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GENUS *JAERA* (ISOPODA, CRUSTACEA) SPREADING IN THE DANUBE BASIN, WITH SPECIAL EMPHASIS ON ROMANIAN STRETCH OF THE RIVER

VIRGINIA POPESCU-MARINESCU

Abstract. The study is worked out as a synthesis of the data concerning Ponto-Caspian isopod *Jaera* spreading in the Danube basin. It is outlined the fact that until 1988 when *J. istri* was mentioned in the Austrian stretch, it was thought that *J. sarsi* and *J. sarsi sarsi* existed in the river.
From the spreading viewpoint, it is mentioned that this isopod spans a wide area, including a number of river lagoons, the areas from the Danube mouths in front of the Sulina Arm as well as the river from the confluence with the Black Sea up to the springs. The richest *Jaera* populations are in the Romanian stretch, particularly in the Iron Gates I and Iron Gates II damlakes.

The genus *Jaera* representatives are the only Ponto-Caspian isopods present in the Danube, including the areas within the river confluence with the sea and the river estuaries (3).

As concerns the origin, Kothé (9) puts forward a number of arguments to support the concept that *Jaera* represents a real Ponto-Caspian relict throughout the Danube basin. But, according to Morduhai-Boltovskoi (11,12) and Bănărescu (personal communication), this isopod is considered as a Ponto-Caspian relict only in the lower part of the Danube while in the higher sections of the river, it is considered as autoimmigrants.

Since 1979, Veuille (28) has reviewed genus *Jaera* and has separated within it three species: *J. sarsi*, *J. istri* and *J. caspica*. Nevertheless, by the time Pockel published his work in 1988 (14) mentioning the occurrence of *J. istri* in the higher Danube, it was thought that in this river *J. sarsi* with *J. sarsi sarsi* sub-species (27) existed. But, no matter whether it is a question of *J.sarsi* or *J.istri*, both of them are fresh water, oxyphilic, rheophilic forms adapted particularly to a pebbly life.

Most of the works referring to genus *Jaera* spreading in the Danube basin were published after 1960, as in 1958, under the aegis of the International Working Group for the Danube study within the International Society of Limnology, a systematic study begun on the whole river course (it is carried out by all the riparian countries).

This work, representing a synthesis, is based both on publication of numerous Romanian and foreign researches and a number of results of personal unpublished studies.

REV. ROUM. BIOL. – BIOL. ANIM., TOME 46, N^{os} 1–2, P. 3–21, BUCAREST, 2001

We present the available data concerning isopod spreading in the Danube basin in the following order: river-lagoons and the areas from the Danube mouths, the Romanian stretch beginning from downstream to upstream, following other Danube stretches.

RESULTS AND DISCUSSION

I. *JAERA* ISOPOD SPREADING IN RIVER-LAGOONS AND THE AREAS FROM THE DANUBE MOUTHS

Isopod presence named as *J. sarsi* in the Danube lagoons, e.g., Chitai, Jalpug, Catlabug, Cugurlui, etc., has been mentioned since 1960 by Morduhai-Boltovskoi (11). In July 1961, Olivari presents isopods from Lazarkin, a fresh water gulf (located at the anterior edge of Chilia arm Delta) inhabiting a muddy substratum, without specifying whether it is a question of *Jaera* or *Asellus* (13).

As concerns the areas at the Danube mouths, in the North part, on the dam stones of Sulina Channel (the man-made extension of Sulina arm into the sea), in 1960, at depths of 1–2 m, *Jaera*, mentioned as *J. sarsi*, constituted the prevailing one in the biocenosis. Therefore, in October 1961, in petrophilic and brackish water biocoenosis from certain portions of the Sulina Channel dam, *Jaera* population was very numerous. There they were found weighing 380 ind/kg (considering the whole amount of material scraped off the dam stones), but the *Balanus* colonies were predominant (4).

A relatively high density of *Jaera* population, i.e. 180 ind/m^2, was found in 1968 (July 30) in Gârla making the link between the Sulina arm and North area (Baia de Nord = Musura Gulf) at 3 m depth, on a muddy-sandy substratum.

II. *JAERA* ISOPOD SPREADING IN THE ROMANIAN STRETCH OF THE DANUBE

Genus *Jaera* occurrence in the Romanian stretch of the Danube relates especially to the rocky or stony substratum, sometimes to the sandy-muddy or the muddy one, water flow of a velocity of minimum 0.5 m/s, a high amount of dissolved in water-oxygen and below 0.5 g‰ salinity.

The presence of isopod named *J. sarsi sarsi* Valk. in the respective river stretch has been reported by M. Băcescu since 1942, when by his investigations it has been found in Porţile de Fier (Iron Gates) section. Even the published work is entitled "Marine fauna traces in the Danube waters, at Cazane and Porţile de Fier" (Iron Gates) (1).

All the works published by 2000, referring to this isopod of the Romanian stretch of the Danube mentioned *J. sarsi* or *J. sarsi sarsi*.

A re-analysis of *Jaera* material from the Danube at the Iron Gates, collected during 1995–1996, allows us to state that *Jaera istri* species occurs in the Romanian stretch of the river.

Starting to present the situation in the river with its last section, the Deltaic one, i.e. towards its confluence with the Black Sea, we point out the fact that on the three Danube Arms: Chilia, Sulina and Sf. Gheorghe, the isopoda spreading is different.

Both on Chilia and on Sf. Gheorghe arms where the dominant substrates are sandy, muddy, clayey, *Jaera* has a limited spreading area. My personal researches carried out in Chilia Arm in 1995 (in Romanian territorial waters) revealed isopod presence in the summer (June) samples at rkm 20, a density below 450 pc/m^2, but with a biomass of 15 mg/m^2 only. This means the specimens are small and habitat under less favourable conditions. On Sf. Gheorghe Arm, although our researches were extended during several years, (from 1960 to 1995), we report this isopod presence in 1965 (March) at rkm 5 (towards the confluence with the sea through the main arm) at the left riparian, on a clayey substrate. Numerical density was low, 5 ind/m^2 and a biomass of 0.3 mg/m^2. In 1966 at 7 rkm, also at the left riparian, on a muddy substrate, the number of individuals was of 20/m^2 and a biomass of 4 mg/m^2. We determined the same figures from the biocoenosis established also at 7 rkm, left riparian, on a muddy substrate, in 1975.

As concerns the Arm Sulina, the least wide and with the lowest flow among arms, where by its canalizing (at the same time with its rectification, the bank pavement and increasing the flow capacity) the man-made stony substrate was created and the water flow velocity increased. Thus, the conditions for *Jaera* development became in the most part of this arm length, more favourable than on the Sf. Gheorghe and Chilia Arms.

But, we outline the fact that in the sector from the Arm Sulina confluence with the sea, i.e. Sulina Channel (marked in hm) with embankments of stone, in the section between hm 54 and mile 0, we found that the number of *Jaera* individuals, was generally low (Table 1).

In this respect, during 1958–1970 at hm 54 and hm 29, the isopod numerical density (in the biocoenosis with *Balanus*) did not exceed tens of ind./ sample or/m^2, with a single exception, when it reached 1320 ind./m^2. This is due mainly to the fact that there are great variations of salinity in different periods of the year (it reached 5.26 g $^0/_{00}$ Na Cl at mile 0; 8.8 g $^0/_{00}$ Na Cl at hm 29 and more than 10 g $^0/_{00}$ NaCl at hm 54) (7).

But one of the river sections of the Romanian stretch, facilitating genus *Jaera* development is the proper Arm Sulina, between miles 0 and 34. On this arm, starting from 1958, our very in – depth (15) researches revealed that the respective isopod constituted one of the biocoenosis components of the stony substratum with the stony-muddy and muddy-stony variants from the river riparian where it had 5.35% of the total of organisms (*Cordylophora, Urnatella* and *Corophiidum* being

dominant). Also, *Jaera* was present in this section and in the intermediate portions between riparian and medial, on a muddy – stony or muddy substratum (where *Polichaeta, Ampharetidae and Oligochaeta*) (Tables 2 and 3).

But we have the most numerous data on genus *Jaera* spreading from the mile 2. There, particularly in 1958–1959, we found that the water flow velocity maintained at an average of 0.8 m/s and salinity was less than $0.5^0/_{00}$ NaCl (15). During the year some salinity fluctuations were recorded (7).

Table 1

Jaera isopod spreading in the section of Danube confluence with Sulina Channel

Date of collecting	Place of collecting	Water depth (m)	Substratum nature	Density number (ind.)	Density biomass (mg)
1	2	3	4	5	
13 IX 1958	hm 54 – N. extreme	5	– shell debris, vegetal detritus	13/q.s.	1.02/q.s.
	– S. extreme	3.5	– vegetal detritus	4/q.s.	0.36/q.s.
	– Intermediate S. Extreme	8	– mud, vegetal detritus	4/q.s.	0.32/q.s.
20 XII 1958	hm 54 – N. extreme	4.8	– stone	8/q.s.	0.52/q.s.
	– S. extreme	3	– mud, vegetal detritus	9/q.s.	0.56/q.s.
27 I 1959	hm 54 – N. extreme	7	– mud, vegetal detritus	1/q.s.	0.08/q.s.
	– S. extreme	4	– stone, mud, vegetal detritus	22/q.s.	1.76/q.s.
4 III 1959	hm 54 – N. extreme	6	– mud, sandy, vegetal detritus	5/q.s.	0.40/q.s.
	– Intermediate N. extreme	7.9	– mud, sandy, vegetal detritus	4/q.s.	0.33/q.s.
	– Middle	9	– mud, sandy, vegetal detritus	1/q.s.	0.08/q.s.
	– S. extreme	5	– stony, vegetal detritus	29/q.s.	3.48/q.s.
13 IX 1959	hm 54 – N. extreme	5	– stone, vegetal detritus	19/q.s.	2.47/q.s.
28 IV 1961	hm 54 – S. extreme	6	– mud, vegetal detritus	20/q.s.	2.13/m^2
8 III 1966	hm 54 – N. extreme	8	– mud, sandy	70/m^2	4.9/m^2
20 VII 1967	hm 54 – S. extreme	3.5	– sticky mud	20/m^2	1.89/m^2
24 X 1970	hm 54 – N. extreme	5	– stone, mud, vegetal detritus	1320/m^2	396/m^2
25 IV 1960	hm 29 – N. extreme	8.5	– mud, vegetal detritus	8/pr.	0.96/q.s.

1	2	3	4	5	
9 VI 1960	hm 29 – N. extreme	3.0	– stone, vegetal detritus	9/q.s.	1.08/q.s.
	– N. extreme	6.7	– stone, mud, vegetal detritus	32/q.s.	4.16/q.s.
	– S. extreme	3.5	– stone	54/q.s.	5.94/q.s.
	– S. extreme	5	– stone	6/q.s.	0.40/q.s.
18 III 1966	hm 29 – N. extreme	8	– stone	35/m^2	4.90/m^2

Legend: N. extreme = Northern extreme; S. extreme = Southern extreme; q. s. = qualitative sample representing the outcome of a dredging on a 25 m length and 0.5 m width section.

From Table 2 analysis it results that although during 1958–1959 our researches at the mile 2 were on a monthly basis, we cannot always indicate exactly the *Jaera* populations numerical density and biomass, since in most cases the samples were qualitative ones. These coordinates are presented in the table as being related to the samples. But, quantitative data at the level of 1967–1974 years (Table 2) show maximal densities of 1000 ind./m^2 with a biomass of 64 mg/m^2 at mile 1 and 2800 ind./m^2 with 840 mg/m^2 at mile 2 (22).

Table 2

Jaera isopod spreading in the Danube on Sulina arm, between miles 0 and 3

Date of collecting	Place of collecting	Water depth (m)	Substratum nature	Density number (ind.)	Density biomass (mg)
1	2	3	4	5	
8 IV 1959	mile 0 – Intermediate right riparian	9	– stone	98/m^2	7.84/m^2
25 IV 1960	mile 0 – Right riparian	9.5	– mud, vegetal detritus	1/pr.	0.07/pr.
	– Left riparian	9	– mud, vegetal detritus	5/pr.	1.5/pr.
6VIII 1968	mile 0 – Right riparian	9.5	– mud, sand, shell debris	1000/m^2	52/m^2
25 IV 1960	mile 1 – Left riparian	9.7	– mud, stone, vegetal detritus	21/pr.	4.8/pr.
	– Middle	12	– sand, mud	9/pr.	2.2/pr.
7 XII 1961	mile 1 – Right riparian	6.5	– mud, stone	6/pr.	2.0/pr.
23 IX 1967	mile 1 – Right riparian	7	– mud, stone, vegetal detritus	340/m^2	17.0/pr.
1 VI 1974	mile 1 – Right riparian	7	– stone, mud	360/m^2	21.6/pr.
26VII 1974	mile 1 – Right riparian	7.5	– mud, stone	20/m^2	3.2/pr.
11 X 1974	mile 1 – Right riparian	6.5	– stone, mud, vegetal detritus	1000/m^2	64/m^2

1	2	3	4	5	
17 V 1958	mile 2 – Right riparian	6	– stone, mud	7/pr.	3.4/pr.
	– Intermediate	7.5	– mud	3/pr.	0.9/pr.
	right riparian	13.5	– sand	6/pr.	1.5/pr.
	– Middle				
	– Intermediate	8	– stone, mud	12/pr.	2.4/pr.
	left riparian	6	– stone, mud	5/pr.	1.1/pr.
	– Left riparian	6.5	– stone, mud	7/pr.	1.9/pr.
17 VI 1958	mile 2 – Right riparian				
	– Intermediate	8	– mud	6/pr.	1.2/pr.
	right riparian	13	– sand	20/pr.	3.9/pr.
	– Middle				
	– Intermediate	7.5	– stone, mud	16/pr.	6.4/pr.
	left riparian	5.5	– stone, mud	160/pr.	13.0/pr.
	– Left riparian	6.5	– stone, mud	14/pr.	2.0/pr.
17 VII 1958	mile 2 – Right riparian	6	– stone, mud	33/pr.	4.5/pr.
	– Left riparian	6.5	– stone, mud	7/pr.	0.9/pr.
16 VIII 1958	mile 2 – Right riparian				
	– Intermediate	8	– mud	2/pr.	0.5/pr.
	right riparian		– mud	32/pr.	4/pr.
	– Intermediate	7	– stone, mud	14/pr.	1/pr.
	left riparian	6	– mud, stone	5/pr.	2/pr.
	– Left riparian	6			
19 IX 1958	mile 2 – Right riparian		– mud	13/pr.	5.3/pr.
	– Intermediate	8			
	right riparian		– mud	4/pr.	0.6/pr.
	– Intermediate	7.5	– stone, mud	24/pr.	7/pr.
	left riparian	5	– mud, stone,		
	– Left riparian	4.5	vegetal detritus	5/pr.	1/pr.
17 X 1958	mile 2 – Right riparian		– sand, vegetal		
		11.5	detritus	23/pr.	5/pr.
	– Middle				
			– sand, mud,		
	– Intermediate	8	shell debris	39/pr.	6/pr.
	left riparian		– stone, mud,		
		4	vegetal detritus	5/pr.	1/pr.
	– Left riparian		– mud, stone	31/pr.	5.7/pr.
		3	– stone, sand,		
18 XI 1958	mile 2 – Right riparian	7.6	mud, vegetal		
	– Left riparian		detritus	5/pr.	1.1/pr.
			– mud, stone	56/pr.	10.6/pr.
19 XII 1958	mile 2 – Intermediate	11	– stone, mud	47/pr.	8/pr.
	right riparian	8.8			
	– Left riparian		– sand, mud		
	– Intermediate	10.8	vegetal detritus	6/pr.	1.1/pr.
	left riparian		– stone, mud, sand	122/pr.	40/pr.
		3			
27 I 1959	mile 2 – Right riparian		– mud, sand,		
	– Intermediate	13	vegetal detritus	30/pr.	6/pr.
	right riparian				

1	2	3	4	5	
	– Intermediate		– mud, sand	4/pr.	1/pr.
	left riparian	10.4	– stone, mud,		
	– Left riparian	9	sand, vegetal		
			detritus	64/pr.	12.2/pr.
			– stone, mud, sand	5/pr.	1.2/pr.
4 III 1959	mile 2 – Right riparian	3.5			
	– Intermediate		– mud	60/pr.	12/pr.
	right riparian	11			
	– Intermediate		– sand, mud	9/pr.	1.8/pr.
	left riparian	9.5	– mud, stone	19/pr.	2.8/pr.
	– Left riparian	9	– stone, mud	70/pr.	21/pr.
6 IV 1959	mile 2 – Left riparian	8	– sand, mud	3/pr.	0.8/pr.
	– Middle	12.3			
1 VII 1959	mile 2 – Intermediate		– mud, vegetal		
	right riparian	10	detritus	1/pr.	0.01/pr.
			– stone, mud	282/pr.	33.84/pr.
	– Left riparian	4	– stone, mud	7/pr.	0.8/pr.
3 VIII 1959	mile 2 – Right riparian	4			
	– Intermediate		– mud, vegetal		
	left riparian	9	detritus	9/pr.	1.5/pr.
	– Intermediate		– sand, vegetal		
	left riparian	11	detritus	3/pr.	0.5/pr.
			– mud, stone,		
30 X 1959	mile 2 – Right riparian	6	vegetal detritus	6/pr.	0.7/pr.
			– sand, vegetal		
	– Middle	11	detritus	1/pr.	0.2/pr.
			– stone, mud,		
	– Left riparian	5	vegetal detritus	256/pr.	45.51/pr.
30 XI 1960					
	mile 2 – Right riparian	1	– stone, mud,		
26 IV 1962			vegetal detritus	40/m^2	3.2/m^2
	mile 2 – Intermediate				
	right riparian	8.5	– mud, vegetal		
20 VI 1971			detritus	4/pr.	0.8/pr.
	mile 2 – Right riparian	5	– mud, sand	2800/m^2	840/m^2
17 X 1958					
	mile 3 – Right riparian	5	– mud, stone	25/pr.	2.3/pr.
	– Intermediate				
	right riparian	9	– mud	48/pr.	9.6/pr.
	– Intermediate				
	left riparian	8.5	– mud, vegetal		
			detritus	14/pr.	2/pr.
18 XI 1958	– Left riparian	6	– mâl	1/pr.	0.05/pr.
	mile 3 – Right riparian	8	– mud, sand,		
			vegetal detritus	6/pr.	0.9/pr.
	– Middle	12.7	– sand	5/pr.	1/pr.
	– Intermediate				
	left riparian	12.1	– sand	7/pr.	1.4/pr.
19 XII 1958	– Left riparian	7.8	– mud	15/pr.	2.8/pr.

1	2	3	4	5	
	mile 3 – Intermediate right riparian	12.7	– sand	5/pr.	1.2/pr.
	– Middle	12.5	– sand	2/pr.	0.3/pr.
	– Intermediate left riparian	9.5	– mud, vegetal detritus	128/pr.	14.9/pr.
	– Left riparian	6	– mud, stone	2/pr.	0.3/pr.
19 III 1966	mile 3 – Left riparian	9	– clay	27/m^2	2.3/m^2

Legend: q.s = qualitative sample representing the outcome of a dredging carried out on a 25 m length and 0.5 m width section

Table 3

Jaera isopod spreading in the Danube on Sulina Arm between miles 9 and 34

Date of collecting	Place of collecting	Water depth (m)	Substratum nature	Density number ind.	Density biomass mg
1	2	3	4	5	
1 VIII 1959	mile 9 – Intermediate right riparian	11	– sand, mud,		
	– Intermediate left riparian	5	– vegetal detritus, stone	2/pr.	0.09/pr.
	– Left riparian	4	– mud,vegetal detritus	2/pr.	0.2/pr.
13 XI 1959	mile 9 – Right riparian	5	– mud, vegetal detritus	1/pr.	0.04/pr.
	– Intermediate right riparian	6 11	– shell debris, vegetal detritus	4/pr.	0.24/pr.
	– Middle		–vegetal detritus	32/pr.	1.92/pr.
	– Intermediate left riparian	5	– mud, vegetal detritus	2/pr.	0.08/pr.
	– Left riparian	4	– mud, vegetal detritus	1/pr.	0.05/pr.
30 XI 1960	mile 9 – Right riparian	4	–vegetal detritus shell debris	14/pr.	0.7/pr.
	– Left riparian	8	– stone, vegetal detritus	7/pr.	0.42/pr.
	– Left riparian	1.5	– clay, vegetal detritus	14/pr. 460/m^2	1.0/pr. 32.2/m^2
25 VI 1960	mile 11 – Intermediate right riparian	10	– stone, mud		
			– stone, shell		

1	2	3	4	5	
18 III 1960	mile12 – Intermediate right riparian	9	debris, vegetal detritus	38/pr.	2.1/pr.
	– Intermediate left riparian	9	– clay, sand, vegetal detritus	2/pr.	0,07/pr.
	– Left riparian	6	– shell debris, vegetal detritus	1/pr.	0,04/pr.
30 XI 1960	mile 13 – Right riparian	7	– stone, detri- tus vegetal	16/pr.	1.3/pr.
	– Left riparian	6	– stone, vegetal	13/pr.	1.2/pr.
8 III 1961	mile 13 – Middle	11	detritus	3/pr.	0.09/pr.
4 VII 1961	mile 13 – Left riparian	4	– stone, mud	12/pr.	1.1/pr.
1 XII 1960	mile 14 – Left riparian	4	– clay	100 ex/m^2	6.2/m^2
			– clay, sand	45/pr.	3.2/pr.
1 VIII 1959	mile 19 – Intermediate right riparian	13	– stone		
	– Intermediate left riparian	9 0.8	–vegetal detritus	2/pr.	0.16/pr.
	– Left riparian	8	– stone	6/pr.	0.5/pr.
13 XI 1959	mile 19 – Right riparian	13	– mud, vegetal detritus	1/pr.	0.06/pr.
	– Middle		– mud, vegetal detritus	40/pr.	2.8/pr.
	– Intermediate left riparian	7	– sand, vegetal detritus, shell debris	3/pr.	0.24/pr.
25 VI 1960	mile 21 – Left riparian	5	– stone, vegetal detritus	129/pr.	9.03/pr.
31 VII 1959	mile 24 – Right riparian	4	– mud, vegetal detritus	15/pr.	1.2/pr.
	– Intermediate right riparian	12	– stone, mud	8/pr.	0.56/pr.
	– Intermediate left riparian	4	– stone, mud	5/pr.	0.35/pr.
11 XI 1959	mile 24 – Intermediate right riparian	10	– stone, vegetal detritus	2/pr.	0.15/pr.
	– Middle	13	– stone, detritus vegetal	187/pr.	15.09/pr.
	– Intermediate left riparian	6 4	– sand, shell debris, vegetal detritus	10/pr.	0.9/pr.
	– Left riparian	3			
19 III 1960	mile 24 – Right riparian		– stone	39/pr.	3.2/pr.
			– mud	22/pr.	1.65/pr.
	– Intermediate	5	– stone, vegetal		

1	2	3	4	5	
	right riparian		detritus	3/pr.	0.21/pr.
		4			
	– Left riparian		– stone, sand, vegetal detritus	6/pr.	0.42/pr.
			– stone, vegetal		
31 VII 1959	mile 34 – Intermediate right riparian	13	detritus	4/pr.	0.28/pr.
		1			
	– Left riparian	7	– stone, vegetal detritus	20/pr.	1.15/pr.
12 XI 1959	mile 34 – Right riparian		– mud	1/pr.	0.04/pr.
	– Intermediate right riparian	10	– stone, mud, vegetal detritus	107/pr.	8.05/pr.
	– Intermediate	8	– stone, vegetal		
	left riparian	3	detritus	18/pr.	1.35/pr.
	– Left riparian	1.2			
25 VI 1960	mile 34 – Right riparian		– mud, sand	1/pr.	0.05/pr.
		3.0	– mud, sand	4/pr.	0.25/pr.
	– Left riparian		– stone, vegetal detritus	5/pr.	0.39/pr.
			– stone, mud, vegetal detritus	14/pr.	1.20/pr.

Legend: q. s. = qualitative sample representing the outcome of a dredging carried out on a 25 m length and 0.5 m width section

On Tulcea Arm, at rkm 62.900 next to Ceatalul Sf. Gheorghe upstream, during 1981–1985, our researches were systematically carried out (through the Danube bifurcation at Ceatalul Izmail resulted the Chilia Arm and the Tulcea Arm. The latter bifurcated at Ceatalul Sf. Gheorghe, resulting the Sulina Arm and the Sf. Gheorghe Arm). The obtained quantitative data show that the isopod presence was a sporadical one. The minimal values, i.e. 20 ind./m^2 with 4 mg/m^2, were found in the first year of the mentioned period, the month of August (on the left riparian, on a muddy-clayey substratum, at a 3 m depth) as well as in April and June 1984, at the same station. But in 1985, in August (on the left riparian too, on a stony-muddy substratum, at a 4 m depth) it was reached a maximum of 640 ind./m^2, but with a biomass of 25.6 mg/m^2.

The researches resumed in 1995, at the same station, on the left riparian too on a clayey-muddy substratum, at a depth of 14.5 m, revealed that *Jaera* reached a maximum also in August, when 600 ind/m^2 with a biomass of 24 mg/m^2 were found.

In the unique Danube at rkm 80 upstream of Ceatalul Izmail, before the river confluence with the Delta (through researches performed systematically, as at rkm 62.900), *Jaera* was found also sporadically. Thus, in 1983 the sole presence of isopod was in August, with 20 ind./m^2 (0.03%) and 10 mg/m^2 (0.01%), on the right

riparian, on a muddy substratum, at a water depth of 6 m. These figures represent an insignificant share of the biocoenosis specific composition dominated by *Polichaeta, Ampharetidae* and *Oligochaeta*. A somewhat better condition was in 1985 (June) by the 40 ind./m^2 (4.17%) and 12 mg/m^2 (0.66%) found also in the right riparian section, on a clayey substratum, at a water depth of 5 m. Here, the biocoenosis, poorer than in 1983, was dominated by *Amphipoda* (19).

