

A new parthenogenetic mayfly (Ephemeroptera:Ephemerellidae:*Eurylophella* Tiensuu) oviposits by abdominal bursting in the subimago

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Abstract. *Eurylophella oviruptis* new species is described and illustrated from female larvae and reared subimagos and imagos collected from swamp streams in North Carolina, USA. Several key morphological characters in the larva place this new species in the *Eurylophella temporalis* group. Genetic comparisons with other eastern North American *Eurylophella* revealed a fixation of alleles unique to *E. oviruptis* at 4 of 19 allozyme loci. *Eurylophella oviruptis* appears to be obligately parthenogenetic because no males were observed in the field or laboratory and eggs taken from subimagos and imagos hatched parthenogenetically (mean hatch rate = 79%). Abdomens of 60% of subimagos reared in the laboratory burst along the ecdysial line of the first 3 tergites immediately after transformation to the subimago at the water surface. Abdominal bursting ruptured the oviducts, released most of the eggs into the water, and left the subimago trapped on the water surface with a large, inflated midgut protruding from the split in the tergites. Subimagos that did not burst (40%) flew from the water surface, molted to the imago on the 2nd day following emergence, and then, without having mated, extruded a ball of eggs that was released into the water. Dissections of *E. oviruptis* and 5 other species of mayflies showed that inflation of the mayfly gut normally occurs at 3 stages: emerging larvae inflate prior to and during the molt to the subimago, subimagos (particularly males) inflate further at the imaginal molt, and female imagos inflate even further at oviposition. Gastrointestinal inflation in mayflies maintains full abdominal distention that might facilitate flight. Abdominal bursting in *E. oviruptis* subimagos appears to be the result of gut inflation well beyond the amount normally associated with this stage. The evolutionary significance (predation, dispersal, demographics) of oviposition by abdominal bursting is discussed.

Key words: taxonomy, parthenogenesis, gastrointestinal inflation, dispersal polymorphism.

Species of *Eurylophella* are common and conspicuous members of the benthic macroinvertebrate community of streams in eastern North America. The most useful morphological characters for species identification are found in the larval stage (McDunnough 1931, Allen and Edmunds 1963). However, identification of *Eurylophella* species is difficult because of the rather subtle differences among species combined with a relatively large amount of intraspecific variation. Funk et al. (1988) used allozyme data from adults to provide a measure of gene flow that enabled an objective assessment of reproductive isolation and, thus, a clearer delineation of species boundaries in this genus. Morphometric data from larval exuviae of specimens identified using allozymes was then used to quantify

intra- vs interspecific variation in larval *Eurylophella* and to provide a basis for a taxonomic revision of the 14 known Eastern North American species (Funk and Sweeney 1994).

Here we describe a new species of *Eurylophella* from eastern North America based on specimens collected from 7 locations in the Neuse, Cape Fear, and Lumber River basins in North Carolina. Morphological and allozyme measures confirm its distinction from and relationship to other known species of eastern North American *Eurylophella* and enable us to amend the taxonomic key of Funk and Sweeney (1994). We also report that this new species is parthenogenetic based on the absence of males at all collection sites and the results of laboratory experiments. In addition, we show that this new species has a novel mode of oviposition that involves the bursting of the abdomen and the release of eggs prior to the subimago leaving

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the water surface. We develop 2 hypotheses regarding this novel behavior: 1) that abdominal bursting is the result of overinflation of the gut at emergence time, and 2) that this unusual form of oviposition is a dispersal polymorphism that gives parthenogenetic females a reproductive advantage by enabling them to avoid losses from predation and other factors during the terrestrial phase, thereby increasing the likelihood of their offspring ending up in suitable habitat.

Methods

Ten unidentified larvae of *Eurylophella* (later found to be *Eurylophella oviruptis* n. sp.) were collected in 1997 from 2 swamp streams in eastern North Carolina by staff from the North Carolina Division of Water Quality (NCDWQ), and 42 more larvae were collected in 2005 and 2006 from 7 locations in the Neuse, Cape Fear, and Lumber River basins. Additional *E. oviruptis* larvae were collected from Back Swamp at SR1003, Robeson County, North Carolina (lat 34.62028°N, long 79.19361°W) on 16 February 2007. These specimens were transported live to the Stroud Water Research Center (SWRC) in Avondale, Pennsylvania, and reared in recirculating, flow-through, 46 × 26 × 20-cm microcosms using water from White Clay Creek (WCC) at ambient photoperiod (adjusted for lat 34.6°N and updated weekly) and a constant temperature (starting at 7°C on 17 February, stepped up gradually to 13°C by 16 March, and held constant thereafter). Larvae were fed fresh algae (a mixed diatom community) cultured on acrylic plates in the greenhouse at the SWRC. WCC was a source of both stream water and natural algal colonization (as described in Sweeney and Vannote 1984). Emerging subimagos were reared to the imago (whenever possible) and stored at -80°C prior to enzyme electrophoresis, which was conducted using techniques described in Funk et al. (1988). Larval exuviae were preserved in ethanol and referenced individually to each reared adult. Oviposition by individuals that reached the imago stage occurred after placing the imago on the water surface of a small (30-mL) museum jar. Eggs were reared in the same jar filled with filtered (0.45-µm pore size) sterilized stream water (Sweeney and Vannote 1987, Funk et al. 2006). Morphological measurements and terminology follow Funk and Sweeney (1994).

A series of fresh dissections were made of last-instar larvae, subimagos, and imagos, including representatives of 3 species of *Eurylophella* and 2 species of *Ephemerella*, to document the occurrence and timing of gastrointestinal inflation in ephemereids. Larvae of *Eurylophella funeralis* were collected from an unnamed

tributary of Buck Run, Chester County, Pennsylvania (lat 39.9228°N, long 75.8119°W), and reared concurrently, and under the same conditions as, the North Carolina material. *Eurylophella verisimilis* were collected from WCC, Chester County, Pennsylvania (lat 39.8589°N, long 75.7839°W), and reared at ambient photoperiod at 15°C. Emerging larvae of *Ephemerella dorothea* and *Ephemerella invaria* were collected at dusk from WCC and immediately placed in 95% ethanol. Adults of these *Ephemerella* species were collected on the wing at dusk from WCC and immediately placed in 95% ethanol. All *Eurylophella* dissections were made on living material. Ethanol-killed *Ephemerella* were dissected within 1 h following collection.

