Thanks for your interest, Bill.

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Synchronous Emergence of *Hexagenia bilineata* Mayflies[1] in the Laboratory[2]

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ABSTRACT

Mass emergences of *Hexagenia bilineata* (Say) from the Upper Mississippi River tend to occur at intervals of about 6–11 days. It has seemed likely that the waves of emergence are indicators that sub-populations or "broods" have developed sympatrically and that the short-lived adults of one emergence peak are sexually isolated by time from adults of preceding and succeeding peaks. However, preliminary experiments with laboratory populations showed that the progeny resulting from eggs laid during the time of one mass emergence will emerge at intervals and en masse over an 11-month period. It seems probable that the broods in the river may include adults from last-instar nymphs of varying ages which have emerged at the same time. Complete sexual isolation, discrete gene pools, and resulting sympatric speciation of the broods therefore seem unlikely.

The general life histories of *Hexagenia* mayflies are well known (Needham et al. 1935, Hunt 1953, Fremling 1960, Swanson 1967). The aquatic nymphs inhabit the silted bottoms of lakes and rivers. *Hexagenia bilineata* (Say) mayflies tend to emerge en masse, and the shores of the Upper Mississippi River are often literally covered by them during periods of maximum emergence. Analyses of more than 500 mayfly collections along the Upper Mississippi River over a 10-year period indicate that mass emergences of *H. bilineata* tend to occur at intervals of about 6–11 days from mid-June through mid-August (Fremling 1964).

Because the adults are extremely short lived, it has seemed probable that the adults of a given emergence peak are sexually isolated, by time, from adults of preceding and succeeding peaks. Therefore, it has seemed likely that the emergence waves are caused by subpopulations, or "broods," which have discrete gene pools and which have developed sympatrically. For example, adults of the July 12 emergence, were thought to give rise to young which would emerge on or about July 12 of the following year. Because the mayfly has a very brief adult life, synchronous emergence has obvious survival value. The purpose of this investigation was to study emergence under semicontrolled conditions in the laboratory.

MATERIALS AND METHODS

General rearing procedures followed the methods described by Fremling (1967). A galvanized stock-watering tank (2.4×0.96 m) was used as a rearing chamber. The tank was divided into 2 compartments (A and B) by a partition. A screened window (25 mesh/cm) at the top of the partition allowed water to circulate between the compartments. During the first 3 months of the experiment the screened partition was covered with polyethylene sheeting to prevent newly hatched nymphs from passing through the screen. A net over each compartment prevented the escape of newly emerged subimagoes.

Eggs were obtained from imagoes which had accumulated beneath a light on the Winona, Minn., river bank on the night of July 12, 1962. Ten cubic centimeters of eggs (ca. 50,000) were placed in compartment A. Another 10 cc of eggs were placed in polyethylene bags of water and were refrigerated at 7°C. Flattum (1963) showed that embryonic development of *Hexagenia limbata* (Serville) eggs virtually ceases at this temperature. After 14 days of refrigeration the eggs were warmed slowly

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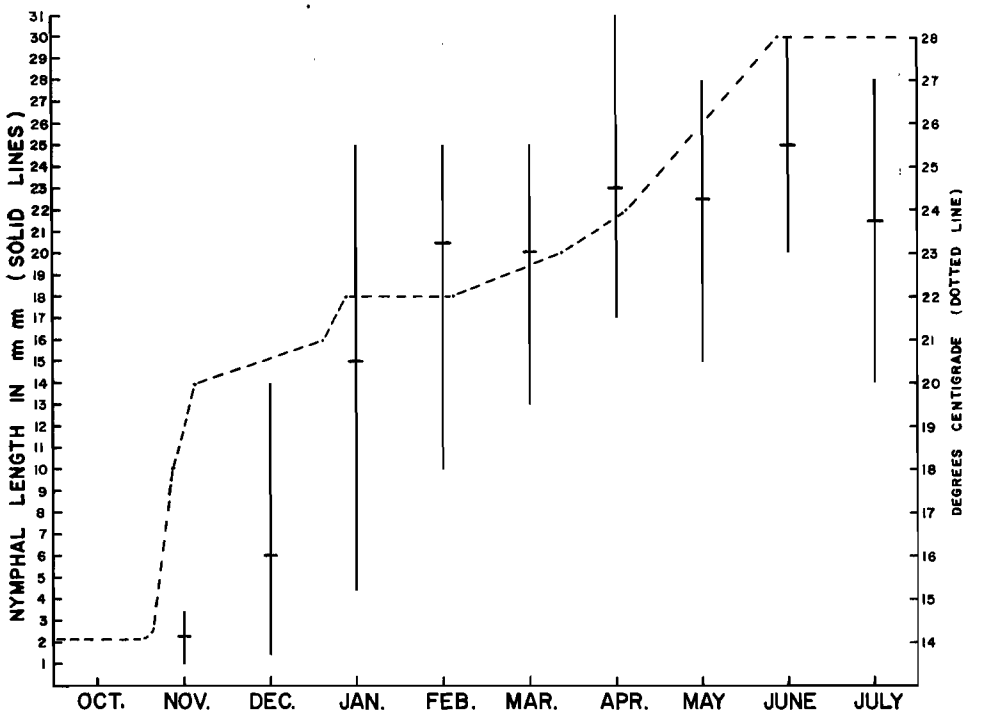

