

## **4 Relationships between the distribution of mayfly nymphs and water quality in the Guadalquivir River Basin (southern Spain)**

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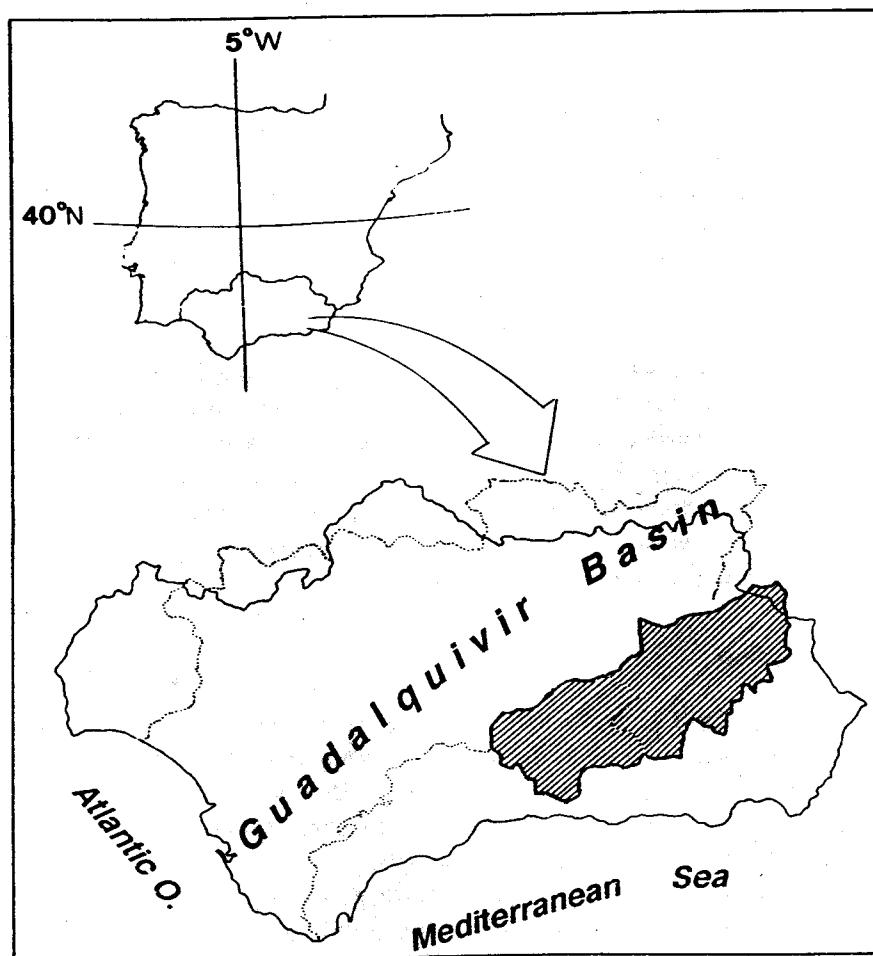
*Seasonal sampling was conducted at 100 sites in the Guadalquivir River Basin (southern Spain) from 1988 to 1990. The biological water quality was assessed using macroinvertebrate communities (the Spanish B.M.W.P. system). We found that the natural mayfly nymphal distribution was affected by pollution. This paper includes the results concerning the degree of intolerance and/or association of taxa (family, genus and species) with respect to five quality classifications, ranging from very good water quality to high pollution.*

### **Introduction**

Mayflies have generally been considered to be very sensitive to pollution (particularly organic loading) (Roback 1974). Thus, mayflies have been widely used as indicators of water quality (Marshall 1980; Brittain 1982) and play an important role in many indices to assess water pollution (Bartsch and Ingram 1966; Ghetti and Bonazzi 1981; Armitage et al. 1983; De Pauw and Vanhooren 1983; Alba-Tercedor and Sánchez-Ortega 1988). Some methods have assessed water pollution by studying only the mayfly composition (Fremling 1970; Sowa 1980). Moreover, these insects have been used largely for toxicological bioassays (Roback 1974; Muirhead-Thomson 1987). However, the old idea that mayflies are sensitive as a group is not true, and detailed results with respect to the tolerance degree of many species have been published (Braasch and Jacob 1976; Hubbard and Peters 1978; Janieva 1979; Russev 1979; García de Jalón and González del Tánago 1986; Hellawell 1986; Thomas 1987; Alba-Tercedor et al. 1991).

