LIFE HISTORY, DEVELOPMENTAL PROCESSES, AND ENERGETICS OF THE BURROWING MAYFLY *DOLANIA AMERICANA*

R.S. Harvey, R.L. Vannote* and B.W. Sweeney*

Savannah River Laboratory
E.I. du Pont de Nemours and Company
Aiken, South Carolina 29801

ABSTRACT

Dolania americana has a two year life history in Upper Three Runs Creek at Savannah River Plant near Aiken, SC. Eggs deposited in early June hatch the following spring; larvae require 12-14 months to mature. Adult metamorphosis is synchronous, with > 95% of individuals emerging during the first week. Females produce an average of 77 eggs per individual; adult size and fecundity are correlated positively. Adult size of both males and females appears to decrease during the emergence period. Caloric measurements on various tissues and developmental stages ranged from 4.63 to 6.55 cal/mg. Eggs had the highest caloric content (avg 6.18 cal/mg); subimaginal molt skins had the lowest (4.63 cal/mg). Caloric content per unit weight of larval tissue increased with larval size. Chemical analysis of eggs revealed a high protein content (50.3%) relative to lipid (25.5) and carbohydrate (9.0) fractions. Weight specific respiration rates for larvae were inversely related to larval size, but correlated positively with water temperature between 6 and 23°C. Larval growth appeared continuous, although seasonal differences in the amount of growth were observed. Net production efficiency for larvae ranged from 5.3 to 29.2% and varied seasonally, averaging 8.1, 16.6, 21.7 and 19.4% for winter, spring, summer and fall, respectively.

* Stroud Water Research Center, Academy of Natural Sciences of Philadelphia, R.D. 1, Avondale, Pennsylvania 19311

INTRODUCTION

Dolania americana Edmunds and Traver is a burrowing mayfly known only from three coastal plain streams in the southeastern United States (Peters and Peters 1977). In Upper Three Runs, a spring-fed blackwater stream in South Carolina (33°23'N; 81°37'W), *D. americana* is the dominant mayfly species inhabiting the sandy substrate. Larvae are very distinct morphologically, being highly adapted for burrowing in sandy substrate (Edmunds and Traver 1959). *D. americana* are predators and feed mainly on chironomid larvae.

Aside from brief field notes accompanying the original taxonomic description of *D. americana* (Edmunds and Traver 1959), only the adult habits and emergence patterns have been reported (Peters and Peters 1977). This paper describes quantitatively: (1) the bioenergetics and developmental dynamics of the larval stage; (2) adult emergence and fecundity; and (3) biochemical characteristics of the eggs. These data are used to delineate both the life history of *D. americana* and the overall importance of this species to energy flow in Upper Three Runs.

METHODS

Larvae were taken at random from all potential habitats throughout the year. Larval collections were made at about two week intervals from September 1972 through August 1974 and also from June 1978 through September 1978. Small larvae (dry weight < 2.0 mg), which were most abundant in fine to coarse (0.1-1.0 mm) sand near the stream edge, were obtained by hand picking through sand collected with a dip net. Large larvae (dry weight > 2.0 mg) were usually collected with a modified Needham scraper (5.0 mm mesh) from coarse sand and fine gravel (11-34 mm) adjacent to the thalweg. Figure 1, derived from transect core samples from Upper Three Runs, shows that *D. americana* are more likely to occur where the sand size is small. Sample size ranged from ≥ 50 when larvae were small to ca. 20 for large larvae.

Larval respiration was measured with a Differential Submarine Respirometer (Gilson 1963). Larvae were collected from the stream, placed immediately in test vessels containing 1.5 cm of washed stream sand and 7 mL of filtered (0.45 µm) stream water, and acclimatised to test conditions for two hours. Test temperatures were with 0.5°C of ambient stream water at the time of collection. One larva was placed in each vessel and oxygen uptake was measured at 30 min intervals for three hours. Carbon dioxide which evolved during respiration was absorbed by a 20% KOH solution. Following the three hour experiment at ambient (AMB) stream temperature, the respirometer water bath was gradually increased 5.0°C over a 48 hour period. Respiration was then measured at 30 min intervals for three hours at AMB + 5.0°C temperature. Larvae used in metabolic studies were

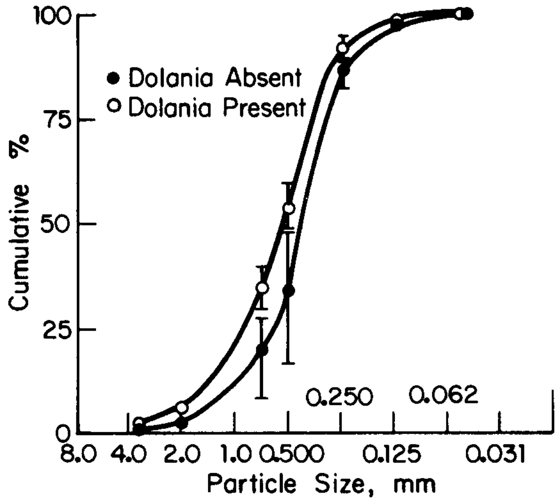

Figure 1. Size distribution of sand from stream transect samples.

killed in ETOH,* sexed, dried at 100°C for 24 hours, and weighed to the nearest 0.1 mg.

