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Running title:

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Abstract

Feeding habits of *Perla grandis* nymphs have been investigated in the Rio Berga, an Apenninic stream of Northwestern Italy. In this study, we analysed gut contents of 50 nymphs of this species, with the aim to investigate feeding preferences. Nymphs were collected from a single riffle, whose benthic coenosis was also determined. We detected a change in the diet during ontogenesis, with small instars feeding mainly on detritus and large instars strictly carnivorous. We also detected the existence of an evident trophic selection: diet was almost entirely dominated by Chironomidae, independently from their availability on the substratum. This finding is discussed on the basis of ecological and ethological considerations.

Keywords: *Perla grandis*, gut contents, diet, Plecoptera, NW Italy

Introduction

The use of benthic invertebrates in biological monitoring has produced an evident increase in the knowledge of taxonomy and systematic of these organisms (Merritt & Cummins 1996). Unfortunately, the knowledge of great part of their ecology is still incomplete. In particular, the analysis of the diet and the study of trophic ecology could represent important source of data, that can be employed to improve our knowledge of the ecology of different species (Elliott 2000), the structure of stream food webs (Huhta *et al.* 1999), and the functional organization of lotic systems (Rosi-Marshall & Wallace 2002).

Plecoptera constitute a numerically and ecologically significant component in freshwater ecosystem, mainly in running waters of all sizes, all over the world (Zwick 2004). For the most part, larvae are either primarily carnivores (feeding largely upon other stream invertebrates), as are many Systellognatha stoneflies and Euholognatha, or primarily leaf shredders and detritivores, as are many Euholognatha (McCafferty 1998; Pattée *et al*. 2000). Large sized stoneflies, such as Perlidae and Perlodidae, represent the main group of predators in many small, fishless lotic systems where they act as top-down control elements in the benthic community (Wipfli & Gregovich 2002). Among this group, feeding seems to have little or no importance in the adult life (Tierno de Figueroa & Sánchez-Ortega 1999; Tierno de Figueroa & Fochetti 2001), because no solid food has been found in the gut of any perlid or large perlodid species (Tierno de Figueroa & Fochetti 2001; Fenoglio & Tierno de Figueroa 2003). Apparently, adults rely on the rich diet of the preimaginal stages (Fenoglio 2003).

Perla grandis (Rambur, 1841) (= *P. maxima* sensu Auctorum nec Scopoli, 1763) is a typical rheophilous-mesothermal species. This species belonging to the Middle-Sud-European chorological group and is widely distributed in the Apennines of northwestern Italy (Ravizza 1974; Ravizza & Ravizza Dematteis 1977; Ravizza Dematteis & Ravizza 1994).

The aim of our study was to investigate the diet of the nymphs of *P. grandis* in a fishless, first order Apenninic stream, where this species constitutes the top predator taxon.

Material and Methods

In the days 09-10 January 2006, 50 *P. grandis* nymphs were collected in a 150 m uniform riffle of the Berga stream (Carrega Ligure, High Borbera Valley, UTM 509.852 - 4.939.795; 780 m a.s.l, fig.1). In the sampling station, Rio Berga is a typical Apennine lotic environment, characterized by elevate slope, coarse riverbed and fast-swallow flowing waters. Riparian vegetation is mainly constituted by *Quercus* sp., *Castanea sativa*, *Alnus glutinosa* and some specimens of *Fagus sylvatica*. In the upper Rio Berga catchment there are no anthropic activities, so that this lotic system shows a good environmental quality, corresponding to an environment without trace of human-inducted alteration (first class in the Italian Extended Biotic Index, Ghetti 1997). Main environmental characteristics and abiotic parameters (measured by Eijkelkamp 13.14 and 18.28 portable instruments) are reported in tab. 1. Samplings were realized early in the morning, because Systellognatha are considered to be chiefly nocturnal feeders (Vaught & Stewart 1974). Moreover, using a Surber net (20 x 20 cm; mesh 255 μm), we collected 13 samples in the same reach to assess the presence and abundance of the taxa of the natural benthic invertebrate population. Samples were preserved in 90% ethanol. In the laboratory, all organisms were counted and identified to genus or species level, except for: Lumbricidae, Lumbriculidae, Niphargidae and early instars of some Diptera, which were identified to family or sub-family level.

Total length of *P. grandis* nymphs was measured (0.1 mm accuracy) and processed to assess food consumption by means of gut content analysis. The most common method to measure patterns of differential predation by aquatic insects is the examination of relative numbers of prey remains in predator stomach contents, and the comparison of those numbers to prey density estimated from samples from the predator's habitat (Peckarsky 1984; Peckarsky & Penton 1989).

