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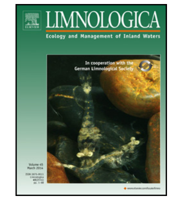


# Fecundity of the mayfly *Ephoron virgo* (Olivier, 1791) (Ephemeroptera: Polymitarcyidae): A long-term study in the River Rhine

Armin Kureck[a,*], Rosita Bieg[a], Rosalinde Wendeler[a], Jost Borcherding[b]

[a] University of Cologne, Zoological Institute, General Ecology & Limnology, D-50674 Cologne, Germany
[b] University of Cologne, Zoological Institute, General Ecology & Limnology, Ecological Research Station Grietherbusch, D-50674 Cologne, Germany

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ABSTRACT

The fecundity of the mayfly *Ephoron virgo* in the River Rhine was studied over a period of twelve years (1992–2003) and compared to four European rivers in 1998. The field data were complemented by mass-rearing in flumes with fresh running river water (1999–2001). The dry mass of a single egg averaged to $4.15 \pm 0.25$ μg, without any significant spatial or temporal differences. Total egg mass per female was found as a reliable measurement of fecundity in *E. virgo* that significantly increased with increasing weight of a female. Within a sample, individual females differed tremendously in egg numbers. This high variability remained even under more homogenous conditions in a densely populated experimental flume. In 1998, fecundity of *E. virgo* in the Rhine tributaries of Main and Neckar was significantly lower than in the rivers Rhine and Lahn, averaging between about 2500 and 4100 eggs per female, respectively. Fecundity did not differ throughout the flight period at the Rhine. Though differences in fecundity between 1992 and 2003 were observed in the Rhine, no clear temporal tendency could be detected. Under extremely high population densities of *E. virgo* in experimental flumes, fecundity was slightly lower compared to parallel field samples. Based on these fecundity data, the decline of the population of *E. virgo* in the Lower Rhine is discussed with respect to some potential environmental factors, giving clear hints that it is not a simple effect of food limitation.



## Introduction

As one of the most important factors to analyse and to predict population dynamics, fecundity data are needed that describe the investment of an organism into reproduction (Sibly and Hone, 2002). While in mammals, birds and reptiles positive relationships between the maternal body weight and the weight of neonates or egg weights are described, poikilotherms primarily increase fecundity per clutch as adult body size increases (for a review cf. Blueweiss et al., 1978). For insects, Honěk (1993) reviewed data across 68 species and predicted a 0.95% increase in median fecundity for each 1% increase in dry body weight. An important assumption for this relationship is that the sizes of the eggs show only low variability across changing female sizes within a species (Honěk, 1993). Though there are a few examples that described an increase in egg size with female weight, other factors like female age or temperature were denoted to influence egg size more than the size of the mother (cf. review of Honěk, 1993). Nevertheless, fecundity in insects is clearly related to the size of the female, which, in turn, may be affected by several environmental factors like latitude or temperature (e.g. Giberson and Rosenberg, 1994; Marshall et al., 2013).

Most mayfly species (Ephemeroptera) lay 500–3000 eggs, but values range from fewer than 100 up to 12 000 (Brittain, 1982). Highest fecundities were found for large mayflies with burrowing larvae and regular mass emergences, e.g. *Hexagenia* and *Palingenia* (Fremling, 1967; Clifford and Boerger, 1974). All *Ephoron* species belong to this group and are well known for their spectacular mass flights in Europe as well as in North America and Japan (Schäffer, 1757; Williamson, 1802; Edmunds et al., 1956; Thew, 1958; Watanabe et al., 1989). After decades of absence, *Ephoron virgo* (Olivier, 1791) came back to the rehabilitated River Rhine in the early 1990s of the last century (Bij de Vaate et al., 1992). On summer evenings from 1990 to 1992, masses of these mayflies affected the traffic near the embankment and provoked great public interest near Cologne (Kureck, 1992, 1996).