A laboratory culture of the parthenogenetic baetid *Centroptilum triangulifer* (McDunnough) from WCC was used to test whether gut inflation is necessary for normal oviposition. Female imagos of this species readily oviposit when placed on a water surface (Sweeney and Vannote 1984, Funk et al. 2006). For a test, gut inflation was prevented by sealing the mouths of individuals with a small amount of methyl-2-cyanoacrylate (Duro Super Glue, Henkel Consumer Adhesives, Avon, Ohio). Gut inflation of these individuals was compared that of control individuals that received a similar amount of glue on the vertex prior to oviposition.

Results and Discussion

I. Species description and taxonomic identification

Previous taxonomic treatments of North American *Eurylophella* Tiensuu include McDunnough (1931) and Needham et al. (1935) (as the “bicolor group” of *Ephemerella*), Allen and Edmunds (1963) (as the subgenus *Eurylophella*), and Funk and Sweeney (1994). The description and key emendation presented below are intended to supplement those of Funk and Sweeney (1994). That work should be consulted for definitions and techniques for the measurement of FWL₁, ITD_{2:7}, ITD_{4:7}, SMT₁, SMT₂, SMT₇, TL₇, MLT_{2:7}, PLP₂, PLP₃ and PLP₉ (Funk and Sweeney 1994; pp. 213–215 and Fig. 1).

Eurylophella oviruptis Funk New Species

Figs 1A–D, 2, 3

Female larva.—Length (exclusive of caudal filaments): average 8.8 mm, range 8.0–9.7. Head: occipital tubercles very small (Fig. 1B), covered with long, sparse setae. Thorax: fore femora slender, average ratio of width to length (FWL₁) = 0.37, range 0.32–0.41. Abdomen: rows of submedian tubercles on segments 1–7 evenly convergent from segment 2 or 3–7 (Fig. 1A).

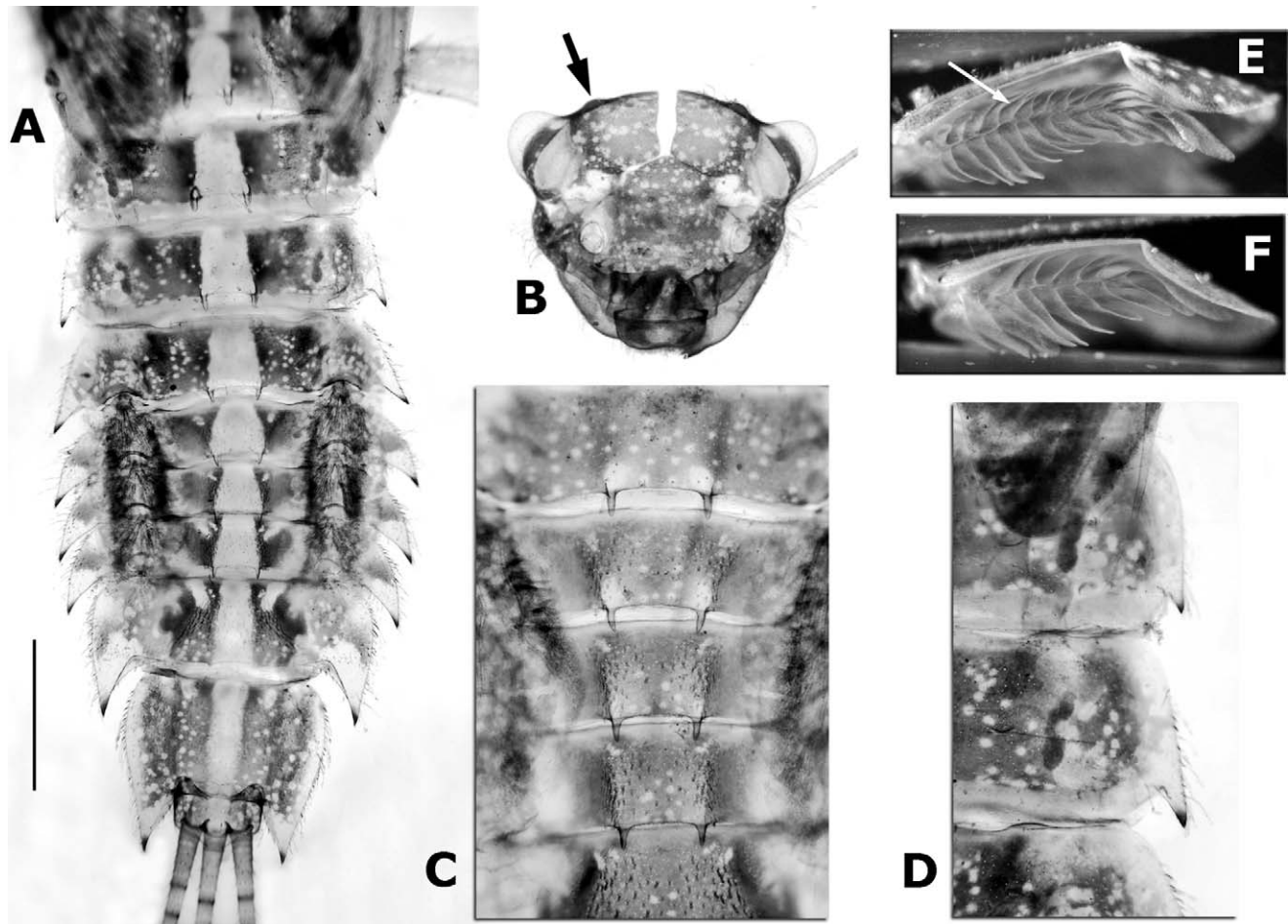


FIG. 1. *Eurylophella oviruptis*, female larval exuviae. A.—Dorsal view of abdomen (bar = 1 mm). B.—Anterior view of head showing occipital tubercle (arrow). C.—Dorsal view of terga 4–7. D.—Dorsal view of posterolateral projections on abdominal segments 2 and 3. E.—*Eurylophella doris*, lateral view of gill 4 (arrow points to dorsal subdivisions of ventral lamella). F.—*Eurylophella prudentalis*, lateral view of gill 4.