The researches of 1995 carried out in summer (June) at rkm 167, in the intermediate section between the right riparian and the middle of the river, revealed an isopod density of 250 ind./m^2 with a biomass of 15 mg/m^2. This crustacean development occurred under the conditions of a muddy-sandy substratum with a *Viviparus* and *Dreissena* rich thanatocenosis at 5.3 water depth and 1.05 m/s flow velocity.

The same year, in summer (June), at rkm 171, *Jaera* populations reached a maximum of 4050 ind./m^2 with 96 mg/m^2 biomass on a muddy-sandy substratum, at 6 m water depth and flow velocity of 1.09 m/s (in a biocoenosis dominated by *Oligochaeta*, *Amphipoda* and *Mollusca*).

At a distance of about 130 rkm upstream at rkm 301, but in 1996, in summer (June) *Jaera* had a density of 200 ind./m^2 and 14 mg/m^2, on a sandy substratum, at 13.5 water depth (in the specific composition of a not too rich biocenosis, dominated by *Oligochaeta*).

In the Danube section, upstream rkm 301 and downstream rkm 804, in 1975 and 1976 we noticed the presence of *Jaera* only at rkm 600 (river Olt confluence) in a small number, on a sandy substratum in a poor biocoenosis (20). The researches performed in 1996 (October) revealed the isopod presence at rkm 624 in a number of 1080 ind./m^2 with 50 mg/m^2 biomass on a sandy substratum.

But in the higher section of the Romanian stretch of the river, *Jaera* proved to have a fairly good spreading. As a matter of fact, the richest populations of this isopod from the entire course of the Romanian stretch of the Danube were found there.

We outline the fact that after 1970, in the section downstream Baziaş and towards upstream Drobeta-Turnu-Severin, by the creation of the two damlakes Iron Gates I and Iron Gates II, major morpho-hydrologic changes, implicitly faunistic ones, took place.

For a better understanding of the fluctuations recorded in isopod populations number, we present the existing data in a chronological order for every section in this region.

In this respect, in the Danube section between rkm 898–931 which includes Iron Gates II lake as well, the researches performed in 1986 point out a good development of crustacean. On a mosaic substratum constituted of stones, sand, mud, vegetal detritus, *Jaera* recorded an average within the section of 2482 ind./m^2

(7.45%) and 547 mg/m^2 (0.55%) (18). The values developed in different points of the section are as follows: 2244 ind./m^2 with 459 mg/m^2 at rkm 898; 1 071 ind./m^2 with 214 mg/m^2 at rkm 901 and 4 131 ind./m^2 with 969 mg/m^2 at rkm 931.

During 1995–1996, the researches resumed between rkm 804 and 911 allowed us to follow *Jaera* populations evolutions after a 10 years period. Analysis of the data presented in Table 4 shows that during 2 years (in the respective section of the Danube), on the dominant stony substratum at rkm 911, values of numerical density of maximum 11 700 ind./m^2, with a biomass of 1 138 mg/m^2 were recorded.

Table 4

Jaera isopod spreading in the Danube between rkms 804–911

Date of collecting	Place of collecting	Water depth (m)	Substratum	Density number (ind.)	Density biomass (mg)
21 VI 1996	km 804 – Left riparian	6.05	– sand with stones	100/m^2	4/m^2
10 VI 1995	km 866 – Intermediate left riparian	20.0	– stone	650/m^2	20/m^2
21 VI 1996	km 866 – Intermediate left riparian	14.1	– sand, stone	100/m^2	4/m^2
9 VI 1995	km 879 – Intermediate left riparian	11.8	– stone	8 500/m^2	711/m^2
21 VI 1996	km 879 – Intermediate left riparian	10.9	– stone	1927/m^2	117/m^2
9 VI 1995	km 911 – Left riparian	14.8	– stone, mud, sand	11 700/m^2	1 138/m^2
20 VI 1996	km 911 – Left riparian	9.65	– stone	35/pr.	1.46/pr.

Legend: q.s. = qualitative sample representing the outcome of a dredging carried out on a 25 m length and 0.5 m width section

More upstream in the Romanian stretch of the river, since 1942–1943 when the Danube was not blocked by the dam, Băcescu mentioned *J. sarsi sarsi* Valk. in the Danube gate. In this respect, the authors outlined the fact that in October 1942, around Kralina, on a stone block the isopod reached about 74 000 ind./m^2 in low waters, under the conditions of a very rich biocoenosis in terms of individuals and species. In contrast, in October 1943, in low waters too, around the rocky ridges up to 1–2 m depth, *Jaera* was found in the specific composition of an exhausted fauna. Also, in 1943, at Cazane and Svinița shallow forms among which *Jaera* were increasingly reduced in number (2).

In 1958, also when the Danube was not blocked by the dam, at rkm 957 (20), *Jaera* was found in a number of 3–12 ind./sample, at 4 m and 45 m depths, in the specific composition of a lithorheophilic biocoenosis (dominated by *Amphipoda* represented by *Corophiidae*). But at the same time, it was determined with a number of 3–13 ind./sample also from psamorheophilic biocoenosis from 3–10 m depths (in which *Amphipoda* were again dominant, but by *Gammaridae*). In 1995, under the conditions of the existing damlake, at rkm 959.5 on a sandy-muddy substratum, at 9.8 m depth, *Jaera* populations were fairly numerous: 500 ind./m^2, but with a biomass of 13 mg/m^2 only. At rkm 967 at the Danube confluence with river Mraconia, in 1958 too, *Jaera* by the 5 ind./ sample constituted a component of the stony substratum biocoenosis at 50 m depth. The water salinity at this period of time in the Danube section between rkm 957–967 ranged between 13.5 and 15.3 mg/l (29).

After a 10 year period, in 1968, the investigations carried out also at rkm 967, revealed a biomass of 11 mg/m^2 generated by a number of 82 ind./m^2 of *Jaera* located on the stony – sandy substratum, at 1–2 m water depth (16).

Following the construction of the Iron Gates I damlake, 2 years after the inundation, i.e. in 1972, also at rkm 967, on the muddy-sandy substratum, isopoda recorded populations' density higher than in 1968, namely 186 ind./m^2 and 60 mg/m^2 in summer and 540 ind./m^2 with 134 mg/m^2 in autumn. In 1973 the species number decreased to 100 ind./m^2 and 30 mg/m^2. In 1981, on the same profile, the annual average was of 16 ind./m^2 only, with 16 mg/m^2 biomass (17). Maxima were recorded at the left riparian in August, with 51 ind./m^2 and 15.3 mg/m^2, on a sandy substratum with shell debris and mud. But on the medial, the maximal figures were higher, i.e. 408 ind./m^2 with 24.48 mg/m^2, respectively in October, on a muddy-sandy substratum.

But in 1986 (at rkm 967 as well) the annual average of *Jaera* populations was of 51 ind./m^2 and 30 mg/m^2 at riparian, and 238 ind./m^2 and 120 mg/m^2 at the medial (21). We outline the fact that in 1986 isopod individuals being big, the biomass was relatively high. The maximal numbers in 1986 were of 153 ind./m^2 with 76 mg/m^2 at riparian and 714 ind./m^2 with 408 mg/m^2 on medial.

All these data show that in the damlake, due to major morpho-hydrological changes which occurred in the region, even in 1986 significant restructuring at biocoenosis' level was taking place (20).

In the Danube, before the construction of the damlake, in 1966, at rkm 998 (Greben) on the stony-sandy substratum, at 1–2 m depth, the isopod had a numerical density of 32 ind./m^2 and a biomass of 11 mg/m^2. At rkm 1007 (at the Danube confluence with the river Elişeva), in 1966 and 1968 *Jaera* populations

varied in terms of numerical density and biomass, ranging between 22 ind./m^2 with 9 mg/m^2 (in 1966) and 117 ind./ m^2 with 23 mg/m^2 (in 1968) (16).

In 1967 Morduhai-Boltovskoi (in his work discussing the particularities of Caspian fauna in the Danube basin) outlined the presence at the Iron Gates of several Ponto-Caspian elements among which, *Jaera* (12).

Also in the most upstream section of the Romanian Danube stretch, at rkm 1042, researches were carried out before and after Iron Gates I damlake construction. Thus, in 1958 (16) *Jaera* was determined at a depth of 3.5 m, on the stony substratum. The figure of 27 ind./sample represented 2.87 % of the total lithorheophilic biocoenosis organisms in the composition of which was found. Following the construction of damlake, in 1978, *Jaera* was determined in the section at the tail of accumulation at rkm 1046. On a substratum with rocks and sand, at 9.1 m water depth, the isopod constituted one of the forms present within benthic biocoenosis (5). After a longer period of time, in 1995 and 1996, in this section at the tail of the Iron Gates I damlake, namely at rkm 1048.7 and rkm 1072.4 *Jaera* populations represented a high number of individuals. The maximum was reached in May 1996, with 37100 ind/m^2 and 1420 mg/m^2 at rkm 1072.4 (Table 5). These last figures show that *Jaera*, although mostly of lithorheophilic shape, developed fairly well in the section between rkm 1048.7 and rkm 1072.4 on a prevailing muddy, sandy, with gravel, substratum.

Table 5

Jaera isopod spreading in the Iron Gates I damlake, between rkm 1048.7 and rkm 1072.4

Date of collecting	Place of collecting	Water depth (m)	Substratum nature	Density number ind./m^2	Density biomass mg/m^2
6 VI 1995	km 1048.7 – Middle	18.3	– mud, sand, gravel	9500	209
30 V 1996	km 1072.4 – Intermediate left riparian	15.5	– mud, sand, gravel	13000	218
13 X 1996	km 1072.4 – Intermediate left riparian	16.2	– mud, sand, gravel	37100	1420

We outline the fact that for the section at rkm 1072.4 we have no available data prior to 1996 in order to refer to the *Jaera* populations development in the area. But, for rkm 1446, in the Danube stretch on Hungarian territory, at 9.4 m depth, on a sandy substratum, during August-September 1978, Cure *et al.* (5) mentioned that *Jaera* was the predominant form within the benthic biocoenosis besides *Asellus aquaticus*.

III. *JAERA* ISOPOD SPREADING IN OTHER DANUBE STRETCHES EXCEPTING THE ROMANIAN STRETCH

Genus *Jaera* presence in other Danube stretches, excepting the Romanian stretch, is mentioned in a number of works (as we have already referred to) published especially after 1960. We point out that for the other Danube stretches until 1988, all the data concerning this isopod refer either to *J. sarsi* or *J. sarsi sarsi.*

We begin to present the data, as for the Romanian stretch, from downstream to upstream the river.

Thus in the Soviet stretch, on Chilia Arm, in 1960 Morduhai-Boltovskoi mentioned *J. sarsi.*

In the Bulgarian stretch, according to data by Russev *et al.* (24) *J. sarsi sarsi* Walk. was found during 1971–1973 between rkms 380 and 945. From Table 6 it results that the highest numerical densities were located towards the two extremes of the studied sector, i.e. at rkms 431 and 845. But, the highest biomass was given by a much smaller number of individuals than the numerical density maximum.

Table 6

Jaera isopod spreading in the Bulgarian Danube stretch (as per Russev *et al.*, 1985)

Date of collecting	Danube km	Distance from the river riparian in m	ind/m^2	g/m^2
1971 summer	581	87 from the right riparian	40	0.04
	713	51 from the right riparian	40	0.02
	834	811 from the left riparian	80	0.01
	845	68 from the right riparian	240	0.05
	845	40 from the right riparian	320	0.05
1973 summer	380	547 from the right riparian	80	0.16
	431	86 from the right riparian	580	0.04

Also, regarding the Bulgarian stretch of the Danube, this isopod is shown to be present among the dominant ones in the Danube section where the water flow had a velocity of 0.310–0.610 m/s (23,24).

As concerns the Hungarian stretch of the Danube, în 1966, Băcescu in his work "Die Kaspische Reliktfauna im pontoazowschen Becken und in anderen Gewässern" (3), using data by Woyanarovich and Dudich mentioned *J. sarsi* as being present from Sulina to Budapest.

In 1967, Dudich (6) in his synthesis on the Danube fauna worked out (based on data from the specialty literature), indicated *Jaera* under the name of *J. sarsi sarsi* Valk., (lithophilic form) as existing throughout the entire Danube, beginning from the delta (Danube Arms) up to Germany, inclusively. The author even outlined this isopod presence in all the river stretches namely: Soviet, Romanian, Bulgarian, Jugoslavian, Hungarian, Slovakian, Austrian and German ones.

In the Slovakian stretch, at Gabcikovo (rkm 1819.7), Kosel enumerated *J. sarsi* among microbenthic forms (8).

In 1967, Morduhai-Boltovskoi (in the same work discussing the particularities of the Caspian fauna in the Danube basin) (12), referring to the conditions in Austrian and German river stretches, enumerated *Jaera* among the Caspian forms passing beyond the frontier with Austria. But, in this case, as well as when he referred to Iron Gates, he mentioned only genus and not the isopod species.

During 1974–1979, Russev (23) undertook a number of investigations concerning the zoobenthos in the sector of the river included between rkms 1950 and 2210. They revealed the isopod presence, mentioned as *J. sarsi sarsi* Valk., at rkm 2147 in the Ottenheim Wilhering damlake as a precentage of 0.11% of the total of animals both at the right riparian and the medial of the investigated river profile. But at rkm 2096, the percentage recorded by this isopod was of 0.03% only.

In 1988, Pöckl (14) was the first author who referring to the Austrian stretch outlined that "so far in the investigated sector, only *J. istri* Veuille was found, endemical in the Danube and as a mass species here and there". Also he mentioned that this species (according to personal communication by Danielopolu) could be found up to the Melc.

Several years later, in 1994, Humpesch and Moog (10) referring to the flora and fauna from the Austrian Danube stretch, on the basis of 1992 material from rkm 1949.18, mentioned *J. istri* in the specific composition of zoobenthos.

As concerns the German stretch of the Danube, ampler data on *J. sarsi sarsi* Valk. are published by Kothé in 1968 (9). In this respect, the author mentioned *Jaera* as being abundant at rkm 2204.5 at a distance of 255 m from the left river riparian, on a coarse sandy and stony substratum, at a water depth of 9 m. But, at 275 m distance from the same left riparian, on a sandy-stony substratum and at 4.40 m depth, the isopod was found in a small number of individuals. As concerns the abundance, somewhat upstream on the river, at rkm 2229.5 *Jaera* populations were classified at degree 5 (i.e. between average to abundant). According to Kothé, *J. sarsi sarsi* is a real Ponto-Caspian relict within the Danube space passing through the forests of Bavaria and Bohemia. He argues against the idea of the isopod emigration from the Black Sea.

Under the same name of *J. sarsi*, this crustacean was mentioned also in the works by Tittizer et al. by 1994 inclusive (25,26). Thus, on the basis of a material from 1987 and 1988, the species was determined at rkm 2396.4 (Kehlheim-Jochenstein section) from the stony and muddy substratum, as a running water species. These authors too enumerate *J. sarsi* among the endemic species in the respective river sector. Isopod consistency during the two studied years was of about 40%.

CONCLUSIONS

1. Until 1988 when *J. istri* was mentioned in the Danube, it was thought that *J. sarsi* with *J. sarsi sarsi* subspecies exist in the river. In the Romanian stretch, where in the past *J. sarsi* and *J. sarsi sarsi* were mentioned, the researches carried out in 2000 on a material from the Iron Gates, confirmed the fact that here *Jaera istri* exists.

2. From the viewpoint of its spreading in the Danube basin, it is present in a number of river lagoons, in the areas at the Danube mouths in front of the Sulina Arm, as well as throughout the whole river length, from the confluence with the Black Sea up to the springs.

3. The richest *Jaera* populations occur in the Romanian stretch, particularly in the two damlakes: Iron Gates I and Iron Gates II.

4. As concerns the origin, there are slightly controversial opinions. In this respect Kothé thinks that throughout the river length *Jaera* is a real Ponto-Caspian relict. But Morduhai-Boltovskoi and Bănărescu think that this isopod is a Ponto-Caspian relict only in the lower part of the Danube whereas in the other sectors of the river, this species is autoimmigrant.

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ON LITTER DECOMPOSITION AND ASSOCIATED INVERTEBRATES

ELENA PREDA, CONSTANTIN CIUBUC, OCTAVIAN CIOLPAN

Abstract. Leaf litter breakdown is a key element of ecosystem functioning in forested headwater streams. In such streams riparian input is the most significant source of energy. The breakdown rate varied between 0.002 and 0.058 units/day for *Fagus sylvatica* and between 0.0031 and 0.0893 units/day for *Alnus incana* litter. There are no significant differences in total associated invertebrates in the two types of litter. Generally, Plecoptera was the dominant group in *F.sylvatica* leaf bags and Chironomidae was dominant in *A.incana* leaf bags. Chironomids assemblages are dominated by *Brillia modesta* in beech litter and by *Rheocricotopus fuscipes* in alder litter. Chironomids use aquatic litter provided by riparian input as refuge against predator species, not directly as food.

INTRODUCTION

Particulate and dissolved organic matter constitutes important energy inputs to most food webs, especially in lotic aquatic ecosystems. While primary production by the autotrophs of running waters can be substantial, much of the energy support of lotic food webs derives from these sources of organic matter (1). In small, heavily shaded streams, there is normally insufficient light to support substantial instream photosynthesis, so energy pathways are supported largely by imported (allochthonous) energy (6). In most small temperate woodland streams, allochthonous, detrital materials from riparian vegetation, mostly leaf litter, make up the largest portion of the energy available for stream organisms (12). Leaf litter breakdown is a key element of ecosystem functioning in forested headwater streams (7, 13). In streams with different chemical background, the process of plant decomposition differs and this is reflected in variation in the amount of benthic fauna. Furthermore, the rate of decomposition is positively temperature dependent (1), such that decomposition is fastest during summer when standing crop is near its lowest seasonal value (12). There is a positive correlation between number of invertebrates and amount of detritus (18).

The aim of this study was to test the hypothesis that populations of stream invertebrates are influenced by litter type and decomposition stage.

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MATERIAL AND METHODS

Valea Cerbului stream is a subsystem of Prahova hydrographic basin located at 45°26'03" latitude and 25°24'50" longitude. Valea Cerbului stream has a length of 7 km and a drainage basin of 18.64 km^2 (16). The stream of 3rd order is about 2 m wide and has a water depth of 10–30 cm.

Valea Cerbului stream is situated in the neighbourhood of the Zoological Station, Sinaia.

The study was carried out from October 1997 to May 1998. On the identified stream two sites were selected according to riparian vegetation types: one site with slow decomposing leaf input, characteristic species *Fagus sylvatica* and one site with fast decomposing leaf input, characteristic species *Alnus incana.*

Leaf bags with *Alnus incana*, weighed in advance, were placed in the stream at the site with fast decomposing leaf input, and leaf bags with *Fagus sylvatica* at the site with slow decomposing leaf input (21 leaf bags for each species) to determine the decomposition rate of these litter types. At regular intervals three of the leaf bags were collected at each sampling date.

Remaining leaf material was cleaned of debris and invertebrates, dried to constant mass, and weighed.

The invertebrates removed from the litter were sorted and identified at order or family level. Only chironomids were identified at species level. In order to perform this activity we used different identification keys (3, 5, 8, 9, 10, 14, 17).

The experiment started in October 1997. The schedule was presented in Table 1.

Table 1

Sampling program of litter decomposition experiment

Sampling time	Start of experiment	T1	T2	T3	T4	T5	T6	T7
Time(wk)	0	1	2	4	8	12	22	27
Time(days)	0	6	14	26	53	90	165	201

Species-specific breakdown rates (k) were then calculated by using an exponential decay model, which assumes that the rate of loss of plant material from the bags is a constant part of the amount of material remaining.

k is the negative slope of the line produced by a linear regression of the natural log of percentage leaf material remaining plotted against time.

k could be calculated using the following formula:

$$W_t = W_i\, e^{-kt}$$

where W_t = dry mass at time t, W_i = initial dry mass, and t is measured in days.

The statistic k is expressed in units day^{-1}.

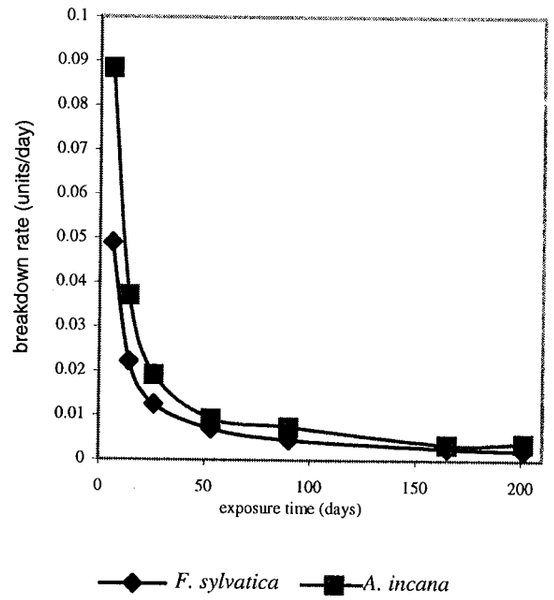


Fig. 1 – Breakdown rate of *A.incana* and *F.sylvatica* litter.

Organic matter combusts at about 550 °C and the remaining material is mineral ash. When the mass of mineral ash is subtracted from initial dry mass, the result is the dry mass of the organic fraction (ash free dry weight – AFDW) of the leaf material.

Chironomidae species assemblages were analysed using Shannon's diversity index and evenness.

Species diversity may be defined as a measure of species composition, in terms of both the number of species and their relative abundance.

Shannon's index of species diversity:

$$H = -\Sigma\, p_i \log p_i,$$

where p_i represents proportion of species "i" from total number of species.

Evenness (J) is computed as:

$$J = H/H_{max}, \qquad H_{max} = \log q,$$

where q is the number of species.

Statistical data analysis was based on the ANOVA model.

RESULTS AND DISCUSSIONS

A. LITTER DECOMPOSITION

Dry weight determination of decaying leaves samples indicated that there was an initial rapid weight loss in the first few months followed by a more gradual decline later. The breakdown rate varied between 0.002 and 0.0580 units/day for *F.sylvatica* and between 0.0031 and 0.0893 units/day for *A.incana* litter.

The rate of leaf breakdown is determined by intrinsic differences among leaves, a number of environmental variables, and the feeding activity of detritivores (4, 1).

F.sylvatica seems similar to *A.incana* in its visible rate of decay. However, it was observed that the *A.incana* litter decomposed faster than *F.sylvatica* litter (Fig. 1).

In the river Speed, a clean river, percentage of ash-free weight losses after six months unguarded exposure of *Ulmus, Acer, Quercus, Alnus* and *Fagus* were 68.9, 56.8, 23.1, 21.8 and 13.0 % respectively. On the other hand, in the polluted river Nith, the corresponding percentage ash-free weight losses after the same period of exposure, and for the same species, were only 54.5, 41.9, 18.5, 10.2 and 3.3 % respectively (18).

In the present study, at the end of experiment, after an exposure time of 201 days, there were losses 79.09 % from alder litter and 57.73 % from beech litter, because processing rates of leaf detritus are temperature dependent.

B. INVERTEBRATES

Generally, the number of taxonomic groups was higher in *F.sylvatica* leaf bags than in *A.incana* leaf bags (Fig. 2). n *F.sylvatica* leaf bags Plecoptera was a dominant group together with Chironomidae and Gammaridae.

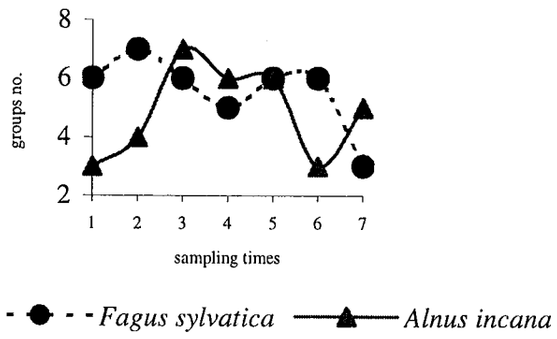


Fig. 2 – The invertebrates groups number associated to *F. sylvatica* and *A.incana* litter.

The Plecoptera numerical relative abundance varied between 29.64 % and 71.15 %. At fifth and seventh sampling times this group was not found in the leaf bags. The Chironomidae numerical relative abundance varied between 10.20 % and 41.67 %. Also, Gammaridae was a dominant group with 37.50 % numerical relative abundance at third and fifth sampling times. At last sampling time, Oligochaeta becomes dominant with 73.77 % numerical relative abundance.

In *A.incana* leaf bags Chironomidae was a dominant group with numerical relative abundance between 28.13 % and 92 %. In the last part of experiment, the dominance was shared between Oligochaeta and Chironomidae. Oligochaeta was on the first place with numerical relative abundance between 46.67 % and 74.05 %.

The connexion between the occurrence of plant material in an advanced stage of degradation as detritus, and the occurrence of benthic invertebrates has been studied by Egglishaw (18). The closest associations between detritus and occurrence were found for Plecoptera and Chironomidae.

The fragmentation of leaves by invertebrate feeding and abrasion constitutes the third stage of leaf breakdown after leaching and microbial colonisation. During the breakdown of autumn-shed leaves in temperate woodland streams, microbial populations play a central role not only in decomposing the leaf substrate, but also in rendering it more palatable and nutritious to consumers.

The feeding activity of detritivores significantly accelerates the decomposition process. Their contribution to the fragmentation of coarse particles through feeding activities and production of feces significantly accelerates breakdown rates and influences subsequent biological processing of the original coarse particulate organic matter (CPOM) inputs. Breakdown rates are higher where invertebrates are more abundant and there is a positive relationship between invertebrate preferences and decay rates (1).

