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NEMATODE FAUNA IN CALCIC BICARBONATE SPRINGS IN THE PROVINCE OF GRANADA (SPAIN)

by
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Summary. Eleven springs were selected from a wider nematological study of 38 mineral-water springs for their low or medium-high conductivity and for the relative abundance of the bicarbonate and calcium ions. The nematode fauna found inhabiting these calcic bicarbonate springs comprised the sort of cosmopolitan species frequently found in rivers or lakes not subjected to extreme physico-chemical conditions. *Eumonhystera barbata* Andrássy 1981, *Monhystrella hastata* Andrássy 1968, *Plectus parvus* Bastian 1865, *Chronogaster typica* (De Man 1921) De Coninck 1935, *Achromadora ruricola* (De Man 1880) Micoletzky 1925 and *Udonchus tenuicaudatus* Cobb, 1913 were positively correlated with bicarbonate or calcium ions.

Studies describing both the physico-chemical conditions of a particular environment and its nematode-fauna are increasing, e.g. Schmitz (1971), Zullini (1976), Eder and Kirchengast (1982), Hodda and Nicholas (1986) and Tudorancea and Zullini (1989), among others.

Highly-salinized environments have been shown to adversely influence both nematode fauna abundance and specific nematode fauna composition, as not all species will tolerate high salinity.

It has previously been shown that an inverse relationship exists between high conductivity values (> 2500 µS/cm) and the number of species, particularly the number of individuals present. Furthermore, in cases of extremely-high salinity (> 8500 µS/cm) there is a total absence of nematodes (Ocaña, 1991a). Tudorancea and Zullini (1989) reported only two nematode species (described for the first time for African lakes) in lakes with a conductivity rate of 15120 and 22470 µS/cm. On the contrary, environments with high numbers of individuals and species have low to medium-high salinity (with conductivity rates between 250-100 µS/cm (Ocaña, 1991a).

In highly-mineralized waters, the ions which are primarily responsible for mineralization are chloride, sulphates and sodium, while in other environments with a low or medium-high level of mineralization the bicarbonate and calcium ions acquire greater relative importance, as described in studies undertaken in the province of Granada (Ropero, 1984).

Of the 38 springs studied in Granada (Spain), from a nematological standpoint 11 were found to show a relative content of bicarbonate and calcium greater than 25% of the anions and cations respectively, and were therefore classified as calcic bicarbonate. The nematode fauna found in these springs is presented in this study.

Area of study and methodology

The 11 calcic bicarbonate springs studied are located in an important interior depression found between the Betic and Subbetic systems (Cruz-SanJulian *et al.*, 1972). The springs are located as follows (Fig. 1): three from the city of Granada (GR1, GR2, GR3), Monachil (MO), Güejar Sierra (GJ), Pitres (PI), Pórtugos (PO), Bérchules (BE), two from Melegís (MG1, MG2) and Huéscar (H1).

The physico-chemical characteristics of the spring waters remain constant over time (Margalef, 1986). Further, as springs are also very homogenous habitats, only one sampling station was established in each spring.

The sampling period extended from April 1983 to March 1984. Two samples were collected from every spring each season of the year (i.e. 8 samples per spring; 88 samples total).

Sediment samples (250 cc) were collected for subsequent extraction of nematodes by a modified version of the Baermann funnel method (Hooper, 1986). Specimens were later fixed in anhydrous glycerin by the Seinhorst method (1962).

Conductivity, pH and temperature values were determined mechanically using a conductometer, pH meter and thermometer, respectively. Bicarbonate and calcium values were determined using volumetric methods, and organic material by oxidation using potassium dichromate in an acid medium, and excesses were subsequently evaluated with ammoniacal iron sulphate (results are expressed as % of organic carbon).

The diversity and evenness indexes used (Shannon: H y J' = H'/H max.) were calculated using Fortran software. Correlation analyses were conducted using the P4m and P3s programs from the BMDP statistic software package

Fig. 1 - Location of calcium bicarbonate springs in the province of Granada.