Adult emergence was monitored by: (1) light trapping at the stream edge; (2) surface drift samples of terminal instar larval molt skins; and (3) drift collections of spent male and female adults. Drift nets (1 mm mesh) were 1 m wide and filtered the top 15 cm of the stream. Two drift nets were deployed: one about 3 m from the stream edge (net 1) and the other at mid-channel (net 2). Drift was sampled daily from 0400 to 0700 h during peak emergence.

Caloric values for eggs, larvae, subimagos and adults were estimated using a Phillipson microbomb calorimeter. The lipid, protein, and carbohydrate content of eggs were estimated by the following methods: (1) lipid-weight loss following a 24 hour hot (50°C) chloroform:methanol (2:1) extraction solvent in a Soxyhlet apparatus; (2) protein-modified Lowry *et al.* (1951) technique (Price 1965); (3) carbohydrate-a phenol-sulfuric acid extraction (Dubois *et al.* 1956).

* The amount of weight loss from ETOH was inversely related to size and ranged from 6.8 to 21.7%. Percent weight loss (y) can be predicted from initial dry weight (x) by:
$y = 24.4 - 0.845x$, correlation coefficient (r^2) = 0.82.

Energy budgets were constructed according to the following equation:

$$A = G + M + R$$

where

A = assimilation = energy consumed in food minus the energy lost by egestion, excretion, and secretion
G = total change in energy value of body materials
M = energy accumulated in molt skins (exuviae)
R = energy metabolically used or released in all ways for all purposes

Growth was measured by changes in biomass and converted to calories using different conversion factors which varied according to size and sex of larvae (see results below). Relative growth rates (mg/mg/day) for each sampling period and season were calculated using an exponential model:

$$\text{Growth Rate} = (\ln W_f - \ln W_i) / t$$

where

W_f = weight at the end of the time period
W_i = weight at the beginning of the time period
t = duration of time period

Molt skin losses during the larval period were estimated by assuming that: (1) each male and female larva molts 13 times (T. Fink, personal communication) while growing from 0.1 mg to about 11 and 25 mg respectively; (2) the first larval molt occurs at 0.15 mg dry weight for both sexes; (3) each larva grows exponentially; (4) larval weight for each successive molt (m + 1) can be predicted from the weight of the previous molt (m) by the following equations: males: $Wt_{(m+1)} = Wt_{(m)} e^{0.336}$, females: $Wt_{(m+1)} = Wt_{(m)} e^{0.383}$, where 0.336 and 0.383 are the average instantaneous growth rates for the entire larval growth period of the fastest growing male and female respectively; and (5) each molt skin weights about 15% of the larva's dry weight at the time of molting. Molt skin losses were converted to calories separately for males and females by using a caloric equivalent of 4.935 cal/mg dry weight.

Respiration costs were estimated for a given size animal and stream temperature using empirically derived regression equations. Total respiration for a given period was obtained by summing daily oxygen consumption for an animal of median weight. Oxygen consumption (μLO_2) was converted to calories by using the oxycaloric equivalent of 4.825×10^{-3} cal per μLO_2 (Brody 1945).

The energy budget reported is for the fastest growing male and female larvae in the subpopulation of Upper Three Runs. For each larval collection, we assumed that the average of the three largest larvae approximated the weight of the fastest growing female and the average larval weight for the entire collection represented the fastest growing male. These assumptions appear valid for *D. americana* because: (1) large (> 3.5 mg) larvae are easily sexed and observations on winter, and spring collections (when most larvae generally exceed 3.5 mg) bear out our assumptions; and (2) rearing data for other mayfly species show that the largest and average sized larvae for each collection made early in the larval growth period are always the largest female and male respectively (Sweeney 1978, Sweeney and Schnack 1977).

The energy budget only includes periods of positive growth. For example, if the mean larval weight decreases or remains the same in consecutive samples then the time interval for calculating growth, respiration, and molt loss is lengthened sequentially until a sample is obtained that exhibits an increase in mean larval weight. This avoids entering negative values for growth into the budget.

RESULTS

Life History

It appears that *D. americana* has a two year life cycle in Upper Three Runs. Adult emergence and egg deposition in 1978 began on June 1 and continued through June 19. Bottom samples collected on June 2 with fine (500 μm) mesh nets revealed many small (0.1-0.5 mg) larvae (Fig. 2B). It is hypothesized that these larvae were not first instar, but rather had hatched in the early spring of 1978 because: (1) dry weight measurements of eggs indicate that the first instar larvae weigh at most 0.1 mg; (2) larvae weighing 0.5 mg were present on the date of first adult emergence; and (3) instantaneous growth rates of small (< 1.0 mg) larvae in June or July (when stream temperatures are highly favorable for growth; Fig. 2A) suggest that at least five weeks would be needed for larvae to grow from 0.1 to 0.5 mg.

