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Research Paper

Trophic Behavior of Two Perlidae Species (Insecta, Plecoptera) in a River in Southern Spain

key words: Dinocras cephalotes, Perla bipunctata, diet, niche breadth, Iberian Peninsula

Abstract

Two Perlidae species, *Dinocras cephalotes* and *Perla bipunctata*, were studied in a river in Spain with the objectives of: a) describing their diets; b) detecting differences between size classes; c) analyzing niche breadth; and d) assessing the degree of niche overlap between both species and size classes. *Dinocras cephalotes* and *Perla bipunctata* nymphs fed mainly on Baetidae and immature Chironomidae. We did not detect significant differences in the quantity of ingested detritus, algae, and leaf pieces. Niche breadth was very low, although both species presented a high niche overlap. *P. bipunctata* seems to select some prey items throughout its nymphal development, whereas *D. cephalotes* augments its diet with other prey items as it grows.

1. Introduction

Insect diets represent one of the most complex misunderstood and underappreciated aspects of entomology (COHEN, 2004). The knowledge of insect feeding habits constitute a key aspect for understanding ecological processes in every terrestrial and freshwater ecosystem (MONAKOV, 2003).

Particularly in freshwater habitats, the feeding of many groups of aquatic insects remains insufficiently studied. In this context, the plasticity of the feeding habits of freshwater insects is revealed in the seasonal, spatial (both on macro- and mesohabitat level) and age-dependent variability of food composition and ecological specificity (LAMBERTI and MOORE, 1984).

Plecoptera constitute a numerically and ecologically significant component in freshwater ecosystems in running waters of all sizes (ZWICK, 2004; FOCHETTI and TIERNO DE FIGUEROA, 2006). Plecoptera have diversified their food habits filling almost all major trophic categories in streams (STEWART and STARK, 2002; MERRITT *et al.*, 2007). Large Perlid nymphs play an important role as the main predators among macroinvertebrates in many European streams (*e.g.*, BERTHÉLEMY and LAHOUD, 1981; ELLIOTT, 2000, 2004; Bo and FENOGLIO, 2005; Bo *et al.*, 2007; FENOGLIO *et al.*, 2007b). Additionally, resource partitioning among different predaceous stonefly species has been investigated (SHELDON, 1980; PECKARSKY, 1984; ELLIOTT, 2003; Bo *et al.*, 2007; THORP *et al.*, 2007).

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The objectives of our study were to 1) describe the feeding habits of two large sized Perlidae species: *Dinocras cephalotes* (CURTIS 1827) and *Perla bipunctata* PICTET, 1833; 2) study the possible differences in the diet between the two species and between size classes of each species; 3) contribute information regarding their niche breadth as predators; and 4) detect the possible existence of resource overlap between both species and size classes.

Dinocras cephalotes is widely distributed in Europe, both in latitude and altitude, and it is a very common stonefly, while *P. bipunctata*, that is also present in Northern Africa (SIVEC and STARK, 2002), is less commonly represented in continental Europe (TIERNO DE FIGUEROA *et al.*, 2003) and reported for the first time in Southern Iberian Peninsula (TIERNO DE FIGUEROA *et al.*, 2005). Both species prefer stony-bed rivers, where they can occasionally coexist occupying a very similar niche. This coexistence makes the study of their trophic biology particularly interesting.

2. Methods

Nymphs of *D. cephalotes* and *P. bipunctata* were collected in the Castril River (Sierra de Castril, Granada, South Iberian Peninsula; 1220 m.a.s.l.; UTM: 30SWG223955; Fig. 1). The Castril River is a tributary of the Guadalquivir basin. The basin substrate is limestone. The water at our sampling station was characterized (during the study period) by temperatures ranging only from 9 to 12 °C, an oxygen saturation >100%, a high stream-flow velocity and a substrate composed mainly by limestone, cobble and gravel.

Both species, *D. cephalotes* and *P. bipunctata*, present a very similar life cycle in the study area. They are merovoltine species (nymphs of different sizes coinciding in the same period) with a spring flight period in that the presence of the highest nymphs of both species occurs in April. Thus, the analysed nymphs were collected in February, March and April, when nymphs with different sizes of both species were simultaneously present.