Average ratio of the distance between paired tubercles on abdominal segment 2 to that on segment 7 ($ITD_{2:7}$) = 1.16, range 1.00–1.32. Average $ITD_{4:7}$ = 1.22, range 1.07–1.32. Distance between tubercles widest on segment 2 or 3, slightly narrower on 1. Average ratio of distance between tubercles on abdominal segment 1 to the length of tergite 1 at midline (SMT_1) = 0.64, range 0.55–0.87. Tubercles on segment 2 widely spaced, average SMT_2 = 0.67, range 0.55–0.84. Spacing of tubercles on segment 7 always distinctly narrower than length of segment at midline, average SMT_7 = 0.79, range 0.72–0.83. Tubercles on 1–4 long and arched in lateral view (similar to fig. 72b in Funk and Sweeney 1994), without scale-like setae, but with scattered fine setae. Tubercles on 5–7 long, sharp, without scale-like setae and with 1 or 2 fine setae. Average ratio of length of caudad protrusion of tubercles on abdominal segment 7 to the length of tergite 7 at midline (TL_7) = 0.23, range 0.17–

0.31. Tubercles on 8 and 9 small. Abdominal segments 5–7 relatively short. Average ratio of length of tergite 2 at midline to that of tergite 7 ($MLT_{2:7}$) = 1.37, range 1.23–1.52. Posterolateral projections on 2 and 3 of medium size (Fig. 1D). Average ratio of length of caudad protrusion of posterolateral projections on abdominal segment 2 to the length of tergite 2 at midline (PLP_2) = 0.09, range 0.05–0.19; PLP_3 = 0.22, range 0.13–0.31. Posterolateral projections on 9 very short, average PLP_9 = 0.45, range 0.37–0.51. Dorsal subdivisions of lower lamella of gill 4 subequal to ventral subdivisions (as in Fig. 2a in Funk and Sweeney 1994) with 6 dorsal and 9 ventral lamellae (as in Fig. 1F). Coloration typical for the genus, with or without a pale median stripe on tergites between submedian tubercles. Sterna 1–8 or 9 each with 4 pairs of small, brown maculae: from center, 1 pair submedian dots; 1 pair of oblique, short submedian dashes nearer anterior

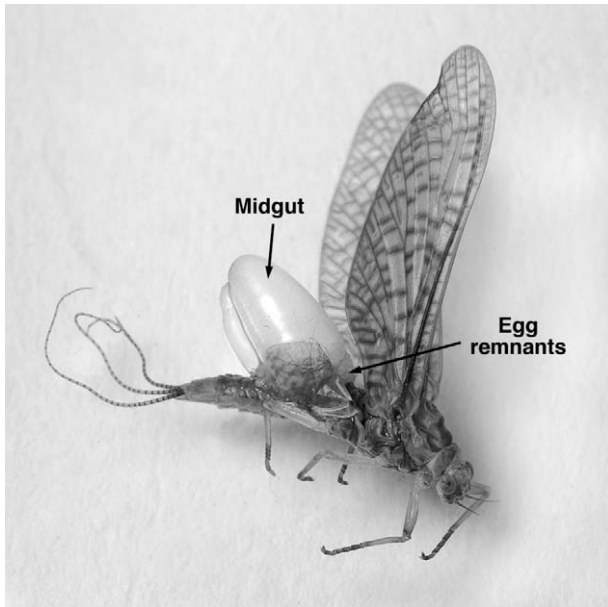


FIG. 2. Female subimago of *Eurylophella oviruptis* after abdominal bursting. A large, inflated midgut and small remnants of ovaries and oviducts can be seen protruding from a split along the midline of the first 3 tergites.

margin; 1 pair slightly of longer sublateral dashes that are parallel to body axis; 1 pair of dots just anterior of sublateral dashes. Tips of posterolateral projections on abdomen blackish (Fig. 1A).

Female subimago.—(description based on living and freshly preserved specimens in alcohol) Length: body 7.6–9.5, forewing 7.5–9.0. Body medium brown. Forewings pale brown. Veins and crossveins, especially in outer region of wing, margined with black, giving a speckled appearance. Hindwing pale brown, without margining. Legs yellowish with blackened tarsi. Caudal filaments dark grey, with joints blackish.

Female imago.—(description based on living and freshly preserved specimens in alcohol) Length: body 7.9–9.7, forewing 7.8–9.1. General coloration light brown with darker brown to blackish maculations. Head with black speckling in occipital area. Compound eyes light greenish grey. Pronotum light brown with dark variegation. Meso- and metathorax mostly light brown, with indistinct darker brown maculation. Wing membrane and veins hyaline except for dark brown coloration dorsally at base of radius on both wings. Legs mostly pale, with narrow brown annulation at the joints of tarsi and paired blackish maculae on dorsal surface of coxae. Tergites light brown with dark submedian maculation and frequently with a pale median stripe. Sternites mostly pale brown, with paired submedian brown dots

and oblique sublateral dashes anteriorly. Caudal filaments pale with dark brown annulations at joints.

Material examined.—

Holotype: Reared female imago (SWRC no. Esw Ba 17), **NORTH CAROLINA**: ROBESON Co., Back Swamp (Lumber River) at SR 1003, 34.62028°N 79.19361°W, collected as larva 16.ii.2007, W.B.Crouch, D.R.Lenat, D.H.Funk, reared at SWRC (tray 601), emerged 31.iii.2007. Imago and larval exuviae in alcohol. Deposited at the Academy of Natural Sciences of Philadelphia.

Paratypes: **NORTH CAROLINA**: PENDER Co., Merrick's Creek, (Cape Fear R) at NC 210, 34.44667°N 77.80333°W, NCDWQ accession no. 7236, 24.ii.1997, 4 female larvae, 1/3 to full grown, D. Penrose; ROBESON Co., Raft Swamp, at SR 1505, 34.81694°N 79.12861°W, NCDWQ accession no. 9778, 06.ii.2006, 9 female larvae, ~1/3 grown, M.Walters, L.Ausley, C.Tyndall, W.B.Crouch. Deposited at the Stroud Water Research Center.