The above hypothesis was tested by dissecting eggs in June 1978 from newly emerged females, fertilizing them with sperm stripped from adult males, and incubating the eggs in glass jars (5.5 cm OD, 6.5 cm deep) containing 100 mL of filtered (0.45 μm) stream water. Jars were kept in a constant temperature water bath at the Stroud Waste Research Center that was adjusted weekly to trace approximately the average annual thermal regime of Upper Three Runs. Mature embryos were observed by November, but eggs did not begin hatching until April 4, 1979. Egg hatch continued through May 25, with first instar larvae being about 0.06 mg dry weight.

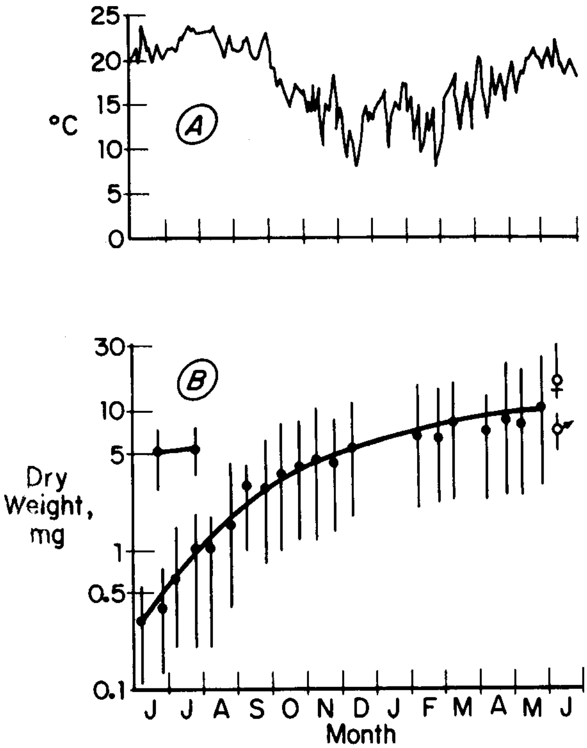

Figure 2. (A) Daily temperature recordings for Upper Three Runs from June 1973-June 1974. (B) Larval growth of *Dolania americana* in Upper Three Runs. Solid dots = mean weight of each collection; vertical lines indicate the range. Male and female signs designate the mean weight and range of male and female subimagos, respectively. Line drawn through sample means is described by: $y = 0.027x - 0.244$ where y is average weight and x is number of days since June 1.

Collections made after June indicate that larval weight increases exponentially during the warm months and growth ends by the following May. Maximum larval weights at emergence were 12 and 34 mg for males and females respectively. Adult emergence was synchronous with most adults on wing during the early part of June. A few intermediate size (range: 2.5-6.0 mg) larvae failed to emerge with the main cohort in June (see Fig. 2B). These remaining larvae showed no indication of adult maturation (e.g. wing bud development, darkened cuticle, etc.) and disappeared from the stream by early August. These larvae apparently contribute little if any to population recruitment in Upper Three Runs because: (1) drift net samples indicate that they probably do not emerge, and (2) if they did emerge, female size would be below the threshold for egg production (i.e. ca. 9 mg).

Calorimetry of Life History Stages

The caloric content of *D. americana* varies substantially with its developmental state (Table 1). Eggs have the highest caloric content (ca. 6.18 cal/mg) while newly hatched larvae have the fewest calories (ca. 5.33 cal/mg). Caloric content per unit weight increases with larval growth due probably to accumulation of stored lipid materials. Mature female larvae contain more lipid material per unit weight than mature male larvae and thus have a higher energy content. Stored energy in mature female larvae is represented largely by mature eggs whose lipid content per unit weight is apparently higher than any other developmental stage.

The energy content of larval molt skins, both male and female, is only about 15-20% lower than for the larvae themselves. This suggests that molting may represent a large energy loss especially since *D. americana* molts about 13 times during larval growth (T. Fink, personal communication) and each molt skin is about 10-15% of the molting larva's dry weight.

Table 1. Caloric content (calories per mg ash free dry weight) of various developmental stages and tissue types for *Dolania americana*.

Dolania americana				
Sample Type	n	Mean	S.D.	Range
Small larvae (♂&♀)	2	5.33	0.22	5.17-5.49
Intermediate larvae (♂&♀)	1	5.80	-	-
Mature ♂ larvae	3	5.68	0.29	5.38-6.08
♂larval molt skin	3	5.09	0.32	4.90-5.48
♂Subimago	3	5.86	0.14	5.72-6.00
♂Subimago molt skin	1	4.63	-	-
Mature ♀ larvae	3	6.09	0.12	5.97-6.26
♀ larval molt skin	5	5.16	0.23	4.91-5.52
♀ Subímago (pre-oviposition)	3	6.03	0.30	5.77-6.36
♀ Subimago (post-oviposition)	2	5.55	0.01	5.54-5.55
Eggs	5	6.18	0.21	5.90-6.43

