PALINGENIA LONGICAUDA (EPHEMEROPTERA, PALINGENIIDAE): FROM MATING TO THE LARVULAE STAGE

PETER LANDOLT[1], MICHEL SARTORI[2] & DENISE STUDEMANN[1]

[1]Entomological Department, Institute of Zoology, University of Fribourg, Pérolles, CH-1700 Fribourg, Switzerland
[2]Musée de Zoologie, Palais de Rumine, C.P. 448, CH-1000 Lausanne 17, Switzerland

The effect of mating attraction on males was studied when visual perception preceded a chemical or a tactile one. The compensation flight behaviour of females is described. Under the presented experimental conditions, *Palingenia* have no embryonal diapause. Nearly all fertilized and about half of the non-fertilized eggs start embryonic development. The development differs in length, 39 days for the fertilized versus 46 days for the parthenogenetically developing eggs. Stages of the embryonic development are shown.

INTRODUCTION

Extended biological studies on *Palingenia longicauda* (OLIVIER) were carried out by RUSSEV (1987) in the Danube river where no Palingenia population had been observed since 1974 (RUSSEV, 1992). The physical and chemical parameters of the water and description of the habitat of *Palingenia* such as granulometry of the sediment in the Tisza were described at the VIIth Conference on Ephemeroptera 1992 in Maine by SARTORI *et al.* (1995) as well as feeding habits, reproduction behaviour of males and females and the egg structure by LANDOLT *et al.* (1995). In the present study, we are concerned with the reproduction behaviour and embryonic development of *P. longicauda.*

MATERIAL AND METHODS

The observations and following investigations originate from three field trips in 1990, 1991 and 1993 to the Tisza river at km 710 in the Hungarian village of Nagyar, near the Ukrainian border. The locality on the Tisza river and the distribution of *Palingenia* are described in SARTORI *et al.* (1995) and LANDOLT *et al.* (1995). The body parts of the females presented to males for mating attraction were attached to thin transparent strings and then allowed to drift on the surface of the river.

The number of females for the flight pattern of the females was estimated from video pictures. Females caught for the respective investigations (egg content, energetic reserves) were captured from a boat in mid-stream. The sink velocity of the eggs was determined by 12 measurements in a 1,5 m long and 10 cm wide cylindrical glass tube. The river depth was measured by a diver.

The non-fertilized eggs were extracted from mature hatching nymphs. The fertilized eggs were extracted from multiple-mated females. The proportions of the eggs starting embryonic development were determined by an aliquot of 100 observed eggs taken from an estimated total of 30'000 incubated eggs. The eggs were incubated near river temperature at 20°C. The pictures of developmental stages of *Palingenia longicauda* and freshly hatched larvulae were taken with a Laborlux S Leitz microscope. The behaviour of the larvulae was observed by video under laboratory conditions.

RESULTS

Mating attraction on males

The attraction of males to lightly coloured objects, already mentioned at the VII International Conference on Ephemeroptera in Maine 1992, and the observation of T. Fink (pers. communication) of sensory structures on our freshly fixed material 1990 led us to verify these hypotheses. The visual perception was tested by presenting males with objects of

Table 1. Mating attraction. Experimental conditions are described in Materiel and methods.

Males presented with	Degree of attraction
Hatching male nymph	slight (±)
Hatching female nymph	present (+)
Female complete body	very high (++++)
head + thorax	moderate (++)
thorax + wings	moderate (++)
thorax + abdomen	slight (±)
head	no (–)
thorax	no (–)
abdomen	no (–)
Female washed with pentane	very high (++++)
Females kept in an opaque perforated box swimming on the water	no (–)
White cigarette filter	present (+)

Ephemeroptera & Plecoptera: Biology-Ecology-Systematics.

different colours and sizes. To check a possible role of pheromones, the males were exposed to hidden females, and females washed in pentane to dissolve possible pheromones.

Freshly hatched females were naturally surrounded on the water surface by up to 20 males forming a flower-like structure (in Hungary named «Tisza virag»). Table 1 summarizes the results of different tests. They support the hypothesis that the first perception of the females by the males is visual. Light coloration of the presented objects e.g. the female nymphs and cigarette filters attracted the males. The larger the female body parts presented to the males, the higher the degree of attraction, and there is no attraction when body segments are too small. Hidden females have no attraction for males whereas females washed with pentane are still highly attractive. Non-volatile chemical substances might have an effect therefore only after close physical contact between male and female. Tactile or chemical stimulation only happens after visual perception.

