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AQUATIC STAGES IN THE DEVELOPMENT OF *HABROPHLEBIA ELDAE* JACOB & SARTORI, 1984 (*)

(*Ephemeroptera Leptophlebiidae*)

INTRODUCTION

Ephemeroptera are entirely aquatic for most of their life cycle. During this period important morphological events, as the formation of both respiratory abdominal gills and wing pads, do occur. Even though the aquatic stages differ from each other in morphological features, changes take place gradually. As a consequence, in spite of the occurrence of well defined subimago and imago instars, the criteria for evaluating stages of larval development are extremely subjective. Many authors have used the term "larvulae", "larvae" and "nymph" without providing any evidence enabling to distinguish them as different stages (see review in CIANCIARA, 1980). In particular the term "nymph" has been utilized to cover either the whole period of aquatic life (EATON, 1983-88; MACAN, 1961; CLIFFORD, 1970), or the period of wing pad growing (LESTAGE, 1921; PLESKOT, 1959), or even stages with fully developed gills (BERTRAND, 1954). More recently seven stages were recognized by HUMPESCH (1979) for *Baetis alpinus* and eight were defined by CIANCIARA (1979) for *Cloeon dipterum*. For both species larvae were defined with respect to larvulae by the presence of fully formed gills, and subsequently staged according to the size of wing pads.

According to CIANCIARA (1980), poorly defined morphological stages of Ephemeroptera have long prevented a comparison of physiological data. In addition, also the influence of environmental factors on mayfly biology, could be better understood through easly distinguishable developmental steps.

The present study was undertaken in order to define the number of developmental stages of *Habrophlebia eldae* Jacob & Sartori, 1984, the revised Italian *H. fusca*. In fact criteria recently utilized in distinguishing stages of *Baetis alpinus* and *Cloeon dipterum* proved inadeguate for a reconstruction of developmental steps of *H. eldae*, as in the latter species wing pad length groups, lead to clustering young and older individuals together.

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Material and Methods

Observations were made on 457 specimens of *Habrophlebia eldae*, collected monthly in seven stations along the Stream Erro (Piedmont) in 1974 - 1975, using a 1 m^2 Surber - type sampler with a 300 μm net. Four samples were taken at each station, equally allocated among rocks, gravel and sand. All original samples were fixed in 6% formaldehyde and each invividual was then washed and stored in an ethyl alcohol-glycerol solution. Morphological details were studied under a Wild M 12 light microscope equipped with a drawing lens. The total length was measured from head to articulation of cerci for each individual.

Results

Criteria proposed for staging *H. eldae*, were initially based on the shape of the rear margins of wing pads (when the wing buds not yet distinguishable), and later on, on the progressive organization of the wings inside the pads. In earlier steps however, also gill morphology was considered, in addition to pad outlines and used as discriminating feature between closely related stages. As in all species of the genus *Habrophlebia*, every gill of *H. eldae* is composed of a lamina divided in two branches each carrying a different number of filaments. Like wing pads and wings, also gill growth is gradual, allowing to subdivide stages into age groups. The youngest individuals of *H. eldae* caught in my samples, have already concluded the larvula stage owing to the presence of gills I to VII.

Nine developmental stages were recognized.

A) Young larva (Plate I, figs. 1a, 1b, 1c; 2a, 2b, 2c): wing buds not visible. At high magnification the outlines of the wing pads are distinguishable on meso- and metathorac. This character together with the different number of filaments carried by the laminae of gills allows to distinguish two sub-degrees of development.

L_I - (Plate I, figs. 1 a, 1 b, 1 c): mesothoracic wing pads with rounded outline at the rear side. Number of filaments on the inner laminae of IV - V gills lower than halfs of those on the exterior one.

L_{II} - (Plate I, figs. 2 a, 2 b, 2 c): mesothoracic wing pads with ondulated rear outline. Number of filaments on the inner laminae of IV - V gills like half of those of the exterior one.

B) Larva (Plate I, figs. 3 - 4): wing buds visible inside the wing pads; two sub-degrees of development were recognized.

L_{III} - (Plate I, fig. 3): mesothoracic wing pads little developed and shaped as two lateral outgrowths envelopping the wing buds. On the mesothorax the rear outline of pads is still rounded and wing buds are barely distinguishable.

