July and 4 August 2003 for 2–3 h during the morning, afternoon, and evening (total ~8 h/day), collecting 30 brook trout in total. We flushed the stomachs of each fish using stream water in a plastic squirt bottle attached to Tygon® tubing (Kamler 2001). Fish regurgitates were preserved in 90% ethanol and analyzed later in the laboratory. We sorted mayflies according to species, sex, and imagoes vs subimagoes, measured body lengths, and dissected females to estimate the percentage of eggs remaining in their abdomens.

Data analysis

To compare the proportion of total females among different oviposition behaviors that still had their eggs when captured in flight (surrogate of risk of aerial predation), in drift nets (drowned), or fish guts (consumed), we performed a general linear model procedure (SAS Institute 2003) for a two-way repeated measures analysis of variance (ANOVA). Our response variable was the arcsine transformed proportion of females with eggs at times T1 through T5 (Sokal and Rohlf 1981). The fixed factors were (1) trapping method with three levels flying, drowning, and eaten by fish, and (2) female oviposition category (five levels). Because "time" was not a significant factor in the within-subject effects (Encalada 2005), we reassigned the time replicates (T1–T5) as true replicates and repeated the analysis as a two-way ANOVA. We performed a posteriori Tukey's Studentized Range [honestly significant difference (HSD)] tests for both factors to interpret specific comparisons.

Finally, we used one-way ANOVA to test for differences in mean size of all females (with and without eggs) captured while flying, drowned, and consumed for 11 mayfly species for which we had sufficient data. We also performed a two-way ANOVA to test if sizes of all mayflies combined differed between females with and without eggs (factor 1) that drowned or were eaten by fish (factor 2). We made a posteriori pairwise comparisons using Tukey's HSD tests when there were significant main effects or interactions.

Results

Mayfly oviposition behaviors

Mayfly flight periods (emergence, swarming, and oviposition) extended from June to October in the upper East River watershed (Fig. 1). However, these patterns may vary among years depending on winter and summer precipitation; high water years usually have earlier and longer emergence periods than low water years (Peckarsky et al. 2000; Encalada 2005). Flight periods of some species were more synchronous (e.g., *B. bicaudatus* and *Cinygmula mimus*), and others extended for longer periods (e.g., *Paraleptophlebia heteronea*). Daily oviposition period of most mayflies was from 0900 to 1700 hours with peak flight activity around midday (1200–1300 hours). Nighttime female catches included very few Ephemeroptera (Encalada 2005). Nonetheless, we observed large male swarms of *Epeorus* spp. from 1700 to 2000 hours (as in Flecker et al. 1988) and also during early morning starting around 0730 hours. Some female *C. mimus* were captured at night, suggesting the possibility of nighttime oviposition (Encalada 2005).

Based on our observations and the published literature (Needham et al. 1905; Elliott and Humpesch 1983), we classified 17 mayfly species into five functional oviposition categories: splashers, bombers, dippers, floaters, and landers (see Fig. 2 and S1 for animations of the different flight patterns).

(1) *Splashers*. (e.g., Fig. 2a) This category included two species, *Ameletus velox* and *Siphlonurus occidentalis*, whose females flew in aggregations. Both species bounced above the water surface while flying upstream. After alighting on the water surface they got carried downstream for 1–1.5 m, discharged all their eggs at once, and then flew away. Most mayflies from this group oviposited in fast velocity (~65 cms^{-1}) locations with no turbulence.

2) *Bombers*. (e.g., Fig. 2b) This category included all species of Ephemerellidae. Egg masses were extruded from the gonopore of the seventh abdominal segment of females during relatively slow downstream or upstream flight, very close to the water surface. Usually the entire egg mass was released from the air; but if the extruded egg mass touched the water surface it was released immediately. Female bombers were observed to drop their eggs in fast and slow velocity places.

3) *Dippers*. (e.g., Fig. 2c, d) This category was very taxonomically diverse, including most species of mayflies in the East River catchment (all species of Heptageniidae, some Baetidae, and one species of Leptophlebiidae). Females flew upstream or downstream above the water surface and dipped their abdomens in the water several times, releasing a few eggs each time. Some species flew very fast covering great distances (e.g., Fig. 2c, *Rhithrogena hageni*), while others flew slowly and dipped at short distances (e.g., Fig. 2d, *Diphetor hageni*).

