

Semivoltinism, seasonal emergence, and adult size variation in a tropical stream mayfly (*Euthyplocia hecuba*)

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Abstract. Life history characteristics of the stream mayfly *Euthyplocia hecuba* (Hagen) (Polymitarcyidae:Euthyplociinae) were studied over a 4-yr period in two tropical streams (Río Tempisquito and Quebrada Marilin) draining primary evergreen forest in northern Costa Rica. Larvae burrow in the substratum of the stream, living under small to large cobbles that are firmly embedded in the stream bottom. *Euthyplocia hecuba* is sexually dimorphic and one of the largest mayflies of Central America, with maximum larval size of 149 mg (dry mass) for females and 35 mg for males. Mature female and male larvae are about 40% larger in Q. Marilin relative to R. Tempisquito. Subimagoes emerge about 1 h before dawn (~0400 h); the molt to the imago (or true adult stage) occurs ~20 min later. Adult emergence and reproduction occurs mainly between June and November. Females mate and then oviposit in riffle areas just before dawn, with the time period gradually changing from 0446 and 0507 h in June to 0508 and 0529 h in October. We found that size of adult males and females gradually declined during the emergence period. Fecundity averaged 984 in October (range 364–2851), with eggs being very large for a mayfly (0.46×0.4 mm; 0.017 mg dry mass). The proportion of adult biomass allocated to eggs (reproductive effort) averaged 0.51 but varied according to female size. Median egg development time was 113, 55, and 31 d at 15, 20, and 25°C. The hatch success of eggs was >90% at 15 and 20°C, only 68% at 25°C, and 0% at 10 and 30°C. First instar larvae are relatively large for a mayfly, having a head width of 0.11 mm and a body length of 1.30 mm. Seasonal changes in larval size distribution suggest that larval development takes ~22 mo to complete and may involve a developmental quiescence or diapause during the first year. A 2-yr life history appears to be the most parsimonious hypothesis given a 2-mo egg development time, the occurrence of two size cohorts of larvae throughout most of the year, and the temporal pattern of maximum and average larval sizes over the course of the sampling period. Our data suggest that seasonality in the developmental dynamics of larvae underlies both the seasonal emergence and decline in dry mass of both adult males and females during the emergence period. The data provide the first evidence for semivoltinism in a tropical mayfly.

Key words: aquatic insect, stream, mayfly, semivoltine, life history, reproductive effort, fecundity, egg development, larval growth, quiescence, diapause.

Mayflies living in temperate streams complete their life cycle in a few weeks to several years depending on the species and geographic location of the population (Brittain 1982). The relative proportion of species in a given mayfly assemblage having short (<1 yr) versus long (≥ 1 yr) life histories varies in part with the annual thermal regime (hence geographic location) of a given river. In general, mayfly species with univoltine (1 yr) and semivoltine (2 or 2+ yr) life histories in a given assemblage seem to be more prominent at higher latitudes (Clifford 1982, Berner and Pescador 1988). For some species, populations are univoltine in the southern and middle part of their temperate range but semivoltine in more northern areas (e.g., *Eurylophella funeralis*, *Eurylophella verisimilis*, and *Stenonema vicarium* in eastern North

America; Newbold et al. 1994). An extreme case appears to be *Hexagenia limbata*, which completes its life cycle in <1 yr in the south (Hunt 1953) but requires >3 yr at the extreme northern part of its range in Canada (Heise et al. 1987, Giberson 1991).

The frequency of species with multivoltine (<1 yr) life histories seems to increase at more southern temperate latitudes (see Clifford 1982 for review). This north to south trend was recognized originally by Clifford et al. (1973) who predicted that most tropical species would have aseasonal, asynchronous multivoltine life histories. Tjonneland had made a similar prediction earlier (1960). Corbet et al. 1974 provided some of the first supporting data for this prediction from tropical streams. Unfortunately, life-history data for mayflies and other aquatic

insects from tropical streams, rivers, and lakes are still too limited to adequately test this prediction. Observations for mayflies in the tropical regions of Australia (Marchant 1982, Campbell 1994) and Africa (Hynes and Williams 1962, Hynes 1975, Gillies and Knowles 1990) are consistent with a short-life-history hypothesis. Jackson and Sweeney (1995) have shown the development time of 35 stream insect species, including five mayflies, from tropical streams in Costa Rica to be <1 to 7 mo.

Two major implications of a geographic gradient in the length of life histories for species making up mayfly assemblages is a parallel gradient in the synchrony of the adult emergence period as well as in the amount of adult size variation during this period. This is because it is known that univoltine and semivoltine species which dominate temperate mayfly assemblages have relatively brief periods of adult emergence, whereas multivoltine species tend to exhibit asynchronous emergence periods (Brittain 1982). In terms of adult mayfly size, univoltine and semivoltine temperate species often exhibit a general decline in adult size during the emergence period (Vannote and Sweeney 1980, Sweeney and Vannote 1981, Brittain 1982). Although multivoltine temperate mayflies also exhibit considerable adult size variation, there seems to be less variation among summer cohorts than among overwintering cohorts (Sweeney and Vannote 1984). Thus, given the above considerations, one might predict *a priori* that tropical mayfly assemblages should be characterized by aseasonal multivoltine species exhibiting asynchronous periods of adult emergence and minimal variation in adult size.

In this paper we show that not all tropical mayflies conform to these general predictions. Specifically, we show that a tropical burrowing mayfly, *Euthyplocia hecuba*, in streams of northern Costa Rica has a life history lasting about two years, distinct seasonality in adult emergence, and variation in adult male and female size during the emergence period. Moreover, the results suggest a developmental quiescence or diapause may be a principal underlying factor affecting these life history characteristics. Although semivoltine mayflies have been reported from subtropical areas (e.g., *Dolania americana*; Harvey et al. 1980), our data appear to be the first evidence for a mayfly life history exceeding one year in the tropics.

Study Sites

Euthyplocia hecuba specimens for life history analysis were collected from two tributaries of the Río Tempisque in Guanacaste Province of northern Costa Rica: Río Tempisquito (10°57'25"N; 85°29'42"W; 580 m elevation) and Quebrada Marilin (10°57'04"N; 85°29'24"W; 600 m elevation). Both drain small stream catchments containing tropical evergreen forest growing on the southwest slopes of an inactive volcano (Volcan Orosi). Detailed physiochemical characteristics of the two streams are presented in Newbold et al. (1995). Both streams have a moderate to steep gradient (8–9%) and are heavily shaded, with substrate dominated by intermediate to large cobble embedded in a sandy matrix. The R. Tempisquito is equivalent in size to a 3rd- to 4th-order Piedmont (USA) stream. Q. Marilin was much smaller, being more like a 1st- or 2nd-order Piedmont stream. Mean wetted channel width and baseflow discharge of the two streams were: R. Tempisquito (5.3 m, ~200 L/s); Q. Marilin (1.9 m, ~7 L/s). Water temperature varied between 19.5 and 23.0°C during the 4-yr study at both streams and showed little diel fluctuation. Photoperiod (light:dark) ranged from 11.45:12.55 h in December to 12.73:11.26 h in June, with a 1.28-h difference between the shortest and longest days.

