

The reproductive life history of the predacious, sand-burrowing mayfly *Dolania americana* (Ephemeroptera: Behningiidae) and comparisons with other mayflies

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Oogenesis in *Dolania* is unique among Ephemeroptera, with one oocyte developing per ovariole, low numbers of ovarioles, and routine resorption of one-third to one-half of ovarioles. Fecundity is 20 times smaller than in mayflies from other families, and mature egg dry weight is approximately 32 times the values of non-Behningiidae mayflies. Seventy percent of the linear growth of maturing oocytes occurs in a 2- to 3-week period during the late penultimate to early final larval stadia. The gut does not atrophy fully, and nonmaturing oocytes remain small and are not resorbed until the other oocytes reach maturity. Thus, resorbing oocytes are probably not an important energy source for the maturing oocytes. Starvation can further reduce *Dolania* fecundity from a normal 100 eggs to 6. Limited oocyte resorption appears common in mayflies. *Callibaetis ferrugineus* female imago, which are ovoviviparous, resorb much of their abdominal muscle. Some ovulation and even egg development may occur in the adult stage of many oviparous species. The thick chorion and thick, sticky fibrous suprachorionic layer of *Dolania*'s egg probably resist damage from sand and fungi for nearly 1 year in a coarse rolling sand habitat. The large egg produces a well-developed first-instar larva 2.5–5 times the length of any other mayfly first instar. The reproductive strategy of *Dolania* and *Behningia*, unlike that of other mayflies, is to produce large eggs and thereby large predatory early-instar larvae capable of exploiting a large size range of prey.

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L'ovogenèse du *Dolania* diffère de celle qui prévaut chez tous les autres Éphéméroptères car il ne s'y développe qu'un ovocyte par ovariole, il y a peu d'ovarioles et de un tiers à la moitié du nombre total d'ovarioles sont ordinairement résorbés. Le taux de fécondité du *Dolania* est 20 fois moins grand que celui des éphémères d'autres familles et la masse sèche de ses oeufs à maturité équivaut à environ 32 fois celle des oeufs des éphémères d'autres familles. Soixante-dix pourcent de la croissance linéaire des ovocytes en voie de maturation se fait en 2 à 3 semaines au cours de l'avant-dernier stade larvaire et au début du dernier stade larvaire. Le tube digestif ne s'atrophie pas entièrement et les ovocytes qui ne se développent pas restent petits et ne sont pas résorbés avant que les autres ovocytes n'atteignent leur maturité. Les ovocytes en résorption ne constituent donc pas une source importante d'énergie pour les ovocytes en voie de maturation. Le jeûne peut contribuer à réduire la fécondité du *Dolania* d'une production normale de 100 oeufs à 6 oeufs. La résorption limitée des ovocytes semble un phénomène commun chez les éphémères. Les imago femelles du *Callibaetis ferrugineus*, qui sont ovovivipares, résorbent une grande partie de leur musculature abdominale. Des ovulations peuvent se produire et même les oeufs se développer chez les adultes de plusieurs espèces ovipares. Le chorion épais et la couche suprachorionique fibreuse, épaisse et collante des oeufs de *Dolania* contribuent probablement à rendre les oeufs résistants au sable et aux champignons pendant près de 1 an dans un milieu où il y a une forte circulation de grosses particules de sable. L'oeuf, de grande taille, produit une larve de premier stade bien développée, de 2,5 à 5 fois la longueur de la larve de premier stade de toute autre espèce d'Éphéméroptère. La stratégie de reproduction du *Dolania* et du *Behningia*, contrairement à celles d'autres Éphéméroptères, consiste à pondre de gros oeufs, et donc à produire des larves de premiers stades de grande taille, prédatrices, capables d'exploiter un large éventail de tailles de proies.

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Introduction

Reproductive strategy in mayflies usually involves the production and oviposition of a large number (500 – 12 000, Clifford and Boerger 1974) of small eggs (about $219 \times 150 \mu\text{m}$) to ensure survival of a few individuals to the reproductive adult stage. In a Canadian brown-water stream, only one subimago mayfly was produced from approximately 934 eggs (average for 11 species, Clifford and Boerger 1974).

In this study, members of the family Behningiidae, *Dolania americana* Edmunds and Traver and *Behningia lestagei* Motas and Băcesco, are shown to follow an alternative reproductive life history strategy in which very small numbers

(approximately 100) of very large eggs ($800 \mu\text{m}$ to 1 mm in length) are oviposited. The importance of large eggs and consequent large early-instar larvae are discussed for *Dolania*, and oogenesis and routine ovariole oocyte resorption are described. Other mayfly species were examined to determine the frequency and role of oocyte resorption in mayfly biology. A brief synopsis of the basic biology of *Dolania* is given below to introduce the reader to this unique group of mayflies.

Dolania is endemic to some coastal plain streams of southeastern United States with coarse shifting sand bottoms, acidic pH, and relatively unpolluted water (see references in Peters in al. 1987). Larvae burrow through the sand in search of

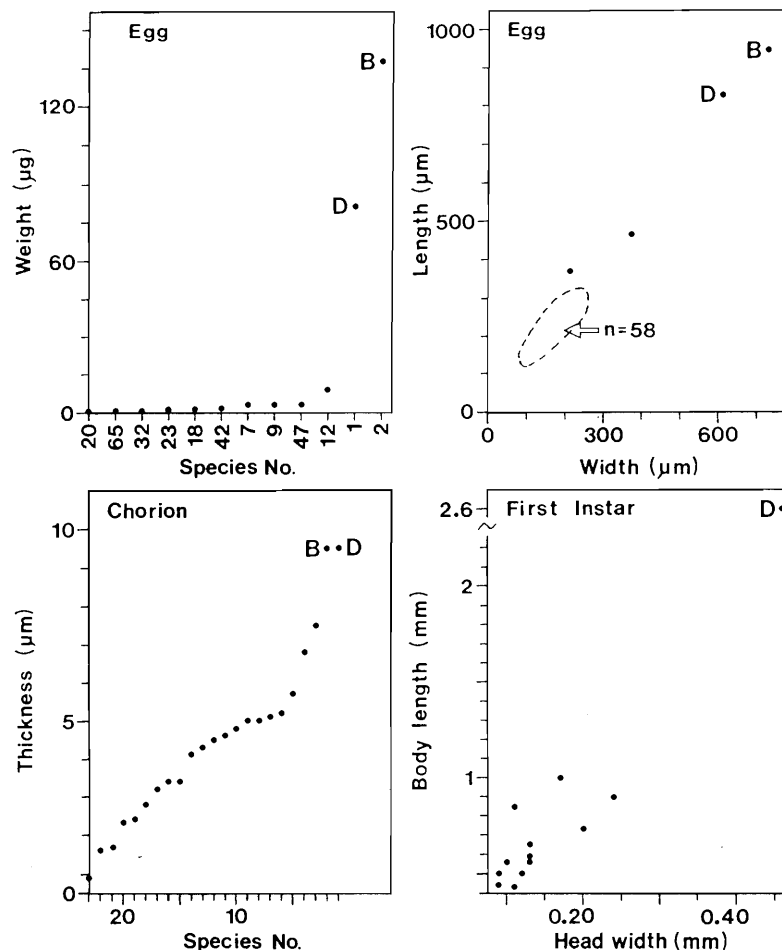


FIG. 1. Comparison of mayfly egg weight, egg size, first instar size, and chorion thickness. Data used to compile this figure can be obtained from Tables 1 and 2 of the supplementary material (see Methods).

Chironomidae (Diptera) larvae and other prey (Tsui and Hubbard 1979).

Dolania has a 2-year life cycle. Eggs require approximately 9–12 months to hatch and larvae mature in 12–14 months (Peters et al. 1987; Harvey et al. 1980; Tsui and Hubbard 1979). Growth of the larvae is rapid in spring and summer and slow in fall and winter; rapid growth resumes in spring shortly before emergence of the adult (Tsui and Hubbard 1979).