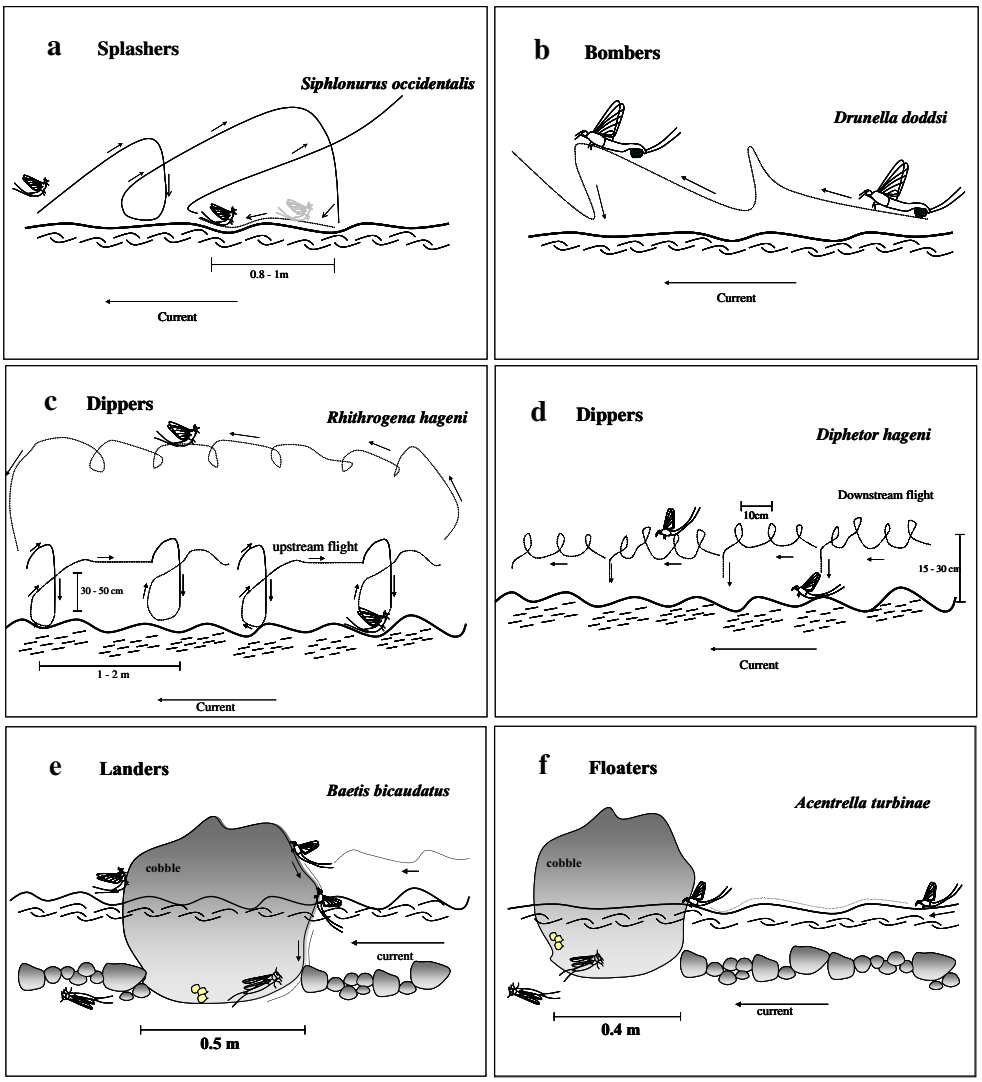


Fig. 2 Oviposition behaviors of different mayflies (Ephemeroptera) observed in the upper East River drainage basin, near RMBL in western Colorado. **a** Splashers (e.g., *S. occidentalis*); **b** bombers (e.g., *Drunella doddsi*); **c**, **d** dippers (e.g., *R. hageni*, *D. hageni*, and *E. longimanus*); **e** landers (e.g., *B. bicaudatus*); and **f** floaters (e.g., *Acentrella turbinae*). See Fig. 1 for a complete list of species showing each behavior.

Heptageniidae had some interesting behaviors not observed in other mayflies. Females and males flew in the same swarms (~80 to >500 individuals). While most males performed pendular flights 3–7 m above the water surface, females flew very close to the water (<1 m). Most remarkably, some males followed females while they were ovipositing and copulated with females between dipping episodes, exhibiting male-guarding behavior that may be similar to some Odonata (Waage 1987). Chases between males were very common, suggesting male–male competition. Females oviposited in relatively fast velocity, turbulent places (riffles). We also observed *Epeorus deceptivus* and *Epeorus longimanus* flying together with *R. hageni* in mixed species swarms.

