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Running title: Life cycle of Plecoptera from an Apenninic stream

Abstract

The life cycles of *Isoperla grammatica*, *Amphinemura sulcicollis* and *Nemoura hesperiae* are studied in a Northern Italy stream, sited in the Apennines. The three species show a univoltine life cycle and their development coincides approximately in the same period of the year, although the one of *I. grammatica* is longer. Possible egg dormancy could exist in the *A. sulcicollis* and *N. hesperiae* life cycles, but this needs to be proved. The three taxa show a spring flight period in the study area. Growth is almost constant through the life cycle, except in *A. sulcicollis* in which an increase is observed at the end of the development. *N. hesperiae* exhibits a faster growth than the other species. In addition, in this paper, the nymph of *N. hesperiae* is described and designed for the first time.

Résumé

Nous avons étudiés les cycles de vie de *Isoperla grammatica*, *Amphinemura sulcicollis* et *Nemoura hesperiae* dans une riviere des Apennins, dans l'Italie septentrional. Les trois espèces ont des cycles univoltins synchronisés a peu prés dans le même période de l'année, même si le cycle de *I. grammatica* est plus long. Un arrêt du développement dans la phase des œufs d'*A. sulcicollis* et *N. hesperiae* est possible, mais celà reste à vérifier. Les trois espèces montrent une période de vol dans le printemps dans l'aire d'étude. La croissance est à peu prés constante dans le temps, avec l'exception de *A. sulcicollis*, chez lequel on peut observer une accélération dans la phase finale. *N. hesperiae* présente une croissance plus rapide que chez les autres espèces. En plus, nous décrivons pour la première fois la nymphe de *N. hesperiae*, qui était inconnue.

Key words: *Isoperla grammatica*, *Amphinemura sulcicollis*, *Nemoura hesperiae*, life cycles, Southern Europe.

Introduction

As pointed out by some authors (Rosenberg 1979; Butler 1984; Williams & Feltmate 1992; Benke & Huryn 2006), information on life cycles and other aspects of life histories of aquatic insects is fundamental to most ecological studies (such as secondary production estimation or population dynamic research) including contemporary researches on the structure and function of aquatic communities and ecosystems, but unfortunately we still lack many data, especially in particular areas. In Italy, studies on life cycles on Plecoptera, one of the most important insect groups in freshwaters, are scarce, especially in the Apennine where only data on a few species are available (e.g. Nicolai & Fochetti 1983; Iannilli *et al.* 2002; Fenoglio *et al.* 2009). Apennine, as other mountain systems from Southern Europe, exhibits particular characters (remarkable flow changes, high temperatures during summer, etc.) that can influence the life cycles of the macroinvertebrate species inhabiting there.

The aim of the present paper is to describe the life cycle of three species inhabiting the Torrente Curone, situated in Northern Apennines: *Isoperla grammatica* (Poda 1761), *Amphinemura sulcicollis* (Stephens 1836), and *Nemoura hesperiae* Consiglio 1960. In the case of the latter, we will also describe the nymph, because it is, up to now, unknown.

I. grammatica and *A. sulcicollis* are species widely distributed by all Europe (Fochetti & Tierno de Figueroa 2004) and data on their biology out of Italy, including on life cycle, are relatively abundant (e.g. Hynes 1941; Maitland, 1966; Lavandier & Dumas 1971; Lillehammer 1988; Malmqvist & Sjöström 1989; Sánchez-Ortega & Alba-Tercedor 1990). On the contrary, *N. hesperiae* is endemic to Italy (Fochetti & Tierno de Figueroa 2004), and its biology is almost unknown. We compare the obtained data for the two former species with those from the bibliography, while for the latter species information is provided for the first time.

Material and methods

Seventeen samplings distributed through the year, from April 2007 to March 2008, were made in the Torrente Curone (Piemonte, Italy; 44° 47' 14''N, 9° 04' 02''E, 320 m a.s.l.), with the aim of collecting Plecoptera nymphs, including the species *I. grammatica*, *A. sulcicollis,* and *N. hesperiae*. The Curone river is situated in the Northern Apennines, a densely forested and scarcely populated area. This stream shows a good environmental quality, reaching the First Class in the Extended Biotic Index system (Ghetti 1997), corresponding to an environment with little human impact. Table 1 shows results of the analysis of main chemical parameters. We measured water temperature by using a HOBO® Water Temp Pro datalogger (0.001 ºC accuracy). Water temperature varied considerably during the study period, with a mean of 11.1 °C, a maximum of 22.3 °C in July and a minimum of 0.004 °C in December (fig. 1). Torrente Curone is a perennial lotic system, with flood increases occurring mainly in autumn and spring. In the study period, width ranged from 3.50 to 4.80 m and depth from 0.17 to 0.60 m. Nymphs were collected with a hand-net (500 μm mesh), while adults were collected with an entomological net and tweezers from the riparian area and in-stream stones. All samples were preserved in 95% ethanol. Total nymph lengths were measured with a Nikon SMZ 1500 stereomicroscope (0.1 mm accuracy). The collection of adults belonging to only one species of the genus *Nemoura* was fundamental for describing the nymph of *N. hesperiae*. Measures were standardized by putting every individual between two glass slides. Life cycles were represented by using size-frequency graphs realised with FiSAT II software (Gayanilo *et al.* 2002).

