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Physico-Chemical Factors that Determine the Distribution of Mayflies and Stoneflies in a High-Mountain Stream in Southern Europe (Sierra Nevada, Southern Spain)

by

Carmen ZAMORA-MUÑOZ, Antonino SANCHEZ-ORTEGA
and
Javier ALBA-TERCEDOR

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The Río Monachil, a high-mountain stream in Sierra Nevada (South of Spain), was investigated. As a consequence of sewage discharges from a ski resort situated at the head of the stream and from populations along its banks, the river is affected by organic pollution.

With the objective of finding what physico-chemical factors determined the distribution of the Ephemeroptera and Plecoptera along the course of the stream, and in what manner they were affected, a stepwise multiple-regression analysis was applied. Through this analysis it was possible to prove that, in general, the distribution and abundance of these two orders of insects in the Río Monachil are influenced by organic pollution. Nevertheless, at the species level other factors assume prime importance, such as temperature, oxygenation and mineralization of the waters, parameters which may or may not be related to pollution.

C. ZAMORA-MUÑOZ, A. SANCHEZ-ORTEGA and J. ALBA-TERCEDOR, Dpto. Biología Animal y Ecología, Facultad de Ciencias, Universidad de Granada, 18071-Granada, España.

INTRODUCTION

Numerous studies in the Iberian Peninsula have used macroinvertebrates to assess the biological quality of freshwaters and have attempted to explain the distribution of the species in terms of their response to pollution (Alba-Tercedor *et al.*, 1986; Alba-Tercedor and Jiménez-Millán, 1987; Alba-Tercedor *et al.*, 1991, in press; Domezáin *et al.*, 1987; García de Jalón and González del Tánago, 1986; González del Tánago *et al.*, 1979; Prat *et al.*, 1983; Puig *et al.*, 1981; Ropero, 1984; Zamora-Muñoz and Alba-Tercedor, 1992).

Nevertheless, although the macroinvertebrates are visibly affected by pollution to a greater or lesser degree (Hart and Fuller, 1974; Hellawell, 1986), it was of interest to establish what physico-chemical parameters significantly determine their distribution throughout a stream. Thus, the present paper examines the physico-chemical factors that best explain the distribution of Ephemeroptera and Plecoptera nymphs in the Río Monachil.

DESCRIPTION OF STUDY AREA

The source of the Río Monachil stream is at 2600 m a.s.l., on the NE face of the Sierra Nevada Mountains (Granada, Southern Spain). It runs a 25 km course, with a snow dependent flow regime (Pulido, 1980), abrupt profile (average slope 8%), a narrow bed, shallow waters and stony substrata, so that muddy margins are very scarce and are only present at lower altitudes. It flows into the left side of the Genil River, at 650 m altitude, below the city of Granada. It runs from its origin to approximately 1500 m altitude over silicates, then over calcareous substrata to 900 m, and finally over sedimentary material until its confluence with the Genil.

Near the source of the Monachil there is a ski resort, which discharges waste waters into the stream without any purification. Farther below, the stream receives the discharges from various towns and residues from agricultural areas, which also use part of its flow for irrigation; and, before it joins the Genil, it receives the sewage effluents from various neighbourhoods of the city of Granada.

MATERIALS AND METHODS

Sampling

Eight sampling stations were established along the course of the stream, above and below different points of contamination (Fig. 1). The sampling stations were monitored seasonally throughout one yearly cycle, between November 1985 and August of 1986.

In situ measurements of temperature, pH and concentration of dissolved oxygen were made. For other factors (conductivity, calcium and magnesium hardness, nitrates, nitrites, ammonium and phosphates) samples of water were gathered in glass bottles, preserved with chloroform, and analysed in the laboratory.

In order to capture and quantify nymphs of mayflies and stoneflies, a Surber sampler (0.36 mm mesh size) was used for consecutive casting, until a length of two meters had been covered, in both the bank and in the central zones.

Full descriptions of this method and the sampling stations, as well as their physico-chemical characteristics, have been given by Zamora-Muñoz and Alba-Tercedor (1992).