Adult emergence is highly synchronous and occurs in about a 2-week period near the end of April to mid-May in Florida (Peters et al. 1987; Peters and Peters 1977) and early June for South Carolina (Harvey et al. 1980; Sweeney and Vannote 1982). Emergence behavior and timing are very similar between the two sites (T. J. Fink, unpublished data).

Methods

Mayfly collections

The main study site for *Dolania* was the "Riverside" locality on the Blackwater River in Okaloosa County, Northwestern Florida, USA, at the Biological Field Station of Florida Agricultural and Mechanical University, 4.5 km northwest of Holt. Sampling dates were December 25–26 in 1981 and January 30, February 27, March 13–14, and April 3–4, 8, 13, 18, and 24, and 27 (subimagos) in 1982. Larvae were collected with kick nets.

We studied numerous other collections from other localities on the Blackwater River and some collections came from Juniper Creek and

the Yellow River. These localities were 28 km or less from the Riverside site and dates ranged from 1971 to 1982. Some samples were collected in late May and June 1978 just prior to and during the adult emergence period from Upper Three Runs Creek (UTR) at the Running Waters Laboratory of the Savannah River Plant, E. I. du Pont de Nemours Company, Aiken County, South Carolina.

Reared first instars of *Dolania* were obtained from fertilized eggs collected early May 1979 from the Blackwater River (five specimens) and June 6, 1978, from UTR (three specimens, courtesy of B. W. Sweeney). All first instars were preserved in 70–80% ethanol and slide mounted for observations and measurements.

Other mayfly species were generally collected near Salt Lake City, Utah, or were obtained from mayfly collections at the University of Utah (G. F. Edmunds, Jr.) and Florida Agricultural and Mechanical University.

Dissections and preservation

Specimens were placed live into preservative and the head and 9th or 10th abdominal segment were cut off to allow fixative penetration. Most specimens were fixed with 10% neutralized formalin or a mixture of 2% glutaraldehyde and 2.5% paraformaldehyde in Millonig's phosphate buffer of pH 7.4. Some specimens were preserved in 70–80% ethanol or Bouin's solution.

Ovarioles were counted by separating or dissecting individual ovarioles from the ovaries with Minutien insect pins. Ovariole and larval measurements were made with microscope ocular micrometers. Volume estimates of oocytes were made using the formula for an ellipsoid (Clifford 1970).

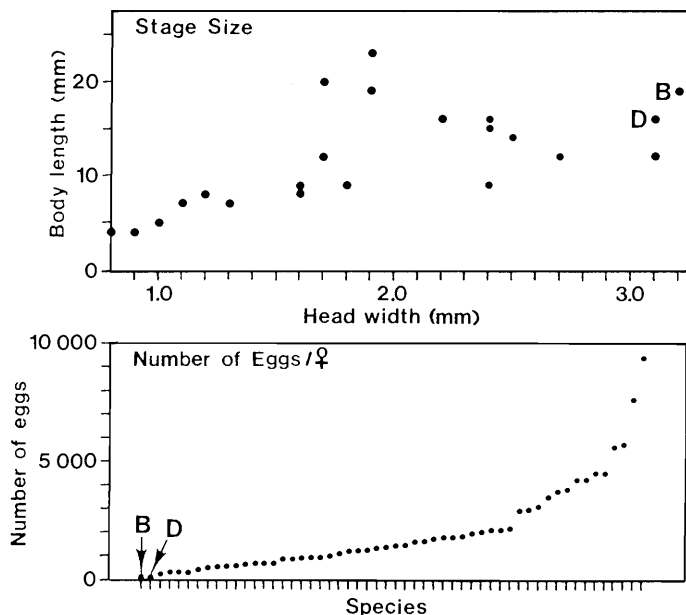


FIG. 2. Comparison of sizes of mayfly stages (adult or mature larval) and the number of mature eggs per mayfly female. Data used to compile this figure can be obtained from Table 1 of the supplementary material (see Methods).

Chorion thickness for mayfly eggs was determined through measurements of sections, broken eggs, and optical sections.

Wet and dry weights

About 10 live larvae were blotted dry and weighed together, soon after collection, on an analytical balance. Usually three groups were weighed per sampling date.

Eggs and larvae were dried for at least 24 h at $\geq 40^\circ\text{C}$ after removal from their alcohol preservative and dry weights were determined on an electrobalance. Eggs were weighed in groups of 10 or more, except for some *Dolania* and all *Behningia* (5 and 10 eggs from two specimens) eggs, which were weighed singly.

Starvation-induced egg resorption

Data were derived from rearings in 1982–1985 for experiments on emergence synchronization and instar duration (see Peters et al. 1987). Larvae were reared until emergence or death, and all egg counts are from adults or from pharate subimagos.

Comparative supplementary data

The egg weight, egg size, chorion thickness, first-instar size, stage size, and fecundity of *Dolania* and *Behningia* (Figs. 1 and 2) are compared with values gathered for many other mayflies from published sources and from examination of specimens. These data, compiled in two tables, are available upon request from the senior author or from the Depository of Unpublished Data, Canada Institute for Scientific and Technical Information (CISTI), National Research Council of Canada, Ottawa, Ontario, Canada K1A 0S2.

Results

Mayfly egg and fecundity comparisons

Behningiidae eggs are the largest known among mayflies (Fig. 1). The dry weight of *Dolania*'s egg was 32 times the average of other nonbehningiid mayfly eggs, and the chorion was 2.4 times thicker than the average for other mayflies (Fig. 1).

Although *D. americana* and *B. lestagei* are large (Fig. 2), their fecundities are among the lowest known in mayflies, with females producing on the average about 21 times fewer eggs than other mayflies (Fig. 2).

In *Dolania*, eggs were found from the metathorax to the seventh abdominal segment. Fecundity correlated highly with female body length ($r = 0.80$, $p < 0.001$, $n = 63$), but poorly with head width ($r = 0.40$, $p < 0.002$, $n = 61$). *Dolania* females displayed about a 1:1 ratio between egg dry weight per female and female dry weight less eggs. A similar relationship occurred in *Leptophlebia cupida* (Say) (Clifford et al. 1979) and may be common in mayflies.

Some *Dolania* female adults and mature final-instar larvae had more empty space in their abdomens than others, and this resulted in the scatter seen in fecundity – body size correlation plots. In 1973, *Dolania* larvae displayed some of the lowest fecundity levels recorded in the Blackwater River. The average number of eggs for 20 specimens was 56 (10 specimens evaluated by Peters and Peters 1977, and 10 in this study) while in 1969, 1971, 1974, and 1975, averages were higher: 120, 99, 79, and 96 eggs, respectively (Peters and Peters 1977). Unfortunately, limited larval size data exist for 1973. Eggs were not tightly packed in the oviducts of some specimens in 1973.