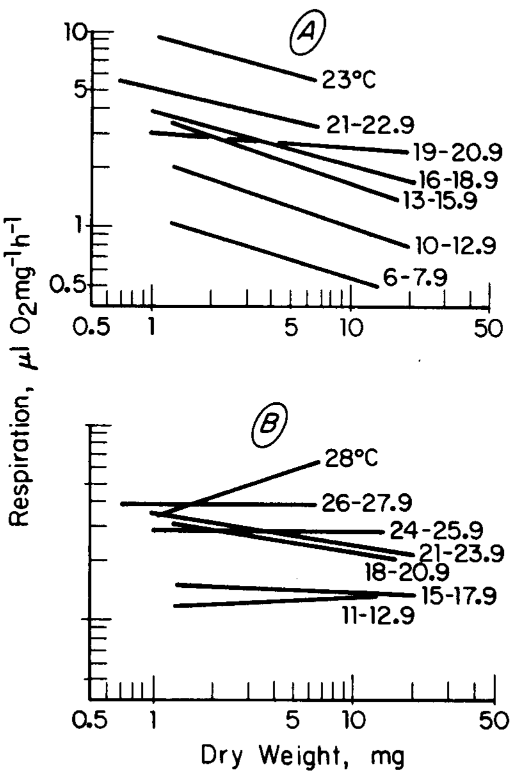

Figure 3. Weight specific respiration rate for *Dolania americana* larvae. Larvae were collected, placed in respiration vessels at ambient stream temperatures, and oxygen uptake measured (Figure 2A); larvae were then gradually acclimated over a 48 hour period to ambient +5°C before taking further measurements (Figure 2B). Two or more experiments within a given temperature range were combined for the regression analysis.

Larval Respiration

Respiration rates were correlated positively with temperature (between 6° and 23°C), but inversely related to body size for any specific temperature (Fig. 3A). Regression coefficients of fitted equations did not vary significantly over the range of test temperatures (Analysis of Covariance: $F_{6,879} = 2.03$*, $p > 0.05$; Table 2). This suggests that the metabolism of small and large larvae responds similarly to changes in temperature. Sex did not appear to alter respiration rates significantly when males and females of similar weight were compared ($p > 0.05$).

For increasing stream temperatures +5°C above ambient, weight specific respiration increased for large animals but decreased for small animals (Figure 3B). This reflects a general tendency for regression coefficients to shift from negative values at ambient temperatures to zero (or even positive values) at ambient plus 5°C. We do not interpret these results as a form of overcompensation or acclimation by small larvae, but rather a type of metabolic collapse associated with stress, particularly among small individuals. The stress involved may be related to the short-term increase in temperature or other factors associated with the experimental design (e.g. food deprivation, build-up of waste materials in test vessels, or prolonged exposure to artificial test conditions).

Table 2. Regression equations (log Y = b log X + log a) for weight specific metabolism (µl O_2 mg^{-1} h^{-1}) of *Dolania americana* at various temperature intervals.

Temperature °C	$b_{Y \cdot X}$	log a	Degrees of freedom	Level of significance
6-7.9	-0.316	0.074	1,022	0.002
10-12.9	-0.371	0.380	1,093	< 0.001
13-15.9	-0.346	0.592	1,229	< 0.001
16-18.9	-0.266	0.590	1,269	< 0.001
19-20.9	-0.087	0.499	1,085	0.112
21-22.9	-0.238	0.721	1,158	< 0.001
23-23.9	-0.284	1.004	1,030	0.024

* Although this F value borders on significance at the 0.05 level, most of the variance in the analysis can be attributed to the 19-20.9°C data. Elimination of the 19-20.9°C data, which do not yield a significant regression, results in more convincing statistics showing that the regression coefficients do not vary significantly (Analysis of Covariance: $F_{5,795} = 0.85$, $p > 0.5$).

Table 3. Partial energy budget for the fastest growing male and female larvae of *Dolania americana* in Upper Three Runs.*