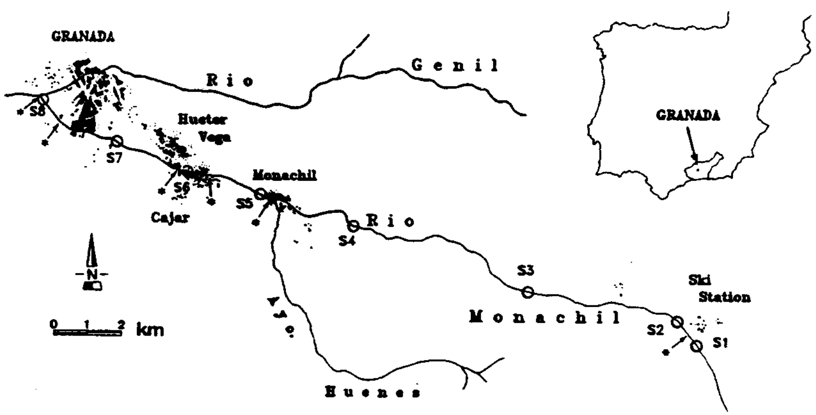

Fig. 1. Course of the Río Monachil, with both sampling stations (S1-S8) and town sites. Arrows and asterisks indicate approximate locations of sewage effluents.

Statistical methods

With the objective of establishing which physico-chemical parameters, or combination thereof, significantly explains the abundance of each species, a stepwise multiple-regression analysis was performed between the number of individuals of each species (dependent variable) and the different parameters measured (independent variables). This type of analysis isolates, from among all the physico-chemical factors, those which significantly influence the distribution of each species, separating some that at first appear to be explained by others (see Sokal and Rohlf, 1979).

Prior to the analysis, in order to normalise the data, a transformation was made of the number of individuals in each species, and of values of phosphate and ammonium, according to the formula $x'=\log_{10}(x+1)$.

Undoubtedly some of the factors that influence the distribution and abundance of the species were not measured. Nonetheless, the position of the independent variables in the best-fit regression model indicated the order of importance of environmental parameters in which a species is found. Thus like Dudgeon (1990) in the Lam Tsuen River, we consider those factors that best fit the model to be "determinants" of species distribution.

At first we included altitude as one of the independent variables, although being a parameter strongly related to other variables it confuses the effect of such factors. Therefore, we have eliminated it.

Logically, the same physico-chemical parameter could explain the distribution of a number of the species studied. To test whether these species showed similar behaviour with respect to the variation of this factor, a covariance analysis (ANCOVA) (Edwards, 1985) was performed. Groups of species were established on the premise that the slope of the line of regression, between the number of individuals of each species and the factor in question, should not differ significantly.

Afterward, among species in the genus *Baetis* that showed different behaviour with respect to a given factor, we tried to demonstrate replacements along the stream. Therefore, the relationship between a species and a given parameter was represented in the first quadrant, and the species to be compared with in the second quadrant. So, in order to fit the hypothesis of the replacement of species along a determined gradient, the slope of the lines of regression must have the same sign and must not be significantly different.

RESULTS

Fifteen species of Ephemeroptera and twelve species of Plecoptera were identified (Table 1). The distribution of most species (10 Ephemeroptera, of which 7 belong to the genus *Baetis*, and 2 Plecoptera) was influenced significantly by at least one environmental parameter. Parameters related to these species, in order of importance, were: water temperature, content of calcium, nitrates, dissolved oxygen, pH and phosphates (Tables 2 and 3).

It should be pointed out that the abundance of the different species of these two orders, in the Río Monachil, could be explained in many cases by only one physico-chemical factor (Table 2). In other cases two (*Ephemerella ignita, Leuctra inermis, Isoperla nevada*) or three parameters (*Baetis alpinus, B. muticus, B. rhodani*) were required for the best-fit regression model.