Compensation flight of the females and drift of the eggs

Fig. 1 shows the behaviour of the females in the compensation flight which is observed only over the water surface in a compact formation. Number 1 indicates the majority (over 90%) of the females that fly upstream up to 4 km in the so-called compensation flight. They have eggs in the abdomen and the energetic reserves are high (Ruffieux, pers. communication). A minor part (about 10%) of the females fly downstream (number 2). Their egg content in the abdomen and energetic reserves are comparable to the females flying upstream. Some females show a particular behaviour (number 3). They fall down on the water surface and start up again several times. These examined females have no eggs in the abdomen and their energetic reserves are nearly depleted (Ruffieux, pers. communication). The presence of different amounts of eggs in the abdomen of flying females and the facility in releasing them when these females are captured, as well as the absence of eggs of exhausted females seem to indicate that the females drop eggs during their flight. Females which fall on the water surface often die there.

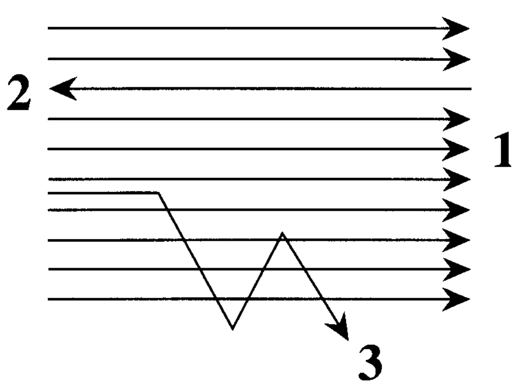


Fig. 1. Flight pattern of the females. Experimental conditions are described in Materiel and methods.

Calculation of the drift of laid eggs was made using the following parameters. The mean sink velocity of the laid eggs amounts to 0,066 m · sec^{-1}, current speed of the river at km 710 is about 1,2 m · sec^{-1} and the mean river depth measures 7,5 m. The eggs reach the river bed after about 19 minutes which corresponds to a drift distance of about 1350 m. The majority of females compensate for the drift of the eggs by flying upstream. The compensation flight distance is at least twice as long as the estimated drift of the eggs.

Embryonic development

Fertilized and non-fertilized eggs are capable of embryonic development (Fig. 2). Nearly all (98%) of the fertilized eggs start their synchronized development. 12% of the eggs develop after 4 days and about 100% after 11 days. The embryonic development lasts 39 days (observed on about 30 larvulae). Only half (52%) of the unfertilized eggs start their developement parthenogenically. The development is delayed and not synchronized (4% of eggs develop after 11 days, about 50% after 30 days). The time of the embryonic development amounts to 46 days (observed on about 30 larvulae).

Figs 3 to 9 show different stages of embryonal development in the egg shell. The incubation temperature at near river temperature leads to

