Diet of *Dinocras cephalotes* and *Perla marginata* (Plecoptera: Perlidae) in an Apennine stream (northwestern Italy)

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Abstract—The feeding habits of nymphs of *Perla marginata* (Panzer) and *Dinocras cephalotes* (Curtis) were investigated in the Rio Orbarina (northwestern Italy). These species are among the largest European carnivorous freshwater invertebrates and they play an important role in the trophic structure of small, fishless Apennine streams. We examined the gut contents of 60 *P. marginata* and 60 *D. cephalotes* nymphs to characterize the diets and evaluate possible feeding differences between the species. In both of these predaceous stoneflies, the diet included vegetable detritus, mainly in the smaller instars. Both species showed trophic preferences, since only a few taxa constituted most of the ingested prey items, independently of their availability in the substratum. Interestingly, there were no clear differences in prey selection between nymphs of the two species.

Résumé—Nous avons étudié les habitudes alimentaires des larves de *Perla marginata* (Panzer) et de *Dinocras cephalotes* (Curtis) dans le Rio Orbarina (nord-ouest de l'Italie). Ces espèces sont parmi les invertébrés d'eau douce carnivores les plus grands d'Europe et elles jouent un rôle important dans la structure trophique des petits cours d'eau sans poissons des Apennins. Notre travail examine le contenu stomacal de 60 larves de *P. marginata* et de 60 de *D. cephalotes*. Le but de l'étude est de définir le régime alimentaire et d'évaluer les différences dans l'alimentation des deux espèces. Le régime alimentaire de ces deux plécoptères prédateurs inclut du détritus végétal, particulièrement chez les plus petits stades. Il existe de nettes préférences trophiques dans le régime des deux espèces : un petit nombre de taxons constitue la majeure partie des proies ingérées, indépendamment de leur disponibilité dans le substrat. Il est intéressant de noter que nous n'avons décelé aucune différence claire dans la sélection des proies entre les larves de ces deux espèces.

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Introduction

Aquatic insects predominate in the trophic structure of streams (Wallace *et al.* 1987; Allan 1995) and their feeding habits have been increasingly studied in the last few decades (Tierno de Figueroa and Sanchez-Ortega 1999; Monakov 2003).

Plecoptera is an important and often dominant order in stream ecosystems (Zwick 2000). The feeding habits of stonefly larvae are varied, as reflected by the great variation in the structure of the mouthparts of different Plecoptera species. Plecopteran detritivorous shredders have been studied to investigate leaf breakdown and allochthonous input processes in lotic systems (Gessner *et al.* 1999; Fenoglio *et al.* 2005*a*), while predaceous stoneflies are a good model for predator–prey studies in streams (Tikkanen *et al.* 1997).

In many small, fishless streams, Plecoptera belonging to the suborder Systellognatha are the dominant predator group, with important roles as top-down control elements of invertebrate communities (Wipfli and Gregovich 2002) and as "ecological engineers" (Zanetell and Peckarsky 1996). Therefore, recent studies have evaluated the trophic roles of these organisms

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in the field (Bo and Fenoglio 2005; Fenoglio *et al.* 2005*b*) and in the laboratory (Elliott 2004).

Gut contents analysis is the most commonly used technique in field studies. It is based on the assessment of undigested and sclerotized prey parts in the stomach of predators and comparison between the prey eaten and the prey available in the natural environment.

The "optimal foraging theory" (Krebs 1978) states that predators include the most profitable prey in their diet in terms of energy content, encounter rate, prey density, handling time, and other factors. Among Perlidae, some species seem to be selective, feeding mainly on a few items (Fenoglio and Bo 2004), while others seem to be more opportunistic (Dudgeon 2000). In this study we examined the diet of the immature stages of two Perlidae species: *Perla marginata* (Panzer, 1799) and *Dinocras cephalotes* (Curtis, 1827). These species attain a final size that places them among the largest European carnivorous freshwater invertebrates, and they play an important role in the trophic structure of small, fishless Apennine streams. The aim of this study was to analyse the diet of *P. marginata* and *D. cephalotes* nymphs of different sizes to test the hypothesis that these species have feeding preferences and to evaluate possible differences in their diets.

