# DISTRIBUTION, ABUNDANCE, AND AGE STRUCTURE OF A POPULATION OF THE BURROWING MAYFLY, *HEXAGENIA LIMBATA*, IN AN OHIO POND[1,2]

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A population of *Hexagenia limbata* (Ephemeroptera: Ephemeridae) was studied from 23 January through 18 September, 1971, in a pond (6134 m²) located 3 km southeast of Oxford, Ohio (Butler County). Nymphs inhabited each of seven different substrates in the pond. Average population abundance during the study period was 473 individuals/m², and average biomass was 2.15 g dry weight/m². Nymphal distribution was influenced by substrate type, dissolved-oxygen concentration, prevailing winds, and water level. Most nymphs in this ecosystem appear to have a two-year life cycle; however, those which hatch from eggs laid early in the emergence period may mature in a single year.

Burrowing mayflies of the genus *Hexagenia* (Ephemeroptera: Ephemeridae) are widely distributed in lakes and rivers throughout much of eastern and northern North America. The nymphs are often abundant in the benthos of these ecosystems and may constitute an important food source for secondary consumers. Recent studies of this genus have ememphasized ecological relations at the population level both in the field (Hunt, 1953; Britt, 1955a, 1955b; Dorris and Copeland, 1962; Carlander, *et al.*, 1967; Swanson, 1967; Craven and Brown, 1969) and in the laboratory (Hunt, 1953; Eriksen, 1968; Fremling, 1970). However, most of the field investigations have focused on populations of *Hexagenia* inhabiting large lakes or rivers, which are difficult to sample effectively and where changes in environmental conditions cannot be easily monitored. In such cases it is often impossible to carry out detailed ecological studies.

Our interest in *Hexagenia limbata* began when a large population of this species was located in a small pond near Oxford, Ohio. This situation gave us an opportunity to study this organism in a small ecosystem, which could be sampled adequately at any time and where the major physical, chemical, and biological factors varied in somewhat limited fashion. This paper summarizes the results of our study, which was designed to determine age structure and abundance of the population, and the influence of different pond characteristics on its distribution.

## STUDY AREA

The burrowing mayfly, *Hexagenia limbata*, occurs in Brandenburg Pond, which is located 3 km southeast of Oxford, Ohio (Butler County). It lies in an area of moderate relief and is situated on a small hill free of agricultural activity. The pond is privately owned, and recreational use is restricted.

The pond is roughly circular, with a diameter of approximately 88 m, a total surface area of 6134 m² (0.61 ha) and a maximum depth of 2.9 m. Its sediments are composed primarily of clay, along with varying amounts of gravel and organic detritus. Runoff into the pond is negligible.

The pond was constructed in 1954 and is spring-fed. The western portion, which is quite shallow, is bordered by a narrow band of willow trees (*Salix* spp.) which seasonally contribute leaves to the littoral areas. A grassy embankment borders the eastern portion of the pond, and the bottom slopes steeply in this

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area. Cattails (*Typha latifolia*) are present around much of the shoreline and represent a major source of autochthonous organic material.

Average transparency of the pond is low (Secchi disc = 60 cm), apparently due in part to a large amount of suspended colloidal clay. Measurements taken during July showed an absence of total nitrates and the presence of phosphates in concentrations of 0.11 ppm at the surface and 0.14 ppm at 2.4 m.

The pond is mesotrophic and has a relatively low macroinvertebrate diversity (richness). *Caenis* sp., the only other mayfly recorded, is occasionally found in very shallow areas. Several species of midges (*Tanypus stellatus*, *Chironomus plumosus*, and *Chironomus attenuatus*) and the mosquito, *Chaoborus punctipennis*, are abundant in substrates which have low densities of *Hexagenia*. Other widely distributed benthic organisms include *Sialis* sp. (Megaloptera), *Physa* sp. and *Helisoma campanulata* (Gastropoda), and *Sparganophilus* sp. (Oligochaeta).

Large-mouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*) were introduced in 1955 and represent the major consumers. *Najas* sp., the only submerged aquatic plant, occupies a narrow band near the shoreline during the winter months, but is found to a depth of 1.5 m in late summer and fall.