Methods

Egg development

Adult females were collected with light traps or in flight with aerial nets just before oviposition. Upon collection, many females already had eggs extruded as two elongated masses at the tip of their abdomen. Each adult was placed in an individual jar (30 mm wide, 40 mm deep) with about 10–20 mL of stream water. All eggs were released by the female after contacting the water surface. Each female was removed within 1–2 h and preserved (frozen or 5% buffered formalin). Jars containing eggs were then placed in water baths at various constant temperatures (10, 15, 20, 25, 30°C ± 1°C) and a 12:12 h (L:D) photoperiod. Eggs were inspected about twice weekly until embryos were observed, and daily thereafter. Newly hatched larvae were counted and removed daily from each jar to measure

hatch success and characterize the temporal pattern of egg hatch for each clutch.

Larval collections

Larvae were collected periodically in a qualitative fashion mainly by inspecting cobble substrates in a 50–200-m reach. The burrowing larvae were usually found associated with the underside of small to large cobbles that were embedded about 25% or more into the sandy substrate. Larvae were collected beneath cobbles embedded everywhere in the stream except deep pools, where only cobbles at the edge contained larvae. The largest larvae were generally found only under very large cobbles (even boulders) whereas smaller larvae generally occurred under smaller cobbles. In general, *E. hecuba* larvae occurred singly under a cobble, even in reaches where densities were high. Cobbles that were not embedded in the sand more than 25% rarely contained *E. hecuba* larvae. We never observed extremely small larvae attached to cobbles.

Some of the larvae included in this study came from six quantitative benthic samples (0.08 m²) taken biweekly from February 1991 to September 1992 in riffle areas of each stream with a D-frame net (236- μ m mesh bag). The D-frame contained two metal guides that extended upstream from the bottom corners to define a 28.5 \times 28 cm sampling area. Macroinvertebrates (including *E. hecuba*) and organic matter were dislodged from the bottom substrata by hand stirring and scrubbing the rocks with a soft brush and then were collected in the downstream net. All netted specimens were preserved in the field in 90% ethanol. Samples were inspected under a dissecting microscope; all *E. hecuba* larvae were counted and sexed, and then head width (across the eyes) was measured. Voucher specimens are at the Stroud Water Research Center.

All specimens were either preserved (90% ethanol or 5% buffered formalin) or frozen (liquid nitrogen). A set of larvae representing a broad size range was collected from both streams, dried (60°C for \geq 48 h), and weighed (Cahn Electrobalance; \pm 1 μ g) to determine the relationship between head width and dry mass. This relationship was used to predict dry mass from head width measurements of all other larval specimens in the study.

Evaluation of seasonal pattern of larval growth and life history

We analyzed temporal patterns in the distribution of larval size data to determine whether the *E. hecuba* populations in Q. Marilyn and R. Tempisquito were multivoltine, univoltine, or semivoltine (Hynes 1970). We initially focused the analysis on female data associated only with the qualitative collections made during the latter part of the study (i.e., mainly January 1992 through November 1994). Quantitative collections were omitted because they were made from a limited area in both streams, generally yielded too few individuals (especially large larvae) on any given date to analyze for the presence or absence of distinct size cohorts, and addressed only a portion of the study period. Male data were excluded because the extreme sexual dimorphism in size tended to obscure patterns.

Larval cohorts for a given sampling date were distinguished based on differences in body size (mass) as well as by the size and color of the wing pads (i.e., well developed and usually dark brown or black versus intermediate to poorly developed and pale). For each cohort, we identified the four largest and four smallest individuals on each qualitative sampling date from January 1992 to November 1994, and then calculated an average size for each of these groups of four larvae as well as an average for all larvae (including the four largest and smallest). When the data were graphed, these averages were connected through time with solid lines (average of the largest four), dotted lines (average of the smallest four), and dashed lines (average of all larvae). These lines represent an estimate of the seasonal pattern of growth for the largest, smallest, and average individual female larva of each cohort.

Once individual size cohorts and patterns of growth for large larvae were distinguished using the above approach, we repeated the analysis using all available female data (i.e., combined larvae from qualitative and quantitative samples). The quantitative samples added considerable data for small larvae. We estimated the growth pattern for the smaller larvae by extrapolating backward (by eye) from the seasonal pattern of growth already established for the larger larvae (as determined above). The extrapolations were guided by the largest and smallest individuals from the quantitative sam-

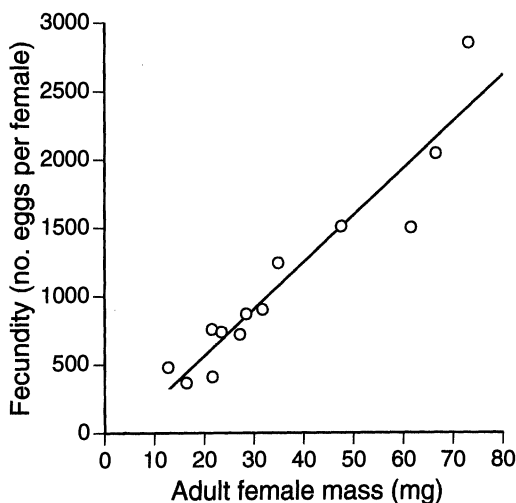


FIG. 1. Individual fecundity of *E. hecuba* in R. Tempisquito as a function of total adult mass (including eggs). The relationship is described by $y = 34.3x - 125.5$, $r^2 = 0.90$.

ples. A more rigorous estimate of the growth trajectory for small larvae is not possible for *E. hecuba* because so few small individuals were collected on any given sampling date.

Instantaneous growth rates were estimated using female data from the qualitative collections by following the change in size for a 1-yr period of the average of the four largest female larvae in a given cohort. This approach was chosen because other studies have shown that the largest individuals in a mayfly population several months before the emergence period are also the largest individuals in the population at the onset of emergence (Sweeney 1978, Sweeney and Vannote 1981). In addition, following growth of the largest larvae in field collections should provide an accurate assessment of growth pattern because their growth pattern was not obscured by seasonal and sexual polymorphisms.

Results

Diel emergence and adult swarming

Adults were abundant in June and October, less common in November, and rare (only one adult) in January over the 4-yr study. Adult *E. hecuba* live only a few hours, with subimagos emerging in the dark at about 0400 h, flying to

streamside vegetation, and molting to the adult about 20 min later. Male imagoes, either alone or in groups of 10–12, have been seen swarming above short (<3 m) streamside plants and limbs of riparian trees that overhang the water. Swarming involves males flying rapidly in a vertically oriented, circular pattern having a diameter of about 0.3 m. Swarms were tight aggregations, occupying a space of about 0.5 m³ immediately above a given plant. Individual males were observed to continue this rapid circular flight pattern for up to 45 min after the peak female oviposition time and well past sunrise. Males often left a given swarm when no females were encountered, flew upstream about 10–20 m, joined another swarm or immediately returned to the original swarm. Males eventually left the swarm site and flew up or downstream without returning. Because males were absent at the swarm sites immediately adjacent to the stream before and during peak female oviposition (i.e., between 0446 and 0507 h in June and 0508 and 0529 h in October based on 4-yr of observations), we suspect that the principal mating swarms occur above the riparian canopy. The swarms we observed appeared to form secondarily as males moved closer to the stream at dawn. Females were never observed entering or leaving swarms near the stream.