We observed two other variations in the oviposition behavior of this group. *P. heteronea* females flew very fast in small groups (6–8 individuals) near the water surface and primarily dipped close to the stream banks in slow or fast velocity water. We never observed males of this species guarding females or mating with them during oviposition. Finally, single *D. hageni* (Baetidae) females flew rather slowly just above the water surface (15–30 cm), and dipped in nonturbulent places, usually near the stream banks.

4) *Landers*. (e.g., Fig. 2e) Females of all observed species of *Baetis* have been reported to land on a rock protruding from the stream in high velocity locations, crawl under water on the downstream side of the rock, and lay one egg mass on the underside (Eaton 1888; Peckarsky et al. 2000). Females face the current during oviposition and lay eggs while moving their abdomens from side to side, resulting in egg masses shaped like tombstones.
5) *Floaters*. (e.g., Fig. 2f) *Acentrella turbida* (Baetidae) females had two alternative oviposition behaviors. Some females landed on fast or slow water surfaces, drifted until they contacted a large rock, then immediately crawled under the rock and laid a small (mean 9 mm^2) irregular egg mass. We suspect that other females released some eggs while drifting and then flew off the water surface because many females caught in horizontal and vertical sticky traps had only a small percentage of eggs remaining in their abdomens. It is interesting to note that we never observed or captured male subimagoes or imagoes of *A. turbida*, suggesting this species may be parthenogenetic.

Preoviposition mortality

The proportion of females with and without eggs captured by all three methods differed with respect to oviposition category (Fig. 3; $F_{14,69}$=9.9; P<0.001). However, the proportions of total females that still had their eggs when captured while flying, in drift nets, or fish guts (consumed) did not vary through the season (no significant time effect; $F_{4,52}$=0.59, P= 0.58 adjusted for sphericity test; SAS Institute 2003).