L_{IV} - (Plate I, fig. 4): mesothoracic wing pads little developed; the wing buds are well separated from the pad walls and a narrow space between the exterior border of pads is present.

C) Larva - nymph (Plate I, figs. 5 - 6): metathoracic wing pads shaped as lateral processes; the occurrence of veins allows to recognize two sub-degrees of development.

$L\text{-}N_I$ - (Plate I, fig. 5): mesothoracic wing pads more readily visible than in L_{IV}; the metathoracic ones scarcely visible. Also in these latter a narrow space is present between wings and pad walls. No veins are visible.

L-N_{II} - (Plate I, fig. 6): veins readily visible on the mesothoracic wings but absent from the metathoracic ones.

D) Nymph (Plate I, figs. 7, 8, 9): both wings with readily visible veins. The degree of folding of both meso- and metathoracic wings inside the wing pads differentiates three stages.

N_I - (Plate I, fig. 7): meso- and metathoracic wings unfolded with well developed venations.

N_{II} - (Plate I, fig. 8): mesothoracic wings are well developed and owing to their growth inside the wing pads, appear particularly folded; the metathoracic ones are unfolded.

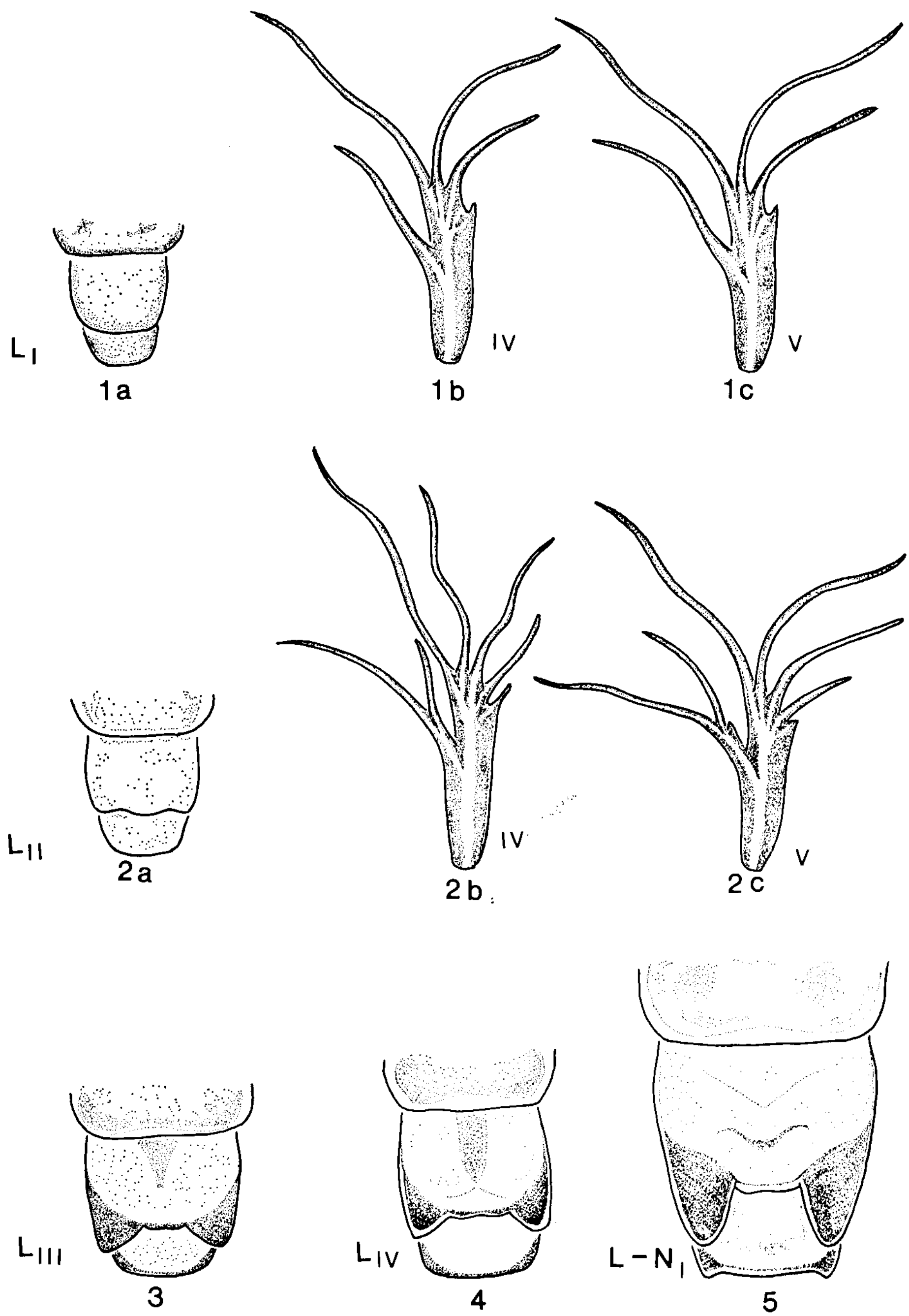
N_{III} - (Plate I, fig. 9): metathoracic wings complete their growth and both wings are strongly folded. The darkness of wing pads preludes to metamorphosis into the subimago.

