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Life history of *Capnia bifrons* (Newman, 1838) (Plecoptera: Capniidae) in a small Apennine creek, NW Italy

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The biology of stoneflies from Apennine mountain system is little known. In the present study, we provide information on the life cycle, secondary production, nymphal feeding and flight period of *Capnia bifrons* (Newman, 1838) in the Albedosa creek (NW Italy). At this study site, the species has a univoltine life cycle, with a relatively short and fast nymphal development period and high annual secondary production. Nymphs feed mainly on detritus but incorporate also other food items in their diet, such as coarse particulate organic matter and fungi. Ontogenetic shift from collector-gatherer to shredder habits is detected. Emergence is in February–April, slightly late compared to northern populations.

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1. Introduction

Plecoptera (Stoneflies) is one of the main components of macroinvertebrate communities in streams and rivers, both in biomass and ecological roles (Hynes 1970, Stewart & Stark 2002). Stoneflies are morphologically relatively homogeneous, but they exhibit a great diversity of life strategies (Fochetti & Tierno de Figueroa 2008). Thus, univoltine, semivoltine and even bivoltine life cycles have been described for different stonefly species. Furthermore, all functional feeding groups are represented in this order (*sensu* Merritt & Cummins 2006), except filterer-collectors (Hynes 1976, Brittain 1990, Lieske & Zwick 2007).

In Europe, Plecoptera biology has been extensively investigated, but some regions have been scarcely studied. This is the case of the Italian Apennine range, an area that encompasses habitats characterized by remarkable flow changes and high summer temperatures. High habitat variability strongly influences life history of macroinvertebrates. Abundant data are available for adult phenology of Apenninic stoneflies (Fochetti & Tierno de Figueroa 2008), while little is known about life cycles (e.g. Iannilli *et al.* 2002, Tierno de Figueroa *et al.* 2009) or nymphal feeding (e.g. Bo & Fenoglio 2005, López-Rodríguez *et al.* 2012). No information at all is available on secondary production, despite its importance for estimating population fitness and role in the com-

munity (Benke & Huryn 2006, Allan & Castillo 2007).

Capnia bifrons (Newman, 1838) is a rheophilous and moderate eurithermic species widely distributed in Europe (Graf *et al.* 2009). It emerges in spring-winter across its range (Graf *et al.* 2009). In the southern part of its distribution area, nymphs can live in intermittent water courses (Puig 1984, Luzón-Ortega & Tierno de Figueroa 2000) and can easily move in the interstitial zone (Bo *et al.* 2006). In northern European populations, nymphs feed mainly on detritus, vegetal fragments and diatoms (Brinck 1949). In general this taxon has been classified as grazer, shredder and collector-gatherer (Graf *et al.* 2009), but little data is available on nymphal feeding preferences. *Capnia bifrons* is almost ovoviviparous (with eggs hatching soon after oviposition in the water), and has a univoltine life cycle with nymphal diapause (Hynes 1941, Brinck 1949, Khoo 1964, 1968, Elliott 1986, Lillehammer *et al.* 1989, Graf *et al.* 2009).

While this species has been well studied in northern Europe (Brinck 1949, Graf *et al.* 2009), little information is available for other areas such as the Apennine range. The aim of this paper is to increase knowledge regarding *C. bifrons* by describing the life history (life cycle, nymphal feeding, secondary production and adult phenology) of a population in the Albedosa creek, and comparing these results with those reported from other European freshwater habitats.