To analyze the dimensional shift in food preference, we separately considered gut contents of smaller nymphs (body length < 15.0 mm) and larger nymphs (body length > 15.0 mm). During the analysis of small specimens, gut content of nymphs was studied using a trasparency technique which has also been used to study feeding in imaginal stages (Fenoglio & Tierno de Figueroa 2003). We use Hertwig's liquid, a modification of Hoyer's liquid, which clears the body wall, thus allowing direct examination of the gut content without dissection. To examine larger specimens we removed guts and the contents of the alimentary canal were analysed by using the transparency method for slides. Fragments of animal prey were identified to the lowest taxonomic level possible. Identification of prey was based on sclerotized body parts, particularly head capsules, mouthparts and leg fragments. We also differentiated four fractions: vegetable matter (diatoms, algae and fungi), animal matter, unidentified organic matter (FPOM-fine particulate organic matter) and mineral matter (sand). During the laboratory-phase of the study we use a NIKON SMZ 1500 light microscope (60-100 x) with JVC TK-C701EG videocamera.

To investigate the existence of feeding preferences, gut contents were compared with the natural composition and abundance of macroinvertebrate communities in the riverbed using the trophic electivity index of Ivlev (1961):

$$E = (ri-pi)/(ri+pi)$$

where ri = the proportion of ingested species and pi = the relative abundance in the benthic community. The index ranges from −1.0 to 1.0. A value of −1.0 means total avoidance, 1.0 indicates preference and 0 indicates indifference. The presence of algae and detritus (e.g. fragments of terrestrial vegetation) was recorded and quantified on a scale of 0-3 (0 = no presence; 3 = the highest abundance class).

Results

We examined the gut contents of 50 *Perla grandis* nymphs; medium length of *P. grandis* immature stages was 19.4 mm, with a minimum of 5.0 mm and a maximum of 27.2 mm. We divided the nymphs in two dimensional classes: “small specimens” (n=13, total length < 15.0 mm) and “large specimens” (n=37, total length >15.0 mm).

In total we collected and identified 1115 aquatic invertebrates belonging to 38 taxa. Taxonomical list and relative abundance are reported in tab. 2. Analysing the entire dataset, we found that 6% of guts contained sand and that 12 % of guts were completely empty. Considering the two dimensional classes, we detected an evident difference: smallest nymphs showed a detritivorous diet (53.8 % of guts), with sporadic ingestions of midges and small mayflies while largest ones are carnivorous, feeding on different invertebrate species (fig. 2). In largest individuals we detected an evident trophic preference for Chironomidae. Midges represented the most abundant item in the diet of *P. grandis*, with a relative importance that is noticeably greater than their abundance in the substratum, and an high value of the Ivlev’s electivity index (47.8 – fig. 3). Gut content analysis revealed that Chironomidae were ingested also entire, without chewing: in facts, we found head capsules and fragments but also 13 complete larvae. Ephemeroptera Baetidae and Plecoptera Leuctridae, also if well represented and abundant in the river bottom, were ingested with a minor frequency, such as Ephemeroptera Heptageniidae, Leptophlebiidae and Trichoptera Hydropsychidae. Other groups, also if well represented in the natural environment, were always avoided, such as different families of Coleoptera (Hydraenidae and Dryopidae, for example) and Crustacea Niphargidae.

Discussion

Many studies noticed that some predaceous stoneflies undergo changes in feeding pattern as they grow, moving from a detritus based diet to a strictly carnivorous alimentation (Bo & Fenoglio 2005). Our study confirms this findings, providing new data about the increase of trophic spectrum during the ontogenesis of great Systellognatha nymphs. The increase in number and type of preys is probably related not only to a dimensional increase of the nymphs (and the related ability in to catch more prey types) but also to an increase in the energetic demand in organisms that don't feed in the adult stage and rely on the preimaginal stages diet to perform gametogenesis.

Another interesting finding of this study is the evidence of clear trophic selection mechanisms: Chironomidae, also if represented only a little component of the benthic coenosis of Rio Berga, resulted the most present item in the food of *P. grandis* nymphs. The importance of Chironomidae as food resource were also underlined in Neotropical environments by Fenoglio (2003) in the genus *Anacroneuria* sp. (Perlidae), and in Neartic environments in *Acroneuria californica* (Perlidae), as reported by Monakof 2003. This latter species feed mainly on Chironomidae, various Ephemeroptera and Trichoptera, but electivity index result positive only for midge larvae.

