Boletín de la Sociedad Entomológica Aragonesa (S.E.A.), nº **46** (2010) : 363−366.

NYMPHAL DIET OF TWO PERLIDAE SPECIES (INSECTA: PLECOPTERA) IN SOUTHERN APENNINES (CALABRIA, ITALY)

Luana Gallo[1], Lucio Lucadamo[1], Manuel Jesús López-Rodríguez[2,3], José Manuel Tierno De Figueroa[2], Tiziano Bo[3] & Stefano Fenoglio[3]

[1] Dipartimento di Ecologia, Università della Calabria, Via P. Bucci, Cubo 6b, 87036 Arcavacata di Rende, Italy.
[2] Departamento de Biología Animal, Facultad de Ciencias, Universidad de Granada, Campus Fuentenueva s/n, 18071, Granada, Spain.
[3] Dipartimento di Scienze dell'Ambiente e della Vita, Università del Piemonte Orientale, Via Teresa Michel, 11, 15121 Alessandria, Italy − fenoglio@unipmn.it

Abstract: The nymphal feeding of *Perla grandis* and *Dinocras cephalotes* was analyzed in a stream from southern Apennines (Argentino stream, Calabria, Italy). Both species behave as predators, feeding mainly on Ephemeroptera Baetidae and Diptera Chironomidae. Data analyses showed high niche overlapping in relation to feeding habits. Shifts on type of prey ingested were just found in *D. cephalotes*, where a slight correlation between size and content in *Baetis* sp. was detected. This study represents the first research on this topic in lotic systems from southern Italy.
Key Words: Stoneflies, *Perla grandis*, *Dinocras cephalotes*, gut contents, Argentino stream

Dieta ninfal de dos especies de Perlidae (Insecta: Plecoptera) en el sur de los Apeninos (Calabria, Italia)
Resumen: Se analizó la dieta ninfal de *Perla grandis* y *Dinocras cephalotes* en un arroyo del sur de los Apeninos (arroyo Argentino, Calabria, Italia). Ambas especies se comportan como depredadoras, alimentándose principalmente de Ephemeroptera Baetidae y Diptera Chironomidae. Los análisis de datos mostraron un alto solapamiento de nicho en relación a los hábitos alimenticios de ambas especies. Tan sólo en *D. cephalotes* se encontraron cambios en las presas ingeridas, en la cual se detectó una ligera correlación entre el tamaño y el contenido de *Baetis* sp. Este estudio representa la primera investigación sobre este tema en sistemas lóticos del sur de Italia.
Palabras clave: Plecópteros, *Perla grandis*, *Dinocras cephalotes*, contenidos digestivos, Río Argentino.

Introduction

In some cold and oxygenated streams stoneflies are one of the top predators, feeding mainly on other benthic invertebrates. In fact, many studies of predation in streams have been conducted on benthic-feeding Plecoptera (Peckarsky, 2006). Particularly in Europe, among the families of this insect order, Perlidae and Perlodidae are those that act mainly as predators (Hynes, 1976; Graf *et al.*, 2009).

Perla grandis Rambur, 1842 and *Dinocras cephalotes* (Curtis, 1827) are two Perlidae species widely distributed in Europe, that are commonly found coexisting in the same lotic environments. Thus, both species prefer streams characterized by cold, high-oxygenated waters, stony-bed rivers and permanent flow (Fochetti & Tierno de Figueroa, 2008).

Data on diet and trophic behaviour of these species are available from studies conducted in different parts of Northern and Central Europe (Berthélemy & Lahoud, 1981; Lillehammer, 1988; Lucy *et al.*, 1990; Elliott, 2003; Bo & Fenoglio, 2005; Cammarata *et al.*, 2007; Fenoglio *et al.*, 2007), but they are scarce in southern Europe. In fact the only study on this topic made in the south of European peninsulas was recently performed in a high mountain river of Southern Spain (Bo *et al.*, 2008). The trophic spectrum of both species includes many macroinvertebrate preys, mainly Ephemeroptera and Diptera but also Trichoptera and other groups, with variations among different populations and ontogenetic shift during the nymphal development.