(Dixon, 1979). Pearson correlation coefficient significance was determined by comparison of the corresponding Rohlf and Sokal (1981) statistical tables.

Results

Bicarbonate, calcium, conductivity, pH, temperature and organic material values obtained for each of the springs studied are listed in Table I.

CO_3H values fluctuated between 90 mg/l in PI and 650 mg/l in BE. Ca showed a minimum value of 59 mg/l in GJ and a maximum value of 191 mg/l in MG1.

Conductivity values varied between 137 μS/cm for PI and 1185 for BE, the latter of which together with GR1 (with a conductivity value of 939 μS/cm) are the only two which showed high conductivity values, according to the criteria used by the United States Salinity Staff (Saura, 1978).

pH values fluctuated from 4.9 to 5.8 in the acid range for springs PI, PO and BE located in the Alpujarra Mountains, as well as a high iron content (14-18 mg/l). The highest basic pH values were 8.1 in MO and H1.

Temperatures in all cases were moderately cold (10-18 °C; the coldest being MO, 10.2 °C) to moderately warm (18-25 °C), in accordance with the Bogomolov criteria (1966). Several springs could be considered as thermal water, e.g. H1, MG1 and particularly MG2, which had the highest temperatures of all the springs studied (24.3 °C average). Those natural water bodies in the province of Granada which are considered as thermal water give a temperature above 19 °C (Cruz-SanJulian, *et al.*, 1972).

Finally, values for sediment organic matter, the last physicochemical parameter studied, were relatively low,

TABLE I - *Values for CO_3H and Ca (mg/l); conductivity (µS/cm); pH; temperature (°C) and organic material in sediment (C.O.) (organic carbon in %), in each of the springs studied.*

Spring	CO_3H	Ca	Conductivity	pH	T	C.O.
GR1	400	127	939	7.6±0.1	15.7±1.2	0.49
GR2	250	55	365	7.8±0.1	15.1±1.7	1.18
GR3	300	64	456	7.6±0.1	15.7±0.2	1.39
MO	200	68	256	8.1±0.1	10.2±0.2	0.10
GJ	200	59	255	7.5±0.1	11.3±0.3	1.77
PI	90	93	137	4.9±0.1	13.2±0.3	1.10
BE	650	127	1185	5.8±0.1	16.4±0.5	1.68
PO	190	110	319	5.3±0.1	15.4±0.1	0.92
MG2	270	80	574	7.3±0.1	24.3±0.2	0.80
MG1	260	191	474	7.7±0.2	21.8±0.3	2.57
H1	190	140	720	8.1±0.1	20.5±2.4	2.68

showing a minimum of 0.1 mg/l in MO and maximum of 2.7 mg/l in H1.

Table II indicates the species from orders Monhysterida, Araeolaimida, Chromadorida and Enoplida including the mean number of individuals found per 250 cc sample. Species belonging to these orders (on which the authors have conducted extensive taxonomical studies over the past years: Ocaña and Zullini 1988; Ocaña, 1990; Ocaña *et al.*, 1990; Ocaña, 1991b; Ocaña 1991c; Ocaña and Coomans, 1991) were the best represented in freshwater habitats. Table III refers to the total number of species found in each spring from orders Monhysterida, Araeolaimida, Chromadorida plus Enoplida, Dorylaimida, Mononchida, Rhabditida, Tylenchida and Aphelenchida.

The total number of species was found to be abundant in the calcic bicarbonate springs, ranging from 8 (MO, PI) to 35 (GR1). Springs GR3, GR2 and PO, MG1 and MG2 showed an equally high number of species, particularly when considering that the average species number was 17.4±7.7. The total number of individuals fluctuated between 14 individuals/250 cc in PI and 2257 individuals/250 cc in GR1.

Of the 45 species from the orders Monhysterida, Araeolaimida, Chromadorida and Enoplida found at all 38 sites studied, 34 appeared in the 11 calcic bicarbonate springs.

