WINTER PREY PREFERENCE OF *PERLODES MICROCEPHALUS* (PICTET, 1833) (PLECOPTERA, PERLODIDAE) NYMPHS IN AN APENNINIC CREEK, NORTHWESTERN ITALY[1]

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ABSTRACT: The feeding habits of *Perlodes microcephalus* nymphs (Plecoptera, Perlodidae) have been investigated in Caramagna, an Apennine creek located in northwestern Italy. This large species is one of the most representative carnivorous stonefly nymphs in this area, where it is a top-bottom predator in many fishless creeks. Despite its ecological importance, little is known about its trophic ecology. In this study, we examined the gut contents of 35 nymphs during the winter of February 2005. We detected an evident trophic preference for the following taxa: Chironomidae (Diptera) as well as Psychomidae, Glossosomatidae, *Hyporhyacophila* sp., and other Trichoptera. This preference appears to be independent of the prey's availability in the substratum. Rheostenic taxa, also abundant and widespread in the substratum, were almost absent or seldom found in the diet of *P. microcephalus*. These results suggest that the trophic preferences of *P. microcephalus* are more dependent on prey microhabitat preference than on prey abundance.

KEY WORDS: *Perlodes microcephalus,* Plecoptera, Perlodidae feeding habit, gut contents, northwestern Italy

Monakov (2003) stated that "there is no discipline in hydrobiology that does not require a study of the feeding and nutrition of aquatic animals." Improving our knowledge about feeding behavior and trophic ecology is indispensable to better understand applied and basic elements of stream ecology. For example, increased human influence in aquatic ecosystems lead to changes in feeding and growth of aquatic invertebrates (Broekhuizen et al., 2001), altering composition and structure of benthic communities. Furthermore, studies about feeding habits take an evident interest in an auto-ecological perspective (Elliott, 2003; 2004). In the last decades, there was a growing interest in the trophic ecology of aquatic insects, especially for some groups such as shredders, for their importance in the metabolism of allochtonous organic inputs (Webster and Benfield, 1986), and predators, for their role as top-down control elements in benthic communities (Molles and Pietruszka, 1987; Wipfli and Gregovich, 2002).

In lentic habitats and low flowing waters, large invertebrate predators are mainly represented by the Odonata, Anisoptera and Zygoptera; Coleoptera, Hydroadephaga, as well as by the Hemiptera, Heteroptera. On the other hand, in lotic systems the dominant predator group is represented by the Plecoptera Systellognatha (Allan, 1995). Among the latter, Chloroperlidae, Perlodidae, and Perlidae have carnivorous nymphs of moderate to large size. In adult Perlidae and large Perlodidae, feeding seems to have little or no importance (Tierno de

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Figueroa and Sánchez-Ortega, 1999; Tierno de Figueroa and Fochetti, 2001). In fact, little if any solid food has been found in the gut of some perlid or large perlodids (Tierno de Figueroa and Fochetti, 2001; Fenoglio and Tierno de Figueroa, 2003). Apparently, adults rely on the rich diet of the preimaginal stages (Fenoglio, 2003).

Perlodes microcephalus (Pictet) (Plecoptera: Perlodidae) is a reophilous mesothermal species with a wide distribution in Europe. As all Perlodidae (Merritt and Cummins, 1996), *P. microcephalus* nymphs are known to be active predators. This species is characterised by a very rapid growth rate, reaching 18-28 mm long in only one year (Hynes, 1993). Previous studies highlighted that *P. microcephalus* feeds mostly at night, mainly on Simuliidae, Chironomidae (both Diptera) and on Ephemeroptera (Berthélemy and Lahoud, 1981; Elliott, 2000). In an experiment conducted in laboratory conditions, Elliott (2003) compared predator-prey interactions of four large-sized, carnivorous Plecoptera nymphs: two Perlodidae (*P. microcephalus* Pictet, 1833 and *Isoperla grammatica* Poda, 1761) and two Perlidae (*Dinocras cephalotes* Curtis, 1827 and *Perla bipunctata* Pictet, 1833). Interestingly, *P. microcephalus* was the most active of the four, with a daily mean prey consumption about three times that each of the others, the highest attack rate, and a generally short handling time.