Measures taken on all specimens classified according to their developmental stages, are reported in Table I.

TABLE I - Dimensions of *H. eldae* in each stage (L_I - N_{III})

STAGE	NUMBER OF INDIVIDUALS	BODY LENGTH (mm) minimum	average	maximum
L_I	32	1,0	1,5	1,8
L_{II}	16	1,4	1,8	2,2
L_{III}	8	2,0	2,3	2,5
L_{IV}	34	1,8	2,3	3,2
L-N_I	67	2,3	2,8	3,9
L-N_{II}	125	2,1	3,7	5,8
N_I	125	3,2	5,0	7,4
N_{II}	13	4,3	5,9	6,8
N_{III}	37	4,3	5,9	7,1

Ephemeropthera association and seasonal fluctuations in abundance were previously recorded in sampling sectors chosen along the Stream Erro (GAINO, 1978). The upper course of this stream derives from rich springs and represents a zone where Ephemeroptera taxocenes are preserved from sewage outlet. Special attention was given to this stream sector chosen as sampling area, at 700 m above sea level, where seasonal changes in the number and structure of Ephemeropteran communities better reflect the life cycle of their components. A total of 201 specimens of *H. eldae*, representing about half of all individuals recorded along the whole stream, were studied. In the montly samples, the average number of specimens in 1 m^2 at different developmental stages (L_I - N_{III}) was calculated (fig. 10). *H. eldae* occurs in maximum number during summer months, with a peak of maximal density in June. Number decreases in September and no specimens were collected during the following months until February. Specimens