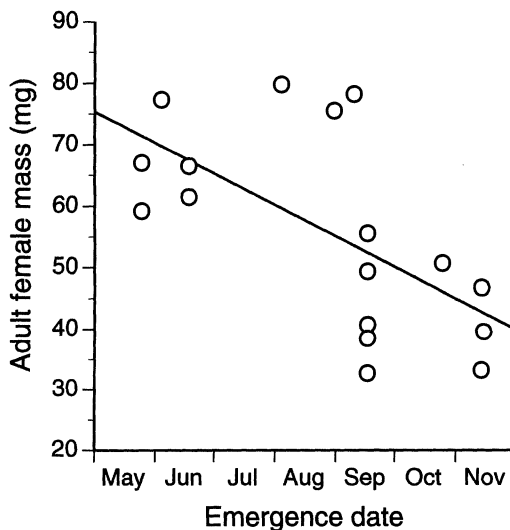


FIG. 2. Linear regression assessing seasonal change in the size of *E. hecuba* adult females captured in light traps or with aerial nets in 1994 at Q. Marlin ($r^2 = 0.38$).

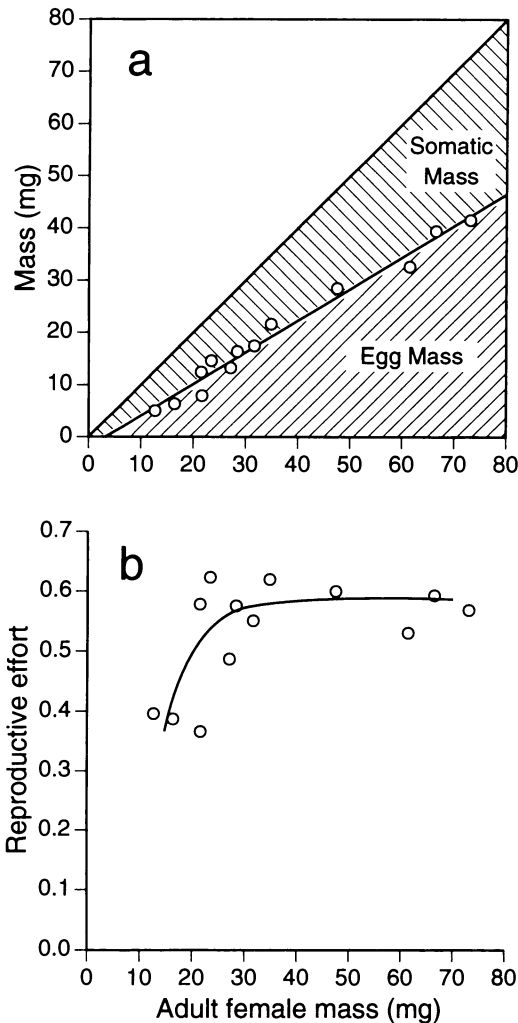


FIG. 3. (a) Allocation of total adult female mass into egg and somatic tissue for *E. hecuba* adults collected from the R. Tempisquito. The regression line ($y = 0.605x - 1.968$, $r^2 = 0.97$) relates egg mass as a function of total female mass (including eggs). Area above the regression line represents the proportion of total adult female mass allocated to somatic tissue. (b) Reproductive effort of *E. hecuba* in R. Tempisquito as a function of adult female mass described by a line fitted by eye.

Oviposition, fecundity, and reproductive effort

Females flew back and forth over the stream, completely extruded a pair of elongated egg masses from their abdomen (one from each oviduct) when they were about 0.5–1.0 m above the stream surface, and immediately flew to the

surface. Oviposition occurred at the upstream end of a riffle. Once a female's wings became wetted during oviposition, she was unable to fly.

Euthyplocia hecuba emerged as subimagos with their full complement of eggs. Individual fecundity was positively correlated with adult female size (Fig. 1), which declined gradually through the emergence period (i.e., May through November; Fig. 2). Average fecundity for individual adults captured in October 1992 was 984 eggs per female (SE = 213) and ranged from 364 to 2851. However, it is likely that average fecundity was substantially higher earlier in the emergence season, when adult females were larger.

The allocation of adult mass into reproductive (egg mass) versus nonreproductive (somatic tissue) structures for *E. hecuba* shows that large adults allocated a greater proportion of their mass to eggs than smaller adults and suggests that few eggs would be produced by a female below a certain size (about 10 mg) (Fig. 3a). The proportion of adult mass that was ac-

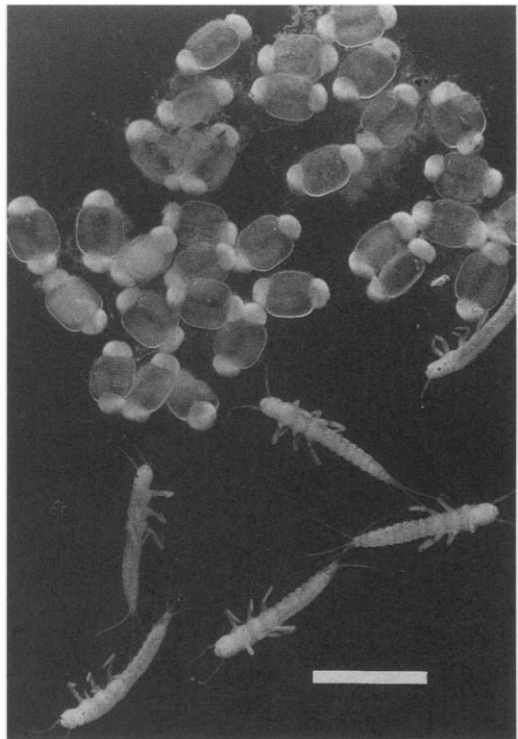


FIG. 4. Eggs and first instar larvae of *E. hecuba*. Scale bar = 1 mm.

TABLE 1. Hatch success and the duration of the embryonic period (development time) for *E. hecuba* eggs kept at various constant temperatures.

Temperature (°C)	Hatch success (%)		Development time (d)			
			First hatch		Median hatch	
	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
10	0		—		—	
15	91.1	(4.5)	106.3	(2.2)	112.6	(2.8)
20	95.4	(1.9)	60.5	(2.3)	54.8	(0.9)
25	67.6	(30.9)	28.6	(0.7)	31.2	(1.3)
30	0		—		—	

tually allocated to eggs (i.e., reproductive effort) in *E. hecuba* averaged 0.51 (SE = 0.029; range 0.36–0.62), although individual reproductive effort varied substantially in a non-linear fashion (Fig. 3b).