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**Figure 1.** Location of the study area — shading indicates the catchment area of the two sub-basins studied.



The ecological requirements and pollution tolerance can vary within a species according to the geographical distribution. The pollution tolerance of many species in southern Spain is not well known. We conducted a study to address this research gap. Specifically, we examined the association between mayfly taxa (family, genus, species) and water quality classes in a drainage basin of southern Spain.

## Materials and Methods

A qualitative, seasonal sampling program (1988-1990) was conducted in two sub-basins (Genil River Basin and Guadiana Menor River Basin), within the Guadalquivir River Basin of southern Spain (Fig. 1). Samples ( $n=744$ ) of macroinvertebrates were taken at 100 stations, every three months, using a kick net sampler. Macroinvertebrates were collected until no new taxa were encountered.

The biological water quality was assessed by the Spanish B.M.W.P.' system. This system is based on a large geographical study in which taxonomic families of macroinvertebrates have been scored according to water quality conditions (Alba-Tercedor and Sánchez-Ortega 1988). This index recognizes five water qualities ranging from 1 to 5 (class I: lightly or unpolluted — good situation; class II: slightly polluted — acceptable situation; class III: moderately polluted — doubtful situation; class IV: heavily polluted — critical situation; and class V: very heavily polluted — very critical situation). These roughly correspond with saprobic classes (x, o, B,  $\alpha$ , p) (Zelinka and Marvan 1961; Sladeczek 1973).

A Kolmogorov-Smirnov test (Siegel 1956) was used to determine if the distribution of each taxon (family, genus, species) with respect to water quality had a random or a specific distribution. Taxa that occurred in  $\leq$  five samples were not considered in the analysis. For those taxa that showed a statistically non-random distribution ( $p < 0.01$ ), the test was repeated in those water quality classes in which taxa occurred. For those taxa that showed a preference for a given water quality class, a  $\chi^2$  2x2 test of independence (corrected by Yate's continuity) was used (Sokal and Rohlf 1969) to determine the statistical significance dependence of a taxon's association with a specific water quality class. Also, the Cramer coefficient (C) was calculated ( $C = (\chi^2/n)/q$ , where  $n$  = total number of samples and  $q$  = minimum value {rows or columns} - 1). This gave a measure of the strength of the association and/or repelling degree when compared to the calculated probability.

## Results

A total of 52 species of Ephemeroptera (Table 1) belonging to 9 families (Table 2) and 21 genera (Table 3) was studied. The total number of samples for each species and each water quality class, and the total number of samples in which each species, genus and family was found within each water quality class, are presented in Tables 1 to 3, respectively.

### Family Level

Most of the families were present within the first three quality classes, except the Baetidae and Caenidae, which showed the highest tolerance degree (Table 2).

**Table 1.** Total number of samples in which each species was present within each water quality class (+ = taxa that occurred in five samples or which showed a random distribution; \* = taxa that showed a statistically non-random distribution; \*\* = species which showed a significant preference with water quality classes where inhabited).