Despite available data on trophic behaviour, no information exists on the role of these species in streams from southern parts of Italy, which present some hydrological peculiarities related to their characteristic climate (e.g. high summer temperatures, low rainfall and seasonal drought).

In the Apennines, the only data available on feeding habits of Perlidae species came from the North (Bo & Fenoglio, 2005; Cammarata *et al.*, 2007; Fenoglio *et al.*, 2007).

The aim of this research is to describe the diet of *P. grandis* and *D. cephalotes* in a typical Calabrian Apenninic stream, comparing the results with previously known data from other geographical areas, as a first step to understand the complex functionality of these peculiar and unstudied lotic systems.

Material and methods

Nymphs of the perlids *P. grandis* and *D. cephalotes* were collected in Argentino stream (Calabria, 39º48'13.05"N, 15º52'23.09"E, 47 m a.s.l.). The Argentino is a small river (18 km long) that originates in the Pellegrino massif, in the Pollino National Park. This stream has torrential character, and very high environmental quality. Dense woodlands (mainly composed by *Quercus cerris*, *Quercus pubescens*, *Castanea sativa* and *Alnus cordata*) cover the catchment. As a typical Mediterranean river, the Argentino has a hydrologic cycle characterized by a maximum flow during spring and water shortages in summer, due to low rainfall and high temperatures.

Samplings were made with a Kick net (250 µm mesh size) in October 3rd 2007. This stream is characterized by good environmental quality. The streambed is composed mainly by rocks (40%) and pebbles (40%), followed by gravel (15%) and sand (5%), with an average width of 6.5 m and an average depth of 30 cm. Macrophytes are absent from the study site, while periphyton is present only locally. The river channel, in the studied reach, is quite natural and is surrounded by well-developed riparian vegetation, constituted mainly by *Salix alba*, *Carpinus betulus*, *Alnus glutinosa* and *A. cordata.* Some abiotic parameters were recorded in the sampling date, and are reported in Table I.

Table I. Abiotic parameters of stream reach during field sampling

Physical and chemical parameters	Mean ± SD
Water temperature (°C)	12.39 ± 1.85
Conductivity (µS/cm)	371.38 ± 22.41
pH	8.05 ± 0.22
O_2 (mg/l)	10.48 ± 0.81
O_2 saturation	101.49 ± 7.91
COD (mg/l)	17.43 ± 7.12
BOD_5 (mg/l)	1.89 ±1.03
Suspended solids (mg/l)	3.17 ± 1.66
Hardness (mg/l $CaCO_3$)	225.02 ± 9.47
Ammonia Nitrogen (mg/l)	7.22 ± 11.18

All macroinvertebrates, in addition to both Perlidae species, were also collected and preserved in 85% ethanol with the aim of describing the community. In the laboratory, all organisms were counted and identified generally to family or genus level (Table II). In laboratory we measured total length (from the tip of labrum to the last urite) of each Perlidae nymph using a Nikon SMZ 1500 stereomicroscope (0.1 mm accuracy) with a JVC TK-C701EG videocamera coupled to a Samsung 36" LCD.

Nymphs were later processed to assess food consumption by means of two methods of gut content analyses. For the small ones (< 10 mm total length), contents of alimentary canal were analysed following the transparency method proposed by Bello & Cabrera (1999) and widely employed in stonefly feeding studies (Derka *et al.,* 2004; Fenoglio *et al.,* 2007; Bo *et al.*, 2008; López-Rodríguez *et al.*, 2009) with slight variations: each nymph was introduced in a vial with Herwitgs´ liquid for 48 hours at 18-25ºC and, afterwards, cleared individuals were collocated on a slide glass with a cover glass on. For large nymphs (> 10 mm total length), we removed the gut after dissected the specimens; the contents of the alimentary canal were extracted and analysed. No differences are obtained using both variations of the method, as previously observed by the authors (Bo *et al*., 2008). Then, in both cases, we used a Zeiss Axiolab microscope for identifying the different components of the gut contents. For identifiable animals, each item was sorted out at the highest possible level and counted. 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. For the rest of content, six items were differentiated: FPOM (fine particulate organic matter), CPOM (coarse particulate organic matter), pollen, filamentous algae, animal matter (unidentifiable animal remains), and sand. For these six items, we quantified the relative abundance that they occupied in the guts estimating the area percentages that they occupied as in Bo *et al*. (2008). Three categories were established from 1 to 3, being 1 scarcely present and 3 maximum occupation.