Egg size and development

Eggs were large ($\approx 0.46 \times 0.4$ mm) and had a distinct "Type I polar cap" (sensu Koss and Edmunds 1974) at both polar regions (Fig. 4). The average dry mass of an *E. hecuba* egg was 0.017 mg (SE = 0.001) as estimated by weighing clusters ($n = 13$) of dry eggs. Although eggs were extruded as a mass in flight and were cohesive while attached to the adult female's abdomen, they separated and dispersed upon contact with stream water. The "polar cap" region of each egg became adhesive after a few hours in the water and eggs readily stuck to one another or to the substratum at that time. It is likely that *E. hecuba* eggs were initially dispersed by the current in riffle areas but eventually became attached to the substrate.

Egg development was slow but continuous for *E. hecuba*, with no evidence of an embryonic diapause. Eggs required about 1–4 mo to hatch in the laboratory depending on temperature (Table 1). Hatch success averaged >90% at 15 and 20°C but only about 68% at 25°C. No eggs hatched at 10 or 30°C. Eggs probably require about 2 mo to hatch in the stream, because water temperatures average about 21.5°C during the year.

Range in larval sizes as evidence for semivoltinism

First instar larvae (Fig. 4) were large relative to other mayflies, having a head width (HW) of

0.11 mm and a length (front of head to end of abdomen) of 1.30 mm. The smallest larva collected from the field had a HW of 0.40 mm. Larval dry biomass can be readily predicted from head width (Fig. 5). Larvae with head widths ≥ 1.18 mm (0.97 mg) were sexed by the presence (males) or absence (females) of abdominal forceps. Sexual dimorphism was marked, with maximum larval size observed in the field being 3.48 mm HW (34.6 mg) for males and 5.20 mm HW (149.1 mg) for females (Fig. 6). Female and male larvae in Q. Marilyn were larger on a given date and seemed to grow to a larger maximum size than those in R. Tempisquito.

Although there is some intra- and intersexual overlap in body size at all times, it was generally possible to discern two cohorts based on size. In Figure 7, we simplify this pattern by presenting only female data from the qualitative collections. The two cohorts are clearly apparent in June (1992–1994) at Q. Marilyn. The size and color of the wing pads indicated that the large-size cohort (i.e., females > 40 mg) and the small-size cohort (i.e., females < 40 mg) were at different developmental stages. There were also two distinct size cohorts for R. Tempisquito in June 1992 but, because larvae do not grow as large in this stream, the separation between the largest and second largest size cohort occurred at about 10 mg.

The seasonal changes in population size structure of *E. hecuba* is more completely elucidated by plotting the mass of female larvae collected from both qualitative and quantitative sampling programs during the 4-yr study for each stream (Fig. 8). The data show, especially for Q. Marilyn, a distinct "gap" in the size distribution of small larvae, providing further evidence for the presence of two, temporally segregated cohorts. Individual larvae less than 0.01

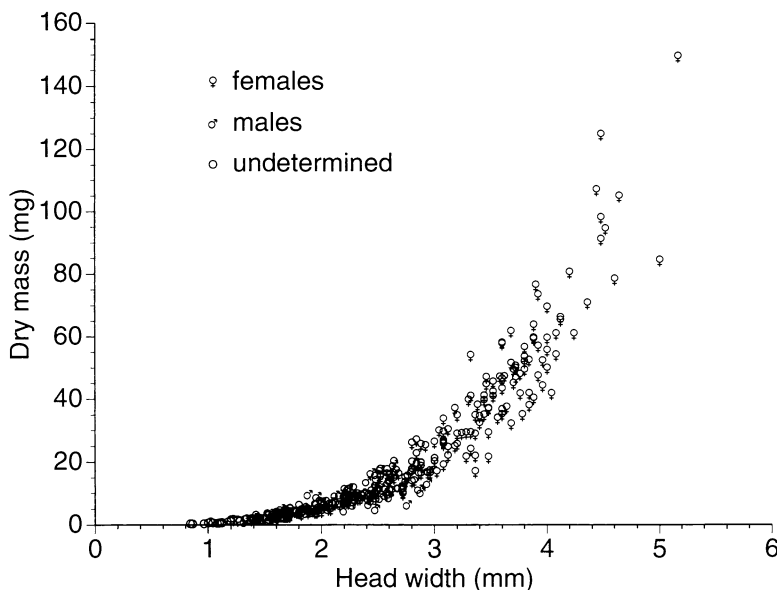


FIG. 5. The relationship between dry mass and head width for individual *E. hecuba* larvae collected from the R. Tempisquito and Q. Marilin. The equation describing this relationship was $y = 0.56x^{3.31}$, $r^2 = 0.96$.

mg in size in Q. Marilin are probably near first instar because individual eggs (including the chorion) average about 0.017 mg dry mass.

Based on these results, we hypothesize that *E. hecuba* has a two-year life history in both streams with adult emergence occurring largely from June through late-October or early-November. For example, eggs laid in June 1991 (Fig. 8) probably hatched in two months, with first instar larvae weighing less than 0.01 mg. By the end of the first year (June 1992), the fastest growing females were about 23 mg in Q. Marilin and 7 mg in R. Tempisquito. During the next year (i.e., from June 1992 to June 1993) the fastest growing females grew to about 112 mg in Q. Marilin and about 78 mg in R. Tempisquito. Thus, according to our hypothesis, adult emergence from eggs laid in June 1991 occurred in June 1993.

Seasonal pattern and rate of growth

Growth rates were estimated by following the change in size for a 1-yr period of the four largest female larvae associated with the second largest cohort starting in June 1992 (Fig. 7). In Q. Marilin, the largest females started at 22.6 mg in June 1992 and increased in average size



FIG. 6. Sexual size dimorphism in *E. hecuba* as demonstrated by the size difference between the largest male (right) and female (left) larvae collected to date from Q. Marilin.