## MATERIALS AND METHODS

*Depth Contours, Temperature, and Dissolved Oxygen.* From 10 April through 3 July, 1971, 2,750 depth measurements were taken with a pole at 1.5-m intervals along line transects. A correction factor was applied to measurements taken in June and July, so that all depths would correspond to 1971 spring conditions of maximum water level.

Water temperature and dissolved oxygen were determined at weekly intervals beginning on 28 March and 15 May, respectively, and terminating on 18 September, 1971. Measurements were taken concurrently at four stations to monitor any spatial differences in these parameters (fig. 1). Readings were taken at the surface and at 0.5-m depth intervals using a Yellow Springs oxygen meter and thermistor. The maximum depths at the four sites (inshore to offshore) were 0.6, 1.2, 1.8, and 2.4 m, respectively.

*Substrate Composition, Population Density and Biomass.* The mayfly population was sampled monthly in January, February, and March 1971, and thereafter on a weekly basis until completion of the study on 18 September 1971. Two hundred and seven samples, equivalent to one sample per 30 $m^2$, were taken with a 15.25-cm Ekman grab (fig. 1). The objective of the sampling program was to take representative samples of nymphs from the entire pond so as to obtain an accurate map of mayfly distribution. Sample sites were systematically predetermined, and no site was sampled more than once during the study period.

Immediately after sampling, the substrate collected in the grab was visually categorized by texture and composition (*i.e.*, clay, gravel, or detritus, or some combination thereof). The substrate was then washed using a 40-mesh screen. Nymphs were hand-sorted and preserved in 10% formalin. Individuals less than 3 mm were not preserved, since efforts to retrieve them during screening and sorting were largely unsuccessful. After several hours of preservation, length measurements to the nearest mm were made from the tip of the frontal process to the base of the caudal filaments. Weights were determined with an analytical balance (Ainsworth Model 10). The organic content of each of the recognized substrate types was determined by ashing representative samples in a muffle furnace at 600 C for five hours.

To express the number of nymphs collected per grab as nymphs per $m^2$, a conversion value of 233 $cm^2$ was used. A mean value of density (number/$m^2$) of nymphs for the entire pond was obtained by averaging the 207 individual samples. This value, when multiplied by pond area, provided an approximation of population size. Number of nymphs per $m^2$ for each substrate type was obtained by averaging all samples taken from a given substrate and by applying the conversion value of 233 $cm^2$.

Estimates of biomass were obtained in a similar manner. A length-weight curve was constructed for a representative group of 50 animals collected and oven-dried in January, 1971. The equation for the curve was determined as: $\text{Log } W = -5.14376 + 2.50678 \text{ Log } L$ (standard error of the slope = 0.14470). For each sample from a given substrate, the total number of nymphs of each size was multiplied by the calculated dry weight. Total weights of all samples from the substrate were averaged to obtain mean biomass per grab. This value was then multiplied by 43 to obtain an estimate of biomass per $m^2$. Values of numbers and biomass represent means for all samples taken from a given substrate throughout the study period. All statistical calculations were carried out with the IBM/360 computer (APL) following the procedures of Woolf (1968).

## RESULTS

*Pond Temperature and Dissolved Oxygen.* Temperature and dissolved oxygen profiles were plotted only for the deepest station (depth = 2.8 m) since, for any given sampling date, spatial and depth