C. CHIRONOMIDS

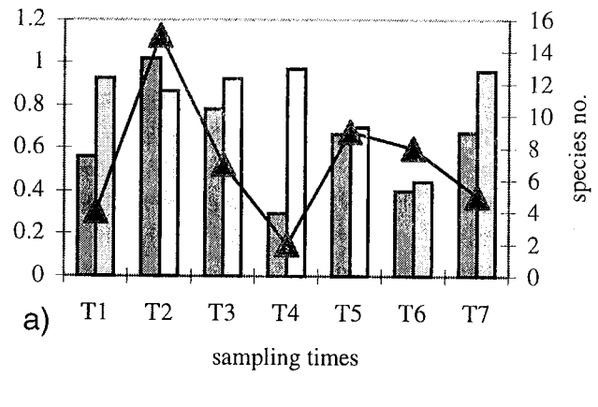
The species composition was different for *F.sylvatica* and *A.incana* litter. In *F.sylvatica* leaf bags at the first three sampling times *Brillia modesta, Brillia longifurca* and *Pseudodiamesa branickii* were dominant species. In the last part of experiment situation changed and *Corynoneura celeripes, Micropsectra praecox, Limnophyes pseudoprolongatus, Limnophyes transcaucasicus, Rheocricotopus fuscipes* became dominant.

In *A.incana* leaf bags in the first part of experiment the dominant species were *Rheocricotopus fuscipes, Prodiamesa olivacea* and *Micropsectra praecox.* In the last part, *Polypedillum convictum* became dominant together with *Rheocricotopus effusus* and *Rheocricotopus fuscipes. Corynoneura scutellata* and *Brillia modesta* were co-dominant species at two sampling times.

The number of chironomids species was generally higher in *A.incana* litter than in *F.sylvatica* litter. At the second sampling time beech litter supported a higher number of species than alder litter.

Based on larval feeding mode benthic invertebrates can be grouped into six general categories: collector-gatherers, collector-filterers, scrapers, shredders, engulfers and piercers, but the most chironomids are not restricted to a single mode of feeding. For this reason it is very difficult to classify species into one of the functional feeding groups.

There are no significant differences, based on the ANOVA model, between chironomids species assemblages associated to different types of litter.



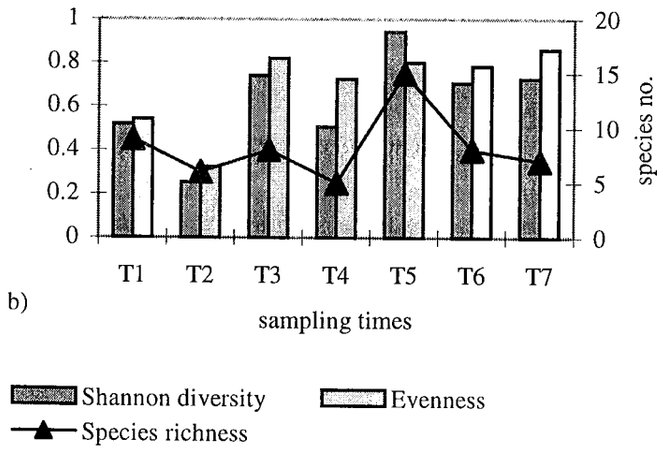


Fig. 3 – Shannon diversity, evenness and species richness of Chironomidae species in *F.sylvatica* leaf bags (a) and *A.incana* leaf bags (b).

Species diversity was higher during the first three sampling times in *F.sylvatica* leaf bags (Fig. 3-a) than in *A.incana* leaf bags. In the second part of the experiment, species diversity increased in *A.incana* litter (Fig. 3-b).

Generally, the evenness of species distribution is inversely related to the overall biological activity (2).

In the first part of the experiment a high biological activity was observed in *A.incana* leaf bags. In the second part when a great part of the litter was lost, the biological activity decreased. However, it increases in *F.sylvatica* leaf bags because this type of litter decomposed slowly. The physical and chemical factors as well as the type and heterogeneity of the substrate are not the only parameters which have an effect on structuring stream chironomid communities (15).

Food quality, independent of quantity, can affect growth rates and densities of aquatic invertebrates (12). Alder is generally considered a high quality food because of its high nitrogen content (12, 11).

Richardson studied the effect of food enrichment on litter invertebrates. The experiment results showed an increase in chironomids and oligochaetes, presumably because of the short generation time of these animals, and very different microbial assemblages from that normally associated with decaying leaf litter. Alternative hypotheses to explain the increased densities in the experiment could be that increasing leaf litter amount provides additional microhabitats for larvae, which reduces interference among larvae and reduces predation rates. An experiment using artificial leaf packs showed no evidence that leaf packs serve as a refuge alone.

CONCLUSIONS

- *Fagus sylvatica* and *Alnus incana* litter have a similar decomposition behaviour even if these species have different breakdown rates.
- *F.sylvatica* litter was colonised by a higher number of invertebrate taxonomic groups dominated by Plecoptera than *A.incana* litter where Chironomidae was a dominant group.
- Chironomids colonise leaf bags with different types of litter. They use aquatic litter from riparian input not only as food, but also as refuge against predator species.
- There are no significant differences between chironomids species assemblages associated to *F. sylvatica* and *A. incana* leaf litter types.

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Department of Systems Ecology and Sustainable Development
Spl. Independenţei 91-95
76201 Bucharest, Romania

PLANKTONIC COPEPOD ROLE IN BASIC ECOLOGICAL PROCESSES OF DANUBE DELTA LACUSTRIAN ECOSYSTEMS. THE COPEPOD CONTRIBUTION TO ENERGY FLOW

LAURA PARPALĂ, VICTOR ZINEVICI

The planktonic copepod associations are considerably involved in basic ecological processes of Danube Delta lacustrian ecosystems, namely: matter and energy circulation, and selfcontrol. These organisms are, on the one side, consumers of energy they need for their activities as well as for their growth and reproduction, and, on the other side, are producers of biomass, an energy source for consumers of different levels. The copepods assure, through their biomass accumulation, a certain production (a part of ecosystem biological production). The acquired energy has different uses: a part of consumed (C) nutrients is digested and assimilated (A), while undigested one is removed through excreta (FU). A part of assimilated nutrients is used for metabolic activities, respectively respiration (R) (oxygen consumption). At last, another part of the assimilated energy is stored resulting in weight (biomass) growth and creation of new individuals, a process named "production" (P). The survey of energy budget of dominant copepod from Roşu Lake, which was carried out in 1980–1996 period, showed that predators had the greatest participation in energy flow. Their parameters of energy budget had greater values than those corresponding to filtrator copepod. The copepods contribute with 13 % to the total zooplankton production.

1. INTRODUCTION

The existence of an ecosystem results, on the one side, from its population relationships and, on the other, on its population interactions with abiotic factors (2, 7, 17). The biological significance of these interactions means that each population must consume some quantity of energy to survive and perform a normal activity. In this process the solar or chemical energy, besides nutrients, is involved in biological flow and transformed in the biomass of living organisms. Thus, the ecosystem may be considered as a production unit of organic matter, namely the organisms themselves, belonging to its biocenosis (21).

The ecosystems are not only substance deposits and transformers, but to some extent, producers too (15, 16). An (aquatic) ecosystem functions as a unit in which organic compounds (biomass) are produced by means of a natural technology (10). The ecological unit of production works like an open system with a limited number of inputs-outputs.

All organisms belonging to a biocenosis consume a certain quantity of energy necessary to their activity, to growth and reproduction. Through the biomass

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accumulations, they yield a certain production (a part of ecosystem biological production), which represents the energy supply to the next trophic levels of consumers of different categories.

The nutrient energy acquired by organism has different uses (19, 20). A part of consumed nutrients (C) is digested and assimilated (A), while the undigested one is removed through excreta (FU). A part of assimilated nutrient energy is used for metabolic activities, respectively respiration (R) (oxygen consumption) (3, 5). At last, another part of the energy is stored in chemical bonds of organic compounds synthesized by organisms themselves, a process resulting in weight (biomass) growth and creation of new individuals, whose name is "production" (P). The above is expressed by the following equation (8, 14), unanimously adopted equation for energy budget evaluation:

$$C = P + R + FU$$

2. MATERIALS AND METHODS

The studies were performed on trophic levels: filtrators and predators, in one of the most representative lakes for deltaic biome – Roşu Lake, in 1994–1996 period.

The copepod contribution to the energy flow of plankton subsystem was determined using the MacFadyen equation (9):

$$C = A + FU \qquad \text{and} \qquad A = P + R$$

where:

C = consumption;
A = assimilation;
FU = feces + urine;
P = production;
R = respiration.

Mention should be made that in the case of P, we used the daily production, so all other parameters of energy budget are referred to 24 h, too.

We determined the copepod daily production by Winberg, Pechen, Shushkina method (20). Assuming that the production is determined, the A value can be calculated:

$$A = \frac{P}{K_2}$$

The K_2 coefficient of Ivlev has in literature the value of 0.35 for filtrators, and 0.30 for predators (6).

The R value was calculated on the basis of A:

$$R = A - P$$

For consumption the next equation was used:

$$C = P\left(\frac{1}{a \times K_2}\right)$$

The "a" coefficient, named assimilation efficiency against consumption, has according to different authors an average value of 0.6 for filtrators and 0.8 for predators. The FU was determined on the basis of C, P, A, R values with the equation:

$$FU = C - A\,.$$

3. RESULTS AND DISCUSSIONS

The results of energy budget analysis have an important contribution in evaluation of the planktonic copepod role in biocenotic economy (3, 11). These data help to better understand the role of biological production, the factors on which it depends, opening the doors for scientific prognosis on biological resources dynamics, on rational amendment and protection of ecosystems (2).

The study of organism communities energetics indicates that the productivity, generally, represents a measure of the energy flow, in other words, the energy quantity against unit of surface and time. We referred production to 24 h, defining practically the daily production.

The parameters of energy budget differed from one trophic level to another, function of structural and functional species particularities and depending on concrete ecological conditions of each ecosystem.

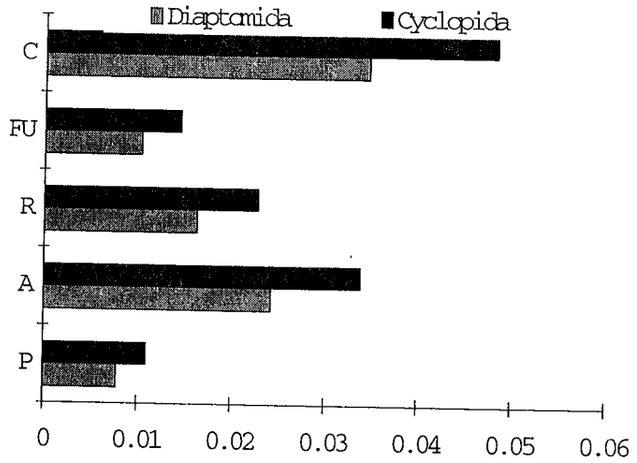
We determined the energy budget of some species from Roşu lake, dominant by structure (individual number and biomass) and production point of view, in 1994–1996 period (Table 1), using the MacFadyen equation (1963). We point out that energy budget parameters were calculated on the basis of obtained biomass (B) (12) and production (P) (13) values, applying the coefficients and equations indicated in literature. We mention that the biomass and production values were expressed in cal/l/24 h, for the species in Table 1. Thus, the calanoid contribution to Roşu lake energy flow is supported mainly by *Heterocope caspia* (2.8 times greater than *Calanipeda aquae-dulcis*). Among cyclopids a particular importance had *Mesocyclops crassus* and *Acanthocyclops vernalis*, constant – dominant species, throughout the 1994–1996 period.

Table 1

Energy flow (cal/l/24h) of some planktonic copepods from Roşu Lake in 1994–1996 period

Species	B	P	A	R	FU	C
Filtrators						
Calanipeda aquae-dulcis	0.03075	0.00435	0.01345	0.00910	0.00575	0.01920
Eurytemora velox	0.05995	0.00685	0.02115	0.01430	0.00910	0.03025
Heterocope caspia	0.10615	0.01240	0.03815	0.02575	0.01630	0.05445
Predators						
Acanthocyclops vernalis	0.06360	0.01055	0.03240	0.02185	0.01390	0.04630
Cyclops vicinus vicinus	0.02635	0.00215	0.00660	0.00445	0.00280	0.00940
Mesocyclops crassus	0.02166	0.03680	0.11320	0.07640	0.04850	0.16170
M.leuckarti	0.03060	0.00470	0.01430	0.00960	0.00595	0.02025
M.oithonoides	0.00780	0.00095	0.00290	0.00195	0.00125	0.00415

All parameters of energy budget had higher values in the case of predator copepods (cyclopids) in comparison to filtrator ones (calanoids) (Fig. 1).



P = Productivity; A = Assimilation; R = Respiration; FU = Excreta; C = Consumption

Fig. 1 – The energy budget of planktonic copepods from Roşu Lake in 1994–1996 period.

Welch (18) proposed the energy budget parameter calculation as consumption percentages for the evaluation of their contribution to energy flow.

The results of our researches in Roşu Lake, and data obtained by different authors are presented in Table 2.

Table 2

The energy budget (%) of some planktonic copepods from Roşu Lake in 1994–1996 period

Species	C	A	P	R	FU
Filtrators					
Calanipeda aquae-dulcis	100	70.05	22.66	47.39	29.95
Diaptomus sciloides (after Comita, 1968)	100	48.80	5.80	43.00	51.20
Eurytemora velox	100	69.92	22.64	47.26	30.08
Heterocope caspia	100	70.07	22.78	47.29	29.93
Predators					
Acanthocyclops vernalis	100	69.98	22.79	47.19	30.02
Cyclops vicinus vicinus	100	70.21	22.87	47.34	29.79
Macrocyclops albidus (after Klekowski and Shushkina, 1966)	100	50.00	25.00	25.00	50.00
Mesocyclops crassus	100	70.00	22.75	47.25	30.00
M. leuckarti	100	70.62	23.21	47.41	29.38
M. oithonoides	100	70.04	22.83	47.21	29.96

Assuming that C = 100%, for above analysed species, the assimilation constituted 70 % or so, an important part of which (47 %) represented respiration, while about 23 % production. The dissimilation processes and undigested food remains, eliminated as feces and urine, represented about 30 % of energy consumption.

In the case of Matiţa and Merhei lakes from the Danube Delta, with a total zooplankton production of 0.327367 cal/l/24 h (23), the copepod contribution was of 14 % (respectively 0.046500 cal/l/24 h). The average production of zooplankton represented 0.271000 cal/l/24 h, for 11 ecosystems, studied in 1980–1996 period, and that of copepods 0.03600 cal/l/24 h, respectively 13% (22). Our data are comparable to those obtained by Alimov *et al.* [1].

The greater assimilation values in planktonic copepods against those of primary producers, bacterioplankton, and even other groups of zooplankton (Ciliata, Testaceea, Rotifera) showed that the greatest part of consumed energy was used in respiration, while for biomass accumulation, to a lesser extent (4, 19) both at filtrator and predator levels (Table 1). In comparison with other mentioned organism groups, the copepods spend more energy for maintenance (basal

metabolism) and movement (nourishment search or pursuing, catching), and consequently had lower efficiency of production.

4. CONCLUSIONS

– As a result of the energy budget survey of dominant copepods from Roşu lake, assuming that C = 100 % resulted: assimilation A = 70 %, from which respiration R = 47 % and production P = 23 %, while feces + urine = 30 %;

– The greatest contribution to energy flow of planktonic copepod associations belongs to predators, whose values of energy budget parameters were greater against those of filtrator copepods;

– The copepod associations had 13% contribution to total zooplankton production from the Danube Delta lakes (1980–1996 period).

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Institute of Biology
Spl. Independenţei 296
Bucharest 79651, P.O. Box 5653
Romania

THE ZOOPLANKTON STRUCTURE IN CIŞMIGIU LAKE (BUCHAREST)

VICTOR ZINEVICI, LAURA PARPALĂ, FLORICA MACOVEI

The seasonal succession of zooplankton structure in Cişmigiu anthropic lake of Bucharest, a remnant of former natural Dura Neguţătorul Lake, located in the flood plain of the Dâmboviţa river, was analysed in 1998–1999 period. The survey of the mentioned community revealed low values of species diversity, numerical abundance and absolute gravimetric abundance, as well as a reduced number of constant forms. On the other side, the wide monthly changes of species diversity determined an annual relatively large taxonomic spectrum. The changes of taxonomic structure during the vegetation period showed that Cişmigiu Lake is a young lacustrian ecosystem from the ecological sequence point of view, as a consequence of the anthropic influences (water discharge and mud drainage in cold seasons, a.o.).

1. INTRODUCTION

The recreation Cişmigiu Lake, located in the same name public garden of Bucharest, represents the anthropic variant of a natural lacustrian basin in the Dâmboviţa river flood plain. The former lake, together with the old course of the Dâmboviţa river, had been subjected to determinant anthropic changes before. The area of the river hydrographical basin had suffered important modifications, too. The disappearance of the forests along the river, placed in a plain, had modified the hydrographical balance, which after that influenced the flow rate of the river and its affluents, and the flood plain.

The structural analysis of zooplankton from Cişmigiu Lake indicated the anthropic character of this lake.

2. MATERIALS AND METHODS

The monthly sample collecting enabled us to analyse the seasonal structural succession of lacustrian zooplankton, in March-November period of 1998–1999 years, in anthropic conditions.

The taxonomic diversity was evaluated on trophic levels and taxonomic groups. The species succession analysis evidenced the constant forms and those with accidental or accessory role.

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The numerical density and biomass were expressed as ex/l and µg wet weight/l respectively. The species dominant from the numerical and gravimetrical point of view are presented, in order to evidence the elements with a determinant role in the structural dynamics of the studied community.

3. THE ORIGIN AND EVOLUTION OF THE LAKE

The consultation of historical, geographical works (1), (2) concerned with the Bucharest city area allowed us to picture an image of the Dâmbovița river with its affluents and lakes, of its flood plain, in natural conditions uninfluenced by anthropic actions.

Before, between the XIIIth and XIXth centuries, the Dâmbovița river had had a meandering line in Bucharest area, with numerous islets (Fig. 1), as C.C. Giurescu (2) described. The river had had several little affluents on both banks, while an actual system of lakes and shallows had existed in its flood plain. The banks had been flanked by groups of willows and alders. Large surfaces had been occupied by forests of secular oaks, which had assured a wet climate, and greater than today debit of Dâmbovița and its hydrographical system.

The cited author mentions that in periods of high river floodings were invaded the Bucharest zones of Grozăveşti, Gorganul or Izvor, the inhabitants had been using boats to go from one house to another. They had fished in remaining shallows and sometimes even in flooded cellars, after passing of the high flood.

The river bed changed periodically. Between river branches existed five islets. The biggest one had a length of above two kilometres in 1791, and was partially inhabited and cultivated with vine and fruit trees.

The river had five affluents in the mentioned area. On the left bank was Bucureştioara (the biggest among them) which sprang from Icoanei shallow. Another small river, whose name was lost, drained the Dura Neguțătorul lake. On the right side, there were Dâmbovicioara, Gârlița and Privalul Cocioc.

Several lakes and shallows were also in the Dâmbovița flood plain. Among these, on the right bank, there were Adânc, Antim, Filaret and Dudescu lakes, Broştenilor swamp, the lake between Grozăveşti and Cotroceni yones (whose nowadays remnant is the small pond from the Botanical Gardens), the shallows near Sf. Elefterie church and Metropolitan Church, and the Privalul Cocioc, too. The Icoanei shallow with reed plots existed on the left bank, in which, "horse with rider may drown together", as the old men told (2). Besides these, Şuțului shallow and Dura Neguțătorul Lake were.

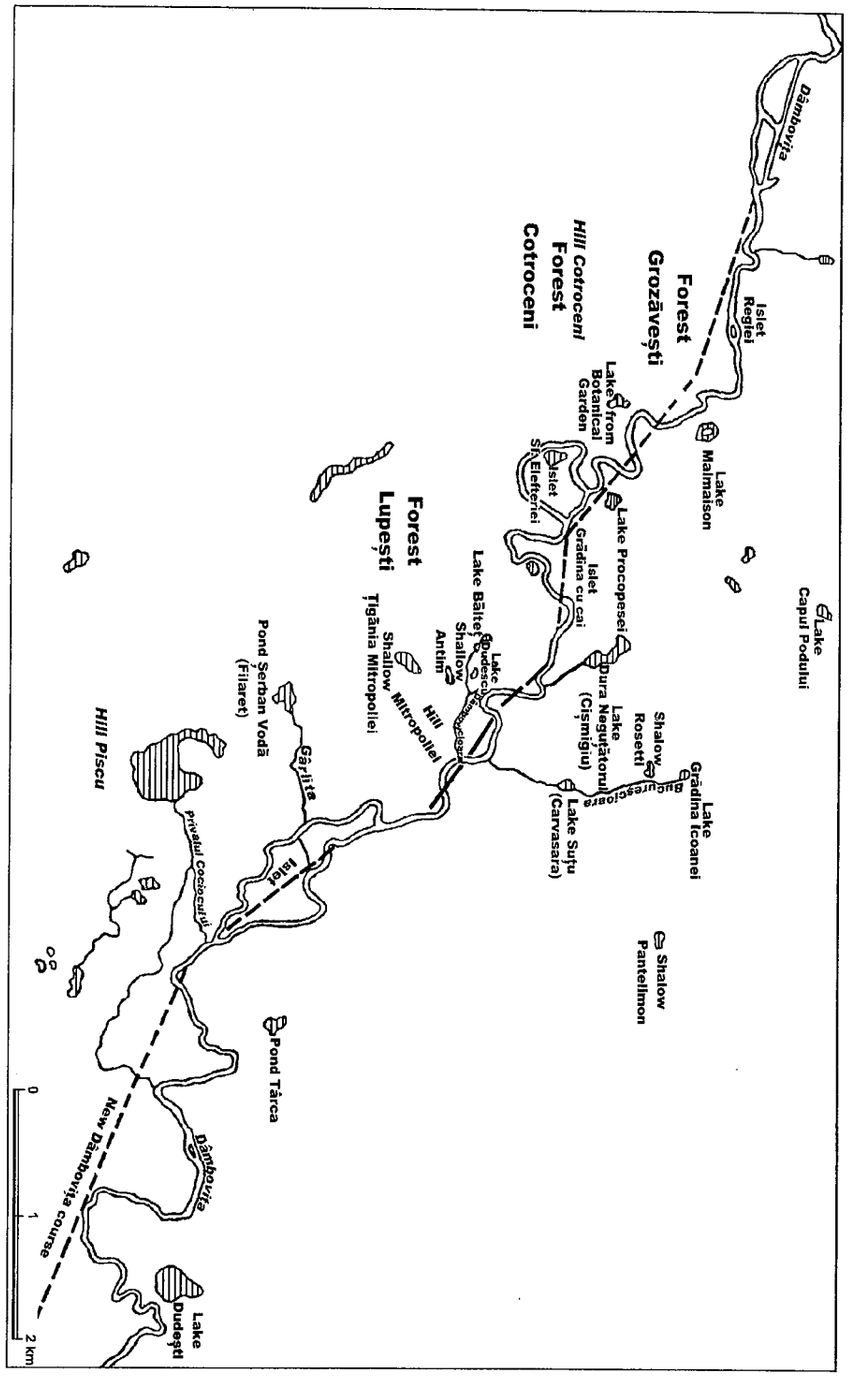


Fig. 1 – The arranged course of the Dâmbovița river in Bucharest town area and its lakes from flood plain (XIII–IXX centuries) relative to unarranged line (after C.C. Giurescu, 1979).

The disappearance of riverbank forests caused a gradual diminution of river and its affluent debit. Some affluents vanished, after territory arrangements and town expansion had been accomplished. The river connections with the flood plain lakes had been interrupted in the course of time and, consequently, the lake surfaces and even their number were reduced. The river line simplified after the meanders and islets had been cleared away, as a result of rectification and flow-deepening works executed in 1880–1883 years (1). At last, a century later, the river debit was partially introduced in a pipe localised under the surface course. To mention that about 1870 year, the river itself was already degraded. An observer of that period described this as follows: "Small depth, meandering like a snake in river bed at street level, dirty, without embankments, with wood ponds maintained in poor condition, had numerous fords, from where water carriers took out the water".

The Dura Neguțătorul lake, first mentioned in the XVIIth century during Matei Basarab voivode, is the ancestor of Cişmigiu lake. Before, it had been much larger than nowadays lake. When the Dâmbovița river had overflowed, often, the lake waters touched the walls of the Sărindar Monastery, situated on the place where the Casa Oştirii was erected later on. The Dura Neguțătorul Lake was surrendered three years for fishing, as it is noted in an original document, namely a concession agreement written in 1855 year. This detail suggests the ecological potential of that period natural ecosystem.

Later on, the lake colmated, becoming a muddy shallow at the beginning of the century. It never emptied because of supplying by subterranean springs. It was surrounded by reed plots and club rush, which sheltered wild dukes. Potra (3) affirms that, "few platforms of planks fixed to pillars touched in lake, were built from one shallow side to another", for urban traffic. Gheorghe Bibescu voivode named a commission for expropriation of shallow surroundings, which had become an infection source, in order to dry it for a public garden plan. This project was actually realised not later than under Barbu Ştirbei ruling, when the actual lake basin had been constructed. A connection channel between the lake and the river was built for water renewal. Later on, this connection was canceled after the channel had been abolished, in accordance with a new conception of water supply and evacuation system.

The denomination of nowadays lake, as well as of the surrounding garden, comes from "Marele Cişmigiu", a name accorded to chief fountain builder who, during Al. Ipsilanti ruling, had built his house in the vicinity of the lake, in its northern part.

4. RESULTS AND DISCUSSIONS

In spite of reduced basin dimensions and of its anthropic character, the zooplankton species diversity revealed an annual taxonomic wide enough spectrum, comprising 71 components (Fig. 2, Table 1). An extremely small number of these (6) had an important role in the ecological balance dynamics of the community, as the frequency analysis had revealed. These were constant forms (frequency > 50 %), while the majority were accidental (frequency < 50 %) or accessory (frequency < 25 %). The high value of annual species diversity was the result of the important taxonomic structure changes from one month to another. Thus, for example, the monthly taxonomic spectrum during vegetation period did not exceed 21 components (March and June), while the minimum value (October) was of 14 components (Fig. 2).