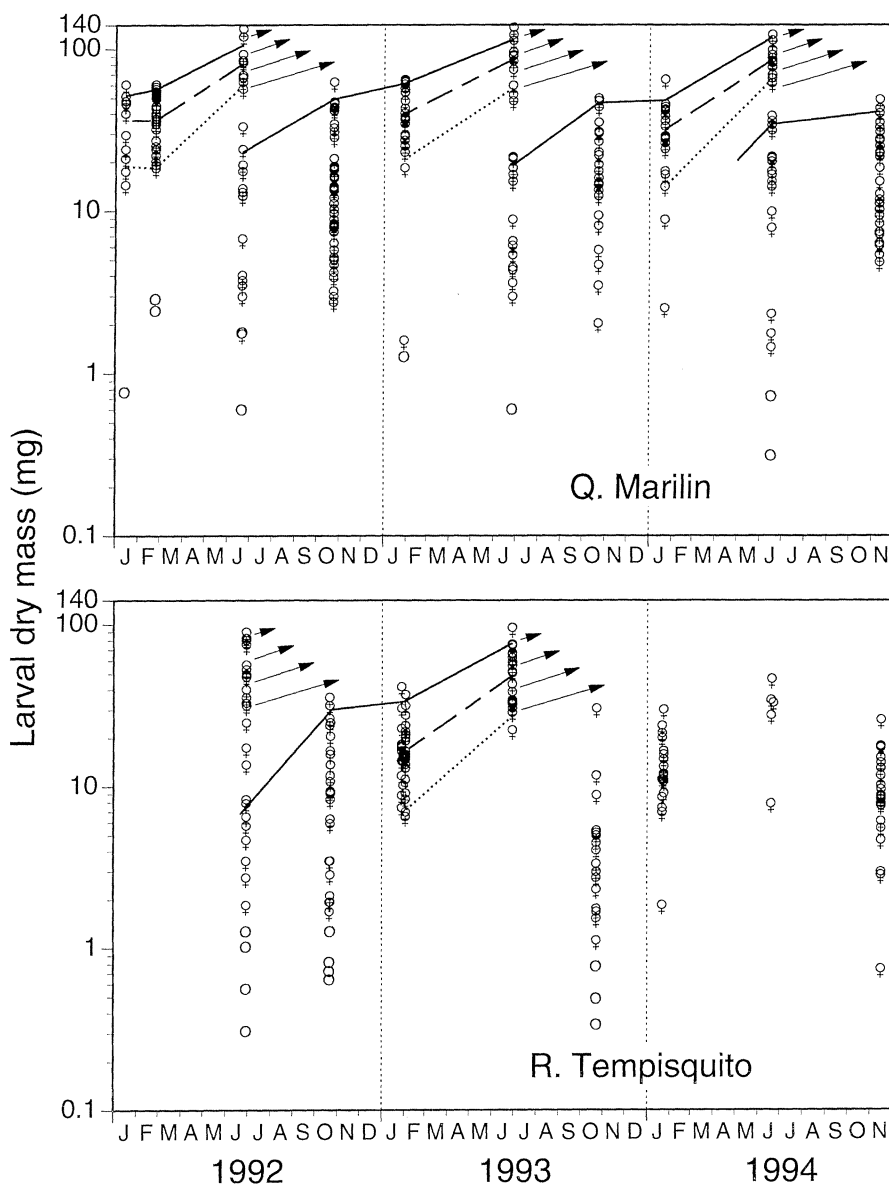


FIG. 7. Individual mass of female larvae of *E. hecuba* collected in qualitative samples during a 3-yr period from Q. Marilin and R. Tempisquito plotted on a logarithmic scale to emphasize the seasonal pattern in population size structure and growth. Open circles represent small larvae presumed (see text) to be females. Heavy solid lines, dashed lines, and dotted lines show the seasonal pattern of growth for the largest larvae, average size larvae, and smallest larvae, respectively, in each cohort (see text). Arrows indicate estimated growth trajectory of variously sized female larvae and the approximate date and size (arrow point) of their emergence from the streams. Lines were not drawn for R. Tempisquito after October 1993 because the larval collection in June 1994 contained only four individuals, effectively precluding accurate estimation of the growth pattern.

by about 90 mg during the subsequent year, which resulted in an instantaneous growth rate of $0.004 \text{ mg mg}^{-1} \text{ day}^{-1}$. Although the largest females in R. Tempisquito were 6.9 mg in June

1992 and grew about 71 mg over the next 12 mo, they had about the same instantaneous growth rate ($0.006 \text{ mg mg}^{-1} \text{ day}^{-1}$) as in Q. Marilin. Based on these growth estimates, the solid

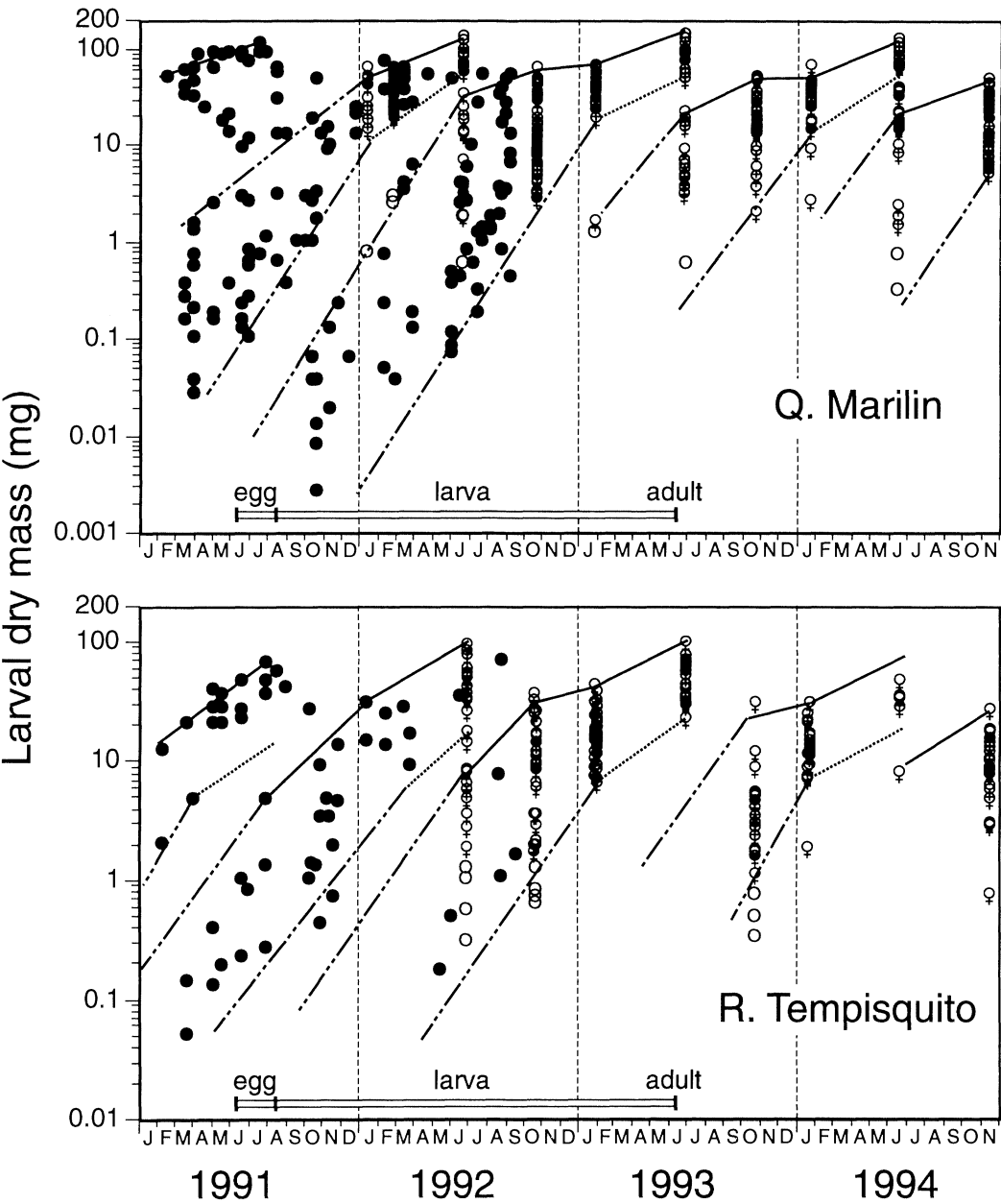


FIG. 8. Individual mass of female *E. hecuba* larvae collected in quantitative (blackened circles) and qualitative samples (⊗ symbols and circles) during a 4-yr period from Q. Marilin and R. Tempisquito plotted on a logarithmic scale. Heavy solid lines and dotted lines show the seasonal pattern of growth for the largest larvae and smallest larvae, respectively, in each cohort (as in Figure 7). The dashed-dotted lines are extrapolations by eye back through time of the estimated seasonal pattern of growth for the largest and smallest females of each cohort (see text).

lines with arrows (Fig. 7) represent our best estimate of the growth trajectory (line) and maximum larval size and date of emergence (arrow head) for individuals in the most mature size cohort present during each July collection.