Materials and methods

Dinocras cephalotes and *P. marginata* nymphs were collected in the Rio Orbarina (570 m a.s.l.) on 24–25 October 2005. This second-order stream in the Regional Natural Park of Beigua is a typical Apennine lotic system with good environmental quality; it is classified as First Class in the Italian Extended Biotic Index (Ghetti 1997), corresponding to an environment with no trace of human-induced alteration. We examined 60 *D. cephalotes* and 60 *P. marginata* nymphs collected in a 200 m riffle. All samplings were conducted early in the morning because Systellognatha are considered to be chiefly nocturnal feeders (Vaught and Stewart 1974). Also, using a Surber net (20 cm × 20 cm, 255 μm mesh), we collected samples in the same reach to assess the presence and abundance of the taxa of the natural benthic invertebrate population. Samples were preserved in 95% ethanol.

In the laboratory, all organisms were counted and identified to the genus level except for Lumbriculidae and early instars of some Diptera and Trichoptera, which were identified to the family level. The total length of *P. marginata* and *D. cephalotes* nymphs was measured (0.1 mm accuracy). Nymphs were later processed to assess food consumption by means of gut contents analysis. Guts were removed and the contents of the alimentary canal were analysed by the transparency method for slides (Faure's fluid). Identification of prey was based on sclerotized body parts, particularly head capsules, mouthparts, and leg fragments. Stewart and Stark (2002) stated that the count of sclerotized fragments (*i.e.*, head capsules or legs) can give a reasonably accurate count of prey consumed. Each head was counted as an individual, as was each leg of the same type (*e.g.*, first right leg of a mayfly).

Gut contents were also compared with the natural composition and abundance of macroinvertebrate communities in the riverbed. Moreover, to analyse a possible shift in food preference according to nymph size, we separated the nymphs into three length classes (*P. marginata*: small, <5.0 mm, $n = 15$; intermediate, 5–15 mm, $n = 21$; large, >15 mm, $n = 24$; *D. cephalotes*: small, <5.0 mm, $n = 9$; intermediate, 5–15 mm, $n = 30$; large, >15 mm, $n = 21$).

Feeding preferences were quantified using the trophic electivity index of Ivlev (1961),

$$E = \frac{r_i - p_i}{r_i + p_i}$$

where r_i is the proportion of ingested species and p_i is the relative abundance in the benthic community, and the electivity index of McCormick (1991),

$$E^* = \frac{W_i - 1/N}{W_i + 1/N}$$

where

$$W_i = \frac{r_i / p_i}{\sum r_i / p_i}$$

and N is the number of food items. Both indexes range from –1 to 1. A value of –1 indicates total avoidance, 1 indicates preference, and 0 indicates indifference.

Analysis of variance (ANOVA) was performed with Systat 8.0 (Wilkinson 1992).

Table 1. Percent relative abundance (% value in the community) of macroinvertebrates collected in the riverbed of the Rio Orbarina (northwestern Italy).

Taxon	Relative abundance (%)	FFG*
Plecoptera		
Leuctra sp.	5.75	Sh
Nemoura sp.	1.56	Sh
Amphinemura sp.	1.20	Sh
Protonemura sp.	0.36	Sh
Isoperla sp.	2.51	P
Perla marginata	7.19	P
Dinocras cephalotes	7.19	P
Ephemeroptera		
Ecdyonurus sp.	4.31	Sc
Torleya major	2.75	Cg
Paraleptophlebia sp.	3.95	Cg
Epeorus sylvicola	8.26	Sc
Baetis sp.	8.38	Cg
Ephemera danica	0.12	Cg
Trichoptera		
Tinodes sp.	0.12	Sh
Sericostoma sp.	7.31	Sh
Hydropsyche sp.	8.02	F
Philopotamus sp.	6.71	F
Hyporhyacophila sp.	0.96	P
Rhyacophila sp.	0.36	P
Odontocerum albicorne	0.48	Sh
Photamophilax cingulatus	0.36	Sh
Limnephilidae	0.12	Sh
Diptera		
Chironomidae	4.07	Cg
Atherix sp.	3.71	P
Tipula sp.	0.60	Sh
Limoniidae	0.24	P
Simuliidae	0.24	F
Odonata		
Onychogomphus forcipatus	0.48	P
Boyeria irene	0.36	P
Coleoptera		
Gyrinidae (larvae)	0.12	P
Elmidae (adults)	0.48	Cg
Haenydra truncata	2.16	Sc
Scirtidae (larvae)	2.16	Sh
Pomatinus substriatum	1.32	Sh
Hemiptera		
Hydrometra stagnorum	0.12	P
Micronecta sp.	0.48	P
Velia sp.	0.12	P
Gerridae	1.32	P
Annelida		
Lumbricidae	0.12	Cg
Lumbriculidae	0.48	Cg
Tricladida		
Dugesia sp.	2.16	P
Gastropoda		
Ancylus fluviatilis	0.24	Sc
Nematomorpha		
Gordius sp.	0.36	P
Arachnida		
Hydracarina	0.72	P