First instar of *Dolania*

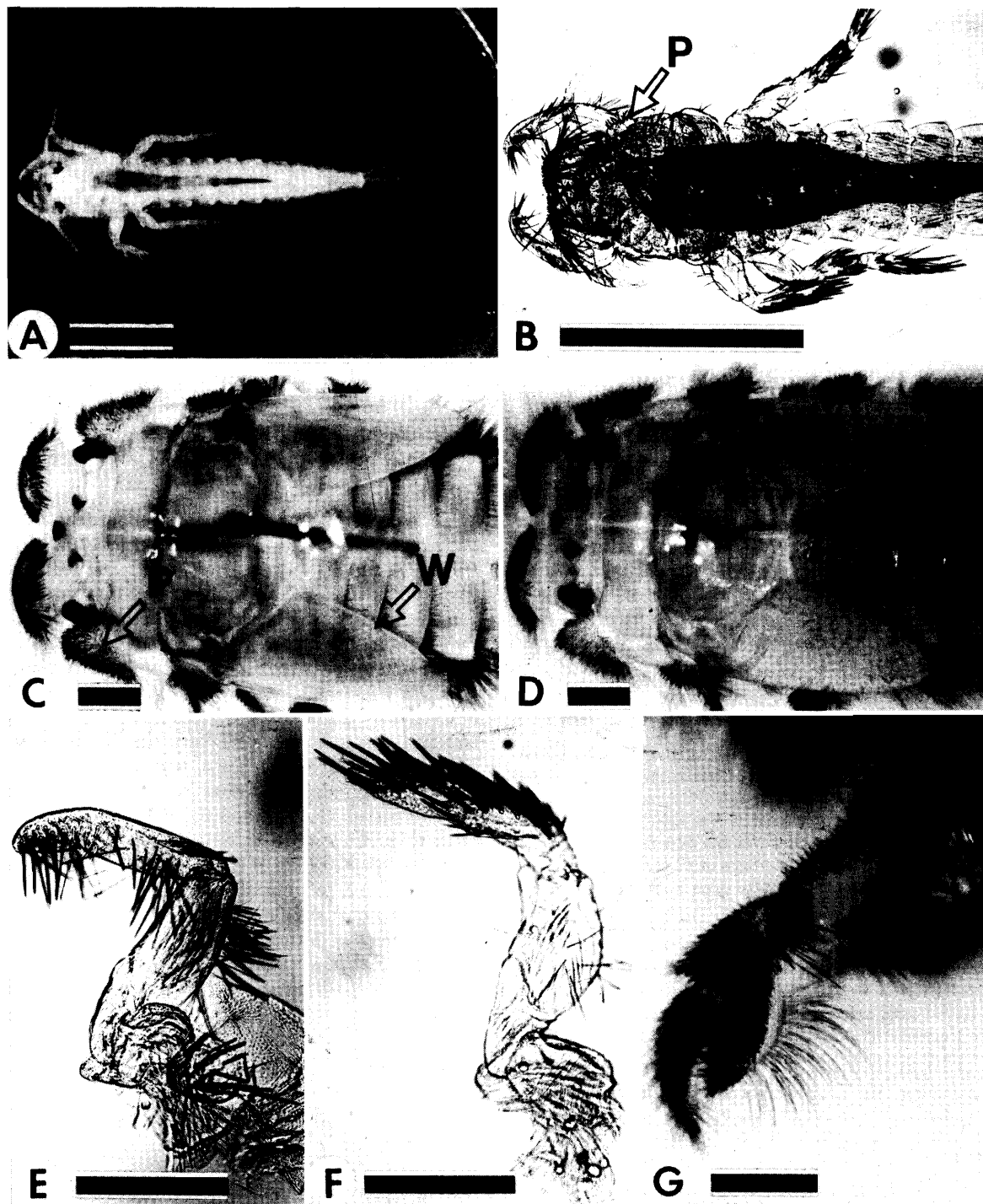
The very large egg of *Dolania* produces the largest (Fig. 1) and most well-developed first-instar larva known in mayflies (Figs. 3A and 3B). Body length and head width were respectively about five and three times larger than the average values for other mayflies. The next largest mayfly first instars were only 1 mm long, compared to 2.6 mm for *Dolania*.

The first instar (which has not been described) was recognizable at a glance as *Dolania* (Fig. 3) (mature larvae have been described by Edmunds and Traver 1959). The heads of first- and final-instar larvae were very similar, both with anterior concentrations of coarse setae, which protect the head during burrowing. Forelegs and hind legs were also similar between the two instars (Fig. 3) and the mesothoracic leg of the first instar (Fig. 3F) can be seen developing along the pattern leading to the cheliped-like leg of later instars (Fig. 3G). The first instar also possessed recognizable beginnings of the coarse-haired prothoracic epaulets (compare Figs. 3B and 3D), which may protect the eyes during burrowing (T. Dolan in Edmunds and Traver 1959, pp. 50–51). Mandibles of both instars lacked molar crushing surfaces, and the apex of the galea lacinia in both was produced apically into a sharp stout spine presumably for stabbing, securing, and manipulating prey. However, labial palpi of mature instars were much more massive than the prothoracic legs, whereas in first instars the reverse was true.

First instars, like other mayfly first instars, lack gills (gills appeared in the second instar), and the coarse protective lateral and sternal abdominal hairs characteristic of later instars. The metathoracic legs were shorter and extended to about the fifth abdominal segment (Figs. 3A and 3B), whereas, in later instars as small as 3.5 mm in length, these legs extended to about the ninth segment and may help to protect the ventral gills on segments 1–7 during burrowing as suggested by T. Dolan for mature larvae (in Edmunds and Traver 1959, p. 51).

Female larval growth

From December 1981 to March 13–14, 1982, there was no significant increase, for female *Dolania* larvae, in body length, sixth abdominal segment width, mesothoracic wing pad length, and total wet weight (one-way ANOVA, $p > 0.05$; Fig. 4). In contrast, a considerable and statistically significant increase ($p < 0.05$) in all measurement variables occurred 3 weeks after the sample of March 13–14 (Fig. 4). This increase correlated



ABBREVIATIONS: B, *Behningia lestaei*; D, *Dolania americana*; DL, dorsal ovariole layer; F, fat body; Fo, follicle; G, gut; I, immature oocyte(s); M, mature oocyte(s); MWP, mesothoracic wing pad; *n*, number of data points; N, nerve cord; Nu, nucleus; O, ovary; Oo, oocyte; P, prothoracic epaulets; R, germarium; W, mesothoracic wing pad; Y, yolk; 6AS, sixth abdominal segment; 6, sixth abdominal segment; 7, seventh abdominal segment. FIG. 3. Comparison of first- and mature-larval instars of *Dolania americana*. (A) and (B) First instars. (C) and (D) Final-instar female larvae. (E) Foreleg of first instar. (F) Mesothoracic leg of first instar. (G) Cheliped-like mesothoracic leg of mature larva. Scale bars: A–D and G, 1 mm; E–F, 250 μ m.

with molts to the penultimate and final larval instars. Many larval exuviae were collected in the sample of March 13–14, indicating a molt to the penultimate instar. Growth of *Dolania* larvae was relatively synchronous; most individuals per sample were similar in size and development. *Dolania* has approximately 15 larval instars (determined through palmen-body analysis, T. J. Fink, unpublished data).

Oocyte growth and development

In 1981–1982, oocyte growth paralleled larval growth. There was little growth in winter and early spring (December – early March), and very rapid growth from mid-March to early–mid-April (Figs. 5 and 6). Oocytes from March 13–14 sample averaged $162 \times 144 \mu\text{m}$ and these dimensions increased three- to four-fold to $600 \times 472 \mu\text{m}$ 25 days later on April 8 (Fig. 5).