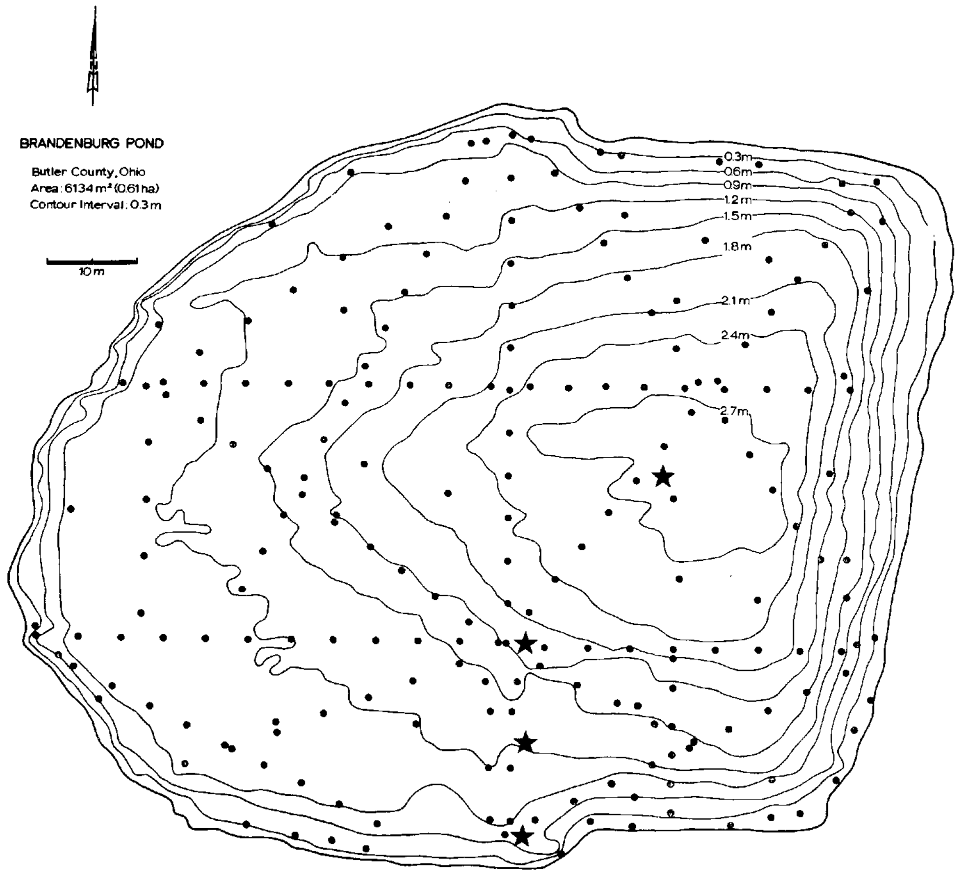


FIGURE 1. Contour map of Brandenburg Pond (interval=0.3 m). Each dot rperesents one benthic sample site; the four stars indicate sites of temperature and dissolved oxygen measurements.

differences were negligible (<0.5 ppm) among the four stations.

Maximum temperatures (26–28° C) were recorded in June and July (fig. 2). The difference between surface readings and those at 2.8 m never exceeded 6° C. Oxygen profiles indicate that stratification began in early June and resulted in anoxic conditions (<0.5 ppm) at depths greater than 2.0 m from June through September (fig. 3). However, during periods of high winds (29 May, 31 July, 28 August, and 18 September), mixing was rapid and complete at all depths. The temperature profiles also reflect these periods of mixing (fig. 2).

*Substrate Composition and Nymphal Density.* Substrate was initially categorized in the field as to texture, composi-

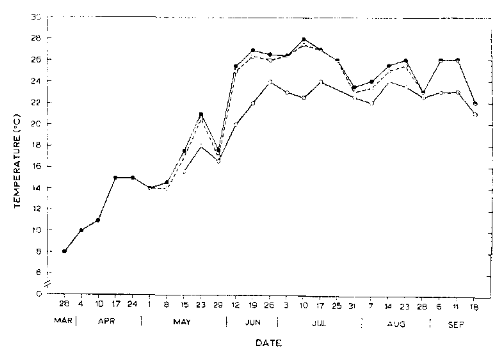


FIGURE 2. Temperature profiles for March-September, 1971.

●———● Surface
○- - - -○ 1.8 m
⊙- - - -⊙ 2.8 m

TABLE 1
*Nymphal density and dry weight biomass in relation to substrate.*[1]