The aim of this study was to investigate the diet of *P. microcephalus* nymphs in an Apenninic creek during the winter, a period in which: a) benthic communities show the greatest diversity and abundance in this area (Fenoglio et al., 2005a) and b) *P. microcephalus* nymphs have a strong energetic need and a high growth rate, before emerging.

METHODS

Between 26-28 February 2005, *P. microcephalus* nymphs were collected in the Caramagna Creek (latitude 44°36' N – longitude 8°32' E; altitude 280 m above sea level; Fig. 1). This lotic system has a good environmental quality, reaching the first class in the Italian Extended Biotic Index (Ghetti, 1997), corresponding to an environment without trace of human-induced alteration. All samplings were carried out in the first hours of the morning, because Systellognatha tend to feed under diminishing light conditions that occur at dusk and dawn (Vaught and Stewart, 1974). We examined 35 specimens, collected in a single uniform 100 m sized riffle. Moreover, using a Surber net (20 x 20 cm; mesh 255 | Ím), we collected thirty samples in the same reach to assess the taxa presence and abundance of the natural population of benthic invertebrates. Samples were preserved in 95 percent ethanol (final concentration) in the laboratory. Later, all organisms were counted and identified to the genus, except for Coleoptera, Hydraenidae; Lumbriculidae, and early instars of some Diptera, which were identified to the family level. *Perlodes microcephalus* nymphs were measured (total length, 0.1 mm accuracy) and processed to assess food consumption by means of gut contents analysis. Guts were removed and the content of the alimentary canal were analysed by the transparency method for slides (Faure's fluid). Identification of prey was based on chitinized body parts, particularly head capsules, mouthparts, and leg fragments.

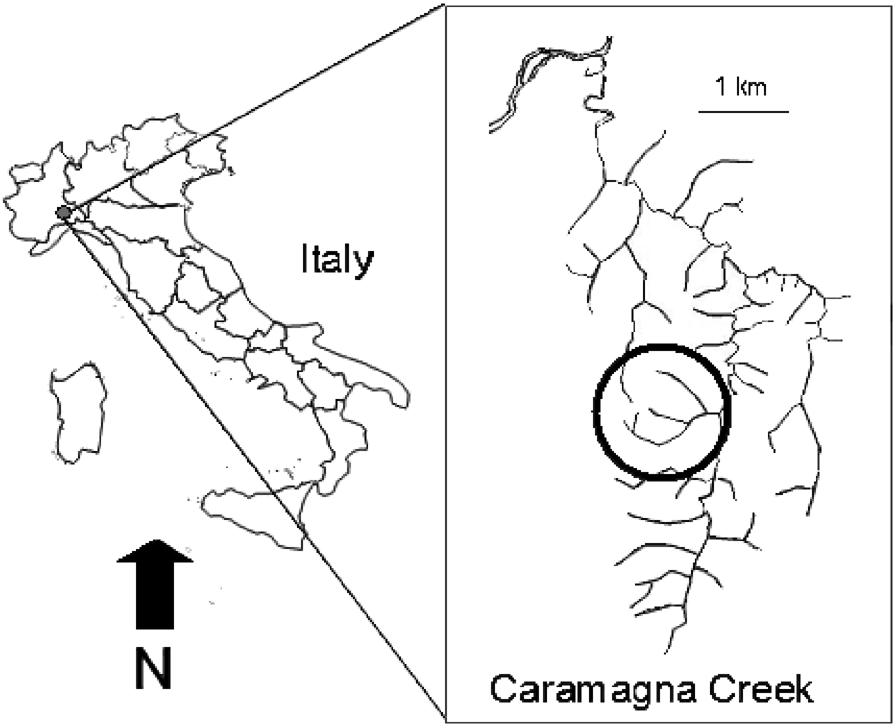

Fig. 1. Caramagna Creek, NW Italy. Circle indicates the sample site.