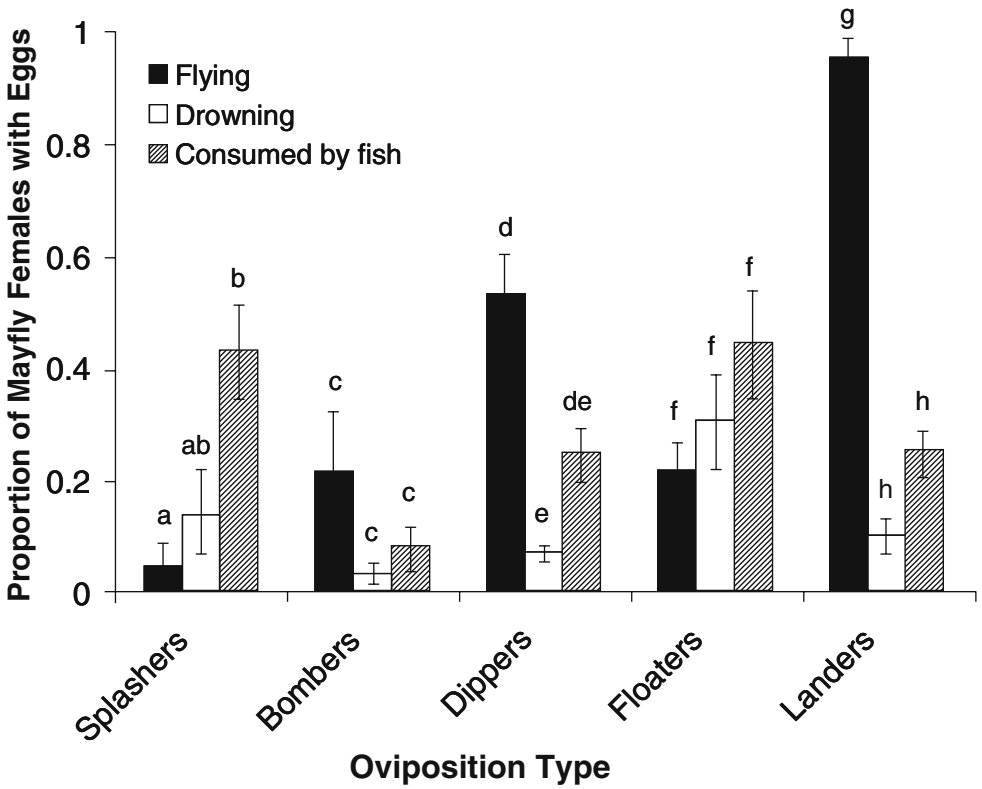


Fig. 3 Average proportion of total mayfly females of each oviposition category that had not yet oviposited their eggs when captured during flight (on vertical and horizontal traps), drowned (in submerged drift nets), and eaten by fish (in fish regurgitates). *Bars* represent ±1 SE. Histograms with *same letters* were not significantly different (Tukey's HSD tests) as a result of pairwise comparisons within each oviposition type. See Table 1 for pairwise comparisons among oviposition types.

Table 1 Summary of a posteriori pairwise comparisons (Tukey's HSD test) among the proportion of females captured with eggs in each "trapped " category (flying, drowned, or eaten by fish) between oviposition types (splashers, bombers, dippers, landers, and floaters) (comparisons within columns)

Factor 2: oviposition type	Factor 1: trapped		
	Flying	Drowned	Eaten by fish
Splashers	a	b	b
Bombers	a	a	a
Dippers	b	b	a
Floaters	a	c	b
Landers	c	b	a

Different letters denote significantly different (alpha=0.05) proportions of females captured with eggs in pairwise comparisons among oviposition types within each trapping method. Groups with the same letter were not significantly different and denote low (a) to high (c) risk behaviors. See Fig. 3.

Therefore, we reran the ANOVA using dates as true replicates. The proportion of females with eggs differed significantly among trapping methods ($F_{2,85}$=12.03, P=0.0001) and among oviposition types ($F_{4,85}$=10.76, P=0.0001). Most importantly, there was a significant interaction term ($F_{8,85}$=12.08, P=0.0001), indicating that variation among trapping methods in the proportion of females that were captured before ovipositing depended on oviposition type.

Pairwise comparisons within each oviposition category showed that splashers were more vulnerable to being eaten by fish before ovipositing than by drowning or while flying (Fig. 3). It is interesting to note that more females than males were observed in fish guts and in some cases only females were found, reflecting their increased vulnerability to predation while ovipositing. In contrast, a significantly higher proportion of dippers and landers captured while flying still had their eggs than those captured in drift nets or in fish stomachs (Fig. 3).

Pairwise comparisons between oviposition categories showed that landers and dippers had a higher proportion of females with eggs caught flying near the stream than any other group (Fig. 3, Table 1). It is interesting to note that almost all (95% percent) female landers captured in sticky traps had not yet oviposited, and most of the 5% captured without eggs were infested with mermithid nematodes. In addition, floaters were more vulnerable to drowning and bombers were the least vulnerable to drowning than other mayfly groups (Fig. 3, Table 1). Finally, brook trout consumed a higher proportion of females with eggs of splashers and floaters than other mayfly groups (Fig. 3, Table 1), making those oviposition behaviors more vulnerable to trout predation.

Variation in mortality with female size

The one-way ANOVA revealed size-dependent variation in overall female mortality for 4 of 11 species of mayflies analyzed (Table 2). While drowned and consumed females of *A. velox* [mean body length (L)=12.5 mm] were larger than their flying counterparts (L=7.5 mm), drowned females of *B. bicaudatus* (L=5 mm), *Ephemerella infrequens* (L=6.4 mm), and *P. heteronea* (L=6 mm) were smaller than females caught while flying (L=8.3, 10, and 11 mm, respectively). Similarly, *B. bicaudatus* females that drifted off rocks before ovipositing were significantly smaller than those who had successfully oviposited.

Finally, for all species combined, females eaten by fish were significantly larger than females that drowned ($F_{1,1}$=19.81, P<0.0001). However, there was no significant difference between sizes of females that died before or after ovipositing ($F_{1,1}$=0.73, P=0.39). It is interesting to note that the interaction term was also not significant ($F_{1,1}$=0.22, P=0.64), indicating that variation in size of females that drowned or were eaten by fish did not depend on whether they had oviposited.