This result led to formulate the hypothesis that midges represent the preferred food item for many Systellognatha: probably they offer a good combination of different elements, such as mobility, dimensions and microhabitat overlap.

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Table 1: Some environmental characteristics and abiotic parameters of stream reach during field sampling

Stream order	1th
Altitude (m asl)	800
Width (cm)	170 ± 20
Depth (cm)	15 ± 5
Water temperature (°C)	3.8 ± 0.5
Air Temperature (°C)	- 2.0 ± 1.0
pH	7.9
Conductivity (µS/cm)	25.0
O_2 (mg/l)	102.0

Table 2: Percent relative abundance (% value in the community) for macroinvertebrates collected in the natural riverbed in the Rio Berga (NW Italy).

Taxa		*relative* **abundance %**	**FFG***
Plecoptera			
Perlidae	*Perla grandis*	4,48	P
	Perla marginata	0,90	P
	Dinocras cephalotes	3,41	P
Perlodidae	*Isoperla* sp.	7,00	P
Leuctridae	*Leuctra* sp.	14,53	Sh
Nemouridae	*Nemoura* sp.	8,70	Sh
	Protonemura sp.	0,18	Sh
	Amphinemura sp.	1,35	Sh
Chloroperlidae	*Chloroperla* sp.	1,08	P
Taeniopterygidae	*Brachyptera* sp.	1,88	Sh
Ephemeroptera			
Heptageniidae	*Ecdyonurus* sp.	7,62	Sc
	Rhithrogena sp.	6,46	Sc
	Epeorus sylvicola	2,06	Sc
Baetidae	*Baetis* sp.	15,61	Cg
Ephemerellidae	*Torleya major*	0,54	Cg
Leptophlebiidae	*Habroleptoides* sp.	5,20	Cg
Trichoptera			
Hydropsychidae	*Hydropsyche* sp.	7,09	F
Philopotamidae	*Philopotamus* sp.	0,18	F
Rhyacophilidae	*Rhyacophila* sp.	0,72	P
Sericostomatidae	*Sericostoma* sp.	0,27	Sh
Odontoceridae	*Odontocerum albicorne*	0,09	Sh
Limnephilidae	*Potamophylax cingulatus*	1,70	Sh
	Undet.	0,18	Sh
Diptera			
Chironomidae	*Tanypodinae*	0,36	P
	Chironominae	1,43	Cg
Tipulidae	*Tipula* sp.	1,08	Sh
Athericidae	*Atherix* sp.	1,61	P
Empididae	*Emerodrominae*	0,09	P
Limoniidae		0,18	P
Coleoptera			
Hydraenidae	*Haenydra devillei*	0,54	Sc
Helodidae (larvae)		0,18	Sh
Dryopidae	*Helichus substriatum*	0,09	Sh
Crustacea			
Niphargidae		1,61	Cg
Annellidae			
Erpobdellidae	*Dina lineata*	0,45	P
Lumbriculidae		0,54	
Lumbricidae		0,09	
Lumbricidae	*Eiseniella tetraedra*	0,36	Cg
Arachnida			
Hydracarina		0,18	P

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

Fig. 1: Rio Berga and sampling station.