Species	Water Quality Classes					N
	I	II	III	IV	V	
+ <i>Acentrella sinaica</i>	1	0	0	0	0	1
* <i>Baetis alpinus</i>	7	3	0	0	0	10
+ <i>B. atrebatinus</i>	0	1	0	0	0	1
+ <i>B. beskidensis</i>	0	2	2	0	0	4
+ <i>B. digitatus</i>	2	3	0	0	0	5
* <i>B. fuscatus</i>	21	33	25	11	0	90
** <i>B. maurus</i>	80	35	7	0	0	122
** <i>B. muticus</i>	102	35	5	1	1	144
+ <i>B. neglectus</i>	3	9	4	1	0	17
** <i>B. pavidus</i>	63	149	164	82	14	472
** <i>B. rhodani</i>	160	143	83	28	5	419
+ <i>B. scambus</i>	2	3	0	0	0	5
** <i>Centroptilum luteolum</i>	42	9	3	0	0	54
+ <i>C. nanum</i>	1	1	0	0	0	2
+ <i>C. pennulatum</i>	2	2	0	0	0	4
* <i>C. gr. pulchrum</i>	5	4	1	0	0	10
+ <i>Cloeon cognatum</i>	7	21	26	13	1	68
+ <i>C. inscriptum</i>	0	0	3	0	0	3
+ <i>C. praetextum</i>	0	0	0	0	1	1
+ <i>C. schoenemundi</i>	3	4	3	0	0	10
+ <i>C. simile</i>	6	6	7	1	0	20
+ <i>C. gr. dipterum</i>	0	0	1	0	0	1
+ <i>C. gr. simile</i>	2	4	0	0	0	6

**Table 1 (continued)**

+ <i>Procloeon</i> sp.	1	6	3	1	0	11
+ <i>P. coccinum</i>	0	0	0	0	1	1
+ <i>Brachycercus</i> sp.	0	1	0	0	0	1
+ <i>Caenis</i> sp. A	0	1	3	0	0	4
+ <i>Caenis beskidensis</i>	4	3	1	0	0	8
** <i>C. luctuosa</i>	69	101	98	47	4	319
** <i>C. pusilla</i>	78	60	36	9	0	183
** <i>Ephemerella ignita</i>	81	41	7	1	0	130
* <i>E. ikonovici nevadensis</i>	2	8	0	0	0	10
* <i>E. maculocaudata</i>	7	7	0	0	0	14
** <i>Torleya</i> cf. <i>belgica</i>	41	5	0	0	0	46
** <i>Ecdyonurus</i> spp.	136	95	52	5	0	288
** <i>Epeorus torrentium/sylvicola</i>	43	15	0	0	0	58
+ <i>Heptagenia coerulans</i>	0	0	0	2	1	3
* <i>Rithrogena marcosi</i>	21	11	4	0	0	36
+ <i>R. goeldlini</i>	3	1	0	0	0	4
+ <i>R. gr. hybrida</i>	1	4	0	0	0	5
** <i>R. gr. semicolorata</i>	34	18	6	1	0	59
** <i>Ephemera danica</i>	48	1	1	0	0	50
** <i>Paraleptophlebia submarginata</i>	50	5	1	0	0	56
** <i>Habrophlebia eldae</i>	28	9	1	0	0	38
+ <i>H. fusca</i>	0	0	0	0	2	2
+ <i>H. lauta</i>	0	0	0	0	1	1
+ <i>Habroleptoides</i> sp.	0	0	0	0	2	2
* <i>Oligoneuriella marichuae</i>	6	7	1	0	0	14
** <i>O. rhenana</i>	26	11	6	0	0	43
+ <i>Oligoneuriopsis skounate</i>	2	8	7	1	0	18
+ <i>Ephoron virgo</i>	1	1	4	0	0	6
* <i>Potamanthus luteus</i>	13	9	8	0	0	30
Total Species Presences	1203	895	573	204	33	2908

**Table 2.** Total number of samples in which each family was present within each water quality class — labelling as for Table 1.