Table 2 Summary of one-way ANOVA testing for differences in the mean body length of female mayflies caught on sticky traps (F flying), in drift nets (D drowned), or in fish regurgitates (C consumed)

SPECIES	*df* (par,error)	*F*	*P* value	Tukey's HSD (α=0.05)
Acentrella turbida	2,80	0.096	0.9085	
Ameletus velox	2,7	7.75	0.0168	F>D=C
Baetis bicaudatus	2,237	5.49	0.0047	F>D=C
Cinygmula mimus	2,76	1.06	0.3509	
Diphetor hageni	2,118	0.82	0.4423	
Epeorus deceptivus	2,40	2.07	0.1391	
Ephemerella infrequens	2,143	6.79	0.0015	F>D>C
Paraleptophlebia heteronea	2,288	7.80	0.0005	F>D=C
Rhithrogena hageni	2,282	0.72	0.4871	
Siphlonurus occidentalis	2,70	0.69	0.5072	

Analyses were performed for each species individually. A posteriori HSD Tukey's tests indicate pairwise comparisons in cases with significant main effects.

Discussion

We observed impressive variation in behaviors of mayflies ovipositing in the East River. Females oviposited by either splashing on the water surface releasing all their eggs, dropping their eggs from the air, dipping their abdomens multiple times releasing a few eggs at a time, landing on rocks and ovipositing on the undersides, or floating downstream while releasing their eggs. Although we estimated ~30 to 40% overall preoviposition mortality of adult females, some mayflies performed better than others, depending on their strategies during oviposition. Bombers had the lowest overall preoviposition mortality, and groups with more contact with surface water (floaters and splashers) were more likely to drown or be eaten before ovipositing (Fig. 3). While landers and dippers had intermediate rates of preoviposition drowning and predation, high rates of capture of egg-bearing females flying near the water suggested the potential for greater exposure to aerial predators.

Effects of oviposition behavior on preoviposition mortality

Seasonal periodicity Flight periods of mayflies were highly seasonal (Fig. 1), which is typical for other sites at high altitudes and latitudes (Elliott and Humpesch 1983), in contrast to extended or aseasonal flight periods observed for mayflies at lower altitudes (Jackson and Fisher 1986) or latitudes (Jackson and Sweeney 1995). Such seasonality has the potential to affect mortality risk of females during oviposition. For example, high discharge early in the summer might constrain the onset of oviposition by increasing the probability of females drowning before ovipositing, especially for those with higher contact with water (e.g., splashers, floaters, and landers). It is interesting to note that the species that oviposited earliest in the season were dippers (Fig. 1), a strategy with relatively low probability of drowning before ovipositing (Fig. 3). On the other hand, ovipositing later in the summer when snowmelt waters recede and water clarity improves could increase the vulnerability of females to trout predation. We observed that species ovipositing latest in the season were dippers, bombers, or landers (Fig. 1), a behavior that was least susceptible to preoviposition trout predation (Fig. 3). Nonetheless, ovipositing too late in the season might affect the probability of finding reasonable oviposition sites and increase the probability of egg desiccation for landers (Encalada and Peckarsky 2006).

Diel periodicity Diel periodicity of oviposition might also be related to the abundance and activity patterns of predators (brook trout, insects, and birds). Insectivorous birds, for example, are more active at dawn and dusk (McCarty and Winkler 1999), which could constrain the diel periodicity of mayfly oviposition. It is interesting to note that females rarely oviposited at dawn or dusk in these streams, with the exception of some Heptageniidae that swarmed and oviposited at dusk (Encalada 2005). However, gut contents of brook trout indicated that they fed on ovipositing females throughout the day from 0800 to 2000 hours. Thus, females could only avoid trout predation if they oviposited at night, which was rare in this study (Encalada 2005).

Aggregation It is interesting to note that some females of dippers, landers, and floaters were observed flying in small groups (~7 to 15 individuals). Although reports of female mayfly aggregations are rare, *Ephemerella* spp. have been observed to form female swarms before oviposition (Pleskot and Pomeisl 1952). Communal oviposition might result from females being attracted to common oviposition sites (Waage 1987; Allan and Flecker 1989), or by association with swarming males (Savolainen et al. 1993). Regardless of the proximate mechanisms explaining this behavior, aggregation during oviposition could ultimately affect preoviposition mortality by reducing individual risk of predation via dilution (Waage 1987; Michiels and Dhondt 1990), predator satiation (Sweeney and Vannote 1982), or predator confusion (Krakauer 1995). Heptageniidae males usually swarm above open canopy, unshaded, and highly reflective sites (Flecker et al. 1988; Allan and Flecker 1989), which makes it difficult for visual salmonid predators to distinguish ovipositing females at the air–water interface against the bright background (Elliott and Humpesch 1983). Alternatively, aggregation during oviposition might attract predators and increase the probability of being eaten (Corbet 1964; McCarty and Winkler 1999). Further data are necessary to test definitively the costs and benefits of female aggregation during oviposition.