| Substrate type | Number of samples | Mean water depth in cm(±SE) | Mean number of nymphs/sample (±SE) | Mean number of nymphs/m² (±SE) | Mean biomass in g dry wt./m² (±SE) |
|---|---|---|---|---|---|
| Dark, large leaves and stems (cattails) | 27 | 94(±9) | 12(±3) | 516(±129) | 2.25(±0.55) |
| East bank[2] | 11 | 118(±18) | 16(±6) | 688(±258) | 2.96(±1.17) |
| West bank[2] | 16 | 76(±5) | 9(±2) | 387(±86) | 1.76(±0.46) |
| Yellow clay, gravel | 44 | 84(±7) | 24(±3) | 1032(±129) | 3.04(±0.36) |
| Light clay | 44 | 137(±6) | 14(±2) | 602(±86) | 2.57(±0.29) |
| Light-textured clay | 13 | 99(±7) | 12(±2) | 516(±86) | 2.04(±0.34) |
| Dark-textured clay | 22 | 158(±5) | 2(±0) | 86(±0) | 1.05(±0.23) |
| Dark clay | 8 | 173(±16) | 3(±2) | 129(±86) | 0.65(±0.34) |
| Black muck | 49 | 231(±6) | 3(±1) | 129(±43) | 1.00(±0.25) |
| NW Quadrant[2] | 3 | 107(±2) | 6(±3) | 258(±129) | 2.55(±1.37) |
| Center of Pond[2] | 46 | 239(±5) | 2(±0) | 86(±0) | 0.90(±0.25) |

[1]SE = standard error of mean.
[2]These data illustrate the large variations in density and biomass among widely-separated areas of the same substrate.

tion, and color, with the latter characteristic being used as an indicator of relative, rather than absolute, differences in organic content. When representative samples of each substrate type were ashed, it was found that the darker substrate did have a higher percentage of organic material (fig. 4). Seven substrate types were identified in the pond, and each contained mayfly nymphs in varying numbers (table 1).

The substrate found in shoreward areas free of cattails was composed primarily of yellow clay and gravel and had the lowest organic content (4–5%). This substrate had the greatest density of nymphs (1032/m²) throughout the study period, and this was further evidenced by numerous burrows visible in the shallow littoral regions where the substrate occurred. The high numbers of nymphs could have reflected egg-laying near the shore during the long emergence period (May-September) since smaller nymphs (<11 mm) were consistently present in this area. This substrate probably predominated in the pond for a short period after its construction.

Cattails were present around much of the periphery, and were a major source of autochthonous plant debris as indicated by the high organic content (10–12%) of the substrate associated with these areas. Nymphal density was relatively high in this substrate (516/m²). Leaves and stems from the cattails become finely divided as they decompose, and the smaller fragments are carried toward the center of the pond during periods of wave action. The process of decomposition continues here with the result that only dark fibrous and particulate material is found in the deeper portion of the pond. Two other sources of plant debris were the willows bordering the west bank and the submerged aquatic plant, *Najas* sp., which was widespread in occurrence during late summer. In

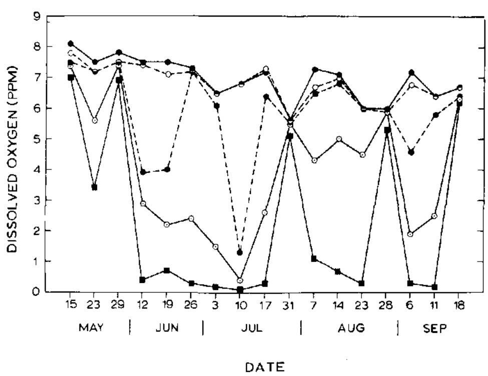


FIGURE 3. Dissolved oxygen profiles for March-September, 1971.
●——● Surface
○- - -○ 1.0 m
●- - -● 1.5 m
⊙——⊙ 2.0 m
■——■ 2.8 m

general, substrate color darkened and organic content increased with depth.

A soft silty substrate with an organic content of 10–12% occurred in the center of the pond. Substrates composed primarily of silt or mud have been shown to be suitable for *Hexagenia*, provided that they are cohesive enough to allow the construction and maintenance of burrows (Neave, 1932; Hunt, 1953; Carlander *et al.*, 1967). The relatively low number of nymphs in this substrate was probably related to periodic absence of sufficient oxygen during the summer (fig. 3). This type of substrate was also found in a small portion of the northwest quadrant of the pond where the depth was only 1.0 m, and where marked oxygen depletion did not occur at any time. Nymphal density (258/m²) at this site was appreciably higher than that observed in the deeper section. It should be noted, however, that only three samples were taken from this location (two in May and one in September), while samples from the deeper section were taken throughout the study period.