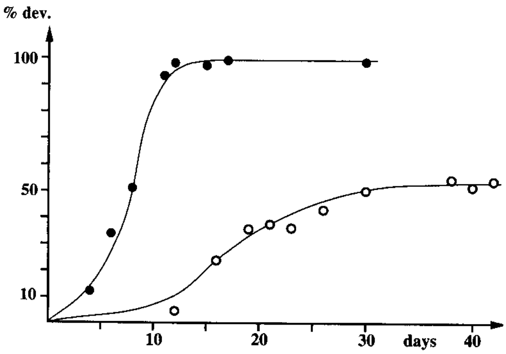
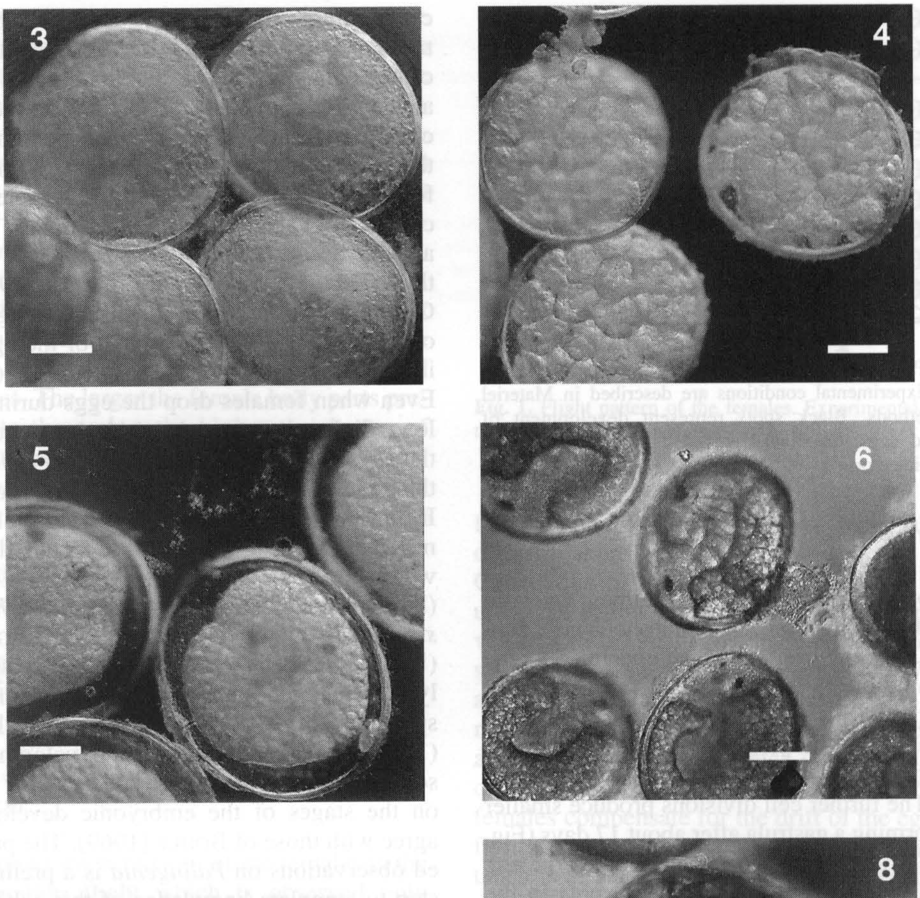


Fig. 2. Proportions of the eggs starting embryonic development. Experimental conditions are described in Materiel and methods. Black spots represent development of fertilized eggs, the blanks represent parthenogenetically developing eggs.

the approximate development times given in days after incubation. The amorphous yolk substance is enclosed in the chorion at the beginning of development apart from a limited lighter area (Fig. 3). After some days, large yolk cells appear. The photograph of Fig. 4 at the stage of about 64 cells is taken after about 10 days. The further cell divisions produce smaller cells forming a gastrula after about 17 days (Fig. 5). Fig. 6 shows the germ band lying on the egg shell after 26 days. The dark eye spots in the cephalic lobe region are well differentiated and the thoracic and abdominal segmentation developed (Fig. 7). The eclosion of the larvulae occurs when the chorion half opens (Figs 8, 9). The length of a freshly hatched larvulae (Figs 10, 11) amounts to about 1200 µm.
Behaviour observations of the larvulae by video pictures show the typical movements of the mouth parts and forelegs as in older filtering larvae. The peristaltism of the intestine with food particles inside is clearly visible, too. The larvulae immediately try to dig a hole in the clay substrate.