FIG. 1.—Relationship between water temperature and mayfly nymphal growth. The length of the solid vertical lines indicates the range of nymphal lengths. The average nymphal length is indicated by a horizontal line on each vertical line. Water temperature is indicated by a dotted line.

to the temperature of the rearing tank and were placed in compartment B. Controls from both groups of eggs hatched in 9–11 days after the initiation of incubation at room temperature. During the first 3 months of the experiment, each compartment was subjected to a 12-hr photoperiod, as it was lighted by two 40-w daylight-type fluorescent tubes to supplement available sunlight during the normal daylight period. From March 18 until April 17 only 2 mayflies emerged, and it was feared that the lights were not sufficient to maintain adequate water temperatures and algal blooms in the compartments. Therefore, on April 17, 1963, a 250-w incandescent bulb with reflector was suspended 20 cm above the water in each compartment and left on 24 hr/day. The only window in the room was blackened at this time so light intensity remained constant.

The 2 nymphal populations were sampled monthly for the first 10 months of the experiment by removing cores from the mud. A tinned can with both ends removed was pushed into the bottom and a spatula was inserted beneath it to lift the core out. Nymphs were removed from the mud by sifting with a soil screen (225 mesh/cm) and by a "salting out" process similar to that used by Lyman (1943). The compartments were checked each morning and each evening for the presence of adults.

RESULTS

Microscopic nymphs were first found November 2, more than 2 months after the control eggs had hatched. A comparison made between the rate of nymphal growth in the rearing tank and the growth reported by Spieth (1938) from a natural environment indicated that the slow rate of growth in the laboratory populations was caused by low temperature (avg 14°C). When the fluorescent lights were replaced by incandescent bulbs as previously described, and the tank temperature was raised, the rate of nymphal growth increased markedly (Fig. 1). Thus, populations A and B began their period of rapid growth at the same time. The retarding effect of refrigerating half

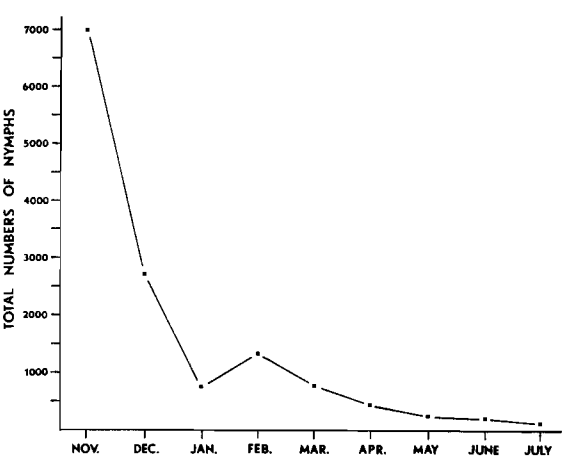

FIG. 2.—Total mayfly nymphal population in rearing compartments A and B as estimated by monthly sampling.