If we observe the results obtained through studying the abundance of the Ephemeroptera as a whole, it can be seen that they are negatively related to phosphates, a parameter that only explained the distribution of *B. rhodani* (Table 2). In turn, the Plecoptera as a group needed a low concentration of nitrates as well as ample aeration of the waters (Table 3).

Table 1. List of species of Ephemeroptera and Plecoptera collected in Río Monachil. No. = Number of individuals.

Stoneflies	No.	Mayflies	No.
Capniidae		Baetidae	
Capnia nigra (Pictet)	22	*Baetis alpinus* Pictet	5462
Leuctridae		*B. fuscatus* (Linné)	12
Leuctra fusca (Linné)	14	*B. maurus* Kimmins	202
L. inermis Kempny	567	*B. muticus* Linné	228
Nemouridae		*B. pavidus* Grandi	21
Amphinemura triangularis (Ris)	3	*B. rhodani* Pictet	4585
Protonemura alcazaba Aubert	3	*B. scambus* Eaton	7
P. meyeri (Pictet)	169	*B. vernus* Curtis	15
Perlidae		*Cloeon cognatum* Stephens	4
Dinocras cephalotes (Curtis)	72	Caenidae	
Perla grandis Rambur	5	*Caenis luctuosa* (Burmeister)	3
P. marginata (Panzer)	125	Ephemerellidae	
Perlodidae		*Ephemerella ignita* (Poda)	324
Isoperla grammatica (Poda	55	Heptageniidae	
I. nevada Aubert	68	*Ecdyonurus* spp.	57
Perlodes microcephalus (Pictet)	3	*Epeorus sylvicola/torrentium* (after Alba-Tercedor)	307
		Rhithrogena marcosi Alba-Tercedor and Sowa	7
		R. gr. *semicolorata* (after Sowa)	2168

Temperature

As Ward (1986) indicated in his study of a Rocky Mountain stream, many researches consider temperature as the major, or one of the major, factors limiting the distribution of Plecoptera and Ephemeroptera. In the Monachil, temperature combined with the calcium content is the most important parameter explaining the distribution of species within the family Baetidae, of *Ephemerella ignita*, and of the Plecoptera, *Leuctra inermis* and *Isoperla nevada*. Furthermore, in all cases temperature was included as the first variable within the best-fit regression models (Tables 2 and 3).

In terms of this parameter, great interspecific differences can be observed, even within the same genus. Thus, a covariance analysis (ANCOVA) performed between the number of individuals of the above species and temperature, produced two significantly different groups: group-1, with species positively related to temperature; and group-2, with species related negatively to temperature (Table 4).

Moreover, two of those species, *Baetis muticus*, and *B. alpinus*, one being represented in the second quadrant, showed slopes with no significant differences (F=2.60; p>0.1). Therefore, we can assume that both species are replaced along the profile, in relation to temperature.

Table 2. Results of regression analysis of mayfly species (Temp = Temperature, °C; Cal = Calcium hardness, mg/l; Nitr=Nitrates, mg/l; Oxy = Oxygen, mg/l; Phos = Total phosphates, mg/l).

Species	Multiple Regression Equation	%R^2	F	P
B. alpinus	y = 19.94 -0.27**Temp** -0.03**Cal** -1.73**pH**	67.91	17.64	<0.0001
B. fuscatus	y = -0.30 +3.91*10^{-2}**Temp**	18.10	5.97	<0.025
B. maurus	y = -0.19 +1.30*10^{-2}**Cal**	15.76	5.05	<0.05
B. muticus	y = -5.35 +0.10**Temp** +0.01**Cal** +0.41**Oxy**	44.13	6.58	<0.005
B. rhodani	y = 1.55 +5.26*10^{-2}**Cal** -2.58**Phos** -1.38**Nitr**	56.55	10.84	<0.0001
B. vernus	y = 2.83 -0.35**pH**	13.93	4.37	<0.05
B. scambus	y = -0.14 +0.02**Temp**	16.42	5.31	<0.05
C. cognatum	y = -0.11 +0.13**Nitr**	14.52	4.59	<0.05
E. ignita	y = -4.23 +0.19**Temp** +0.27**Oxy**	38.11	8.01	<0.0025
R. gr. *semicolorata*	y = -3.87 +0.49**Oxy**	19.79	8.90	<0.025
TOTAL	y = 12.73 -6.19**Phos**	24.79	8.90	<0.01