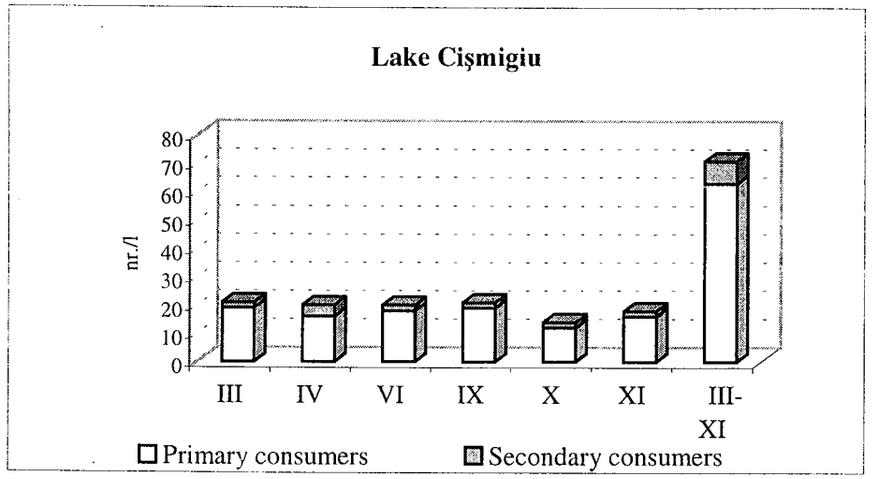


Fig. 2 – Diversity seasonal dynamics of Cişmigiu lake zooplankton (taxon number).

The last characteristic reflected the existence of a young lacustrian ecosystem, from an ecological sequence point of view, characterised by very variable environment conditions. The ecosystem youth is artificially maintained through water and mud drainage in winter months. The temporal variations of zooplankton taxonomic spectrum (Fig. 2, Table 2) in Cişmigiu lake were a consequence of dominance of the species characterised by a reduced generation period (a feature of Ciliata, Testacea and Rotifera group), too. On the contrary, the longer lifespan species (cladocers and copepods) had a reduced representation. In

the copepod case, the juvenile stage individuals prevailed, while the adults were extremely rare, as a result of environment factor variations.

Table 1

Zooplankton taxons from Cişmigiu Lake and their frequencies

Taxonomic structure	Constant elements	Accidental and accessorial elements
1	2	3
PRIMARY CONSUMERS		
Ciliata		
Bizonula parva		+
Codonella cratera		+
Cyclidium glaucoma		+
Glaucoma scintilans		+
Links lateralae		+
Opryoglena collini		+
Philasterides armata		+
Strombidium viridae		+
Strombilidium gyrans		+
Vorticella convallaria		+
V. microstoma		+
Testacea		
Arcella arenaria		+
Centropyxis ecornis		+
C. discoides		+
C. spinosa		+
Difflugia corona		+
D. globulosa		+
D. oblonga		+
Rotifera		
Adineta gracilis		+
Brachionus angularis		+
B. calyciflorus anuraeiformis	+	
B. cayciflorus dorcas		+
B. diversicornis		+
B. forficula		+
B. leydigi tridentatus		+
B. quadridentatus brevispinus		+
B. quadridentatus clunorbicularis		+
Colotheca pelagica		+
Conochilus unicornis		+
Euchlanis deflexa		+

Table 1 (continued)

1	2	3
E. dilatata	+	
Epiphanes clavulata		+
Filinia limnetica		+
Gastropus stylifer		+
Habrotrocha crenata		+
H. solida		+
Hexarthra fennica		+
H. mira		+
Keratella cochlearis tecta		+
K. ticinensis		+
K. valga		+
Lecane arcuata		+
L. clasterocerca		+
L. luna		+
L. lunaris		+
Lepadella quadricarinata		+
Mytilina ventralis		+
Polyarthra minor		+
P. remata	+	
Pompholyx sulcata		+
Proales micropus		+
Rotaria neptunia		+
Synchaeta oblonga		+
S. pectinata		+
Trichocerca dixon-nuttalli		+
T. fusiformis		+
T. pusilla		+
T. tenuior		+
Cladocera		
Bosmina longirostris		+
Ceriodaphnia pulchella		+
Chydorus sphaericus		+
Daphnia galeata		+
Copepoda		
Nauplial forms *Copepoda* g.sp.	+	
I-III st. Copepodites *Cyclopida* g.sp.	+	
Harpacticoida g.sp.		+
Secondary consumers		
Ciliata		
Dileptus anser		+
Trachelius ovum		+

Table 1 (continued)

Rotifera		
Asplanchna brigtwelli		+
A. herricki		+
A. priodonta	+	
Copepoda		
IV–V st. Copepodites *Cyclopida* g.sp.		+
Cyclops furcifer		+
Mesocyclops leuckarti		+

Table 2

Monthly taxonomic structure of Cişmigiu lake zooplankton (taxon number)

Systematic components \ Month	III	IV	VI	IX	X	XI	Σ III-XI
C_1	**19**	**16**	**18**	**19**	**12**	**16**	**63**
CILIATA	3	2	1	2	1	4	11
TESTACEA	3	1	1	3	-	3	7
ROTIFERA	11	7	13	12	9	7	38
CLADOCERA	1	3	-	-	-	-	3
COPEPODA	1	3	3	2	2	1	3
C_2	**2**	**4**	**2**	**2**	**2**	**2**	**8**
CILIATA	-	-	-	-	-	2	2
ROTIFERA	1	2	1	2	2	-	2
COPEPODA	1	2	1	-	-	1	3

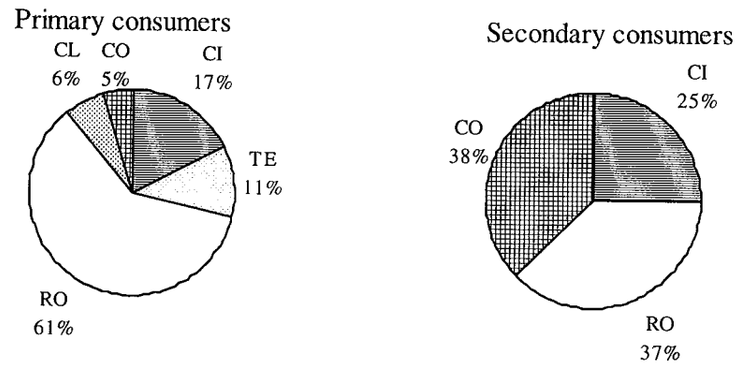


Fig. 3 – Taxonomic structure of Cişmigiu Lake zooplankton (%).

Of a total of 71 taxonomic components, 63 belonged to primary consumers, the remaining (8) being secondary ones (predators) (Fig. 2, Table 1). The taxonomic spectrum of the first trophic level reflected the Rotifera group dominance (60.32 %), while in the case of the second level, the dominance is provided both by Rotifera and Copepoda (37.50 % each) (Fig. 3).

The absolute numerical abundance was characterised by reduced values of monthly averages (177 ex/l in May, 1356 ex/l in March), as well as in the case of average calculated for the entire vegetation period (1356 ex/l), comparatively to temperate zone lakes. The unusual situation of the annual maximum value, at the beginning of vegetation period, is due to the numerical explosion of ciliats (Table 3). The analysis of two trophic level contribution to seasonal averages showed the marked dominance of primary consumers (92.58 %) against secondary ones (7.42 %) (Fig. 4).

Table 3

Monthly numerical density (ex/l) of Cişmigiu Lake zooplankton, on taxonomic groups and trophic levels

Systematic components \ Month	III	IV	VI	IX	X	XI	Xa III-XI
C_1	**1356**	**340.7**	**479.3**	**571.5**	**666.6**	**174.3**	**598.1**
CILIATA	1296	133.3	3.6	42.9	17.0	134.5	271.2
TESTACEA	8.2	3.1	1.4	0.3	-	5.0	3.0
ROTIFERA	51.6	54.5	186.3	479.67	637.6	21.8	238.6
CLADOCERA	0.1	3.8	-	-	-	-	0.7
COPEPODA	0.1	146	288	48.63	12.0	13.0	84.6
C_2	**0.12**	**201.7**	**15.1**	**53.9**	**14.9**	**2.0**	**47.9**
CILIATA	-	-	-	-	-	1.5	0.2
ROTIFERA	0.1	198	7.9	53.9	14.9	-	45.8
COPEPODA	0.02	3.7	7.2	-	-	0.5	1.9

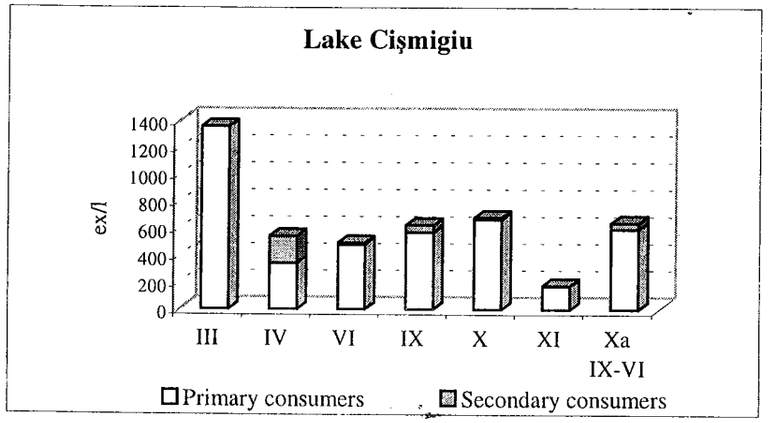


Fig. 4 – Absolute numerical abundance seasonal dynamics of Cişmigiu Lake zooplankton (ex/l).

The numerical structure analysed for the entire March-November period, reflected the ciliate (45 %) and rotifer dominance (40 %), in the case of primary consumers, and that of rotifer (96 %) in the case of secondary ones (Fig. 5).

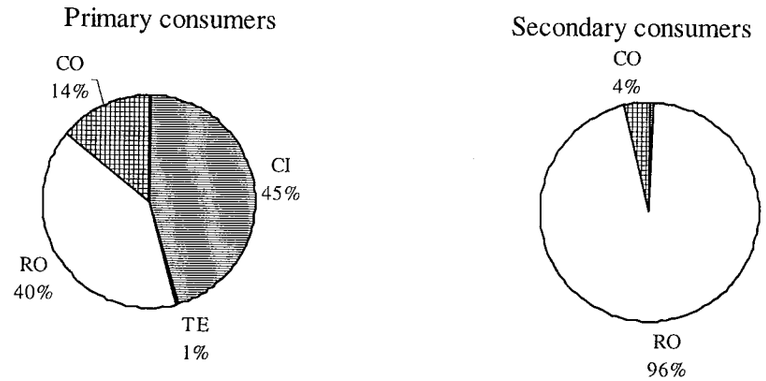


Fig. 5 – Numerical structure of Cişmigiu lake zooplankton (%).

Of the total taxonomic spectrum, only 14 components (9 belonging to primary consumers and 5 to secondary ones) had, from the numerical and gravimetrical point of view, a peculiar role in the ecological balance dynamics of Cişmigiu lake zooplankton. Of these 14 elements, the copepod dominance, especially of nauplial stages (in the case of primary consumers) and *Asplanchna priodonta* (in the case of secondary ones) dominance, from the numerical and gravimetrical point of view, lasted the longest time during the vegetation period (Table 4).

Table 4

Numerical and gravimetrical dominant elements of Cişmigiu Lake zooplankton

Species composition	Numerical dominance						Gravimetrical dominance					
Month	III	IV	VI	IX	X	XI	III	IV	VI	IX	X	XI
PRIMARY CONSUMERS												
Ciliata												
Cyclidium glaucoma	+											
Opryoglena collini							+					
Strombidium viridae		+										
Strombilidium gyrans						+						+
Vorticella microstoma	+						+					
Rotifera												
Brachionus alyciflorus anuraeiformis										+		
Keratella cochlearis tecta				+	+							
Keratella ticinensis			+									
Synchaeta oblonga					+						+	
Synchaeta pectinata											+	
Trichocerca dixon-nuttalli				+								
Copepoda												
Nauplial forms		+	+			+			+	+		+
I-III st. Copepodides *Cyclopida* g.sp.								+	+			
Harpacticoida g.sp.								+				
SECONDARY CONSUMERS												
Ciliata												
Dileptus anser						+						+
Rotifera												
Asplanchna herricki				+	+					+	+	
Asplanchna priodonta	+	+	+	+	+		+	+	+	+	+	
Copepoda												
IV–V st. Copepodides *Cyclopida* g.sp.		+	+			+		+	+			+
Mesocyclops leuckarti	+						+					

The absolute gravimetrical abundance was characterised by reduced values too. This is valid in the case of monthly data (67–2799 μg wet weight/l November, April respectively) as well as in that of entire vegetation period average (916 μg/l) (Fig. 6).

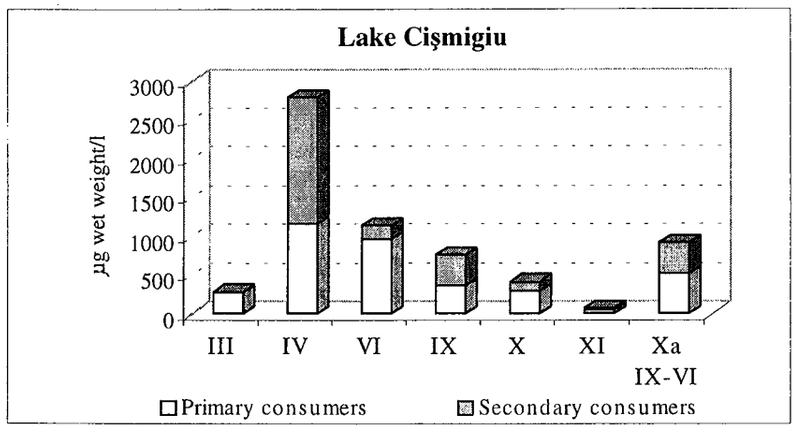
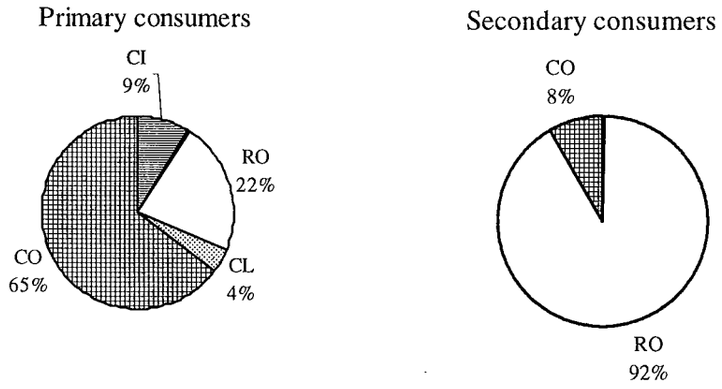


Fig. 6 – Absolute gravimetrical abundance seasonal dynamics of Cişmigiu Lake zooplankton (µg wet weight/l).

One of the causes which generated this situation was the low contribution of bigger size forms. The gravimetric ratio, primary consumers/secondary consumers (57.04/42.96) showed an especially large proportion of the latest ones, which allow us to assume an omnivorous feed of secondary consumers (Fig. 5).

The gravimetrical structure analysed for the entire vegetation period evidenced the determinant role of juvenile copepods (65%) for primary consumers, and rotifers (65%) for secondary ones (Fig. 7).



CL = Ciliata; TE = Testacea; RO = Rotifera;
CL = Cladocera; CO = Copepoda

Fig. 7 – Gravimetrical structure of Cişmigiu Lake zooplankton (%).

6. CONCLUSIONS

– The zooplankton of anthropic Cişmigiu Lake is characterised by low monthly values of species diversity, numerical abundance and absolute gravimetric abundance and by a reduced number of constant forms;

– On the contrary, the marked monthly changes of species diversity determined a relatively large annual spectrum;

– The wide variations of taxonomic structure during the vegetation period reflected the existence of a young lacustrian ecosystem, by ecological sequence point of view, as a consequence of anthropic influences (water emptying, the mud drainage in cold season a.o.).

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Institute of Biology
Spl. Independenţei 296
Bucharest 79651, P.O. Box 5653
Romania

CHRYSOMÉLIDÉS AVEC UN STATUT INCERTAIN DANS LA FAUNE DE LA ROUMANIE (*COLEOPTERA*: *CHRYSOMELIDAE*)

AURELIAN LEONARDO ILIE

22 species having an "uncertain status", recorded in the Romanian fauna, are presented. Some of them are erroneous mentions, others represent accurate signals, but the respective species have disappeared due to natural or anthropogenic causes.

1. INTRODUCTION

Grâce au relief extrêmement varié, ainsi qu'à la diversité des conditions climatiques, la faune des chrysomélidés de Roumanie est particulièrement riche en espèces intéressantes de point de vue zoogéographique.

En comparaison avec d'autres familles de coléoptères ou même d'autres ordres d'insectes, les listes faunistiques concernant les chrysomélidés de Roumanie ne sont pas nombreuses, la plupart de celles-ci étant élaborées pendant la première moitié du vingtième siècle. Elles contiennent généralement des espèces de Transylvanie, les données concernant les espèces d'autres provinces du pays étant assez peu représentées.

Dans l'intervalle 1992–2001, on a recommencé les études faunistiques, étant publiées des listes faunistiques de diverses zones de Transylvanie, d'Olténie et de Dobroudja (Al. Crişan, A.L. Ilie, etc.).

Un pourcentage relativement insignifiant du total des espèces signalées en Roumanie sont considérées comme ayant un «statut incertain». Ce fait est dû à quelques déterminations erronées, à quelques présences accidentelles ou à quelques déterminations correctes concernant des espèces disparues grâce aux influences anthropiques ou naturelles.

2. MATÉRIEL ET MÉTHODES

Ce travail représente une tentative d'élaboration de la liste des espèces avec «statut incertain», résultée à la suite de cumul des données trouvées dans la littérature roumaine et étrangère et des recherches personnelles, dans l'intention de servir comme point de départ pour les futures recherches.

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On a utilisé la nomenclature existante [10] mais en ce qui concerne la dispersion des chrysomélidés dans la région paléarctique on a consulté [14].

3. RÉSULTATS

Les chrysomélidés étudiés appartiennent à 10 genres, totalisant 22 espèces. On présente par la suite la liste de ces espèces en mentionnant les données et les auteurs qui ont signalé les espèces, les années de leur publication et les localités de récolte.

Sous-famille *Donaciinae* Kirby, 1837

Donacia (Donacia) brevicornis Ahrens, 1910

Bielz, 1887; localité Sibiu

C'est la seule mention dans la faune roumaine, bien que ce soit une espèce nordique et central-européenne; en Hongrie elle est considérée aussi comme une espèce rare [9]. On nécessite reconfirmations.

Sous-famille *Clytrinae* Kirby, 1837

Labidostomis propinqua Falderman, 1837

Montandon, 1906; localité Comana

Espèce existante dans le Caucase, en Turquie, en Grèce et au sud-est de la Bulgarie. Probablement, c'est une présence accidentelle dans la faune roumaine.

Labidostomis rugicollis Lefévre, 1872

Fleck, 1904; localité Comana

Espèce existante au sud de la Russie. Signalée en Roumanie probablement par une détermination erronée.

Labidostomis lusitanica Germar, 1824

Crişan, Bonea, 1995; localité Arcalia

La récolte d'un seul exemplaire (juillet 1993) et la distribution zoogéographique de cette espèce seulement dans la zone méditerranéenne suggèrent sa présence accidentelle en Roumanie.

Lachnaia pubescens Dufour, 1763

Fleck, 1904; localités Comana et Bucarest

Espèce répandue seulement à l'ouest de la zone méditerranéenne. Sa présence dans la faune roumaine est exclue.

Coptocephala floralis Olivier, 1808

Hurmuzachi, 1904; localité Scăeni-Prahova

Espèce répandue exclusivement en Espagne et dans les îles Baléares. Sa présence est exclue dans la faune roumaine.

Sous-famille *Cryptocephalinae*, Gyllenhal, 1813

Pachybrachys limbatus Ménétriés,1836

Bielz,1887; localité Cibin

Bien que l'espèce soit répandue dans toute la Péninsule Balkanique, c'est la seule mention dans la faune de notre pays. On attend reconfirmations.

Cryptocephalus (Proctophysus) cyanipes Suffrian, 1847

Petri, 1912; localité Postăvarul

Espèce répandue dans les Alpes françaises et en Italie. Sa présence est exclue dans la faune roumaine.

Cryptocephalus (Burlinius) macellus Suffrian, 1860

Warchalowski, 1991 – carte, dans le midi et l'ouest de la Roumaine

Bien que ce soit une espèce répandue dans la partie sud, centrale et ouest de l'Europe, jusqu'à présent, il n'existe pas de données dans la faune roumaine. On attend confirmations.

Cryptocephalus (Asiopus) flexuosus Krynicki, 1834

Gruev, Tomov, 1984; au sud de la Dobroudja

Espèce répandue dans le Caucase, la région de la Mer Noire. Il n'existe pas de mentions dans la littérature roumaine, mais sa présence dans la faune roumaine est possible.

Cryptocephalus (Cryptocephalus) albolineatus Suffrian, 1847

Bielz, Petri, 1912; Massif Făgăraş

Cette mention est basée probablement sur une détermination erronée. L'espèce est répandue seulement dans les Alpes.

Cryptocephalus (Cryptocephalus) duplicatus Suffrian, 1847

Warchalowski, 1991 – carte, Roumanie
Espèce répandue en Asie mineure et en Bulgarie, son existence est possible même dans la faune roumaine.

Sous-famille *Chrysomelinae* Latreille, 1802

Timarcha montana Fairmaire, 1825

Fleck, 1904; région Prahova
Espèce répandue en Macédoine, Bosnie-Herzégovine. Sa présence sur notre territoire national est discutable.

Timarcha tenebricosa moravica Bechyné, 1945

Kaszab, 1962; Péninsule Balkanique
La présence de cette espèce en Roumanie est possible.

Sous-famille *Alticinae* Kutchera, 1859

Phyllotreta variipenis Boieldieu, 1859

Fleck, 1904; localité Babadag
Espèce répandue dans la zone méditerranéenne et dans le midi de l'Asie mineure. Sa présence dans la faune roumaine est peu probable.

Phyllotreta parallela Boieldieu, 1859

Fleck, 1904–1905; localités Bucarest et Rucăr
Espèce exclusivement méditerranéenne. Détermination probablement erronée; sa présence en Roumanie est exclue.

Longitarsus (Longitarsus) pallidicornis Kutchera, 1863

Kuthy, 1900; massif Pietrosu Rodnei
Espèce central-européenne. On nécessite reconfirmations.

Longitarsus (Longitarsus) corynthius Reiche, 1858

Petri, 1912; localité Sighişoara
Espèce exclusivement méditerranéenne et sud-européenne. Sa présence dans la faune roumaine est exclue.

Longitarsus (Longitarsus) gracilis Kutchera, 1864

Warchalowski, 1991 – carte; Roumanie
Bien que cette espèce soit répandue dans presque toute l'Europe, il n'existe pas d'autres mentions pour la Roumanie. On nécessite confirmations.

Longitarsus (Longitarsus) rectilineatus Foudras, 1860

Warchalowski, 1991 – carte; Roumanie
Espèce centrale et sud-européenne. Quoiqu'elle n'ait pas été signalée en Roumanie, sa présence sur le territoire national est possible.

Sous-famille *Cassidinae* Gyllenhal, 1813

Cassida (Hypocassida) meridionalis Suffrian, 1844

Fleck, 1904; localité Cernavodă
Espèce répandue dans la partie occidentale de la zone méditerranéenne. Sa présence dans la faune roumaine est exclue et probablement qu'elle a été signalée sur une détermination erronée.

Cassida (Cassida s.str) deflorata Suffrian, 1844

Fleck, 1904; localités Bucarest et Băneasa
La même situation que pour l'espèce précédente.

4. DISCUSSIONS

Parmi les espèces signalées jusqu'à présent dans la faune roumaine, la présence de 9 espèces est exclue, 2 espèces sont considérées comme des présences accidentelles, 7 espèces constituent des présences possibles, tandis que l'existence des 4 espèces nécessite reconfirmations pour le territoire national.

En ce qui concerne l'espèce *Donacia brevicornis* Ahr., probablement que sa mention en Roumanie représente l'extrémité sud. Cette espèce est plus fréquente dans le nord de l'Europe et plus rare dans son centre, ce qui pourrait expliquer «le statut incertain» de cette espèce dans notre pays.

De plus, pour l'espèce *Pachybrachys limbatus* Mén., signalée dans la localité Cibin, la Roumanie pourrait représenter son extrémité nordique, l'espèce étant répandue dans toute la péninsule Balkanique.

De point de vue zoogéographique, les espèces méditerranéennes sont prédominantes – 8 (35%), suivies par celles central-européennes et balkaniques, chacune par 3 espèces (13,5%), européennes, ouest-européennes, sud-européennes et Asie mineure, caucasiennes, chacune par 2 espèces (9%).

5. CONCLUSIONS

Les chrysomélidés analysés comptent au total 22 espèces. Parmi celles-ci, 9 espèces n'appartiennent pas à la faune roumaine, tandis que 2 sont accidentelles; le reste des espèces nécessitent confirmations ou reconfirmations pour le territoire national. De point de vue zoogéographique on constate la prédominance des espèces méditerranéennes.