We also compared gut contents with the natural composition and abundance of macroinvertebrate communities in the riverbed. Feeding preferences were quantified using the Electivity Index by Ivlev (1961),

$$E = (r_i - p_i) / (r_i + p_i).$$

In this formula, r_i = the proportion of ingested species, p_i = the relative abundance in the benthic community, and N = the number of food items. This index ranges from -1 to 1. A value of -1 means total avoidance, 1 indicates preference and 0 indicates indifference.

Table 1. Percent relative abundance (% value in the community) for macroinvertebrates collected in the natural riverbed.

Taxon	FFG	%	Taxon	FFG	%
Arthropoda: Insecta			Limoniidae	P	0.54
Plecoptera			Tabanidae	P	0.15
Isoperla sp.	P	0.73			
Capnia bifrons	Sh	0.48	**Hymenoptera**		
Leuctra sp.	Sh	0.73	*Agriotypus armatus*	P	0.04
Nemoura sp.	Sh	0.30			
Brachyptera sp.	Sh	21.07	**Coleoptera**		
Perlodes microcephalus	P	0.77	*Helichus substriatus*	Sh	0.73
			Hydraenidae	Sc	1.17
Ephemeroptera			Dytiscidae	P	0.02
Paraleptophlebia sp.	Cg	0.02	Gyrinidae (larvae)	P	0.58
Ecdyonurus sp.	Sc	1.36	Elminthidae (larvae)	Cg	0.15
Baetis sp.	Cg	7.41	Elminthidae (adults)	Cg	0.15
Caenis sp.	Cg	0.11	*Esolus* sp.	Cg	0.06
Habrophlebia sp.	Cg	1.23	Helodidae (larvae)	Sh	0.37
Habroleptoides sp.	Cg	0.28	Hydrophilidae	P	0.02
Ephemera danica	Cg	0.04			
Centroptilum luteolum	Cg	0.32	**Odonata**		
Torleya major	Cg	0.02	*Orthetrum* sp.	P	0.09
			Calopteryx sp.	P	0.06
Trichoptera			*Onychogomphus* sp.	P	0.06
Sericostoma pedemontanum	Sh	0.06			
Limnephilidae	Sh	0.71	**Arthropoda: Arachnida:**		
Potamophylax cingulatus	Sh	0.06	**Hydracarina**	P	0.37
Psychomidae	Cg	0.13			
Goeridae	Cg	0.02	**Mollusca: Gastropoda**		
Leptoceridae	Cg	0.06	*Lymnaea peregra*	Sc	0.06
Glossosomatidae	Sc	0.80			
Hyporhyacophila sp.	P	0.04	**Mollusca: Bivalvia**		
Hydropsyche sp.	F	1.04	*Pisidium* sp.	F	0.02
Wormaldia sp.	F	0.06			
Beraeidae	Cg	0.02	**Platyhelmintes: Turbellaria:**		
Odontocerum albicorne	Sh	0.30	**Tricladida**		
			Dugesia sp.	P	7.41
Heteroptera					
Micronecta sp.	Cg	3.82	**Nematoda**		
			Mermithidae	P	0.02
Diptera					
Stratiomyidae	P	0.06	**Annelida**		
Atherix sp.	P	0.09	Lumbriculidae	Cg	0.26
Anopheles sp.	Cg	0.02	Naididae	Cg	0.17
Simuliidae	F	30.22	*Eiseniella tetraedra*	Cg	1.60
Dixidae	P	0.04	Lumbricidae	Cg	0.82
Chironomidae	var.	10.41	Tubificidae	Cg	0.13
Ceratopogonidae	P	0.78			
Psychodidae	P	0.09	**Nematomorpha**		
Tipulidae	Sh	1.10	*Gordius* sp.	P	0.09

(*) FFG: functional feeding groups (Cg=collectors-gatherers; F=filterers; P=predators; Sc=scrapers; Sh=shredders; var. = various, mainly Cg and P).

To analyze the dimensional shift in food preference, we separately considered gut contents of smaller nymphs (body length < 20.0 mm) and larger nymphs (body length > 20.0 mm). The preference for individual prey taxon was evaluated between the two length classes using indicator species analysis, computed by the INDVAL 2.0 software (Dufrêne, 1998). Indicator species analysis is a randomization-based test that compares the relative abundance and relative frequency of occurrence of taxa to find indicator species assemblages characterizing groups of samples.