*FFG, functional feeding groups: Cg, collectors–gatherers; F, filterers; P, predators; Sc, scrapers; and Sh, shredders (see Merritt and Cummins 1996).

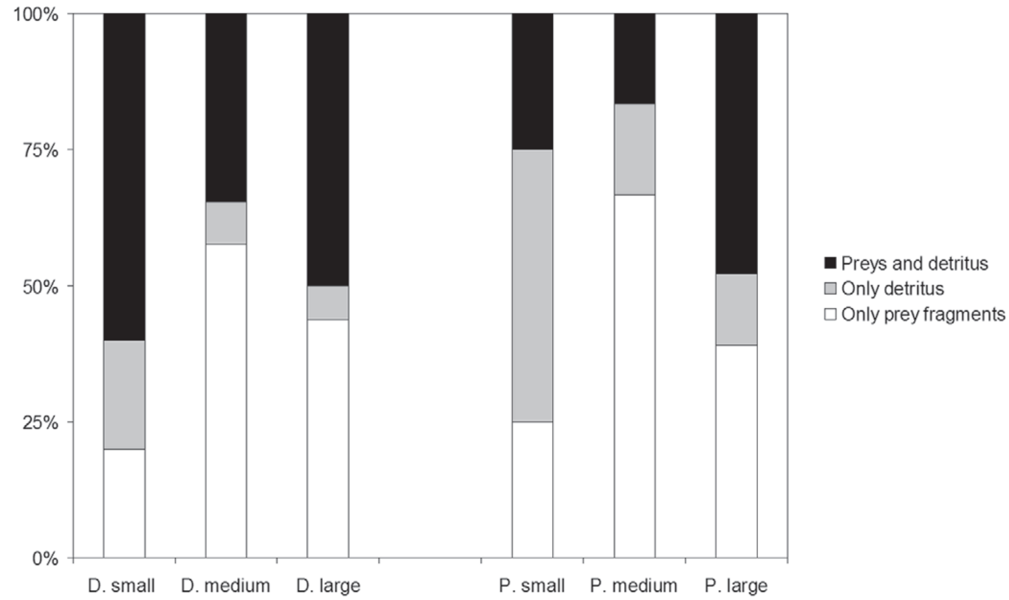
Results

In total, we collected 835 stream invertebrates belonging to 44 taxa (see Table 1). We examined the gut contents of 60 *D. cephalotes* and 60 *P. marginata* nymphs.

Most of the food ingested consisted of insect larvae, but algae and vegetable fragments were found in a large number of specimens of both species. Figure 1 shows the percentages of guts with prey fragments, with detritus only, and with detritus and prey fragments for the different size classes of the two species. There were no significant differences between the two species in the number of prey fragments ingested (ANOVA: $F_{1,118} = 0.034$, $P = 0.56$) or the number of prey taxa (ANOVA: $F_{1,118} = 1.12$, $P = 0.29$). Because of difficulties in quantifying and comparing detritus consumption, the statistical analysis was performed only on prey ingestion data. Vegetable detritus was found in 35% of *P. marginata* nymphs and 40% of *D. cephalotes* nymphs. There was no significant difference in the presence of detritus between the two species (ANOVA: $F_{1,118} = 0.034$, $P = 0.61$). Small nymphs had larger amounts of vegetable detritus than large ones, even though its presence was not significantly different among nymphs of different sizes, both in *P. marginata* (ANOVA: $F_{2,57} = 2.39$, $P = 0.06$) and in *D. cephalotes* (ANOVA: $F_{2,57} = 0.14$, $P = 0.29$).