The univoltin life cycle of *E. virgo* has already been described (Ibañez et al., 1991; Kureck and Fontes, 1996). It is quite similar for all *Ephoron* species (e.g. Edmunds et al., 1956; Britt, 1962; Aoyagi et al., 1998). First instar larvae lack filter-feeding structures

* Corresponding author at: Im Auel 13a, 51491 Overath, Germany.
*E-mail address:* armin.kureck@t-online.de (A. Kureck).

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and live among sand and gravel in the interstices. Wantzen (1992) found small larvae deep in the sediment of the Rhine. Possibly the early instars use comb-like structures on their mandibles to feed on the biofilm (Kureck and Bieg, 2001). As gills and complex filter structures develop, the larvae start burrowing and filter feeding in U-shaped burrows in the sediment of medium to large-sized rivers. Pennate setae on the mouthparts retain particles of as small as 1 μm (Kureck and Bieg, 2001). The development of mouthparts and gills is similar in *Ephoron shigae* and *Ephoron eophilum* (Aoyagi et al., 1998) and *Ephoron leukon* (O'Donnell, 2009). In our laboratory *E. virgo* ingested small algae (e.g. *Clamydomonas* sp.) as well as fine suspensions of different organic material (yeast, nettle powder, fish food, A. Kureck, unpublished data).

The natural food of the larvae in the River Rhine seems to be a mixture of small organic particles (seston) including bacteria and algae. Friedrich and Pohlmann (2009) described a massive decrease in the chlorophyll content and changes in the phytoplankton community in the Lower Rhine since 1990 that were expected to affect higher levels of the food web. In addition, tremendous changes in the communities of benthic invertebrates were observed that primarily depend on the invasion of filter feeding molluscs (e.g. *Corbicula*, Bij de Vaate and Greijdanus-Klaas, 1990) and many Crustacean species (e.g. *Corophium*, Den Hartog et al., 1992; *Dikerogammarus*, Van Riel et al., 2006), especially after the opening of the Rhine-Main-Danube Canal (Bij de Vaate et al., 2002). All these species must be assumed as potential competitors on the same (and decreasing) food resources also used by *E. virgo*. Thus, the observed population decline of this native species after the last impressive mass flights at the Lower Rhine in 1992 maybe seen in correlation to decreasing food conditions and/or an increasing number of competitors. Nevertheless, assuming lower amounts of food may result either in a direct decrease of the numbers of individuals within the population or a decrease in size, and consequently in fecundity of the individual.

So far, published data on the fecundity of *E. virgo* are rare and gave no clear picture: Reaumur (1742) counted 700–800 eggs while Joly (1876) found only 500 per female, and of course, these data cannot be used as a reliable estimation of fecundity on *E. virgo* at the Lower Rhine under the actual environmental conditions. Consequently, our first aim was to describe fecundity data of *E. virgo* at the Lower Rhine, to analyse potential changes over time and to get a first impression if differences exist to other rivers. These data may serve in future assessments of population dynamics in the River Rhine and other rivers across Europe. In the second step we conducted first experiments in which we reared larvae of *E. virgo* under field-like conditions, however, excluding competitors and predators. The obtained fecundity data of these experiments and the field data were finally used to discuss the potentials reasons for the observed decline of *E. virgo* in the Lower Rhine during the last two decades.

## Methods

### *Sampling and counting of eggs, determination of dry mass*

Adult *E. virgo* are attracted by light. Soon after contact with a surface, the females extrude their eggs in two elongate yellow batches similar to small rice grains. These egg batches sink in water. The tiny threads of the polar caps uncoil and stick to the surface of any object they strike. Some egg batches were collected dry and stored as compact, dry grains. They were dried at 60 °C, stored in a desiccator for several days and weighed to the nearest microgram. Only complete, unbroken egg batches were used to calculate the number of eggs. Weighed pieces of these egg batches were immersed in water to separate and count the eggs (300–900 eggs per piece). The total egg numbers per female were calculated from these subsamples (cf. Table 1).