Diversity and evenness indexes were additionally calculated for each of the springs at each sampling date. Table IV illustrates the minimum and maximum values among the 8 samples, presented by both indexes for each spring. Important differences are found between sampling dates, and only springs GR1, GR2, GR3, GJ and MG2 showed some constancy in the nematode fauna present throughout the sampling period (presenting non-zero diversity and evenness values throughout the indicated period of study). Zero diversity and evenness values are due to the fact that only one species appears for the corresponding sampling date.

For the purposes of establishing some type of relationship between the nematode fauna from those orders which are characteristic of the habitat studied (i.e. Monhysterida, Araeolaimida, Chromadorida and Enoplida), the different species were correlated, using the Pearson rank correlation, with ions and the following parameters: CO_3H, Ca, organic carbon, conductivity, pH and temperature. Table V shows only positive correlation values ($r > 0.52388$); in this case, no species were found to be positively correlated with either pH or temperature. After having calculated correlation values, *Plectus parvus* and *Achromadora ruricola* showed a positive correlation coefficient with bicarbonate, while *Eumonhystera barbata*, *Monhystrella hastata*, *Chronogaster typica* and *Udonchus tenuicaudatus* correlated positively with calcium. Moreover, *P. parvus* and *A. ruricola* were the only species to positively correlate with conductivity, whereas *Monhystera stagnalis* was the only species to show a positive correlation with organic material.

Discussion

On the basis of the results reported in the present study and those described by Ocaña (1991a), highly-salinized waters show a lower relative importance for the bicarbonate and calcium ions. According to Margalef (1986), in cases in which the highest percentage of anions is composed of weak acids (i.e. mainly bicarbonates as is most commonly found) a positive correlation between conductivity and total alkalinity is seen whereas in continental water where conductivity generally ranges between

TABLE II - *Species of the orders Monhysterida, Araeolaimida, Chromadorida and Enoplida found in the calcium bicarbonate springs studied, including the number of individuals per species per sample (= 250 cc of sediment) in each spring.*

Order	Nematode species	GR1	GR2	GR3	MO	GJ	PI	BE	PO	MG1	MG2	H1
Monhysterida	*Monhystera stagnalis* Bastian, 1865	1										7
	M. paludicola De Man, 1881		585							9	97	33
	Eumonhystera longicaudatula (Garlach et Riemann, 1973) Andrássy, 1981					14						
	E. vulgaris (De Man, 1880) Andrássy, 1981		16									
	E. andrassyi (Biró, 1969) Andrássy, 1981	50	214	36						7	2	59
	E. pseudobulbosa (Daday, 1896) Andrássy, 1981			9	26	12		2	4	1		
	E. filiformis (Bastian, 1865) Andrássy, 1981	8	40	9				10	4		4	
	E. dispar (Bastian, 1865) Andrássy, 1981	1		14							1	
	E. barbata Andrássy, 1981			22	2							
	Monhystrella hastata Andrássy, 1968		3	1		188		1	1	1		101
	M. macrura (De Man, 1880) Andrássy, 1981										15	
Araeolaimida	*Plectus palustris* De Man, 1880		1									
	P. aquatilis Andrássy, 1985	1330	127	31	5	4	2	130				
	P. parvus Bastian, 1865	30	6	10	3			13	3			
	P. geophilus De Man, 1880	2		8				3	176	3		
	P. parietinus Bastian, 1865				10			1				
	Chronogaster tipica (De Man, 1921) De Coninck, 1935	2	1			2					5	
	C. cameroonensis Heyns et Coomans, 1983	2								1		
	Rhabdolaimus terrestris De Man, 1880			6								
	Aphanolaimus aquaticus Daday, 1894						1					
Chromadorida	*Prodesmodora circulata* (Micoletzky, 1913) Micoletzky, 1925	1										
	Achromadora ruricola (De Man, 1880) Micoletzky, 1925	4					5			1		
	A. micoletzkyi (Stefanski, 1915) Van der Linde, 1938	2	8							1	8	
	Ethmolaimus pratensis De Man, 1880					2						
Enoplida	*Prismatolaimus intermedius* (Bütschli, 1873) De Man, 1880	8		30		1			2		2	
	P. dolichurus De Man, 1880		1	5								
	Udonchus tenuicaudatus Cobb, 1913	1									2	
	Trischistoma monohystera De Man, 1880	13	28	8								
	Alaimus primitivus De Man, 1880			5								
	Ironus elegans Colomba et Vinciguerra, 1979			13								
	Odontolaimus aquaticus Schneider, 1937					110						
	O. chlorurus De Man, 1880					754						
	Tobrilus pellucidus Bastian, 1865	10	7									
	T. granatensis Ocaña et Zullini, 1988	192	598	1							7	10