Mate guarding An unusual behavior for mayflies observed in this study was contact and noncontact guarding of females by male Heptageniidae during flight. Males were commonly observed to harass females and force repeated matings during female dipping episodes. Because mayfly eggs are fertilized as they travel from the oviducts to the genital duct (Needham et al. 1935), repeated mating in species with dipping oviposition behavior may ensure paternity in these species. Similar harassment behavior has been observed in Odonata resulting in ~95% fertilization precedence by the last male to mate (e.g., Michiels and Dhondt 1990). In some species of Odonata male guarding has been reported to decrease female mortality risk (Fincke 1986; Michiels and Dhondt 1988). However, male presence might also increase female reproductive costs related to time and energy expenditure during oviposition (Michiels

and Dhondt 1990). The fitness costs or benefits of mate-guarding behaviors are not known in mayflies.

Site selection The oviposition behavior of mayflies in general has been described as nonselective (Hinton 1981), given that females (except for landers) do not precisely control the destination of their eggs. Studies of terrestrial herbivorous insects suggest a strong evolutionary link between female oviposition preference and offspring performance (Thompson 1988; Craig et al. 1989; Ohgushi 1992; Price 1994). However, selective oviposition behavior by mayflies may have little influence on the survival of their offspring because larvae are highly mobile generalist grazers capable of selecting their own foraging habitat (Richards and Minshall 1988; Palmer 1995). Consequently, we argue that mayfly oviposition behavior should be under stronger selection pressure to minimize female preoviposition mortality and maximize egg survival than to maximize offspring performance.

Nonetheless, we observed some site selection in all oviposition strategies, potentially resulting in tradeoffs between minimizing female mortality and maximizing egg survival. For example, most females in all categories of female behaviors oviposited in fast velocity locations (Encalada 2005), which could have both costs (increase probability of preoviposition drowning of females) and benefits related to increasing egg dispersal. Egg dispersal by stream current might reduce the probability of mass predation on eggs, or maximize unpredictability of offspring dispersion (Thompson and Pellmyr 1991). Although eggs of most mayflies have adhesive devices to prevent drifting long distances (Needham et al. 1935; Hinton 1981), eggs of *Palingenia longicauda* have been reported to drift as far as 2.5 km from the oviposition site (Russev 1959). Drifting eggs could also end up in suboptimal environments for development, such as trapped in sediment, locations vulnerable to egg desiccation, or suboptimal temperatures (Elliott and Humpesch 1980). Therefore, the lander strategy provides the most benefits in terms of increasing the probability of egg survival (Encalada and Peckarsky 2006), while trading off the observed costs of potentially high preoviposition predation.

Egg release strategies vs predation risk Terrestrial stages of aquatic insects have been shown to provide important food subsidies for terrestrial predators (Murakami and Nakano 2001; Sabo and Power 2002). In fact, Nakano and Murakami (2001) showed that aquatic prey represent 25.6% of the annual total energy demand of the entire bird assemblage in a forested stream. One study showed that mayflies comprised more than 50% of the diets of a population of tree swallows during the summer months (Wayland et al. 1998). Other studies found that densities of emerging insects were highly correlated with densities of flycatchers (Gray 1993), as well as with spatial distribution and abundances of lizards (Sabo and Power 2002). We observed tree swallows, flycatchers, and warblers commonly feeding on swarming male mayflies and on females during oviposition flights. Thus, our sticky trap data and observations suggest that some of the observed oviposition strategies (landers and dippers) could have high potential costs in the form of bird predation on egg-bearing females. However, direct comparisons of aerial predation of mayflies are needed to draw more definitive conclusions regarding the relative fitness costs and benefits among the different oviposition behaviors and how they affect variability in preoviposition mortality.

Another aspect of female oviposition behavior that could affect female survival and egg survival is whether eggs were released all at once (e.g., splashers and bombers) or a few at a time (e.g., dippers, floaters, and landers). Laying a few eggs at a time may increase the probability some eggs would be released before females died, while the "all eggs in one basket" strategy may reduce time of exposure of egg-bearing females to predation by aerial predators. Our data were not consistent with this argument even though female splashers, which lay all their eggs at once, had the lowest probability of being caught in aerial traps before ovipositing (Fig. 3). However, bombers, the other group that lay all their eggs at once, had similar probabilities of aerial capture to floaters (Fig. 3), which release a few eggs at a time.