The nymphs were extracted from samplings carried out with a kick net (250 μ m mesh size) monthly from July 2004 to July 2005. All macroinvertebrates, in addition to both perlid species, were collected and preserved in 70% ethanol with the aim of describing the benthic community to the family level (except Oligochaeta and Hydracarina).

Sixty nymphs of *D. cephalotes* and sixty nymphs of *P. bipunctata* representing all size categories were selected. In the laboratory we measured total length (dorsally, from head apex to apical segment of abdomen) of each nymph with the ocular micrometer of an Olympus stereomicroscope $(10\times)$. For better precision, before measuring them, all individuals were pressed flat using a microscope slide.



Figure 1. Map of the Iberian Peninsula with the study area indicated by the dark dot.

Nymphs were processed to assess food consumption by means of two gut content analyses. For some of the small individuals (<15 mm total length), the contents of the alimentary canal were analysed following the transparency method proposed by BELLO and CABRERA (1999) and widely employed in stone-fly feeding studies (TIERNO DE FIGUEROA and SÁNCHEZ-ORTEGA, 1999; DERKA *et al.*, 2004; FENOGLIO *et al.*, 2007a): each nymph was singularly placed in a vial with Herwitgs' liquid for 48 hours at 18–25 °C and, afterwards, cleared individuals were placed on a glass slide with a cover slip. For large nymphs (>15 mm total length) and some of smaller size classes, the guts were dissected and the contents of the entire alimentary canal were extracted and analysed. As previously observed by the authors, no differences were observed using either method. When possible, identifiable gut content items were sorted to the family level and counted. For the remaining contents, five categories were used: (1) Fine Particulate Organic Matter (FPOM), (2) leaf pieces, (3) algae, (4) animal matter (unidentifiable animal remains >1 mm), and (5) sand. For these five categories, we estimated subjectively the area percentages that they occupied in the guts previously squashed.

Statistical analyses were performed with Statistica 7.1 (STATSOFT, 2005). For each category of gut contents, mean, standard deviation, range and presence (both absolute and relative) were calculated. Absolute presence indicates the number of individuals containing a given gut content item.

We compared both species and size classes regarding the prey consumed. Levins's index for niche breadth (LEVINS, 1968) was also calculated, and the Hurlbert's standardization (HURLBERT, 1978) was applied. The scale of the latter index varies between 0 and 1: the higher the value the higher the niche breadth. The Levins's index (B) and the Hurlbert's standardization (B_A) are calculated as follows:

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

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

where:

 p_j = fraction of items in the diet belonging to food category *j*, and n = number of possible resource states (items).

Regarding other components of the diet (FPOM, leaf pieces, algae, animal matter, and sand), we employed a Kolmogorov-Smirnov's test due to normality assumptions were not achieved. This test is more reliable than others available for small sample sizes and for those situations in which both variable distributions are different (TOWNSEND, 2002; GUISANDE GONZÁLEZ *et al.*, 2006).

In order to assess niche overlap as predators (considering only ingested prey items) between species and also between size classes we used Simplified Morisita Index proposed by HORN (1966):

$$C_{H} = \left[2\sum_{i}^{n} p_{ij} \cdot p_{ik}\right] / \left[\sum_{i}^{n} p_{ij}^{2} + \sum_{i}^{n} p_{ik}^{2}\right]$$

where:

 C_H = Simplified Morisita Index of niche overlap between species *j* and *k*, p_{ij} = proportion resource *i* (in number of items) is of the total resources used by species *j*, p_{ik} = proportion resource *i* (in number of items) 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.

3. Results and Discussion

Forty-six taxa at family level (except Oligochaeta and Hydracarina) were collected by the kick net method in the sampling station during the study period (Table 1).

Nine of the 60 *D. cephalotes* nymphs and 22 of the 60 *P. bipunctata* nymphs had completely empty guts. Tables 2 and 3 present the diet composition of both species. Both species exhibit typical predatory behaviour, but ingest relatively high quantity of detritus (FPOM), leaf pieces, and algae. Some of this material may be incidentally ingested during prey capture. Both *D. cephalotes* and *P. bipunctata* apparently augment their diet with non-animal matter. This has mentioned previously by STEWART and STARK (2002) and MONAKOV (2003). The presence of

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Table 1.	Benthic	community	of	the	sampling	station	in	the	Castril	river	of	southern
					Spain.							