L I
1a
IV
1b
V
1c
L II
2a
IV
2b
V
2c
L III
3
L IV
4
L - N I
5

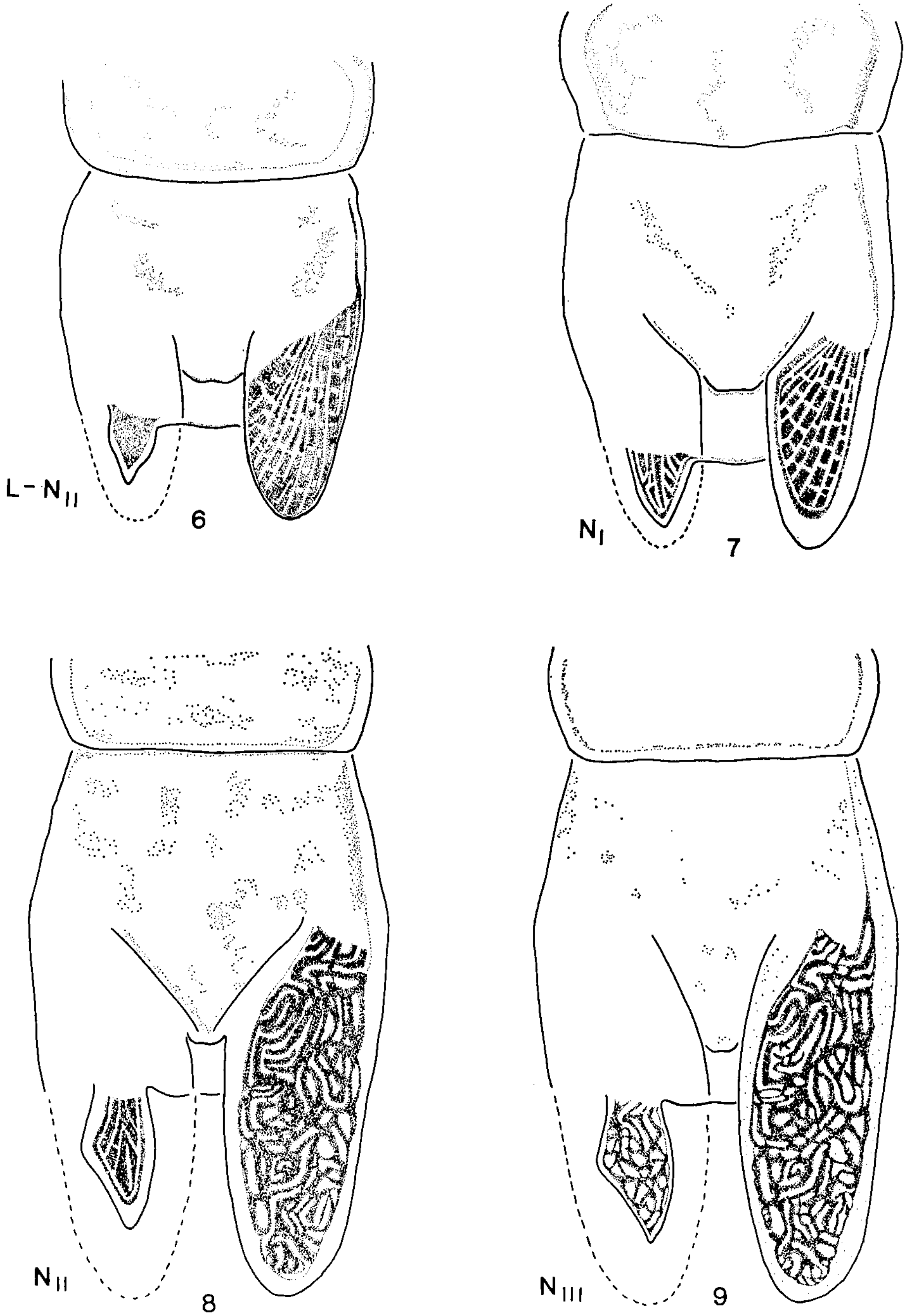


PLATE I - Figs. 1 - 9: Aquatic stages of *Habrophlebia eldae*: gradual wing pads and wing organization as criteria to distinguish young larvae (L_I - L_{II}), larvae (L_{III} - L_{IV}), larva - nymphs (L-N_I - L-N_{II}) and nymphs (N_I - N_{III}). For L_I - L_{II} stages, the different morphology of IV - V left gills is pointed out.

at the stages of young larva, larva and larva - nymph appear still in August; early stages were collected in June together with those precurring the nymphal one. The following rapid growth provides the pre-emergent nymphs found in July.

Discussion

The morphological study carried out on *Habrophlebia eldae*, allowed to distinguish different steps during the aquatic life of this mayfly on the basis of both wing pad shape, and organization of the wings inside pads.

In the early larval period wing buds are not visible and the different shape of mesothoracic wing pads allowed to distinguish two age groups. Larval time is marked by wings buds whose organization inside the metathoracic wing pads allows to identify two main age groups. The larva - nymphal period is characterized by the lateral outgrowth of the metathoracic wing pads; wing vein organization distinguishes two age groups. The late nymphal period is marked by the well developed veins and complete folding of the wings, owing to their growth inside the wing pads. These events significantly differenciate three age groups.

Comparing all the identified stages, morphological processes involved in the organisation of the wings seem coordinated with the gradual growth of the wing on the abdomen. On the contrary there was no constant positive relationship between wing pad length and developmental stages. In fact, testing this parameter in order to classify specimens of *H. eldae* into age groups resulted in clustering together individuals of different ages. As a conseguence the development of wing pads did not allow to distinguish in *H. eldae* different aquatic stages. It is important to note that in natural conditions growth patterns may be influenced by environmental variables (Bertrand, 1954; Macan, 1961) and that according to Clifford (1970) similar body sizes may correspond to different stages of development. During the aquatic cycle of *H. eldae* body dimensions are in agreement with such observations, because differences in length were not significant in conforming size classes.

