
21 **Biological studies of *Palingenia longicauda* (Olivier) (Ephemeroptera: Palingeniidae) in one of its last European refuges — Feeding habits, ethological observations and egg structure**

Peter Landolt¹, Michel Sartori², Christian Elpers¹ and Ivan Tomka¹

¹University of Fribourg, Institute of Zoology, Department of Entomology, CH-1700 Fribourg, Switzerland and ²Museum of Zoology, P.O. Box 448, CH-1000 Lausanne 17, Switzerland

*Analysis of the gut contents of *Palingenia longicauda* (Olivier) shows the composition of the filtered food: over 90 per cent was particulate organic material, about 5 per cent was algae with some bacteria and small clay granules. The behaviour of the larvae from emergence to the winged stages, the moult of the male subimagos and their following patrolling flight are described. Observations of the mating behaviour and the compensation flight of the females are included. The egg structure is shown by scanning electron micrographs.*

Introduction

Recently, Russev (1987) gave an overview of observations of *Palingenia longicauda* (Olivier) and described his investigations on the ecology and the life history of populations in the Danube River. The formation of flower-like arrangements on the water surface (Tisza virag) of up to 30 specimens of *P. longicauda* and the sound of the flying animals fascinate scientists and fishermen. The extraordinary short, well-synchronized emergence period after a three-year maturation period aroused our curiosity. We present some supplementary results and particular observations but also questions that arose during two field trips to Hungary.

Materials and Methods

One- to three-year-old larvae and specimens of the winged stages were captured at the Tisza River in Nagyar (Hungary) near the Ukrainian border at the end of June

1990 and at the beginning of July 1991 (Sartori et al. 1994). The larvae were taken with an apparatus used by fishermen, a metallic cylindrical tube (length 55 cm and diameter 24 cm) fixed on an eight-metre-long wooden handle. The animals were preserved in alcohol (75 per cent) or in ten per cent formaldehyde buffered with MgCO_3 .

The mouthparts were prepared for the scanning electron microscopy (SEM) as described in Elpers and Tomka (1992). The gut contents were extracted by dissection from specimens preserved in formaldehyde, then dehydrated by successively placing in alcohol (100 per cent), a 1:1 mixture of acetone:alcohol (100 per cent) and acetone. The samples were subsequently filtered on a micropore filter with a pore size of 0.45 μm and immediately air-dried. The eggs released from females were stored in formaldehyde. Preparation of samples for scanning electron microscope (SEM) observations were carried out as described in Studemann et al. (1987).

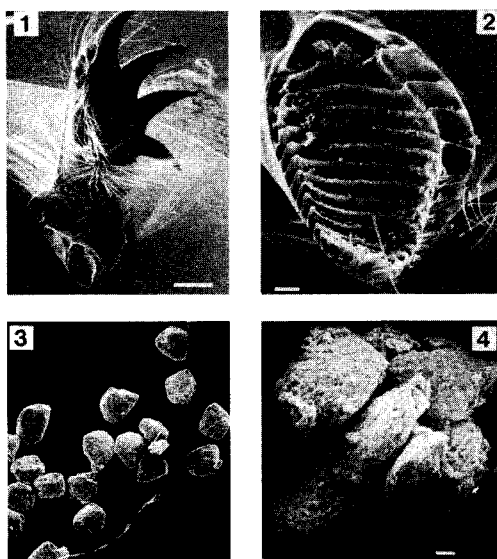
Results

Feeding

Palingenia longicauda possesses mandibles with large, chitinized lateral incisors (Fig. 1). The inner molar region of the mandibles is used to remove the water from filtered food particles. The water flows off through the parallel grooves (Fig. 2) by the pressing movements of the mandibles. The food particles form compact, flattened, discoidal food pellets (Fig. 3). When the pellets are about 50 μm thick, they pass through the oesophagus to the intestine. The size of the food pellets is about 283 x 227 x 50 μm (Fig. 4). The gut contents of one- or two-year-old larvae are characterized by a large number of these food pellets. The estimated volume of one food pellet amounts to about 0.003 mm^3 . The parallel structures on the surface of the pellets (Fig. 3) are impressions of the ridges on the molar part of the mandible.