	Community
Tricladida	Planariidae
Annelida	Oligochaeta Erpobdellidae
Mollusca	Ancylidae
Arachnida	Hydracarina
Amphipoda	Gammaridae
Ephemeroptera	Baetidae Ephemerellidae Ephemeridae Heptageniidae Leptophlebiidae
Odonata	Cordulegasteridae
Plecoptera	Chloroperlidae Leuctridae Nemouridae Perlidae Perlodidae
Hemiptera	Gerridae Hydrometridae Nepidae Veliidae
Coleoptera	Dytiscidae Elmidae Gyrinidae Haliplidae Hydraenidae
Trichoptera	Brachycentridae Hydropsychidae Hydroptilidae Lepidostomatidae Limnephilidae Psychomyidae Rhyacophilidae Sericostomatidae
Diptera	Blephariceridae Ceratopogonidae Chironomidae Culicidae Dixidae Empididae Limoniidae Psychodidae Simuliidae Stratiomyidae Tabanidae Tipulidae

D. cephalotes	Ν	Mean	Std. Dev.	Range	Presence	Presence (%)
Absolute %	60	35.25	34.26	100.00		
Ephem. undet.	51	0.10	0.46	3.00	3.00	5.88
Baetidae	51	0.31	0.65	3.00	12.00	23.53
Heptageniidae	51	0.06	0.24	1.00	3.00	5.88
Leptophlebiidae	51	0.02	0.14	1.00	1.00	1.96
Trich. undet. with case	51	0.12	0.43	2.00	4.00	7.84
Trich. undet. without case	51	0.24	0.84	5.00	6.00	11.76
Rhyacophilidae	51	0.02	0.14	1.00	1.00	1.96
Limnephilidae	51	0.02	0.14	1.00	1.00	1.96
Elmidae larvae	51	0.02	0.14	1.00	1.00	1.96
Dipt. undet.	51	0.02	0.14	1.00	1.00	1.96
Chironomidae	51	0.24	0.47	2.00	11.00	21.57
Stratiomyidae	51	0.04	0.20	1.00	2.00	3.92
Simuliidae	51	0.02	0.14	1.00	1.00	1.96
FPOM %	51	12.31	20.91	100.00	16.00	31.37
Leaf pieces %	51	7.12	16.58	66.00	9.00	17.65
Animal matter %	51	16.84	23.29	100.00	21.00	41.18
Algae %	51	11.65	20.69	66.00	14.00	27.46
Sand %	51	0.10	0.36	2.00	4.00	7.84

Table 2. Gut content of *D. cephalotes* nymphs. Presence indicating number of individuals containing that item; % Presence indicating number of individuals containing that item divided by the total number of individuals with gut content ×100.

sand in the guts could be a consequence of the ingestion of Trichoptera with their cases or incidental ingestion. The more common prey in *D. cephalotes* guts was Baetidae followed by Chironomidae and Trichoptera without cases. For *P. bipunctata*, the most abundant prey was also Baetidae followed by other Ephemeroptera and Chironomidae. These results coincide with those pointed out previously for both species in different parts of their distribution area (BERTHÉLEMY and LAHOUD, 1981; LILLEHAMMER, 1988; LUCY *et al.*, 1990; ELLIOTT, 2003). The presence of sclerified prey such as Stratiomyidae (Diptera) and larvae of Elmidae (Coleop-

Table 3. Gut content of *P. bipunctata* nymphs. Presence indicating number of individuals containing that item; % Presence indicating number of individuals containing that item divided by the total number of individuals with gut content ×100.