The morphogenetic processes involved in the growth of meso- and metathoracic wing pads are similar, but the first wings, owing to their earlier budding, complete their organization before the second ones. As a consequence, even though mesothoracic wing pads are well developed, nymphs cannot undergo metamorphosis into subimago until the metathoracic ones are full formed.

As is well known, during their postembryonic life, Ephemeroptera increase in body size and differentiate through the process of moulting that allows passage from an instar to the next one. Instar determination is particularly difficult and also Palmén's body method seems to have a limited use in screening field-collected specimens (see Fink, 1980 for a review). Therefore number of instars was calculated on reared individuals (Brittain, 1976; Cianciara, 1980; Degrange, 1959; Illies & Masteller, 1977: Pescador & Peters, 1974) and changes in morphology were designated as broad stages. In fact, morphological events occuring during the long aquatic life cycle of mayflies are not exactly correlated with the number of moults. As a consequence, the gradual organization of both wing pads and wings provides for *H. eldae* a readily evident character, allowing

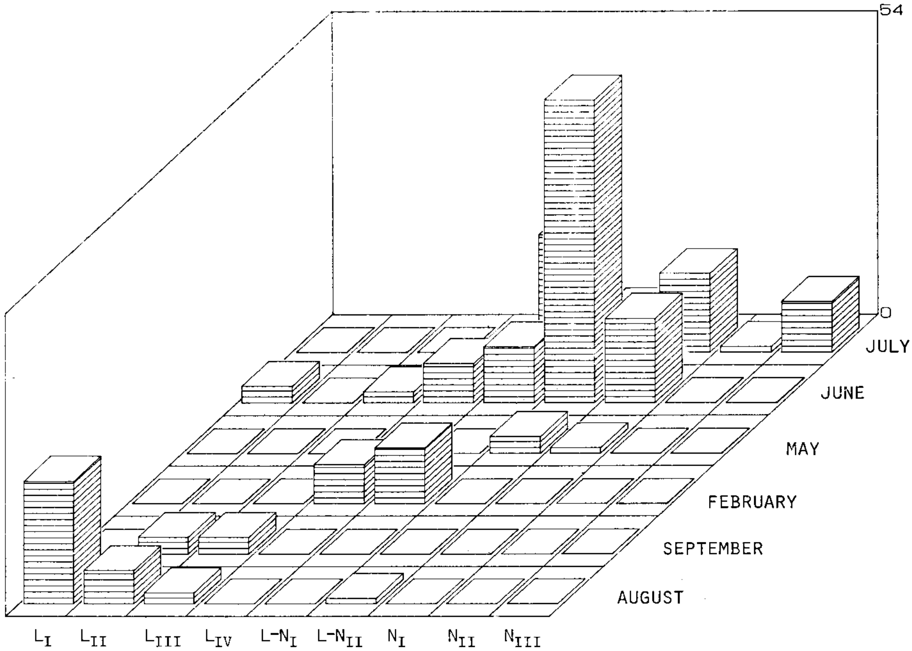


Fig. 10 - Number of specimens of *Habrophlebia eldae* at different developmental stages (L_I - N_{III}) monthly collected in the tract of the Stream Erro arising from springs.

to distinguish nine different postlarvula stages, each of them probably passing through a larger number of moults.

Criteria here illustrated represent a new approach to the reconstruction of developmental degrees and could possibly be taken in some account to estimate the aquatic stages of other species of Ephemeroptera.

As for the life cycle of *H. eldae*, the low frequency of identified stages and the absence of this species in several monthly samples, allow to put forward some considerations. Like other species of *Habrophlebia*, also *H. eldae* seems to be a summer monovoltine species (GRANDI, 1960). In the examined sector of the Stream Erro, emergence probably occurs in July, as indicated by the finding of pre-emergent forms in this month. Young larval stages collected in August might derive from quickly etched eggs. In fact, according to LANDA (1968), the eggs of another summer species, *H. fusca* (LANDA, 1957; MACAN, 1961), etch in 18 days only and the following diapause at the stage of "older larvae" allows them to overwinter. Also in *H. eldae* the long-living larvae might grow slowly and perhaps overwinter in diapause.