Seasonal relationship of larval size and adult emergence

During the 4-yr study, a consistent seasonal pattern was observed of maximum female and

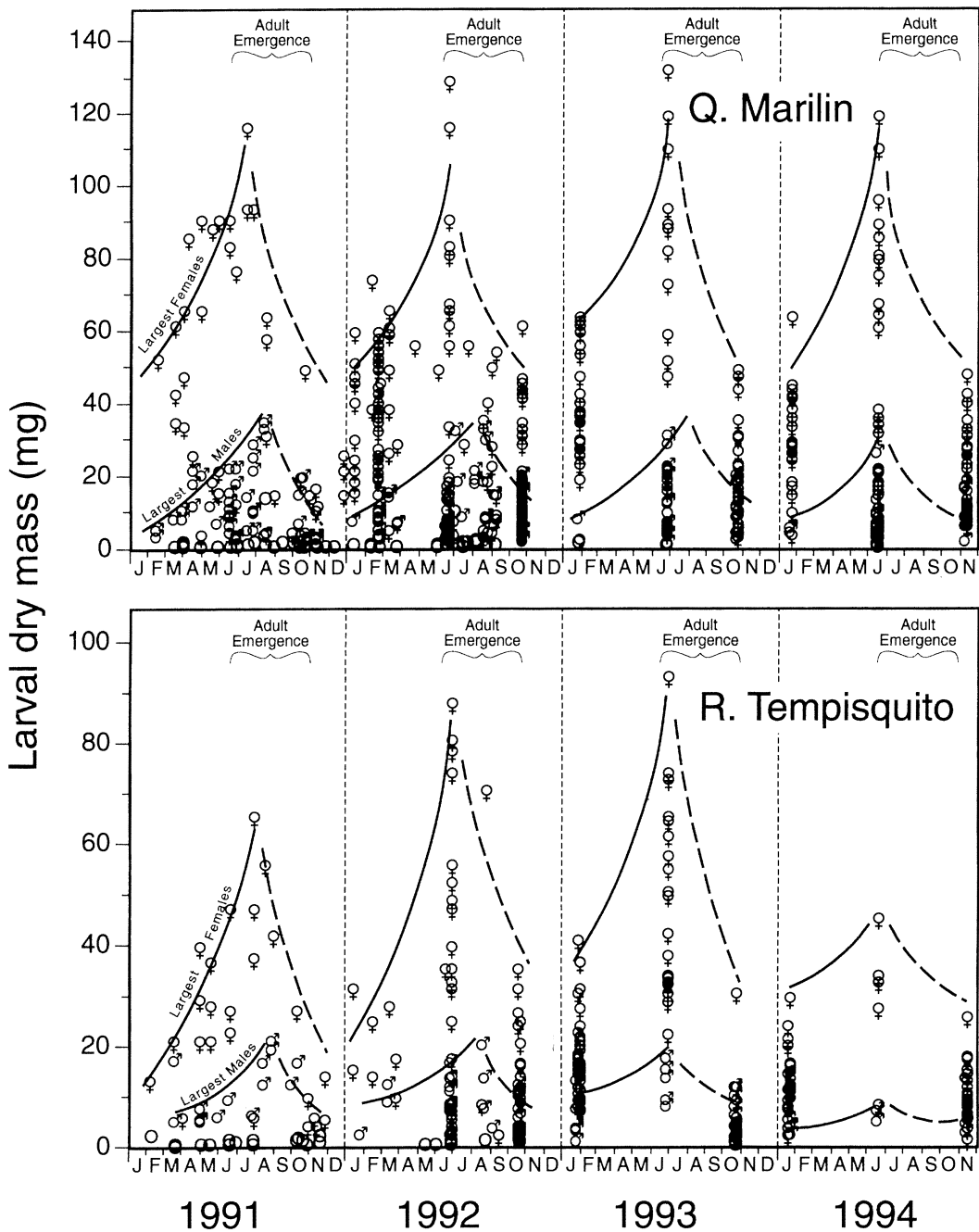


FIG. 9. Individual mass of *E. hecuba* larvae collected during a 4-yr period from Q. Marilin and R. Tempisquito plotted on an arithmetic scale to emphasize the seasonal changes in size for large male (δ) and female (φ) larvae in the streams. The solid and dashed lines have been fitted by eye. The curved solid lines describe the approximate growth pattern of the largest female (upper line) and male (lower line) larvae from January through August of each year. The curved dashed lines approximate the seasonal decline in maximum larval size for females (upper line) and males (lower line) during the period of adult emergence. Larvae too small to sex are shown as open circles.

male larval size gradually increasing from December through July and then gradually decreasing thereafter through November (Fig. 9). This latter period of declining female and male larval sizes corresponded well with the time when adults emerge from the stream as well as when the size of adults gradually diminished (see Fig. 2 for Q. Marilin). The seasonal relationship between changes in larval size, adult emergence, and adult size is demonstrated further by the following observations in R. Tempisquito. In June 1993, large mature larvae were abundant in field collections and adults were observed in reasonable quantities. In late October 1993, however, aggregations of swarming adult males were absent and only a few isolated adult males (small in size) were observed and captured. No adult females were observed during the period. An extensive collection of larvae made on 23 and 27 October 1993 revealed that only two larvae (a male and a female) were mature and ready to emerge. Both larvae were small (male: 8.45 mg; female: 29.03 mg), being <50% of the maximum larval size observed earlier (July) in this stream. All remaining larvae in the collection were small (<10 mg dry mass), immature (wing pads not well developed or black), and appeared to need substantially more growth and development prior to emerging. Subsequent observations and collections during a 10-d period in January 1994 confirmed a complete lack of both adults and/or mature larvae ready to emerge.

Discussion

Systematic integrity of E. hecuba in the study streams

Aquatic insects of tropical streams are relatively unknown, especially at the species level. Our studies of the mayflies of R. Tempisquito and Q. Marilin have revealed the occurrence of two or more congeneric species for at least seven genera (e.g., *Leptohyphes*, *Tricorythodes*, *Farroses*, *Thraulodes*, *Acerpenna*, *Baetodes*, and *Moribaetis*). Obviously, the presence of morphologically cryptic species is a concern in any study of stream insects. We have ruled out this possibility for *E. hecuba* in our study by examining the genetic structure of the populations using protein electrophoresis (Sweeney, unpublished data). Results show that individuals from the

two study streams are part of a single panmictic population. We conclude from these data that our results and the following discussion pertain to a single species of *Euthyplocia*.