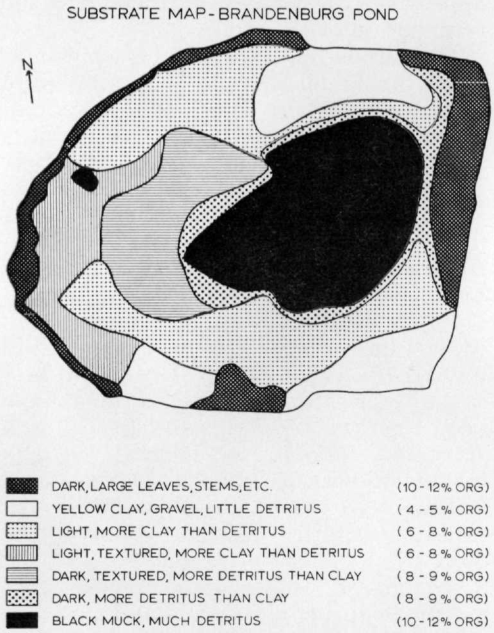


FIGURE 4. Map of substrate types and distribution. Percentage organic contents of the substrates are given in parentheses.

The western half of the pond contained a textured clay substrate which ranged in color from yellow to black and lacked gravel. This substrate appeared flaky after screening. Throughout the study period winds were predominantly from the southwest. The trees and shrubs which surround this half of the pond probably act as an effective windbreak, thus minimizing wave action in this area. The reduced wave action, together with a gentle slope and the absence of gravel, prevented the occurrence of the more extreme conditions found in the eastern sector of the pond. As a result, molar action and silt accumulation were most likely reduced in this area. Nymphs avoided the dark-textured clay for some unknown reason, and density was low (86/m²). Mean water depth over this substrate was 1.6 m, and low concentrations of dissolved oxygen (1.2 ppm) occurred only in July. On the other hand, nymphal density in the light-textured clay averaged 516/m² throughout the study period.

Nymphal density decreased with increasing depth, a condition that may have been related to low oxygen-levels in the deeper areas during the summer. In general, *Hexagenia limbata*, Chironomidae, and *Sialis* sp. were more numerous in the eastern half of the pond, perhaps due to the influence of the prevailing southwest winds and their effects on emergence and subsequent egg-laying. For example, there were marked differences in the density of mayfly nymphs between the substrates associated with cattails on the east and west shorelines (east—688/m²; west—387/m²).

Movements of nymphs among substrates were not investigated since the sampling design precluded collection of such data. Migratory movements probably occur, however, during periods of low oxygen in the deeper water and during low-water levels, when substrate along the shoreline is exposed.

*Population Density and Biomass.* A total of 2,350 nymphs was collected during the study period, the mean number of nymphs per grab being 11 (range = 0–97). Average standing crop of *Hexagenia limbata* in the pond was 473 per m², or roughly 2,800,000 individuals. This estimate did not include nymphs less than

3 mm and did not take into account aspects of the population such as emergence, oviposition, and mortality. Population size prior to the emergence period, which began in May, was estimated at 2,300,000.

Mean biomass was 2.15 g dry weight per m$^2$, or 13.2 kg. By dividing mean biomass per m$^2$ (2.15 g) by mean density per m$^2$ (473), an average dry weight per nymph of .0045 g was obtained, which corresponded to a body length of 13 mm (derived from equation for length-weight curve).

*Population-Age Structure.* One- and two-year life cycles have been documented for *Hexagenia limbata.* Hunt (1953) studied the growth of nymphs produced through artificial insemination and showed that temperature directly affects the length of the life cycle, with little or no nymphal growth occurring below 10° C. Fremling (1970) reported that *H. limbata* grows slowly during the winter, but does not complete final-instar development. Different investigators have extended these findings by documenting two-year life-cycles in northern latitudes (Neave, 1932; Swanson, 1967) and one-year life cycles in southern latitudes (Craven and Brown, 1969). The values in figure 2 suggest that growth in Brandenburg Pond resumed or accelerated about 17 April, when water temperature approached 15° C, and continued past the termination of the study.