The composition of the food pellets in the gut consisted of particulate organic material (POM) (Fig. 5), small clay particles (Fig. 6), algae and bacteria. The dominant component (over 90 per cent) of the pellet is POM. There are no preferential parts of animals or plants visible. The size of the filtered particles suggests the manner of filtering: too big and too small particles are lost, only the appropriately sized particles (Fig. 5) are caught by the filter apparatus of the larvae. The small stone grains (Fig. 6) eventually break open some of the algae. The proportion of the algae was estimated at about 5 per cent of the filtered material. The structure of most of the diatoms is not changed during ingestion (Figs. 7 and 8). Bacteria are sometimes visible on smooth surfaces (Fig. 10). The guts of one- or two-year-old larvae are full, whereas the guts of the mature nymphs are already empty and difficult to observe.

Figures 1-4. Mandible and nutritional pellets. 1) right mandible of *P. longicauda*, apical view; 2) molar region of the right mandible, top view; 3,4) nutritional pellets extracted from the gut of *P. longicauda*; scale lines: 1, 3: 500 μm ; 2,4: 50 μm .



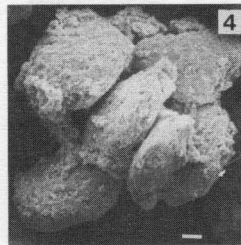
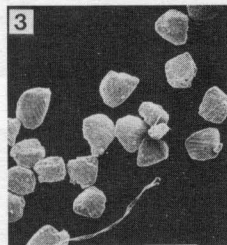
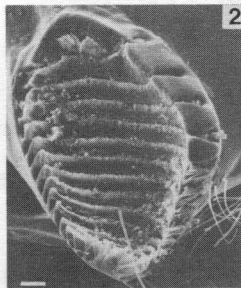
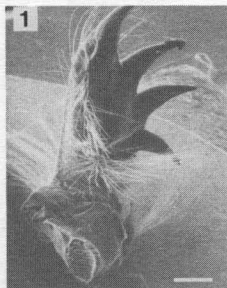
Ethological Observations

The larvae dig their U-shaped burrows in the wall of the outer curve of the river loop (Sartori *et al.* 1994) in a water depth down to five m. One gallery is formed by two parallel, horizontal tubes of about 17 cm in length, connected to each other by a semi-circular tube. The opening of the parallel tubes is slightly orientated towards the flow direction of the river.

The period of the mass emergence takes several days, normally at the end of June/beginning of July. Table 1 shows the run of events during an emergence event. At the beginning, at about 7:30 p.m. (time of sunset), only males hatch. The mature nymphs swim actively to the water surface up to ten m from the river bank and emerge within a few seconds. Many of them are eaten by fish (e.g., sturgeon).

The male subimagos fly immediately to the river bank and attach themselves to the nearest support (on the ground, on leaves). The subimagos grip the leaves with the middle and hind legs. The onset of moulting can be identified by the dull colouration on the abdomen accompanied by a rhythmic peristaltic muscle

Figures 1-4. Mandible and nutritional pellets. 1) right mandible of *P. longicauda*, apical view; 2) molar region of the right mandible, top view; 3,4) nutritional pellets extracted from the gut of *P. longicauda*; scale lines: 1, 3: 500 μm ; 2,4: 50 μm .



Figures 5-10. Components of the nutritional pellets. 5) particular organic material (POM); 6) a fine clay particle with POM; 7-9) diatoms; 10) bacteria; scale lines: 5 : 10 μ m; 6-10: 5 μ m.