Additional material: **NORTH CAROLINA**: JONES Co., Beaverdam Creek, (Neuse R) at SR 1002, 35.06750°N 77.27694°W, NCDWQ accession no. 7249, 25.ii.1997, 5 female larvae, 1/2 to nearly full grown, D. Penrose; JONES Co., Tuckahoe Swamp, at SR 1142, 35.03194°N 77.57944°W, NCDWQ accession no. 9574, 23.ii.2005, 1 female larva, full grown, E. Fleek; ROBESON Co., Little Raft Swamp, at SR 1323 (Industrial Road), 34.83472°N 79.19028°W, NCDWQ accession no. 9779, 06.ii.2006, 19 female larvae, ~1/3 grown, W.B.Crouch, C.Tyndall, M.Walters; Back Swamp, at SR1003, 34.62028°N 79.19361°W, NCDWQ accession no. 9781, 2/7/06, 12 female larvae, ~1/3 grown, W.B.Crouch, L. Ausley, C.Tyndall; Gum Swamp, (Lumber R) at SR 1312, 34.71746°N 79.27011°W, NCDWQ accession no. 9794, 2/24/06, 1 female larva, S. Beaty.

Range.—To date, *E. oviruptis* has been collected only from 7 swamp streams in the Southeastern Plains and Middle Atlantic Coastal Plain US Environmental Protection Agency Level III ecoregions eastern North Carolina. Very early-season collections from similar habitats elsewhere in the southeast USA could eventually show its range to be more extensive.

Diagnosis.—In the key provided by Funk and Sweeney (1994), the well developed dorsal subdivisions of the ventral lamella of gill 4 and the convergent rows of submedian tubercles on abdominal segments 2 or 3–7 in *E. oviruptis* larvae will lead to couplet 3. The very small occipital tubercles will tend to place *E. oviruptis* with *Eurylophella prudentialis* in that couplet. Both *E. prudentialis* and *E. oviruptis* have only 6 dorsal subdivisions of the ventral lamella of gill 4 (Fig. 1F) compared with 9 or more in *Eurylophella temporalis*, *Eurylophella poconoensis*, and *Eurylophella doris* (Fig. 1E)

(this difference between *E. prudentialis* and the other *E. temporalis* group species was overlooked in our 1994 treatment [Funk and Sweeney 1994]). However, *E. oviruptis* can be distinguished easily from *E. prudentialis* by the wider spacing of submedian tubercles on segment 7 (greater SMT₇), greater reduction in length of segments 5–7 (greater MLT_{2:7}), shorter posterolateral projections on segment 9 (smaller PLP₉) and larger body size. Substitution of the following for couplet 3 in the key provided by Funk and Sweeney (1994) will facilitate identification:

3. Six dorsal subdivisions present on ventral lamella of gill 4 (Fig. 1F); occipital tubercles small in both sexes (Fig. 1B; figs. 56 and 57 in Funk and Sweeney 1994), sometimes barely perceptible in males..... 3a

Nine or more dorsal subdivisions present on ventral lamella of gill 4 (fig. 1E); occipital tubercles usually large in both sexes (figs. 19, 20, 50, 52, 61, and 62 in Funk and Sweeney 1994) (occasionally small in *E. poconoensis*; figs. 49 and 51 in Funk and Sweeney 1994)..... 4

- 3a. Submedian tubercles on abdominal segment 7 narrowly spaced, average SMT₇ = 0.58 (range 0.49–0.65); relative length of abdominal segments 5–7 longer, average MLT_{2:7} = 1.09 (range 0.93–1.19); posterolateral projections on abdominal segment 9 longer, average PLP₉ = 0.61 (range 0.54–0.70); body length of full-grown larva 6.5 to 8.0 mm; submedian tubercles on 1–3 thin, often pointed, and relatively straight in side view (fig. 72a in Funk and Sweeney 1994); widespread in eastern North America..... *prudentialis*

Submedian tubercles on abdominal segment 7 more widely spaced, average SMT₇ = 0.79 (range 0.72–0.83); relative length of abdominal segments 5–7 shorter, average MLT_{2:7} = 1.37 (range 1.23–1.52); posterolateral projections on abdominal segment 9 shorter, average PLP₉ = 0.45 (range 0.37–0.51); body length of full-grown larva 8.0 to 9.7 mm; submedian tubercles on 1–3 thicker, dorsally arched in side view (as in fig. 72c in Funk and Sweeney 1994); known only from Southeastern Plains and Middle Atlantic Coastal Plain ecoregions of the southeastern United States *oviruptis*

Discussion.—The well developed dorsal subdivisions of lower lamella of gill 4 and the apparent convergence of the rows of submedian tubercles from tergites 2 or 3 to 7 (i.e., ITD_{2:7} > 1.0) place this species in the *E. temporalis* group of Funk and Sweeney (1994) (see couplet 2 in key). The spacing of the submedian

tubercles on abdominal segment 7 (average SMT₇ = 0.79), while somewhat wider than is typical for the *E. temporalis* group (SMT₇ in other *E. temporalis* group species averages <0.75) is, nevertheless, lower than most other *Eurylophella* species and, thus, also suggests a closer relationship to other members of the *E. temporalis* group. However, the relatively shortened abdominal segments 5–7 in *E. oviruptis* (as indicated by an average MLT_{2:7} of 1.37) is not typical of the *E. temporalis* group (see fig. 77a of Funk and Sweeney 1994), but rather more like the members of the other 3 eastern North American groups (*Eurylophella lutulenta*, *E. funeralis*, and *E. bicolor*). In addition, the posterolateral projections on abdominal segment 9 in *E. oviruptis* are shorter (relative to the length of segment at middle), on average, than any other eastern North American *Eurylophella* (see PLP₉ above).

Larval color characters can be troublesome because of the large amount of intraspecific variation present in *Eurylophella* species, but 2 features in *E. oviruptis* distinguish it from other members of the *E. temporalis* group. Although the small sternal maculae described above are present in most *Eurylophella* to varying degrees, these structures are distinctly blackish (especially the sublateral dashes) in other members of the *E. temporalis* group, whereas they are brown in *E. oviruptis* (as they are in other *Eurylophella* species). Also, the tips of posterolateral projections on the abdomen are distinctly darkened in *E. oviruptis* (Fig. 1A). Although this condition is present in many other species of *Eurylophella* (especially members of the *E. bicolor* group), the tips of the posterolateral projections are darkened only slightly, or not at all, in other *E. temporalis* group species.