TABLE III - *Total number of species of each order found in each spring.*

Order	GR1	GR2	GR3	MO	GJ	PI	BE	PO	MG1	MG2	H1
M, A, C, E	17	14	17	4	10	3	7	6	8	10	5
Dorylaimida	5	9	4	2	6	1	4	5	2	2	2
Mononchida	–	2	1	–	1	1	1	1	1	1	–
Rhabditida	10	2	8	–	2	1	3	7	8	5	3
Tylenchida	2	–	1	1	2	1	1	2	2	2	–
Aphelenchida	1	–	2	1	–	1	1	1	1	1	–
TOTAL	35	27	33	8	21	8	17	22	22	21	10

TABLE IV - *Variation range for 8 samples taken in sampling period of diversity index and evenness in the springs studied.*

Spring	Index	
	Diversity	Evenness
GR1	1.47-3.47	0.43-0.90
GR2	1.82-2.72	0.51-0.82
GR3	1.00-3.81	0.80-1.00
MO	0.00-1.70	0.00-0.92
GJ	1.09-2.00	0.43-0.77
PI	0.00-1.92	0.00-1.00
BE	0.00-2.41	0.00-0.99
P0	1.40-3.09	0.83-1.00
MG1	0.00-2.72	0.00-0.93
MG2	0.68-3.52	0.46-1.00
H1	1.04-2.65	0.62-0.94

50 µS/cm and 500-1000 µS/cm, the bicarbonate and calcium ions show a greater relative importance.

Venkateswarlu and Das (1980) reported a positive correlation between water hardness and the population of benthic nematodes in the Moosi River (India); (all values for water hardness were under 240 mg/l and expressed as mg/l of CO_3Ca^-).

Other nematological studies on rivers in which water conductivity values are assumed to be normal (< 1000 µS/cm) and whose nematode fauna could be taken into consideration in the present discussion, correspond to those by Zullini (1974, 1975, 1976), Colomba and Vinciguerra (1979) or Eder and Kirchengast (1982). In several cases, the springs studied were also subjected to pollution, a problem which is not considered in the present study. However, no mention of bicarbonate and calcium content is made in these studies, although the authors assume on the basis of Margalef's criteria (1986) that both ions are relatively well-represented. Only Colomba and Vinciguerra (1979) state that the course of the Anapo River flows over calcareous terrain.

Very few data are available on conductivity in nematological studies on lakes. This type of information, for example, is not mentioned by Prejs and Papinska (1983) in their study on various lakes situated in the NE of Poland, nor for the Polish lakes sampled by Prejs (1977a, 1977b), or by Wasilewska (1973). Other lakes such as Lake Balaton, Hungary (Biró, 1969) or Lake Neusiedlersees (Schiemer, 1978) are saline lakes showing conductivity values of > 1500 µS/cm. Several of the African lakes sampled by Tudorancea and Zullini (1989) which were not salty, showed conductivity values similar to those which could be reported for a river (224-868 µS/cm), while others were extremely salty reaching values of 40810 µS/cm.