Family	Water Quality Classes					N
	I	II	III	IV	V	
** Baetidae	175	210	173	89	16	663
** Caenidae	121	134	113	48	4	420
** Ephemerellidae	109	46	7	1	0	163
** Ephemeridae	48	1	1	0	0	50
** Heptageniidae	150	110	55	5	0	320
** Leptophlebiidae	68	12	2	0	0	82
** Oligoneuriidae	34	26	13	1	0	74
+ Polymitarcidae	1	1	4	0	0	6
* Potamanthidae	13	9	8	0	0	30

Most distribution frequencies corresponded to water quality classes 1 and 2. Polymitarcidae (distributed within classes 1 to 3) appear to be randomly distributed with respect to pollution and to be tolerant to organic pollution. Potamanthidae showed a non-distinctive preference for classes 1 to 3 (Table 2).

The preference analysis within each water quality class (Table 4), revealed that most taxonomic families showed a significant level of association with class I waters. The tolerance degree increased from Ephemeridae and Leptophlebiidae to: Heptageniidae, Ephemerellidae, Oligoneuriidae, Caenidae, Baetidae and Potamanthidae.

#### Genus Level

It was not possible to determine any particular preference for seven genera (*Acentrella*, *Procloeon*, *Brachycercus*, *Heptagenia*, *Habroleptoides*, *Oligoneuriopsis* and *Ephoron*). In addition, *Potamanthus* showed a non-distinctive preference for classes I to III (Table 3).

**Table 3.** Total number of samples in which each genus was present within each water quality class — labelling as for Table 1.

Genus	Water Quality Classes					N
	I	II	III	IV	V	
+ <i>Acentrella</i>	1	0	0	0	0	1
** <i>Baetis</i>	175	208	168	84	15	650
** <i>Centropilum</i>	48	15	5	0	0	68
** <i>Cloeon</i>	15	31	31	14	1	92
+ <i>Procloeon</i>	2	6	3	1	0	12
+ <i>Brachycercus</i>	0	1	0	0	0	1
** <i>Caenis</i>	121	134	113	48	4	420
** <i>Ephemerella</i>	85	44	6	1	0	136
** <i>Torleya</i>	41	5	0	0	0	46
** <i>Ecdyonurus</i>	136	95	52	5	0	288
** <i>Epeorus</i>	43	15	0	0	0	58
+ <i>Heptagenia</i>	1	2	0	0	0	3
** <i>Rhithrogena</i>	46	23	9	1	0	79
** <i>Ephemera</i>	48	1	1	0	0	50
+ <i>Habroleptoides</i>	2	0	0	0	0	2
** <i>Habrophlebia</i>	30	9	1	0	0	40
** <i>Paraleptophlebia</i>	50	5	1	0	0	56
** <i>Oligoneuriella</i>	32	18	7	0	0	57
+ <i>Oligoneuriopsis</i>	2	8	7	1	0	18
+ <i>Ephoron</i>	1	1	4	0	0	6
* <i>Potamanthus</i>	13	9	8	0	0	30

**Table 4.** Preferences of families for water quality classes (Sig. = association or avoidance expressed with "+" or "-", and the significance level - with 4,3,2, or 1 sign depending on whether the significance level "p" was <0.01, <0.05, <0.1, or higher — non-significant —, respectively; C = Cramer's index).

Family	Water Quality Classes				
	I Sig. C	II Sig. C	III Sig. C	IV Sig. C	V Sig. C
** Baetidae	++++0.036	++++0.046	++++0.015	----0.015	----0.424
** Caenidae	++++0.019	+++0.008	++0.005	----0.013	----0.100
** Ephemerellidae	++++0.185	+0.002	----0.046	----0.034	
** Ephemeridae	++++0.205	----0.023	----0.018		
** Heptageniidae	++++0.226	++++0.013	----0.019	----0.105	
** Leptophlebiidae	++++0.238	----0.010	----0.030		
** Oligoneuriidae	++++0.029	+0.002	+0.002	----0.015	
+ Polymitarcidae	(*)	(*)	(*)		
+ Potamanthidae	(+)	(+)	(+)		

Species of the genera *Baetis*, *Cloeon* and *Caenis* inhabited heavily polluted waters (classes IV and V). *Ephemerella* and *Rhithrogena* were found once in heavily polluted waters (class IV). The remaining species were present within the better quality classes.