Eurylophella oviruptis has been collected only in “swamp streams” (NCDWQ designation) in eastern North Carolina. These streams normally have no visible flow during a part of the year (usually summer). *Eurylophella oviruptis* is a very early-season species; all larval specimens we have seen have been from collections made in late winter, and adult emergence occurs in early to mid March. Its restricted range, lack of males, and likely disappearance from streams before the vernal equinox probably account for it having been overlooked until recently. Most collections of *E. oviruptis* examined also contained *E. doris*, the latter being at a distinctly earlier developmental stage. *Eurylophella doris* collected together with *E. oviruptis* from the type locality did not begin to emerge until 2 wk after the last *E. oviruptis* emerged.

Etymology.—The specific epithet *oviruptis* is a combination of the Latin *ovum* for egg and *eruptum* for bursting forth, a reference to its unusual mode of oviposition.

II. Genetic analysis using allozymes

A total of 19 allozyme loci were scored in 12 individuals, including 6 subimagos that had burst immediately after emergence (see *Mode of oviposition in E. oviruptis* below) and 6 imagos that had laid their eggs normally. Fifteen loci were monomorphic and 4 were polymorphic: GPI, 6PGD, TRI, and PRO. Seven multilocus genotypes were identified using the 4 polymorphic loci. These multilocus genotypes probably represent clones because all obligately parthenogenetic mayflies tested to date have proved to be clonal (Funk et al. 2006; DHF, unpublished data for *Ameletus ludens*, *Ephemera varia*, *Centroptilum minor*, *Procloeon viridoculare*, *Dipheter hageni*, *Acerpenna macdunnoughi*). Of the 4 multilocus genotypes for which >1 individual was identified, 2 included both individuals that had burst as subimagos and individuals that oviposited normally. Observed heterozygosity in *E. oviruptis* ranged from 0.05 to 0.16 (mean = 0.08), values quite comparable to other *Eurylophella* species (Funk et al. 1988). *Eurylophella oviruptis* had alleles found in various other eastern North American *Eurylophella* species at 15 of the loci scored, but were fixed for unique alleles at 4 other loci: HEX, 6PGD, G6PDH and AAT1. These results indicate an absence of recent gene flow between *E. oviruptis* and other eastern North American species.

III. Parthenogenesis

Fifteen clutches of eggs were tested for parthenogenetic egg hatch. Five were remnants (20–50 eggs) dissected from the protruding oviducts of burst subimagos and 10 were full clutches laid by imagos (one such clutch was counted and found to have 1450 eggs). Seven clutches (2 from burst subimagos and 5 laid by imagos) were incubated at ambient photoperiod and stream temperature of WCC. The water temperature during the incubation period averaged 11.2°C (range 5.6–17.8). Onset of hatching began, on average, at day 43 (range 40–47). Parthenogenetic hatch rate was high, averaging 86% (range 63–98). The other 8 clutches (3 from burst subimagos and 5 laid by imagos) were incubated at ambient photoperiod and room temperature in our wet laboratory (mean = 16°C, range 13–18). For these clutches, onset of hatching began, on average, at day 23 (range 17–29). Parthenogenetic hatch rate averaged 72% (range 21–93). No thermograph data are available for the sites where *E. oviruptis* has been collected, but temperatures experienced by *E. oviruptis* eggs in the field probably were higher than those of WCC, but somewhat lower than our room temperature. Therefore, onset of hatching is likely to be somewhere between 21 and 43 d.

Obligate thelytokous (all female) parthenogenesis is known in certain populations of one other *Eurylophella* species, *funeralis* (McDunnough) (Sweeney and Vannote 1987). Although sexual populations might be found eventually, *E. oviruptis* now appears to be the only entirely parthenogenetic species of *Eurylophella*.

IV. Mode of oviposition in *E. oviruptis*

Eurylophella oviruptis larvae emerged from the water surface at the margins of our flow-through rearing tanks in a fashion similar to other members of the *E. temporalis* group, but unlike most other *Eurylophella* species (whose larvae usually crawl part or all the way out of the water before transforming to the subimago). Although other species of *Eurylophella* reared in our laboratory emerge at predictable times of day (late afternoon in most species) over a relatively narrow 1- to 2-h period, emergence of *E. oviruptis* occurred any time from mid-morning to late afternoon. The abdomens of 30 of the 50 *E. oviruptis* reared burst along the center of the first 3 tergites moments after the subimago was free of the larval exuviae, and both oviducts ruptured and released most of their eggs into the water. A large portion of an inflated midgut and remnants of the oviducts were left protruding through the tergites (Fig. 2). All but 2 of these bursters were ensnared on the water surface (see below) and died within a few hours. The 2 bursters that escaped the water surface were recovered from the netting placed over the rearing trays; both failed to molt and died as subimagos within a few hours. The remaining 20 individuals did not burst their abdomens, even though some had trouble escaping the water surface. Eighteen of these individuals were successfully reared to the imago (time until imaginal molt was 36–48 h at 20°C). Oviposition by 14 of these imagos occurred after they were placed on the water surface in a small jar. These imagos extruded eggs in the usual ephemereid way (through the genital opening) into a large mass (Fig. 3) that they then released into the water. Presumably, eggs would normally be extruded in flight and then released by females either over the water or at the water surface following a rapid descent, as we have observed in *E. funeralis* and *E. doris*, and as has been described previously for other ephemereidids (Needham et al. 1935, Edmunds et al. 1976).

Movement into and out of water by mayflies is a challenge because of the surface tension of the water. A dense covering of hydrofuge hairs aids the transition of mayflies to a terrestrial existence by helping the emerging subimago avoid entrapment on the water surface (Edmunds and McCafferty 1988). Eggs have the opposite problem—they must break through the

water surface to reenter the aquatic habitat. Our observations of oviposition in a variety of mayfly species suggest that a surfactant is released with mayfly eggs that breaks the surface tension and facilitates their entry into the water. For example, when placed on water, imagos of *Centroptilum triangulifer* begin extruding eggs (Sweeney and Vannote 1984, Funk et al. 2006). Just as the first eggs touch the water surface, the imago can be seen riding the wake of the breaking surface tension. A similar phenomenon was observed in *E. oviruptis* imagos that oviposited on the water surface in our jars. All but 2 of the burst subimagos of *E. oviruptis* ($n = 30$) that were reared were left ensnared on the water surface, on their sides with the inflated midgut and remnants of the ovaries beneath the water surface, apparently because of the action of surfactants released with the eggs. In contrast, none of the 20 subimagos that did not burst were ensnared.