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École Obedeanu
Rue Brestei 40, 1100, Craiova

INFLUENCE OF THE THRIPS (INSECTA: THYSANOPTERA) POPULATIONS ON THE GREENHOUSE PLANTS, WITH THE VIEW OF CONTROLLING THEM BY BIOLOGICAL CONTROL

LILIANA VASILIU-OROMULU

The work presents the study of thrips populations from the cultures of vegetables and flowers, under directed conditions. This problem is of wide concern worldwide.
For a correct management of controlling this Thysanoptera species causing great economic damage to cultured plants in greenhouses, the ecology and structure of its populations must be known.

Key words: *Frankliniella occidentalis*, structural indices, biological control.

INTRODUCTION

In the greenhouses of Romania, besides *Frankliniella occidentalis* Pergande, 1895, other species have also been mentioned before 1990: *Parthenothrips dracanae* (Heeger, 1854); *Heliothrips haemorrhoidalis*, Bouche, 1833; *Hercinothrips femoralis* (O.M. Reuter, 1891), *Thrips tabaci*, Lindeman, 1888, but the first species invaded the greenhouses eliminating most of the other species mentioned earlier.

In Europe, new thrips species have already been observed, such as *Thrips palmi, Chaetanaphothrips orchidii, Leucothrips nigripennis, Echinothrips americanus,* and *Acantothrips orchidaceus*.

The American specialists from Burlington University (Vermont) created a site named "Thripsnet" with the participation of over 450 scientists involved in thrips studies, most of them interested in the issue of WFT (Western Flower Thrips), common denomination of *Frankliniella occidentalis*, main pest species of Thrips in nature, in California and in greenhouses, both in America and in Europe.

During the past two years, worldwide, the subject of our study was also approached by many scientists among whom Jude Benisson (UK), G. Moritz (Germany), M. Roditakis (Greece), Margaret Skinner (USA), G. Vierbegen (The Netherlands), I.Vannien (Finland), Helen Myksvoll Singh (Norway), H. Benstrom, H. Larsson (Sweden), L. Acosta (Spain), T. Roman, A. Szabo, D. Mihăescu (Romania).

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The population ecological indices were approached with the purpose of the biological control of the WFT thrips populations, particularly *Amblyseius cucumeris* (Acarina, Phytoseidae), and to reveal the manner of transmission of TSWV virus (Tomato Spotted Wild Virus). Bedsides this virus, *Frankliniella occidentalis* may also transmit other tospoviruses such as TCSV (Tomato Chlorotic Spot Virus), GRSV (Groundnut Ring Spot Virus) and INSV (Impatiens Necrotic Spot Virus). The attack produced by WFT and TSWV together has a synergic effect.

Other thrips growing in greenhouses and which have been established as carriers for tospoviruses are: *Frankliniella fusca* (TSWV, INSV), *Frankliniella intonsa* (TSWV), *Frankliniella schultzei* (TSWV, TCSV, GRSV, the dark form, and the light form just TSWV, TCSV,), *Thrips palmi* (TSWV), *Thrips setosus* (TSWV, WSMV– Watermelon Silver Mottle Virus), *Thrips tabaci* (TSWV).

In late 1999, a new species of *Frankliniella zucchini* Nakahara & Monteiro was discovered, which is vector for ZLCV virus (Zucchini Lethal Chlorotic Disease), for *Cucurbita pepo*.

The viruses belong to Genus Tospovirus, Fam. Bunyaviridae.

Frankliniella occidentalis Pergande 1985 belongs to S/Ord. Terebrantia, Trib. Thripini, S/Trib. Thripina, Fam. Thripidae.

This is a flower, leaf, and predator species (unlike most Thysanoptera species, which are phytophagous); in warmed greenhouses it may be found all year long, in nature only during late spring-autumn. It is spread on about 150 plant species outside the greenhouse, but the latter populations disappear due to the winter cold, the site from where the infection spreads again being the plants and soil of the greenhouse.

Species *Frankliniella occidentalis* was identified in nature in California, where, due to the warm climate, it perpetuates through numerous generations on fruit trees and crops.

It arrived in Europe from America on plant products carrying eggs or larvae. It was observed starting with 1986 (zur Strassen in Germany), 1988 (Van de Vrie in The Netherlands), 1990 (Vasiliu-Oromulu in Romania).

MATERIAL AND METHOD

In Romania, *Frankliniella occidentalis* was studied in Comaico greenhouse, warmed in winter, in Codlea, Işalniţa, Iernut, Vidra and in a private greenhouse. The crop plants grown in the greenhouse, used to collect thrips samples, were aubergines, tomatoes, cucumbers, onion and gerbera, freesia, carnation, roses, Alstroemeria among flowers.

The structural index used to analyze the thrips populations was numerical abundance of WFT specimens inhabiting plants (for which the numerical density could not be calculated) such as carnation buds. Numerical density is the number of WFT specimens on cm^2 of leaf area from plants infested with thrips.

The data obtained by Vasiliu-Oromulu and Burlacu (1970) in the study of respiration metabolism were used for biomass density of the thrips populations, starting from the average weight of the specimens, 0.000038 g fresh matter.

RESULTS AND DISCUSSION

DIVERSITY OF THRIPS SPECIES IN THE GREENHOUSE DEPENDING ON THE TROPHIC SUPPORT

Insects were sampled from plants growing in different locations in the greenhouse in order to assess the specific presence. To Thysanoptera, the plant species are both the spatial, the trophic and reproductive niche. This is why the relation between Thysanoptera and plants is very strong, up to feeding on a single plant species (monophagia); oligophagia (Thysanoptera species growing on the species of the same plant genus is also known) but polyphagia is widespread.

The thrips are known as opportunistic insects which, when a certain vegetal food is missing, pass to other species or even become predators feeding on eggs, larvae or adult specimens of other invertebrata.

The large number of samples show that in this greenhouse there are thrips belonging to just one species, *Frankliniella occidentalis*. This means that in the greenhouses warmed in winter (Codlea, Iernut, etc.) only this species grows and mass replicates.

Unlike this greenhouse, the samples collected from greenhouse not warmed in winter (Vidra and a private greenhouse, Giurgiu county) show a wide taxonomic spectrum, 3 to 12 thrips species.

The conclusion is that in environmentally controlled areas, *Franklinellia occidentalis* is the only thrips species growing there, displaying a grate polyvoltinism inducing both directly by sucking the cell juice and indirectly by retransmission of TSWV virus, important economic damages to the greenhouse; this is why the greenhouse management removed some crops strongly infected or closed some areas for complete pest control (Codlea).

NUMERICAL ABUNDANCE

Assessing the thrips populations is of particular importance, both theoretical and applied, because it provides information on the size and gradation of the particular coenosis. Thus, few specimens were observed in carnation, most of them in the stage of buds, in average 5.5 individuals/plant. The reason for this low number of thrips specimens resides in the abiotic conditions unfavorable to the growth of insects, particularly the low temperature, about 18°C, which was

unfavorable to the replication of *Frankliniella occidentalis;* at the same time, before planting in the sand beds, the cuttings were treated with remnant insecticides. However, the greenhouse soil was not treated by thorough fumigation, although the soil is a continuous source of new infestation of plants, part of the life cycle of the thrips taking place in the soil.

Quite low numerical abundance was observed in freesia, both in leaves and particularly in flowers. The average number of 7 specimens/plant is considered as not causing important economic damage.

The tolerance level in flowers is considered as 10 to 12 specimens/flower, while in vegetables it is considered as 50 to 55 specimens/leaf (according to Bournier, 1987). Recent investigations have identified a higher maximum level than the one found by the French scientist.

A further structural index was calculated for the vegetable crops.

NUMERICAL DENSITY/cm^2

Minimal numerical density was observed in the cucumber crop, maximal in aubergines and average in tomatoes. Damages were observed in these crops, which is why we did a specimen count per cm^2 to determine the intensity of thrips attack. Thus, the average number of specimens/cm^2 was 2.5 in the cucumber leaves and 1.5 in the cucumber flowers, 5.5 in the aubergine leaves; very few specimens were observed on the aubergine flowers and only in those recently bloomed. The average density in tomato leaves was 3.0 specimens/cm^2, while very few specimens were detected on the tomato flowers, and only on the flowers towards the top of the plants.

BIOMASS DENSITY

Biomass density of the thrips populations was determined on the main greenhouse crops: 0.95 mg/cm^2 in cucumber leaves and 0.57 mg/cm^2 in cucumber flowers; 2.09 mg/cm^2 in aubergine leaves; 1.17 mg/cm^2 in tomato leaves.

Thus, the biomass density of Thysanoptera on greenhouse crops was significantly different according to the site (leaves, flowers) and according to the trophic support (different vegetables). As with the numerical abundance, the biomass values of Thysanoptera are clearly higher in aubergine crops, minimal in cucumber and intermediary in tomatoes.

VERTICAL DISTRIBUTION OF THE THRIPS POPULATIONS ON VEGETABLE CROPS

All vegetable crops grown in greenhouse are cultivated on props, which allow a proper development of plants, an optimal illumination of the vegetative parts and a strong plant growth on the vertical, supporting the fruits irrespective of their weight. The vertical distribution of the *Frankliniella occidentalis* populations shows that the number of specimens increases with the height of the leaves.

In the aubergine crop the peak numerical abundance of thrips specimens occurred at the height of 120 cm from the soil: 326 specimens/leaf, decreasing to 308 specimens/leaf at 100 cm and to 225 specimens/leaf at the height of 70 cm from the soil.

In the cucumber crop, the average numerical abundance was 130 specimens/leaf at the height of 106 cm from the soil, increasing to 195 specimens/leaf towards the top of the plant, at the height of 160, because the young cells, just developed, are the preferred food of the thrips populations.

In the flower crops, where the thrips populations inhabit preferably the flowers (nevertheless, they can also be found on leaves), there was no such vertical distribution of the thrips, because the flowers are all at just about the same height and the differences are not significant.

CORRELATION OF THE SIZE OF THRIPS POPULATIONS WITH THE ABIOTIC FACTORS OF THE GREENHOUSE

The importance of the local abiotic factors to the development of insect populations in the greenhouse and in nature is well established.

The entire biological cycle of these greenhouse thrips, WFT, is influenced by temperature and moisture. Generally, in greenhouses, moisture is constant and temperature varies slightly: 18–18.5°C in carnation, freesia, fichus and Alstroemeria greenhouses and 24 to 26°C in vegetable greenhouses (Table 1).

The increase of temperature by just 2 degrees C determined the even faster replication of the insects. This factor influences the number of thrips generations per year, the favourable conditions leading even to 12 to 14 generations per year, which intensifies the damage produced by these insects.

ASSESSMENT OF THE DAMAGE PRODUCED BY THRIPS TO GREENHOUSE CROPS

The damage brought by thrips and TSWV to greenhouse crops is assessed by the necrotic spots appearing on the flower petals in carnation, freesia, gerbera, roses and Alstroemeria and on the fruits of aubergines, cucumbers and tomatoes. All these changes of aspect make the products unmarketable or decrease their price bringing serious losses to the greenhouse.

Table 1

Numerical density of the thrips populations on vegetable leaves according to temperature

Plant	°C	Number of leaves	Average number of thrips specimens per leaf
Cucumbers	24	10	130
	26	10	156
Aubergines	24	10	286
	26	10	326
Tomatoes	24	10	14
	26	10	20

Here is the damage produced by thrips to some crops (Table 2):

Table 2

Intensity of thrips attack on greenhouse vegetable crops

Plant	Level of attack
Cucumbers	xx
Aubergines	xxx
Tomatoes	x

xxx very strong
xx strong
x weak

The economic damages, from 25–75% of the crops, due to the thrips attacks and TSWV transmitted only by larval stage of this insect, emphasize the importance of the global management of integrated control of *Frankliniella occidentalis.*

The work is a scientific fundamental base of the introduction, for the first time in our country, of the biological control with *Amblyseius cucumeris* (Acarina, Phytoseidae), which ensure realizing of ecological crops.

CONCLUSIONS

Frankliniella occidentalis is found in large counts on aubergine greenhouse crops, in lower counts in tomato, cucumber, greenhouse crops in gerbera, roses, carnation and in very low counts freesia flower plants.

The vertical distribution of the thrips populations peaks towards the top of the plants and decreases on the leaves located close to the basis of the plants.

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Institute of Biology
Bucharest
Email: biologie@ibiol.ro

CLUTCH SIZE AND TIMING OF REPRODUCTION IN A POPULATION OF *LACERTA AGILIS* FROM ROMANIA

SZILÁRD NEMES

Clutch size was studied in a population of sand lizards, *Lacerta agilis* from Transylvania, Romania. A total of 31 female sand lizards were collected opportunistically in their breeding period from 1995 through 2000. Pregnant females carried between 3–8 eggs and the mean clutch size was 5.9 ± 1.3. Clutch size was positively correlated with female body length. Clutch size did not vary significantly among years. Females that carried eggs in lower development stages were not smaller than females that had eggs in a final development stage.

INTRODUCTION

Reproduction has a paramount importance to lizards not only in the obvious sense of producing new generations but also due to its strong effects on lizards' natural history. Body shape, foraging mode, predator escape tactics have a strong relationship with reproduction (20, 21, 22, 23); pregnancy strongly affects the female lizards' behaviour as well (1, 5, 8,19).

The sand lizard, *Lacerta agilis*, is a medium sized oviparous lizard widely distributed all over Europe. Females lay up to 12 eggs in June, incubation time lasts from 30 to 60 days depending on environmental temperature (11). Not all adult females get pregnant each year (17) and a second clutch in a year is very uncommon (10).

The aim of the present note is to provide comparative information on clutch parameter in a sand lizard population from Transylvania, Romania. The relationship of maternal body length with clutch size and timing of reproduction is discussed.

MATERIALS AND METHODS

Clutch size and maternal body length were recorded from an examination of 31 female sand lizards collected opportunistically in their breeding period from 1995 through 2000 in the nearby meadows of Sf. Gheorghe town (Transylvania, Romania). All pregnant females were found death in the field out of which 11 road killed, 14 killed by humans and at 6 lizards the cause of death remains unknown. Prior to dissection all lizards were measured (SVL: snout went length) with dial

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callipers to the nearest 0.1 mm in a standardised manner. Eggs development stages were determined on the 14 human killed lizards collected on 9th of June 2000 using Fuhn's (9) classification:

(1) ovules in ovaries, 2 mm diameter;
(2) ovules in ovaries, spherical shape, from 4 mm to 5.5 mm diameter;
(3) oviductal eggs, from 5.5 mm to 8.5 mm diameter;
(4) white oviductal eggs, spherical shape, presence of the membrane, up to 12 mm diameter.

Basic statistics was computed for the data (means, standard deviation SD, standard error SE, and coefficient of variation CV). Regression between female body length and clutch size was computed on Log 10 transformed data as both variable has non normal distribution (Kolmogorov-Smirov test, $P > 0.15$). Difference in body length among females in different reproductive stage was tested by Wilcoxon sign test. Friedman tests were performed to test the significance of the difference in clutch size among years. Years 1996 and 1999 were excluded due to the fact that in both years only one specimen was examined. The standard criteria of statistical significance were $\alpha = 0.05$ for all tests, except for the regression test where $\alpha = 0.01$ to avoid false significant relationships. Statistical analysis followed Précsényi (16).

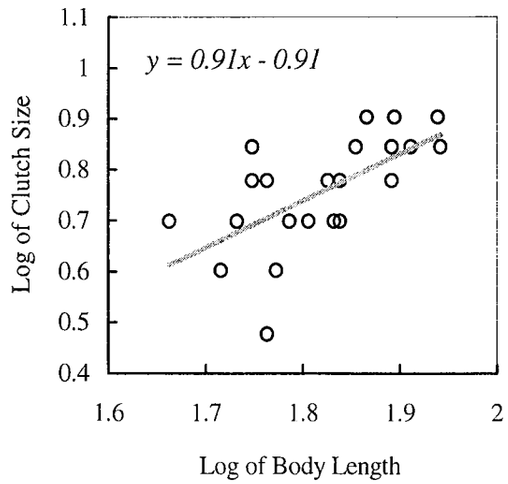

Fig. 1 – The positive relationship between female Sand Lizards body size and clutch size.

RESULTS AND DISCUSSIONS

Females with oviductal eggs had a mean clutch size of 5.9 ± 1.3 ranging between 3 to 8 eggs (Table 1). Clutch size did not vary significantly during the

years ($\chi^2 = 0.97$, d.f. = 26, $P = 0.78$). Two females did not carry any eggs. There is a significant relationship between female body length and clutch size ($r^2 = 0.42$, $F_{1,27} = 19.39$, $P < 0.001$, n = 29) (Fig. 1), larger females tend to produce larger clutches. Large clutches did not always assure a higher number of surviving hatchlings, large clutches of small eggs are balanced by survival selection favouring large offspring (12, 17). Sand lizards'clutch size exerts a strong negative relationship on hatchling size, this because the relative clutch mass (clutch mass divided by maternal body mass) did not correlate with maternal body length (13). This is in contradiction with previous hypotheses according to that the reptiles reproductive output is induced by the optimal energy allocation among maintenance, growth and reproduction (6, 7). Only 8 out of 14 lizards had oviductal eggs in the 4th stage while 6 were in the 3rd stage of development. There is no significant difference in body length between females that carried eggs in the 3rd or 4th stage ($z = -0.736$, $P = 0.46$). Usually females in better physical condition lay their clutches earlier (14), early reproductions allow a longer growth period of offspring before their first hibernation which assures a higher survival success (2).

Table 1

Summary statistics of body length and clutch size for 29 female Sand Lizards

	Mean	SD	SE	CV	Range
Body length	67.7	11	2	16.3	46 – 87.5
Clutch size	5.9	1.3	0.2	22	3 – 8

The studied sand lizard population has a similar mean clutch size like other populations from different areas (3, 4, 18). True that at the northern limit of the species distribution area, in a population from Sweden, Olsson and Shine (14) found a much higher mean clutch size (8.76), but this corresponds to the general trend of producing larger clutches at higher latitudes and elevations (15, 23).

With respect to the large distribution area published data gives only limited information on overall variation of sand lizards population characteristics. More studies are necessary for comparative data from populations in little studied parts of the species' distribution area. The data presented in this study of sand lizards agrees with other similar studies from different areas (3, 4, 13, 14, 18) with a few exceptions due to the latitudinal variations of the study site.

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Str. Gábor Áron 28/28/3
Sf. Gheorghe, Ro – 4000, Romania
E–mail: nemessz@cii.educv.ro

CELL DEATH IN RAT BRAIN AFTER LOW-DOSE EXPOSURE TO PARAOXON

ZOIA MĂRCULESCU*, ADRIANA POCIU*, V.A. VOICU**, M.E. HINESCU***

Toxic compounds can induce cell-damage having the hallmarks of apoptosis, depending on dose, duration of exposure, mechanism of action, and susceptibility of each individual cell type (16, 39, 40, 41). We hypothesized that among other tissular targets, certain cerebral regions may be very susceptible to the effects of low-dose POX (the main active metabolite of the organophosphorus compound parathion) (34, 36). The biological material was examined by both electron microscopy and flow cytometry. Data presented suggest that exposure to low-dose (1 mg/kg body weight) of POX may alter tissular homeostasis in the brain. Electron microscopy revealed a mosaic-like set of ultrastructural changes, from early apoptotic stages, up to characteristic end-stages of cellular necrosis. Flow cytometry allowed measurement of DNA strand breaks associated with apoptosis (when present), based on detection of PI (propidium iodide) fluorescence, after DNA extraction.
Activation of cell death programme may therefore be a one (but not the only) of the mechanisms of cellular toxic effects at low-dose exposure to paraoxon, when there is evident clinical outcome.

Keywords: Paraoxon, apoptosis, electron microscopy, flow-cytometry

1. WHY ARE THE EFFECTS OF LOW-DOSE PARAOXON TO BE EXAMINED?

Under physiological circumstances, the homeostasis of many cellular systems is characterized by an equilibrium between the rate of cell proliferation and cell death. Alteration of this balance may be involved in tissue pathology, being the critical end-point of injury inflicted by many toxins (21, 29, 31, 37). At the same time controlled cell death may represent a significant response to xenobiotics (13). It has been previously shown that toxic substances, natural or synthetic, could trigger cell death through apoptosis (4, 25, 26).

Among the toxic compounds with large scale use, the organophosphorous compounds are known to induce cholinesterase inhibition. The clinical effects generated by high doses have been extensively studied. A lot of antidote formulas are available, although still not active enough (1, 3, 4, 5, 6). However, cellular mechanisms of action at low doses of these compounds are less known. The use of organophosphorous compounds in industry (as soaking agents or additives for lubricants), in agriculture (as chemical manure, insecticides or acaricides), or for military purposes may explain why one may consider important to understand the consequences of low-dose exposure (1, 18, 27).

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Theoretically, depending on the administered dose, on the duration of the exposure to xenobiotic and on the susceptibility of each cellular type, the histopathological changes could be different (from an intense tissular hyperemia, up to typical morphological changes of apoptosis and/or cellular necrosis, nowadays termed "oncosis" (2, 23, 38, 43).

The degree of tissular damage provoked by an organophosphorous compound depends on dose and time of assessment. The main goal of the present study was to establish the relative importance (if any) of apoptosis (programmed cell death) among the other morphological changes provoked in rats, after exposure to low-dose of paraoxon (POX).

2. MATERIAL AND METHODS

Male Wistar rats (180 g ± 20 g) were used (6 animals per group) in all studies. The rats were housed in laboratory with standard rat diet and tap water ad libitum. The animals were administered intraperitoneally, daily one dose of paraoxon ($C_{10}H_{14}$ NO_6P) – 1 mg/kg body weight, for different periods of times (6 h, 24 h, 48 h, 72 h up to 10 days). At the end of experiment, the animals were euthanasied and biological materials were processed for examination by electron microscopy or flow-cytometry.

Investigations involving animals have been conducted in conformance with the National Regulations concerning the use of animals in research and education.

Electron microscopy. After 5 % glutaraldehyde tissue fixation, 1 % osmium tetraoxide post-fixation and embedding in Epon 812 (according to a standard protocol), tissue pieces, from hippocampus and cerebellar cortex, were examined on an electron microscope LEO 912 OMEGA (30).

Flow cytometry. Archival samples of formalin-fixed and paraffin-embedded nervous tissue, obtained from POX treated rats, were evaluated by flow cytometry. The Robinson-method for DNA analysis of paraffin-embedded tissues was applied (14, 15, 32). Sections of 50 µm thickness were cut, deparaffinized, rehydrated and dilacerated with a 0.5 % pepsin solution (*p*H 1.5). Thereafter, the single cell suspension ($1–2 \times 10^6$ cell/ml) was processed using DNA-Prep Reagents Kit (Coulter) according to the manufacturer's instructions. This DNA-Prep Kit consists of two components. DNA-Prep LPR (Lysing and Permeabilizing Reagent) is a reagent for permeabilizing cellular membranes and DNA-Prep Stain is a propidium iodide stain for DNA.

Finally, 500 µl PI solution (DNA-Prep Stain) was added to 100 µl sample permeabilized with 100 µl DNA-Prep LPR.

DNA content was then measured on a Coulter EPICS XL flow cytometer using a standard "DNA ANALYSIS" protocol (System II Software).

The protocol combined acquisition of signals on two-parameter data plots (displaying as "contour density plot") and one-parameter histograms (as frequency distribution of PI-red fluorescence-FL3).

3. RESULTS AND DISCUSSION

Electron microscopy. A wide range of ultrastructural changes (from mild to severe) were present in the examined samples. Neuronal lesions were similar in hippocampus and cerebellar cortex (in both Purkinje and granular cells).

At 6 h post-POX intoxication pathological changes were suggestive for apoptosis, despite the fact that some other cell death types were also present. The most frequent ultrastructural change (sometimes very pronounced) consisted in shrinkage of nuclear envelope (Fig. 1A). Nuclear shape underwent same distortion. Chromatin condensed under the nuclear membrane, appearing as densely different size granular hemilunar caps (Figs. 1B and 1C). In some neurons of the Purkinje layer the necrotic phase becomes visible; pyknotic nucleus is evident (Fig. 1D).

At 24 h after first POX intoxication the number of apoptotic neurons was reduced (Fig. 2B). There are present other kinds of ultrastructural changes: compaction of cytoplasm and nucleus (Fig. 2D), appearance of dense chromatinian bodies (Figs. 2A and 2C).

After 72 h from the first dose of POX administration one may frequently observe nuclei with dark and dense chromatin clumps (Figs. 3A, 3B, 3C). Certain Purkinje cells had nuclear dense peripheral chromatin condensation and osmiophilic granules (Fig. 3D). Normal or slightly dilated cisternae of rough endoplasmic reticulum are presented in Fig. 3. Some ultrastructural changes more characteristic for oncosis were also present: dilatation of organelles, ribosomes disintegration, damaged mitochondria (Fig. 3A), multivesicular bodies (Fig. 3C), vacuolar dilatations of Golgi apparatus and aggregation of cytoskeletal filaments (Fig. 3D).

After 8 days from the first dose of POX specific ultrastructural aspects (shrinkage of nucleus due to wrinkle of nuclear membrane) for apoptosis were less frequent. In Fig. 4D "shadows" of apoptotic bodies are presented.

Flow cytometry. Extensive DNA fragmentation during apoptosis can be determined by the measurement of DNA content using an intercalating DNA fluorochrome, such as propidium iodide – PI (excitation at 488 nm and fluorescence emission at > 600 nm) (7, 8, 11).

The permeabilized apoptotic cells show a low DNA stainability (11, 17, 19, 22, 28), resulting in a distinct region below the G_0/G_1 peak in a cellular cycle curve (Fig. 5).

The histogram A (Fig. 5) represents the negative control (untreated animals), allowed the determination of the appropriate positioning of diploid normal cell acquisition channel (channel no. 191).

Figs. 5A and B show DNA fluorescence histograms of PI-stained nervous cells: untreated control (A) and treated with dexamethasone (positive control) (B). Apoptotic cells are found in the "sub-G_0/G_1" peak. The degree of DNA fragmentation in apoptotic cells was different in early *versus* late stages of apoptosis. The number of apoptotic and dead cells increased in a time-dependent manner.