2. Material and methods

The study was conducted in the Albedosa creek (44°41'14" N, 8°43'19" E; 190 m a.s.l.), a small tributary of the Orba river in NW Italy. The climate is temperate-Mediterranean, with high autumnal rainfalls, and warm dry summers. The stream flows through a small valley in a secondary deciduous forest. The riparian vegetation is dominated by *Robinia pseudoacacia* (L., 1758), *Alnus glutinosa* Gaertn, 1790, *Corylus avellana* L., 1753, scattered *Castanea sativa* (Miller, 1768) and *Quercus* spp. In the sampling station, the channel width was about 2.5–3.5 m, with a mean depth of 25–30 cm. The stream substrate consisted mainly of sand (40%), gravel (30%), cobbles (20%), and boulders (10%). Samples were collected randomly from every microhabitat using a Surber sampler (area 0.0625 m^2, mesh size 250 μm). Specimens were collected every 15 days between 28.V.2009 and 15.IV.2010. Water temperature was measured during the sampling period with a data logger (Hobo water temperature Pro) and ranged from 0.5 °C in winter to 31.0 °C in summer (mean 11.1 °C). *Capnia bifrons* nymphs were collected and preserved in 85% ethanol. Adults were collected from the riparian vegetation and from the stones manually and with a sweep net.

Total body length and pronotum width of each nymph were measured with a precision of 0.01 mm using a binocular microscope equipped with a micrometer. High correlation existed between both measures (Gamma correlation = 0.82, $p < 0.05$), so we used only total length for all the analyses. Nymphs were classified in 1 mm length size intervals. The life cycle was represented by means of size-frequency graphs obtained with FiSAT II software (Gayanilo *et al.* 2002).

To study the diet of the nymphs, we used the method proposed by Bello and Cabrera (1999), which is widely applied for the study of Plecoptera nymphal feeding (e.g. Fenoglio *et al.* 2009, Kozáčeková *et al.* 2009). Nymphs were cleared by immersion in Hertwigs' liquid in an oven at 65 °C for approximately 24 hours. Hertwigs' liquid is a modification of Hoyer's liquid, which clears the body wall, thus allowing direct examination of the gut content without dissection. Subsequent to the clearing process, specimens were mounted on slides and the gut contents examined (Bo *et al.* 2012). The percentage of total gut content was measured at 40× magnification (as percentage of total area occupied by the contents in the whole digestive tract), while the relative abundances of food items in the gut content was observed at 400× (as percentage area occupied by each component of the total gut contents). The relation between gut content and nymphal size was tested using Gamma correlation.

To assess the secondary production of *C. bifrons*, we used the size-frequency method, which is the most appropriate when nymphs of several sizes cohabit at the same time in the stream (i.e. there is a high overlap in nymphal size in each sampling date; Hynes & Coleman 1968,

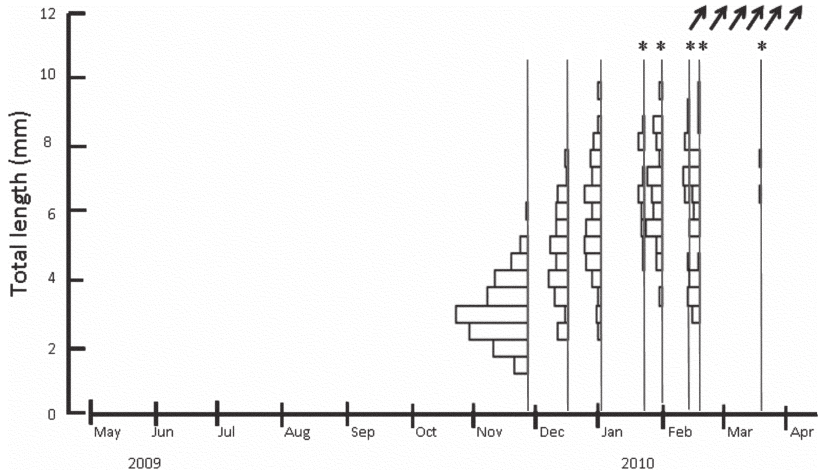

Fig. 1. Life cycle of *Capnia bifrons* in the Albedosa creek, NW Italy. Asterisks indicate the presence of mature nymphs and arrows indicate the presence of adults.