P. bipunctata	Ν	Mean	Std. Dev.	Range	Presence	Presence (%)
Absolute %	60	23.25	28.27	100.00		
Ephem. undet.	38	0.24	0.63	3.00	6.00	15.79
Baetidae	38	0.32	0.70	3.00	8.00	21.05
Heptageniidae	38	0.11	0.31	1.00	4.00	10.53
Trich. undet. with case	38	0.03	0.16	1.00	1.00	2.63
Trich. undet. without case	38	0.03	0.16	1.00	1.00	2.63
Chironomidae	38	0.24	0.49	2.00	8.00	21.95
Gammaridae	38	0.05	0.32	2.00	1.00	2.63
FPOM %	38	24.34	25.16	100.00	22.00	57.89
Leaf pieces %	38	2.61	9.02	33.00	3.00	7.89
Animal matter %	38	13.05	22.52	100.00	12.00	31.58
Algae %	38	6.08	12.96	33.00	7.00	18.42

Compared categories	Simplified Morisita Index value					
Dc vs. Pb	0.817					
Dc < vs. Dc >	0.542					
Pb < vs. Pb >	0.897					
Dc < vs. Pb <	0.861					
Dc > vs. Pb >	0.602					
Dc < vs. Pb >	0.844					
Dc > vs. Pb <	0.636					

Table. 4. Simplified Morisita Index values for niche overlap (Dc = *D. cephalotes*; Pb = *P. bipunctata*; < = shorter than 15 mm; > = longer than 15 mm). N = 23 (Dc >), 37 (Dc <), 17 (Pb >), 43 (Pb <).

tera) in three different individuals of *D. cephalotes* can probably be associated with an engulfer condition and hunting behaviour. The presence of these kinds of prey has been considered occasionaly for other European Perlidae, such as *Perla grandis* (RAMBUR, 1842; BERTHÉLEMY and LAHOUD, 1981).

When comparing the percentage of leaf pieces, algae, and detritus (FPOM) between species and between large and small nymphs of the same species, we did not find significant differences (Kolmogorov-Smirnov's test, P > 0.05 for every case). This result, perhaps reflecting differences in size classes, differs from those pointed out for other species of the superfamily Perloidea (FULLER and STEWART, 1977, 1979; GRAY and WARD, 1979; GILLER and MALMQVIST, 1998). One possible explanation for this is that larger size classes may ingest less detritus and other plant material. Previous studies in *D. cephalotes* and *P. bipunctata* are not in accord on this topic, as some populations seem to change their herbivorous-carnivorous habits, and others may not (BERTHÉLEMY and LAHOUD, 1981; LUCY *et al.*, 1990).

Although the niche breadth was very low in all cases, we found greater values for *D. cephalotes* than for *P. bipunctata* when comparing individuals representing all size classes $(B_A = 0.108 \text{ and } 0.071, \text{ respectively})$. The same situation occurred for large nymphs $(B_A = 0.105 \text{ and } 0.072, \text{ respectively})$ but not for the small ones, that present approximately the same value $(B_A = 0.049 \text{ and } 0.051, \text{ respectively})$. A smaller niche breadth (inferred from the reduced number of consumed prey) for *P. bipunctata* was suggested, but not completely demonstrated due to the low sample size, only 14 studied nymphs of *P. bipunctata*, in a study in an Ireland stream (LUCY *et al.*, 1990). This could not be explained by differences in foraging activity between both species, as experimentally demonstrated by ELLIOTT (2000). Despite the relatively low number of individuals, our results seem to support the general pattern suggested by LUCY *et al.* (1990). Obviously a higher number of studied individuals probably would help clarify this uncertainty. The greater similarity in niche bread between small nymphs of both species could be a consequence of a physical limitation due to mouth size, as reported in other predaceous macroinvertebrates and also in carnivorous fishes (GILLER and MALMQVIST, 1998).

Both species as a whole present a high niche overlap (Table 4), as could be expected from their morphological, ecological and ethological similarity (ELLIOTT, 2000). Nevertheless, large nymphs of *D. cephalotes* showed a lower niche overlap with regards to all the other size classes (Table 4), no matter the prey species. This result does not completely support the findings of Lucy *et al.* (1990) for different size classes of *D. cephalotes*. Our results suggest that *D. cephalotes* is more opportunistic in that it seems to utilize additional prey items as it grows, whereas *P. bipunctata* seems to maintain its selection of specific prey items.

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