DISCUSSION

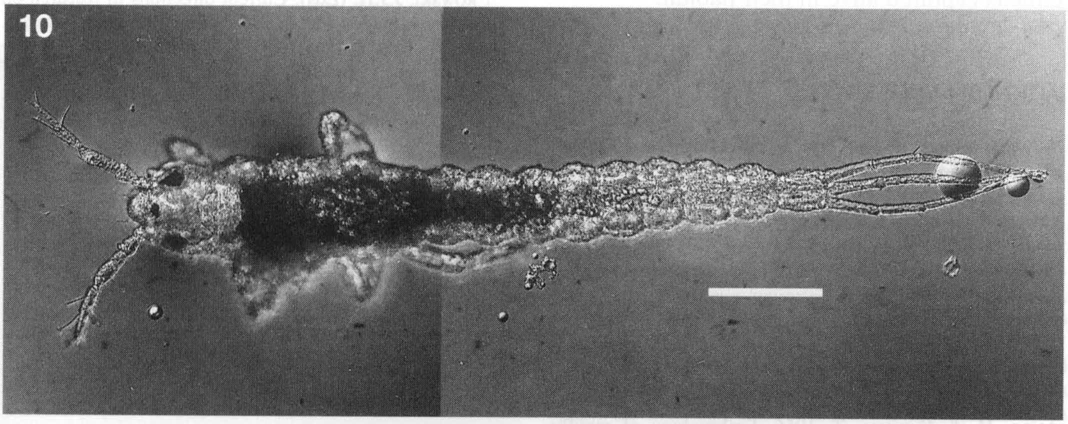
The observation of putative sensory structures on the anterior wing veins of males of *P. longicauda* and *P. fuliginosa* (Fink & Andricovics, 1997) could explain the role of chemical substances acting from a short distance. As we noted 1993 (Landolt *et al.*, 1995) on the behaviour of the successful male before and during copulation, responses on tactile or chemical stimuli happen. Once positioned in the water under the female, the male holds the fore wings of the female with his forelegs and curves his abdomen to the female genital apparatus and rubs his head frantically on the thorax of the female (Landolt *et al.*, 1995).
Our observations on compensation flight and oviposition behaviour and drift of the eggs are in agreement with those of Russev (1987). Even when females drop the eggs during their long flight upstream, the drift downstream of the eggs is compensated and in such a way that the population is safeguarded in a river locality. Brittain (1982) in his review on the biology of mayflies mentionned the absence of detailed work on egg development. Ando & Kawana (1956) studied the embryology of *Ephemera strigata* by external observations. Degrange (1960) described the eggs, eclosion and specially parthenogenesis of many Ephemeroptera species. Bohle (1969) and Tsui & Peters (1974) described embryonic development of some other Ephemeroptera species. Our results on the stages of the embryonic development agree with those of Bohle (1969). The presented observations on *Palingenia* is a preliminary step to complete knowledge of this subject. In *Palingenia*, nearly all fertilized eggs start embryonic development, whereas only half of the non-fertilized eggs start parthenogenetical development. Fertilization seems to stimulate and coordinate the beginning of development compared with the development of non-fertilized eggs. Contrary to the conclusions of Brittain (1982), the different number of males and females in *Palingenia*, parthenogenesis could have an importance in the population dynamics. Determination of the sex by analysis of the caryotypes in males and females will clear the role of fertilization. Fertilized eggs perhaps produce males and parthenogenetically developing eggs females. *Palingenia* do not undertake an embryonic diapause. Under the presented experimental conditions, the feeding behaviour of the larvulae suggests that the newly hatched larvulae have only few embryonal nutritional reserves and they are obliged to feed immediately after hatching.

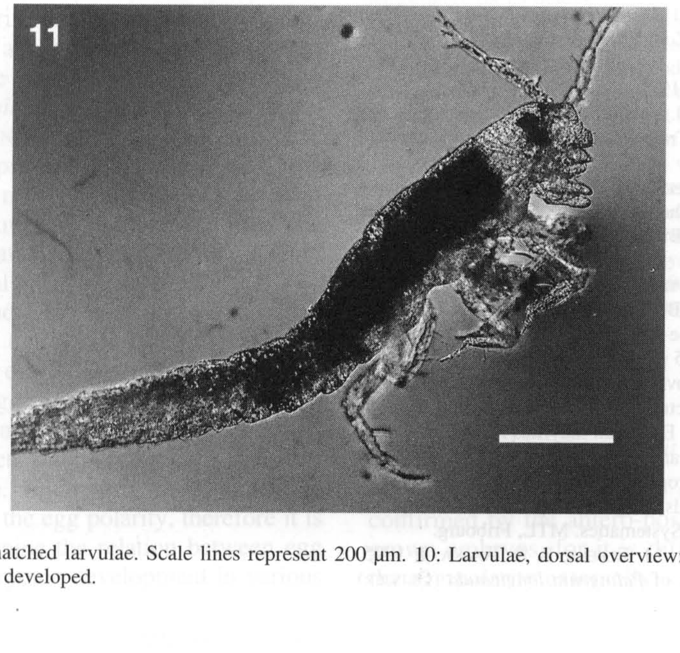


Figs 3 - 9. Developmental stages of *Palingenia longicauda*. Scale lines represent 100 µm. 3: Eggs starting embryonic development; 4: Stage of 64 cells; 5: Stage of gastrula; 6: Embryo: appearance of the eyes (black marks); 7: Embryo in detail: segmentation of different body parts; 8: Hatching larvulae; 9: Empty egg shells.

ELLIOTT & HUMPESCH (1983) and ELLIOTT *et al.* (1988) summarized what was known about egg hatching, effect of temperature on the egg development, hatching success and length of development. Complementary studies will clear up external influences such as temperature on the development, the proportion of hatching larvulae as well as a possible diapause later in the larvula stage for *Palingenia*.