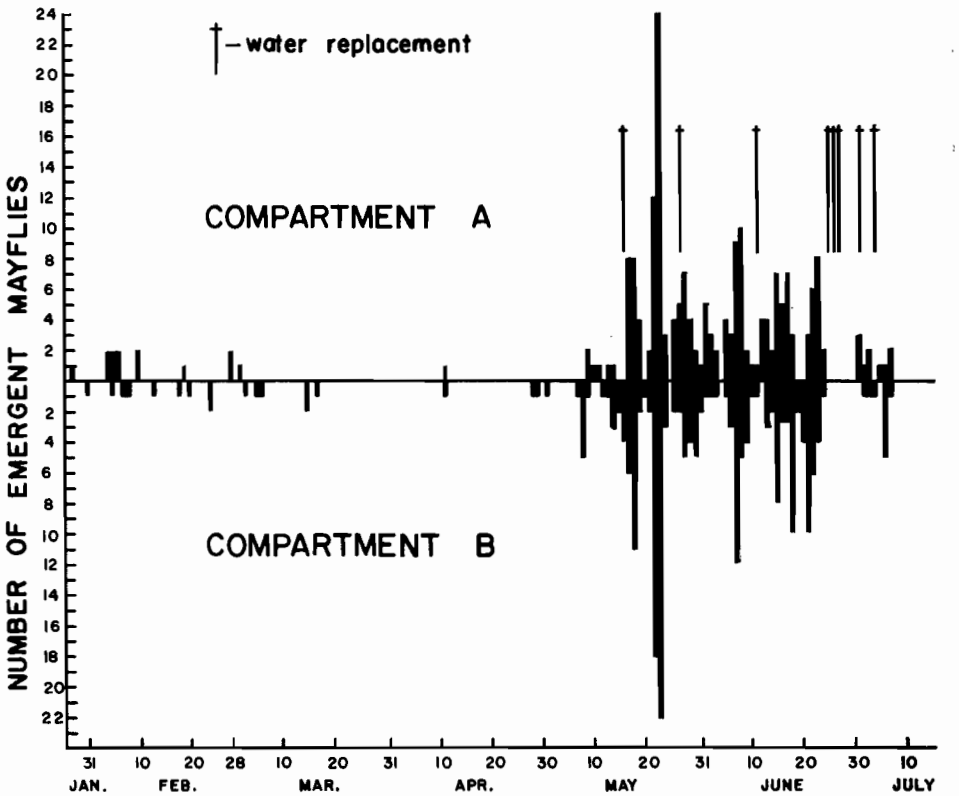

FIG. 3.—Total daily mayfly emergences from compartments A and B.

the eggs was apparently offset by low water temperature during the first 3 months of the experiment. Growth rates were not uniform among the nymphs, and on January 15, just 13 days prior to the 1st emergence, the nymphs ranged in size from 4.5 to 25 mm (Fig. 1).

Bottom sampling showed a rapid decline in total numbers of nymphs through July, when core sampling was discontinued (Fig. 2). Emergence accounted for much of the decline after May 1. The apparent increase in numbers of nymphs in February is unexplained, although it may result from recruitment because of delayed hatching of eggs. Delayed hatching in other ephemeropterans has been observed (Pleskot 1961).

The 1st subimago emerged from compartment A on January 28, 1963, and 3 days later a subimago emerged from compartment B (Fig. 3). During the next 3 months, sporadic emergences of 1 or 2 individuals at a time occurred in both compartments. Only 8 insects emerged during the 60-day interval from March 7 to May 7, and it was feared that the dense populations were stressed by factors such as insufficient food, electrolyte accumulation, or by ectocrine suppression such as that reported for tadpoles (Richards 1958, Rose 1960) and snails (Berrie and Visser 1963). On May 15, all the water in the tank was drained away and replaced with algae-rich water from a storage tank. It seemed likely that the replacement of the old water caused the large emergences which soon followed. However, it may be noted from Fig. 3 that subsequent emergences were not coincidental with complete water replacements. Mass emergences occurred synchronously from both compartments at intervals of 4–6 days from May 7 through July 7 (Fig. 3). There was no tendency for emergences to occur mainly at night, as they do from the river, even during the period when the lights were turned on and off with normal daylight (Fig. 4).

On July 12, 1963, a complete census was made of the nymphs in compartments A and B. Compartment A contained 48 nymphs which ranged from 15 to 25 mm long. Compartment B contained 31 nymphs which ranged from 14 to 28 mm. Nymphs from compartments A and B were consolidated into a single new tank where they continued to emerge sporadically, in small numbers, until December 21. During the entire experiment 479 adults were produced from 2.3 m^2 of substrate. Of 432 insects which were sexed, 103 were males and 329 were females.

The continued presence of small nymphs suggests the possibility that the adults were reproducing in the rearing compartments. Because *H. bilineata* mayflies have an elaborate mating behavior pattern (Fremling 1960) which involves swarming and sight recognition of the female flight pattern by

the male, it seems very unlikely that mating and subsequent oviposition of fertile eggs could have occurred in the rearing chamber. The large percentage of females suggests the possibility of parthenogenetic reproduction. Attempts were made on 3 occasions to hatch eggs from female imagoes which had emerged in the tank. None of the eggs from 12 ♀ hatched, but eggs which were artificially inseminated hatched readily. The number of eggs produced by each of 8 laboratory-reared females varied from 380 to 6300. The average number produced (2500) was considerably below the 7100 average reported from mayflies collected in the wild (Fremling 1960). However, the possibility remains that parthenogenetic development occurred in a portion of the original eggs which were collected in the wild.