Mineralization of the water

Of all the chemical parameters measured that are related to the mineralization of the water, only the calcium content was a determining factor in the distribution of four baetid species. For *Baetis maurus* this was the only parameter that could significantly explain its distribution (Table 2). *B. rhodani* and *B. muticus* are similar to this species in relation to calcium, but *B. alpinus* is inversely related to water mineralization in the Río Monachil (Table 4). In addition, it is shown that *B. rhodani* replaces *B. alpinus*, depending on the concentration of calcium, along the profile of the river (F=0.76; p>0.1).

pH

The variations in pH in the Río Monachil were not sharp. Nevertheless these changes affected the distribution of two species of Ephemeroptera, *Baetis alpinus* and *B. vernus* (Table 2). Although both species were influenced negatively by pH level, they behaved differently toward this factor (Table 4).

Table 3. Results of regression analysis of stonefly species. For abbreviations, see Table 2.

Species	Multiple Regression Equation	%R^2	F	P
L. inermis	y = 2.98 -0.15**Temp** -0.75**Nitr**	32.54	6.27	<0.01
I. nevada	y = 1.81 -0.09**Temp** -0.47**Nitr**	30.61	5.73	<0.01
TOTAL	y = -2.82 -2.70**Nitr** +0.92**Oxy**	30.85	5.80	<0.01

Dissolved oxygen

The concentration of dissolved oxygen in the water was the only factor for the distribution of *Rhithrogena* gr. *semicolorata* in the Río Monachil. This factor, along with others, influenced the distribution of *Baetis muticus* and *Ephemerella ignita* (Table 2) and, as mentioned earlier, the distribution of Plecoptera as a whole.

In the three species of mayflies, the relationship between abundance and concentration of oxygen was positive, and they did not behave toward this parameter in significantly different manners (Table 4).

Nitrogen content

Of the three parameters measured, nitrates, nitrites and ammonium, only the first was found to be a determining factor for the distribution of the two species of Ephemeroptera, *Cloeon cognatum* and *Baetis rhodani* (Table 2). Likewise, the concentration of nitrates had a negative influence on the general distribution of Plecoptera, and particularly on *Leuctra inermis* and *Isoperla nevada* (Table 3). The behaviour of these species toward this factor separates them significantly from the two Ephemeroptera, which showed a positive average slope (Table 4).

It is noteworthy that nitrate influence upon *B. rhodani* is a positive factor when considered alone, but becomes a negative factor when considered along with the other parameters measured (Tables 2 and 4).

Phosphates

The concentration of phosphates was the factor that best explained the distribution of all Ephemeroptera in the Río Monachil. Also, in a negative manner, it interferes as a second variable in the best-fit model for *Baetis rhodani* (Table 2).

DISCUSSION

All along the Río Monachil organic pollution negatively influences the distribution of Ephemeroptera and Plecoptera as a whole, and more specifically the distribution of *Leuctra inermis*, *Isoperla nevada* and *Baetis rhodani*. The first two were captured only at the head of the river (sampling stations 1 and 2) where, as we had already observed (Zamora-Muñoz and Alba-Tercedor, 1992), the discharges from the ski resort were one of the determining factors for the abundance of these species. Moreover the capturing of *L. inermis* and *I. nevada* during the summer was rare or non-existent due to their summer life cycle (García de Jalón and González del Tánago, 1986; Sánchez-Ortega, 1986). This could explain the negative

Table 4. Grouping of species according to their behaviour toward physico-chemical factors (ANCOVA; see Material and Methods).