The mean numbers of prey categories found in the guts were 1.13 (SD = 1.15, min. = 0,

Fig. 1. Percentages of guts with prey fragments, with detritus, and with preys and detritus for each of three size classes of *Perla marginata* (P.) and *Dinocras cephalotes* (D.).



max. = 5, n = 60) in *D. cephalotes* and 0.92 (SD = 1.08, min. = 0, max. = 4, n = 60) in *P. marginata*. Comparing the number of elements of each prey type in the guts, we found no significant differences between the two stonefly species in all three size groups (ANOVA: small nymphs, $F_{1,22} = 0.18$, $P = 0.67$; intermediate nymphs, $F_{1,49} = 0.065$, $P = 0.80$; large nymphs, $F_{1,43} = 0.25$, $P = 0.62$).

Comparison of the diets with the composition of the bottom community via the Ivlev and McCormick electivity indexes produced some interesting results. Chironomidae were the most selected prey of both species: larvae of this family were found in 28.3% of *P. marginata* nymphs and 25.0% of *D. cephalotes* nymphs, and both indexes indicated a high preference (Fig. 2 and Table 2). Ephemeroptera parts were the second most abundant item in the guts: undetermined mayflies, Baetidae, and Leptophlebiidae were found in 33.3% of *P. marginata* nymphs and 26.7% of *D. cephalotes* nymphs. Interestingly, some taxa were abundant and widespread in the natural environment but less represented in the diets of the two stonefly nymphs, particularly large organisms such as Rhyacophilidae, Philopotamidae, and Odonata and organisms living in particular microhabitats, such as aquatic Hemiptera.

Discussion

Recent studies have demonstrated that many Perlidae are not strictly carnivorous: especially in their first instars, their diet includes vegetable detritus and algae (Gray and Ward 1979; Fenoglio 2003). In agreement with those results, we found that *D. cephalotes* and *P. marginata* nymphs feed on vegetable detritus in the Rio Orbarina; interestingly, although detritus was most abundant in smaller nymphs, the larger ones also consumed significant amounts of it.

Although our results refer to a single sampling campaign, we think that they are of general interest because the investigated species are semivoltine and, by analysing different size groups, we collected data covering the entire postembryonic development of the two species. Our comparison of the diets with the composition of the natural community indicated that carnivorous stoneflies prefer small rather than large prey and sedentary rather than mobile prey (Allan and Flecker 1988). Different factors could play important roles in this context: size (*e.g.*, in the case of Trichoptera: Philopotamidae) and (or) predaceous abilities (*e.g.*, in the case of Odonata and Diptera: Athericidae) may increase the handling time and discourage attacks, while mobile prey might easily avoid attacks. On the other hand, encounter rate is one of the most important factors influencing

Fig. 2. McCormick's electivity index for the macroinvertebrate taxa in the diet of *Perla marginata* and *Dinocras cephalotes* nymphs of Rio Orbarina. A value of –1 indicates total avoidance, 1 indicates preference, and 0 indicates indifference.