RESULTS

In total, 30 Surber samples were collected, including 4629 aquatic invertebrates belonging to 63 taxa. The gut content of 35 *P. microcephalus* nymphs was examined. The list of taxa and their relative abundance are listed in Table 1. The mean abundance of stream benthic community was 3856.7 individuals/m^2 ± 632.6 SE.

In Table 2, we report the list of taxa found in the *P. microcephalus* guts. The most important prey in the guts were Chironomidae (Diptera): they constituted 43.2 percent of total ingested items, and they were present in the 74.3 percent of examined guts. Other important prey were Trichoptera, particularly Psychomidae, Glossosomatidae, and Limnephilidae.

Table 2. Indicator values, abundance, and fidelity for prey taxa found in smaller and larger *P. microcephalus* guts.

Species	IndVal	Smaller nymphs	Larger nymphs	*P*
Glossosomatidae	43.33	4/4	51/9	<0.01
Chironomidae	44.74	122/12	98/14	n.s.
Psychomyidae	27.83	37/8	35/11	n.s.
Plecoptera und.	26.32	0/0	7/5	n.s.
Ephemeroptera und.	5.79	0/0	3/3	n.s.
Trichoptera und.	49.98	41/13	84/15	n.s.
Simuliidae	9.76	6/2	2/2	n.s.
Hyporhyacophila sp.	6.25	1/1	0/0	n.s.
Heptageniidae	12.18	1/1	4/3	n.s.
Brachyptera sp.	15.08	2/1	6/4	n.s.
Baetis sp.	3.39	1/1	1/1	n.s.
Limnephilidae	6.60	1/1	2/2	n.s.

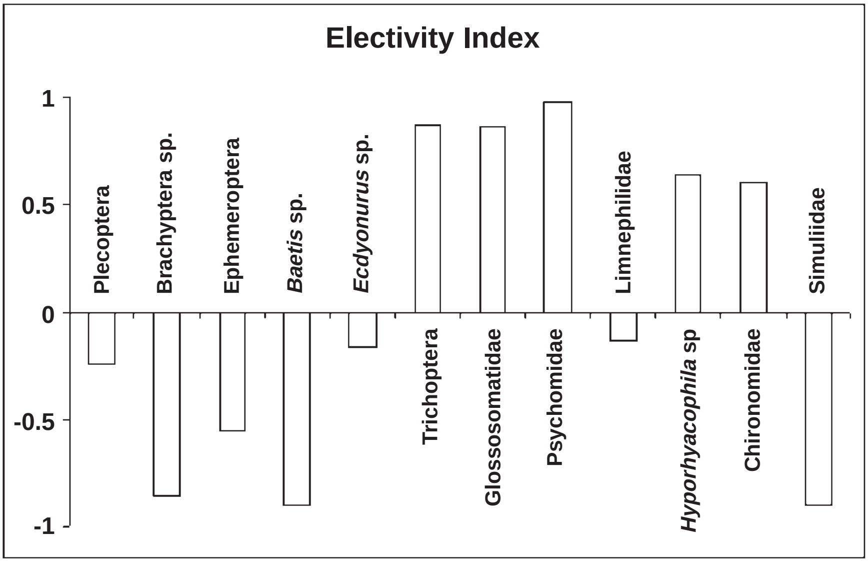

Fig. 2. Electivity index (E*) for macroinvertebrate taxa in the *P. microcephalus* nymphs diet in the Caramagna Creek.

Comparing gut contents of the two dimensional classes (smaller nymphs, n=16; larger nymphs, n=19), we detected no significant quantitative difference (i.e.=number of preys consumed; ANOVA $F_{1,33}$=0.51, P=n.s.) but interestingly we noticed a significant qualitative difference (i.e.=number of taxa ingested; ANOVA $F_{1,33}$=4.95, $P<0.05$), with larger nymphs feeding on a wider range of preys. In the guts of larger individuals we found 3.7 ± 0.29 (mean ± SE) prey taxa, while in smaller ones 2.7 ± 0.33 (mean ± SE). IndVal analysis detected that larger individuals showed a significant preference for Glossosomatidae, considering both number of items and number of guts.

