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## Life cycle and nymphal feeding of *Besdolus ravizzarum* (Plecoptera: Perlodidae), a threatened stonefly

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### Abstract

We have studied some aspects of *Besdolus ravizzarum* biology in the Apennines. This stonefly has a very localized distribution in southeastern France and northern Italy. This species is considered threatened. For this reason it is especially relevant to improve knowledge about its natural history, previously unknown. Despite the large size of nymphs, this species shows a univoltine life cycle. The growth is slow but uniform from October to April or May when the short flight period occurs. The analysis of the gut contents indicates this species is a predator, feeding mainly on Baetidae and Chironomidae. It is notable that the development of this carnivorous species through cold periods coincides with the season with higher richness and density of potential prey in Apennine streams. Further studies are needed to improve knowledge of the distribution of the species and to start planning effective conservation efforts.

### Key words:

conservation, life history, northern Italy, stonefly, threatened species, trophic role

## Introduction

The stonefly fauna of Italy is composed of 160 species distributed in 22 genera belonging to seven families; thus, Italy is one of the European countries with the greatest Plecoptera diversity and highest number of endemics ([Fochetti & Tierno de Figueroa, 2008](#)). Unfortunately, data on the biology and ecology of many species are scarce or even absent, despite this information being fundamental for conservation efforts targeting threatened species ([Fochetti & Tierno de Figueroa, 2006](#)).

This is the case of *Besdolus ravizzarum* [Zwick and Weinzierl, 1995](#). Currently, the genus *Besdolus* Ricker, 1952 comprises five European species. One species, *B. ravizzarum* was recently described from a few localities in northern Italy and southeastern France ([Zwick & Weinzierl, 1995](#)). This species was previously recorded as *Dictyogenus ventralis* (Pictet, 1841) in Italy by [Ravizza and Ravizza Dematteis \(1976\)](#), indicating that this species inhabits typical Apenninic streams with summer drought. These authors also noticed that mature nymphs prefer areas with filamentous algae and indicated an adult flight period extending from May to June. In his general guide to Italian Plecoptera nymphs, [Consiglio \(1980\)](#) included this taxon among the potamal stoneflies. More recently, [Tierno de Figueroa and Fochetti \(2001\)](#) found that *B. ravizzarum*, as with other large Perlodidae and Perlidae, do not ingest food during the adult stage. Nothing more is known about the natural history of this species, which, due to the scarcity and patchiness of its populations, can be considered extremely vulnerable ([Fochetti et al., 1998](#)). Moreover, the conservation of populations is particularly necessary because of the altitudinal distribution of the species within mainly lowland and midland running waters, which represent the most altered lotic systems ([Zwick, 1992](#); [Fochetti & Tierno de Figueroa, 2006](#)).

The aim of this study is to increase knowledge on some aspects, such as life cycle and nymphal feeding, of *B. ravizzarum* from a newly discovered population in Piemonte Apennines, which is presently the most northern population known for Italy.

## Materials and methods

*Besdolus ravizzarum* nymphs were collected every 20 days from the Torrente Curone (Piemonte, Italy; 44° 47' 14" N, 9° 04' 02" E, 320 m a.s.l.), a northern Apennine stream, from April 2007 to March 2008. Dense woodlands cover the catchment. This lotic system has good water quality, reaching First Class in the Extended Biotic Index system ([Ghetti, 1997](#)),

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corresponding to a stream without substantial human impact. In its lowland part, the stream enters a more populated area and the environmental quality of waters decreases dramatically. We collected 17 samples through the year, but nymphs of *B. ravizzarum* only appeared in nine of the samples. In the same stream reach, water temperatures were measured with a datalogger (HOBO® Water Temp Pro, 0.001°C accuracy) (Fig. 1) and they were used to calculate accumulated day temperatures between two sampling dates. Water temperature varied considerably during the study period, with a mean temperature of 9.43°C, a minimum of 0.004°C in December and a maximum of 22.3°C in July. Other physico-chemical parameters (conductivity, dissolved oxygen, pH, and flow speed) were measured on each sampling date using Eijkelkamp 13.14 and 18.28 portable instruments (Table 1). During the sampling period, the width of the stream varied from 3.50 to 4.80 m, and the channel depth ranged from 0.17 to 0.60 m. Torrente Curone is a permanent stream, with floods occurring mainly in autumn and spring, but contrary to other streams where *B. ravizzarum* has been previously collected (Ravizza & Ravizza Dematteis, 1976) this water course lacks summer droughts.