**Table 1**
Mean egg weight (μg dry weight) and mean number of eggs of *Ephoron virgo* (SD = standard deviation) in the River Rhine and other rivers. *n* = number of egg batches analysed in detail (cf. Materials and methods section).

| River and Year | Egg weight (μg) | SD | Eggs per female | SD | *n* |
|---|---|---|---|---|---|
| Rhine 1992 | 4.11 | 0.29 | 2333 | 708 | 12 |
| Rhine 1998 | 4.17 | 0.25 | 4104 | 1113 | 54 |
| Main 1998 | 4.15 | 0.22 | 2809 | 718 | 18 |
| Neckar 1998 | 4.09 | 0.20 | 2437 | 811 | 20 |
| Lahn 1998 | 4.05 | 0.25 | 4058 | 1413 | 12 |
| Dordogne 1998 | 4.17 | 0.27 | 3423 | 467 | 10 |
| Rhine 1999 | 4.25 | 0.18 | 2666 | 1108 | 5 |
| Flume 1999 | 4.16 | 0.27 | 2541 | 721 | 41 |
| Total mean | **4.15** | **0.25** | **3194** | **1180** | **172** |

Eggs from the Rhine population were collected from the left shore of the river in Cologne near the floating Ecological Rhine Station of Cologne University at Rhine-km 684.5 between 1992 and 2003. In summer 1998, eggs and females were also collected at the River Main near Rumpenheim at km 47–48 (09. and 13.08.1998), the River Neckar near Ladenburg at km 14.2 (15.08.1998) and the River Lahn near Bad Ems (20.08.1998). All of these rivers are tributaries of the Rhine. Additionally, we obtained some dry egg batches which had been collected at the Dordogne River in France in 1998. We checked how much mass they could have lost during prolonged dry storage by repeatedly weighting 13 egg packets. These controls showed only marginal weighing errors and a negligible loss of mass of 0.2% after one month and 2.9% after three months of storage.

In 1998, also the dry mass of females was determined by this means. Since females captured at light traps soon extrude their large egg batches, we used only females without eggs. (The egg masses filling almost the complete abdomen and accounting for the largest part of the female body mass were weighed separately.)

### *Experimental data*

For comparison we also collected eggs from a population reared in a flume with fresh-running Rhine water in 1999, 2000 and 2001, respectively. Our method of collecting and storing the eggs, used since 1992, is described by Greve et al. (1999) and has also been used for studies on *E. leukon* (O'Donnell, 2009).

Most of the eggs (>95%) were fertilised. They developed within a month at room temperature to a resting stage with five eye spots and spend the winter in an obligate diapause comparable to that of *Baetis vernus* (Bohle, 1969). This type of development is documented in other *Ephoron* species as well (Edmunds et al., 1956; Britt, 1962; Giberson and Galloway, 1985; Watanabe and Takao, 1991). We kept the dormant embryos alive for up to two years at 4 °C. Once returned to room temperature, they hatched at any season. Fremling (1967) collected eggs of *Hexagenia* mayflies with a similar method and used them for mass-rearing. *Hexagenia* developed without a diapause.

We transferred young larvae before the middle of May to a flume in the Ecological Rhine Station Cologne. The flume was supplied with fresh running water from the Rhine via short pipes. Temperature and food content (plankton and seston) were comparable to that of the river. Potential predators were excluded by a 0.3 mm stainless steel mesh in the inlet tank. Adults emerged between mid-August and mid-September. The duration of the emergence period was comparable to the flight period in the field, but was delayed by two to three weeks. In the flume (6 m long, 0.1 m wide), we kept the larvae in densities of at least 2265–2560 $m^{-2}$. These densities were calculated from 1536 emerging adults in 1999 and 1359 in 2000