MALE LARVAE

	°C		mg		Total calories per median size larva				%	$mg\ mg^{-1}\ d^{-1}$
Date	Average Temp.	No. Days	Average Weight (s.d.)	Median Weight	Growth	Respiration	Exuvia	Assimi-lation	Net Production Efficiency	Instantaneous Growth Rate
June 7			0.314 (0.101)							
	20.8	15		0.354	0.419	2.127	0.291	2.837	24.9	0.015
June 22			0.395 (0.169)							
	21.1	15		0.529	1.436	5.625	0.414	7.475	24.7	0.034
July 7			0.663 (0.324)							
	21.7	15		0.870	2.205	8.221	0.577	11.003	25.2	0.032
July 22			1.077 (0.441)							
	23.0	31		1.334	2.770	33.598	1.999	38.367	12.4	0.013
Aug. 22			1.591 (0.988)							
	20.8	31		2.155	6.212	28.031	1.667	35.980	22.0	0.017
Sep. 22			2.721 (1.563)							
June 7 - Sep. 22	21.6	107		1.517	13.042	77.602	4.968	95.612	18.8	0.020
Sep. 22			2.721 (1.563)							
	20.4	15		3.180	4.817	15.740	2.294	22.851	31.1	0.019
Oct. 7			3.641 (1.822)							
	17.3	15		3.890	2.509	18.341	0.000	20.850	12.0	0.008
Oct. 22			4.139 (1.855)							
	15.4	16		4.321	1.656	17.688	3.072	22.419	21.1	0.005
Nov. 7			4.504 (2.463)							
	14.4	30		5.129	6.308	39.543	0.000	45.851	13.7	0.008
Dec. 7			5.755 (2.979)							
Sep. 22 - Dec. 7	16.4	76		4.238	15.293	91.312	5.366	111.971	18.4	0.009
Dec. 7			5.755 (2.979)							
	12.9	62		6.234	5.024	79.015	4.441	88.425	10.7	0.003
Feb. 7			6.893 (3.501)							
	12.1	28		7.610	6.179	27.783	0.000	33.957	18.2	0.006
Mar. 7			8.328 (4.289)							
Dec. 7 - Mar. 7	12.7	90		7.038	11.203	106.793	4.441	122.434	12.7	0.004
Mar. 7			8.328 (4.289)							
	16.1	46		8.493	1.172	94.761	6.292	102.225	7.3	0.001
Apr. 22			8.659 (6.155)							
	18.4	30		9.913	11.895	81.677	8.261	101.833	19.7	0.008
May 22			11.168 (6.784)							
Mar. 7 - May 22		76		9.748	13.067	176.438	14.554	204.058	18.3	0.004

* Net production efficiency (%) was obtained by dividing the sum of growth and exuvia energy by total assimilation for a given period. See text for description of other parameters.

Table 3. (Continued) Partial energy budget for the fastest growing male and female larvae of *Dolania americana* in Upper Three Runs.*

					FEMALE LARVAE					
	°C		mg		Total calories per median size larva				%	mg mg^{-1} d^{-1}
Date	Average Temp.	No. Days	Average Weight (s.d.)	Median Weight	Growth	Respiration	Exuvia	Assimi-lation	Net Production Efficiency	Instantaneous Growth Rate
June 7			0.574 (0.018)							
	20.8	15		0.677	1.084	3.844	0.503	5.431	29.2	0.020
June 22			0.781 (0.079)							
	21.1	15		1.155	4.038	10.205	0.740	14.983	31.9	0.044
July 7			1.530 (0.090)							
	21.7	15		1.739	2.184	13.937	1.110	17.231	19.1	0.016
July 22			1.948 (0.112)							
	23.0	31		3.124	12.862	61.269	3.997	78.129	21.5	0.025
Aug. 22			4.300 (0.360)							
	20.8	31		5.333	11.508	59.802	3.405	74.715	19.9	0.012
Sep. 22			6.366 (0.550)							
June 7 - Sep. 22		107			31.676	149.057	9.755	190.488	21.7	0.022
Sep. 22			6.366 (0.550)							
	20.4	15		7.366	10.938	33.951	0.000	44.889	24.3	0.018
Oct. 7			8.366 (1.101)							
	17.3	15		8.499	1.468	32.533	4.885	38.886	16.3	0.002
Oct. 22			8.633 (1.106)							
	15.4	16		9.616	10.855	31.814	0.000	42.669	25.4	0.012
Nov. 7			10.600 (1.757)							
	14.4	30		10.933	3.664	64.867	7.402	75.933	14.5	0.002
Dec. 7			11.266 (1.193)							
Sep. 22 - Dec. 7	16.4	76			26.925	163.165	12.287	202.377	19.4	0.007
Dec. 7			11.266 (1.193)							
	12.9	62		13.649	2.640	131.984	10.363	144.987	8.9	$<$ 0.001
Feb. 7			16.033 (1.877)							
	12.1	28		16.249	2.540	44.901	0.000	47.441	5.3	$<$ 0.001
Mar. 7			16.466 (2.369)							
Dec. 7 - Mar. 7	12.7	90			5.180	176.885	10.363	192.428	8.1	0.004
Mar. 7			16.466 (2.369)							
	16.1	46		19.999	41.907	175.486	0.000	217.393	19.3	0.007
Apr. 22			23.533 (2.357)							
	18.4	30		24.364	9.762	172.289	17.910	199.961	13.8	0.002
May 22			25.196 (0.600)							
Mar. 7 - May 22		76			51.669	347.775	17.910	417.354	16.5	0.006

* Net production efficiency (%) was obtained by dividing the sum of growth and exuvia energy by total assimilation for a given period. See text for description of other parameters.

Larval Growth

Larval growth appeared continuous for *D. americana*, although seasonal differences in the amount of growth occurred (Table 3). The magnitude and efficiency of growth were greatest in non-winter months when stream temperatures exceeded 15°C. Females generally grew more efficiently and faster than males during all seasons. The daily caloric requirement per female larva was usually about twice the amount estimated for male larvae. Instantaneous growth rates appeared to be inversely related to body size and positively correlated with stream temperatures for both sexes. Energy lost from respiration greatly exceeded that used in net production (growth plus exuviae) for most time periods. Net production efficiency was highest for small animals growing at temperatures above 20°C. Maximum net production (cal/day) per individual occurs in spring for both sexes. The data do not show when maximum net production occurs for the subpopulation as a unit in Upper Three Runs because larval density estimates were not made.