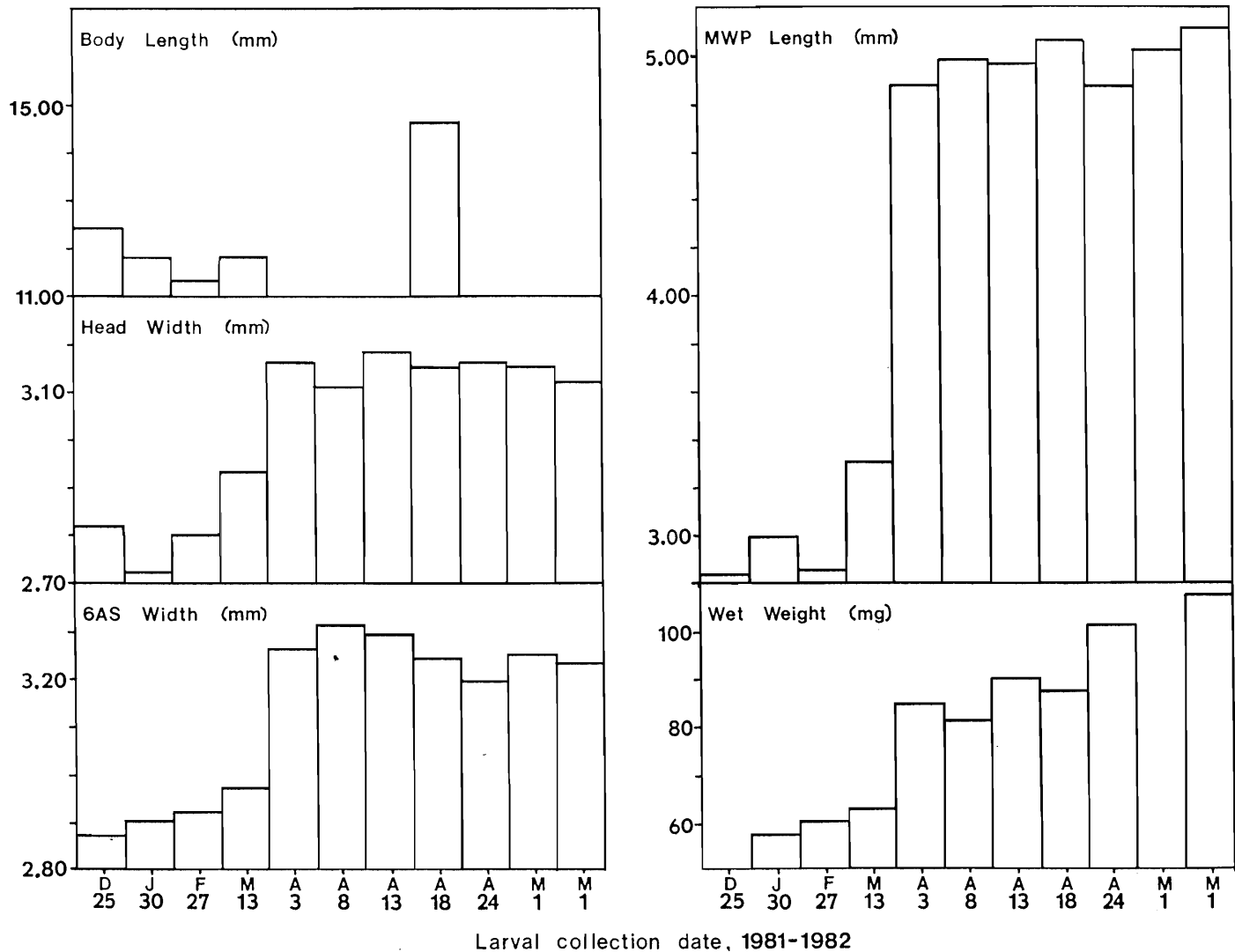


FIG. 4. Growth of *Dolania americana* female larvae. Specimens were collected from the Riverside locality from December 25, 1981 to April 24, 1982, and from Juniper Creek at Red Rock (first bar) and the Blackwater River at Red Oak (second bar) on May 1.

Ovaries from specimens of March 13–14 occupied less than 10% of the abdominal cross-sectional area, while those of April 3–4 and later occupied nearly 100% exclusive of the gut (Figs. 6G–6J). The largest oocytes of April were about twice as large as the largest of March, and by April 8, over 40% of oocytes were mature or nearly mature in size (around $750 \times 600 \mu\text{m}$). A similar pattern of growth occurred in other years (1971–1981). From December to March, oocytes were covered by masses of fat body anchored into place by tracheae and tracheoles. Fat body removal was difficult without destroying some underlying oocytes, and many specimens were dissected in order to obtain complete or relatively complete ovaries. In contrast, abdomens (segments 1–7) of early to mid-April specimens were filled with oocytes (Figs. 6C–6E, 6I–6J) tightly juxtaposed, and very little fat body remained. Mature-sized oocytes at that time were extremely brittle since they were not yet protected by a completely formed chorion.

In the last larval instar, chorion deposition, subimaginal wing development, and the degeneration of the gut were coordinated. Many oocytes were full-sized when the wing pads were still brownish-translucent. Initial chorion formation began when wing pads showed the venation of the developing (white)

subimaginal wings (Fig. 3C); at this time the gut remained full-sized with food and sand. When half to two thirds of the mature-sized oocytes had completed chorion formation, wing pads soon began to swell and display folded subimaginal wings, and the gut, still full, became reduced in width. Chorion formation was complete on all, or nearly all, mature-sized oocytes when wing pads were very white, swollen, and subimaginal wing folding was beginning. Continued folding of the wings correlated with further width reduction and emptying of the gut. Complete egg maturation (including suprachorionic layer formation) and ovulation were associated with tightly folded subimaginal wings and swollen wing pads (Figs. 3D, 6F), indicating impending emergence and gut degeneration. This pattern of chorionic, suprachorionic, and wing pad development was noted for all years (1971–1981) for which appropriate specimens were available.

Timing of events in 1982 at Riverside

Vitelline membrane was discernible in females of April 8, signalling the end of vitellogenesis. Chorion formation began around April 13 and was generally completed by April 18. The suprachorionic layer was completed in some females around

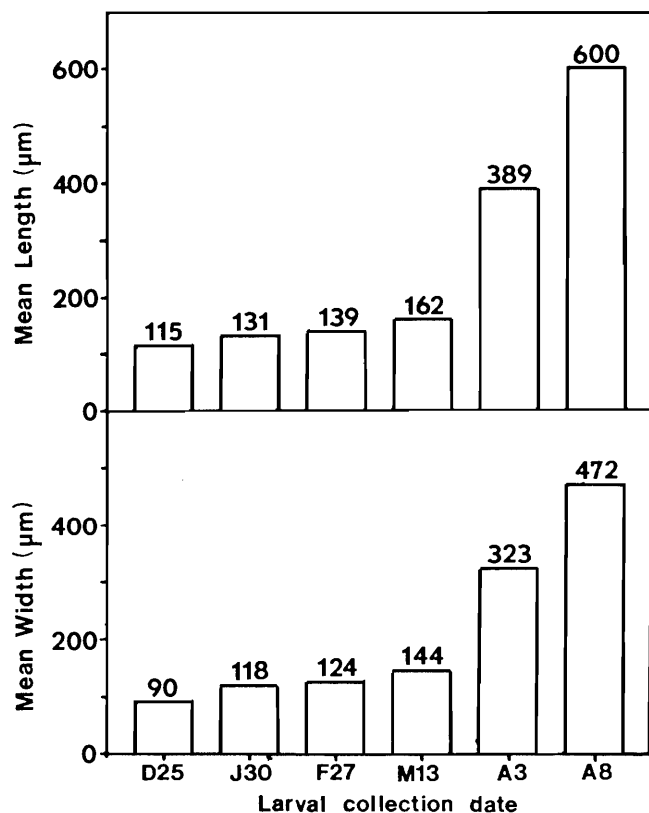


FIG. 5. *Dolania americana* oocyte growth, December 25, 1981, to April 8, 1982.

April 18 and in most others by April 24. Follicle cells began to degenerate immediately after suprachorionic layer formation, and ovulation soon occurred.

Ninety percent of females collected on April 18, 1982, ($n = 31$) possessed full guts, while 32 and 100%, respectively, of females collected on April 20 ($n = 19$) and April 24 ($n = 20$) contained atrophied guts. Males ceased feeding before females. Eighty-eight percent of males collected on April 20 ($n = 17$) contained atrophied guts. Red Oak specimens collected on May 1, 1982, included 86% of females ($n = 64$) with full to slightly reduced guts, while 82% of males ($n = 34$) had atrophied guts.

Adult emergence in 1982 at Riverside began with a few males appearing on April 21. The first large emergence of both males and females occurred on April 28. The largest emergence occurred on May 3 and the last on May 11. Peak emergence at Red Oak occurred on May 16.

Routine oocyte resorption

Dolania routinely resorbs one third to half of its developing oocytes (Soldán 1979c). Resorbing oocytes might serve to store nutrients until needed by those oocytes that eventually mature. To test this hypothesis, it was necessary to ascertain the extent and timing of oocyte resorption, the size and development of resorbing and maturing oocytes at the beginning of resorption, and the timing of gut degeneration (end of feeding) and fat body depletion.