The finding of young larval stages in June needs some additional discussion, owing to the lack of adults inside box emergence traps during the previous months. It could be hypothised that these developmental stages derive from delayed egg etching. Eggs might overwinter in diapause owing to drop in water temperature and shortening of the day. As it is well known, these parameters can influence both the growth and emergence (BRITTAIN, 1976; CIANCIARA, 1980; LAVANDIER, 1981; MACAN, 1957; MACAN & MAUDSLEY, 1966; PLESKOT, 1961). It is worth stressing that CAMPBELL (1980) evidentiated diurnal variations in the activity of mayflies. The absence of specimens of *H. eldae* for five months, could be related to a light intensity factor controlling the larval movement.

Delayed egg etching could represent an adaptative device ensuring species survival under unfavorable environmental conditions. The long-living winter larval stages and the new etched ones, would then live together. This peculiar life cycle entails different growth rates in specimens of *H. eldae* and as a consequence wing-pad size does not allow to reconstruct stages of development in this species.

Scanning electron microscopy analysis carried out on the egg shell of *H. eldae* showed a chorionic pattern of ridges arising from the surface (GAINO & MAZZINI, 1984). Each ridge is composed of columns separated from each other by chambers containing mucous material (MAZZINI & GAINO, 1985). Mucous envelops represent an important adhesive device for the eggs, allowing their adhesion to the substrate. Mucus is particularly important when eggs lack the more differentiated chorionic attachment structures frequently observed on shell surface in Ephemeroptera (GAINO & MAZZINI, 1987; GAINO *et al.*, 1987). Egg mucous coat of *H. eldae*, while preventing egg drift, allows survival during winter months. Egg etching could trigger inception through higher temperature (BRITTAIN, 1975). It is worth stressing that a univoltine cycle has been reported already for Italian specimens of *H. fusca* (probably *H. eldae*; see BELFIORE & GAINO, 1984) by MARCHETTI *et al.* (1967), together with an egg diapause lasting eight months.

In conclusion, the life cycles of the Ephemeroptera are strongly related to environmental conditions which can influence development, growth rate and emergence. As a consequence, species adopt different strategies according to different conditions (CIANCIARA, 1979; BRITTAIN, 1974; 1980). The life cycle of *H. eldae*, involving both long-living overwintering larvae and egg diapause, confirm that the Ephemeroptera, owing to their gradual metamorphosis, have retained a high plasticity and a high adaptive potential.

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ABSTRACT

The examination of 457 specimens of *Habrophlebia eldae* Jacob & Sartori, 1984, collected monthly along a year in the Stream Erro (Piedmont, Italy), allowed to distinguish nine postlarvula stages.

Meso- and metathoracic wing pad shape, wing bud organization and gradual formation of wings were applied as morphological criteria in evaluating the degrees of development in this species.

A sector of the Stream Erro preserved by sewage outlet, has been chosen to study the occurrence of specimens at different developmental stages in order to evidentiate the life cycle of *H. eldae.* Data suggest a generation per year with both larval and egg overwinter. This strategy might allow survival of this species also in non ideal habitat conditions.

RIASSUNTO

Stadi acquatici dello sviluppo di Habrophlebia eldae *Jacob & Sartori, 1984* (Ephemeroptera Leptophlebiidae).

L'esame di 457 esemplari di *Habrophlebia eldae* Jacob & Sartori, 1984, raccolti mensilmente e per un anno nel Torrente Erro (Piemonte), ha permesso di distinguere nove stadi postlarvula.

La forma delle pteroteche meso- e metatoraciche, l'organizzazione degli abbozzi alari e la graduale formazione delle ali sono state utilizzate come criteri morfologici nel valutare il grado di sviluppo della specie.

Una zona del Torrente Erro preservata da carichi inquinanti fu scelta per studiare la presenza di esemplari a differenti stadi di sviluppo allo scopo di evidenziare il ciclo di *H. eldae.* I dati suggeriscono un ciclo monovoltino con uova e larve che superano l'inverno. Questa strategia potrebbe permettere a questa specie di sopravvivere anche in condizioni ambientali non ottimali.

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