Statistical analyses were performed with R software (R Development Core Team, 2009). Median, mean, standard deviation, minimum, maximum, presence (i.e. number of guts that contained a given item) and % presence (i.e. percentage of guts that contained a given item were calculated). None of the variables followed a normal distribution (Kolmogorov-Smirnov with $p< 0.05$ in every case) so non-parametric tests were used. Kendall *tau* was used to assess correlations between total length and the presence of prey in the guts.

Table II. Number of individuals of each taxa collected in the stream.

Argentino stream		N
Plecoptera		
Leuctridae	*Leuctra* sp.	79
Nemouridae	*Protonemura* sp.	51
Perlidae	*Perla grandis*	42
	Dinocras cephalotes	52
Perlodidae	*Isoperla* sp.	1
Ephemeroptera		
Baetidae	*Baetis* sp.	586
Heptageniidae	*Ecdyonurus* sp.	6
	Epeorus sp.	55
	Rhithrogena sp.	56
Ephemerellidae	*Ephemerella* sp.	62
Leptophlebiidae	*Habroleptoides* sp.	2
Trichoptera		
Limnephilidae		59
Sericostomatidae	*Sericostoma* sp.	124
Odontoceridae	*Odontocerum* sp.	10
Hydropsychidae	*Hydropsyche* sp.	342
Rhyacophilidae	*Rhyacophila* sp.	7
Philopotamidae	*Wormaldia* sp.	4
	Philopotamus sp.	4
Glossosomatidae		3
Diptera		
Chironomidae	undet.	63
	Tanypodinae	3
Simuliidae		17
Tabanidae		5
Tipulidae		5
Ceratopogonidae		7
Stratiomyidae		3
Athericidae	*Atherix* sp.	40
Limoniidae		98
Empididae		2
Dixidae	*Paleodixa* sp.	1
Blephariceriidae		4
Coleoptera		
Elmidae	adults	21
	larvae	25
Dryopidae	*Pomatinus substriatus*	5
Hydraenidae	adults	45
Gyrinidae	larvae	3
Helodidae	larvae	1
Nematomorpha		
Gordiidae	*Gordius* sp.	1
Platelminta		
Dugesiidae	*Dugesia* sp.	5
Oligochaeta		
Lumbriculidae		14
Lumbricidae	undet.	14
	Eiseniella tetraedra	5
Naididae		3
Aracnida		
Hydracarina		2
Gastropoda		
Hydrobioidea	undet.	14
Lymnaeidae	*Lymnaea* sp.	1
Ancylidae	*Ancylus fluviatilis*	10
Nematoda	undet.	1

Table III. Diet characterization of the studied species in the sampling site.