The extent of oocyte resorption in 1982 was similar to that reported by Soldán (1979c). The average number of oocytes for winter specimens (December 1981 – February 1982) was 196.9 ($n = 12$, $\sigma_{n-1} = 39.2$, percentage coefficient of variation = 21.2) while, for April 24, the average was reduced by 45% to

108.8 ($n = 12$, $\sigma_{n-1} = 16.0$, percentage coefficient of variation = 14.7) and only mature or near-mature oocytes were present. The average number of oocytes was reduced by 38% when comparing specimens of December 1981 – April 18, 1982 ($n = 22$: December 25–26, 2; January 30, 6; February 27, 4; March 13–14, 5; April 3–4, 1; April 8, 1; April 18, 3) with those of April 24 ($n = 12$). In three specimens of April 18, an average of 40% of oocytes were very small and would have been resorbed before emergence of the subimagos. Resorbing oocytes were observed in one or more specimens in 7 of 11 years between 1971 and 1981 where suitable specimens were available.

Ovaries from all sampling dates, except very late April when only mature oocytes remained, exhibited a wide range in size of oocytes (Fig. 7). By April 8–18, 1982, two classes of oocytes remained: small oocytes that would have been resorbed and large mature-sized oocytes that would have been oviposited.

Many oocytes that would have been resorbed apparently stopped growing at least by the middle of March in 1982, or approximately 1.5 months prior to subimaginal emergence. Many of these oocytes never developed beyond the stage reached by December or January oocytes; they remained very small (90–130 µm in length) and vitellogenesis was very incomplete (compare oocytes of Fig. 8A–8C). Some oocytes attained fairly large maximum linear measurements of up to 400–600 µm, but these oocytes constituted less than two thirds the volume of a mature oocyte. Similar results occurred in other years.

Resorption of oocytes in 1982 occurred around April 18. Some specimens of April 18 possessed only mature-sized oocytes and empty collapsed follicles, while others possessed mature-sized and very small oocytes. In 1982, no small oocytes remained by April 24 and, in other years, no small oocytes were present in a comparable stage of larval development (i.e., larva nearing emergence with swollen wing pads and folded subimaginal wings within, Figs. 3D and 6F).

The beginning of resorption coincided with the maturation of the remaining oocytes. Resorbing oocytes constituted 38–45% of the total number of oocytes per female, but were very small and collectively constituted only an estimated 1–5% of the total egg volume. Approximately 2 weeks before emergence, *Dolania* oocytes that were less than half to two thirds the volume of a mature oocyte would probably be resorbed. Rarely, some eggs matured at very small sizes. In two female larvae from UTR, two very small eggs with complete chorions were found in one female and a similar egg in the other. The volumes of these eggs were 39 to 57% of the volume of an average *Dolania* egg.

Resorption of germaria

Germarium size was relatively constant during oocyte development, except for very small December oocytes and mature-sized oocytes. The germaria of maturing oocytes became reduced to the width of terminal filaments. Germarium resorption coincided with chorion deposition.

Starvation-induced resorption

Egg number was negatively correlated with the number of days since collection, for larvae reared for 3 weeks to 70 days ($r = -0.74$, $p < 0.001$, $n = 24$). These larvae were in the penultimate and early last instar when collected, which is when most oocyte growth (approximately 70%) and development occurs. Any stress experienced by these larvae, such as starvation (i.e., an inadequate laboratory diet), apparently resulted in oocyte resorption beyond the normal amount (as low as six mature eggs).

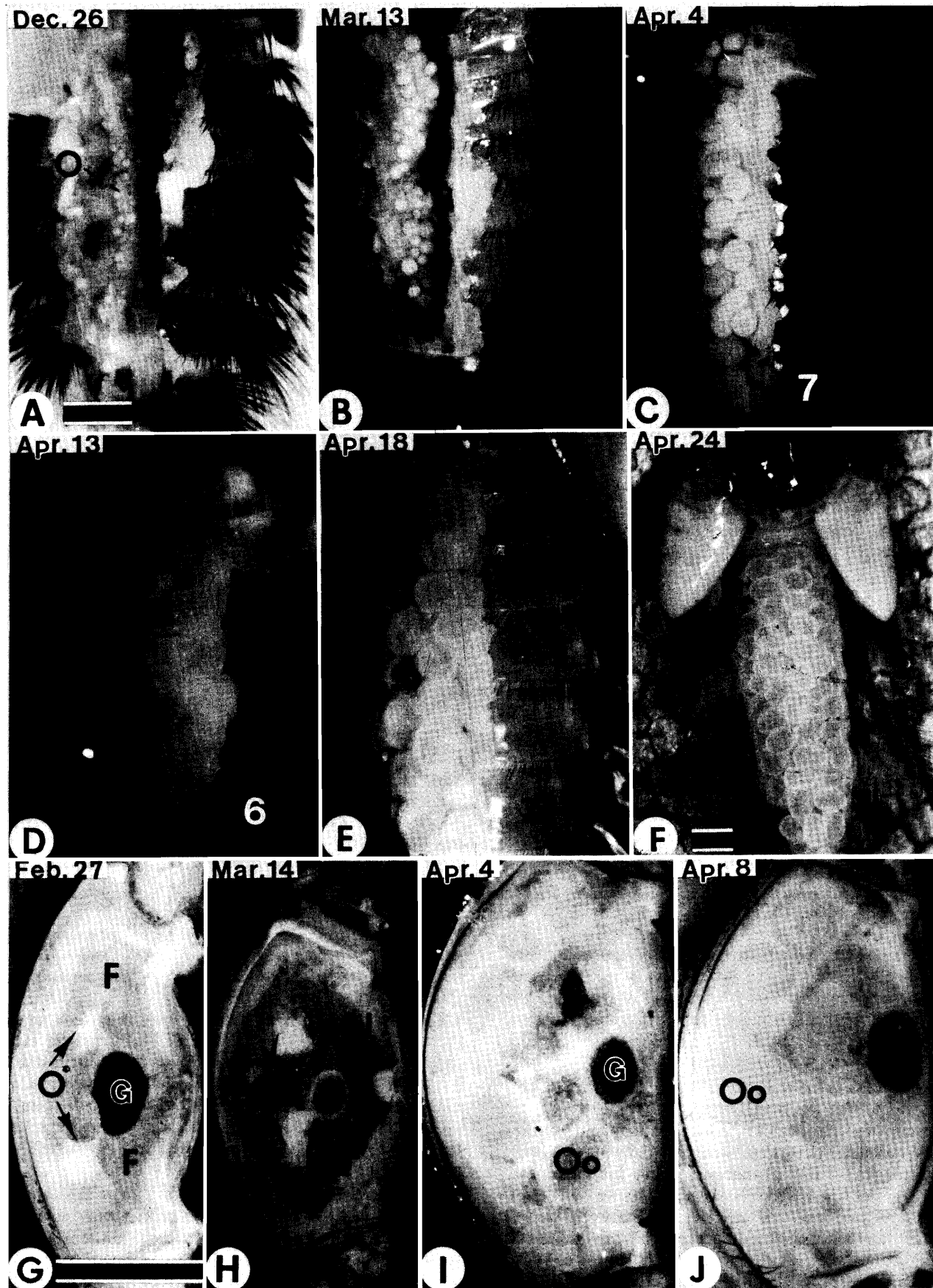


FIG. 6. *Dolania americana* oocyte growth and fat body depletion. (A) to (F) Dorsal view of abdominal segments 1-6 or more for larvae collected December 26, 1981, to April 24, 1982. (G) to (J) Transverse sections through fifth abdominal segments of larvae collected February 27 to April 8, 1982. All scale bars (SB) represent 1 mm; SB in A for A-E; SB in G for G-J.