Results and discussion

Description of the nymph of Nemoura hesperiae *Consiglio 1960*

Nymph of *N. hesperiae* reaches a maximum total length of 7.8 mm (fig. 2). It presents a general colour pattern brown-yellowish, with the final uroterga slightly lighter than the previous ones, and with pale ventral parts. The colour of the head is dark, with a light area between ocelli and around the compound eyes. The pronotum, slightly trapezoidal (width/length ratio 1.3-1.6, mode 1.5), has abundant long bristles on its edges, measuring until 1/10 of the pronotum width and 1/7 of the pronotum length. Mesonotum is also hairy, with hairs even longer than those of the pronotum. The femurs present long bristles, particularly long in the distal part, but extending to the femur base. The tibiae have hard hairs disposed in four rows. The uroterga are hairy, with some particular long bristles reaching a 4/10 of the tergum length and a 1/8 of the tergum width, but with long bristles along all the distal edge. The cerci present long hair crowns in each article junction. The nymph of *N. hesperiae* looks like that of *N. mortoni* Ris 1902 by its hairy aspect, but the former has not the clear bristle rings around all the legs typical of *N. mortoni*. Moreover, in *N. mortoni* leg bristles are not extended to the proximal part as it is in *N. hesperiae*. The pronotum shape is also different between both species, not trapezoidal and with a higher width/length proportion in *N. mortoni*. Adult collections through all the year confirm the existence of only one species of the genus *Nemoura*, *N. hesperiae,* in the torrente Curone.

Despite its nymphal similarity with *N. mortoni*, *N. hesperiae* belongs to the *flexuosa-marginata* group (sensu Zwick 1970), whose species validity has been demonstrated by Fochetti (1992). The nymphs of *N. hesperiae* differ from the other nymphs of this group described up to now present in Italy by their hairy aspect.

Life cycles

Isoperla grammatica shows a univoltine life cycle in the study area, as in other European rivers (Hynes 1941, 1961; Lillehammer 1988; Malmqvist & Sjöström 1989; Sánchez-Ortega & Alba-Tercedor 1990), with nymphs present in the benthos from November to mid June (fig. 3). Nevertheless, it is possible that the cycle begins one or two months earlier because, as pointed by Elliott (1991), the incubation period lasts 36 days at 16ºC (the optimum temperature for this species), similar to the mean temperature found in our study area during the summer period. This life cycle can be catalogued as slow-seasonal according to Hynes (1970). The presence of a great nymph size range is outstanding, especially pronounced during the last months of its cycle, before the emergence, that can be explained partially by the sexual dimorphism, more accused in the final part of the species development and, in part, by the existence of a long recruitment period, previously pointed for this species in other areas (Lillehammer 1988; Sánchez-Ortega & Alba-Tercedor 1990). Nevertheless, we did not detect a prolonged flight period for this species in the study area (the flight period extends from May to June). The growth is approximately constant through the nymph development. The presence of only one nymph in September is not significant, and probably corresponds to one asynchronized individual.

The life cycle of *A. sulcicollis* is also univoltine (fig. 4), with nymphs in the benthos from November to April-May. A univoltine life cycle for this species seems to be the general pattern, as pointed out in other European localities (Hynes 1961; Maitland 1966; Lavandier & Dumas 1971; Lillehammer 1988; Fjellheim & Raddum, 2008). The life cycle could be catalogued as fast-seasonal (Hynes 1970) if an egg dormancy period exists during the summer. If not, the cycle should be catalogued as slow-seasonal. Thus, more studies should be carried out to clarify this topic. In the study area the flight period of this species ranges from mid April to June, not extending during the summer as in other Italian areas (Consiglio 1980). The growth is approximately constant through the nymph development, except at the end, when the growth increases.

Nemoura hesperiae also presents an univoltine life cycle (fig. 5), very similar to the previous ones. In fact, the possible existence of a dormancy period would also need to be proved. The presence of a single, asynchronized individual in June is not significant. In the studied stream, *N. hesperiae* shows a spring flight period, with collections in April and May, similar to the previously registered in the rest of its distribution area (Fochetti & Tierno de Figueroa 2009). The growth through the nymphal development in this species is faster than in the previous taxa.

In general it is outstanding that the three studied species develop mainly during the colder months, probably related to the extreme summer temperatures that low quote Appennic streams can reach. Moreover, it has been reported that stoneflies can grow at very low temperatures, even near 0 ºC (Raušer 1962; Hynes 1970).

A complete knowledge of the life cycles of aquatic insects is necessary for attending other ecological researches such as, for instance, secondary production studies. In them, a proper understanding of the biology of the species is a fundamental first step to design samplings, obtain results and discuss them (Benke & Huryn 2006). Also, from a conservation point of view, to understand life cycles let us monitoring and designing conservation plans based on the strategies that species present in different life stages to cope with natural and anthropogenic disturbances (Rosenberg 1979; Butler 1984; Lytle 2008).

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Caption to figures

Figure 1. Water temperature variation during the study period.

Figure 2. Nymph of *Nemoura hesperiae*; A) cercus; B) head, pronotum and mesonotum; C) leg; D) urotergites.

Figure 3. Size-frequency graph representing the life cycle of *I. grammatica* at the studied area.

Figure 4. Size-frequency graph representing the life cycle of *A. sulcicollis* at the studied area.

Figure 5. Size-frequency graph representing the life cycle of *N. hesperiae* at the studied area

Table 1. Main physico-chemical parameters of the Torrente Curone during the studied period (mean ± se) .

Parameter	Values
pH	8. 97 ± 0.22
Conductivity (mS)	330.0 ± 13.3
Dissolved oxygen (mg/l)	7.95 ± 0.13
Flow speed (m/s)	0.67 ± 0.14
Water temperature (°C)	11.01 ± 6.50