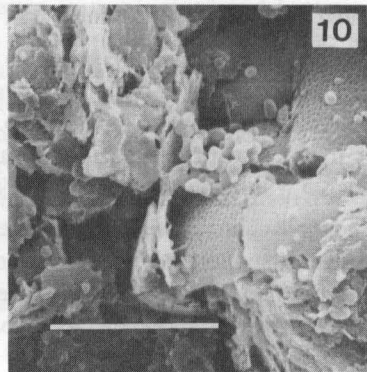
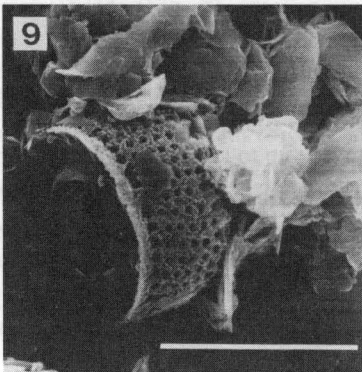
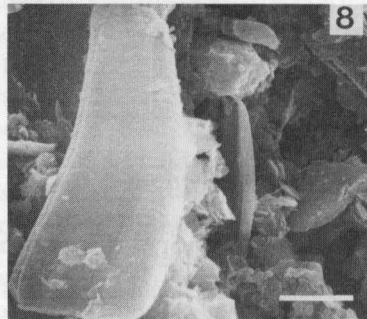
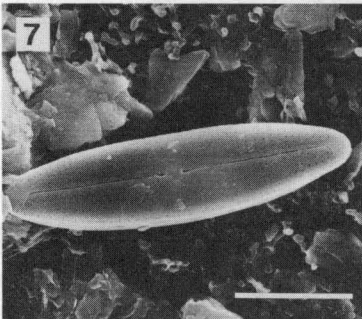
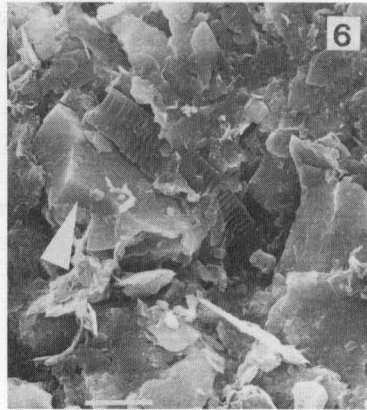
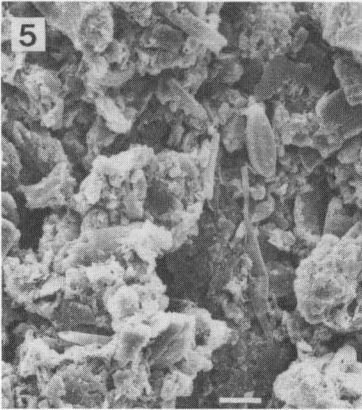


Table 1. Comparison of the reproduction behaviour of males and females of *P. longicauda*.

TIME (minutes)	MALES	FEMALES
	larval stages: 3 years	larval stages: 3 years
0	hatching of the male subimagoes	
0.5	moulting to the male imagoes (on the river bank)	
5	patrolling flight of the males (10 cm over the water)	
35		hatching of the females
		mating
60		compensation flight
	males exhausted	oviposition

contraction. The animals spread their wings and the skin ruptures in the prothorax region. The abdomen and the wings are pulled out of the subimaginal skin. Halfway through the transformation, the specimen tips over backwards, its head hanging downwards. In this position, the moulting process is assisted by gravity, and the rest of the wings and the abdomen are released. With the active fluttering of the wings, the male imago pulls out the caudal filaments and flies back to the river. Moulting to the imaginal stage takes up to five minutes. For the following patrolling flight, the males fly about ten cm over the water surface, and normally up to 20 m parallel to the bank. The patrolling flight to and fro extends from about ten m to 150 m.

The mass of the female subimagos emerge about 20 to 40 minutes after the males. Microscopic preparations of the wings show microtrichia all over the wing surface of the male subimagos, freshly hatched females and females on the compensation flight, whereas the wings of the male imagoes lack these structures. This observation demonstrates that for *P. longicauda* the females stay in the subimaginal stage.

Males even try to copulate with female larvae (even with cigarette-ends). Up to 20 males try to clutch at one female subimago on the water surface. The arrangement of several specimens on the water surface looks like a flower, in Hungary known as "Tisza virag". The copulating male is positioned under the female and holds the base of her wings with his forelegs. He curves his abdomen for mating and rubs his head against the thorax of the female frantically. The copulation takes up to ten seconds. The incorrectly positioned, highly excited males clutch the wings or the abdomen of the female with their forceps.

When a female is able to separate herself from the males, she flies towards the middle of the river. It is possible that pursuing males mate with the female in flight. Mated females are still attractive to males. We observed attempts by several different males to copulate with one female. In an experiment, up to 30 different males tried to copulate with a female held by a thin string. Only when the female is more than 20 m off the bank, can she safely join other females on their compensation flight. No male is observed in the huge mass of females that fly upstream in a compact formation, from two to 20 m over the water. Towards the end of an emergence period, the females are more numerous and the males exhausted. Nevertheless, many unmated females undertake the compensation flight. The oviposition takes place during the compensation flight. The females drop the eggs on the water surface. During all this time, no winged predator was observed. After the end of a mass flight (about 1.5 hours after emergence of the males), the river bank and the vegetation are covered with the male subimaginal skins and the water surface with nymphal skins and exhausted males.