Oviposition was not observed in the field. However, we feel confident that abdominal bursting in *E. oviruptis* is not an artifact of laboratory conditions because: 1) during 25 y of using the identical systems for rearing thousands of *Eurylophella* mayflies representing multiple populations of all 14 other eastern North American species (Funk et al. 1988) (including 3 other species/population combinations concurrent with the rearing of *E. oviruptis*), we have never before observed abdominal bursting; and 2) 2 individual *E. oviruptis* that did not burst at emergence were confined to the water surface for 4 and 5 h, respectively. Neither burst and both were subsequently reared to the imago and oviposited normally. This latter observation also suggests that abdominal bursting is not a behavioral response to a condition encountered at emergence (such as an inability to escape the water surface) but rather appears to be developmentally programmed.

V. The role of gut inflation in *E. oviruptis* and other mayflies

The alimentary canal of mayflies undergoes anatomical changes from the larva to the imago, coincident with the cessation of feeding and the nearly complete atrophy of the mouthparts and their associated musculature. The most conspicuous change involves inflation of the midgut by the swallowing of air, facilitated anteriorly by the formation of a 1-way valve at the junction of the fore- and midgut and posteriorly by a plug of food debris at the junction of the mid- and hindgut (Harker 1999). Gut inflation in adult mayflies was noted by several European workers in the late 19th and early 20th centuries (e.g., Palmén 1884, Fritze 1888, Sternfeld 1907). According to Pickles (1931), its function was generally considered by these

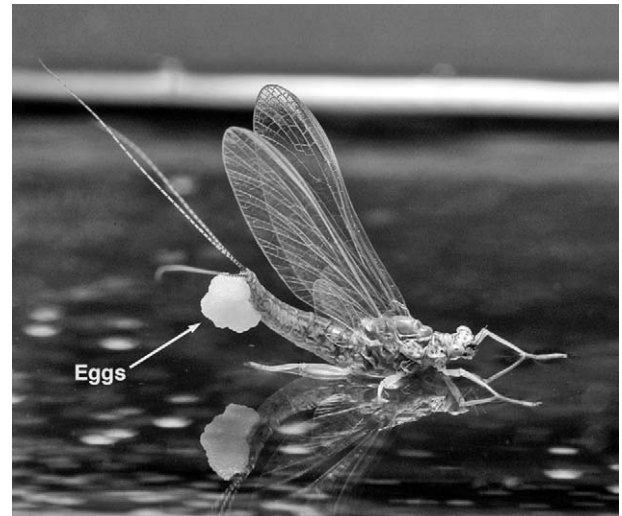


FIG. 3. Female imago of *Eurylophella oviruptis* on water surface with eggs fully extruded, just before their release into the water.

and other early workers to be an adaptation to an aerial existence, whereby the imago would be able to alter its specific gravity by inflating or deflating the gut. However, Palmén (1884) considered the function of the imaginal gut to be connected primarily with the “ejection of genital products” (Pickles 1931). Harker (1999) studied the fore- and midgut of all stages of *Cloeon dipterum* and concluded that gut inflation in subimagos and imagos functions primarily in reducing the volume of the haemocoel and in channeling the haemolymph, and probably plays no significant role in reproductive processes or the mechanism of flight. Harker (1999) also suggested that inflation of the midgut in subimagos might supply the pressure needed to split the subimaginal cuticle during the final molt.

All 30 *E. oviruptis* that burst as subimagos had a conspicuously inflated midgut protruding from the split in their abdomens (Fig. 2), an observation that strongly suggests a causative role for gut inflation in abdominal bursting. Two additional observations suggest that gut inflation also plays an important role in normal mayfly oviposition. First, although it is well documented that adult mayflies do not feed (Brittain 1982) and that, prior to oviposition, the internal volume of adult female mayflies consists almost entirely of eggs and flight muscles (Needham et al. 1935, Brinck 1957), we have observed that following oviposition, the internal volume of female mayflies consists largely of air trapped in the gut. Second, stereoscopic observation of imagos in the process of extruding egg masses (including several species of baetids as well as *E. oviruptis* and *E. funeralis*) reveals

waves of what appear to be peristaltic contractions in the head and the region between the head and prothorax ("neck") that we interpret as the swallowing of air. The latter is consistent with morphological observations by Sternfeld (1907), who described the development of musculature surrounding the foregut in the head region of the imago and suggested that it "might function as a powerful pump for forcing air into the mesenteron [= midgut]" (Pickles 1931).

Most of the published observations cited above were made by morphologists using material that had been fixed in various preservatives, and then embedded and sectioned. Gases might or might not remain in tissues prepared in this way, depending on the type of preservative and the length of storage prior to dissection. To document the occurrence and timing of gastrointestinal inflation in ephemereids more thoroughly, the following dissections were made on living (in the case of *Eurylophella*) or freshly killed (in the case of *Ephemerella*) specimens.

Eurylophella verisimilis (McDunnough).—Last instar larvae in the white wing pad stage (viz., just prior to the black wing pad stage, recognizable by swollen wing pads with subimaginal wing features visible beneath) had guts still full of larval food ($n = 2$ males, 4 females). However, midguts of larvae in the black wing pad stage ($n = 1$ male, 3 female) were empty, presumably because of cessation of feeding 1 or 2 d earlier. *Eurylophella verisimilis* larvae usually crawl part way out of the water prior to emergence, spending several minutes to $\sim\frac{1}{2}$ h in this position before transforming. Larvae dissected just as they crawled out ($n = 2$ males) had a somewhat enlarged gut cavity (compared with pre-emergent black wing pad stage larvae), with no food or air in it. Larvae that had been part way out of the water for several minutes ($n = 3$ males, 1 female) showed the beginnings of gut inflation, with an air bubble visible in the anterior portion of the midgut extending in some cases as far back as the 3rd abdominal segment. Freshly transformed subimagos ($n = 4$ males, 6 females) all had air in the midgut. In the case of males, this air was estimated to be $\sim 20\%$ of the body volume. In females, the relative volume of air was noticeably less ($< 10\%$). In both male and female subimagos, the inflated portion of the midgut was restricted to the anterior region and extended no further back than the 3rd abdominal segment. Male imagos ($n = 5$) had noticeably greater volumes of air in the gut than subimagos. The inflated portion of the midgut extended further distad than in subimagos, and it appeared that air pressure in the gut was responsible for maintaining the extension and rigidity of the abdomen. Guts of female imagos (prior to oviposition;

$n = 6$) contained a small air bubble (similar to subimagos) that was confined to the anterior portion.