We have not, however, resolved conclusively whether the species present in our study streams is definitely *E. hecuba* as described originally from southern Mexico by Hagen (1861). An electrophoretic comparison of individuals from the two study streams with those from other streams in the Guanacaste region reveals genetic isolation between populations of *Euthyplocia* (Sweeney, unpublished data). The degree of genetic differentiation observed within this small region of Costa Rica raises the possibility of more than one species in the general region of Central America. Seasonal and diel emergence data also suggest that the *Euthyplocia* in our study streams in northern Costa Rica may be a different species from the *Euthyplocia hecuba* reported from Panama (Wolda and Flowers 1985). In Panama, emergence occurred in the evening and was observed throughout the year whereas in Costa Rica emergence occurred in the early morning and was seasonal. The diel differences are especially significant because our studies of other Costa Rican mayfly species have revealed that no species exhibits both evening and morning emergence and reproductive activities (Jackson and Sweeney 1995). This diel specificity in timing of emergence and reproduction has also been observed in other tropical environments (Edmunds and Edmunds 1980). However, until the genetic structure of *E. hecuba* from Panama and the type locality can be studied, we must continue to refer to individuals in our two study streams as *E. hecuba* because it is presently the only *Euthyplocia* species described from this region of Central America.

Egg size, embryonic development time, and reproductive effort

The eggs of *E. hecuba* are significantly larger than all other mayflies studied to date in Costa Rica as well as most other species in the western hemisphere. Only the eggs of the predaceous mayfly *D. americana* are bigger and heavier than those of *E. hecuba* (Fink et al. 1991). The eggs of another predaceous mayfly, *Siphonisca aerodromia*, are slightly smaller than *E. hecuba* (Gibbs and Mingo 1986). It has been hypothesized that the large eggs of both *D. americana* and *S. aerod-*

romia are highly adaptive for these two predaceous mayflies because they result in unusually large first instar larvae that can readily exploit a much wider size-range of prey than if they were of average mayfly size (Fink et al. 1991). Although mature *E. hecuba* larvae are large, gut analysis has revealed only detritus and no evidence of carnivory.

Incubation time for *E. hecuba* eggs is at least a month longer than the average time of 19 d (SE = 1 d) at 20°C for the other 19 mayfly species studied to date from the two study streams (Jackson and Sweeney 1995; Sweeney, unpublished data). The two temperate mayflies with comparable large eggs (*D. americana*: Harvey et al. 1980; *S. aerodromia*: Gibbs and Mingo 1986) have much longer embryonic periods (>7 months) than *E. hecuba*, but an embryonic diapause seems to be involved in at least one of them (K. E. Gibbs, University of Maine, personal communication). We do not believe that diapause underlies the relatively long incubation period of *E. hecuba* because we observed continuous, albeit slow, embryonic development in this species. We attribute the long embryonic period for *E. hecuba* to very slow development and/or the additional time needed to produce a large offspring.

Although *E. hecuba* eggs are large, the average fecundity that we measured in October (\bar{x} = 984) was much higher than for the other mayfly species with large eggs (e.g., *D. americana*: \bar{x} = 76 [Harvey et al. 1980]; *S. aerodromia*: \bar{x} = 394 [Gibbs and Mingo 1986]). The discrepancy between *E. hecuba* and the other two species is even greater than this because we showed that adult females of *E. hecuba* are actually at or near their smallest average size (hence less fecund) in October and reproductive effort tends to decrease near the lower limit of adult size *E. hecuba*. This latter phenomenon has been reported for one other mayfly (Sweeney et al. 1986) but does not seem to occur in all aquatic insect species (Butler and Walker 1992). One possibility is that the relationship between reproductive effort and adult female size is actually asymptotic (i.e., reproductive effort is fairly constant above a certain body size but declines rapidly below this threshold). For *E. hecuba*, reproductive effort consistently scaled above 0.5 for females between 30 and 75 mg in mass but was highly variable and generally <0.5 for females smaller than 30 mg. Similarly, data for the mayfly *Lep- tophlebia intermedia* show a fairly constant re-

productive effort of about 0.3 for females between 3.5 and 6.5 mg, but a lower effort for smaller females (Sweeney et al. 1986). In other words, reproductive effort may be compromised in mayflies only in extremely small females.

Rate of larval growth and development

The rate of growth and development of *E. hecuba* remains speculative because we have not successfully reared this species in the laboratory. Other, much smaller species of mayflies from our study streams required 1–6 mo at 20°C to grow from first instar to the adult in the laboratory and exhibited growth rates averaging 0.107 mg mg⁻¹ d⁻¹ over the duration of the rearings. For example, developmental times and growth rates, respectively, for the four species were: *Acerpenna*, 28 d, 0.220 mg mg⁻¹ d⁻¹; *Lep- tohyphes*, 82 d, 0.086 mg mg⁻¹ d⁻¹; *Tricorythodes*, 86 d, 0.073 mg mg⁻¹ d⁻¹; and *Thraulodes*, 159 d, 0.050 mg mg⁻¹ d⁻¹; calculated from Jackson and Sweeney 1995). Thus, the larval development time for *E. hecuba* appears to be substantially slower, with the embryonic development alone requiring as much time as the entire larval stage of at least one other mayfly occurring in these streams. Moreover, we estimate that in order for *E. hecuba* to complete larval growth faster than hypothesized (e.g., in one year instead of two), larval growth rate would have to average about 0.028 mg mg⁻¹ d⁻¹ for the larval period in either Q. Marilyn or R. Tempisquito. Although this growth rate is lower than those estimated above for the multivoltine tropical mayflies studied in these streams, it is about six times faster than the maximum growth rate (0.004 mg/mg/d) estimated for *E. hecuba* based on seasonal changes in larval size measured in the field. We conclude, therefore, that the 2-yr life history hypothesis for a given cohort is the most parsimonious interpretation given the length and rate of egg development, the overall magnitude of larval growth required to achieve the adult biomass observed in the field, and the actual pattern of larval growth estimated from field collections.

Adult-size variation

For univoltine and semivoltine species, the first adults to emerge are the largest and size gradually declines through the emergence pe-

riod. This has been reported for many temperate species of both mayflies (Sweeney 1978, Vannote and Sweeney 1980, Sweeney and Vannote 1981, Brittain 1982) and stoneflies (Hynes 1976, Moreira and Peckarsky 1994) as well as for at least two species of tropical stoneflies (Froehlich 1990). We have also observed that adult metamorphosis can occur successfully over an extremely large range of female and male sizes for *E. hecuba*. For example, captured adult females in Q. Marlin ranged in size from about 33 to 79 mg. This seasonal variation in both mature larval and adult size is undoubtedly important because larval size structure is a critical population characteristic that affects population dynamics and resource use as well as community processes (Lomnicki 1988, Latto 1992), and adult size is directly related to reproductive effort in mayflies. Stearns (1992) suggested that body size often represents a compromise between age at maturity and reproductive investment.