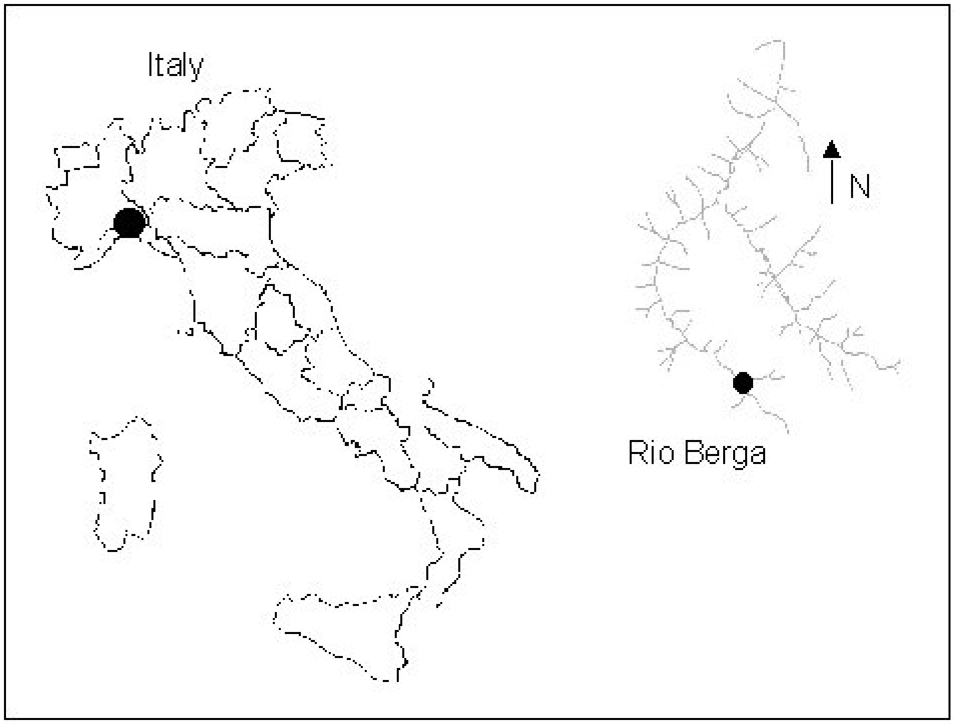

Fig. 2: Relative abundance of prey items in the *Perla grandis* nymphs.

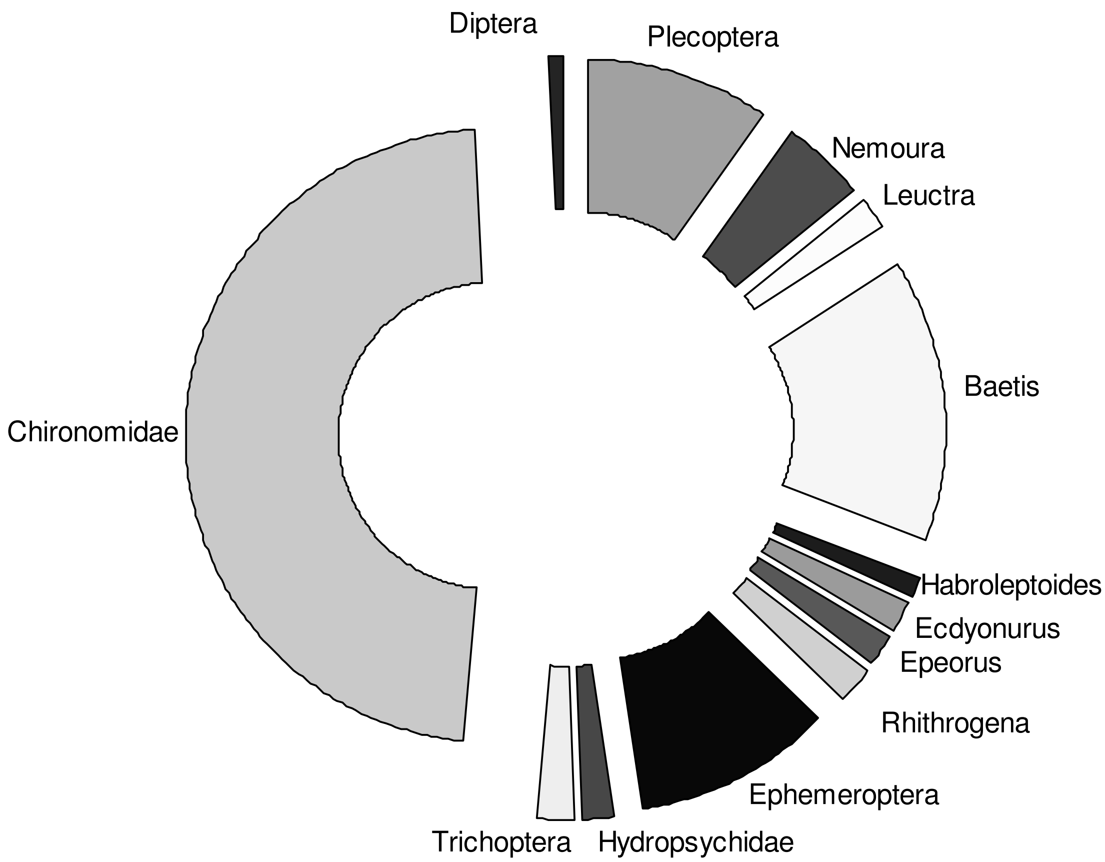

Fig. 3: Electivity index (E*) for the macroinvertebrate taxa in the guts of the three size groups of *Perla grandis* nymphs.