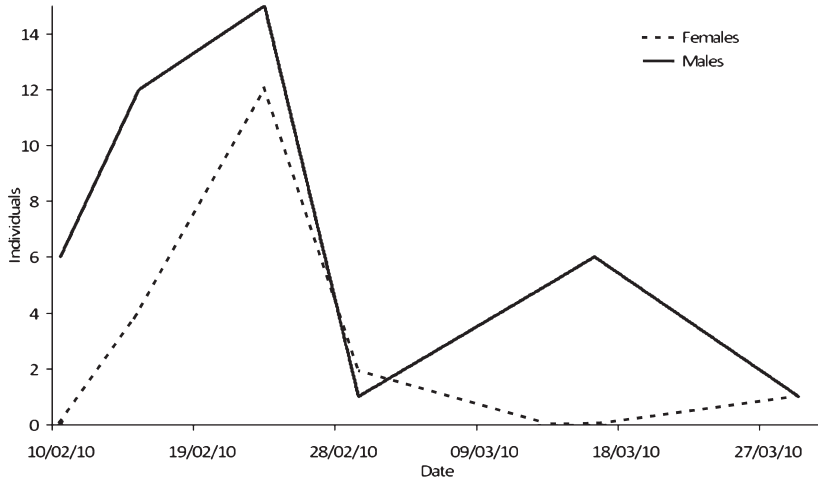

Fig. 2. Numbers of *Capnia bifrons* adults collected during its flight period.

Hamilton 1969, Benke 1979, Benke & Huryn 2006). Annual secondary production was calculated using the cohort production interval (CPI) correction of Benke (1979). To estimate nymphal biomass from total length, a regression line was calculated using 20 formalin-preserved specimens. After drying at 60 °C for 24 hours, they were placed in a desiccator for 1 hour and weighed to the nearest 0.001 mg using a Mettler M3 microbalance.

Dry weight of all specimens was calculated using the following equation:

$$DM = aX^b \quad (1)$$

or, in natural logarithmic form:

$$Ln(DM) = Ln(a) + b\ Ln(X) \quad (2)$$

where DM = individual dry mass, X = total length, a = intercept of the regression, and b = slope of the regression.

All the statistical analyses were performed using STATISTICA v. 7.1 software (StatSoft 2005).

3. Results

The life cycle of *C. bifrons* in the Albedosa creek was univoltine (Fig. 1), with an average nymphal growth period of four months. Nymphs were first collected in November, while none were found in benthic samples from previous months. Adults were collected from mid-February to the end of March (Figs. 1 & 2). In our samples males were more abundant than females (Fig. 2).

Nymphs fed mainly on fine detritus, but also

Table 1. Gut contents of the nymphs of *Capnia bifrons* as proportion (%) of the gut volume filled with food (N=103) and proportions of different components of the total food volume in cases with food in the gut (N=82, i.e. 21 guts were empty).

	Mean	SD	Min.	Max.
Guts with food	43.01	32.10	0	100
Detritus	55.49	25.01	0	98
Hypha	3.55	6.62	0	50
Spores	1.77	3.51	0	20
CPOM [1]	15.63	22.71	0	100
Pollen	0.70	3.39	0	30
Diatoms	5.89	11.06	0	40
Mineral matter	16.98	17.36	0	65

1 CPOM: coarse particulate organic matter.

ingested a considerable amount of coarse particulate organic matter (CPOM, Table 1). Other components, such as diatoms, fungi (both hyphae and spores), and pollen were poorly represented in the diet. Additionally, a relatively large quantity of mineral matter was found, even more abundant than that of the CPOM. Gut content varied with size. Larger nymphs ingested less detritus and more CPOM, mineral matter and fungal hyphae (Table 2).

Table 2. Gamma correlation coefficients (G) between body size (total length, mm) and the numbers of different gut content items of the nymphs of *Capnia bifrons*. Asterisks indicate values significant at $p < 0.05$ (N = 103).