For *E. hecuba*, the pattern of adult size during the emergence period suggests that reproductive success may be higher if an individual emerges mostly with the main component of the cohort (i.e., seasonally) even if it means emerging at a substantially reduced size and fecundity. In this regard, the difference in average adult size between those individuals emerging early in the emergence period versus those emerging later is related to, and may in part reflect, the probability of surviving and successfully locating a mate after the additional growth period needed to achieve maximum size and fecundity. In other words, the mechanism underlying the emergence of an individual at less than maximum size and fecundity has been refined evolutionarily by the probability of lower reproductive success if/when emergence is postponed.

We hypothesize that the decline in adult size during emergence results from an innate propensity for individuals to undergo metamorphosis during a specific time of the year. Thus, small individuals of a given cohort compromise additional growth in order to emerge during the same general time period as the rest of the cohort. The temporal pattern of declining adult size of this species results from the emergence of progressively smaller individuals. We propose, therefore, that the key factor(s) underlying the seasonality of adult emergence also underlies much of the adult size variation in this

species. This is consistent with the observations of others that the stimulus to emerge hurries the smaller individuals on so that they do not attain full size (e.g., Khoo 1968, Froehlich 1990).

At least three alternative explanations account for the decline in larval and adult size during emergence. The first is that environmental conditions such as food quantity or quality decline during the June to November period, which in turn affects the final growth potential of maturing larvae. We presently do not know exactly where, when, how, or on what *E. hecuba* larvae do most of their feeding. We do know from limited gut analysis that the digestive tract is generally filled with very fine organic matter containing a few algal (diatom) remnants. A food-limitation problem seems unlikely, however, for at least three reasons: (1) levels of periphyton (chlorophyll *a* and phaeophyton) on benthic substrates in the two streams are lowest during the rainy season (June–November period), as are inputs of coarse detritus, but neither exhibit a gradual decline during this period (J.D. Newbold, Stroud Water Research Center, unpublished data); (2) the quantity of dissolved organic carbon that can support bacterial productivity is greatest during the June to November period (Newbold et al. 1995); and (3) there is good evidence from the larval weight-structure data that the immature larval cohort of *E. hecuba* present during the June to November period is actually increasing in size, with weight-specific growth rates equal to or greater than those measured for the largest larvae of the mature cohort during the preceding January to June period (i.e., when large larvae were doing much of their growth).

A second explanation for the decline in larval and adult size during the emergence period of *E. hecuba* is that temperature, and its effects on bioenergetics, is the principal underlying factor. This explanation has been proposed previously for temperate species (Sweeney and Vannote 1978, Vannote and Sweeney 1980) and suggests that warmer temperatures near the end of the emergence period of spring- and summer-emerging species may increase metabolic costs disproportionately over growth for larvae and result in metamorphosis at a smaller than normal size. However, this explanation seems unlikely because the decline in adult size during the emergence period of *E. hecuba* occurs in tropical streams where water temperature remains almost constant throughout the year.

A third explanation is that the genotypes of large individuals emerging at the beginning of the emergence period are superior with respect to growth than the genotypes of small individuals emerging later in the period. In other words, small adults reflect inherent genetic constraints on the growth potential of certain individuals. We know of no data that bear directly on this issue. However, this explanation seems unlikely for *E. hecuba* because weight-specific growth rates measured for the smallest individuals in a cohort are equal to those for the largest individuals. Moreover, the explanation requires an assumption that after many thousands of generations exposed to natural selection, the reproductive advantage of the large-body genotypes would not completely overwhelm the genotypes that result in larvae capable of growing to only 20% of maximum size. We find this assumption difficult at best to entertain.

Seasonality in life history characteristics

Clifford (1982) predicted that most tropical mayflies would characteristically have short, aseasonal life histories. Although this generally seems to be true in *Q. Marilin* and *R. Tempisquito* (Jackson and Sweeney 1995), it appears that at least one species, *E. hecuba*, has a relatively long life history, a larval size structure characterized by year classes, and a distinct seasonal pattern of larval growth, adult emergence, and reproduction. It is especially significant that the seasonal pattern of adult emergence, broad size range of female and male larvae present at the onset of adult emergence, and gradual decline in the size of female and male adults during emergence are three characteristics that the tropical *E. hecuba* shares with univoltine temperate species.

The synchrony of the adult emergence period in some temperate mayfly species has been shown to follow as a direct result of a developmental quiescence that is sensitive to temperature (Newbold et al. 1994). We hypothesize that a developmental quiescence or diapause is also involved in the seasonality of adult emergence and adult size variation of *E. hecuba*. Support for this hypothesis comes from the fact that adult emergence appears to be more time-dependent than size-dependent. Thus, near the end of the emergence period, females in the two streams often undergo metamorphosis at a

size equal to or less than one year old females that are immature (i.e., have undeveloped wing pads). This suggests that one year old, actively growing females are in a state of developmental quiescence or diapause with respect to development (i.e., maturation of adult tissues).

If a developmental quiescence or diapause necessarily underlies and promotes the 2-yr life history and associated seasonal characteristics, it is unlikely that temperature is involved in initiating or ending this condition because of the thermal constancy (range $<3^{\circ}\text{C}$ per year) of *Q. Marilin* and *R. Tempisquito*. A more likely environmental cue would be photoperiod, even though seasonal variation in daylength is only about 1.5 h. Wolda (1988) discusses some of the proximate causes of insect seasonality in the tropics but points out that there are very few tropical insects for which the life cycle is sufficiently known to enable careful analysis. Although elucidation of the factor(s) involved in *E. hecuba*'s life history goes beyond this study, our results clearly show that not all mayfly species in the tropics have aseasonal, short life-history characteristics.

Perspective

The observation that adult size of *E. hecuba* declines significantly during the emergence period in thermally constant streams where a concomitant seasonal decline in food resources does not seem to occur has significant implications for the interpretation of life-history patterns for univoltine and semivoltine temperate mayfly species (as well as other groups). First, it suggests that the principal explanations for adult size variation in temperate species may be wrong. In other words, individual differences in growth rate (genetic or otherwise), seasonal changes in the quantity or quality of food, and magnitude and pattern of temperature during the emergence period may all play, at best, a modifying role in the level of adult size variation observed in the field. We propose that most of the variation in size of adults reflects the combined effects of: (i) random processes affecting egg development and/or the growth of early instar larvae; and (ii) the synchrony of the emergence period as produced by the developmental constraints of the species' univoltine or semivoltine life history. Thus, random factors that result in delayed egg hatching (e.g.,

sedimentation of eggs, brief periods of low temperature, etc.) or slow larval growth during the early instars (e.g., chance colonization of habitat with poor food, parasitism, disease, etc.) will tend to create a broad size-range of larvae by the time the population is halfway through its life cycle. The developmental constraint of all individuals completing their cycle in exactly one or two years and emerging more or less synchronously necessarily limits the period available for smaller individuals to achieve full size and almost guarantees substantial size variation among emerging adults.

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