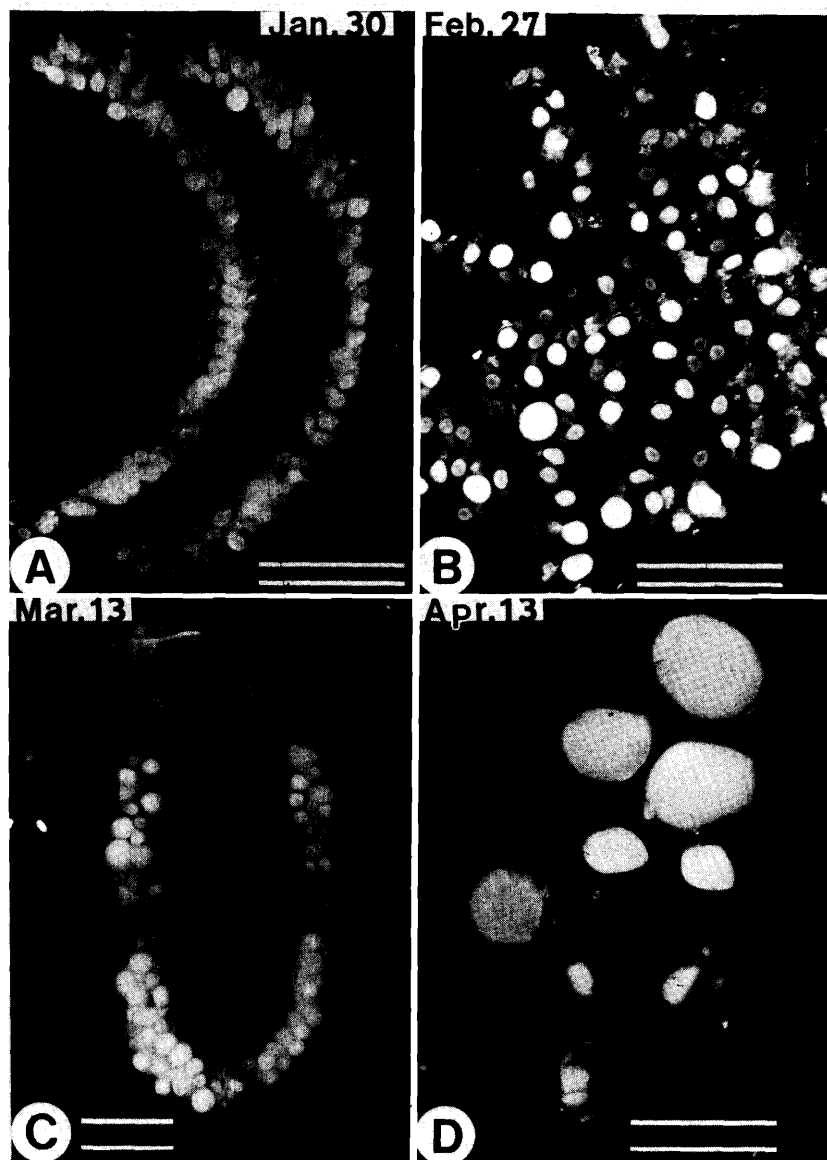


FIG. 7. *Dolania americana* oocyte size variability, January 30 to April 13, 1982. (A) and (C) Entire ovaries. (B) and (D) Separated ovarioles. All scale bars represent 1 mm.

There was no correlation between egg number and number of days since collection of 15–16 days or less ($n = 63$), because most of these specimens were near the end of the final larval stadium in which feeding ceases and eggs are completely mature.

Oocyte resorption in other mayflies *Heptageniidae*

Resorption of some oocytes is probably common in mayflies, because very small oocytes are observed frequently in subimagos and imagos. In four pharate subimagos and three newly molted imagos of *Rhithrogena robusta* Dodds, we observed an extensive layer of dorsal ovarioles (Fig. 9A) that obscured the much larger number of ovulated oocytes. These ovarioles each contained about three mature oocytes and three to four small resorbing oocytes (Figs. 9B–9C). In one imago, we estimated that 22% of the oocytes would be resorbed in an ovary that contained 2813 mature, ovulated oocytes and 257 ovarioles with unovulated mature and immature oocytes. Four adults that lived 7 days each (subimago + imago) in the laboratory

possessed only mature, ovulated oocytes. Oocyte resorption, ovulation, and perhaps some development may have occurred in these specimens.

In two or more adults each of *Stenacron interpunctatum* (Say), *Cinygmula mimus* (Eaton), and *Pseudiron centralis* McDunnough some multi-oocyte ovarioles contained oocytes too small to mature. In two subimagos of *S. interpunctatum*, many mature, unovulated oocytes had very small or undetectable polar coils (mature eggs possess a very large single-stranded coil at each pole and much smaller single-stranded coils near the poles of the egg).

Siphonuridae

Through the entire length of each ovary in an imago of *Siphonisca aerodromia* Needham, a dorsal concentration of resorbing oocytes, comprising 25% of all oocytes ($n = 669$), was observed. Fifty-six percent of all oocytes were ovulated, while 19% were mature-sized but unovulated and without a complete chorion. Two final-instar larvae of the sand-dwelling mayfly *Anaetris eximia* Edmunds also possessed resorbing oocytes. Two final-instar larvae of the omnivorous *Siphonurus*

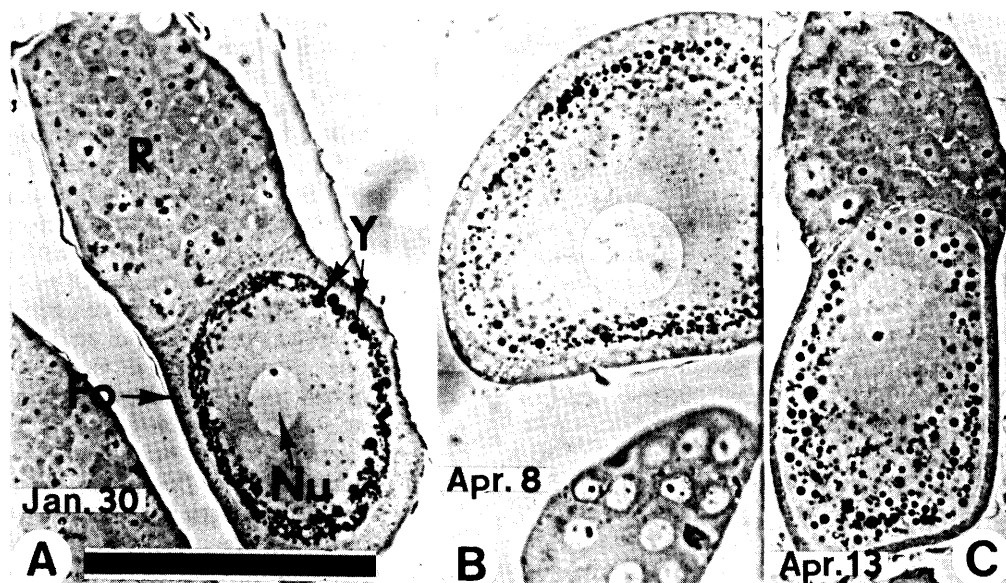


FIG. 8. Small early vitellogenic oocytes of *Dolania americana*, January 30 to April 13, 1982. Scale bar (in A for A–C): 100 μ m.