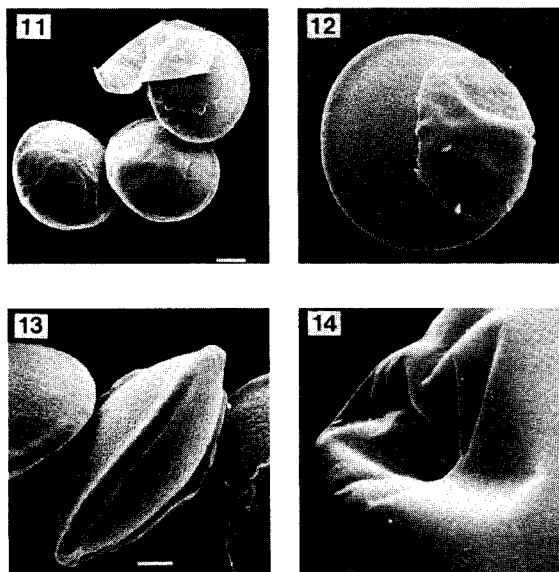
Egg Structure

The diameter of the discoidal egg of *P. longicauda* amounts to 361 μm and the width is about 150 μm (Figs. 11, 12). The surface of the chorion is rough in contrast to the smooth polar cap. The thin polar cap covers about one third of each side of the egg (Fig. 12). The egg of *P. longicauda* lacks any other attachment structures. The edge of the egg is thicker (Fig. 13). The micropyle is situated on the edge in the polar cap region and its diameter is about five μm .

Discussion

Palingenia longicauda are filtering larvae that live in galleries like *Ephemera* sp. (Strenger 1970, 1975). The forelegs are used for digging the galleries. Food particles on the hair of the forelegs are combed out and transported to the first maxillary palpi, and then to the mandibles. The mouthparts never have contact with the ground (Strenger 1973). The direction of the galleries against the water flow results in a water current in the tube. This current transports food particles for

Figures 11-14. Eggs of *P. longicauda*. 11, 12) eggs, lateral view; 13) egg, slice view; 14) micropyle; scale lines: 11: 500 μm ; 12, 13: 50 μm ; 14: 5 μm .

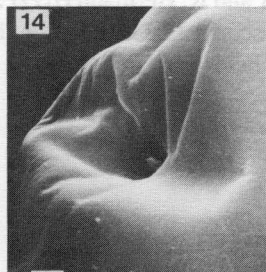
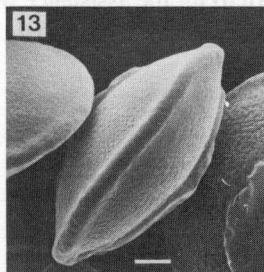
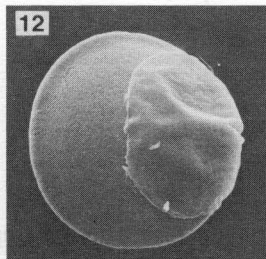
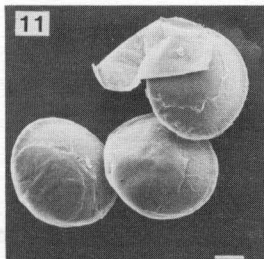


passive filtering. The intact algae and the scattered accumulation of bacteria on POM particles support the filter theory.

Emergence *en masse* requires a coordination of stimuli for the beginning of the event. Water temperature as well as light intensity can act as stimulating factors: the mass flight takes place when the water depth decreases to a level over the galleries sufficient to guarantee a certain light intensity and when the temperature is over 18°C (Sartori et al. 1994). These observations agree with the results of Csonkya (1973): a high level of oxygen is required before the transformation. The intake of oxygen is stimulated by light as well as by temperature. Curiously, the females hatch after the males. Other stimulation factors must control this phenomenon (secreting chemical substances or mechanical drumming of the male larvae?).

The hairy structures on the wings of females and of subimago males of *P. longicauda* confirm the observation of Edmunds and McCafferty (1988) that females of all Old World members of the Palingeniidae remain subimagos. The mating behaviour is interesting, too. Males appear to have a strong attraction for light-coloured objects. The first contact is visual: the lighter females or light artificial objects attract the males. Next, the male rubs his head on the thorax of the female. Perhaps a chemical substance on the body of the female increases the excitement of the males.

Figures 11-14. Eggs of *P. longicauda*. 11, 12) eggs, lateral view; 13) egg, slice view; 14) micropyle; scale lines: 11: 500 μm ; 12, 13: 50 μm ; 14: 5 μm .



The dimensions of the egg, 361 μm in diameter and 150 μm in width, agree well with the results of Russev (1987). It would be interesting to find out what happens to the unfertilized eggs of the unmated females. A supplementary field trip will clear up some questions but will raise others.

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