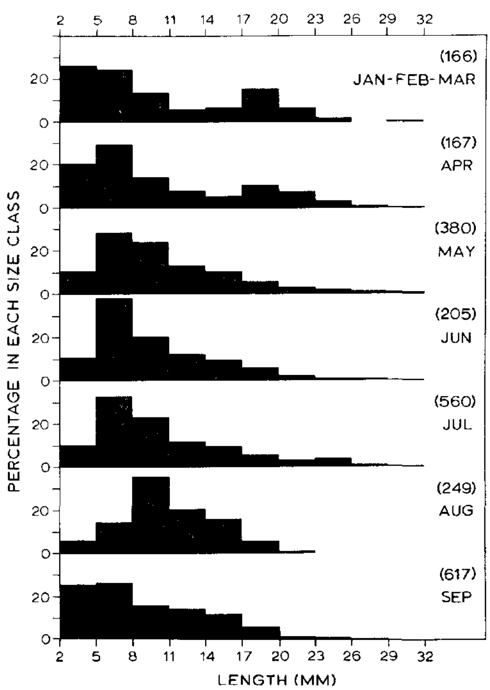


FIGURE 5. Length-frequency histograms for *Hexagenia limbata*. Numbers in parentheses represent sample sizes.

Population-age structure is shown in figure 5. Small nymphs (2–5 mm) were present throughout the study, and final-instar nymphs occurred in all months except August. If it is assumed that growth occurs at temperatures above 10° C, then the bimodal population of nymphs present from January through April is indicative of a two-year life cycle for at least a portion of the population. Nymphs hatching from eggs deposited in May, June, or early July would probably undergo sufficient growth to mature in a single year. Similar observations have been reported by Swanson (1967) for a *Hexagenia* population located on the boundary between South Dakota and Nebraska at a latitude of 43°00′N. In contrast, Oxford, Ohio is at a latitude of 39°30′N.

Systematic studies of emergence were not made during late spring and summer, but observations of shed exuviae did indicate that emergence occurred continuously from 1 May through 18 September. The highest numbers of individuals emerged in June and in mid-August. This long emergence period is consistent with the findings of Peters and Warren (1966) in northwestern Arkansas (latitude = 36°00′N).

## DISCUSSION

Throughout the spring and summer months, mayfly nymphs inhabit all substrate types in varying numbers (86–1032/m$^2$). The variations in density suggest that they prefer certain substrates, though other factors could also account in part for the observed density levels.

For example, low dissolved-oxygen at depths greater than 2.0 m may cause death or migration of nymphs from otherwise suitable substrates. Britt (1955a) observed dead nymphs of *Hexagenia limbata* in areas of Lake Erie where dissolved oxygen was 0.7 mg per liter. Laboratory studies of the same species indicated that death usually occurs at concentrations less than 0.8 cc per liter (=1.2 ppm) (Eriksen, 1968). Hunt (1953) concluded that, at oxygen levels below 1.0 ppm, nymphs become hyperactive and abandon their burrows. In this study, summer stratification resulted in bottom oxygen-levels of less than 0.5 ppm in the center of the pond (fig. 3). The absence of sufficient dissolved oxygen could explain the low numbers of nymphs ($86/m^2$) in the soft, silty substrate found in this area. If locomotor activity occurs at low oxygen concentrations, it is probable that nymphs experiencing gradual depletion of dissolved oxygen over deep sediments would have sufficient time to migrate to inshore areas with adequate oxygen levels. The observed high densities of organisms in the yellow clay substrate near the shoreline could reflect this kind of movement.

However, only certain portions of the yellow clay substrate were habitable during June, July, and August, since evaporation lowered the water level about 15 cm. This resulted in the exposure of roughly 1.0 m of substrate around the shoreline. Under these circumstances, the nymphs inhabiting this area must relocate or perish. Such movements would not appreciably alter the overall distribution pattern in the pond, but would tend to concentrate the organisms in those areas of yellow clay substrate covered with water during the entire summer.

Passive movements could also explain some of the variation in nymphal distribution. During the summer months, when southwest winds prevailed, nymphs were more abundant in the eastern half of the pond. Published observations indicate that local concentrations of *Hexagenia* adults are largely dependent on wind direction, and that emerging subimagoes are carried by wind currents (Lyman, 1944; Fremling, 1970). The same events were observed during emergence in the pond, with even the slightest breeze being of sufficient strength to influence the initial flight of the subimagoes.

Water depth did not appear to influence mayfly distribution to any great extent. This was not surprising, as the littoral zone of the pond was quite shallow and encompassed almost its entire area. In other habitats, *Hexagenia limbata* has been found at depths up to 21 m (Hunt, 1953).

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