After the preference analysis within each water quality class (Table 5), species from nine genera (*Centroptilum*, *Torleya*, *Epeorus*, *Ephemera*, *Habrophlebia*, *Paraleptophlebia*, as well as *Ephemerella*, *Rhithrogena* and *Oligoneuriella*) showed a high significance level of association with the highest quality waters (class I). *Ecdyonurus* and *Caenis* were associated with classes I and II; *Baetis* were associated with classes I to III (Table 5). *Cloeon* species showed a high significance level of association with moderately polluted waters (class III); the lack of any association with better water qualities could be explained because species of this genus tend to

**Table 5.** Preferences of genera for water quality classes — labelling as for Table 4.

Family	Water Quality Classes				
	I Sig. C	II Sig. C	III Sig. C	IV Sig. C	V Sig. C
+ <i>Acentrella</i>	(*)				
** <i>Baetis</i>	++++0.042	++++0.047	++++0.009	----0.023	----0.370
** <i>Centroptilum</i>	++++0.120	-0.001	----0.014		
** <i>Cloeon</i>	-0.003	+0.002	++++0.014	+0.000	----0.009
+ <i>Procloeon</i>	(*)	(*)	(*)	(*)	
+ <i>Brachycercus</i>		(*)			
** <i>Caenis</i>	++++0.190	+++0.008	++0.005	----0.013	----0.100
** <i>Ephemerella</i>	++++0.185	+0.002	----0.046	----0.034	----0.020
** <i>Torleya</i>	++++0.153	----0.009			
** <i>Ecdyonurus</i>	++++0.194	+++0.007	----0.012	----0.085	
** <i>Epeorus</i>	++++0.116	-0.000			
+ <i>Heptagenia</i>	(*)	(*)			
** <i>Rhithrogena</i>	++++0.077	+0.000	----0.010	----0.016	
** <i>Ephemera</i>	++++0.205	----0.023	----0.018		
+ <i>Habroleptoides</i>	(*)				
** <i>Habrophlebia</i>	++++0.080	-0.000	----0.013		
** <i>Paraleptophlebia</i>	++++0.190	----0.014	----0.021		
** <i>Oligoneuriella</i>	++++0.046	+0.000	----0.006		
+ <i>Oligoneuriopsis</i>	(*)	(*)	(*)	(*)	
+ <i>Ephoron</i>	(*)	(*)	(*)		
* <i>Potamanthus</i>	(+)	(+)	(+)		

Table 6. Preferences of species for water quality classes — labelling as for Table 4.

Genus	Water Quality Classes				
	I Sig. C	II Sig. C	III Sig. C	IV Sig. C	V Sig. C
* <i>Baetis alpinus</i>	(+)	(+)			
* <i>B. fuscatus</i>	(+)	(+)	(+)	(+)	
** <i>B. maurus</i>	++++0.193	+0.000	---0.035		
** <i>B. muticus</i>	++++0.294	-0.001	---0.054	---0.037	---0.019
** <i>B. pavidus</i>	++++0.098	++++0.009	++++0.103	+++0.007	---0.075
* <i>B. rhodani</i>	++++0.152	++++0.021	---0.013	---0.069	---0.084
** <i>Centroptilum</i>					
<i>luteolum</i>	++++0.124	-0.004	---0.013		
* <i>C. gr. pulchrum</i>	(+)	(*)			
** <i>C. luctuosa</i>	-0.000	++0.004	++++0.017	-0.000	---0.053
** <i>C. pusilla</i>	++++0.064	+0.003	-0.003	---0.025	
** <i>Ephemerella ignita</i>	++++0.174	+0.001	---0.040	---0.032	
* <i>E. ikonovici</i>					
<i>nevadensis</i>	(+)	(+)			
* <i>E. maculocaudata</i>	(+)	(+)			
** <i>Torleya cf. belgica</i>	++++0.153	---0.009			
** <i>Ecdyonurus</i> spp.	+++0.194	+++0.007	---0.012	---0.085	
** <i>Epeorus</i>					
<i>torrentium/sylvicola</i>	++++0.0116	-0.000	---0.025		
* <i>Rithrogena marcosi</i>	(+)	(+)	(+)		
** <i>R. gr. semicolorata</i>	++++0.050	-0.000	---0.008	---0.011	
** <i>Ephemera danica</i>	++++0.205	---0.023	---0.018		
** <i>Paraleptophlebia</i>					
<i>submarginata</i>	++++0.190	---0.014	---0.021		