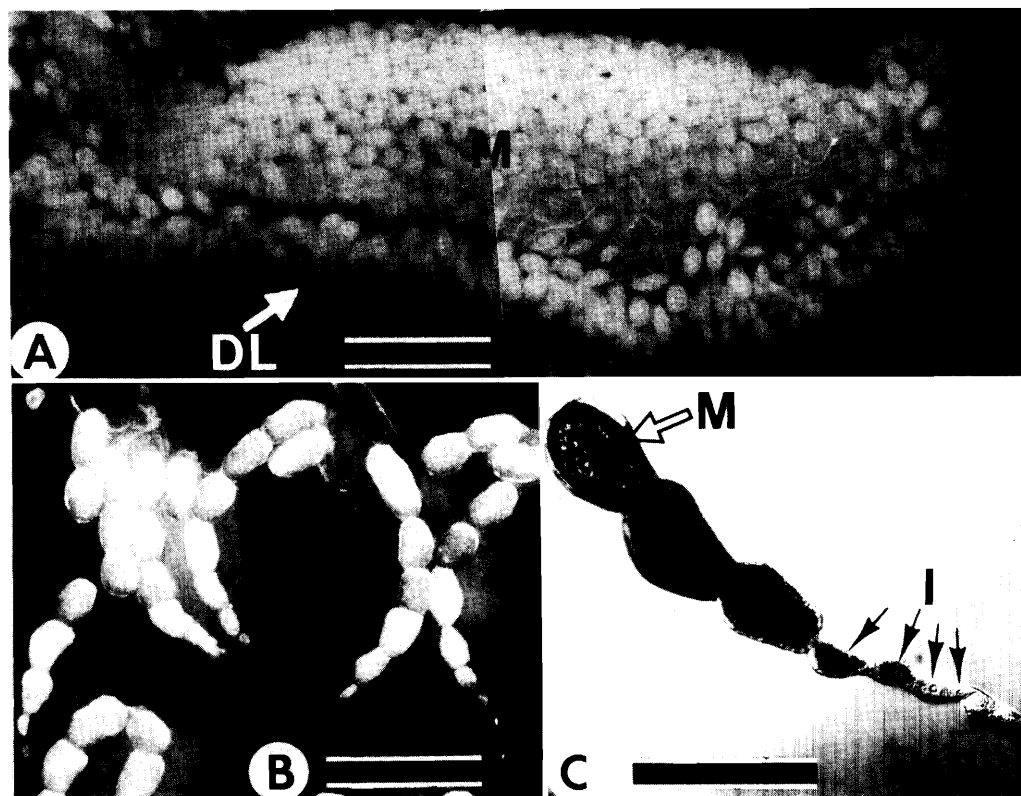


FIG. 9. Oocyte resorption in *Rhithrogena robusta* imago (Heptageniidae). (A) One complete ovary (SB, 1 mm). (B) and (C) Ovarioles (SB, 500 and 250 μ m, respectively).

occidentalis (Eaton) possessed a large number of resorbing oocytes. One specimen contained 5729 mature (87%) and 846 resorbing (13%) oocytes. A very small number of resorbing oocytes were found in the single mature larva of *Acanthametropus* sp.

Baetidae

In a female imago of *Baetis* sp., we observed many ovarioles with small resorbing oocytes.

In the ovoviviparous *Callibaetis ferrugineus* (Walsh), dissections of five subimagos and two newly molted imagos revealed a large amount of empty space in the abdomen, significant muscle masses throughout the abdominal terga and sterna, and eggs from the anterior mesothorax to the sixth or seventh abdominal segment. The number of eggs per female from four of the subimagos averaged 728 and one female imago possessed 433 first instars ready to be oviposited. Eggs from the four subimagos averaged $134 \times 128 \mu$ m in size, while eggs from the

ovipositing imago were about $381 \times 188 \mu\text{m}$. Thus the empty space in subimaginal and newly molted imaginal abdomens allows for growth of the eggs. All 13 female imagos that oviposited had very little abdominal muscle. Nutrition for the developing embryos may come in part from resorbing muscle tissue, and probably from some of the very small amount of fat body remaining in newly molted imagos. It is unknown if oocyte resorption also occurs.

Discussion

Reproductive strategy in Dolania

Dolania and *Behningia* pursue an unusual mayfly reproductive strategy of producing a very small number of very large eggs. Fecundity in Behningiidae (about 70–100 eggs) is approximately 21 times less than that of other mayflies, and the eggs are the largest known among mayflies, at 800–1000 μm in length. Eggs of *Dolania* are about four times the length and width, and 32 times the dry weight, of the average values of non-behningiid mayflies.

The large egg produces a very large and well-developed first-instar larva. In the laboratory, first instars molted to the second instar without feeding (J. G. Peters, unpublished data). Tsui and Hubbard (1979) showed that larvae of 0.7 mm head width, probably second instars, ate Chironomidae. Large early-instar size makes a large range of prey sizes available.

In another predacious mayfly, *Siphonisca aerodromia*, eggs are larger ($460 \times 340 \mu\text{m}$) than the average mayfly egg, but are more numerous (313–486, mean = 394) and not nearly as large as Behningiidae eggs. Early instars feed on detritus, but shift to prey as larval head width approaches 1 mm (Gibbs and Mingo 1986).

Eggs of *Dolania* and *Behningia* have the thickest chorions of any measured mayfly eggs (Fig. 1). The thick chorion, aided by a thick suprachorionic adhesive layer and attached sand grains may help resist damage from sand and fungi during a 9- to 12-month period in a "coarse rolling sand habitat" (we do not know where the majority of eggs remain in the stream and how they avoid being washed downstream). However, large eggs and thick chorions are not necessary for survival in this habitat, as demonstrated by the relatively small eggs of other coarse-sand dwellers such as the mayflies *Pseudiron centralis* and *Homoeoneuria* spp. and the Chironomidae.

Ongoing studies of J. G. Peters, W. L. Peters, and C. LeDuc are investigating the fate of *Dolania* eggs through the guts of their fish predators, where large tougher eggs may have a survival advantage.

Reasons for low fecundity in Dolania

Low fecundity of *Dolania* is made possible in three ways. The number of ovarioles of young larvae is about only 200 (100 per ovary), while most other mayflies have a greater number. Each ovary of *Paraleptophlebia submarginata* (Stephens), *Ephemera danica* Muller, and *Caenis robusta* Eaton consisted of 250–500 ovarioles and those of *Oligoneuriella rhenana* (Imhoff) and *Ecdyonurus torrentis* Kimmins consisted of 150–300 ovarioles, although an ovary of the ovoviviparous *Cloeon* included only 90–200 ovarioles (Soldán 1979b).

The most important factor for low fecundity in *Dolania* is that a maximum of one oocyte matures per ovariole. Other mayflies typically develop 5–7 oocytes per ovariole (e.g., *P. submarginata*, *E. torrentis*) or as many as nine (*E. danica*, *C. robusta*) (Soldán 1979a). However, ovoviviparous species like *Cloeon dipterum* (Linnaeus) (Soldán 1979a), and probably *Callibaetis* sp., develop only 1–2 oocytes per ovariole.

The germarium of *Dolania* is similar to those of other mayflies in that it appears to contain many small primary and young oocytes (terminology of Chapman 1982, see Fig. 219A on page 337) (Figs. 8A–8C). In some unknown way, these oocytes in all mayflies are inhibited from developing into mature oocytes.

Fecundity is further reduced by routine resorption of about one third to half the original number of oocytes in the vitellaria. Resorbing oocytes may have little or no importance as a nutrient source for the maturing oocytes because they generally remain very small and are not resorbed until other oocytes are mature, and larval feeding continues until oocyte maturation. *Dolania*, like many other insects (Bell and Bohm 1975), may also resorb oocytes when female larvae are starved. Fecundity of some starved *Dolania* females was only 6 to 11 eggs. Routine egg resorption also occurs in other insects (Bell and Bohm 1975; Highnam et al. 1963; Trepte and Trepte-Feuerborn 1980; Spradbery and Schweizer 1981), but usually not to the extent seen in *Dolania*. Resorption of all ovarioles, and even the oviducts, has been observed in two heptageniid mayfly species in response to the ectoparasitic chironomid *Symbiocladius rhithrogenae* Zavrel (Codreanu 1939; Soldán 1979d).

The extent of resorption in *Dolania* may be temporal and site-specific, in response to local food resources and other environmental conditions. Fecundity of *Dolania* from South Carolina is only about 77 eggs (Harvey et al. 1980). In the Blackwater River, specimens at the upstream site (Red Oak) have greater fecundity than Riverside specimens. Recent counts from nine female imagos from the Yellow River in northwestern Florida showed an average fecundity (at 169 eggs per female) almost 40% greater than at Riverside. Fecundity in 1973 at Riverside was lower than during other years.