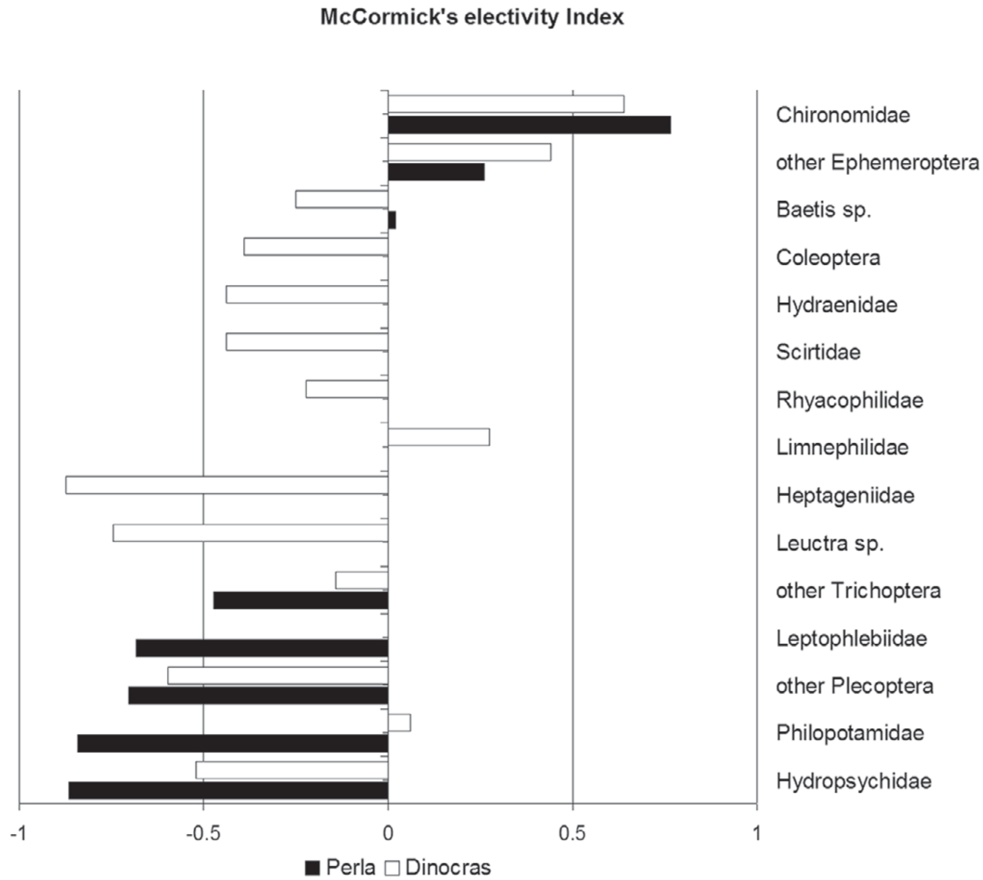


Table 2. Ivlev's index values for *Perla marginata* and *Dinocras cephalotes* nymphs.

Prey	*P. marginata*	*D. cephalotes*
Hydropsychidae	–0.82	–0.40
Philopotamidae	–0.79	0.20
Other Plecoptera	–0.62	–0.49
Leptophlebiidae	–0.59	0.00
Other Trichoptera	–0.35	0.01
Leuctra sp.	0.00	–0.67
Heptageniidae	0.00	–0.83
Limnephilidae	0.00	0.40
Rhyacophilidae	0.00	–0.07
Scirtidae	0.00	–0.31
Hydraenidae	0.00	–0.31
Coleoptera	0.00	–0.25
Baetis sp.	0.16	–0.11
Other Ephemeroptera	0.39	0.55
Chironomidae	0.82	0.72

predator–prey interactions in aquatic invertebrates (Sih 1993; Tikkanen *et al.* 1997). Nevertheless, the most common insects were not consumed at a greater rate, while some organisms, albeit well represented in the environment, inhabited particular microhabitats and were almost absent in the diets of the two species.

Elliott (2000, 2003) compared the gut contents of four large Systellognatha (*D. cephalotes*, *Perla bipunctata* Pictet, 1833, *Perlodes microcephalus* (Pictet, 1833), and *Isoperla grammatica* (Poda, 1761)) and stated that these species are active nocturnal predators with no clear differences in prey selection. This may be particularly evident in environments with rich and abundant prey communities. Our study confirms this finding, providing evidence that large carnivorous stoneflies may have common characteristics in prey selection: they seem to prefer some prey, such as Chironomidae and different families of Ephemeroptera. This overlapping of the diet could be related to the adoption of similar hunting strategies (sit-and-wait ambush strategy, Elliott 2000), to prey behaviour (Elliott 2004), or to phylogenetic constraints related for example to the semivoltinism of these Perlidae.

In the Rio Orbarina, *P. marginata* and *D. cephalotes* are the largest predators, and their activity could have a top-down effect on the invertebrate community. Future studies should investigate whether the presence of fish changes the diet and feeding habits of these Systellognatha.

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