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Nymphal biology of *Ephoron virgo* (Olivier, 1791) (Ephemeroptera, Polymitarcyidae) in an Apenninic river (NW Italy)

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Abstract

Life cycle, feeding habits and nymphal density of a population of *Ephoron virgo* from the Bormida river (Northwestern Italy) were studied. Nymphs were present in the river from the beginning of May until August, with the highest density of nymphs recorded at the end of May - beginning of June. The life cycle was univoltine and the nymphal growth was fast. During the life cycle, an obligatory egg diapause occurs and individuals remain in this stage for up to nine months. Nymphs fed mainly on detritus, although mineral matter constituted a considerable percentage of the gut contents, predominantly in smaller nymphs.

Key words: Bormida river, feeding habits, life cycle, Mayflies, Southern Europe.

Ephoron virgo (Olivier 1791) is a representative of Polymitarcyidae, a relatively small family of Ephemeroptera that includes 7 genera and 84 species worldwide, with just 1 genus and 5 species in the Palearctic (Barber-James *et al.* 2008). This species, despite its wide European distribution, was extirpated from many rivers during the last century, because of human induced alteration of environmental conditions and is currently considered threatened in Italy (Thomas & Belfiore 2004; Buffagni *et al.* 2009). The aim of the present study is to describe the nymphal biology of *E. virgo* in a north-western Italian river. Surber samplers (0.5 m^2 area, 250 μm mesh size) were collected monthly for a year (2011) from a site in the Bormida River (Alessandria, NW Italy, 44°53'04.51" N - 8°38'05.76" E, 87 m a.s.l.), and specimens were preserved in 70% ethanol. After the first *E. virgo* nymphs were collected (May 2011), sampling was

performed weekly until all nymphs emerged (August 2011). During this period, mean water temperature was 22.5 ± 1.83 °C, mean oxygen content was 10.3 ± 3.30 mg/l (88.4 ± 20.2 % saturation), mean pH was 7.03 ± 0.52, and mean conductivity was 339.8 ± 54.6 µS/cm. Total length and pronotum width of nymphs were measured with a Nikon SMZ 1500 stereomicroscope (0.10 mm accuracy). Measurements were standardized by placing each nymph between two slides. The representation of the life cycle by means of size-frequency graphs was produced using FiSAT II ver. 1.2.0 software (Gayanilo *et al.* 2002).

Nymphal diet of *E. virgo* was determined by observing the gut contents of 35 individuals of different sizes (mean total length: 9.66 mm ± 4.22 SD). Each specimen was cleared using Hertwigs´ liquid and then heated in an oven at 65ºC for 24 hours, following the methodology of Bello and Cabrera (1999), used in other studies of Ephemeroptera nymphal feeding (e.g. López-Rodríguez *et al.* 2009). The percentage of the absolute gut content (at 40x) was estimated as the proportion of digestive tract occupied by dietary items. The relative gut content (at 400x) was calculated by measuring the area of gut content occupied by each dietary item.

Data were analyzed using STATISTICA software (version 7.1; StatSoft, 2005). Normality of each variable was assessed with the Kolmogorov–Smirnov test. All the variables were not normally distributed (Kolmogorov-Smirnov with $p<0.05$ for all the variables); therefore, nonparametric statistics were used. Gamma correlation was used to test for an association between total length and pronotum width, and size and percentages of diet components. It is reported that Gamma correlation is the most appropriate correlation test when a high degree of range overlap exists among variables (Guisande González *et al.* 2006).

The highest density of nymphs occurred at the end of May - beginning of June (Fig. 1). Afterward, a gradual decrease was observed, mainly due to natural mortality and, at the end of the study period, to adult emergence. From the end of May to the end of the study, variation of nymphal density through the life cycle was comparable with that reported for a population of *E. virgo* in Northern Spain (Cid *et al.* 2008).

Pronotum width and total body length were highly correlated (Gamma correlation = 0.89; $p<0.05$), so that only total body length was used in the analysis (Fig. 2). Nymphal development of *E. virgo* lasted three months, corresponding to a univoltine seasonal life cycle (*sensu* Hynes 1970). The growth rate was very fast and approximately constant through the nymphal development. A wide range of nymphal sizes was observed in all collecting events, probably due to size differences between male and female. After mating and oviposition, eggs are laid in the river and undergo a 9 months obligatory diapause. Egg diapause has been reported for this and other *Ephoron* species (e.g. Watanabe & Ohkita 2000; Cid *et al.* 2008). Similar values of diapause duration, exceeding eight months, have been reported for this species in Germany (Kureck & Fontes 1996) and for others *Ephoron* species in other areas. Embryonic development is variable and can last up to ten months (in Watanabe & Ohkita 2000). The relatively constant nymphal growth rate found in our study contrasts that found by Cid *et al.* (2008) for this species in the lower Ebro River (Spain). In the Spanish population growth was steady from May to early July then increased until late July.

The analysis of the gut contents indicates that detritus is the fundamental component of *E. virgo* nymphal diet (Table 1). Mineral matter constituted a considerably percentage of the gut contents, but this was probably due to incidental

ingestion. Fungi and coarse particulated organic matter (CPOM) were present in the diet in low quantities. Body size and % detritus were positively correlated (Gamma correlation test = 0.43; $p < 0.05$) and body size and % mineral matter were negatively correlated (Gamma correlation test = -0.49; $p < 0.05$). No significant correlation with body size was found for the other items.

It is reported in other studies that nymphs of *E. virgo* are active filter-feeders living in U-shaped tunnels in the riverbed (Tachet *et al.* 2000). Our results show that the species is mainly detritivorous. It is likely that *E. virgo* is a filterer-collector. However, evidences from our study indicate that this species could be a gatherer-collector (considering the high presence of mineral matter, CPOM and fungi in its diet). Controversies in the assignation of organisms to a main functional feeding group (FFG, Merritt and Cummins 2006) have been pointed out for other Ephemeroptera species in the family Ephemeridae (in López-Rodríguez *et al.* 2009). Moreover, it is possible that mature nymphs filter detritus from the water column, while small nymphs collect it from the substrate (ingesting more mineral matter).

Studies on the natural history of lotic insects supply valuable information on both the autoecological characteristics of aquatic species and on the functional organization of ecological systems in rivers (Fenoglio *et al.* 2005; Bottová *et al.* 2012).

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	N	Mean	SD	Minimum	Maximum
% absolute	35	50.43	21.87	10.00	90.00
% detritus	35	89.13	15.32	47.90	99.90
% CPOM	35	1.12	1.25	0.00	4.00
% fungi	35	0.15	0.36	0.00	2.00
% mineral matter	35	9.92	14.78	0.10	50.00

Table 1. Nymphal gut contents of *E. virgo*.

Figure legends

Figure 1 Density (ind/m^2) of *E. virgo* in the Bormida river during the period in which the nymphs are present.

Figure 2 Life cycle of *E. virgo* in the Bormida river.