DISCUSSION

Behavioral and ecological studies about stream macroinvertebrates predation mechanisms are attracting a growing interest in the last decades. In field conditions, most studies analyzed prey choice by means of the examination of gut contents (Fuller and Stewart, 1977; Allan, 1995; Peckarsky, 1996). In particular, prey selection is becoming a key element in this context: different studies focused on the reasons why some prey species are captured in preference to others. This could depend by how frequently predators and prey encounter, which is the probability of an attack and the level of the attack success. In an elegant laboratory study Tikkanen et al. (1997) demonstrated that, for a Perlodidae species, encounter rates were poor predictors of the preferences for different prey categories. Frequently encountered preys, such as Ephemeroptera Baetidae, were only rarely ingested, while other more stationary organisms, such as Simuliidae and Nemouridae, were rarely encountered but when they were, they were captured with high success.

Comparing gut contents with the array of available prey living on and among substrates, we detected some interesting elements. The electivity index showed that some taxa were preferred or avoided independently from their availability in the riverbed. Although some groups were abundant and widespread on the river bottom, they were virtually absent in the diet. Noticeably, rheostenic organisms living in epilithic microhabitats, such as Simuliidae and Heptageniidae were little present, while we detected a strong preference for taxa living among and below substratum elements, such as Chironomidae, Psychomyidae, Glossosomatidae, and others. Our study, according to the results of other studies concerning Systellognatha diet (Siegfried and Knight, 1976; Berthélemy and Lahoud, 1981), confirms that Chironomidae are the most important component in the carnivorous stonefly diet: also if electivity index shows an evident preference for some taxa (e.g.: Psychomyidae) independently from their availability, Chironomidae represent the most common prey item in the guts of *P. microcephalus.* The mean abundance of stream communities agree with the findings of other studies conducted in the same area (Fenoglio et al., 2005b).

Many studies, conducted in the field by analysis of gut contents, revealed a good correlation between what is eaten and what is available (Allan, 1995). Allan and Fleckner (1988) noticed that the rank order of prey taxa in the diet of the large sized *Hesperoperla pacifica* Banks 1900 (Perlidae) is analogous to the prey rank order in the benthos.

Interestingly, our study supports the hypothesis that *P. microcephalus* shows an evident trophic preference, feeding mainly on medium-sized, less mobile organisms, and avoiding taxa inhabiting fast-flowing waters. Our data may also indicate the growth of the trophic spectrum over time, as noticed in other Systellognatha (Femminella and Stewart, 1986) with larger nymphs hunting and consuming a higher number of taxa.

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LITERATURE CITED

Allan, J. D. 1995. Stream ecology. Structure and function of running waters. Chapman & Hall. London. 388 pp.

Allan, J. D. and A. S. Fleckner. 1988. Prey preference in stoneflies: a comparative analysis of prey vulnerability. Oecologia 76: 495-503.

Berthélemy, C. and M. Lahoud. 1981. Régimes alimentaires et pièces buccales de quelques perlodidae et perlidae des Pyrénées (Plecoptera). Annales de Limnologie 17: 1-24.

Broekhuizen, N., S. Parkyn, and D. Miller. 2001. Fine sediment effects on feeding and growth in the invertebrate grazers *Potamopyrgus antipodarum* (Gastropoda, Hydrobiidae) and *Deleatidium* sp. (Ephemeroptera, Leptophlebiidae). Hydrobiologia 457: 125–132.

Dufrêne, M. 1998. IndVal or how to identify indicator species of a sample typology? Version 2.0. User's Guide and application published at: http://mrw.wallonie.be/dgrne/sibw/outils/indval/home.html.

Elliott, J. M. 2000. Contrasting diel activity and feeding patterns of four species of carnivorous stoneflies. Ecological Entomology 25:26-34.