ADULT EMERGENCE

Drift collections of larval molt skins, which are shed at metamorphosis suggest that 98% of the individuals emerged synchronously in a seven day period (Table 4). Adult emergence in Upper Three Runs began each day at about 0530 h (sunrise) and ended about 0615 h. Observations support Peters and Peters (1977) contention that *D. americana* are sensitive to small changes in light intensity occurring just before sunrise. During the peak emergence period, nine flow-through microcosm streams were stocked with mature male and female larvae. Adult emergence among microcosms was synchronous and could be initiated earlier in the morning than normal by artificially lighting the microcosms for one hour early in the morning, then darkening them again, and re-exposing the trays to natural sunrise (Table 5).

The sex ratio at emergence was skewed towards males according to data from drifting larval molt skins, but towards females for drifting adults (Table 4). The skewed sex ratio for drifting adults has also been reported for other *D. americana* subpopulations (Peters and Peters 1977). A predominance of females among drifting adults may be an artifact because adult males fail to return to the river after mating due to intense predation by bats, birds, etc. while patrolling for females. The skewed sex ratio of drifting cast molt skins could result if female skins sink after floating a shorter distance than males due to their heavier weight (avg. = 2.787 vs. 1.804 mg). We suggest, therefore, that skewed sex rations based on drifting adults and larval skins may be artifacts, especially since larval collections made prior to emergence (late May) indicate a 1:1 sex ratio.

Table 4. Number and sexual composition of cast larval molt skins and dead adults collected in drift nets on the Upper Three Runs. Net #1 = near right bank; Net #2 = mid-channel.

Date	Larval Skins					Adults			
	Net #1		Net #2		Net #2	Net #1		Net #2	
	Male	Female	Male	Female	Cumulative %	Male	Female	Male	Female
6-3-78	18	4	28	15	1.5	-	-	-	-
6-4-78	47	45	279	187	17.8	-	-	51	60
6-5-78	1	-	30	-	18.9	-	-	1	-
6-6-78	290	240	329	264	39.7	-	-	45	61
6-7-78	(discontinued)		5	8	40.2	(discontinued)		-	-
6-8-78			821	626	91.1			160	199
6-9-78			121	93	98.6			12	18
6-10-78			5	4	98.9			-	-
6-11-78			7	11	99.5			-	-
6-12-78			2	-	99.6			-	-
6-13-78			4	2	99.8			1	-
6-14-78			3	1	99.9			-	-
6-15-78			-	-				-	-
6-16-78			-	-				-	-
6-19-78			1	-	100.0			-	-
Totals	356	289	1635	1211		-	-	269	338

Table 5. Influence of artificial and natural lighting on the onset of diel emergence for *Dolania americana*

	Laboratory Trays		Upper Three Runs	
Date	Lights	First Emergence	Sunrise	First Emergence
6-3-78	0400	0401	0518	0534
6-4-78	0330	0333	0518	0540
6-5-78	0416	0417	0518	0536
6-6-78	0230 (0530)	0233 (0533)	0518	0542

The mean weight of drifting larval skins appears to decrease for both sexes during the emergence period (Fig. 4). Since larval skin weight is correlated positively with the weight of the larva (and thus emerging adult), these data suggest that adult size of both sexes decrease through the emergence period. Thus, the relative contribution of the last emerging females to population recruitment may be decreased considerably by: (1) reduced size and fecundity; and (2) decreased probability of mating successfully.

Adult Fecundity

The number of eggs per female averaged about 77 and ranged from 44 to 148 (Table 6). There was no significant difference between mature larvae and subimagos when comparing the average number of eggs, weight per egg, and total egg weight per individual. The quantity of nonreproductive tissue appeared higher in female larvae than adults (10.9 vs. 7.201 mg); but when the average weight of the cast larval skin (2.787 mg) is subtracted from the larval weight, the differences are negligible (7.299 vs. 7.201 mg). About 56% of the total dry weight of an emerging female is eggs. After ovipositing, adult females weigh about the same as adult males.

A highly significant linear relationship was observed for fecundity as a function of female dry weight (Fig. 5). Since there were no significant differences between the fecundity dry weight regressions for subimagos and larvae (Analysis of Covariance: $F_{1,37} = 0.73$, $p > 0.05$), the two data sets were combined into a single regression. Although *D. americana* produces fewer eggs per female than has been reported for any other mayfly, indivudual eggs are at least ten times larger and heavier than reported for any other

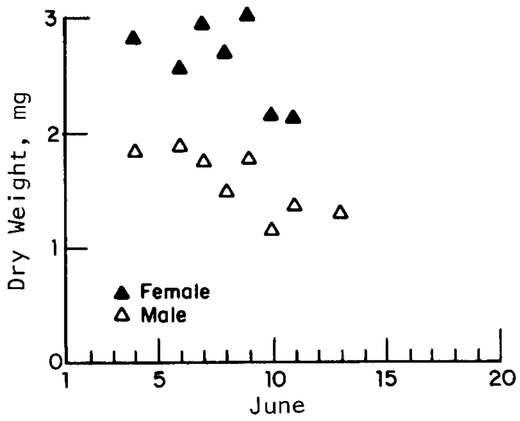

Figure 4. Average weight of male (Δ) and female (▲) larval molt skins collected from Upper Three Runs in drift nets during the adult emergence period. Molt skins were counted and weighed collectively for each date; the variance of each mean is unknown.