Nymphs were collected with a hand-net (500 µm mesh) early in the morning, because Systellognatha nymphs are considered to be chiefly nocturnal feeders (Vaught & Stewart, 1974). Adults were collected with an aerial net beating the riparian vegetation and directly picked from the stones with forceps, from transects of approximately 5 m along both edges of the stream. All samples were preserved in 95% ethanol. Total length and third right femur length of all nymphs were measured with a Nikon SMZ 1500 stereomicroscope (0.1 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 made by using FiSAT II software (Gayaniilo *et al.*, 2002). The individual growth rate was expressed as the difference between arithmetical means of individual body lengths for two adjacent sampling dates. We examined the gut contents of 100 *B. ravizzarum* nymphs. Guts of nymphs were dissected to assess food consumption. Contents of the alimentary canal were dispersed in Faure's fluid on microscope slides and identified using a microscope. Identification of prey was based on sclerotized body parts, particularly head capsules, mouth parts and leg fragments. As pointed out by Stewart and Stark (2002), the count of sclerotized fragments (i.e., head capsules or legs) can give a reasonably accurate count of prey consumed.

## Results and discussion

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## Life cycle

We collected and examined 155 nymphs of *B. ravizzarum*. To study the life cycle, we used only total length, because this variable was significantly correlated to the third femur length (Gamma correlation = 0.80;  $P < 0.05$ ). As shown in [Fig. 2](#), nymphs were detected in the benthos for 8 months, from October, when the smallest nymphs appeared, to April and May, when mature nymphs (and also adults) were found. Thus, the detected flight period is slightly advanced in relation to that previously reported by [Ravizza and Ravizza Dematteis \(1976\)](#), occurring in May and early June, but, as pointed out by these authors, this could be related to the lower elevation. The species is univoltine, with a slow seasonal life cycle following [Hynes' \(1970\)](#) classification. Growth rate was uniform with no significant correlation between growth and accumulated day temperatures (Spearman  $R = 0.26$ ;  $P > 0.05$ ). As pointed out by [Hynes \(1970\)](#), many species of stoneflies that develop mainly during the cold season do not show an evident dependence on temperature, and the developmental temperature threshold of Plecoptera can be very low, even near 0°C ([Raušer, 1962](#)). Eggs laid at the end of spring probably hatch at the end of summer without diapause. It is remarkable that *B. ravizzarum* can complete its life cycle in only 1 year despite its large final size (17.0–22.0 mm). Univoltinism has been also observed in some *Perlodes microcephalus* (Pictet, 1833) populations, that can develop in 1–2 years depending on the existence of embryonic diapause ([Berthélemy, 1979](#)), and it is suggested that *Isogenus nubeculum* Newman, 1833, another rare and extremely threatened large-sized Perlodidae, has also a 1-year life cycle ([Lillehammer, 1988](#)). Otherwise, smaller Perlodidae, for example different species of *Isoperla* Banks, 1906 are often univoltine ([Lillehammer, 1988](#); [Stewart & Stark, 2002](#); [Tierno de Figueroa et al., 2003](#)).

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## Feeding behavior

Nine percent of all guts were completely empty, 40% contained detritus, 16% algae, 7% vascular plant fragments, and 4% sand. Nevertheless, animal remains constituted the most abundant items, present in 70% of guts ([Table 2](#)); in particular, the most important prey group in the guts was Baetidae (Ephemeroptera): they constituted 38.9% of total ingested items, and they were present in 40% of the examined guts, followed by Diptera Chironomidae (26.8% of total items, 24% of examined guts). Other major items were the mayfly family Heptageniidae, the caddisfly family Hydropsychidae, and some Plecoptera (such as *Nemoura* sp., *Brachyptera* sp. and *Isoperla* sp.).