Eurylophella funeralis (McDunnough).—Many local populations of this species are parthenogenetic and, as such, female imagos usually extrude their eggs when placed on the surface of water (Sweeney and Vannote 1987). Larvae from a parthenogenetic population in an unnamed tributary to Buck Run, Chester County, Pennsylvania, were reared in the laboratory to the imago. Dissection of 4 female imagos that had not oviposited revealed some inflation in the anterior portion of the midgut extending to about the 2nd or 3rd abdominal segment. Four other imagos were placed on water in small museum jars and observed under a stereomicroscope. During egg mass extrusion, what appeared to be peristaltic contractions were observed in the head and prothorax. Dissection of these individuals following oviposition revealed that the entire midgut was inflated and occupied the volume that had been filled by eggs.

Eurylophella oviruptis.—As described above, the entire midgut of all 30 burst subimagos was inflated as in Fig. 2. However, 2 intact (nonbursting) subimagos had only small air bubbles confined to the anterior region of the midgut, similar to those described for *E. verisimilis* above. Dissection of 3 imagos that had not oviposited revealed small air bubbles in the anterior region of the midgut, similar to those seen in subimagos. The entire midgut was inflated following oviposition in 8 dissected imagos.

Eurylophella dorothea Needham and *Eurylophella invaria* (Walker).—Both species emerge at the water surface in midstream around dusk. Larvae swimming to the surface to emerge from WCC were captured in a net and quickly transferred to 80% ethanol. Subimagos of this species emerge from the larval skins within a few seconds of surfacing, and all had begun to molt by the time they were killed in ethanol. Dissections of individuals ($n = 5$ male, 8 female *E. dorothea*; 4 female *E. invaria*) whose transformation had been halted at the earliest point (viz., that had split the larval cuticle along the thoracic ecdysial line, but whose wings and heads were still in the larval exuviae) revealed that most (4 of 5 male and 7 of 8 female *E. dorothea*) had already swallowed enough air to produce a small air bubble in the anterior portion of the midgut. All individuals that had freed the subimaginal wings or head capsules had air bubbles in the anterior portion of the midgut ($n = 8$ male and 12 female *E. dorothea*; 2 male and 4 female *E. invaria*). These bubbles were similar in size to bubbles in subimagos captured on the wing ($n = 8$ male and 14 female *E. dorothea*; 2 male and 5 female *E. invaria*). For both species, inflation extended from just behind the head to about halfway

through the abdomen in male subimagos. The volume of air in female subimago midguts was less extensive, with only a small portion of the gut in the anterior 2 or 3 abdominal segments inflated. Examination of male imagos revealed more extensive inflation than was observed in subimagos ($n = 5$ *E. dorothea*, 3 *E. invaria*). Inflation in female imagos that had not oviposited was similar to that found in subimagos ($n = 12$ *E. dorothea*, 5 *E. invaria*). Following mating, both species hover over riffles while they extrude their entire egg mass. Once extruded, the egg masses (balls) are dropped into the stream either from the air, or when the female drops briefly to the water surface. Eighteen *E. dorothea* and 12 *E. invaria* females captured after having dropped their eggs were dissected. In all cases the entire midgut was inflated, effectively displacing the volume that had been occupied by the eggs (i.e., most of the female's body volume from just behind the head to the 7th abdominal segment). The remains of the ovaries were appressed to the dorsal surface of the abdominal cavity.

We interpret the results of these dissections as follows. Larvae stop feeding 1 or 2 d before emergence, during which time the mouthpart musculature degenerates and the gut is evacuated. Thus, black wing pad stage larvae have guts that are largely empty of food but might appear distended with water. Beginning when larvae first crawl out of the water to emerge (in species that do this), or right at emergence (in species that emerge at the water surface, including *E. oviruptis*), larvae begin to swallow air, inflating the midgut. Males inflate their midguts further when they molt to the imago, and the pressure of air trapped in the midgut is largely responsible for the extension and rigidity of the imaginal abdomen. In females, little or no additional inflation occurs in the imago until oviposition, when air volume in the gut increases substantially, such that air volume displaces egg volume as eggs are extruded from the genital opening.

The conspicuous protrusion of a large inflated midgut (Fig. 2) strongly suggests that air pressure in the gut is responsible for abdominal bursting in subimaginal *E. oviruptis*. This pressure causes the abdomen to burst at its weakest point along the ecdysial line on the first 3 tergites, ruptures the oviducts (which are located dorsal of the gut in this region) and releases eggs into the water. In mayfly imagos (including *E. oviruptis*), a similar degree of gut inflation normally coincides with extrusion of the eggs through the gonopore (see above). The question is why, in the case of *E. oviruptis* subimagos, inflation results in abdominal bursting rather than egg extrusion through the gonopore. Microscopic examination of the gonopore in *E. oviruptis* subimaginal exuviae

revealed a short (less than the length of segment 7), dead-ended chamber opening between sternite 7 and 8 whose connection to the oviducts is presumably completed at (but not before) the imaginal molt. Thus, it appears that eggs cannot flow through the genital opening of an *E. oviruptis* subimago.

The coincidence between gut inflation and normal egg extrusion in female mayflies and the fact that inflation is powerful enough to cause abdominal bursting in *E. oviruptis* subimagos (whose eggs apparently cannot escape through the gonopore) suggested to us that gut inflation might provide the mechanism for normal egg extrusion through the gonopore of mayfly imagos. The parthenogenetic baetid *Centroptilum triangulifer*, a species whose oviposition occurs immediately when imagos are placed on a water surface (Sweeney and Vannote 1984, Funk et al. 2006), was used to test this hypothesis. Live dissections of subimagos ($n = 5$) revealed the presence of a small bubble in the midgut comparable to bubbles in the ephemereids described above, except that the air tended to be distributed more evenly through the midgut (rather than confined to the anterior region as in the ephemereids). Preovipositional imagos ($n = 5$) had bubbles similar in size and distribution to subimagos, whereas postovipositional imagos ($n = 6$) had much larger bubbles, indicating inflation during oviposition. To test whether inflation was necessary for oviposition, swallowing of air during oviposition was prevented by gluing the mouths shut on 8 imagos (and placing a similar quantity of glue on the vertices of the heads of 4 controls). Following placement of the imagos on water, all 12 individuals extruded what appeared to be a normal-sized egg mass (i.e., the entire contents of both oviducts) while swallowing or attempting to swallow air (as indicated by repeated contractions visible in the head and neck region). However, during egg extrusion, the abdomens of individuals whose mouths had been glued shriveled markedly, whereas the outward appearance of controls did not change. Subsequent dissections confirmed that: 1) the oviducts of both groups were largely devoid of eggs (although 3 from the glued-mouth treatment had some [<50] eggs left in the oviducts); and 2) individuals from the glued-mouth treatment had been unable to inflate their guts beyond the amount observed in preovipositional imagos, whereas guts in the control group had very large bubbles occupying the thorax and entire length of the abdomen.