Table 6. Average dry weight and fecundity of *Dolania americana* females collected from Upper Three Runs from June 3 through June 6, 1978.

	Subimagos*	Larvae+
	Avg.± S.D.	Avg. ± S.D.
Nonreproductive tissue wt (mg)	7.201±1.628	10.086±2.489
Egg wt (mg)	9.182±2.580	9.581±3.304
Total female wt (mg)	16.390±3.238	19.671±5.521
Egg number per female	75±20	79±25
Wt per egg (mg)	0.122±0.009	0.120±0.011

* sample size = 18
\+ sample size = 22

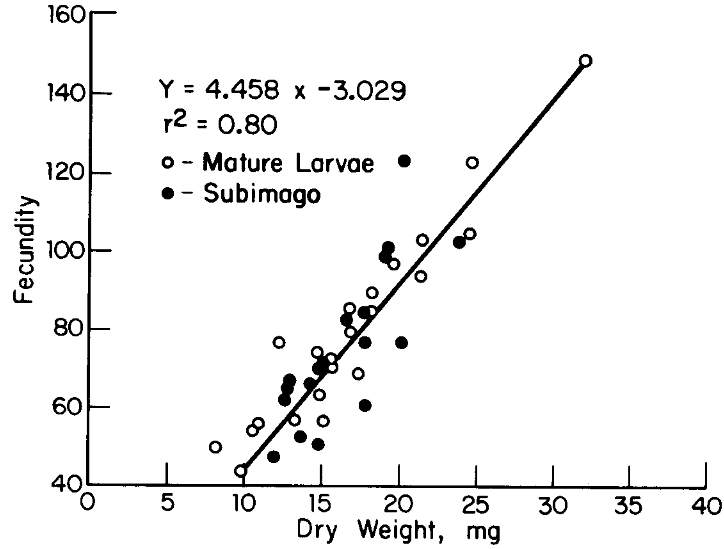

Figure 5. Fecundity of *Dolania americana* as a function of individual dry weight. Open circles = mature larvae; closed circles = subimago.

species. The adaptive significance of a low fecundity-large egg reproductive strategy is unclear, but may be related more to the species trophic habit (i.e. predator) than to the shifting, sandy nature of the habitat.

Egg Quality

Biochemical analysis revealed that eggs were mainly protein (65.5%) and lipid (25.2%) by weight, with small amounts of carbohydrate (9.0%; Table 7). Although similar data on other aquatic species are not available for comparison, the results agree well with the limited published data for terrestrial insects (Singh and Sinha 1977). Assuming the non-lipid dry component is used primarily for synthesis rather than respiration and the average dry weight of one egg is 0.12 mg (see Table 6), the maximum dry weight of first instar larvae would be 0.08 mg.

Table 7. Chemical composition of *D. americana* eggs.

Nutrient Chemical	n	Mean %	Range
Carbohydrate	5	9.0	7.6-12.8
Lipid	3	25.2	24.7-25.6
Protein	6	65.5	58.7-69.1

DISCUSSION

Although a two year life cycle has been suggested for other species of burrowing mayflies (Bartholomae and Meier 1977, Hynes 1970), the two years are spent mainly in the larval stage. In contrast, *D. americana* remains in the egg for most of the first year and as a larva the second. The ecological significance of a ten month embryonic period is unclear, particularly since the substrate of Upper Three Runs is loose, shifting sand. The egg of *D. americana*, however, appears adapted to endure a prolonged embryonic period because: (1) eggs contain a large supply of nutrient chemicals needed for embryonic development; (2) the chorion is thick and seems very resistant to mechanical breaking or puncturing; and (3) the intact chorion is not readily infected by aquatic fungi. The large size of the egg may also be highly adapted because it yields a large first instar larva. This assumes that larval mortality is inversely related to size and increased larval size expands the size range of potentially exploitable prey.

For most insect species, egg quantity and quality are generally considered to be important life history parameters that are related directly to the probability of the average animal to survive during the period between egg deposition and larval maturation. Our analyses of the size and number of eggs for *D. americana* suggests that individual mortality for eggs and larvae is probably very low relative to other mayfly species. Since the degree of egg mortality would seem to be correlated positively with the duration of the egg stage, one might expect strong selection against a prolonged egg diapause. Our results however, on the bioenergetics of larval development indicate that if there was no diapause and larvae hatched in June (as opposed to April), they would never achieve sufficient growth by the following June to emerge with more than a few eggs, if they could emerge at all. The intermediate sized larvae which fail to emerge with the remaining subpopulation of Upper Three Runs may result from a few individuals each generation failing to undergo an embryonic diapause and hatching prematurely.