Elliott, J. M. 2003. Interspecific interference and the functional response of four species of carnivorous stoneflies. Freshwater Biology 48: 1527-1539.

Elliott, J. M. 2004. Prey switching in four species of carnivorous stoneflies. Freshwater Biology 49: 709-720.

Femminella, J. W. and K. W. Stewart. 1986. Diet and predation by three leaf-associated stoneflies in an Arkansas mountain stream. Freshwater Biology 16: 521-538.

Fenoglio, S. 2003. Feeding habits of *Anacroneuria* nymphs (Plecoptera Perlidae). Bollettino della Società Entomologica Italiana 135: 15-17.

Fenoglio, S. and M. Tierno de Figueroa. 2003. Observations on the adult feeding of some *Neoperla* and *Anacroneuria* species (Plecoptera, Perlidae). African Entomology 11: 138-139.

Fenoglio, S., T. Bo, P. Agosta, and G. Malacarne. 2005a. Temporal and spatial patterns of coarse particulate organic matter and macroinvertebrate distribution in a low-order Apennine stream. Journal of Freshwater Ecology. In press.

Fenoglio, S., T. Bo, P. Agosta, and M. Cucco. 2005b. Mass loss and macroinvertebrate colonisation of fish carcasses in riffles and pools of a NW Italian stream. Hydrobiologia 532: 111-122.

Fuller, R. L. and K. W. Stewart. 1977. The food habits of stoneflies (Plecoptera) in the upper Gunnison River, Colorado. Environmental Entomology 6: 293-302.

Ghetti, P. F. 1997. Manuale di applicazione dell'Indice Biotico Esteso. Provincia Autonoma di Trento Press, Trento, Italy. 222 pp.

Hynes, H. B. N. 1993. A key to the adults and nymphs of the British stoneflies (Plecoptera), with notes on their ecology and distribution. Freshwater Biological Association Publ., Ambleside, U.K. Third Edition. 92 pp.

Ivlev, V. S. 1961. Experimental ecology of the feeding of fishes. Yale University Press. New Haven, Connecticut, U.S.A. 302 pp.

Merritt, R. W. and K. W. Cummins. 1996. An introduction to the aquatic insects of North America. Kendall/Hunt. Dubuque, Iowa, U.S.A. 862 pp.

Molles, M. C. and R. D. Pietruszka. 1987. Prey selection by a stonefly: the influence of hunger and prey size. Oecologia 72: 473-478.

Monakov, A. K. 2003. Feeding of freshwater invertebrates. Kenobi Productions. Ghent, Belgium. 373 pp.

Peckarsky, B. L. 1996. Predator-prey interactions. pp. 431-451. *In*, F. R. Hauer and G.A. Lamberti (Editors). Methods in Stream Ecology. Academic Press, New York, U.S.A. 669 pp.

Siegfried, C. A. and W. Knight. 1976. Prey Selection by a Setipalpian Stonefly Nymph, *Acroneuria (Calineuria) californica* Banks (Plecoptera: Perlidae). Ecology 57: 603-608.

Tierno de Figueroa, J. M. and A. Sanchez-Ortega. 1999. Imaginal feeding of certain systellognathan stonefly species (Insecta, Plecoptera). Annals of the Entomological Society of America 92: 218–221.

Tierno de Figueroa, J. M. and R. Fochetti. 2001. On the adult feeding of several European stoneflies (Plecoptera). Entomological News 112: 130–134.

Tikkanen, P., T. Muotka, and A. Juntunen. 1997. The roles of active predator choice and prey vulnerability in determining the diet of predatory stonefly (Plecoptera) nymphs. Journal of Animal Ecology 66: 36-48.

Vaught, G. L. and K. W. Stewart. 1974. The life history and ecology of the stonefly *Neoperla clymene* (Newman) (Plecoptera: Perlidae). Annals of the Entomological Society of America 67: 167–178.

Webster, J. R. and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics 17: 567-594.

Wipfli, M. S. and D. P. Gregovich. 2002. Export of invertebrates and detritus from fishless headwater streams in southeastern Alaska: implications for downstream salmonid production. Freshwater Biology 47: 957-969.