	Dinocras cephalotes								*Perla grandis*							
	N	Min	Median	Mean	SD	Max	Presence (N)	Presence (%)	N	Min	Median	Mean	SD	Max	Presence (N)	Presence (%)
Protonemura sp.	44	0.000	0.000	0.045	0.211	1.000	2	4.545	33	-	-	-	-	-	-	-
Plecoptera undetermined	44	-	-	-	-	-	-	-	33	0.000	0.000	0.030	0.174	1.000	1	3.030
Baetis sp.	44	0.000	0.000	0.682	1.177	6.000	16	36.364	33	0.000	0.000	0.879	1.386	6.000	15	45.455
Ephemeroptera undetermined	44	0.000	0.000	0.068	0.255	1.000	3	6.818	33	0.000	0.000	0.273	0.517	2.000	8	24.242
Heptageniidae	44	-	-	-	-	-	-	-	33	0.000	0.000	0.091	0.522	3.000	1	3.030
Hydropsychidae	44	0.000	0.000	0.023	0.151	1.000	1	2.273	33	-	-	-	-	-	-	-
Philopotamidae	44	0.000	0.000	0.068	0.255	1.000	3	6.818	33	-	-	-	-	-	-	-
Trichoptera undetermined (larvae)	44	0.000	0.000	0.068	0.255	1.000	3	6.818	33	-	-	-	-	-	-	-
Trichoptera undetermined (pupae)	44	0.000	0.000	0.023	0.151	1.000	1	2.273	33	0.000	0.000	0.030	0.174	1.000	1	3.030
Chironomidae	44	0.000	0.000	0.864	1.456	6.000	19	43.182	33	0.000	0.000	0.970	1.357	5.000	15	45.455
Simuliidae	44	-	-	-	-	-	-	-	33	0.000	0.000	0.030	0.174	1.000	1	3.030
Stratiomyidae	44	0.000	0.000	0.023	0.151	1.000	1	2.273	33	-	-	-	-	-	-	-
Limoniidae	44	0.000	0.000	0.045	0.211	1.000	2	4.545	33	-	-	-	-	-	-	-
Diptera undetermined (larvae)	44	0.000	0.000	0.023	0.151	1.000	1	2.273	33	-	-	-	-	-	-	-
Diptera undetermined (pupae)	44	0.000	0.000	0.023	0.151	1.000	1	2.273	33	-	-	-	-	-	-	-
Elmidae	44	0.000	0.000	0.023	0.151	1.000	1	2.273	33	-	-	-	-	-	-	-
Animal matter	44	0.000	1.000	0.909	0.858	3.000	28	63.636	33	0.000	1.000	1.030	0.918	3.000	22	66.667
CPOM	44	0.000	0.000	0.091	0.291	1.000	4	9.091	33	0.000	0.000	0.182	0.584	3.000	4	12.121
FPOM	44	0.000	0.000	0.341	0.608	3.000	13	29.545	33	0.000	0.000	0.424	0.561	2.000	13	39.394
Sand	44	0.000	0.000	0.250	0.534	2.000	9	20.455	33	0.000	0.000	0.182	0.465	2.000	5	15.152
Pollen	44	-	-	-	-	-	-	-	33	0.000	0.000	0.061	0.242	1.000	2	6.061
Algae	44	0.000	0.000	0.045	0.211	1.000	2	4.545	33	-	-	-	-	-	-	-

Levin's index for niche breadth (Levins, 1968) was also calculated, and the Hurlbert´s standardization (Hurlbert, 1978) was applied (Krebs, 1999). The scale of the latter index varies between 0 and 1: the higher the value the higher the niche breadth. Though no quantitative samples were collected, we calculated relative abundances of prey and so calculated these indexes according to relative abundances. The Levin's index (B) and the Hurlbert's standardization (B_A) are calculated as follow:

$$B = 1 / (\sum p_j^2)$$

$$B_A = (B-1) / (n-1)$$

where: p_j= fraction of items in the diet belonging to food category j, and

n= number of possible resource states (items)

In order to assess niche overlap as predators (considering only ingested prey items) between species we used Simplified Morisita Index proposed by Horn (1966) (Krebs, 1999), assuming relative abundances from the qualitative sampling:

$$C_H = [2\sum_i^n p_{ij} \cdot p_{ik}] / [\sum_i^n p_{ij}^2 + \sum_i^n p_{ik}^2]$$

where C_H = Simplified Morisita Index of niche overlap between species j and k,

p_{ij} = proportion resource i is of the total resources used by species j,

p_{ik} = proportion resource i is of the total resources used by species k,

This index ranges from 0 to 1, where 0 means no overlap, and 1 means total niche overlap.