The sub-G_0/G_1 peak was evident at 6h after first administration of POX (Fig. 5C). The histograms D, E and F corresponding to samples taken at 24 h, 48 h and respectively 72 h after exposure to POX show the most significant fluorescence (broken DNA).

After 4 days since first POX dose, the intensity and frequency of sub-G_0/G_1 peaks were more reduced (unshown data).

Typical cytometric scattergrams of control and POX treated rats shown in Fig. 6b present concomitant multiple cell death forms.

Light scatter is altered during cell death reflecting morphological changes (swelling or shrinkage, rupture of the plasma membrane, chromatin condensation, nuclear fragmentation or shedding of apoptotic bodies) (9, 10, 24, 33). The bivariate contour plots show forward scatter *vs.* PI-red fluorescence. Three regions are defined by PI staining: PI-negative (living cells), PI-dim (apoptotic cells) and PI-bright (late apoptotic or dead cells).

Cytometric data about cellular DNA content are in accord with the electron microscopic examination of the biologic material sampled in similar circumstances.

4. INSTEAD OF CONCLUSIONS

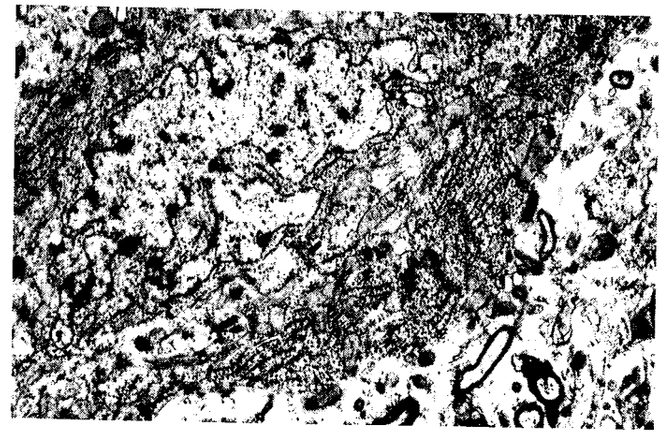
Identification and measurement of cell death after exposure to a xenobiotic remains a difficult task.

Ultrastructural and cytometrical data presented show that low dose exposure to POX may disturb the cellular homeostasis in the nervous tissue of rats without any clinical outcome.

Short time repeated exposure to the toxic compound may be associated with a mosaic-like ultrastructural pattern of cell damage.

In the first hours after exposure, it has been observed an important increase in cellular changes, similar to early stages of apoptosis. These damages may co-exist with ultrastructural changes, specific to other forms of cell death.

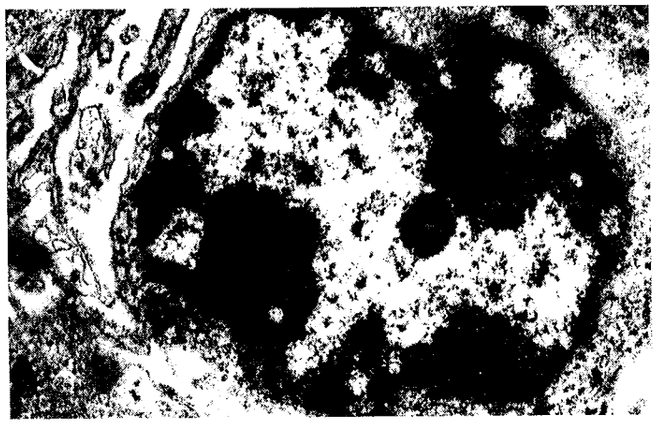
In later stages of the extended exposure to the toxic agent, when the apoptotic index is diminished, this co-existence of cellular damage, through different types of cell death, seems more obvious.



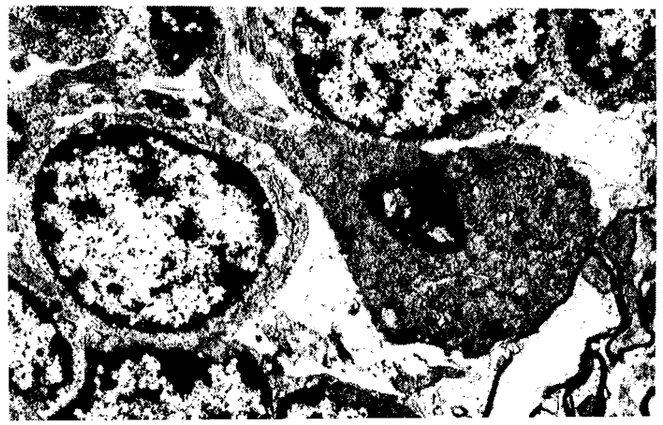
A

B

Fig. 1 – Electron micrographs of cells from the rat brain: hippocampus (A, B), cerebellar cortex (C, D), 6 h after exposure to low dose of POX. Early apoptosis with convolution of nuclear shape (A), condensation of chromatin to form peripheral marginated chromatin (B, C, D). Note decreased cell volume, increased cell density and pyknotic nucleus; by–stander cells (granular neurons) have normal ultrastructural features (D). Magnifications: A: × 6800, B: × 8500, D: × 6800.

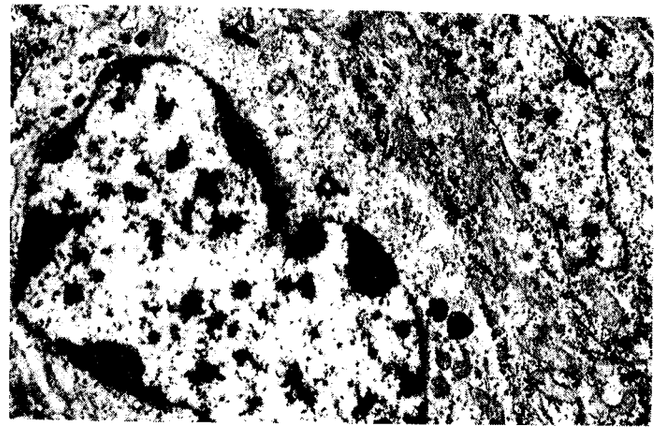


C

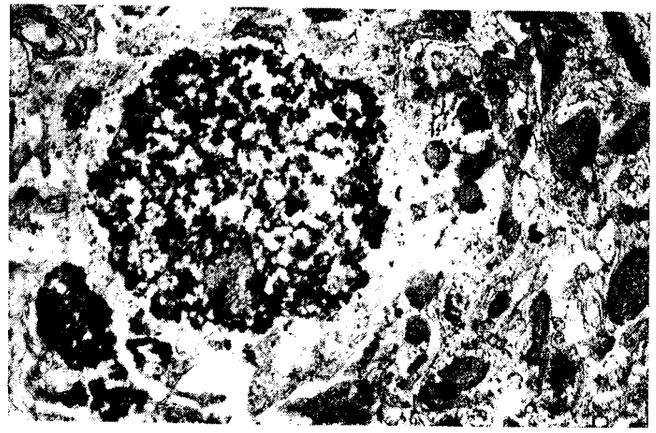


D

Fig. 1

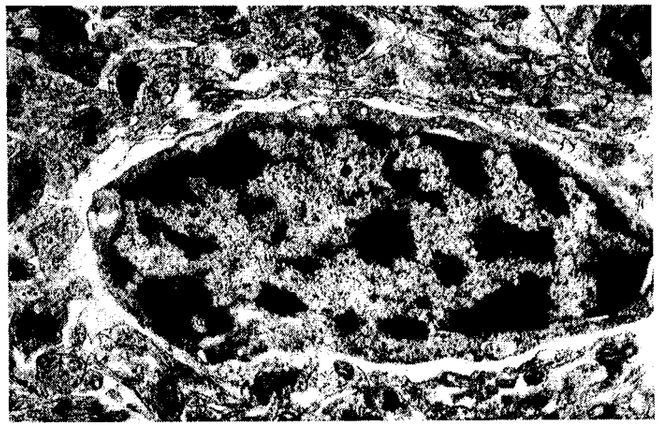


A



B

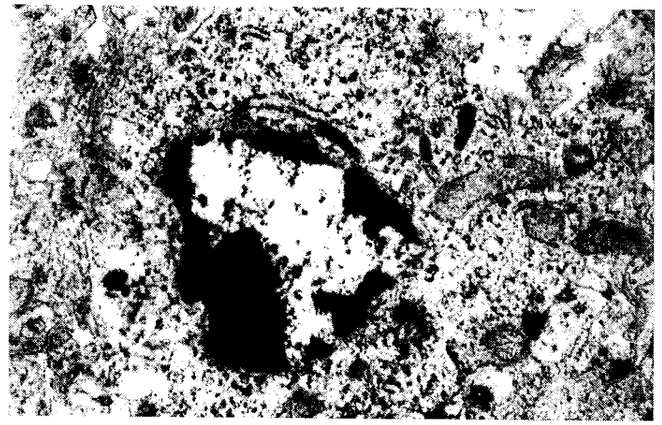
Fig. 2 – Electron micrographs of cells from the rat brain: hippocampus (A, B), cerebellar cortex (C, D), 24 h after first exposure at a low dose of POX.
The condensation of chromatin is associated with convolution of the nuclear envelope (A).
Apoptotic cell with typical morphological characteristics (B).
Chromatin is condensed under the nuclear membrane (C).
Purkinje cell with a pyknotic nucleus and cytoplasm that shows mitochondria, rough endoplasmic reticulum, some multivesiculate bodies and primary lysosomes (D).
Magnifications: A, B and C: × 10710, D: × 6800.



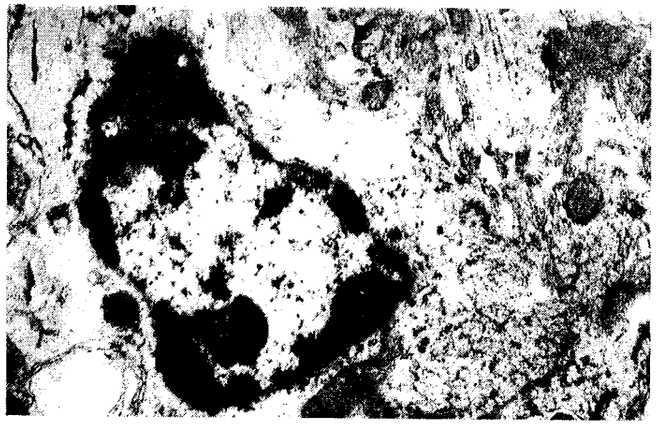
C

D

Fig. 2

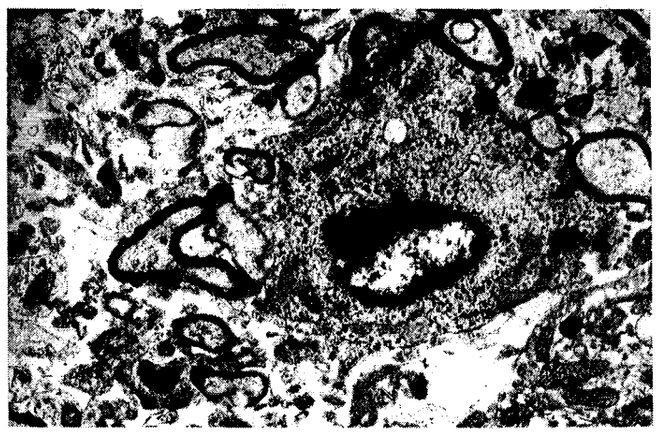


A

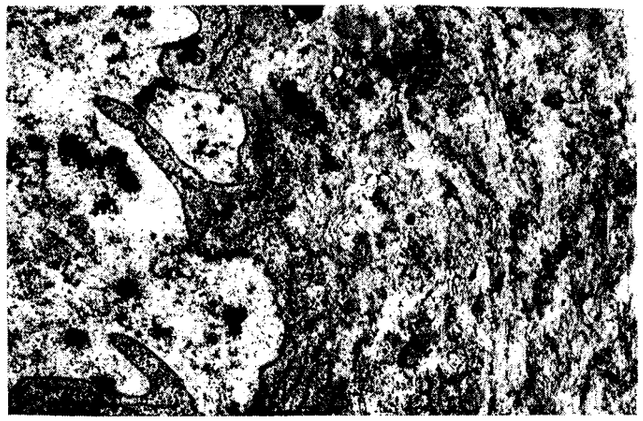


B

Fig. 3 – Electron micrographs of cells from the rat brain: hippocampus (A, B, C), cerebellar cortex (D), 72 h after first exposure at low dose of POX.
The ultrastructural changes detected in the hippocampus consist in:
large masses of condensed chromatin material and shrunken nuclear envelope (A, B).
Rarefied cytoplasm is visible into an astroglial cell (B) and pyknotic nucleus with condensed chromatin around the inner nuclear membrane is present into an oligodendroglial cell (C).
A Purkinje cell with very wrinkled nucleus, intact nuclear membrane, light condensed chromatin; cytoplasmic organelles are represented by: rough endoplasmic reticulum, dilated Golgi apparatus, clusters of ribosomal particles, aggregated cytoskeletal filaments.
Magnifications: A and B: × 17000, C and D: × 10710.

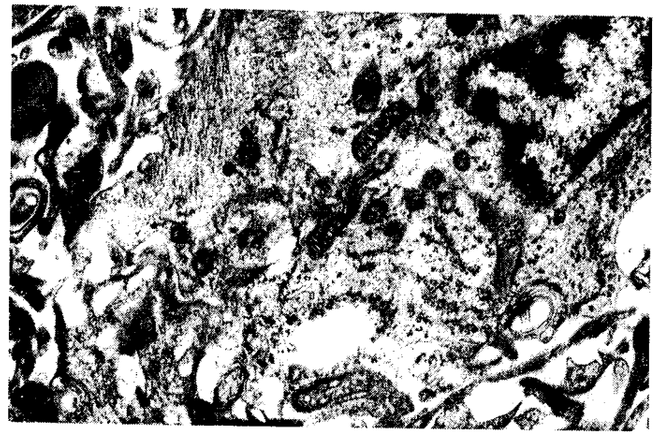


C

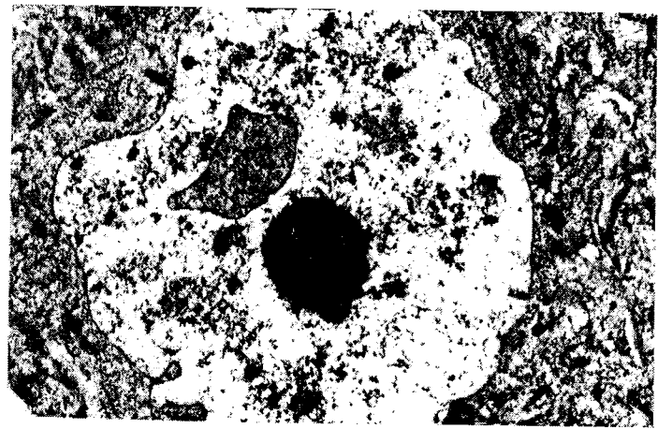


D

Fig. 3

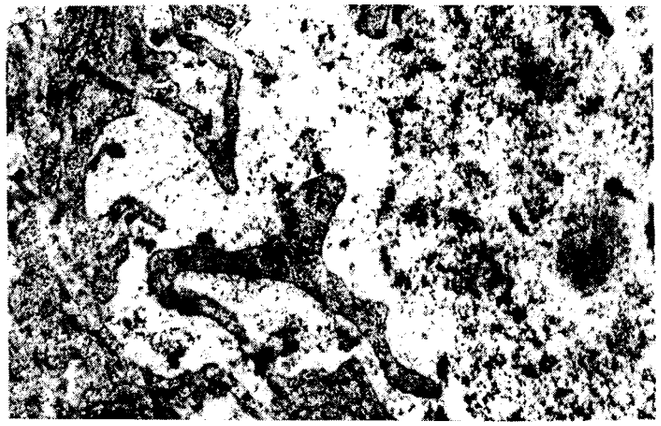


A



B

Fig. 4 – Electron micrographs of cells from the rat brain: hippocampus (A), cerebellar cortex (B, C, D) 8 days after first exposure at low dose of POX.
Changes at this time include: decreased cellular volume, intact mitochondria, aggregation of cytoskeletal filaments, myelin-like structures and condensed chromatin (A). Significant shrinkage of nucleus. Numerous ribosomal particles are rearranged into marked wrinkles (B, C). The nucleolus has a central localization (B). D: Cell "shadows" of apoptotic bodies.
Magnifications: A: × 13600, B and D: × 6800, C: × 8500.



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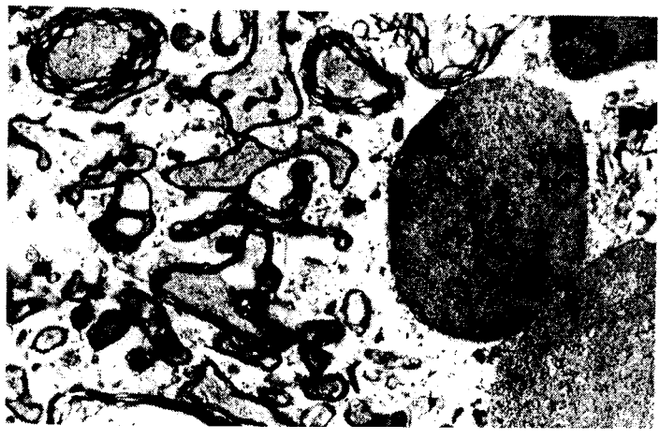

D

Fig. 4

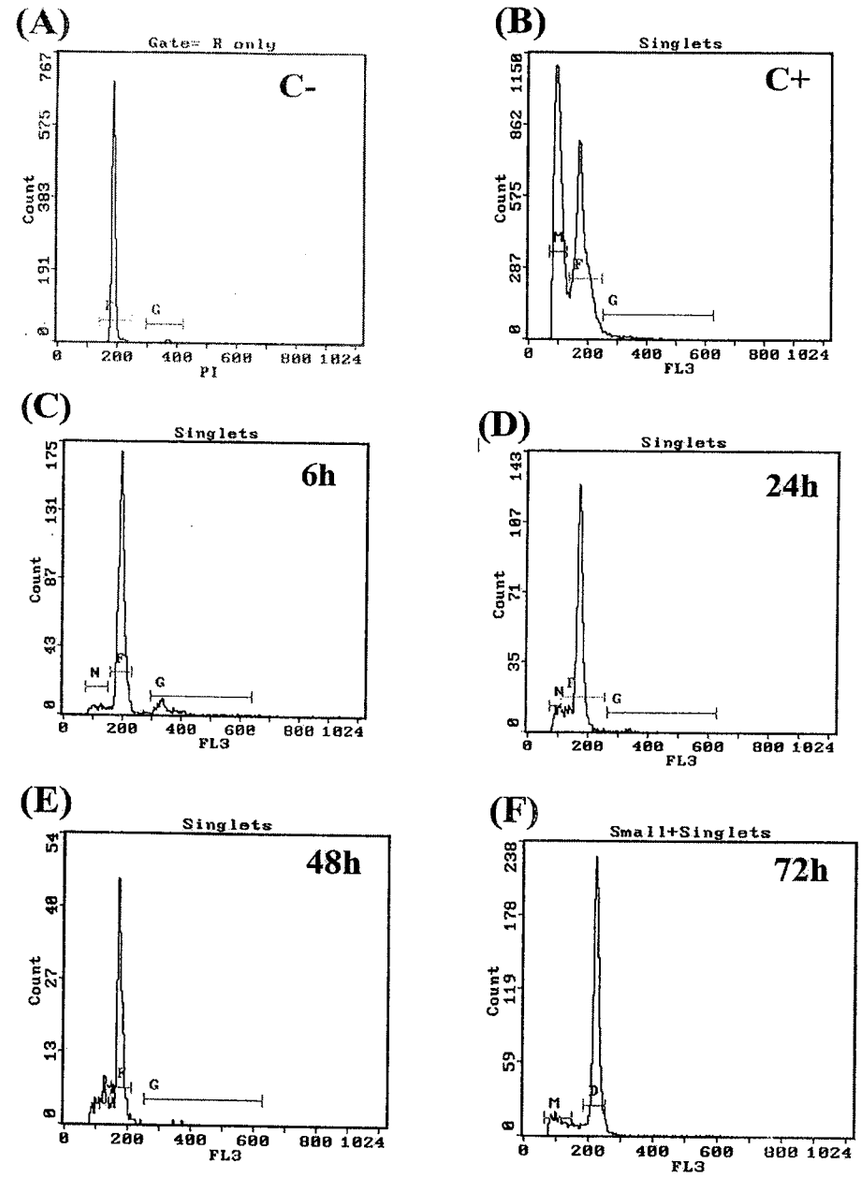

Fig. 5 – DNA content frequency histograms obtained from rat brain paraffin-embedded specimens: A: untreated control, B: treated with dexamethasone, C, D, E, F: histograms corresponding to cells from groups 6 h, 24 h, 48 h and 72 h, after first exposure. Cellular DNA was stained with propidium iodide (PI). Apoptotic cells are found in the "sub-G0/G1" peak.

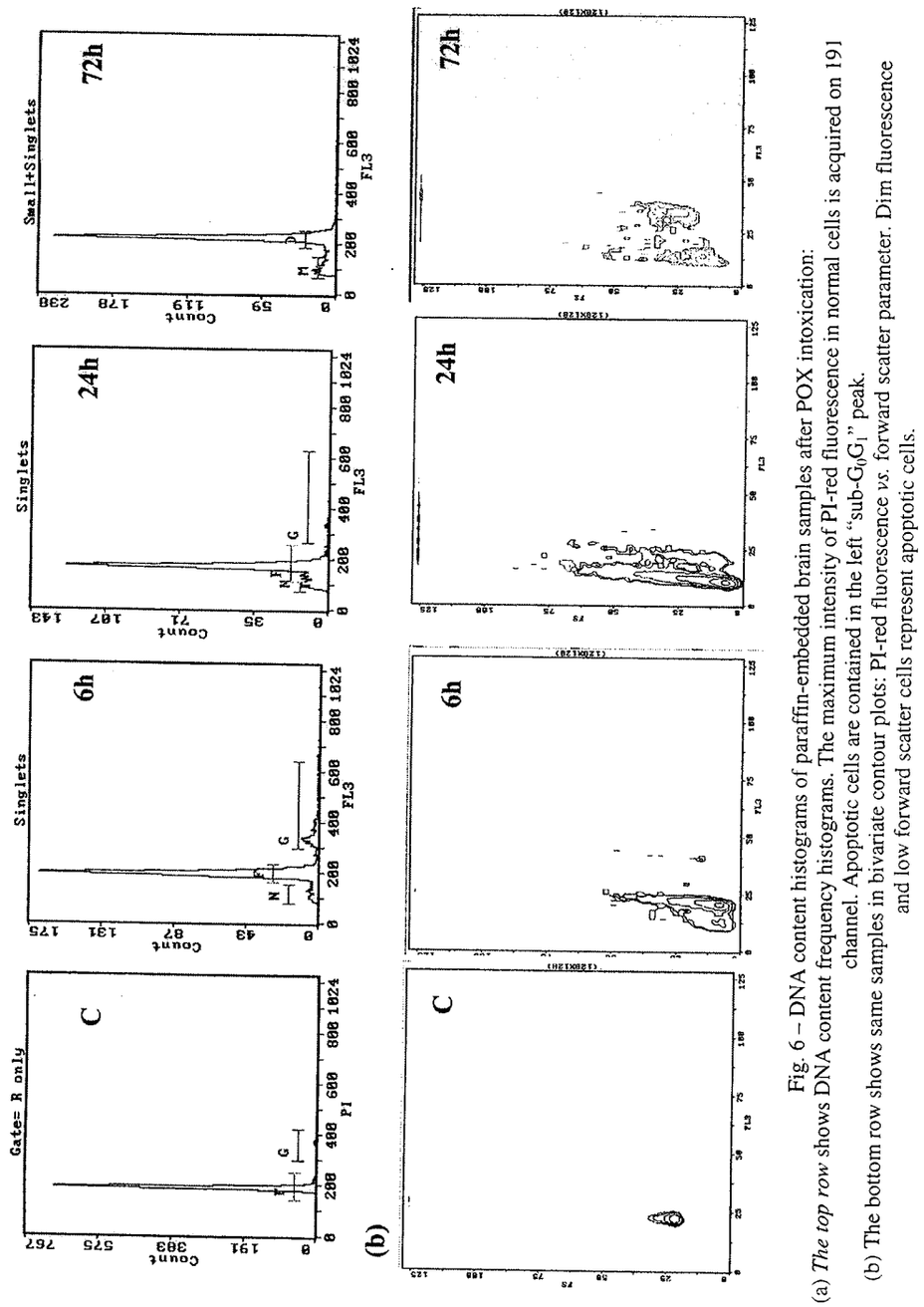

Fig. 6 – DNA content histograms of paraffin-embedded brain samples after POX intoxication:
(a) *The top row* shows DNA content frequency histograms. The maximum intensity of PI-red fluorescence in normal cells is acquired on 191 channel. Apoptotic cells are contained in the left "sub-G_0G_1" peak.
(b) The bottom row shows same samples in bivariate contour plots: PI-red fluorescence *vs.* forward scatter parameter. Dim fluorescence and low forward scatter cells represent apoptotic cells.

Electron-microscopy data presented support the recent idea that programmed cell death can take many forms: apoptosis, necrosis, "apoptosis-like programmed cell death" and "necrosis-like programmed cell death" (20), as well as the toxicologic pathologists's concern about the nomenclature of cell death (21, 42).

Flow cytometry allowed us the detection of the DNA strand breaks as apoptosis markers in both, freshly isolated, and paraffin-embedded, specimens of brain tissue.

The data from the present study do not exclude the fascinating hypothesis that excessive programmed cell death in organs of vital importance may lead to "phenoptosis" (programmed death of an organism) (35).