The nematode fauna studied by the various authors mentioned could have physico-chemical characteristics comparable to those found in the calcic bicarbonate springs in the province of Granada. The data on the number of species, percentage of species from Monhysterida, Araeolaimida, Chromadorida and Enoplida, the number of species common to these former studies and the author's research, and variations in the diversity and evenness indexes (when possible) are given in Table VI.

Data on the number of individuals in the present study cannot be compared with other studies as the superficial or volume sample units used were very different. For example, Wasilewska (1973) found in the littoral region of Lake Mikoljskie a mean of 117 ind/cm^2 = 29 ind/cm^2 individuals. Zullini (1974) found in the sands of the Po River a variation in the number of individuals of between 178-606 ind/200 l of filtrated interstitial water. In another study by Zullini (1975) a variation of 172-185 ind/300 l was reported. Prejs (1977a) found a variation of 1-1160 ind/m^2 in the number of individuals in different lakes in Poland. Therefore, the existing data are not very significant when drawing conclusions about the environment. It is worth mentioning the conclusion drawn by Ocaña (1991a), in a study comparing conductivity and the number of individuals present in 38 springs (i.e. 38 mediums which in this case were methodologically homogenous) in which it was

TABLE V - *Positive correlation values (r > 0.52388) obtained for species and parameters studied.*

Nematode species	CO_3H	Ca	Conductivity	C.O.
Monhystera stagnalis				0.55682
Eumonhystera barbata		0.61300		
Monhystrella hastata		0.70346		
Plectus parvus	0.62649		0.63549	
Chronogaster typica		0.58843		
Achromadora ruricola	0.88807		0.87629	
Udonchus tenuicaudatus		0.75003		

TABLE VI - *Total number of species, number of species for orders [Monhysterida (M), Araeolaimida (A), Chromadorida (C), Enoplida (E)], number of common species found in calcium bicarbonate springs, diversity and evenness indexes from the papers cited in the text.*

Author	Total Sp.	Sp (M, A, C, E)	Common Sp.	Index	
				Diversity	Evenness
Wasilewska (1973)	10	9 (90%)	3 (33%)	–	–
Zullini (1974)	31	16 (51%)	6 (38%)	1.12-3.37	–
Zullini (1975)	32	13 (41%)	7 (52%)	3.49-3.62	–
Zullini (1976)	70	25 (36%)	13 (52%)	2.52-3.97	0.53-0.68
Prejs (1977)	42	32 (76%)	11 (34%)	0.00-2.90	–
Colomba *et* Vinciguerra (1979)	46	21 (46%)	11 (52%)	0.55-3.56	–
Eder *et* Vinciguerra (1982)	33	20 (61%)	9 (45%)	–	–
Prejs *et* Papínska (1983)	17	15 (88%)	5 (33%)	0.00-1.44	–
Tudorancea *et* Zullini (1989)	12	7 (58%)	1 (15%)	0.00-1.54	–

deduced that the number of individuals is greater in those springs not subjected to extreme physico-chemical conditions, which is the case of the calcic bicarbonate springs.

A parameter which the number of individuals would be expected to depend upon is organic material content in sediment, but no correlation whatever has been found between either (Ocaña, 1987). Only *Monhystera stagnalis* showed a positive correlation with organic material.

The data given in Table VI on the number of nematode species found by different authors in the environment samples, show that the total number of species oscillated between 10 (Wasilewska, 1973) and 70 (Zullini, 1976), although the mean of the data compared is approximately 30. The total number of species in the springs studied in the province of Granada (Table III) ranged between 8 (MO) and 35 (GR1). GR1, GR2, GR3, GJ, PO, MG1 and MG2 showed a total number of species which was similar to the mean found in other continental aquatic habitats.

The number of species for orders *Monhysterida*, *Araeolaimida*, *Chromadorida* and *Enoplida* ranged between 7 and 32, i.e. 90% of the total number of species reported by Wasilewska (1973) and 36% of those reported by Zullini (1976). Zullini (1976) conducted a nematological study of the Seveso River which is subjected to a high degree of pollution and in which, therefore, the number of species from order *Rhabditida* increased considerably. The presence of this taxon of saprobacteriophagus feeders negatively affects the percentage of species from the above-mentioned orders present with regard to the total number of species.