Results

In total 48 macroinvertebrate taxa were collected and identified in the sampling station (Table II). Regarding Perlidae, we analyzed 94 specimens, 42 *P. grandis* and 52 *D. cephalotes.* Because both perlid species are semivoltine (Fochetti & Tierno de Figueroa, 2008; Graf *et al.*, 2009), different size classes were present. Some kind of gut content was found in 33 *P. grandis* and in 44 *D. cephalotes*. The main prey of both species were Chironomidae and *Baetis* sp. (Table III), though *D. cephalotes* fed on a total of 13 different prey while *P. grandis* did it on a total of seven. A slight positive correlation was found between size and presence of *Baetis* sp. in the gut of *D. cephalotes* (Kendall´s *tau*= 0.24, p< 0.05).

Apart from animal matter, both species ingested also detritus, fine particulate organic matter (FPOM), coarse particulate organic matter (CPOM), pollen, algae and sand. FPOM and sand were frequently ingested by both species, and also CPOM was highly present in the guts of *P. grandis* nymphs (Table III).

The niche breadth of both species was very low, a little bit higher in *P. grandis* than in *D. cephalotes* (B_A = 0.325 and 0.216, respectively). *Dinocras cephalotes* used more frequently just two resources (Chironomidae and Baetidae) and *P. grandis* three (Chironomidae, Baetidae and other undetermined Ephemeroptera). Moreover they had a very high niche overlap (Simplified Morisita index= 0.977).

Discussion

Despite these species have been already studied in some lotic systems of the European hydrographic network, this study supposes the first approach done in a typical low altitude stream from southern Italy. These streams are characterized by particular environmental conditions as high water and air temperature fluctuations, almost inexistent slope and so low water velocity.

As previously pointed out, both species fed mainly in the same two resources, Chironomidae and Baetidae, which were present in more than 35-40 % of the studied guts, thought several other prey items constituted part of their diet. In the case of *P. grandis*, also undetermined Ephemeroptera were relatively important in the diet. Despite quantitative samplings were not carried out, Baetidae seems to be the most abundant macroinvertebrate taxa in the stream, but Chironomidae are not very abundant (Table II). Thus, these species would feed mainly on one of the highest abundant potential prey (Baetidae) and in other relatively little abundant (Chironomidae), probably due to the facility of capture of these organism (Diptera of small size and little capacity of scape). These results coincide widely with those previously found in other studies in European perlids (Berthélemy & Lahoud, 1981; Lillehammer, 1988; Lucy *et al*., 1990; Elliott, 2003; Fenoglio *et al*., 2007) where Chironomidae and *Baetis* sp. were the main eaten prey. Concretely, the scarce studies made in Northern Apenninic streams show similar ingestion patterns (Bo & Fenoglio, 2005; Cammarata *et al*., 2007), although with some differences. Thus, *P. grandis* (the only species coinciding with those of our study) in the North ingested a higher proportion of Chironomidae, and a relatively lower quantity of *Baetis* sp. and other Ephemeroptera (Cammarata *et al*., 2007; Fenoglio *et al*., 2007).

Regarding the niche breadth, its low value depends on the very low number of prey consumed (two or three respectively for *D. cephalotes* and *P. grandis*), but, as these prey coincide, the niche overlap is very high. These results are similar to those found in a study conducted in Southern Iberian Peninsula with *D. cepahlotes* and *P. bipunctata* Pictet, 1833 (Bo *et al*., 2008).

The relatively high proportion of guts that contained FPOM and sand (and CPOM in *P. grandis*) could be explained as incidental ingestion when predating or as part of the gut content of the prey. Nevertheless, some Perloidea predators also ingest vegetal matter and detritus to complete their diets (Stewart & Stark, 2002), and this could be the case.

Cited references

BELLO, C.L. & M. I. CABRERA 1999. Uso de la técnica microhistológica de Cavender y Hansen en la identificación de insectos acuáticos. *Bol. Entomol. Venez.*, **14**: 77-79.

BERTHELEMY, C. & M. LAHOUD 1981. Regimes alimentaires et pieces buccales de quelques Perlodidae et Perlidae des Pyrenées (Plecoptera). *Ann. Limnol.*, **17**: 1-24.

BO, T. & S. FENOGLIO 2005. Age-related shift in the diet of *Perla marginata* in a woodland Apenninic creek of NW Italy (Plecoptera: Perlidae). *Entomol. Gen.,* **28**: 147-154.