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**Army Center for Medical Research*
P.O. Box 12-128, Bucharest, Romania
***Department of Clinical Toxicology and Pharmacology,*
"Carol Davila" University of Medicine and Pharmacy
– Army Center for Medical Research
****Department of Cell Biology, "Carol Davila",*
University of Medicine and Pharmacy;
– Army Center for Medical Research

IMMUNOCYTOCHEMICAL LOCALIZATION OF α-TUBULIN IN OOCYTES OF *Rana ridibunda*

OTILIA ZĂRNESCU

The organization of cytoplasmic microtubules during oogenesis of edible frog, *Rana ridibunda,* was examined by immunoperoxidase technique using an anti-α-tubulin antibody. In the oogonia and early previtellogenic oocyte immunolabeling was associated with the entire cytoplasm. In the larger previtellogenic oocytes α-tubulin form a complex and disordered-appearing network surrounding the nucleus and extended between the germinal vesicle and the cortex. Microtubules are more concentrated in the cortical cytoplasm. In the early vitellogenic oocytes, microtubules begin to orientate towards the oocyte cortex and perinuclear. As vitellogenesis progresses, microtubules form a filamentous framework that extends radially. These radial bundles are less distinct in the future vegetal half. Moreover, numerous microtubules are concentrated in the perinuclear cytoplasm. At the end of vitellogenesis, the microtubules were detected as bundles that radiate from the germinal vesicle to the animal cortex. Microtubules also surround the oocyte nucleus.

INTRODUCTION

Studies made mainly using oocytes of African frog, *Xenopus laevis*, but including oocytes from other species of both anurans and urodeles, have shown that amphibian oocytes contain all major classes of cytoskeletal elements found in animal cells, including microtubules (8, 15, 22), actin microfilaments (6) and intermediate filaments (7, 14, 18, 19, 23).

Although the cytoskeleton of amphibian oocytes has been implied in different developmental functions, including specification of animal-vegetal axis and localization of maternal RNAs (4, 5, 17, 26, 27), our understanding of the oocyte cytoskeletal organization was limited at *Xenopus laevis* (1, 3, 10, 12, 25). Moreover, the studies carried out on somatic cells show that the microtubules are required to maintain the distribution of the late endocytic compartment in its juxtanuclear position and facilitate the delivery of the ligands to the degradative compartment (2). From this point of view the amphibian oocyte is the ideal model for the study of the endocytosis because the major source of the components required for embryo growth is the yolk, formed during the differentiation of the oocyte by the uptake of yolk proteins, mainly vitellogenin (24).

The goal of this study is to localize α-tubulin in developing oocytes of *Rana ridibunda*.

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MATERIALS AND METHODS

Fragments of *Rana ridibunda* ovaries were fixed in 3 % glutaraldehyde in 0.1 M phosphate buffered saline, *p*H–7.4 (PBS), with 1 % dimethylsulphoxide (DMSO), overnight, at 4 °C. After washing in PBS, samples were then transferred in 2 % glycine in PBS, to quench the free aldehyde groups, dehydrated and embedded in paraffin.

Slides were given several 10-min rinses in 0.1M PBS and sequentially incubated in methanol:H_2O_2 (9:1) to remove endogenous peroxidase (30 min), PBS plus 10 % normal rabbit serum to remove non-specific background staining (1 h), mouse monoclonal anti-α-tubulin, primary antibody (Sigma), diluted 1:1000 (overnight, at 4 °C), rabbit anti-mouse IgG peroxidase conjugate (Sigma), diluted 1:200 (1 h, at room temperature). Each incubation step was followed by four 5-min rinses in PBS. To visualize the primary antibody binding sites, the slides were incubated for 5–15 min in a solution of 3,3'-diaminobenzidine (0.05 %) and 0.015 % hydrogen peroxide, dissolved in 0.1 M PBS.

The controls for immunohistochemical reaction were performed by incubating sections with: (1) normal mouse serum, instead of specific antiserum and (2) antiserum pre-absorbed with the respective antigen (10–100 μg/ml). These procedures were carried out overnight at 4 °C. The results of these controls were negative.

RESULTS

Edible frog ovary contains oogonia and follicles at different stages of growth. In post-mitotic oocytes, clustered in "nests", immunolabelling is intense throughout the cytoplasm (Fig. 1)

Small previtellogenic oocytes (28 μm in diameter) are spherical, with a centrally located germinal vesicle (nucleus) and relatively little ooplasm. In these oocytes immunolabelling was observed as a dense array throughout the cytoplasm (Fig. 2).

In the larger previtellogenic oocytes (50–300 μm in diameter), α-tubulin forms a complex and disordered-appearing network surrounding the nucleus and extended between the germinal vesicle and the cortex (Fig. 3). Microtubules are more concentrated in the cortical cytoplasm.

Early vitellogenic oocytes (300–500 μm in diameter) are characterized by the onset of vitellogenesis. These oocytes are round with a large yolk free cytoplasm surrounding the centrally placed germinal vesicle. Microtubules begin to orientate towards the oocyte cortex and perinuclear (Fig. 4).

As vitellogenesis progresses microtubules form a filamentous framework that extends radially (Fig. 5). These radial bundles are less distinct in the future vegetal

Fig. 1 – A nest of post-mitotic oocytes showing the intense cytoplasmic immuno-staining. × 1960.

Fig. 2 – Early previtellogenic oocytes with a dense array of microtubules throughout the cytoplasm. × 1960.

Fig. 3 – In the larger previtellogenic oocytes α-tubulin appearing as a disordered network in entire cytoplasm and more concentrated in the cortex (arrow). C-cytoplasm; N-nucleus. × 1960.

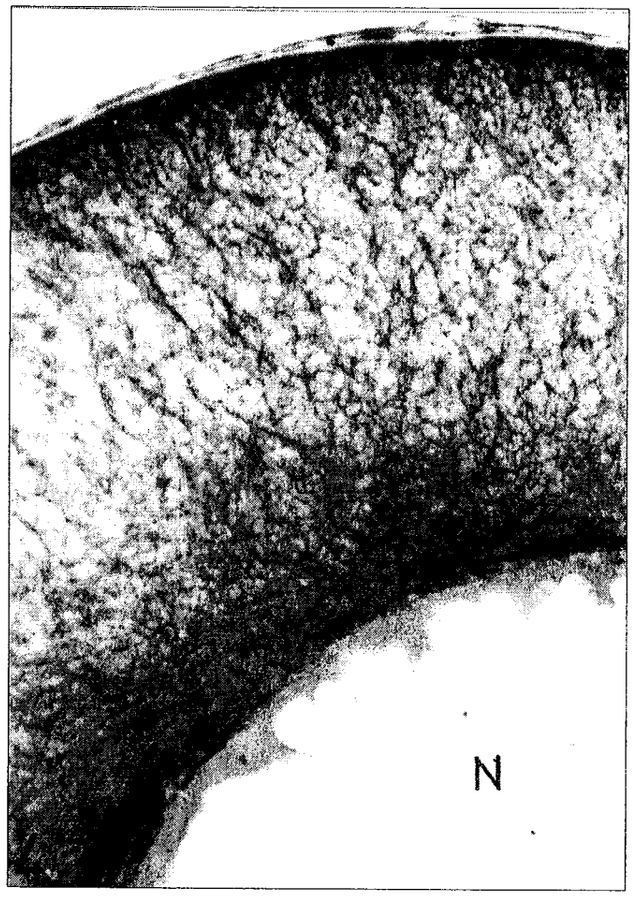

Fig. 4 – In the early vitellogenic oocytes microtubules begin to orientate towards the oocyte cortex and perinuclear. N-nucleus. × 1960.

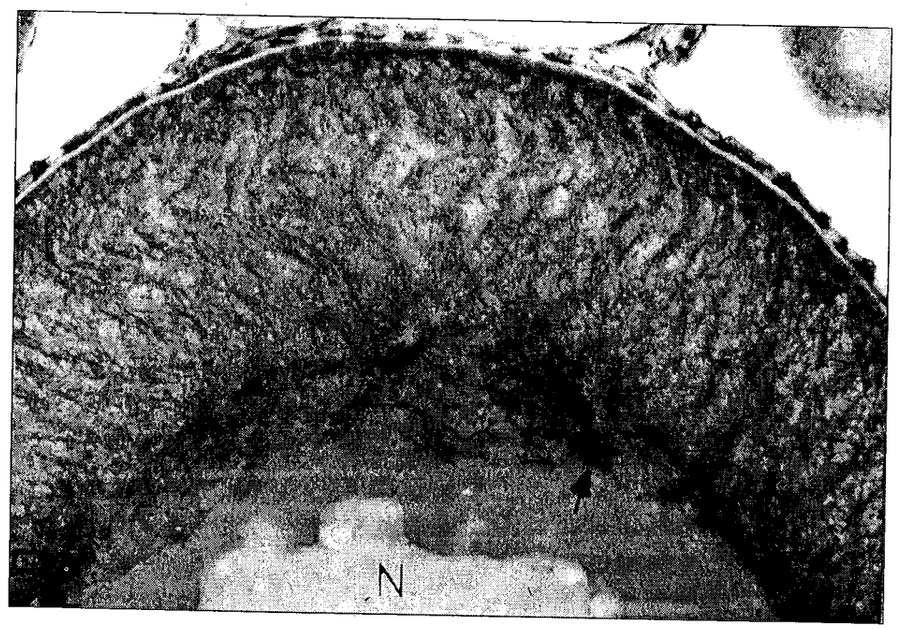

Fig. 5 – Most cytoplasmic α-tubulin in oocytes from mid vitellogenesis are orientated radially and perinuclear (arrow). N-nucleus. × 1225.

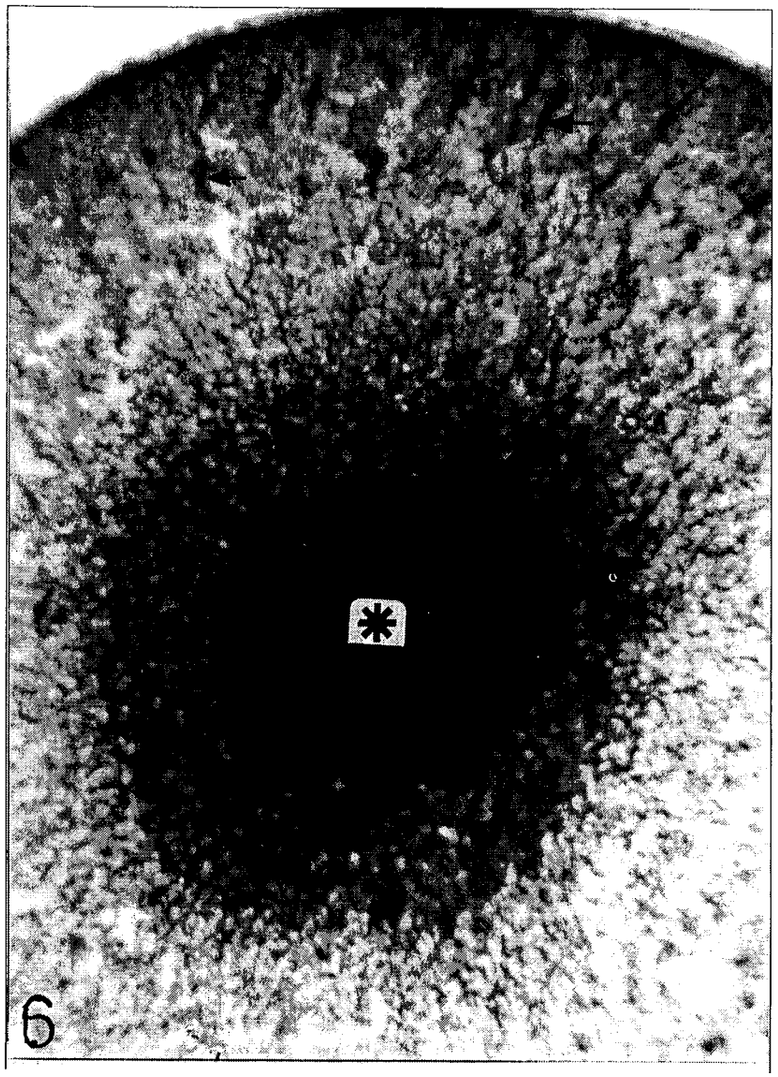

Fig. 6 – In fully grown oocytes α-tubulin was detected as bundles (arrows) that radiate from germinal vesicle to the cortex surrounding oocyte nucleus (*). × 1225.

half. Moreover, numerous microtubules are concentrated in the perinuclear cytoplasm.

At the end of vitellogenesis (1108 μm in diameter) the microtubules were detected as bundles (also called radii) that radiate from the germinal vesicle to the animal cortex (Fig. 6). Microtubules also surround the oocyte nucleus.

DISCUSSION

The pattern of microtubules in the oocyte of *Rana ridibunda* varied both temporally and spatially during the oogenesis. Temporal changes included an increase of density of microtubules throughout the oocyte cytoplasm. Spatial changes involved orientation of microtubules in growing oocytes.

Throughout the previtellogenic phase the dense array of microtubules observed in the oogonia and small oocytes was replaced in the middle and late previtellogenic oocytes by the complex and disordered-appearing network surrounding the nucleus and extended between the germinal vesicle and the cortex. Immunocytochemical studies on the distribution of microtubules in oocytes of *Xenopus laevis* indicated that this transition is accompanied by two significant changes in the organization of cytoplasmic microtubules: (a) dispersal of microtubules throughout the oocyte cytoplasm and (b) inactivation of the maternal microtubule-organizing center, or centrosome. The presence of a large number of acetylated (stable) microtubules in early previtellogenic oocytes suggests that the dispersal of microtubules accompanying this transition occur, at least in part, through a redistribution of existing microtubules rather by a process of disassembly and reassembly (10).

In the early previtellogenetic oocyte of *Rana ridibunda* did not form the mitochondrial cloud, characteristic for *Xenopus*. From this point of view results from this study are in agreement with those carried out with *Rana pipiens* and *Ambystoma sp.*, which lack mitochondrial masses and contain microtubules arrays comparable in all other respects to those of stage I *Xenopus* oocytes (11).

In the vitellogenic oocytes microtubules form a radial filamentous framework, extending from the perinuclear cytoplasm towards the oocyte cortex. These radial bundles are less distinct in the future vegetal half. Moreover, numerous microtubules are concentrated in the perinuclear cytoplasm. Recent studies indicated that in these stage's oocyte, microtubules are orientated with their minus-ends in the cortex and plus-ends extending inward the germinal vesicle (12). These observations suggest that the cortex plays a major role in establishing the radial organization of microtubules. Many of the microtubules from the animal hemisphere are highly acetylated. In contrast, microtubules in the vegetal hemisphere appear less ordered and are acetylated to a lesser degree (8). The reorganization of the cytoplasmic microtubules during stage IV is accompanied by

the redistribution of γ-tubulin in the oocyte cortex (9), suggesting that cortical γ-tubulin might play a role in polarization of the microtubule array during this stage.

At the end of vitellogenesis the microtubules were detected in cytoplasmic corridors that radiate from the germinal vesicle to the animal cortex. Microtubules also surround the oocyte nucleus. It has been proposed that the cytoplasmic corridors link the cortex with the inner cytoplasm and provide pathways for movement of organelles to and from the nucleus. Also, it is possible that organelles and other components travel along these cytoskeletal components from the nucleus to their destination in the cortex, or that endocytotic vesicles use them to travel from the cell surface to their cytoplamic destination (20). Moreover, a recent study demonstrates that in *Xenopus* oocytes early to late endosomal transport requires microtubules (21). Gard (9) postulates that the microtubules in stage VI oocytes are oriented with their minus-end in the cortex and plus-end towards the germinal vesicle. This orientation is opposite that found in most somatic cells. Moreover, western blots reveal that a single stage VI *Xenopus* oocyte contains a pool of α and β-tubulin sufficient to assemble more than 1.5 km of microtubules (13, 16). Moreover, as much as 20 % of the cytoplasmic pool of tubulins in these oocytes are present as sedimentable polymer (8, 16).

In conclusion, distribution of microtubules in the growing oocytes of *Rana ridibunda* shows a similar pattern with those observed by other authors at *Xenopus laevis*.

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Faculty of Biology, Spl. Independenţei 91–95, Bucharest
E-mail: otilia@dbio.ro

IN VITRO POLYMOLECULAR AGGREGATION AND ULTRASTRUCTURAL CHARACTERIZATION OF THE OSTEOARTHRITIC CARTILAGE PROTEOGLYCANS

ANIŞOARA CÎMPEAN[1], LUCIA MOLDOVAN[2], MARINELA FLAMÂNZEANU[2], CLAUDIA NICOLAE[2]

Our present study focuses on the osteoarthritic proteoglycan isolation from the osteoarthritic cartilage samples of the femoral condyle in different stages of damage and their *in vitro* polymolecular aggregation and ultrastructural characterization. Proteoglycan extraction with 4M guanidine hydrochloride and their ultracentrifugal purification under dissociative conditions were performed. Their electrophoretic analysis showed the appearance of two proteoglycan monomer subpopulations in the fibrillated cartilage. This could be the result of alteration of molecules interacting to form aggrecan macromolecule and of disturbance of biosynthetic and degradative chondrocyte activities at this level. The electron micrograph of proteoglycan aggregates obtained by the combination of ultracentrifugation fractions representing proteoglycan monomers, hyaluronic acid and link protein revealed a higher alteration of the *in vitro* polymolecular aggregation of proteoglycans originating from fibrillated osteoarthritic cartilage.

1. INTRODUCTION

Articular cartilage is a highly specialized connective tissue with a biomechanical function that is suited to bearing compressive load. It consists of a large, highly expanded extracellular matrix (ECM) that is maintained by a sparse population of cells, named chondrocytes.The properties of this tissue depend on the structure and organization of the macromolecules (collagens and proteoglycans) in the ECM. The collagen (COL), mainly type II, but also types IX and XI, forms a dense fibrillar network that is embedded in a high content of proteoglycans (PGs).

Osteoarthritis (OA) is a heterogeneous and multifactorial disease characterized by the progressive degeneration of the hyaline cartilage as a result of modulation of the chondrocyte phenotype and ECM degradation.

Numerous studies [1], [2] examined the changes in composition of OA cartilage PGs as an indicator of synthetic and degradative ability of chondrocytes. Besides aggrecan loss that mainly implies its cleavage by most of matrix metalloproteinase (MMP) members, changes at the level of glycosaminoglycan (GAG) constituents were found. Thus, as pathological process advances, a decrease in keratan sulfate (KS) content and chondroitin-6-sulfate/chondroitin-4-sulfate (C6S/C4S) ratio occurs [3].

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Proteoglycans have a high hydrodynamic dimension and they superpose and interpenetrate both between them and with collagen. Articular cartilage collagen has a fine spacing (100 nm) and this is further divided by PGs into pores of less than 5 nm. PG diffusion through these extremely fine pores is considerably slower down by their sizes, bigger PGs diffusing much slower than those of smaller sizes.Thus, the diffusion barrier represented by the dense collagen network can constitute a serious matter when one intends to purify PGs from cartilage, particularly of human origin.

Aggrecan, the prevalent PG in articular cartilage, consists of a large number of chondroitin sulfate (CS) and keratan sulfate (KS) glycosaminoglycans covalently bounded to a core protein [4]. This PG monomer has the ability to form aggregates in the ECM by their binding to hyaluronic acid (HA), an interaction stabilized by a glycoprotein (GP) named link protein (LP) [5].

The objectives of the present study were:

– isolation and electrophoretic characterization of PGs from OA cartilaginous tissue of macroscopically normal and fibrillated aspects;

– comparative study concerning *in vitro* reconstruction of proteoglycan aggregates.

2. MATERIALS AND METHODS

Proteoglycan extraction under dissociative conditions. PG extraction from OA cartilage was performed with 4M guanidine hydrochloride (GuHCl), 50 mM Tris-HCl, *p*H 7.4 in the presence of protease inhibitors (0.01 M EDTA – Na_2, 0.1 M 6-amino hexanoic acid, 5μg/ml pepstatin A) at 4 °C for 24 h. Wet tissue: solvent extraction ratio was of 1:10. Supernatants containing PGs were separated from the undigested tissue by centrifugation at 5 000 rpm for 30 minutes.

Density gradient ultracentrifugation.The GuHCl extracts were dialyzed against 7 × vol. 50 mM Tris-HCl *p*H 7.4, and subjected to cesium chloride (CsCl) density gradient ultracentrifugation under dissociative conditions. Solid CsCl was added to the resulted extracts, up to a final density of 1.5 g/ml. Centrifugation was performed at 4000 rpm and 4 °C for 24 h, in a Beckman TL100 centrifuge. Under these conditions, the molecules were distributed according to their molecular weights (MW). From each tube, 6 fractions, each of 1 ml, were collected with a Pasteur pipette, and they were numbered from 1 to 6, beginning with the bottom one (Fig. 1). Each fraction was dialysed against distilled water and was analyzed regarding its uronic acid and protein concentrations.

Determination of uronic acid content. Uronic acid content was determined by Bitter and Muir's method with orcinol [6], based on uronic acid oxidation in a furfurol derivative that together with orcinol give a green colour chromatophore of

maximal absorption at 665 nm. Oxidation occurs at 100 °C in the presence of HCl and $FeCl_3$.

The samples (20 μg) were mixed with 3 ml orcinol reagent (prepared from 31 ml HCl conc., 30 mg $FeCl_3$ and 80 mg orcinol) and were incubated for 40 min, at 100 °C. After the samples were cooled, the absorbance was determined at 665 nm. Uronic acid values were determined by using a standard curve constructed with glucuronic acid (0–0.15 μmol/ml).

Polyacrylamide gel electrophoresis of proteoglycans in the presence of sodium dodecylsulfate. Electrophoretic characterization of PGs isolated from the two zones of the studied cartilaginous tissue was carried out according to Vilim and Krajickova's method [7] using 1.32–10 % gradient gels. All the samples were dialysed prior utilization. Samples (4 μl) containing PGs (2 μg) were mixed with an equal volume of the sample buffer (0.06M Tris-HCl, *p*H 6.8, 2 % SDS, 10 % glycerol and 5 % mercaptoethanol), incubated at 100 °C for 5 min and applied to gel. Sample migration was carried out for 4 h, at 24 mA, together with a high MW marker that was stained with Coomassie Brilliant Blue R-250. After electrophoresis, the gels were three times washed with 40 % methanol, 8 % acetic acid, in order to remove SDS traces. The gels were stained with 0.001 % Alcian Blue solution in 40 % methanol, 8 % acetic acid.

Proteoglycan aggregates assembly for electron microscopic characterization. PG fractions purified by ultracentrifugation under dissociative conditions: F I, F IV and F VI, respectively, were combined at a ratio of 50:1:0.4 resulting a final PG concentration of 0.6 mg/ml. Reaggregation was carried out by this mixture dialysis against PBS for 18 h at 4 °C. The resulted mixture was combined with 1M ammonium acetate solution containing 0.1 % cytochrome c, adjusted to *p*H 5 with acetic acid, so that the final concentration of PG solution was 5 μg/ml. In order to reveal proteoglycan aggregates in electron microscopy, nickel grids were used. They were floated for 15 min at room temperature, on drops from the previously prepared solution and afterwards on drops of 0.3 M ammonium acetate, *p*H 5. The grids were then washed with distilled water (at least 2 washes, 1 min each), air-dried and counterstained with 4 % uranyl acetate solution. Aggregates were revealed by the means of a Philips EM 208S electron microscope.

3. RESULTS AND DISCUSSION

Proteoglycan fraction by density gradient ultracentrifugation under dissociative conditions. After the cartilage pieces were extracted in 4M GuHCl, supernatant densities containing PG monomers, HA, LP and other components were adjusted to 1.5 g/ml with solid CsCl. They were separated by ultracentrifugation under dissociative conditions at 40 000 rpm for 24 h, in an

ultracentrifuge Beckman TL 100. Under these conditions, molecules were distributed depending on their molecular weights in 6 fractions (Fig. 1).

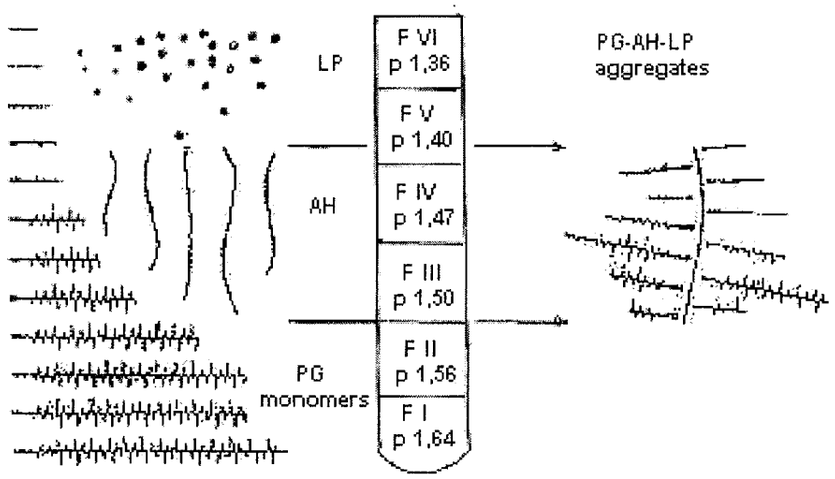


Fig. 1 – Distribution of macromolecules from OA cartilage extract obtained under dissociative conditions (with 4M GuHCl) along CsCl gradient, during ultracentrifugation.

After centrifugation, the density of the collected fractions was determined and it varied between 1.64 at the tube bottom and 1.36 at the higher part of gradient. The fractions were then analyzed as concerns their PG content (uronic acid dosing by orcinol method) and protein concentration by Sheffield's method [8]. The results are shown in Table 1.