Females were also vulnerable to trout predation before ovipositing. Allan (1978) reported that 14% of emerging aquatic insects were eaten by brook trout in a nearby stream; and adult forms of aquatic insects made up more than 20% of invertebrate biomass in diets of coho salmon in an Alaskan stream (Allan et al. 2003). However, previous studies do not provide information about fish predation on ovipositing aquatic insects. Our data showed that terrestrial stages of aquatic insects constituted a large proportion of the diets of brook trout (~30 to 40% from June to September), a third of which was ovipositing females. Furthermore, preoviposition predation rates varied with the amount of time females spent on the water surface (Fig. 3). Bombers, which do not contact the water surface, had the lowest vulnerability to fish predation, and splashers and floaters, which drift on the water surface while ovipositing, had the highest rates of brook trout predation. However, we do not know whether mayfly eggs are viable after passing through the digestive track of fish, as has been seen in some aquatic invertebrates (Jarnagin et al. 2000). It is interesting to note that most lander females consumed by brook trout had already laid their eggs, indicating a reduced cost due to fish predation for females that have this type of oviposition behavior.

A very high proportion of female landers captured flying close to the stream surface still had their eggs, most of the other 5% with no eggs captured in sticky traps were infested with mermithid nematodes. Parasitized females do not develop eggs and, when ready to oviposit, extrude the mermithid instead (Flecker and Allan 1988; Vance 1996). These data suggest that lander females rarely flew again after ovipositing under a rock, as corroborated by experimental releases of females in the in-stream wooden flumes, which showed that 97% of drifting females did not settle on rocks once they were in the water column.

Tradeoffs involved in female size

Size at oviposition may affect the probability of female survival and determines the fecundity of survivors (Elliott and Humpesch 1983; Peckarsky et al. 2001). Small individuals of three species (*B. bicaudatus*, *P. heteronea*, and *E. infrequens*, a lander, dipper, and bomber, respectively) were more vulnerable to drowning than large ones, suggesting another cost to being a small female in these groups. In contrast, large individuals of *A. velox* females (splashers) were more vulnerable to drowning than small ones. Females of all species combined that were eaten by fish were significantly larger than females that drowned, which could partially offset the general fecundity benefit to insects of being a large female. This pattern may be explained by larger prey being more profitable or more visible to predators (cf. Li et al. 1985), or as an experimental artifact of size-selective predation (i.e., fish could have eaten the larger females before they were caught in the drift nets). Large egg-bearing females may also be more conspicuous to bird predators. In a study of diet selectivity of tree swallows, McCarty and Winkler (1999) showed that selectivity for large insects increased with insect abundance, a pattern that holds among insectivorous birds in general (Hespenheide 1971). Therefore, variation in the size of ovipositing female mayflies may reflect tradeoffs between vulnerability to predation, drowning and fecundity.

In summary, preoviposition mortality of female mayflies by drowning and by fish predation was substantial and dependent on their type of oviposition behavior. Females that did not touch the water surface had the lowest risks of fish predation and drowning before ovipositing. In contrast, females with longer exposure to the water surface had the highest preoviposition rates of mortality. Diel and seasonal timing of oviposition, aggregation behavior, variation in the numbers of eggs released at one time, and female size all affected female mortality, with potential for offsetting benefits in egg survival. Such information about factors affecting female mortality during oviposition and the consequences of female behavior for egg or larval survival is necessary to understand the adaptive values of different oviposition behaviors.

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Declaration The authors of this manuscript declared that the experiments in this study comply with the current USA laws.

Electronic supplementary material

Below is the link to the electronic supplementary material. S1Power-point presentation showing animations of oviposition behaviors of different mayflies (Ephemeroptera) observed in the upper East River drainage basin, near RMBL in western Colorado. A. Splashers (e.g., *Siphlonurus occidentalis*), B. Bombers (e.g., *Drunella doddsi*), C. Dippers (e.g., *Rhithrogena hageni, Diphetor hageni, Epeorus longimanus*), D. Landers (e.g., *Baetis bicaudatus*), E. Floaters (e.g., *Acentrella turbinae*). (To activate the animations, click on "ppt presentation" or the F5 key, and use the "return" key to move through the different animations (PPT 232 KB).

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