Although annual or seasonal production of *D. americana* was not estimated in Upper Three Runs, the resource requirements of individual larvae can be obtained from the energy budget. We estimate the minimum daily caloric intake (i.e. assimilation/day) of a male and female larva growing during the summer - fall - winter - spring period to be 0.88 - 1.46 - 1.35 - 2.68 and 1.77 - 2.65 - 2.13 - 5.49 cal/day, respectively. Assuming *D. americana* eat mainly chironomid larvae that average about 0.15 mg and have a caloric equivalent of about 5.0 cal/mg, the number of prey consumed per day would range from 1.2 to 3.5 for males 2.3 to 7.3 for females depending on the season and stage of larval development.* Although the daily caloric intake per animal increases during larval development (except in winter), resource exploitation by the subpopulation as a whole may actually decrease or remain in a quasi-equilibrium throughout the year. To approach an equilibrium, larval mortality would have to be about 75% during the growing season. Higher mortality, which seems reasonable, would decrease the amount of resources used by large larvae relative to earlier stages.

RESUME

Le cycle évolutif de la *Dolania americana* dans le Upper Three Runs Creek de la Savannah River Plant près d'Aiken, en South Carolina, est de deux ans. Les oeufs pondus au début de juin éclosent le printemps suivant; les larves mettent de douze à quatorze mois à atteindre à leur plein développement. La métamorphose des larves à l'état adulte se produit en même temps, l'éclosion de plus de 95% des éphémères ayant lieu au cours de la première semaine. Les femelles pondent en moyenne 77 ouefs chacune; il y a corrélation entre la taille de l'adulte et sa fécondité. Les mâles et les femelles adultes semblent rapetisser pendant la période de métamorphose. La teneur en calories des divers tissus durant toute la durée du développement s'établit entre 4.63 à 6.55 cal/mg. Ce sont les oeufs qui ont la plus forte teneur en calories (moyenne 6.18 cal/mg) et la mue au stade subimaginal la plus faible (4.63 cal/mg). La teneur en calories par unité de poids du tissue larvaire augmente proportionnellment à la taille de la larve. L'analyse chimique des oeufs révèle une forte teneur en protéines (50.3%) contre 25.5% de lipides et 9% de glucides. Le taux de respiration relatif au poids des larves et inversement proportionnel à leur taille, mais directement proportionnel à la température de l'eau qui se situe entre 6 et 23°C. La croissance des larves semble être continue, bien que le rythme de croissance diffère de saison en saison. La capacité totale de production des larves se situe entre 5.3 et 29.2% avec les

* These values may be quite conservative because in this analysis we did not include the energy expended in the actual search and capture of prey.

variantes saisonnières s'établissant en moyenne à 8.1, 16.6, 21.7 et 19.4% en hiver, au printemps, en été et à l'automne respectivement.

ZUSSAMENFASSUNG

Dolania americana hat eine zweijährige Lebensgeschicte im Upper Three Runs Creek der Savannah River Plant nahe bei Aiken, South Carolina. Anfang Juni abgelegte Eier werden im folgenden Frühjahr ausgebrütet. Larven brauchen 12 bis 14 Monate, um zu reifen. Die Metamorphose der ausgewachesenen Tiere ist synchron, wobei 95% der einzelnen Fliegen während der ersten Woche zum Vorschein kommen. Die Weibchen produzieren durchschnittlich je 77 Eier. Größe und Fekundität der reifen Tiere sind positiv aufeinander bezogen. Ausgewachsene Tiere beider Geschlechter scheinen während der Periode des Auftauchens an Größe abzunehmen. Kalorienmessungen an verschiedenen Geweben und Entwicklungsstadien schwankten von 4.63 cal/mg bis 6.55 cal/mg. Eier hatten den höchsten Kaloriengehalt (durchschnittlich 6.18 cal/mg). Subimaginale Häutungsschalen hatten den niedrigsten Kaloriengehalt. Der Kaloriengehalt pro Einheit von Larvengewebe nahm mit der Larvengröße zu. Eine chemikalische Analyse von Eiern ergab einen hohen Gehalt an Protein (50.3%) im Verhältnis zu Lipoid (25.5%) und Kohlehydrat Bruchteilen (9.0%). Die gewichtsspezifischen Respirationsraten standen in entgegengesetztem Verhältnis zur Larvengröße, waren jedoch positiv auf die Wassertemperaturen zwischen 6 Grad und 23 Grad C. bezogen. Das Wachstum erschien stetig, obwohl Unterschiede, die durch die Jahreszeit bedingt waren, in der Wachstumsrate beobachtet wurden. Die Netto Produktionswirksamkeit für die Larven reichte von 5.3% bis 29.2% und variierte jahreszeitlich, mit Durchschnittswerten von 8.1% für Winter, 16.6% für Frühling 21.7% für Sommer und 19.4% für Herbst.

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