		F	
	Average Slope	**Within Species**	**Within Groups**
Temperature			
Group-1: *B. fuscatus, B. muticus, B. scambus, E. ignita*	+0.07+/-0.03	2.58; p>0.1	
			13.10; p<0.01
Group-2: *B. alpinus, L. inermis, I. nevada*	-0.15+/-0.04	0.88; p>0.1	
pH			
Group-1: *B. alpinus*	-2.17	-	
			5.10; p<0.05
Group-2: *B. vernus*	-0.35	-	
Nitrates			
Group-1: *B. rhodani, C. cognatum*	+0.16+/-0.03	1.74; p>0.1	
			4.17; p<0.025
Group-2: *L. inermis, I. nevada*	-0.50+/-0.11	0.27; p>0.1	
Calcium hardness			
Group-1: *B. rhodani, B. maurus, B. muticus*	+0.02+/-6.0*10^{-3}	2.03; p>0.1	
			4.17; p<0.025
Group-2: *B. alpinus*	-0.04	-	
Oxygen			
Single group: *B. muticus, E. ignita, R. gr. semicolorata*	+0.30+/-0.10	1.39; p>0.1	

influence of temperature.

Baetis rhodani and *Cloeon cognatum* are abundant species in zones rich in vegetation (Alba-Tercedor, 1984; Macan, 1979; Wiederholm, 1984) and are classified by Hellawell (1986) as tolerant of moderate pollution. This agrees in part with our findings, but in the case of *B. rhodani*, our analysis method pinpointed the factors which truly determine the distribution of this species in the Río Monachil. That is, the population of this species increases with greater mineralization of the water, but decreases when the concentration of nutrients is excessive.

Although the remaining species of Ephemeroptera that were significantly related to physico-chemical parameters at certain sites showed affects of organic discharges (Alba-Tercedor *et al.*, 1991; Zamora-Muñoz and Alba-Tercedor, 1992), their distributions were due to another series of factors. Thus, while *B. alpinus* is considered intolerant of pollution (García de Jalón and González del Tánago, 1986; González del Tánago and García de Jalón, 1984; Hellawell, 1986) since it inhabits the higher parts and the origins of watercourses (Alba-Tercedor *et al.*, 1991; Müller-Liebenau, 1969; Vinçon and Thomas, 1987), it showed tolerance in the Río Monachil because the determining factors of its distribution were temperature, calcium content, and pH.

Previously, in the lower course of other rivers in the Sierra Nevada we have found that *B. alpinus* was displaced by *B. maurus*, a species similar in morphology and directly influenced by mineralization of the waters (Alba-Tercedor, 1983). Although in the Río Monachil this replacement is also seen (Alba-Tercedor *et al.*, 1991; Zamora-Muñoz and Alba-Tercedor, 1992), there was no statistical significance ($F=6.85$, $p<0.01$); nevertheless there was significance with another species of the same group in relation to calcium: *B. rhodani*. Similarly, *B. alpinus* was replaced by *B. muticus* in relation to water temperature.

Baetis vernus is a species that always occurs in sparse numbers in the Sierra Nevada and, as in the Río Monachil, it is restricted to high altitude (Alba-Tercedor *et al.*, 1991, in press). These characteristics, such as a significant preference for acidic waters, cause a behaviour toward pH different from that of *B. alpinus*.

Some authors have associated *Baetis* species of the group *fuscatus* (*B. fuscatus* and *B. scambus*) with mineralization of waters (Macan, 1979). Nevertheless, the affinity of *B.* gr. *fuscatus* for waters with high temperature in the Río Monachil confirms the observation of Vinçon and Thomas (1987) in the Western Pyrenees. Similarly, they considered *Ephemerella ignita* eurythermic. These three species, which are grouped according to temperature, probably owe their behaviour to the fact that they have only one summer generation (Alba-Tercedor, 1984, 1990).

Some of the parameters that we used in the analysis may not be the direct determinants for the distribution of a species. In this way the positive relationship between *Rhithrogena* gr. *semicolorata* and the concentration of dissolved oxygen is probably related with the increase in swiftness of the current, since it is a torrential species (Hynes, 1970).

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