To investigate the presence of ontogenetic shifts of the diet, nymphs were divided into three size classes: “small” ( $n = 42$ , total length  $< 13.0$  mm), “medium sized” ( $n = 29$ , total length between 13.0 and 17.0 mm) and “large” ( $n = 29$ , total length  $> 17.0$  mm). Even the smallest specimens (6.5–7.7 mm) were partly carnivorous, with animal remains found in guts. Apparently, no differences were evident in the amount of non-animal components among nymphs of different size classes; interestingly, with the increase of size *B. ravizzarum* nymphs consume a broader range of prey items (Fig. 3). It is probably that this age-related shift could be associated with the fact that larger-sized predators can handle and feed on a greater spectrum of types of prey, including larger prey. The presence of plant fragments and algae in some specimens was not related to the nymphal size. Large-sized nymphs also ingested small amounts of plant detritus and diatoms. This finding supports the hypothesis that a certain level of trophic omnivory is more common in predatory stream insects than previously supposed (Lancaster *et al.*, 2005). It is interesting that this species shows trophic selection patterns similar to that found in many other large-sized Systellognatha (Lucy *et al.*, 1990): Baetidae and Chironomidae seems to represent the preferred food items of large carnivorous stoneflies, such as *D. cephalotes* and *P. bipunctata* (Bo *et al.*, 2008), *Perla grandis* (Cammarata *et al.*, 2007), *Dyctiogenus fontium* (Fenoglio *et al.*, 2007) and *D. alpinus* (Fenoglio & Bo, 2004). Many factors could influence this evident trophic preference, such as encounter rate and microhabitat overlapping, prey/predator size ratios, handling time, attack and capture success, and profitability. From the size-frequency graph (Fig. 2) of *B. ravizzarum*, it appears that the nymphal growth of this predatory species is on-going throughout the cold-water season, a time when benthic communities in the Apennine lotic systems express the highest abundance and diversity (Fenoglio *et al.*, 2002).

In conclusions, the main findings of this work are that this threatened species is a univoltine stonefly, with conspicuous final size and diet predominantly carnivorous. This species seems to be well adapted to Apennine low-altitude mountainous streams, where summer is characterized by strong increase of water temperatures and conspicuous reduction of stream discharge. Further studies are needed to improve knowledge of the distribution of this menacing species, to investigate the existence of ecological corridors among isolated populations and to start planning effective conservation efforts.

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**Fig. 1** Water temperature variation during the study period.

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**Fig. 2** Size-frequency graph representing the life cycle of *B. ravizzarum* at the studied area.

**Fig. 3** Number of prey types in the guts of the three age groups of *Besdolos ravizzarum* nymphs (mean  $\pm$  SE; differences are not statistically significant).

**Table 1.** Main physico-chemical parameters of the Torrente Curone during the studied period (mean  $\pm$  SE).

Parameter	Values
Conductivity (mS)	330.0 $\pm$ 13.3
Dissolved oxygen (mg/L)	7.95 $\pm$ 0.13
pH	8.97 $\pm$ 0.22
Flow speed (m/s)	0.67 $\pm$ 0.14
Water temperature ( $^{\circ}$ C)	9.43 $\pm$ 0.15

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**Table 2.** Gut contents of *Besdolos ravizzarum* nymphs ( $n = 100$ ) at the studied area. Presence (%) indicates percentage of nymphs with guts containing this kind of item.

	Number of items	Presence (%)
<i>Baetis</i> sp.	77	40
Chironomidae	53	24
Ephemeroptera	28	24
Heptageniidae	17	11
Simuliidae	7	5
Hydropsychidae	4	4
Plecoptera	4	4
<i>Isoperla</i> sp.	3	3
<i>Brachyptera</i> sp.	2	2
Trichoptera	2	2
<i>Nemoura</i> sp.	1	1