DISCUSSION

Although the variables in this preliminary experiment were many, some postulations can be offered. It seems obvious that emergence peaks may not represent genetically isolated broods as was thought previously. Since, under laboratory conditions, the progeny resulting from eggs laid at the time of 1 mass emergence may emerge over a prolonged period (almost 11 months under laboratory conditions), it seems possible that emergences resulting from different periods of oviposition in the river may overlap. The broods may include last-instar nymphs of varying ages which emerge at the same time. If this be true, the gene pools of the broods are not isolated and eventual sympatric speciation is unlikely. However, the artificial conditions imposed on the laboratory populations may have inhibited the tendency for each to exhibit a single synchronous emergence.

The possibility exists that the apparent differential growth rates exhibited by the nymphs may result from delayed hatching of eggs. Perhaps the growth of small nymphs is inhibited by ectocrine substances secreted by larger nymphs. Thus, the interval between emergence peaks may be the time required to complete the last nymphal instar. A remarkable coincidence of emergence occurred in compartments A and B (Fig. 3). Because there was a water connection between the 2 compartments, the coincidence must have resulted from either a common external stimulus operating contemporaneously over the whole system, or the stimulus of a water-borne pheromone. The factor(s) which synchronizes the emergences is still unkown. Current experiments, with adequate controls, are aimed at determining the possible effects of light, water change, crowding, ectocrine suppression, and water-borne pheromones.

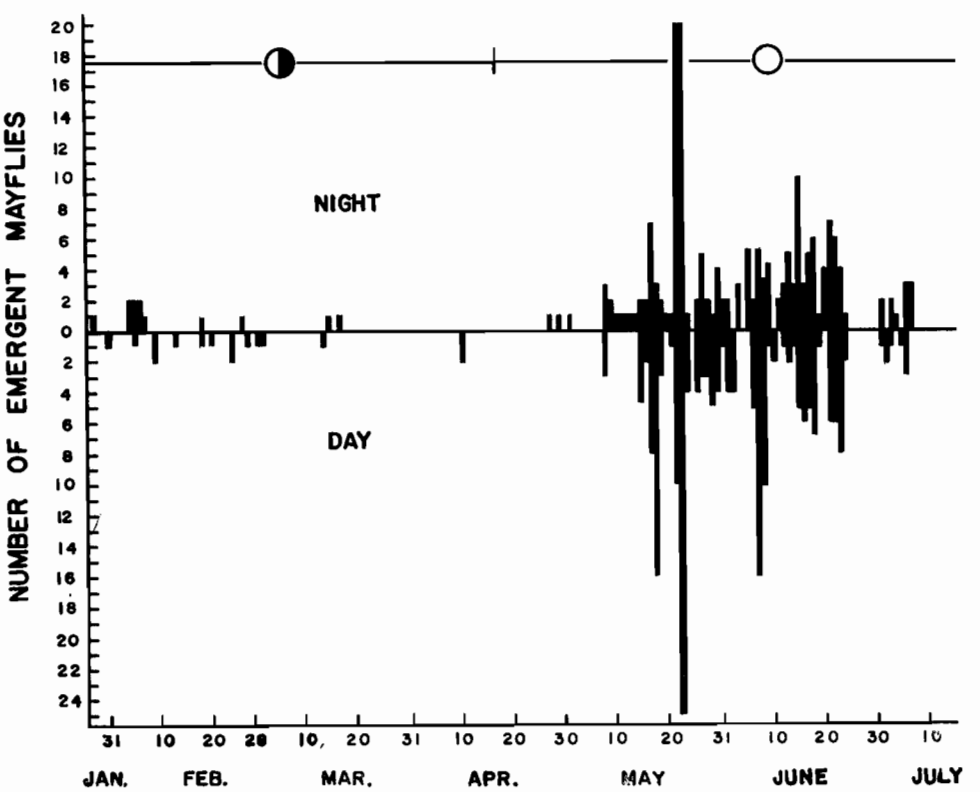

FIG. 4.—Relationship between rate of mayfly emergence and time of day. The half-darkened circle indicates 12 hr of darkness and 12 hr of light. The open circle indicates continuous light.

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