The number of nematode species found to be common in both papers cited in Table VI and in the calcic bicarbonate springs of the province of Granada range from 1 (Tudorancea and Zullini, 1989) to 13 (Zullini, 1976), i.e. between 15% and 52% of the total number of species found from the 4 orders studied here. Tudorancea and Zullini's study (1989) analyzed a series of African lakes in which of the 12 species found in Lakes Zwai, Awassa, Abaya and Koka (the only lakes with conductivity values < 1000 μS/cm), half (6) are new to Science, which has a logical negative affect on the possibility of there being common species. With the exception of this study, the percentage of common species is high, ranging between 33-52%.

Finally, Table VI illustrates the fluctuations in the diversity (H) and evenness indexes on the different sampling dates and for each of the different sampling stations. It should be mentioned that the diversity index for the Po and Seveso Rivers (Zullini, 1975, 1976) shows great consistency. Moreover, the total absence of nematode fauna at particular stations or on specific dates, seen in the Granada springs, is quite usual. This occurrence is shown as a zero diversity index and in many cases is the result of the physical instability of the sediment submerged underwater which can be affected by large currents of water which disturb the superficial layer of the sediment in which nematodes are found, either by affecting the water supply or by alterations caused by the indirect or direct impact brought about by man (Ocaña, 1987). These are the same factors which produce zero evenness values. The evenness values obtained by Zullini (1976) are very similar to those obtained for springs GJ or GR2.

Conclusions

It is confirmed that the bicarbonate and calcium ions in waters with low to medium-high conductivity are more consistently present than other anions and cations.

Most of the species found in environments with these characteristics, as the case of the 11 calcic bicarbonate springs studied in the province of Granada (Spain), are cosmopolitan species which have been described on numerous occasions in other continental aquatic habitats.

One exception, however, is *Tobrilus granatensis* which to date has only been found in the Granada springs (Ocaña and Zullini, 1988). *Chronogaster cameroonensis*, previously reported in the soil of a region in Cameroons (Heyns and Coomans, 1983), or *Ironus elegans*, described for the first time in the Anapo River, Italy (Colomba and Vinciguerra, 1979) and also reported in Spain on three occasions (Ocaña, 1991b), could also be considered as a cosmopolitan species, although more information would have to be compiled to confirm this. On the other hand, *Eumonhystera barbata*, found in swampy sediment in a stalagmite cave in Hungary (Andrássy, 1981), in several springs and in the Monachil River (Granada) (Ocaña, 1990), could equally be considered a cosmopolitan species. *Udonchus tenuicaudatus* could be a species which preferentially inhabits hot water (Schneider, 1937; Ocaña, 1991c) and highly-saline environments (not above 1500 µS/cm) in which the calcium ion is well represented (see Schiemer, 1978).

Besides *Eumonhystera barbata* and *Udonchus tenuicaudatus*, *Monhystrella hastata* and *Chronogaster typica* also appear to show a preference for habitats with a high calcium content. The same can be said for *Plectus parvus* and *Achromadora ruricola* with regard to bicarbonate, and *Monystera stagnalis* with regard to organic material.

Furthermore, the mean number of species from the orders Monhysterida, Araeolaimida, Chromadorida and Enoplida found in environments which are comparable with the calcic bicarbonate springs of the province of Granada is approximately 30, i.e. the same number of similar species found in springs GR1, GR2, GJ, PO, MG1 and MG2. In addition, the number of species found to be common in both the calcic bicarbonate springs of Granada and other comparable habitats was generally between 33-53%. With regard to the environments analyzed in the present study, the total absence of nematodes on various sampling dates or at several sampling stations is not unusual, although on the whole the diversity and evenness indexes for these mediums tend to be more consistent than those for other environments subjected to extreme physico-chemical conditions.

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