BO ,T., S. FENOGLIO, M.J. LÓPEZ-RODRÍGUEZ & J.M. TIERNO DE FIGUEROA 2008. Trophic behavior of two Perlidae species (Insecta, Plecoptera) in a river in southern Spain. *Internat. Rev. Hydrobiol.*, **93**: 167-174.

CAMMARATA, M., S. FENOGLIO, M.J. LÓPEZ-RODRÍGUEZ, T. BO & J.M. TIERNO DE FIGUEROA 2007. Prey selection of *Perla grandis* (Rambur, 1841) nymphs (Plecoptera: Perlidae) in Borbera stream (NW, Italy). *Boletín de la Sociedad Entomológica Aragonesa (S.E.A.)*, **41**: 271-274.

DERKA, T., J.M. TIERNO DE FIGUEROA & I. KRNO 2004. Life cycle, feeding and production of *Isoptena serricornis* (Pictet, 1841) (Plecoptera, Chloroperlidae). *Internat Rev. Hydrobiol*. **89**: 165-174.

ELLIOTT, J. M. 2003. A comparative study of the functional response of four species of carnivorous stoneflies. *Freshwat. Biol.*, **48**: 191-202.

FENOGLIO, S., T. BO, M. PESSINO & G. MALACARNE 2007. Feeding of *Perla grandis* nymphs (Plecoptera: Perlidae) in an Apennine first order stream (Rio Berga, NW Italy). *Ann. Soc. Entomol. Fr.,* **43**: 221-224.

FOCHETTI, R. & J.M. TIERNO DE FIGUEROA 2008. *Plecoptera. Fauna d'Italia*. Edizioni Calderini de Il Sole 24 ore, Milano.

GRAF, W., A.W. LORENZ, J.M. TIERNO DE FIGUEROA, S. LUCKE, M.J. LOPEZ RODRIGUEZ & C. DAVIES 2009. *Distribution and ecological preferences of Europea freshwater organisms. Volume 2. Plecoptera.* Schmidt-Kloiber A., Hering D. (eds). Pensoft Sofia-Moscow.

HORN, H.S. 1966. Measurement of "overlap" in comparative ecological studies. *Am. Midl. Nat.*, **100**: 419-424.

HURLBERT, S.H. 1978. The measurement of niche overlap and some relatives. *Ecology,* **59**: 67-77.

HYNES, H.B.N. 1976. Biology of Plecoptera. *Ann. Rev. Entomol.* **21**: 135-153.

KREBS, C.J. 1999. *Ecological methodology*. Addison Wesley Longman.

LEVINS, R. 1968. *Evolution in changing environments: some theoretical explorations*. Princeton University Press, Princeton, N. J.

LILLEHAMMER, A. 1988: Stoneflies (Plecoptera) of Fennoscandia and Denmark. *In*: Fauna Entomologica scandinavica, 21. Scandinavian Science Press Ltd., Leiden, Sweden.

LÓPEZ-RODRÍGUEZ, M.J., J.M. TIERNO DE FIGUEROA, S. FENOGLIO, T. BO & J. ALBA-TERCEDOR 2009. Life strategies of 3 Perlodidae species (Plecoptera) in a Mediterranean seasonal stream in Southern Europe. *J. N. Am. Benthol. Soc.*, **28**: 611-625.

LUCY, F., M.J. COSTELLO & P.S. GILLER 1990. Diet of *Dinocras cephalotes* and *Perla bipunctata* (Plecoptera, Perlidae) in a south-west Irish stream. *Aquat. Insects*, **12**: 199-207.

PECKARSKY, B.L. 2006. *Predator-prey interactions*. Pp. 561-583. *In:* Hauer F.R. and Lamberti G.A. (eds) Methods in stream ecology 2nd ed. Elsevier Amsterdam.

R DEVELOPMENT CORE TEAM 2009. *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna. http://www.R-project.org.

STEWART, K.W. & B.P. STARK 2002. *Nymphs of North American stonefly genera (Plecoptera)*. 2nd ed. The Caddis Press, Columbus, Ohio.