Table 1

Uronic acid and protein concentrations of the fractions resulted following proteoglycan ultracentrifugation under dissociative conditions

Fraction No.	Uronic acids (mg/ml)		Protein (mg/ml)	
	Intact cartilage	Fibrillated cartilage	Intact cartilage	Fibrillated cartilage
Fr.I	1.924	2.050	0.95	1.15
Fr.II	1.580	1.720	1.30	1.58
Fr.III	0.780	0.838	1.50	2.00
Fr.IV	0.630	0.675	1.90	2.04
Fr.V	0.252	0.580	2.50	2.57
Fr.VI	0.170	0.230	3.00	3.10

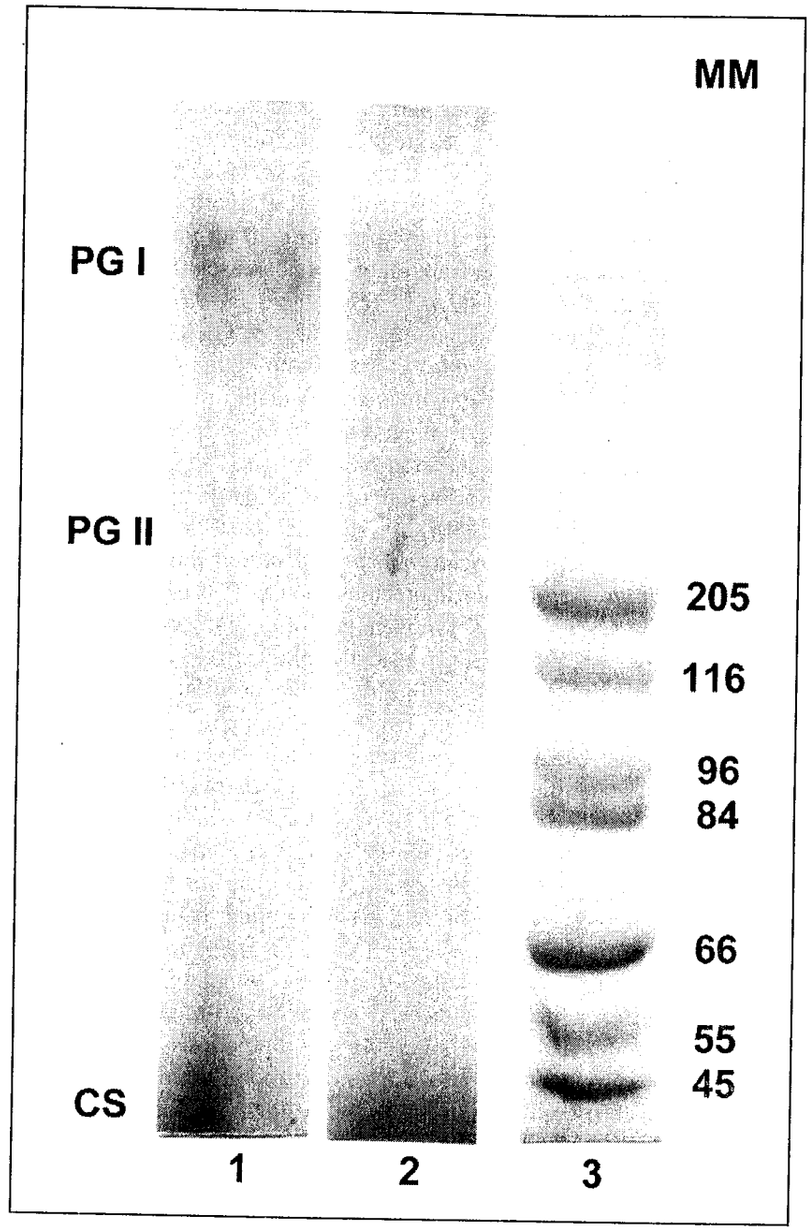


Fig. 2 – SDS-PAGE analysis in (1.32–10%) PAA concentration gradient, of PGs from macroscopically normal (1) and fibrillated (2) OA cartilage. Electrophoresis was performed comparatively with a Sigma marker of high MW.

In both types of cartilaginous tissue, the highest quantity of PG monomers occurs in the fractions I and II, which have a high aminoacid content.

The other fractions in the higher part of CsCl gradient contain HA and LP in various proportions, required by aggregates' reconstruction. Fraction IV is constituted mostly of LP with the highest protein concentration.

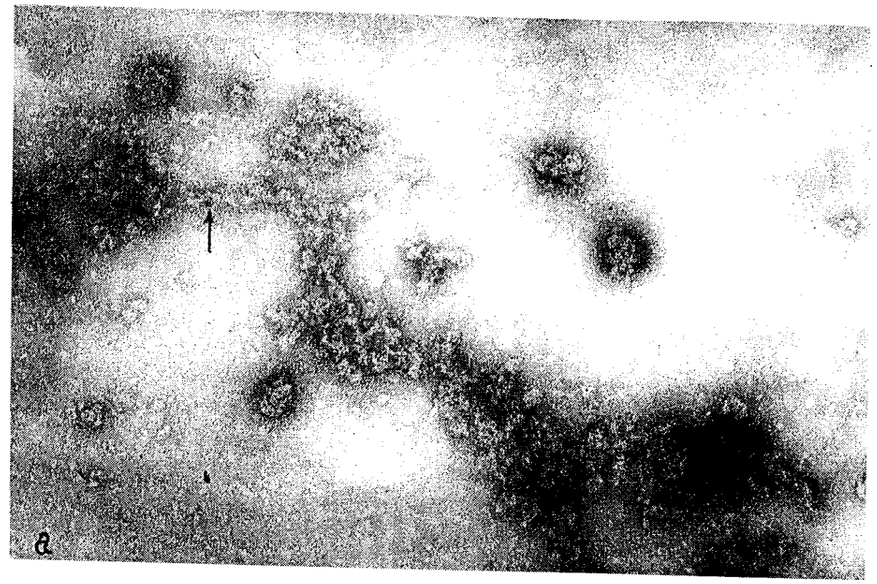
By a previous study, Bayliss [9] followed the effect of the two CsCl concentrations (p = 1.5 g/ml and p = 1.69 g/ml) on extract yield recovery ratio of PGs from articular cartilage and he found that this decreases from 90 % in the case of 1.50 g/ml density, to 50 % for 1.69 g/ml density.

Therefore, in our studies we selected 1.5 as optimal density value of CsCl, at which a good PG monomers' separation from protein components was performed. It seems that 1.69 g/ml CsCl density values are efficient only for the separation of macromolecules originating from young tissues. In our experiment, the extracted matrix components originate from an adult cartilaginous tissue and it is known the fact that during the cartilage maturation and aging, a significant increase in protein contents of aggregated PGs occurs [10].

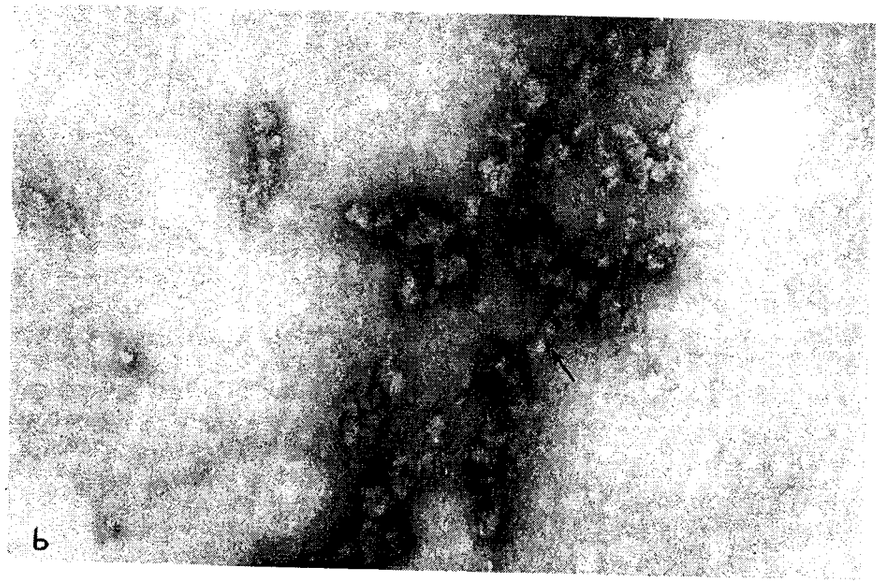
Electrophoretic characterization of proteoglycans from human osteoarthritic cartilage. Large macromolecules such as PGs cannot be separated in polyacrylamide (PAA) gels of homogeneous concentration. The experience achieved in many years in this respect demonstrates that this type of macromolecules are separated either in PAA concentration gradient gels, or in composite gel (agarose-acrylamide). In this context, we performed the electrophoresis in 1.32–10% PAA concentration gradient. Samples containing PGs, extracted from the two studied cartilage zones, were co-migrated with a high MW standard (Sigma). Also, in every sample, CS (0.5 μg) was embedded as a GAG standard. The electrophoretic profile obtained by this system is shown in Fig. 2.

Analysing the electrophoregrams in Fig. 2 one observes that PGs from human intact cartilage migrate as a single wide electrophoretical band (PGI) that remains in the higher part of the gradient, due to their high molecular weights (400–630 kDa). The band that migrated more rapidly in all the samples was the CS standard, added to sample buffer, which being of MW < 30 kDa, co-migrated with migration front. In the case of fibrillated cartilage, two subpopulations of PGs appear: PGI and PGII. The latter one has an electrophoretic mobility higher than PGI that occurs also in a macroscopically intact cartilage, and migrates to 210–260 kDa MW range. This molecule heterogeneity in tissue extracts from human OA cartilage, mainly suggested by its diffuse electrophoretic migration, as a spot, can be considered as a result of molecule proteolytic processing and, obviously, of its *in vivo* degradation, particularly in the case of fibrillated zone.

Electron microscopical evaluation of proteoglycan aggregates formed in vitro. In order to reveal *in vitro* proteoglycan aggregates' formation, it was used a mixture composed of three fractions separated by ultracentrifugation under



a)



b)

Fig. 3 – Electron micrographs of aggregated PGs, originating from macroscopically intact OA cartilaginous tissue. Arrow: HA molecule. a) × 30 000; b) × 50 000.

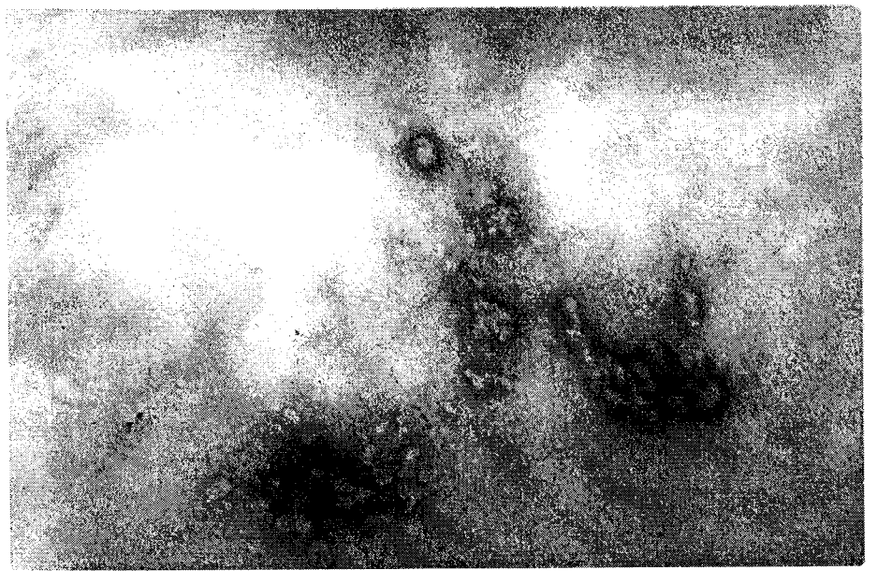


Fig. 4 – Electron micrographs of partially aggregated PGs originating from fibrillated OA cartilaginous tissue (× 50 000).

dissociative conditions: F I (containing PGs monomers), F IV (containing HA) and F VI (consisting mainly of LP). After solution dialysis against PBS, the resulted precipitates were processed for an electron microscopic study. PG aggregates were revealed with cytochrome c.

Electron micrographs revealed the occurrence of high PG aggregates, mainly in the case of the samples originating from macroscopically intact cartilage (Fig. 3a, 3b), being conspicuous HA chain to which there are laterally attached monomer proteoglycan subunits, creating a glass "brush" appearance. Electron micrographs obtained in the case of solutions derived from fibrillated cartilage revealed the existence of smaller aggregates or unaggregated, individual monomers (Fig. 4). Tight package of these aggregates, in both studied cases, suggests the occurrence of some changes compared to *in vivo* formation of proteoglycan aggregate.

Kahn *et al.* [11], who investigated *in vitro* reaggregation of proteoglycan components originating from nasal bovine cartilage, noticed that even in the case of normal cartilage, there is no reaggregation similar to that existing *in vivo*. That can be due to some PG-PG interactions that occur between PG-LP complexes determining a higher package of PG monomers along HA molecule.

Nevertheless, the differences noticed as concerns PG aggregates' sizes and packages in the two studied zones of OA cartilage suggest a greater alteration of PGs in OA injured zone.

4. CONCLUSIONS

Electrophoretic analysis of PGs extracted from the fibrillated zone of OA cartilage and purified by ultracentrifugation in density gradient demonstrated the appearance of two monomer subpopulations: PGI and PGII. PGII monomer is characterized by an electrophoretic mobility higher than that of PGI existing also in a macroscopically normal cartilage. Both PGs migrated, as a wide, diffuse band into the range of 400–630kDa MW (PGI) and 210–260 kDa MW (PGII), showing the heterogeneity of this molecule in tissue extracts from human OA cartilage, which can be the result of its *in vivo* degradation, particularly in fibrillated zone.

Electron micrographs of proteoglycan aggregates obtained by the combination of fractions F I (PG monomer), F IV (HA) and F VI (LP) resulted from density gradient ultracentrifugation under dissociative conditions, at a ratio of 50:1:0.4 respectively, showed the occurrence of some smaller aggregates or of some unaggregated, isolated monomers in the case of PGs originating from fibrillated OA cartilage. The noticed differences concerning the sizes and packages of proteoglycan aggregates, between the two studied zones of OA cartilage, suggest a higher alteration of PGs in the region with OA lesions and in the process of PG monomer aggregation. These could be the result of *in vivo* alteration of the

constituting GAGs, or/and of HA and LP molecules interacting in order to form the aggrecan molecule, as a result of disturbance of biosynthetic and degradative chondrocyte activities at this level.

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[1] *Faculty of Biology, 91–95 Spl. Independenței, Bucharest*
[2] *National Institute for Biological Sciences, Bucharest*

SEMINOLIPID, A NEW INSTRUMENT FOR STUDIES ON PHOSPHOLIPASE A_2

ADRIAN IGA, SILVIA IGA, NATALIA ROŞOIU, SEPPO RÖNKKÖ

Phospholipase A_2 was purified from bovine seminal vesicle fluid and its substrate was 1-palmitoyl-2-[1-^{14}C]-linoleoyl-sn-3- phosphatidylethanolamine. Seminolipid (1-O-alkyl-2-O-acyl-*sn*-3-(ß-D-galactopyranozil 3'-sulfate)glycerol) was purified from boar testis. The interaction between seminolipid and phospholipase A_2 was determined due to the structural similarity of lecithin to glycoglycerolipids. Low concentrations of seminolipid enhanced the activity of the enzyme while high concentrations were inhibitory. A modulatory role for glycolipids on phospholipase A_2 might be inferred.

INTRODUCTION

Phospholipase A_2 (PLA_2) (EC 3.1.1.4) hydrolyses carboxyl ester link of fatty acid in C-2 of glycerol within lecithin, leading to lysolecithin. The respective enzyme also acts on phosphatidylethanolamine, choline plasmalogen and other phosphatides. The first studies on PLA_2 enzymes have centered on snake venom PLA_2s, the reason being the fact that PLA_2 enzymes are extremely abundant in snake venoms and are easily purified. PLA_2 enzymes have also been identified and purified from a diversity of other sources including bovine, swine and human pancreas (Verheij *et al.*, 1983), human synovial fluid aspirates from rheumatoid and osteoarthritis patients (Parks *et al.*, 1990; Seilhammer *et al.*, 1989). Classification of PLA_2 enzymes took into consideration factors like structural and sequence characteristics. Pancreatic PLA_2 enzymes belong to group I, whereas the human secretory non-pancreatic PLA_2 is a group II enzyme. PLA_2 enzymes from snake venom belong alternatively to group I or II (Dennis, 1997). PLA_2 enzymes have been involved in a range of diseases including rheumatoid and osteoarthritis, asthma, acute pancreatitis, psoriasis, multiple organ failure, septic shock, and adult respiratory distress syndrome (Vadas *et al.*, 1993; Michaels *et al.*, 1994). The physiological and potential disease role of human secretory PLA_2-II have been the topic of several review articles (Mukherjee *et al.*, 1994; Pruzanski and Vadas, 1991; Chang *et al.*, 1987). The reaction products of PLA_2 activity are a free fatty acid and a lysophospholipid. The fatty acid is often arachidonic acid, the precursor for inflammatory mediators such as thromboxanes, leukotrienes, and prostaglandins (Flower and Blackwell, 1976; Sommers *et al.*, 1992). Lysophospholipids are the precursor of platelet activating factor (Mukherjee *et al.*, 1992).

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The suggestion that PLA_2 enzymes play a role in diseases process has intensified research on PLA_2 inhibitors. They were purified from numerous sources including plant, fungi, and bacteria (Cuellar *et al.*, 1996; Hyodo *et al.*, 1995; Lindahl and Tagesson, 1997).

In the present paper, the interaction between seminolipid and phospholipase A_2 was studied taking into consideration that seminolipid (1-O-alkyl-2-O-acyl-*sn*-3-(ß-D-galactopyranozil 3'-sulfate)glycerol) is a structural analog of lecithin and phosphatidylethanolamine. It is quite evident that the upper part of the seminolipid, i. e., 1-O-alkyl-2-O-acyl-*sn*-3-glycerol, is similar to the corresponding moiety of glycerophospholipids.

MATERIALS AND METHODS

Seminolipid has been purified from boar testis (Iga and Şerban, 1988; Iga and Iga, 1996) according to the following sequence: chloroform-methanol-water extraction, Folch partition, adsorption chromatography on Florisil, ionic exchange chromatography on DEAE-Sephadex A-25, partition chromatography on silicic acid. Thin layer chromatography was used to monitor the seminolipid purification and its chemical constituents identification and determination to assert the respective structure.

Phospholipase A_2 was purified from bovine seminal vesicle fluid and its substrate was 1-palmitoyl-2-[1-^{14}C]-linoleoyl-sn-3-phosphatidylethanolamine. Enzymic activity has been determined by measuring radioactivity of released [1-^{14}C]-linoleic acid after its separation from initial phospholipid.

RESULTS AND DISCUSSION

Seminolipid purity attained at least 95–97 % as proved by thin layer chromatography in three solvent systems as well as by densitometry (Iga and Iga, 1996).

The main results concerning seminolipid influence on phospholipase A_2 are presented in Fig. 1. It is quite evident that low concentrations of seminolipid (0.1–10 µM and even 50 µM) played an activator role for phospholipase A_2 from bovine seminal vesicle fluid, whereas high concentrations (100 µM and higher) were inhibitory. Interestingly, enzymatic activity at a concentration of 5 µm in seminolipid presented a minimum (Fig. 1). A modulatory role of seminolipid, and probably of other glycoglycerolipids, on phospholipase A_2 might be envisaged. In the last decade, a remarkable structural diversity of glycoglycerolipids has been

discovered (Kojima *et al.*, 1990, 1991): Galß1-3'- , Galα1-6Galß1-3'-, Galß1-6Galß1-3'-, [Galß1-6]$_2$-Galß1-3'-, Galß1-6Galα1-6Galß1-3'-, [Galß1-6]$_3$-Galß1-3'- and [Galß1-6]$_2$-Galα1-6Galß1-3'-diacylglycerol.

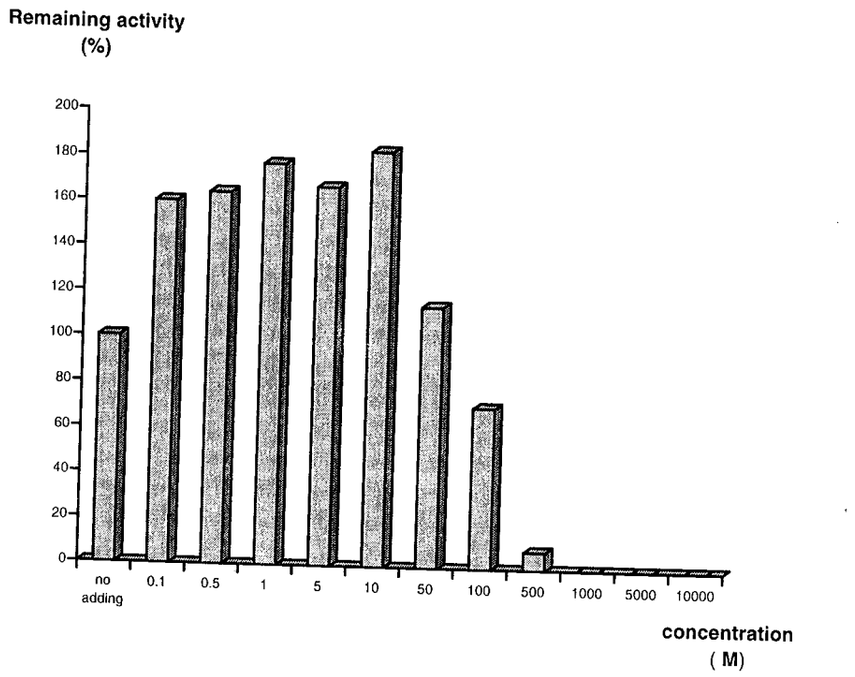

Fig. 1 – Influence of seminolipid on phospholipase A_2 purified from bovine seminal vesicle fluid.

A PLA_2 inhibitor isolated from *Notechis ater* serum (NAI) proved to have inhibitory action on the following enzymes: notexin from *Notechis scutatus* (common tiger snake), taipoxin from *Oxyurans scutellatus* (Australian taipan), bee venom PLA_2, bovine pancreatic PLA_2, human secretory PLA_2. NAI IC_{50} values determined by using fluorometric assay with 10pPC (1-hexadecanoyl-2-(1--prendecanoyl)-sn-glycero-3-phosphoglycerol) as substrate were as follows: 0.13 nM (notexin), 0.89 nM (taipoxin), 2.4 nM (*C. atrox* PLA_2), and 0.16 nM (bee venom PLA_2). The IC50 for bovine pancreatic PLA_2 calculated using fluorometric assay with 10pPG as substrate was 32.3 nM. The IC50 values found for bovine pancreatic PLA_2 and human secretory PLA_2, by using the mixed micelle assay, were 12,050 nM and 287.3 nM, respectively. Such results constituted valuable data for evaluating the affinity of NAI for PLA_2 enzymes (Dennis, 1997).

Carbohydrate moieties are a common feature of snake PLIs and as a consequence NAI was found to cross-react with a range of PLA2 enzymes. It was found that inhibitory activity of deglycosylated NAI against group I, II and III venom PLA2 enzymes was not affected *in vitro* by glycosylation status and that deglycosylated NAI was still as active NAI on all PLA2 enzymes tested. A similar result was reported for the PLI isolated from *Laticauda semifasciata* serum (Ohkura *et al.*, 1999). At the same time, it became clear that NAI was able to inhibit all PLA_2 enzymes against which it was tested, using a variety of substrates and assay conditions.

A comparison was made between cobra venom PLA_2, on the one hand, and PLA_2 from procine pancreas, *Crotalus adamanteus* (rattlesnake) venom, and bee venom, on the other hand, concerning the kinetics of phospholipid hydrolysis. The enzyme from *Naja naja naja* (cobra) venom was the only one, which was found to be activated significantly by phosphorylcholine-containing compounds when hydrolyzing phosphatidylethanolamine. The cobra venom PLA_2 was also the only one in which these activators induced protein aggregation (Plückthun and Dennis, 1985).

Prostaglandin biosynthetic response of murine $P388D_1$ macrophages was delayed when the cells were exposed to bacterial lipopolysaccharide for prolonged periods of time that was dependent on induction of the genes coding for Group V secretory PLA_2 and cyclooxygenase-2. It was noticed that lipopolysaccharide-induced arachidonic acid metabolite release in $P388D_1$ macrophages was strongly attenuated by the $P2X_7$ purinergic receptor antagonists periodate-oxidized ATP, and this was accompanied by suppression of the expression of both enzymes. Moreover, extracellular nucleotides were found to stimulate macrophage arachidonic acid mobilization with a pharmacological profile that involved the participation of the $P2X_7$ receptor and that it was inhibited by periodate-oxidized ATP. These results demonstrated altogether the coupling of the $P2X_7$ receptor to the arachidonic acid cascade in $P388D_1$ macrophages and implicated the participation of this type of receptor in LPS-induced arachidonic acid mobilization. In fact the expression of P_2 purinergic receptors that bind extracellular adenine nucleotides containing two or three phosphates has been shown to serve as a marker of macrophage activation and differentiation in response to lipopolysaccharide (Balboa *et al.*, 1999).

It must be added that a separate test meant to inquire the possible cleavage of seminolipid by bovine seminal vesicle fluid indicated negative results.

CONCLUSIONS

1. A complex interaction between seminolipid (1-O-alkyl-2-O-acyl-*sn*-3-(ß-D-galactopyranozil 3'-sulfate)glycerol) and phosholipase A_2 from bovine seminal vesicle fluid has been demonstrated.

2. Low concentrations of seminolipid (0.1–10 µM and even 50 µM) significantly activated phosholipase A_2 while higher concentrations were inhibitory.

3. Seminolipid was not cleaved by the enzyme in conditions where lecithin was rapidly cleaved.

4. Interesting is also the activating influence of seminolipid on phosholipase A_2 from bovine seminal vesicle fluid was noticed.

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Institute of Biology
Spl. Independenţei nr. 126, P.O.Box 56–53,
Bucharest 79651, Romania

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