Table 6. (continued)

** <i>Habrophlebia eldae</i>	++++0.070	-0.000	---0.012
* <i>Oligoneuriella</i>			
<i>marichuae</i>	(+)	(+)	(+)
** <i>O. rhenana</i>	++++0.040	-0.000	-0.003
* <i>Potamanthus luteus</i>	(+)	(+)	(+)

inhabit medium and lower courses (generally moderately polluted) and are absent in headwaters (with waters in good conditions).

### Species Level

Although nine species were found in highly polluted waters (classes IV and/or V) (Table 1), the total number of each species present within the water quality classes decreased noticeably from class I to V (Table 1, bottom). Whereas some tolerant species do exist, mayflies are restricted by pollution. Although many species can inhabit a very high range of water quality classes (Table 1), the significance level of association is more restricted (Table 6). Thus, the highest tolerance degree corresponds to *B. pavidus*, significantly associated with classes I to IV, and to *C. luctuosa*, highly associated with moderately polluted waters (class III). Four species (*C. gr. pulchrum*, *R. marcosi*, *O. marichuae* and *P. luteus*) were randomly distributed within classes 1 to 3. Seven species (*B. alpinus*, *B. maurus*, *B. rhodani*, *C. pusilla*, *E. ikonovici nevadensis*, *E. maculocaudata* and *Ecdyonurus* spp.) were associated with good or slightly polluted waters (classes I-II), and others (*B. muticus*, *C. luteolum*, *E. ignita*, *Epeorus torrentium/sylvicola*, *R. gr. semicolorata*, *E. danica*, *H. eldae*, and *O. rhenana*) were significantly associated with non-polluted waters (class I).

### Discussion

In general our results agree with previous findings for mayfly species pollution tolerance, but results from our statistical analysis indicate that there were some exceptions. Our findings suggest that *B. alpinus* should not be considered strictly as an intolerant species (Janieva 1979; Hellawell 1986; García de Jalón and González

del Tánago 1986) but as a slightly tolerant species (as previously suggested Alba-Tercedor *et al.* 1991). According to Sladecek (1973), *E. danica*, *C. luteolum* and *R. gr. semicolorata* behave as intolerant species in this region and not as tolerant species, as indicated by other authors (Russev 1979; Hellawell 1986; García de Jalón and González del Tánago 1986). *Oligoneuriella rhenana* occurred in water quality classes I-III, agreeing with the findings of other researchers (Sladecek 1973; Russev 1979; García de Jalón and González del Tánago 1986). Moreover, *O. rhenana* had a preference for non-polluted waters (class I). Also, *E. ignita* behaved as an intolerant species, in agreement with Sladecek (1973) but in disagreement with authors who considered this species to be indifferent (García de Jalón and González del Tánago 1986) or at least tolerant (Russev 1979; Hellawell 1986) to pollution.

In summary, Baetidae (*B. pavidus*) and Caenidae (*C. luctuosa*) exhibited the highest pollution tolerance of all mayflies identified in the Guadalquivir River Basin of southern Spain. Other taxonomic families of mayflies occurred at river sites characterized by unpolluted to slightly polluted waters.

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