Items	G	Items	G
Detritus	–0.22*	Pollen	–0.04
Hyphae	+0.17*	Diatoms	+0.01
Spores	+0.13	Mineral matter	+0.21*
CPOM [1]	+0.23*		

1 CPOM: coarse particulate organic matter.

Dry mass (DM) was related to body length (X) by the following equation:

$$\text{LnDM} = -5.40 + 2.65(\text{LnX}) \quad (3)$$
$$(r^2 = 0.68, F_{1,18} = 38.40, p < 0.05)$$

Annual secondary production for this population was equal to 144.10 $mgDWm^{-2}year^{-1}$, considering a cohort production interval of four months. Annual production/biomass rate was 12.03 $year^{-1}$, while cohort production/biomass ratio (P/B) was 4.01.

4. Discussion

The life cycle of *C. bifrons* in the Albedosa creek is consistent with the univoltine pattern reported from other areas of Europe (Graf *et al.* 2009). However, differences in the phenology of the cycle have been detected. In Sweden, nymphs were found in samples from September to March (Brinck 1949) that corresponds to a nymphal development period of about 7 months; 2 months longer than what we recorded. This is probably related to the lower water temperatures of the stream where the Swedish population of *C. bifrons* was studied. Similar differences are present when our data is compared to other northern populations (in Graf *et al.* 2009). For example, Khoo (1968) found that young nymphs went into diapause in June, during the warmest period of the summer, when the water temperature was between 9.5 and 13.5 °C, and that diapause ended in September. Lillehammer (1988) discussed that both temperature and photoperiod had an influence on the diapause duration. As this species is ovoviviparous (e.g. Khoo 1968, Lillehammer *et al.* 1989), nymphs hatched from eggs laid in the spring in the Albedosa creek would enter diapause and spend several months at this stage. Nymphs would exit diapause at the end of the fall and concentrate growth in few months (Fig. 1).

The life history data of *C. bifrons* from Albedosa creek supports the findings of Khoo (1964,

1968). This author reported that a special type of early fast life cycle is present in winter stoneflies with a larval diapause, after determining that *C. bifrons* goes into diapause during the spring in its fourth or fifth instar due to rising temperature and/or increasing day length (Hynes 1970).

The flight period of *C. bifrons* reported from northern Europe is mainly spring and winter (Graf *et al.* 2009), although Lillehammer (1988) recorded a more delayed flight period in Fennoscandia, with adults collected from March to June. In the Albedosa catchment, emergence occurs mainly during winter, as reported for the rest of Italy, probably because of the higher temperatures of lower latitudes (Fochetti & Tierno de Figueroa 2008). Our results regarding nymphal feeding are in line with data reported by Brinck (1949) for northern European populations. This author found a wide trophic spectrum for this species, with detritus as the main food component. The population from the Albedosa creek also feeds mainly on detritus, and can be considered a collector-gatherer. Most individuals contained large amounts of mineral matter, which is probably ingested accidentally while feeding on sediments. We detected an ontogenetic shift in the feeding habits of this species. Small nymphs fed prevalently on detritus, while large individuals consumed less detritus and more hyphae, CPOM and mineral matter. Thus, we can speculate that this species shifts from being a collector-gatherer to a shredder along nymphal growth. The high amount of hyphae and spores in large size specimens indicates that most CPOM is ingested from conditioned leaves (dead leaves which are colonized by microorganisms).

Annual secondary production in this population is relatively high for a small size stonefly, but it is in the range of other collector-gatherers according to Benke (1993). Regarding annual P/B, our data show a slightly higher rate than that found in other collector-gatherers (Benke, 1993). However, it is an intermediate value when compared to aquatic invertebrates in general (Huryn & Wallace 2000). The relatively high annual secondary production and P/B of the Albedosa creek population could be related to its elevated growth rate. This was also reflected in the relatively high value of cohort P/B that implies a fast turnover of biomass. Cohort P/B ratio was similar to the one found in an acidified spring area of Slovakia by Krno (1998), who considered a CPI of 190 days. Thus, in our study site, despite a relatively longer nymphal development period, biomass turnover was greater, and thus the fitness of the species in this creek seems higher.

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