Resorption of germaria of maturing oocytes occurs while oocytes are reaching maturity. Because *Dolania* females are still feeding, the nutritional input of resorbing germaria may be slight.

Resorption and adult oocyte development in other mayflies

Considering the hundreds or thousands of eggs developing in most mayflies, it should not be surprising that a small percentage of oocytes may be resorbed or never reach maturity. Some of the most distal oocytes in some ovarioles may not have time to complete development before subimaginal emergence. It may be advantageous for females to continually develop new oocytes near their emergence time because weather conditions that delay emergence may allow further egg development and greater fecundity. In most mayfly species examined, some evidence of resorbing oocytes was found. Soldán (1979a, 1979b, 1979d) also found that resorption of the youngest and smallest oocytes in an ovariole is common. Oocyte resorption in *Rhithrogena robusta*, and perhaps some other mayflies, may also be routine as in *Dolania*. In *R. robusta*, large numbers of dorsal ovarioles contained many unovulated mature and immature oocytes and these oocytes were either ovulated or resorbed during an adult period of 7 days in the laboratory. Immature oocytes may serve as a food source during the potentially long adult life.

Callibaetis female imagos can live for 1 week or 2 (Berner 1941; Edmunds 1945; Gibbs 1979), during which time eggs develop within the female to the first instar larva. Apparently some nutrition for these developing embryos comes from resorbing abdominal muscle tissue because females cannot feed and very little fat body remained from their larval development.

Another potential source of nutrition would be resorbing oocytes, but this remains to be investigated.

Oocyte development, except in ovoviparous species, is believed to occur in the larval stage because of the relatively short duration of the adult stages (Smith 1935; Soldán 1979a, 1979b). This is true in species with very short adult lives (less than 2 h), such as *Dolania*, *Ephoron*, and *Caenis*. In other species, some egg development and ovulation apparently occurs in the subimaginal or imaginal instar. In many mayflies, the adult stage lasts 1 to 2 days and, in this period, it is possible that some eggs complete the development of the chorion and (or) suprachorion. In *Drunella doddsi* Needham and *Drunella grandis* (Eaton), eggs with the largest polar caps were not ovulated until the adult stage (T. J. Fink, unpublished data). Polar attachment coils on some unovulated *Stenacron interpunctatum* eggs appeared to be lacking or were very small in subimagos and imagos. A fairly large number of oocytes in *Rhithrogena robusta* females may complete development in the imago. A protracted adult life may achieve a slightly higher fecundity by providing enough time for any late-developing oocytes. However, in most species, the majority of oocytes are probably matured and ovulated in final-instar larvae.

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- BELL, W. J., and BOHM, M. K. 1975. Oosorption in insects. *Biol. Rev. Cambridge Philos. Soc.* **50**: 373–396.
- BERNER, L. 1941. Ovoviparous mayflies in Florida. *Florida Entomol.* **24**: 32–34.
- CHAPMAN, R. F. 1982. *The insects structure and function*. 3rd ed. Harvard University Press, Cambridge, MA.
- CLIFFORD, H. F. 1970. Analysis of a northern mayfly (Ephemeroptera) population with special reference to allometry of size. *Can. J. Zool.* **48**: 305–316.
- CLIFFORD, H. F., and BOERGER, H. 1974. Fecundity of mayflies (Ephemeroptera), with special reference to mayflies of a brown-water stream of Alberta, Canada. *Can. Entomol.* **106**: 1111–1119.
- CLIFFORD, H. F., HAMILTON, H., and KILLINS, B. A. 1979. Biology of the mayfly *Leptophlebia cupida* (Say) (Ephemeroptera: Leptophlebiidae). *Can. J. Zool.* **57**: 1026–1045.
- CODREANU, R. 1939. Recherches biologiques sur un chironomide, *Symbiocladius rhithrogenae* (Zav.), ectoparasite «cancérigène» des éphémères torrenticoles. *Arch. Zool. Exp. Gen.* **81**: 1–283.
- EDMUNDS, G. F., JR. 1945. Ovoviparous mayflies of the genus

- Callibaetis* (Ephemeroptera: Baetidae). *Entomol. News*, **56**: 169–171.
- EDMUNDS, G. F., JR., and TRAVER, J. R. 1959. The classification of the Ephemeroptera. I. Ephemeroidea: Behningiidae. *Ann. Entomol. Soc. Am.* **52**: 43–51.
- GIBBS, K. E. 1979. Ovoviviparity and nymphal seasonal movements of *Callibaetis* spp. (Ephemeroptera: Baetidae) in a pond in southwestern Quebec. *Can. Entomol.* **111**: 927–931.
- GIBBS, K. E., and MINGO, T. M. 1986. The life history, nymphal growth rates, and feeding habits of *Siphonisca aerodromia* Needham (Ephemeroptera: Siphonuridae) in Maine. *Can. J. Zool.* **64**: 427–430.
- HARVEY, R. S., VANNOTE, R. L., and SWEENEY, B. W. 1980. Life history, developmental processes, and energetics of the burrowing mayfly *Dolania americana*. In *Advances in Ephemeroptera Biology, Proceedings of the 3rd International Conference on Ephemeroptera*, Winnipeg, July 4–10, 1979. Edited by J. F. Flannagan and K. E. Marshall. Plenum Press, New York. pp. 211–230.
- HIGHNAM, K. C., LUSIS, O., and HILL, L. 1963. Factors affecting oocyte resorption in the desert locust *Schistocerca gregaria* (Forsk.). *J. Insect Physiol.* **9**: 827–837.
- PETERS, J. G., PETERS, W. L., and FINK, T. J. 1987. Seasonal synchronization of emergence in *Dolania americana* (Ephemeroptera: Behningiidae). *Can. J. Zool.* **65**: 3177–3185.
- PETERS, W. L., and PETERS, J. G. 1977. Adult life and emergence of *Dolania americana* in northwestern Florida (Ephemeroptera: Behningiidae). *Int. Rev. Gesamten Hydrobiol.* **62**: 409–438.
- SMITH, O. R. 1935. The eggs and egg-laying habits of North American mayflies. In *The biology of mayflies*. Edited by J. G. Needham, J. R. Traver, and Y.-C. Hsu. Comstock Publishing Co. Inc., New York. pp. 67–89.
- SOLDÁN, T. 1979a. Spermatogenesis and oogenesis in mayflies (Ephemeroptera). In *Proceedings of the second conference on Ephemeroptera*. Edited by K. Pasternak and R. Sowa. Państwowe Wydawnictwo Naukowe, Warszawa-Kraków. pp. 267–271.
- 1979b. The structure and development of the female internal reproductive system in size European species of Ephemeroptera. *Acta Entomol. Bohemoslov.* **76**: 353–365.
- 1979c. Internal anatomy of *Dolania americana* (Ephemeroptera: Behningiidae). *Ann. Entomol. Soc. Am.* **72**: 636–641.
- 1979d. The effect of *Symbiocladius rhithrogenae* (Diptera, Chironomidae) on the development of reproductive organs of *Ecdyonurus lateralis* (Ephemeroptera, Heptageniidae). *Folia Parasitol. (Prague)*, **26**: 45–50.
- SPRADBERY, J. P., and SCHWEIZER, G. 1981. Oosorption during ovarian development in the screw-worm fly, *Chrysomya bezziana*. *Entomol. Exp. Appl.* **30**: 209–214.
- SWEENEY, B. W., and VANNOTE, R. L. 1982. Population synchrony in mayflies. A predator satiation hypothesis. *Evolution* (Lawrence, Kans.), **36**: 810–821.
- TREPTE, H.-H., and TREPTE-FEUEBORN, C. 1980. Development and physiology of follicular atresia during ovarian growth in the house fly, *Musca domestica*. *J. Insect Physiol.* **26**: 329–338.
- TSUI, P. T. P., and HUBBARD, M. D. 1979. Feeding habits of the predaceous nymphs of *Dolania americana* in northwestern Florida (Ephemeroptera: Behningiidae). *Hydrobiologia*, **67**: 119–123.