This experiment demonstrated that midgut inflation, while coincident with oviposition, is not directly responsible for normal egg extrusion. However, inflation does appear to be responsible for full

distention of the abdomen in both males and females (especially following oviposition in the latter). We observed that postovipositional female imagos whose mouths had been glued shut (and thus, whose abdomens were shriveled) had difficulty flying. Three of these individuals repeatedly crash-landed within several centimeters of where they took flight, an observation suggesting that full abdominal distention might be necessary for competent flight. In nature, most mayflies extrude their eggs in flight, and many species extrude and then deposit eggs repeatedly in small batches. Thus, it appears that maintenance of full abdominal distention (afforded by gastrointestinal inflation) might be necessary for proper flight during this process.

VI. The significance of abdominal bursting

For bursting subimagos of *E. oviruptis*, gut inflation at emergence continues far beyond the normal point (i.e., the amount that results in normal extension of the abdomen and leaves a small bubble in the gut, as in other ephemereids and nonbursting *E. oviruptis*). Our experiment with *C. triangulifer* suggests that inflation is not necessary for the normal extrusion of eggs. Nevertheless, it seems possible that if overinflation occurred in a preovipositional imago, the result might be the extrusion of eggs through the gonopore rather than abdominal bursting. Perhaps overinflation in the subimago causes the abdomen to burst at its weakest point (along the ecdysial line) because the connection between the gonopore and the oviducts is not yet complete. The ovaries and oviducts lie dorsal to the gut at this point (and for most of their length). As the abdomen bursts, so do the fragile oviducts, thereby releasing most of the eggs.

In sexual mayflies, abdominal bursting in the emerging subimago would result in release of unfertilized eggs because mating generally occurs only in the imago. In the few sexual species where females do mate and lay eggs as subimagos (e.g., *Ephoron*, *Dolania*), mating occurs in flight, not at the water surface at emergence. Thus, selection against overinflation at emergence in sexual species is likely to be strong. However, an obligate parthenogen such as *E. oviruptis* does not require egg fertilization, and such bursting behavior might increase the likelihood of successful oviposition by avoidance of the risks associated with: 1) leaving the water and flying to nearby vegetation (predation by birds and other aerial predators in flight), 2) resting for the 36 to 48 h needed to prepare for the subimaginal molt (risk of desiccation and exposure to terrestrial predators), 3) molting to the imago (a substantial number of mayflies die during

this transformation; DHF, personal observation), 4) locating and entering a courtship swarm and mating, and 5) dispersal and ovipositional flight (exposure to aerial and aquatic predators). One important disadvantage to abdominal bursting might be the rather severe limitations it imposes on dispersal. Eggs released at the water surface are likely to travel some distance downstream between the time they are released and hatching because *Eurylophella* eggs lack the adhesive polar caps found in many other ephemereids (Koss and Edmunds 1974) and 20+ d are required for incubation in *E. oviruptis*. *Eurylophella* larvae are poor swimmers, so upstream migration is likely to be minimal. Thus, with the effective loss of flight, dispersal between watersheds is much less likely, and the net movement within a watershed is likely to be in a downstream direction. This limitation might explain why a substantial number (~40%) of *E. oviruptis* individuals do not burst as subimagos, but rather follow the normal mayfly pattern described above (except for mating).

The existence of both bursting and nonbursting individuals of *E. oviruptis* could represent a dispersal polymorphism because bursting individuals of *E. oviruptis* are effectively flightless and the propensity to burst appears to be determined by something other than conditions experienced at emergence (viz., genetics or conditions experienced by earlier life stages). The frequency of alternate dispersal morphs in other insects (e.g., macropterous vs brachypterous or apterous individuals) is determined by genotype, environmental variation, or most often, a combination of the 2 (Zera and Denno 1997). Bursting in *E. oviruptis* might have a genetic component, but our data do not show a significant correlation with multilocus genotype (i.e., clone), so an environmentally induced component to this polymorphism is likely.

Our study is the first to document oviposition by this sort of abdominal bursting in a mayfly. However, G. F. Edmunds, Jr (University of Utah) and M. Lawson (Henry's Fork Anglers, Last Chance, Idaho) (unpublished manuscript provided to the senior author by Edmunds in 1993) found an undescribed, and presumably parthenogenetic, species of *Serratella* in the Frying Pan River (Colorado, USA) that appeared to have similar abdominal bursting in the emerging subimago. However, in contrast to bursting in *E. oviruptis*, where most of the female's eggs are released into the water at the time of bursting, in each of the 32 *Serratella* specimens examined (collected from the water surface and preserved in alcohol), all or nearly all of the eggs were still contained in 2 masses (corresponding to the 2 oviducts) protruding from the rupture along the midline of the first 3 tergites.

Retention of eggs by bursting *Serratella* would seem to expose these eggs to a very high predation risk (these mayflies were first discovered by fly fishermen, and are considered to be an important hatch on the Frying Pan). Thus, Edmunds (personal correspondence) postulated that, for this sort of abdominal bursting to be a viable ovipositional strategy in the Frying Pan *Serratella*, eggs would have to be capable of surviving a trip through a trout gut.

The fact that abdominal bursting has been observed in 2 ephemerellid species, both parthenogenetic but residing in separate genera, suggests a possible propensity for bursting by overinflation in the subimago in ephemerellids in general. Presumably selection against such behavior is strong in sexual species because eggs released in this manner would be unfertilized. But for parthenogens, abdominal bursting in the subimago (by at least some portion of individuals in the population) might represent a significant advantage by enabling many individuals to deposit their eggs in suitable habitat while avoiding some of the mortality risks associated with normal postemergent mayfly behavior.

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