Sex ratio and mating ratio seem to be contradictory. First, the observed sex ratio of over 300 mature male and female nymphs is about 1:1, second, the observed number of flying males is lower than that of females and third, there are fewer mated females than unmated ones. These observations can be explained by the following hypotheses. First, the males are more likely to be preyed on by fish because they hatch about one hour earlier than the females and are therefore more exposed to fish than females. The fish have finished feeding when the female nymphs hatch.





Figs 10, 11. Freshly hatched larvulae. Scale lines represent 200 µm. 10: Larvulae, dorsal overview; 11: Larvulae, lateral view: mouthparts well developed.

Second, at the end of the daily emergence period, the remaining males are exhausted and not able to fertilize the great number of emerging females. Third, only half of the non-fertilized eggs start embryonic development and not all of these achieve successful embryonic development.

Many questions about the development of *Palingenia* arose after three expeditions. Further investigations on the determination of sex, the proportion of successful embryonic development and external influences on the length of development are only possible on specimens captured alive in their habitat.

ACKNOWLEDGEMENTS

We wish to thank Prof. Gerolf Lampel for his support and remarks concerning the manuscript. We are indebted to Laurence Ruffieux for assistance in the field and for energetic content investigations, Shandor Szilagy, Nagyar, for his hospitality and Dr. Tom Fink for interesting discussions. Sheila Werhonig-Watson and Hubert Gachoud are thanked for their technical assistance.

REFERENCES

ANDO, H. & KAWANA, T. 1956. Embryology of mayfly (*Ephemera strigata* EATON) as studied by external observation. Kontyû 24: 224-232.

BOHLE, H.W. 1969. Untersuchungen über die Embryonalentwicklung und die embryonale Diapause bei *Baëtis vernus* CURTIS und *Baëtis rhodani* (PICTET) (Baëtidae, Ephemeroptera): Zool. Jb. Anat. 86: 493-575.

BRITTAIN, J.E. 1982. Biology of Mayflies. Ann. Rev. Entomol. 27: 119-197.

DEGRANGE, CH. 1960. Recherche sur la reproduction des Ephéméroptères. Trav. Lab. Hydrobiol. Piscic. Grenoble 51: 7-193.

ELLIOTT, J.M. & HUMPESCH, U.H. 1983. A Key to the adults of the British Ephemeroptera. Scientific Publications of the Freshwater Biological Association Ambleside 47, 101 p.

ELLIOTT, J.M., HUMPESCH, U.H. & MACAN, T.T. 1988. Larvae of the British Ephemeroptera. Scientific Publications of the Freshwater Biological Association Ambleside 49, 145 p.

FINK, T.J. & ANDRICOVICS, S. 1997. The presumed role of wing sensory structures in the unique mating behaviour of the endangered European mayflies *Palingenia longicauda* (OLIVIER) and *Palingenia fuliginosa* (GEORGI) (Insecta, Ephemeroptera). 326-331. *In:* LANDOLT, P. & SARTORI, M. (Eds). Ephemeroptera & Plecoptera: Biology-Ecology Systematics. MTL, Fribourg.

LANDOLT, P., SARTORI, M. ELPERS, E. & TOMKA, I. 1995. Biological studies of *Palingenia longicauda* (OLIVIER) (Ephemeroptera: Palingeniidae) in one of its last European refuges-Feeding habits, ethological observations and egg structure. 271-281. *In:* CORKUM, L.D. & CIBOROWSKI, J.J.H. (Eds). Current directions in research on Ephemeroptera. Canadian Scholar's Press Inc., Toronto.

RUSSEV, B. 1987. Ecology, life history and distribution of *Palingenia longicauda* (OLIVIER) Ephemeroptera. Tijd. Entomol. 130: 109-127.

RUSSEV, B. 1992. Threatened species of Ephemeroptera (Insects) from Bulgaria. Lauterbornia 9: 13-17.

SARTORI, M., LANDOLT, P., LUBINI, V. & RUFFIEUX, L. 1995. Biological studies of *Palingenia longicauda* (OLIVIER) (Ephemeroptera: Palingeniidae) in one of its last European refuges-Abiotic characteristics and description of the habitat. 263-272. *In:* CORKUM, L.D. & CIBOROWSKI, J.J.H. (Eds). Current directions in research on Ephemeroptera. Canadian Scholar's Press Inc., Toronto.

TSUI, PH. T. & PETERS, W. L. 1974. Embryonic development, early instar morphology, and behaviour of *Tortupus incertus* (Ephemeroptera: Polymitarcyidae). Fla. Entomol. 57: 349-356.